WCRC - 7

Book of Papers

WORLD COTTON
RESEARCH CONFERENCE-7

INTERNATIONAL COTTON ADVISORY COMMITTEE

ICRA
INTERNATIONAL COTTON RESEARCHERS ASSOCIATION

4-7 October 2022, Cairo, Egypt

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PREFACE

The World Cotton Research Conference is held once every four years in different cotton-growing countries. Cairo is hosting the seventh edition. Previous conferences were held in Australia (1994), Greece (1998), South Africa (2003), the USA (2007), India (2011), and Brazil (2016). With my presence at this conference, I can proudly declare that I am fortunate to have attended all seven WCRCs. Each of the previous conferences exuded its own charm. I enjoyed all of them. They not only provided a platform for the global cotton fraternity to meet but enabled cotton researchers across the world to develop friendships and professional linkages. I am personally aware of several international collaborative research projects that were conceptualized at the conferences and eventually reached fruition to benefit the cotton world.

Einstein once said 'most people say that it is the intellect which makes a great scientist. They are wrong. It is character.' Conferences such as these help us to meet these characters with character. We publish our research as scientific papers. We read papers. We may know the names but not the characters behind the names. It is research conferences which allow us to meet fellow scientists, interact and learn their insights, their passion, their curiosity, and their wisdom. This conference, the WCRC brings us together so that we can present our work, meet our fellow cotton scientists in person and discuss with them to develop professional bonds.

WCRC was the brainchild of Dr. Rafiq Chaudhry, former Head technical information section of the ICAC. He initiated the WCRC and spearheaded six conferences successfully over 22 years. Dr. Rafiq also created the International Cotton Researchers Association (ICRA) as a body of scientists who could conduct the WCRC. The first six WCRCs were conducted by the ICAC. This seventh edition is a joint effort of the ICAC and ICRA. Four leaders, Dr. Michel Fok, former Chair of ICRA, Dr. Mohamed Negm, the current Chair of ICRA, Dr. Eric Hequet, Vice-Chair and Dr. Khalid Abdullah, President of ICRA Secretariat, Pakistan, deserve accolades for coordinating all the conference activities on the ground. The International organizing committee, chaired by Mr. Kai Hughes, Executive Director, ICAC supported the conference well. My colleagues Mr. Mike McCue, Ms. Caroline Taco, Ms. Lorena Ruiz, and members of the ICRA Executive committee chipped in many times to ensure the success of the conference.

We are grateful to Dr. Kater Hake, Vice President, Cotton Incorporated for the tremendous technical support and for helping with platinum sponsorship from Cotton Incorporated and Cotton leads. The ICAC and ICRA are grateful to the Ministry of Trade and Industry, H.E. Ahmed Samir, and Mr. Mohamed Khedyr, Chairman of CATGO, for hosting the conference. We thank Dr. Khalid Schuman, Executive Director, Egypt Cotton Association, and Wael Olma, Chairman Egypt Cotton Association for sponsoring World Cotton Day. We thank all the sponsors for their generosity.

Publishing this 'Book of Papers' wasn't an easy task. Dr. Michel Fok, Dr. Khalid Abdullah, Dr. Mohamed Negm and Dr. MV Venugopalan did a stupendous job as Chief Editors. The subject matter Editors worked hard to maintain quality and conformity to the WCRC standards. Editors of Plant breeding and genetics, Agronomy, Crop Protection, and Molecular Biology had to work more than other Editors, because of the large volume of papers in these disciplines. I am personally gratified to acknowledge Dr. Sabesh, Editor, Information Technology and Socio-economics, for rendering extraordinary help in not only editing articles but also personally type-set and creating this volume.

As organizers, we braved through COVID-19 and through several anxious times to reach where we are today with the conference. Dr, Mohamed Negm and his team deserve a standing ovation for retaining their cheer irrespective of all the turbulences. I congratulate them and would like to thank all the participants of the WCRC-7 for making it a grand success.

-Keshav Kranthi
Chief Scientist, ICAC
Secretary General, ICRA

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Contents of Book of Papers

CPD - Index for Earliness Evaluation and an Algorithm for Estimation of Missed Experiment in Cotton Research

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Abstract

Background *Early maturing is an important breeding objective in upland cotton improvement. The criteria for earliness were (1) Early flowering days based on germination to first flowering (EFD). (2) Fraction of first picking to the total seed cotton yield (FFP). (3) Earliness Bartlett's index (EBI) and finally (4) Earliness index of combined picking and day (CPD) which has been presented as a new and complete earliness index in this paper.*

Results *In the combined analysis of the combined experiments in different years and locations, it may be missed in one of the experiments. For example in the earliness study of combined experiments in the two years and three locations, we collected data in three regions except for the second year of the third location. Therefore, missed data were estimated and imputed by a new algorithm, and then six experiments were subjected to a combined analysis of variance.*

Keywords: *Cotton, Missed experiment, Earliness, Index*

Background

Earliness is also one of the most important objectives of cotton breeders. Early maturing cultivars not only enhance cropping intensity but also increase the income of the farmers. Early maturing cultivars cause less fertilizer, irrigation, and insecticide applications and avoid cotton crops from white fly attack, disease buildup, soil moisture depletion, and frost damage. Early harvesting of early maturing cultivars also leaves fields and facilities for the next cultivation (Hosseini, 2017a).

Early maturing also has a relative component when it interacts with management practices. For example, in the rainy regions planting may be delayed to coincide with the bloom period with increased rainfall conditions. Delaying in planting also will minimize boll weevil reproduction following their spring emergence from diapauses (Hosseini, 2017b).

The criteria for earliness were (1) Early flowering days based on germination to first flowering (EFD). (2) Fraction of first picking to the total seed cotton yield (FFP). (3) Earliness Bartlett's index (EBI) and finally (4) Earliness index of combined picking and day (CPD) which has been presented as a new earliness index in this paper. A comparison of the four mentioned formulae shows the effects of weight and time in the last accurate formulae (Hosseini, 2017c). If the earliness criteria of cotton cultivars were compared in the inappropriate condition of cotton growth, the environmental and management suppression factors like soil moisture, soil fertility level, pest pressure, temperature, and cloudy weather can affect earliness criteria which are controlled by additive quantitative genes despite of dominant qualitative genes. (Hosseini, 2020).

In addition to the CPD index, a new algorithm has been also offered for the estimation of missed experiments in the combined analysis of multi-experiments in the different locations and years in this paper.

Results and discussion

Comparison of the three mentioned formulae as FFP, EBI, and CPD in this research showed that the last formulae despite FFP and EBI indexes which are made from the only weight of seed cotton yield have been made by both weight of seed cotton yield and days to picking. For this reason, it can show the genetic effect of earliness very well and also separate the effect of management and environmental effects that are suppressing genetic effects. For example in the current research CPD index despite the FFP and EBI is showing the varieties such as new earliness genotype as a late-planted or planted timeliness in the first and second ranking of earliness (Table 1).

Table 1 Results of ANOVA to characterize early maturing cotton cultivars

SOV	DOF	EFD	FFP	EBI	CPD	Yield(Kg/h)
Replication	3	0.667	19.628	0.000	62.11	1048422.6
Variety	6	121.036**	462.255**	0.012**	6011.79**	992047.5*
Error	18	0.639	30.610	0.001	53.797	342356.1
CV%		1.71	6.58	3.01	5.77	16.37
Duncan's Mean Comparison		7 = 55.00 A 6 = 53.75 A 5 = 45.50 B 4 = 45.25 B 3 = 44.50 B 2 = 42.00 C 1 = 41.00 C	1 = 94.81 A 7 = 93.34 AB 2 = 91.00 AB 5 = 82.94 AB 4 = 81.92 B 3 = 81.36 B 6 = 63.32 C	1 = 0.9714 A 7 = 0.9667 A 2 = 0.9550 A 5 = 0.9147 A 4 = 0.9096 A 3 = 0.9068 A 6 = 0.8166 B	1 = 182.7 A 2 = 167.3 B 7 = 144.5 C 4 = 109.3 D 3 = 108.6 D 5 = 103.5 D 6 = 74.25 E	1 = 4305 A 3 = 3939 AB 4 = 3917 AB 7 = 3470 ABC 2 = 3355 ABC 5 = 3222 BC 6 = 2853 C

Varieties: 1= New earliness genotype, 2 = Sindose-80, 3 = Varamin, 4 = Oltan, 5 = Sahel, 6 = Arya, 7 = Late planted new earliness genotype.

EFD = Early Flowering Days, FFP = Fraction of First Picking to the total seed cotton yield,
EBI = Earliness Bartlett's index, CPD = Earliness index of Combined Picking and Days

*, ** = Significant at 0.05 and 0.01 probability levels, respectively.

A combined analysis of variance (Gomez et al., 84) is carried out if the errors of each experiment at the locations and years are homogeneous. Therefore, for testing the homogeneity of error variances, each experiment at a given location or year is analyzed separately using Bartlett's Chi-square test and both tests allowed combined multiple analyses of variance (Table 2). Estimated values make the error sum of square zero in the individual experiment and minimum in the combined multiple analysis of variance.

$$\text{Hartley's Ratio Test} = \frac{S^2_{max}}{S^2_{min}} = \frac{92.686}{34.788} = 2.66$$

$$\text{Bartlett Test} = \frac{1}{1+\frac{1}{3(k-1)}\left(\frac{1}{df_i}+\frac{1}{df_i}+\frac{1}{df_i}+\frac{1}{df_i}+\frac{1}{df_i}-\frac{1}{df_p}\right)}\left[df_p \times lnS^2_p - \sum\left(df_i \times lnS^2_i\right)\right]$$

$$S^2_p = \frac{\sum(df_i \times S^2_i)}{df_p}$$

$$S^2_p = \frac{\sum(df_i \times S^2_i)}{df_p} = \frac{3837.45}{75} = 51.165$$

$$x^2 = \frac{1}{1+\frac{1}{3(5-1)}\left(\frac{1}{15}+\frac{1}{15}+\frac{1}{15}+\frac{1}{15}+\frac{1}{15}-\frac{1}{75}\right)}[75 \times 3.935 - 289.845] = 5.147$$

Table 2. Hartley and Bartlett's chi-square test for homogeneity of error variances.

Sources	DF	Tehran		Moghan		Mashhad		Bartlett's index	Hartley's index
		Year 1	Year 2	Year 1	Year 2	Year 1	Missed		
Replications	3	29.167	27.375	78.486	184.732	60.723	10.562		
Treatments	5	468.267	469.542	314.242	235.367	514.267	227.715	5.147	2.66
$MS_E = S^2_i$	df_i=15	34.788	36.808	92.686	50.189	41.356	0.000		
CV%		7.14	7.35	18.86	18.36	8.18	0.000		

χ^2 for 4 degrees of freedom in 95% probability = 9.49
Harley's Ratio table for (5,15) in the 95% probability is 4.37

Table 3. Mean squares from ANOVA for earliness (%) in cotton cultivars

Sources	DF	MS	F-Values	Prob	CV%
Year	1	2384.694	55.9265	0.0000	
Location	2	18378.813	421.0247	0.0000	
Year ×Location	2	590.965	13.8595	0.0000	
R(YL)	18(- $\frac{rt}{l}$ = $\frac{4\times6}{6}$ =14)	65.079(83.673)	1.5262	0.0992	9.79
Earliness	5	1360.95	31.9174	0.0000	
Year × Earliness	5	69.111	1.6208	01626	
Location × Earliness	10	235.663	5.5268	0.0000	
Year × Location ×Earliness	10	161.382	3.7848	0.0003	
Error	90 (- rt = 66)	42.64(58.145)			

A combined analysis of variance shows significant differences among the cultivated cultivars and varieties, location, year × location, year ×treatment, and year × location ×treatment and non-significant between location × earliness effects for earliness (Table 3). Missed value estimations caused the values of $\frac{rt}{l}$ = $\frac{4\times6}{6}$ =14 and rt = 66 deducted from R(YL) and Error and F-test should be done after the adjustment of the two above-mentioned values. Varamin, Moghan, and Mashhad sites in the two different years had specific different environments for specific cotton cultivars and these sites are considered as a non-random sample from the population. Therefore their effects considered are fixed effect models (Table 4).

Duncan's mean comparison is carried out by three categories of comparisons: two means normal (2-N), one mean normal and other mean including missed value (1-M), and both two means including missed values (2-M) (Hosseini, 2012). Their least significant ratios for required ranges are computed by related following formulae and briefed in (Table 5).

$S_{\bar{x}}$ Estimation for two normal means:

$$S_{\bar{x}} = \sqrt{\frac{MS_E}{r(l-1)}} = \sqrt{\frac{58.145}{4(6-1)}} = 1.705$$

$S_{\bar{x}}$ Estimation for one missed mean:

$$S_{\bar{x}} = \sqrt{\frac{MS_E\left(\frac{2}{r(l-1)}+\frac{t}{r(r-1)(t-1)(l-1)}\right)}{2}}$$

$$S_{\bar{x}} = \sqrt{\frac{58.145\left(\frac{2}{4(6-1)}+\frac{6}{4(4-1)(6-1)(6-1)}\right)}{2}} = 1.868$$

$S_{\bar{x}}$ Estimation for two missed means:

$$S_{\bar{x}} = \sqrt{\frac{MS_E\left(\frac{1}{(l-1)}+\frac{1}{(l-1)}\right)}{2}} = \sqrt{\frac{\frac{2MS_E}{(l-1)}}{2}} = \sqrt{\frac{\frac{2\times58.145}{(6-1)}}{2}} = 3.411$$

Table 4. Critical values at a 1% significance level for Duncan's multiple range test in N= normal and M= missed value condition of means.

DF = 66	2	3	4	5	6	7	8	9	10	11	12	13	14
SSR	3.75	3.91	4.02	4.11	4.16	4.22	4.26	4.3	4.33	4.38	4.4	4.43	4.45
LSR.2-N	6.398	6.668	6.858	7.009	7.095	7.197	7.265	7.333	7.387	7.475	7.555	7.553	7.587
LSR.1-M	7.010	7.306	7.514	7.679	7.773	7.885	7.960	8.034	8.093	8.189	8.277	8.275	8.331
LSR.2-M	12.8	13.340	13.721	14.023	14.193	14.398	14.534	14.671	14.778	14.954	15.114	15.111	15.179

Duncan's mean comparison shows that new earliness genotype-II and I allocated the first ranking of the earliness percentage with 75.75% and 71.83% in three regions respectively. In the three studied sites, Varamin had the best ranking of 82.56% of earliness and for interaction effects of treatment × location genotype-I and II with 94.75% and 91.25% respectively, and interaction effects of

cultivar and varieties in the three regions and two years genotypes-I had the best ranking with 94.75%. More comparisons are available in Table 5.

Table 5. Duncan's mean comparison of earliness (%) in cotton cultivars.

Source	Mean	Rank	Source	Mean	Rank	Source	Mean	Rank
Year	1= 70.74	A	Location	1= 82.56	A	Y× L= (1×1)	82.58	A
	2= 62.60*	B		3= 72.63*	B	2× 2	82.54	A
				2= 44.81	C	2× 1	78.58	A
						3× 2	66.67*	B
						1× 2	51.04	C
						3× 1	38.58	D
	2= 75.75*	A	L×T(1×1)	94.75	A	Y×L×T = (1×1×1)	94.75	A
Earliness	1= 71.83*	AB	1×2	91.25	A	2×1×1	94.74	A
	4 = 68.71*	ABC	3×2	84*	B	1×3×2	92.25	A
	3= 68.13*	ABC	1×5	82.88	B	2×1×2	91.50	A
	5= 60.08*	BC	1×4	82	B	1×1×2	91	A
	6= 55.5*	C	1×3	80.88	B	1×3×3	87.5	B
			3×3	77.75*	B	1×3×4	83.75	B
			3×4	76.25*	B	1×1×5	83	B
			3×1	73.63*	B	2×1×5	82.75	B
			1×6	63.63	C	1×1×4	82	B
			3×6	63.13*	C	2×1×4	81.9	B
			3×5	61*	C	1×1×3	81.25	B
			2×2	52	D	2×1×3	80.5	C
			2×4	47.88	D	2×3×2	75.75*	C
			2×1	47.13	D	1×3×1	75.5	C
			2×3	45.75	D	2×3×1	71.75*	D
			2×6	39.75	E	1×3×6	70.5	D
			2×5	36.38	E	2×3×4	68.75*	D
						2×3×3	68*	D
						2×1×6	63.75	E
						1×1×6	63.5	E
						1×3×5	62	E
						1×2×2	60	E
						2×3×5	59.9*	E
						1×2×4	59.8	E
						2×3×6	55.75*	F
						1×2×1	55	G
						1×2×5	48	H
						2×2×3	46	H
						1×2×3	45.5	H
						2×2×2	44	H
						2×2×6	41.75	H
						2×2×1	39.25	I
						1×3×1	37.75	I
						2×2×4	35.75	I
						2×2×5	24.75	J

Conclusion

A comparison of the two conventional methods along with the new earliness index (CPD) showed the effects and deficiency of one of the time or weight factors in the FFP as a prevalent index and EBI, EFD methods respectively, but playing roles of both weight and time in the CPD accurate index makes it complete index for evaluation of different earliness genotypes and cultivars by expression of their real gene effects beyond of environmental effects like different soil moisture, soil fertility level, pest pressure, temperature, and cloudy weather. These environmental effects can affect earliness criteria which are controlled by additive quantitative genes despite dominant qualitative genes. For this reason, the CPD index is the best formulae for the estimation of earliness in cotton for illustrating the genetic effect of earliness very well and also separates the effect of management and environmental effects which are playing as suppressing factors (Hosseini, 2017a). For example, if we are comparing the two new

earliness genotypes by the same FFP= 0.75 but two different first picking dates as P1= 4 months and 5 months after germination for the genotype of I and II respectively, and last pickings have been done for both 7 months after germination. For adjustment of the FFP formulae, in the CPD index, we are affecting time factors 7(30) ÷ 4(30) = 1.75 and 7(30) ÷ 5(30) = 1.4 of the first and second genotype to their FFP as following which is showing genotype-I is more earliness than the Genotype II.

Genotype I = 0.75 × 1.75 = 1.31 × 100 = 131

Genotype II = 0.75 × 1.4 = 1.05 × 100 = 105

In the present study FFP index ranked the New Earliness Genotype (1 = 94.81) with sindose-80 (2 = 91.00) in the same class but the CPD index which is affected by both weight and time factors put the above-mentioned genotypes (1 = 182.7 and 2 = 167.3) in separate classes.

The main purpose that trials carried out on the specific sites simultaneously was to find compatible early maturing cotton genotypes for particular regions of Varamin, Moghan, and Mashhad. Analysis of variance showed significant differences among treatments for earliness trait and related Duncan's mean comparison showed that newly developed genotypes had the first ranking of early maturing.

Methods

In this study, evaluation of two early maturing cotton genotypes viz. Hosseini-I and Hosseini-II which had been developed by hybridization and backcross methods are being reported. An initial cross between Sindise-80 as an early maturing parent and Red leaf was attempted in 2005. It was followed by selection for superior and early maturing segregants till 2010. In 2011, early maturing genotypes from this cross were hybridized with Sindose-80, and selection for superior and early maturing genotypes was conducted till 2015. The new early maturing genotype identified from this cross has been named genotype I. The same processes have been done between sindose-80 and Bulgare-557 and its early maturing genotype was identified II (Hosseini, 2014).

Bulgare-557× Sindose-80 (2005)

▼

Selection (2006-2010)

▼

Back cross: New earliness genotype× Sindose-80 (2010)

▼

Selection (2011-2015)

▼

New earliness genotype

The criteria and indexes which have been calculated for evaluation of earliness were; (1) Early flowering days based on days from germination to first flowering (EFD). (2) Fraction of first seed cotton picking to the total seed cotton yield (FFP). (3) Earliness Bartlett's index (EBI) and finally (Bartlett, 1979). (4) The new earliness index of combined picking and day (CPD) which is combined by both days to picking and weight of seed cotton picking as weighted new earliness index. The four formulae are computed as followings:

Early Flowering Days (EFD)

$$\text{Fraction of First Picking (FFP)} = \frac{P_1}{P_1+P_2+\cdots\ldots\ldots+P_n} \times 100$$

And Earliness is measured by adopting Bartlett's (1973) given as under:

$$\text{Earliness Bartlett's Index (EBI)} = \frac{P_1+(P_1+P_2)+(P_1+P_2+P_3)+\cdots\ldots\ldots+(+P_n)}{n(P_1+P_2+\cdots\ldots\ldots+P_n)}$$

Earliness Index of Combined Pickings and Days (CPD)

$$= \left\{\frac{P_1}{P_1+P_2+\cdots\ldots\ldots+P_n}\right\} \times \left\{\frac{Number\ of\ days\ to\ last\ picking}{Number\ of\ days\ to\ first\ piking}\right\}$$

Or

$$(CPD) = \left\{\frac{P_1}{P_1+P_2+\cdots\ldots\ldots+P_n}\right\} \times \left\{\frac{Number\ of\ days\ to\ last\ picking}{Number\ of\ days\ to\ first\ piking}\right\} \times 100$$

Where (P1, P2, Pn) is the weight of seed cotton picked during the first and second and n is the total number of pickings.

Among the test entries, “early” genotypes I and II were ready for first picking about an average of 110 days after germination. The first picking of the remaining varieties was conducted about 169 days after germination and the last picking of all varieties was accomplished about 182 days after germination.

Related to the second issue or offering an algorithm for estimation of missed experiments in the combined analysis, the new earliness genotypes identified from the above-mentioned crosses were compared with four native cotton cultivars in RCBD with four replications in the three regions of Varamin, Moghan, and Mashhad. The followed formula in this study was the FFP method for earliness comparison based on the approved project. Early maturing data were collected from Varamin, Moghan, and Mashhad regions during 2016-2017, except for the second year of the Mashhad region which is considered a missed experiment and is subjected to values estimation. Missed values of the lost experiment as the sixth experiment ($Exp'_{l=6}$) were estimated and imputed using available data (Table 6). Therefore data set was completed as:

$$\text{Missed plots estimation} = X'_{lij} = \frac{(t\sum_{i.j}X + r\sum_{li.}X) - \sum_{l...}X}{rt(l-1)}$$

$$X'_{611} = \frac{6(379+\cdots+302)+(488+\cdots+454)-(1982+\cdots+1886)}{4\times6\times(6-1)} = 73$$

$$X'_{612} = \frac{6(364+\cdots+369)+(488+\cdots+454)-(1982+\cdots+1886)}{4\times6\times(6-1)} = 77$$

$$X'_{621} = \frac{6(379+\cdots+302)+(483+\cdots+459)-(1982+\cdots+1886)}{4\times6\times(6-1)} = 70$$

$$X'_{622} = \frac{6(364+\cdots+369)+(483+\cdots+459)-(1982+\cdots+1886)}{4\times6\times(6-1)} = 74$$

$$\vdots$$

$$X'_{646} = \frac{6(254+\cdots+282)+(513+\cdots+477)-(1982+\cdots+1886)}{4\times6\times(6-1)} = 56$$

Table 6. Observed and estimated data of six cotton genotypes and cultivars for earliness (%)

$Exp_{l=1}$		T_1	T_2	T_3	T_4	T_5	T_6	
	R_1	$X_{lij=111}$ = 94	X_{112} = 89	77	73	90	65	$X_{11.}$ = 488
Year = 1	R_2	X_{121} = 96	91	68	87	79	62	$X_{12.}$ = 483
Site = 1	R_3	96	92	90	82	77	61	$X_{13.}$ = 498
	YR_4	93	92	90	86	86	X_{146} = 66	$X_{14.}$ = 513
		$X_{1.1}$ = 379	$X_{1.2}$ = 364	$X_{1.3}$ = 325	$X_{1.4}$ = 328	$X_{1.5}$ = 332	$X_{1.6}$ = 254	$X_{1..}$ = 1982
$Exp_{l=2}$		T_1	T_2	T_3	T_4	T_5	T_6	
	YR_1	$X_{lij=211}$ =95	X_{212} = 91	76	72	90	66	$X_{21.}$ = 490
Year = 2	YR_2	X_{221} = 95	91	68	88	79	62	$X_{22.}$ = 482
Site = 1	YR_3	96	92	89	82	76	60	$X_{23.}$ = 495
	YR_4	93	92	89	86	86	X_{246} = 67	$X_{24.}$ = 513

		$X_{2.1}$ = 379	$X_{2.2}$ = 366	$X_{2.3}$ = 322	$X_{2.4}$ = 328	$X_{2.5}$ = 331	$X_{2.6}$ = 255	$X_{2..}$ = 1981
$Exp_{l=3}$		T_1	T_2	T_3	T_4	T_5	T_6	
	YR_1	$X_{ij=311}$ =69	X_{312} = 68	48	60	48	33	$X_{31.}$ = 326
Year = 1	R_2	X_{321} = 39	59	46	50	37	49	$X_{32.}$ = 280
Site = 2	R_3	49	57	40	74	65	37	$X_{33.}$ = 322
	R_4	63	56	48	56	42	X_{346} = 32	$X_{34.}$ = 297
		$X_{3.1}$ = 220	$X_{3.2}$ = 240	$X_{3.3}$ = 182	$X_{3.4}$ = 240	$X_{3.5}$ = 192	$X_{3.6}$ = 151	$X_{3..}$ = 1225
$Exp_{l=4}$		T_1	T_2	T_3	T_4	T_5	T_6	
	R_1	$X_{ij=411}$ =53	X_{412} = 48	55	37	37	46	$X_{41.}$ = 276
Year = 2	R_2	X_{421} = 39	51	47	51	19	31	$X_{42.}$ = 238
Site = 2	R_3	35	39	39	25	23	46	$X_{43.}$ = 207
	R_4	30	38	43	30	20	X_{446} = 44	$X_{44.}$ = 205
		$X_{4.1}$ = 157	$X_{4.2}$ = 176	$X_{4.3}$ = 184	$X_{4.4}$ = 143	$X_{4.5}$ = 99	$X_{4.6}$ = 167	$X_{4..}$ = 926
$Exp_{l=5}$		T_1	T_2	T_3	T_4	T_5	T_6	
	R_1	$X_{ij=511}$ =66	X_{512} = 89	84	84	58	73	$X_{51.}$ = 454
Year = 1	R_2	X_{521} = 76	92	88	89	47	67	$X_{52.}$ = 459
Site = 3	R_3	79	94	92	85	78	68	$X_{53.}$ = 496
	R_4	81	94	86	77	65	X_{546} = 74	$X_{54.}$ = 477
		$X_{5.1}$ = 302	$X_{5.2}$ = 369	$X_{5.3}$ = 350	$X_{5.4}$ = 335	$X_{5.5}$ = 248	$X_{5.6}$ = 282	$X_{5..}$ = 1886
$Exp'_{l=6}$		T'_1	T'_2	T'_3	T'_4	T'_5	T'_6	
	R'_1	$X'_{ij=611}$ =72.98	X'_{612} = 76.88	69.28	69.83	61.23	56.58	$X'_{61.}$ = 406.8
Year = 2 '	R'_2	X'_{621} = 69.95	73.85	66.25	66.8	58.2	53.55	$X'_{62.}$ = 388.6
Site = 3 '	R'_3	72.45	76.35	68.75	69.3	60.7	56.05	$X'_{63.}$ = 403.6
	R'_4	72.02	75.92	68.32	68.87	60.27	X'_{646} = 55.62	$X'_{64.}$ = 401
		$X'_{6.1}$ = 287.4	$X'_{6.2}$ = 303	$X'_{6.3}$ = 272.6	$X'_{6.4}$ = 274.8	$X'_{6.5}$ = 240.4	$X'_{6.6}$ = 221.8	$X'_{6..}$ =1600
Sites	Site.1: Varamin		Site.2: Moghan			Site.3: Mashhad		
Y Treatments	T_1 = genotype I	T_2 =Genotype II	T_3 = Varamin	T_4 = Oltan	T_5 = Sahel	T_6 = Arya		

After testing the homogeneity of individual experiment's error variances using Hartley and Bartlett's Chi-square method, data were subjected to the combined analysis of variance following (Little et al., 2002] and (Snedecor et al., 1989) by a fixed model of treatment (Steel et al., 1997). Earliness Means was compared with Duncan's multiple range tests.

References

1. Gomez, K. W. and Gomez A.A. (1984). Statistical procedures for agricultural research. John Wiley & Sons. 680p.
2. Hosseini, GH. (2012). Applied Parametric Statistics. Rahnama press. ISBN: 978-964-367-503-5.p.118.
3. Hosseini, GH. (2014). Estimation of genetic parameters for quantitative and qualitative traits in cotton cultivars (Gossypium hirsutum L. & Gossypium barbadenseL.) and new scaling test of additive – dominance model. Journal of plant molecular breeding. Vol 2. No. 1. 45-63.
4. Hosseini, GH. (2017a). Earliness and suppression of its additive genetic effects by environmental and management factors in cotton (Gossypium hirsutum L.). International Journal of Agricultural Policy and Research. 5 (7).pp.125-128.
5. Hosseini, GH. (2017b). Evaluation of elite lines of upland cotton (Gossypium hirsutum L.) for earliness in Iran. Agricultural Research Journal. 54 (4).pp.578-579.
6. Hosseini, GH. (2017c). New earliness index and integration of earliness additive genes in the new genotype of cotton (Gossypium hirsutum L.). Int. J. Life. Sci. Scienti. Res., 3(3).pp.1016-1019.
7. Hosseini, GH. (2020). Evaluation of two early maturing cotton genotypes in three ecological regions of Iran. Agricultural Research Journal. 57 (1).
8. Little, R.J.A. and Rubin, D.B. (2002). Statistical Analysis with Missing Data, 2nd edition, New York: John Wiley
9. Snedecor, G.W., and W.G. Cochran. (1989). Statistical Methods. 8th ed. Iowa State Univ. Press, Ames.p.503.
10. Steel, R.G.D., J.H. Torrie, and D.A. Dickey. (1997). Principles and Procedures of Statistics: A biometrical approach. McGraw-Hill, New York.p.481.

Adaptation of Advanced High-Yielding Cotton Lines to Subtropical Conditions in Argentina

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Abstract

*Cotton is mainly produced in the north of Argentina, principally in the provinces of Chaco, Santiago del Estero, and Santa Fe, with stand out by planted area and by production. One of the main aims of breeders and agronomists is to recommend to farmers new agriculture production alternatives that are stable under different environmental conditions and minimize the risk of falling below a certain yield level. The objective of this study is to examine the adaptation to the subtropical northern region of Santa Fe (Argentina) of advanced cotton lines generated by the INTA national breeding program. Six advanced cotton lines from the INTA germplasm bank and three commercial varieties were evaluated in three different environments. The statistical design was completely randomized blocks for the three environments, with 3 replications for each genotype. The evaluated character was cotton fiber yield in kg*ha-1. The additive main effects and multiplicative interaction model (AMMI) were used to analyze the stability and adaptability of cotton lines. Significant differences among genotypes, environments, and GE interaction for fiber cotton yield were found. The main effect of the environment accounted for more variation than the main effect of genotypes and the GE interaction. The first multiplicative term of the AMMI model (PC1) explained 83.7 % of the GE interaction. The most stable genotype was SP 1623 and the one with the highest adaptability was SP 41255. The inter-annual difference in the same location and the difference between different locations in the same season were observed. The results showed that the best-advanced cotton line in terms of fiber cotton yield was SP 41255. It also presented adaptability and potential stability. The results also indicate that the AMMI analysis may be incorporated as a selection criterion in the routine of cotton breeding programs.*

***Keywords**: Fibre Cotton Yield, Genetic Improvement, Adaptation, AMMI Analysis, GE Interaction*

Background

Cotton is mainly produced in the north of Argentina, principally in the provinces of Chaco, Santiago del Estero, and Santa Fe, with stand out by planted area and by production (MAGYP, 2020). Different climatic and soil conditions are found in these regions; therefore, differential behaviors of the genetic materials tested under different environmental conditions are to be expected. Yield responses among certain agricultural production alternatives (genotypes, agronomic treatments, and cropping systems), when evaluated in different environments, are known as interaction. This interaction is part of the behavior of the genotype or agronomic treatment and confounds its observed mean performance with its true value (Zobel et al., 1988). A significant portion of the resources of crop breeding and agronomy programs are used to determine this interaction through replicated multilocation trials (Crossa, 1990). Consequently, the identification of cultivars with greater phenotypic stability is a strategy to reduce the effect of genotype-environment interaction (Silva Filho et al., 2008).

Crossa (1990) reviewed some of the conventional statistical analyses and stability methods for yield trials. The author presented methodologies for analyzing multilocation trials as well as multivariate analyses for assessing yield stability; such as Analysis of Variance, Joint Linear Regression, Multivariate Analyses of Multilocation Trials, AMMI Analyses, and other analyses. The additive main effect and multiplicative interaction (AMMI) method integrate analysis of variance and principal components analysis into a unified approach. It can be used to analyze multilocation trials (Crossa, 1990; Zobel et al., 1988). Additive main effects for genotypes and environments are first fitted by the analysis of variance. Then, multiplicative effects for genotype by environment interaction are calculated by principal components analysis. The biplot of the model helps to visualize the overall pattern of

response as well as specific interactions between genotypes and environments (Crossa, 1990; Duarte and Vencovsky, 1999; Zobel et al., 1988). Previous studies used this model for cotton crops (Farias et al., 2016; Maleia et al., 2017,2019; Morello et al., 2008; Pretorius et al., 2015; Silva Filho et al., 2008; Souza et al., 2006).

One of the main aims of breeders and agronomists is to recommend to farmers new agriculture production alternatives (genotypes, agronomic treatments, and cropping systems) that are stable under different environmental conditions and minimize the risk of falling below a certain yield level (Crossa, 1990). The National Institute of Agricultural Technology (INTA) has a national breeding program that generates new varieties with increasing performance for production, quality, and disease resistance (Poisson et al., 2005; Royo et al., 2007). The objective of this study is to examine the adaptation to the subtropical northern region of Santa Fe (Argentina) of advanced cotton lines generated by the INTA national breeding program.

Results

The joint ANOVA analyses (Table 1) revealed a significant difference among genotypes (<0.01), environments (<0.001), and GE interaction (<0.01) for fiber cotton yield. This result was due to the edapho-climatic conditions or the agronomic management inherent to each environment that influences the analyzed character. The main effect of environment accounted for more variation than the main effect of genotypes and the GE interaction, with 75.3 %, 15.8 %, and 8.8 % sum of squares for environments, cultivars, and GE interaction, respectively (unexposed data). Table 1 also shows the multiplicative decomposition (AMMI) of the GA interaction. This showed significant differences ($p < 0.001$) in the first multiplicative term of the AMMI model (PC1) due to the inconsistency in the performance of the genotypes in the different environments evaluated and explained 83.7 % of the detected interaction. The PC1 scores (Table 2) assist in understanding interaction, improving the accuracy of yield estimates, and increasing the probability of successfully selecting cultivars with higher yields. Table 2 also shows the fiber performance ranking of the genotypes tested and the significant differences among them (Tukey's test), demonstrating that the genotype also influenced the cotton fiber yield. The most stable genotypes, with less contribution for the GE interaction captured by the axis PC1, were SP 1623 (PC1= -0.3266), SP 187 (PC1= -1.9412), SP 1276 (PC1=1.9774), and the commercial variety NuOpal (PC1= 0.4051). Among the advanced cotton lines, SP 41255 (1085.8 kg*ha-1) and SP 1623 (961.8 kg*ha-1) showed a higher value than the average fiber cotton yield, indicating those were the most adaptable.

Biplot Graph (Figure 1) shows the first PC scores versus the cultivar and environment means. Stability was interpreted from the ordinate axis (PC1 axis), with scores close to zero considered stable genotypes. Adaptability was interpreted from the abscises axis, where the means of genotypes and environments are plotted (Gauch and Zobel, 1988). Therefore, genotypes furthest from the origin contribute more to the interaction, such as the DP 1238 and SP 6582 genotypes. Combinations of genotypes and environments with PC1 scores of the same sign have specific positive interactions. Consequently, specific adaptations to environments could be observed in the DP 1238 genotype to environments E1 (Reconquista 2016-2017) and E2 (Campo Hardy 2017-2018), and specific negative adaptation with environment E3 (Reconquista 2017-2018). In regards to the environments, E1 showed the highest mean value for cotton fiber yield (1199.1 kg*ha-1). In the same season (2017-2018), the Reconquista location had a higher environmental mean value (823.6 kg*ha-1) than Campo Hardy (806.6 kg*ha-1). In the Reconquista location, there was an interannual variation; consequently, in the biplot graph seasons, 2016-2017 and 2017-2018 (E1 and E3) are dispersed in different quadrants.

Table 1: Joint ANOVA and AMMI GA for fiber cotton yield.

Effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Environments	2	2657333	1328666	101.042	< 2e-16 ***
Genotypes	8	312650	39081	2.972	0.00873 **
Blocks(Environments)	6	245514	40919	3.112	0.01175 *
Environments x Genotypes	16	558302	34894	2.654	0.00465 **
PC1	9	467282	51920	3.948	0.00081 ***
AMMI1 Residuals	7	91020	13002	0.988	0.45063
Total Residuals	48	631185	13150		

Significances: *** 0.001 ** 0.01 * 0.05

Table 2: Yield of cotton fiber and contribution of the first component of the AMMI model.

Effect		Fibre Yield (kg*ha^{-1})	PC1
Genotypes	SP 41255	1085.8 a	2.6048
	NuOpal	989.1 ab	0.4051
	SP 1623	961.8 ab	-0.3266
	SP 6582	939.4 ab	7.1640
	DP 1238	934.4 ab	-17.3419
	SP 187	925.1 ab	-1.9412
	SP 152	909.6 b	3.8903
	SP 1276	876.4 b	1.9774
	DP 402	866.1 b	3.5679
Environments	Reconquista 2016-2017	1199.1	-5.8961
	Reconquista 2017-2018	823.6	16.0346
	Campo Hardy 2017-2018	806.6	-10.1385
Grand Mean		943.1	

PC1 = Principal Component 1; Tukey test: different letters indicate significant differences ($p < 0.05$)

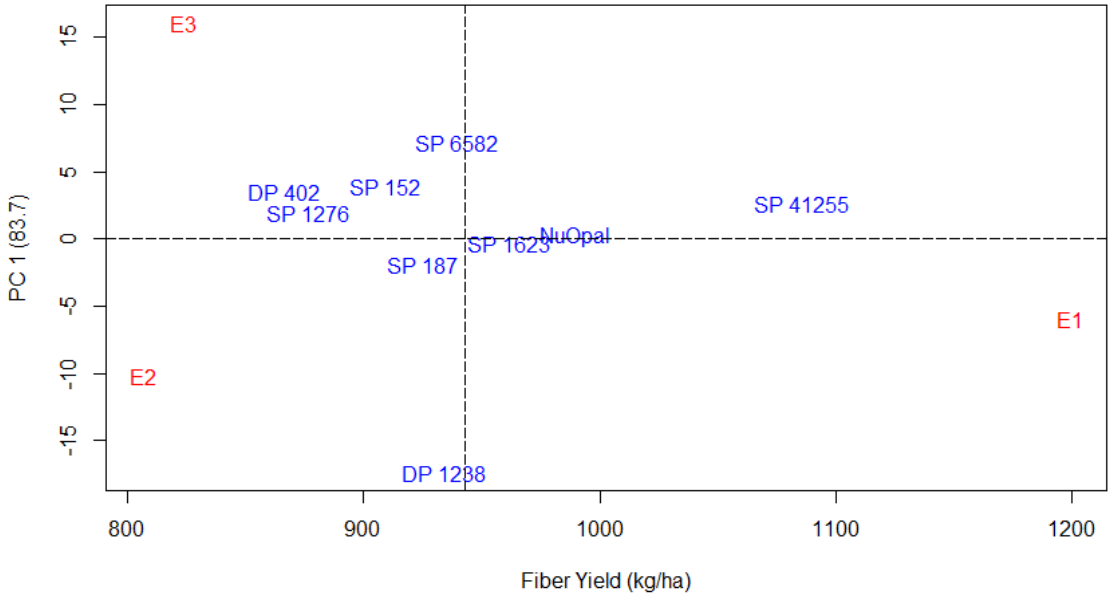

***Fig 1**:Graphical representation of AMMI1 biplot (mean vs. first axis of GA interaction, PC1) for fibre yield data (kg * ha^{-1}) of nine cotton genotypes (DP 1238, DP 402, NuOpal, SP 6582, SP 1276, SP 1276, SP 1623, SP 187 and SP 41255) tested in three environments: E1: Reconquista (2016-2017), E2: Campo Hardy (2017-2018) and E3:Reconquista (2017-2018).*

Discussions

Genotype, environment, and GE interaction effects were also observed by Morello et al. (2008), Pretorius et al. (2015), Farias et al. (2016), and Maleia et al. (2017) evaluating cotton genotypes in multi-environmental trials. In agreement with them, the GE interaction accounted for less variation than the main effect of the environment, showing that the environment had a greater effect on fiber cotton yield than either genotype or GE interaction. The significant GE interaction shows that some genotypes had better performance in one environment and low performance in others, which provides a change in their performance standard under the environmental variation; this is often observed in a complex (multigenic) trait such as fiber cotton yield (Maleia et al., 2019; Maleia et al., 2017).

In the decomposition of GE interaction into principal components (Table 1), only the first (PC1) was significant. Thus, the first interaction axis (PC1) was sufficient to describe the pattern associated with the GA interaction. The same result was obtained by Morello et al. (2008) whereas other studies found significance for the first two multiplicative terms (PC1; PC2) of the model (Maleia et al., 2017; Pretorius et al., 2015; Silva Filho et al., 2008; Souza et al., 2006)

The AMMI Biplot Graphic (Figure 1), shows that SP 41255 and SP 1623 had a higher fiber cotton yield than the average value among the advanced cotton lines. Standing out the advanced line SP 41255 had the highest average for the trait. The lower contribution for the GE interaction captured by the axis PC1 was for SP 1623, SP 187, SP 1276, and the commercial variety NuOpal; where SP 1623 presented the lowest value. Therefore, the genotype with the highest adaptability was SP 41255 and the one with the highest stability was SP 1623.

In the biplot graph, it was also observed that the studied environments (E1, E2, and E3) were located in different quadrants. This is due to variations in different edapho-climatic conditions and different agronomic management. On the one hand, a year-on-year difference was observed in environments E1 (Reconquista 2016-2017) and E3 (Reconquista 2017-2018), mainly due to climatic differences with greater water availability and global radiation in the E1 environment (Scarpín et al., 2018). Maleia et al. (2017) found similar results when studying the GE interaction of different genotypes in Mozambique. On the other hand, the differences in environmental means obtained between different locations in the same season (E2 and E3) could be attributed to differences in agronomic management, soil type, and climatic conditions (mainly water availability).

Conclusions

The results showed that the best-advanced cotton line in terms of fiber cotton yield was SP 41255. It also presented adaptability and potential stability. The AMMI analysis is an important tool to determine production levels with high adaptability and stability in cotton lines. The results indicate that it may be incorporated as a selection criterion in the routine of cotton breeding programs.

Methods

Genotypes, location, and seasons

Six advanced cotton lines from the INTA Sáenz Peña germplasm bank were evaluated: SP 1276, SP 152, SP 1623, SP 187, SP 41255, SP 6582, and three commercial varieties NuOpal BG RR, DP 1238 BG RR, and DP 402 BG RR. The genotypes were evaluated by comparing them during 2 seasons (2016-2017 and 2017-2018) at the Agricultural Experiment Station of the National Institute of Agricultural Technology (INTA) in Reconquista (29°15'56.19"S, 59°44'32.14"O) and 1 season (2017-2018) in Campo Hardy (28°7'19.69"S, 59°17'35.06"O). Three different environments were evaluated through the combination between locations and seasons.

Experimental design and management

The statistical design was completely randomized blocks for the three environments, with 3 replications for each genotype. It was sown manually in 4 rows at 52 cm spacing in 12.5 m2 plots, with the two central rows being considered for harvesting and evaluation. The planting density in each plot was equivalent to 180,000 plants*ha-1. Fertilizer was applied according to the soil analysis of each site. Both weeds and pests were controlled according to commercial practices.

Evaluation of variables

The evaluated character was cotton fiber yield in kg*ha-1. At the time of harvest, the production of seed cotton yield per plot was obtained, and the cotton harvested was ginned turn out, obtaining the percentage of fiber and, subsequently, the yield of cotton fiber. For the analysis of the data, the fiber yield corresponding to a moisture content of 12% was used.

Statistical analysis

The additive main effects and multiplicative interaction model (AMMI) were used to analyze the stability and adaptability of cotton lines. The analysis initially explains the main effects of genotypes and environments through a conventional analysis of variance and later partitions the source of variation corresponding to the GE interaction, through a multivariate analysis of principal components (CPI, CP2,

CPn). Tukey´s test was also used for testing the significant differences between genotypes. The AMMI method and the generated two-dimensional graph (biplot) were performed using the R (2019) software detailed by Onofri and Ciriciofolo (2007), following the next linear mathematical model:

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^{p} \lambda_k \gamma_{ik} \alpha_{jk} + \varepsilon_{ij}$$

Where, Y_{ij} = Mean yield of the i^{th} genotype in the j^{th} environment; μ = Grand mean; g_i = i^{th} genotype; e_j = j^{th} environment; k = Number of PCA axes retained in the model; λ_k = Singular value for the PCA axis, k; γ_{ik} = i^{th} genotype PCA score for the axis, k; α_{jk} = j^{th} environment PCA score for the axis, k; and ε_{ij} = Residual.

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References

1. Crossa, J., 1990. Statistical analyses of multilocation trials, Advances in agronomy. Elsevier, pp. 55-85.
2. Duarte, J., Vencovsky, R., 1999. Interação genótipos x ambientes: uma introdução à análise AMMI. Ribeirão Preto: Sociedade Brasileira de Genética.
3. Farias, F., De Carvalho, L., Da Silva Filho, J., Teodoro, P., 2016. Biplot analysis of phenotypic stability in upland cotton genotypes in Mato Grosso. Embrapa Algodão-Artigo em periódico indexado (ALICE).
4. Gauch, H.G., Zobel, R.W., 1988. Predictive and postdictive success of statistical analyses of yield trials. Theoretical and Applied genetics 76, 1-10.
5. MAGYP, 2020. Ministerio de agricultura ganaderia y pesca.
6. Maleia, M., Jamal, E., Savanguane, J., João, J., Teca, J., 2019. Stability and Adaptability of Cotton (Gossypium Hirsutum L.) Genotypes under Multi Environmental Conditions in Mozambique. J Agron Agri Sci 2, 017.
7. Maleia, M.P., Raimundo, A., Moiana, L.D., Teca, J.O., Chale, F., Jamal, E., Dentor, J.N., Adamugy, B.A., 2017. Stability and adaptability of cotton ('Gossypium hirsutum'L.) genotypes based on AMMI analysi. Australian Journal of Crop Science 11, 367.
8. Morello, C., Duarte, J.B., Da Silva Filho, J.L., Freire, E.C., Suassuna, N.D., 2008. Interação genótipo x ambiente em linhagens de algodoeiro desenvolvidas para condições de cerrado. Embrapa Algodão-Artigo em periódico indexado (ALICE).
9. Onofri, A., Ciriciofolo, E., 2007. Using R to perform the AMMI analysis on agriculture variety trials. R News 7, 14-19.
10. Poisson, J., Bonacic, I., Royo, O., Ibalo, Y., Sosa, M., Peterlin, O., 2005. Mejoramiento genético de algodón. Año Agrícola 2004/2005. Reunión Anual del Proyecto Nacional Algodón. 2. 2005 08 18-19, 18 y 19 de agosto de 2005. Sáenz Peña, Chaco. AR.
11. Pretorius, M., Allemann, J., Smith, M., 2015. Use of the AMMI model to analyse cultivar-environment interaction in cotton under irrigation in South Africa. African Journal of Agriculture 2, 76-80.
12. R, T.C., 2019. A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2019. .
13. Royo, O.M., Poisson, J., Bonacic, I., Montenegro, A., Ibalo, S., Mazza, S., Giménez, L., 2007. Direction of cotton breeding in Argentina, Plant Breeding and Genetics World Cotton Research Conference-4, Lubbock, Texas, USA.
14. Scarpín, G., Winkler, H.M., Dileo, P., Roeschlin, R., Paytas, M., 2018. Condiciones ambientales en el norte de Santa Fe, Campaña 2017/18. Publicación anual APPA. .
15. Silva Filho, J.L.d., Morello, C.d.L., Farias, F.J.C., Lamas, F.M., Pedrosa, M.B., Ribeiro, J.L., 2008. Comparação de métodos para avaliar a adaptabilidade e estabilidade produtiva em algodoeiro. Pesquisa Agropecuária Brasileira 43, 349-355.
16. Souza, A.A.d., Freire, E.C., Bruno, R.d.L.A., Carvalho, L.P.d., Silva Filho, J.L.d., Pereira, W.E., 2006. Estabilidade e adaptabilidade do algodoeiro herbáceo no Cerrado do Mato Grosso e Mato Grosso do Sul. Pesquisa Agropecuária Brasileira 41, 1125-1131.
17. Zobel, R.W., Wright, M.J., Gauch, H.G., 1988. Statistical analysis of a yield trial. Agronomy journal 80, 388-393.

Coping for Sustainable Cotton Production through Developing of Climate Resilient Cotton Varieties in Mutant's Background in Pakistan

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Abstract

Cotton has special significance in the Asia Pacific region as a leading fiber crop with its cultivation on 20.5 million hectares in three major cotton-producing countries i.e. China, India, and Pakistan. Pakistan is one of the most affected countries by climate change, with its disastrous effects of extreme periods of heat stress i.e. 40-50 % fruit abortion during 2013-14 and 33 % shortfall in Cotton Production during 2016-17. The devastating effects of climate change on cotton production in Pakistan can be further visualized during the year 2019, 25 % shortfall in area, 40.2 % in yield, and 25 % in yield per acre against the year 2004. Poor resilience of main grown GMO cotton varieties against extreme periods of heat stress is considered the main cause of this drastic fall. Using the approach of induced mutation breeding, NIAB Faisalabad, Pakistan has demonstrated its capabilities in developing cotton mutants that can withstand the changing climatic conditions. The results of thermos-tolerant cotton varieties in the background of mutants derived (i.e. NIAB-878, NIAB-545, NIAB-1048, NIAB-444, NIAB-1089, NIAB-1064, NIAB-1042 in comparison i.e. FH-142 and FH-Lalazar for their phenological & physiological traits conferring heat tolerance will be presented in the said event. NIAB-878 excelled in heat tolerance by maintaining the highest anther dehiscence (82 %) and minimum cell injury percentage (39 %) along with illustrating maximum stomatal conductance (27.7 mmol CO2 m-2s-1), transpiration rate (6.89 µmol H2O m-2s-1), net photosynthetic rate (44.6 mmol CO2 m-2s-1) and physiological water use efficiency (6.81 mmol CO2/ µmol H2O). The author would like to share the gradual adaptation potential (above 41 % share in seed production) of these climate-resilient cotton varieties among the farming community with their ability to sustain their yield under the changed climatic scenario with recovery against biotic & abiotic stresses.

***Keywords**: Sustainable, Climate change, cotton productivity, Phenological & Physiological traits*

Background

Cotton has a special significance and plays an important role in the economies of Australia, China, India, Iran (the Islamic Republic), Myanmar, Pakistan, Viet Nam, and Bangladesh. This leading fiber crop is grown on 20.5 million hectares in the three main cotton-producing countries of the Asia and Pacific region i.e. China, India, and Pakistan, with their annual contribution of about 60-65 % of total world cotton production. Bangladesh, Myanmar, Viet Nam and Iran (the Islamic Republic of), have a very nominal role in total world cotton production. However, emerging demands from Viet Nam and Bangladesh for their cotton mill use signifies the increased role of cotton production in the economy of regional countries. The huge yield gap differences exist among the top three cotton-producing countries of the region (i.e. China;1484 Kg/ha, India; 529 Kg/ha, and Pakistan; 700 Kg/ha), but also among other countries of the region (i.e. Viet Nam;453 Kg/ha, Bangladesh; 608.0 Kg/ha, Iran (the Islamic Republic of); 594 Kg/ha and Myanmar; 653 Kg/ha). These yield differences amongst regional countries are being further aggravated due to changing climate associated with higher temperature stresses. Pakistan is likely to be the country that is most affected by climate change as far as agriculture and cotton production are concerned. Year-to-year variation in yields of the cotton crop due to climate change is not only impacting the farming industry negatively but is also straining the positive development of cotton-based industries in the region. The cotton belts in countries like Bangladesh, China, Iran (Islamic Republic), and Pakistan, are located in the high-temperature zone, where the maximum temperature often exceeds 40°C during the cotton growing season. This increasing trend of temperature inhibits the

growth of the plant, causes increased photorespiration, leads to poor control of insects/pests, and enhances the requirements for inputs like irrigation and fertilizer with a higher cost of production. The disastrous effects of an extreme period of heat stress are very prominent in Pakistan during the cotton growing season 2013-14, there was early termination of the crop with 40-50 % abortion of fruiting parts. The quality of lint and cotton seed is affected in general, while seed cotton yield per hectare is particularly affected due to changing climatic conditions (high temperatures/heavy rainfalls). The sensitivity of commercial cotton varieties against extreme periods of heat stress coupled with enhanced requirements for inputs like water and fertilizer and poor seed germination is considered as major factors/causes of erratic trend of cotton production in the region. The impact of global warming on cotton production (Anonymous, 2013a, 2013b & 2013c), effects of unexpected periodic episodes of extreme heat stress on cotton in China (Liu, et al., 2006; Zhou et al., 1996) India (Anonymous. 2013c) and Pakistan are gaining significance as emerging challenges for researchers and cotton producers in coming years. Reduced pollen viability (Burke et al., 2004), the physiological response of cotton to high temperatures (Bibi et al,2003), less fertilization efficiency (Snider et al., 2009), reduced boll size and seed number per locules (Pettigrew, 2008), increased fruit shedding (Hodges et al., 1993; Reddy et al., 1991a &1999) are the main effects associated with reduction cotton yield associated due to extreme periods of heat stress. Screening of cotton cultivars for high temperature (Liu, et al.,2006), use of cellular membrane thermostability for heat tolerance in upland cotton (Rahman et al., 2004), genetic diversity for stomatal conductance in Pima cotton (Radin et al.,1994), multi-level determination of heat tolerance in upland cotton under the field conditions (Cottee, et al., 2007 & 2010), screening of upland cotton under the field conditions (Karademir et al, 2012),

High-temperature stress on floral development and yield of cotton (Oosterhuis et al, 2008 & 2011) influence and emphasize high temperature, breeding for heat tolerance in cotton to evolve heat tolerant cotton varieties (Singh et al, 2007), and multi-model projections of future climate and climate change impacts uncertainty assessment for cotton production in Pakistan (Rahman et al, 2018) are well documented in early findings. Using the approach of induced mutation breeding, NIAB, Faisalabad, Pakistan has demonstrated its capabilities in developing cotton mutants that can withstand the changing climatic scenario of heat stress. So far, NIAB has developed 14 cotton cultivars through the use of induced mutation including the famous cultivar of NIAB-78, NIAB-Krishma, NIAB-111, and most recently, two high-yielding and fine fiber cotton varieties i.e. NIAB-KIRAN and NIAB-878, NIAB-545 & NIAB-1048. The cotton mutants, developed through the use of induced mutation, showed enhanced resilience against high temperatures under the field conditions and also showed significant variations in their rooting length after days of sowing (i.e. 30,60,90,120 and 150). Thus, the results of cotton mutants for their various phenological and physiological traits studied under the current investigation suggest, that these cotton mutants developed in the background of the induced mutation can sustain their yield against the changing climatic scenario of high temperature being presented under the present studies.

Results

The results of various phenological attributes conferring heat tolerance i.e. fruit retention capacity/ number of bolls, locules, and several seeds up to 10th sympodia of the plant, plant height, squares/flowers, and several bolls formed /opened days after sowing (15 days' interval), yield /plant (g) up to 10th sympodia, seed coat (%), embryo and %, Protein % and final yield data (Kg ha-1) for the studied cotton genotypes are given in the Tables-1, 2 &3. Data regarding various physiological parameters conferring to heat tolerance; such as relative cell injury, electrical conductivity, anther dehiscence, pollen viability, gas exchange characteristics (i.e. stomatal conductance, transpiration rate, net photosynthesis rate, and physiological water use efficiency) are given in Figures 2-12.

From the given data in Table 1, it is evident that maximum fruit retention capacity up to 10th sympodia was shown by NIAB-1089 (56 %) followed by NIAB-545 (47%), NIAB-878B (46%), and NIAB-1011/48 (42%) against standard varieties (i.e. FH-142 & FH-Lalazar) with very less fruit retention capacity (25%). The highest number of bolls, locules, and several seeds up to the 10th sympodia of the plant (21 bolls; 79 locules and 357 seeds) was shown by NIAB-1089, followed by NIAB-444 (17 bolls; 69 locules and 336 seeds) and NIAB-545 (16 bolls; 65 locules and 316 seeds) as compared to standard varieties i.e. FH-142 (9 bolls; 36 locules and 189 seeds) & FH-Lalazar (7 bolls; 29 locules and 141 seeds). The

highest value of yield per plant (g) up to 10th sympodia was revealed by NIAB-1089 and NIAB-444 (40 g) followed by NIAB-545 (35 g) and NIAB-878B (32 g) about 45 to 57 % higher than standard varieties (i.e. FH-142; 22 g & FH-Lalazar; 17 g).

Data recorded for other plant phenological traits days after planting (DAP) with 15-day intervals is given in Table 2. NIAB-1042 showed the highest value for plant height (171 cm) against the minimum plant height value of 147 cm for cotton genotype NIAB-444. For all other genotypes, height values ranged from 149 to 168 cm. NIAB-1011/64 produced a maximum number of bolls (92) at 150 DAP followed by NIAB-1089 (89) and NIAB-545 (88) against FH-Lalazar with a minimum number of bolls (65) and FH-142 (84). Data on flowers per plant were consistent for all genotypes except FH-Lalazar which showed 3 flowers at an interval of 75 DAP. Flowering was completely ceased in FH-Lalazar at 135 DAP. Data on the number of squares per plant yielded interesting results because all genotypes showed variation even at 150 DAP. There was an increase in the number of squares for all genotypes between 75 DAP and 90 DAP. After this interval number of squares started to decline to 135 DAP. After this interval, all genotypes showed an increase up to 150 DAP and ranged between 6-10 squares per plant. NIAB-1011/64 surpassed all genotypes with 82 opened bolls per plant against FH-Lalazar (60) and FH-142 (75) after 150 DAP also as given in Table-3. From the given data in Table-3, it is also evident that NIAB-1011/89 showed the maximum number of unopened bolls (11) followed by NIAB-1011/64 (10) against FH-Lazar (5) and FH-142 (7) after 150 DAP. From the yield results data as given in theTable-1, it is also evident that NIAB-1011/48 surpassed all the genotypes by illustrating the highest yield of 5477 Kg ha-1 followed by the yield of NIAB 1011/64 (5475 Kg ha-1) and NIAB-878B (5384 Kg ha-1) against the yield of FH-142 (4465 Kg ha-1) and FH-Lalazar (3328 Kg ha-1) respectively.

From the data values given in Figure-1, it is evident that the maximum seed coat percentage (42%) was shown by NIAB-878B, followed by NIAB-1011/48 (40%) against the minimum seed coat percentage value of FH-Lalazar (37%). For embryo percentage, NIAB-1089 showed a maximum portion of the embryo (4.76%) followed by NIAB-545 (4.39%) and NIAB-1011/64 (4.27%) as compared to standard FH-Lalazar which showed 3.18% embryo. Regarding seed protein percentage, NIAB-1011/48 showed maximum content (28 %) followed by NIAB-1011/64 (27.13 %) and NIAB-1089 (27.04 %) against FH-Lalazar was 24.59% also given as Figure-1.

Table-1 Results of various morphological, yield, and fruit retention traits for determining heat tolerance in cotton genotypes studied at NIAB, Faisalabad 2016-17

Traits name	NIAB-545	NIAB-878	NIAB-1089	NIAB-1011/64	NIAB-444	NIAB-1042	NIAB-1011/48	FH-142*	FH-Lalazar*
1st Syp-node number	13	12	12	12	11	14	13	10	11
1st Syp-node height (cm)	23	23	24	25	25	27	26	20	20
Syp-height bearing 1st intact boll (cm)	30	36	29	37	31	41	37	37	32
TFP-up to 10th sympodia of the plant	35	29	35	32	47	29	26	28	27
TIP-up to 10th sympodia of the plant	17	13	19	10	18	11	11	7	7
SPs-up to 10th sympodia of the plant	18	16	15	22	29	18	14	21	20
Fruit Retention Capacity (%)	47	46	56	30	38	38	42	25	25
TNB-up to 10th sympodia	16	16	21	12	17	12	11	9	7
TNL-up to 10th sympodia	65	60	79	47	69	46	46	36	29
TNS-up to 10th sympodia	316	282	357	225	336	230	223	189	141
Yield (g) up to 10th sympodia of the plant	35	32	40	26	40	26	24	22	17
Plant Height (cm)	160	168	149	160	147	171	162	164	168
Number of Bolls m^{-2}	916	853	891	892	871	926	948	782	723
Yield (Kg ha^{-1})	5004	5384	4997	5475	4745	5121	5477	4465	3328

Syp-Sympodia, TFP- Total Fruiting Points, TIP- Total Intact Points, SPs-Shedding Points, FRC-Fruit Retention Capacity, TNB-Total number of bolls, TNL-Total number of locules, TNS-Total number of seeds

* for control/standards

Table-2: Results of different phenological parameters studied in cotton genotypes at NIAB, Faisalabad -2016-17

Genotypes	Days after planting (75)				Days after planting (90)				Days after planting (105)			
	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P
NIAB-545	93	14	6	38	106	32	6	47	128	56	6	35
NIAB-878	93	7	4	42	108	23	6	39	146	48	7	36
NIAB-1089	89	14	5	39	99	32	6	45	127	57	6	31
NIAB-1011/64	92	13	5	43	102	29	7	49	129	56	6	36
NIAB-444	92	12	5	42	101	26	6	40	120	50	5	32
NIAB-1042	99	9	4	38	116	28	6	46	150	48	8	39
NIAB-1011/48	95	10	4	38	108	26	6	41	130	52	7	29
FH-142*	95	9	5	43	112	26	7	43	140	47	9	30
FH-Lalazar*	86	9	3	40	101	21	6	45	128	40	5	34

Table 2 (Cont)

Genotypes	Days after planting (120)				Days after planting (135)				Days after planting (150)			
	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P
NIAB-545	149	63	3	16	152	83	1	3	160	88	1	10
NIAB-878	161	56	2	13	162	70	1	3	168	74	1	7
NIAB-1089	139	70	1	14	145	86	2	5	149	89	0	7
NIAB-1011/64	149	64	3	18	155	86	2	5	160	92	1	6
NIAB-444	141	57	3	20	145	72	2	4	147	78	1	7
NIAB-1042	162	65	2	15	162	79	1	2	171	85	1	10
NIAB-1011/48	150	50	2	15	157	73	1	4	162	74	1	7
FH-142*	153	57	3	16	156	75	1	3	164	84	1	10
FH-Lalazar*	151	50	4	17	152	64	0	3	168	65	1	10

PH = Plant Height, NB/P = Number of bolls/plant, NF/P = Number of flowers/plant, NS/P = Number of squares/plant

* for control/standards

Table-3: Phenological data on the number of unopened and opened bolls per plant with 15 days intervals at NIAB, Faisalabad-2016-17

Genotypes	Days after planting (105)		Days after planting (120)		Days after planting (135)		Days after planting (150)	
	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant
NIAB-545	43	12	35	29	20	63	7	81
NIAB-878	41	6	37	20	18	53	6	69
NIAB-1089	46	12	37	33	24	63	11	78
NIAB-1011/64	46	10	39	25	27	58	10	82
NIAB-444	38	12	31	27	23	49	7	71
NIAB-1042	42	6	43	22	17	63	5	80
NIAB-1011/48	42	10	29	22	25	49	9	65
FH-142 *	36	11	37	21	21	56	7	75
FH-Lalazar *	30	10	33	17	21	43	5	60

* for control/standards

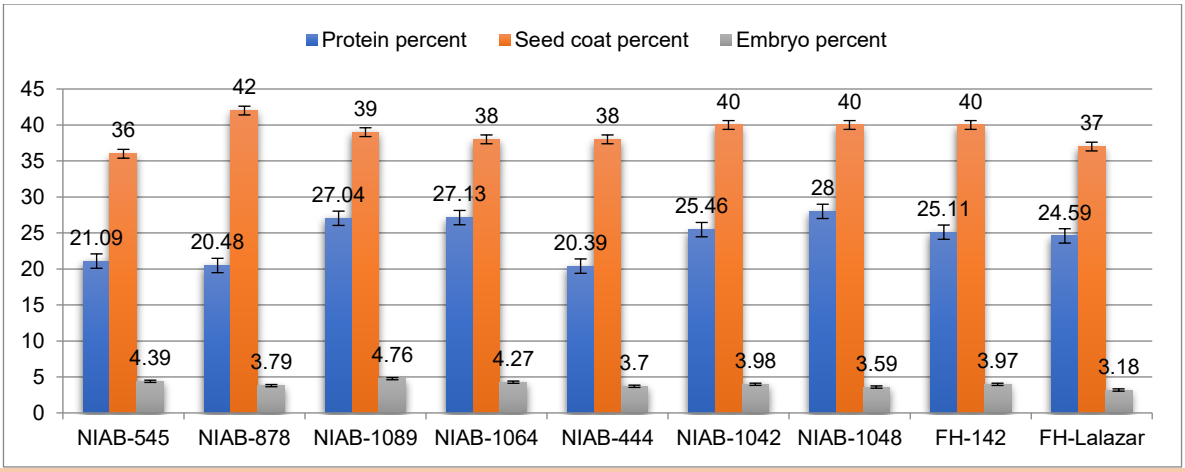

Figure-1: Results for the analysis of seed traits for determining heat to lerance in cotton genotypes at NIAB, Faisalabad-2016-17

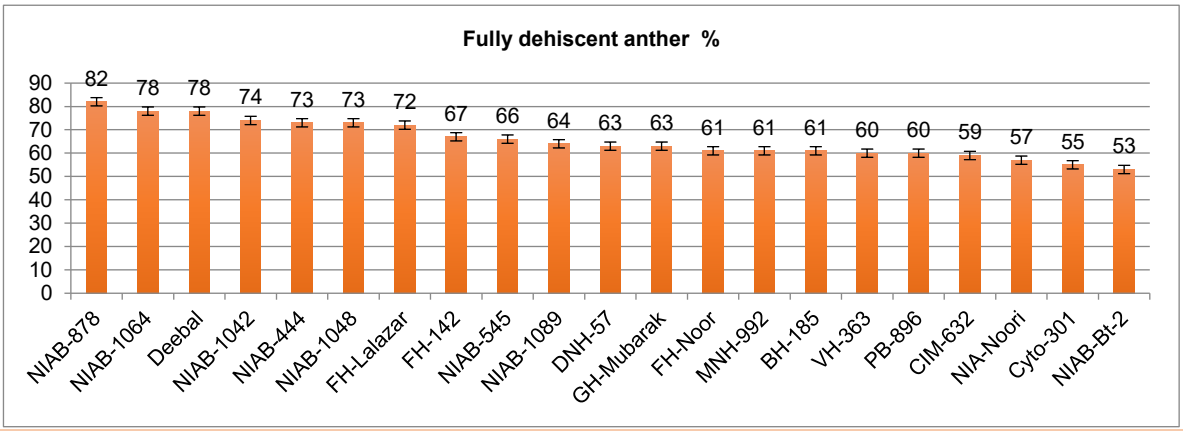

Figure- 2: Dehiscent anther in percent related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

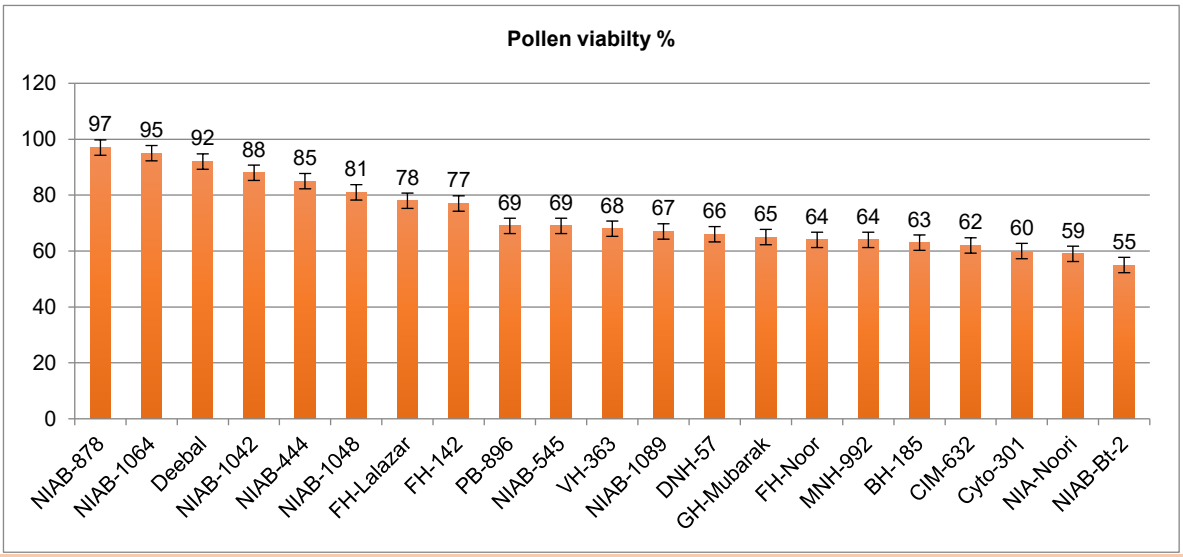

Figure- 3: Pollen viability in percent related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

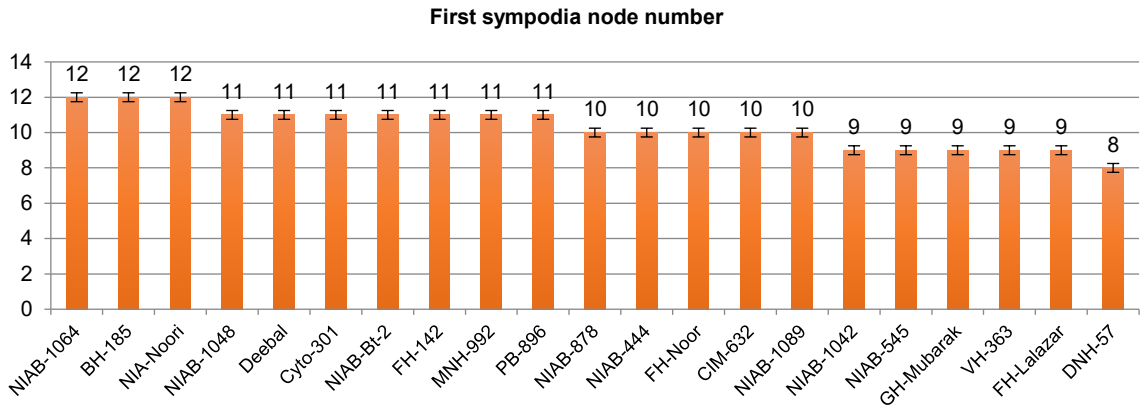

Figure- 4 : First sympodia node number related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

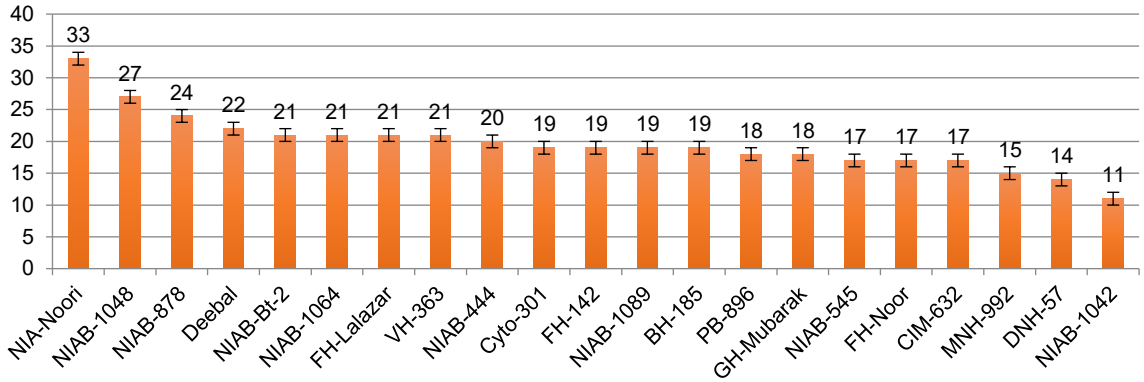

Figure- 5 : Sympodia node number bearing 1st effective boll related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

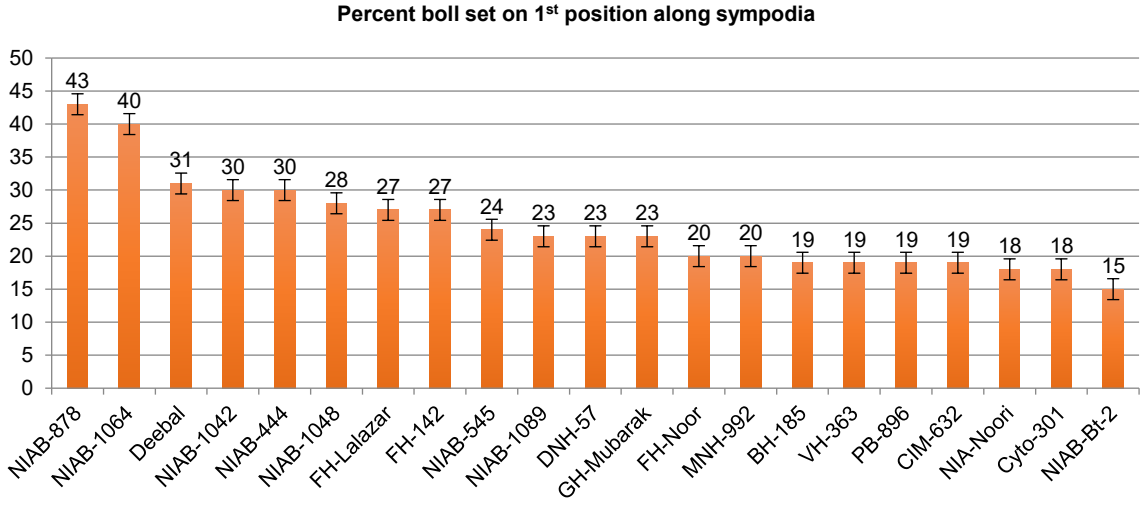

Figure- 6 : Percent boll set on 1st position along sympodia related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

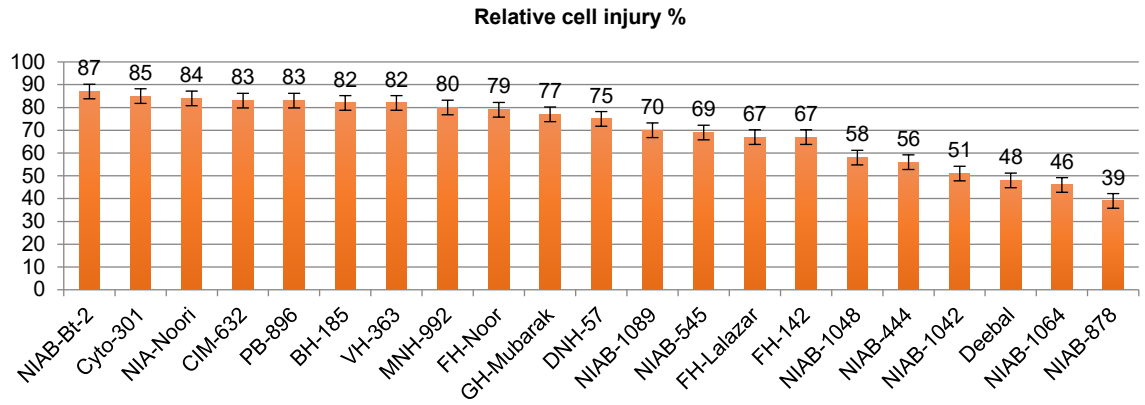

Figure- 7: Relative cell injury related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

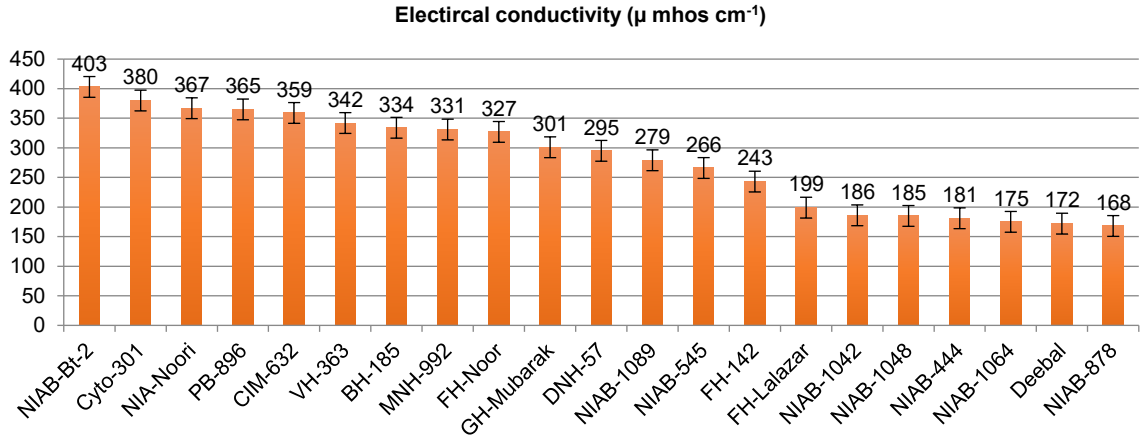

Figure- 8 : Electrical conductivity related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

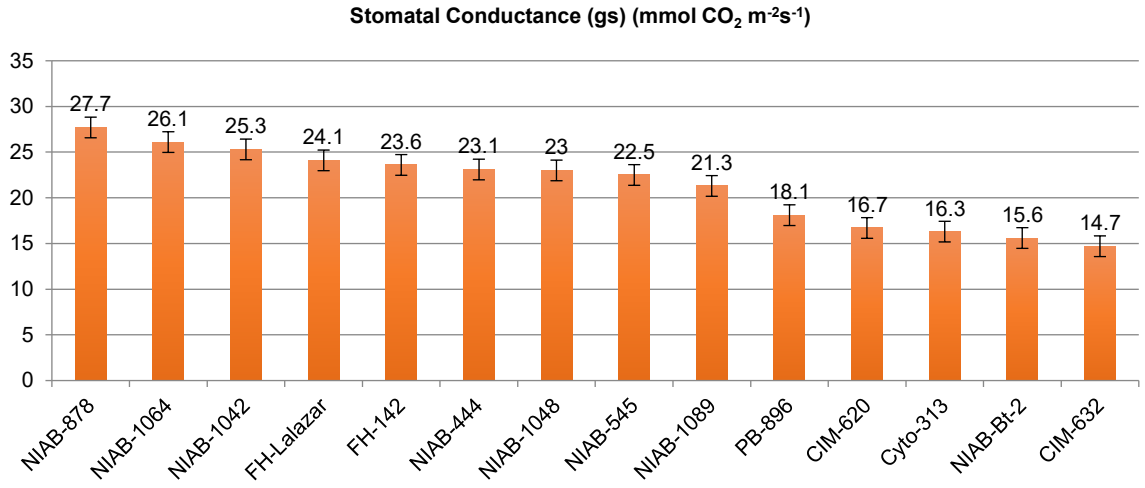

Figure- 9 : Stomacal conductance related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

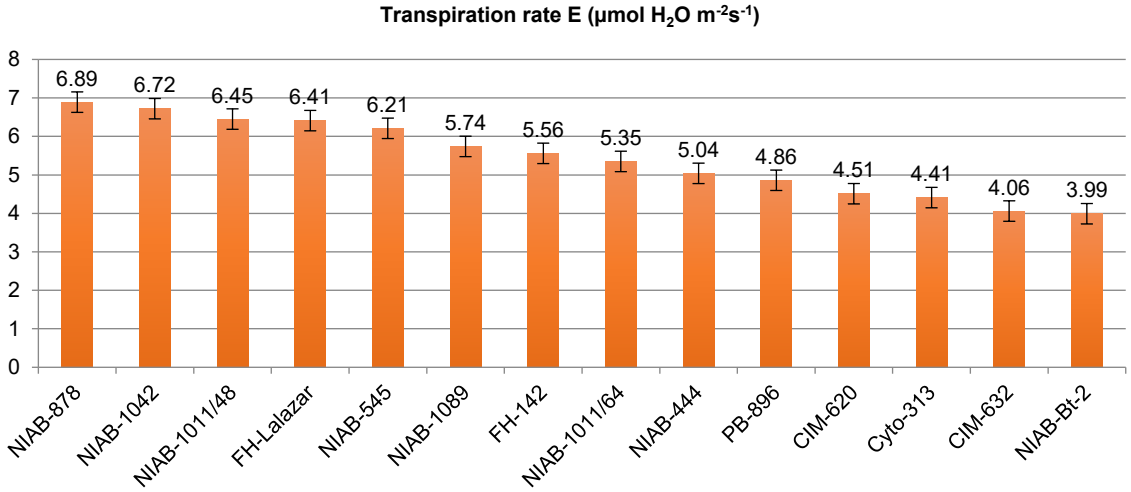

Figure- 10: Transpiration rate related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

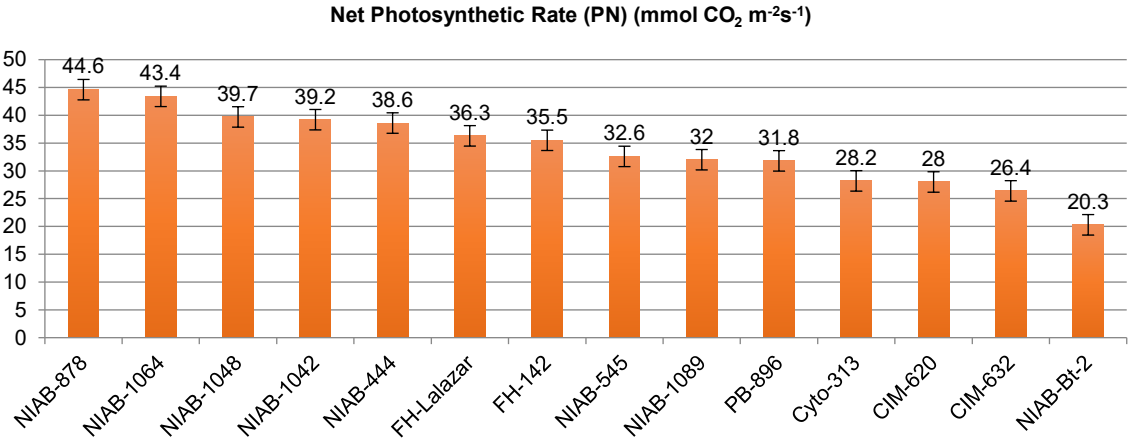

Figure- 11: Net photosynthetic rate related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

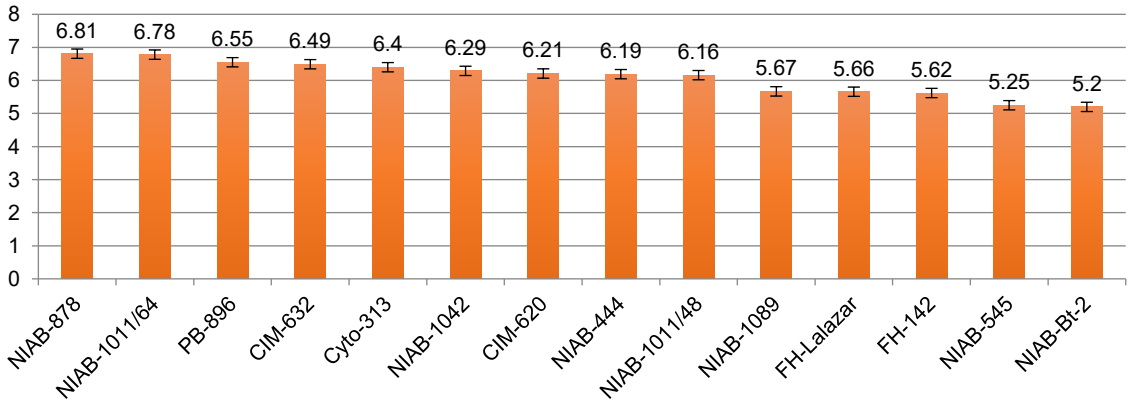

Figure- 12: Physiological water use efficiency related to heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

Data regarding various physiological parameters conferring heat tolerance are given as Figures 2-8. Data given in Figure 2 illustrates that NIAB-878B excelled in heat tolerance by maintaining the highest anther dehiscence (82%), pollen viability (97%), and lowest cell injury percentage (39 %) as compared to other genotypes and standard varieties. The lowest anther dehiscence (53 %), pollen viability (55 %), and maximum cell injury percentage (87 %) were shown by NIAB-Bt-2 and found to be the most susceptible genotype to heat stress. Data regarding gas exchange characteristics like stomatal conductance (gs), transpiration rate (E), and net photosynthetic rate (PN) varied among the genotypes as given in Figures 9-12. The stomatal conductance (gs) varied from 21.3 to 27.7 m mol CO2 m-2 s-1, transpiration rate (E) from 5.04 to 6.89 µ mole H2O m-2s-1 and net photosynthetic rate (PN) from 32.0 to 44.6 m mol CO2 m-2s-1. The physiological water use efficiency (PN/E) varied from 5.25 to 6.81 m mol CO2/µ mol H2O in different genotypes. Amongst the evaluated genotypes, NIAB-878B maintained the highest values of net photosynthetic rate and physiological water use efficiency under the prevailing high-temperature conditions. NIAB-878B also surpassed all genotypes by manifesting the maximum value of stomatal conductance (27.7 mmol CO2 m-2s-1), transpiration rate (6.89 µmol H2O m-2s-1), net photosynthetic rate (44.6 mmol CO2 m-2s-1) and physiological water use efficiency (6.81 mmol CO2/ µmol H2O) under prevailing high-temperature conditions. NIAB-Bt-2 considered the most susceptible genotype to heat tolerance showed minimum stomatal conductance (15.6 mmol CO2 m-2s-1), transpiration rate (3.99 µmol H2O m-2s-1), net photosynthetic rate (20.3 mmol CO2 m-2s-1) and physiological water use efficiency (5.20 mmol CO2/ µmol H2O) among all studied genotypes.

Discussion

The studies were conducted in identifying/screening of thermos- tolerant and sensitive cotton genotypes, under the prevailing high temperature under the field conditions using various morphological and physiological attributes conferring heat tolerance in cotton. Amongst 07 advanced cotton genotypes i.e. i.e. NIAB-878, NIAB-545, NIAB-1011/48, NIAB-444, NIAB-1011/89, NIAB-1011/64, NIAB-1011/42 (F8 generation) along with 02 controls (i.e. FH-142 and FH-Lalazar) evaluated for their responses to high temperature under controlled conditions revealed that maximum fruit retention capacity, the highest number of bolls/locules and number of seeds, maximum seed cotton yield per plant up to 10th sympodia of the plant was shown by NIAB-1011/89 against standard sensitive cotton varieties (i.e. FH-142 & FH-Lalazar). The standard cotton varieties illustrated very less fruit retention capacity, the lowest number of bolls/ locules/ number of seeds, and yield per plant up to 10th sympodia of the plant showing their inability to survive under the prevailing high temperature with ultimate loss/shedding of bolls. Fruit retention ability up to 10th sympodia of the genotypes studied was used as a marker in the development of heat tolerant cotton mutants, due to the reason that the reproductive organs of the plant are mostly affected under the peak of high temperature (July-August) up to this part of the plant. In July – August day (40 0C +) and night temperatures (27 0C +) often exceed the optimal limit. The maximum seed coat percentage value was shown by NIAB-878, followed, by NIAB-1011/48 against the minimum seed coat percentage value of the FH-Lalazar standard. The data, when compared with other studied parameters, showed that seed coats in cotton might act as environment protectants, against high temperatures in cotton particularly, in protecting of development of cotyledons in developing bolls of the cotton plant. Stronger the development of cotyledon, the stronger the development of seed vigor with an ultimate gain weight of seed cotton per boll, resulting in vigorous germination and crop growth stand. Embryo percentage studies results of genotypes showed that the maximum portion of the embryo was shown by NIAB-1011/89, followed by NIAB-545 in comparison with standard FH-Lalazar reflecting, the least effect of high temperature in the development of the embryo during the peak hours of high temperature (July –August).

Higher values of anther dehiscence/pollen viability and lower values for cell injury percentage makes the cotton plant most resilient against high-temperature tolerance. NIAB-878B excelled in heat tolerance by maintaining the highest values for anther dehiscence, pollen viability, and minimum cell injury percentage as compared to other genotypes and standards i.e FH-142 & FH-Lalazar. NIAB-878 also surpassed all the genotypes & standards by manifesting maximum stomatal conductance, transpiration rate, net photosynthetic rate, and physiological water use efficiency under prevailing high-temperature conditions with its ability to survive under the water deficit conditions under the high-temperature stress. Based on the overall performance of cotton genotypes regarding heat tolerance under controlled conditions, NIAB-878B, NIAB-1011/89 and NIAB-1011/48 were found as tolerant and can better withstand the heat stress as compared to susceptible genotypes i.e. FH-142 and FH-Lalazar.

Secondly based on the results of cotton genotypes for their screening for physiological, gas exchange characteristics, phenological traits, seed related parameters under the field conditions advanced line NIAB-878B was found to be resilient against high temperature as compared to standards. The said material developed against high-temperature stress is proving of extreme worth in making of successful cultivation of cotton in heat-prone cotton growing areas of the country like Pakistan, with maximum return to end-users.

Conclusion

Current studies were conducted during the year 2016-17 in identifying/screening of thermo- tolerant and sensitive cotton genotypes, under the prevailing high temperature of the field conditions using various morphological and physiological traits conferring heat tolerance in cotton. Amongst evaluated cotton mutants, NIAB-1011/89 revealed that maximum fruit retention capacity, the highest number of bolls/locules and number of seeds, maximum seed cotton yield per plant up to 10th sympodia of the plant against the standard sensitive cotton varieties (i.e. FH-142 & FH-Lalazar). The standard cotton varieties illustrated very less fruit retention capacity, the lowest number of bolls, locules/ number of seeds, and yield per plant showing their inability to survive under the prevailing conditions of high temperature with ultimate loss/shedding of bolls and yield (Kg/ha) at maturity. The maximum seed coat percentage was shown by NIAB-878B, followed, by NIAB-1011/48 against the minimum seed coat percentage value of the FH-Lalazar standard. The studied parameters showed that seed coats in cotton might act as an environment protectant against high temperature in cotton particularly, during the development of cotyledons at the cotton boll development stage. The developed vigorous cotyledons, ultimately lead to the gain weight of seed cotton per boll with vigorous germination and crop growth stand. Further maximum portion of the developed embryo in NIAB-545 in comparison with standard FH-Lalazar reflects, the least effect of high temperature in the development of the embryo during the peak hours of high temperature (July –August) in Pakistan.

The results of physiological studies showed that NIAB-878B excelled in heat tolerance by maintaining the highest values for anther dehiscence, pollen viability, and minimum cell injury percentage as compared to other genotypes and standards i.e FH-142 & FH-Lalazar. Higher values of anther dehiscence/pollen viability and lower values for relative cell injury percentage make the cotton plant most resilient against high-temperature tolerance. NIAB-878 surpassed all the genotypes & standards by manifesting maximum stomatal conductance, transpiration rate, net photosynthetic rate, and physiological water use efficiency under prevailing high temperature of field conditions, with its ability to survive under the water deficit conditions under the high-temperature stress. Based on the overall performance of cotton genotypes regarding heat tolerance under field conditions, NIAB-878B, NIAB-1011/89 and NIAB-1011/48 were found as tolerant and can better withstand the heat stress as compared to susceptible genotypes i.e. FH-142 and FH-Lalazar. The said material developed against high-temperature stress, also proving of extreme worth in making of successful cultivation of cotton in heat-prone cotton growing areas of the country like Pakistan with maximum return to end-users.

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Materials and Methods

Seven advanced cotton mutant genotypes i.e. NIAB-878, NIAB-545, NIAB-1011/48, NIAB-444, NIAB-1011/89, NIAB-1011/64, NIAB-1011/42 along with 02 controls (i.e. FH-142 and FH-Lalazar) were evaluated for their responses to high temperature under field conditions at Nuclear Institute for Agriculture & Biology in RCBD. Data regarding morphological/ phenological parameters conferring heat tolerance was recorded. The same set of genotypes was also evaluated for their heat tolerance, under field conditions at Central Cotton Research Institute, Multan using physiological attributes. Sowing of

genotypes at Nuclear Institute for Agriculture & Biology was done in the 1st week of May during 2016-17, whilst sowing of material at Central Cotton Research Institute Multan was done in the month of mid-April (to coincide their fruiting phase with the hottest period of high temperature of the season). Data regarding various other phonological traits i.e. plant height, number of squares/flowers, and total bolls formed/opened/unopened were recorded on five guarded plants of each genotype in four repeats after 75 days of planting with 15 days intervals up to 150 days was recorded.

Data regarding physiological parameters that potentially contribute towards heat tolerance in cotton-like relative cell injury, electrical conductivity, anther dehiscence, pollen viability, gas exchange characteristics (stomatal conductance, transpiration rate, net photosynthesis rate, and physiological water use efficiency), and some morphological characteristics like; 1st sympodia node number/its height, sympodia node number bearing 1st effective boll, sympodia node height bearing first effective boll, percent boll set on the first position along sympodia, percent boll set on the second position along sympodia, total fruiting positions/ intact points, number of locules and seeds/boll up to 10th sympodia of the genotypes were also recorded. Porometer was also used to measure leaf transpiration rate, diffusive resistance, relative humidity, leaf temperature, etc. Gas exchange characteristics (stomatal conductance, transpiration rate, net photosynthesis rate, and physiological water use efficiency) were also computed from the data generated by Porometer. At maturity, data were also recorded on plant population, plant height, number of bolls per m2, yield potential, average yield per plant, etc. Analysis of seed parameters that may contribute toward heat tolerance was also done. Seed protein contents were measured by using the Micro Kajeldhal method as reported by AOAC (1990). Seed coat percentage, seed embryo percentage, and their indexes were calculated as follows. Agronomic practices were kept uniform throughout the experiment period. Seed coat & seed embryos percentages were calculated by using the formula:

i. Seed Coat (%) = (Coat dry weight/seed dry weight) × 100
ii. Seed Embryo (%) = (Embryo dry weight/seed dry weight) × 100
iii. Seed Coat Index (SCI): SCI = Coat dry weight/Seed dry weight
iv. Embryo Index (EI): EI = Embryo dry weight/Seed dry weight

References

1. Anonymous. 2013a. Global warming and cotton production: ICAC, press release, 03 Oct, 2013. (https://www.icac.org).
2. Anonymous. 2013b. Cotton Crop Assessment Reports, 2013-14.
3. Anonymous. 2013c. Impact of heat and other stresses on boll development in cotton plant (www.cicr/org.in).
4. Bibi A. C., D. M. Oosterhuis, R. S. Brown, E. D. Gonias and F. M. Bourland. 2003. The physiological response of cotton to high temperatures for germplasm screening. In Oosterhuis, D. M. (ed.) Sum. of Arkansas cotton research. Arkansas Agric. Exp. Stn., Fayetteville, AR 521:87-93.
5. Burke, J., J. Velten, M. J. Oliver. 2004. In vitro analysis of cotton pollen germination. Agron. J. 96:359–368.
6. Cottee, N. S., D. K. Y. Tan, M. P. Bange, J. T. Cothren, and L. C. Campbell. 2010. Multi-level determination of heat tolerance in cotton (Gossypium hirsutum L.) under Field Conditions. Crop Sci. 50:2553-2564.
7. Cottee, N. S., D. K. Y Tan, J. T. Cothren, M. P. Bange, and L. C. Campbell. 2007. Screening cotton cultivars for thermo tolerance under field conditions. In: Proc. 4th World Cotton Research Conference. Lubbock, TX, USA, 10-14th September.
8. Rahman H. M, A. Ahmad, X. Wang, A. Wajid, W. Nasim, M. Hussain, B. Ahmad, I. Ahmad, Z. Ali, W. Ishaque, M. Awais, V. Shelia, S. Ahmad, S. Fahd, M. Alam, H. Ullah, G. Hoogenboom. 2018. Multi-model projections of future climate and climate change impacts uncertainty assessment for cotton production in Pakistan. Agricultural and Forest Meteorology; Vols. 253–254, 94-113
9. Hodges, H. F., K. R. Reddy, J. M. McKinion, and V. R. Reddy. 1993. Temperature effects on cotton. p. 1-15. Mississippi Agricultural & Forestry Experiment Station, Mississippi State, MS.
10. Karademir E., C. Karademir, R. Ekinci, S. Basbag and H. Basal. 2012. Screening cotton varieties (Gossypium hirsutum L.) for heat tolerance under field conditions. African Journal of Agricultural Research 7(47): 6335-6342.
11. Liu, Z., Y. Yuan, S. Liu, X. Yu, and L. Rao. 2006. Screening for high-temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. J. Integr. Plant Biol. 48:706-714.
12. Oosterhuis D. M. and Snider J. L. 2011. High temperature stress on floral development and yield of cotton: stress physiology in cotton. In: Oosterhuis, D. M. (ed.), Stress physiology in cotton, 1-25. The cotton foundation, Cordova, Tennessee.
13. Oosterhuis D. M., F. M. Bourland, A. C. Bibi, E. D. Gonias, D. Loka and D. K. Storch. 2008. Screening for temperature tolerance in cotton. In Oosterhuis, D. M. (ed.) Summaries of Arkansas cotton research. Arkansas Agric. Exp. Stn., Fayetteville, AR. 573:37-41.
14. Pettigrew, W. T. 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. Crop Sci. 48:278–285.
15. Radin J. W, Z. M. Lu, R. G. Percy and E. Zeiger. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc Natl. Acad. Sci. USA 91, 7217–7221.
16. Reddy K. R., G. H. Davidonis, A. S. Johnson and B. T. Vinyard. 1999.Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. Agron. J 91, 851-858.

17. Reddy K. R., H. F. Hodges and V. R. Reddy. 1992a. Temperature effects on cotton fruit retention. Agron. J. 84: 26-30.
18. Reddy V. R, K. R. Reddy and H. F. Hodges. 1991a. Temperature effects on growth and development of cotton during the fruiting period. Agron. J. 83, 211-217.
19. Rehman H, S. A. Malik and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermo stability. Field Crops Res. 85, 149-158.
20. Singh R. P., P. V. Prasad, K. Sunita, S. N. Giri and K. R. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. Adv. Agron. 93: 313-385.
21. Snider, J. L., D. M. Oosterhuis, B. W. Skulman and E. M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in Gossypium hirsutum. Physiol. Plant. 137:125-138.
22. Zhou G.Y, F. X. Yang, X. Q. Fu, X. L. Wang. 1996. The influence of climatic differences in the Yangtze River valley on cotton yield and fiber quality. China.

4

Determination of Genetic Diversity for Yield and Fiber Quality Traits among Some Cotton Genotypes (*Gossypium Barbadense L.)*

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Abstract

Genetic diversity is an important target for plant breeding programs to determine the power of genetic gain. Genetic variation, principle component analysis, cluster analysis, and path analysis were used to analyze thirty cotton genotypes belonging to Gossypium barbadense L, in a randomized complete block design with three replications during two growing seasons. Analysis of variance for ten quantitative yield and fiber quality traits was highly significant indicating variation between these traits across genotypes. Principle components analysis is used to partition total variation into ten principal components. Three out of ten Eigenvalues were more than unity with 81.5% of the total cumulative variance among thirty cotton genotypes for all traits. All the studied traits had longer trait vectors which reflected a total high amount of variation. In the score plot, seven genotypes located on the polygon vertex showed more variation and less similarity with other genotypes. While the genotypes close to each other on the graph had a narrow genetic background and lower dissimilarity each other. Correlation through the biplot graph showed a significant and positive association between all the studied yield traits and among fiber strength, fiber length, and uniformity ratio because the traits vector for these traits was <90o. The PCA and cluster analysis classified thirty genotypes into eleven clusters with a few differences depending on the performance expression rather than origin or pedigree. Cluster analysis and PCA could be used for the selection of desirable recombinants for various traits to generate greater variability in future cotton breeding programs. Path analysis indicated that lint yield per plant had the highest direct effect on seed cotton yield per plant followed by boll weight, whereas the other traits had a small direct effect. These outcomes could help the cotton breeder to identify the superior genotypes characterized by specific traits to be utilized in future hybridization breeding programs.

Keywords: *Cotton, yield, Principle components analysis, Cluster analysis, Genetic diversity and Path analysis.*

Background

Diversity in plant genetic resources (PGR) provides an opportunity for plant breeders to develop new and improved cultivars with desirable traits, which include both preferred farmers' traits (yield potential and large seed, etc.) and preferred breeders' traits (pest and disease resistance and photosensitivity, etc.). The genetic analysis of quantitative traits is the main target for any plant breeding program, which can lead to a systemic method of design and planning plant breeding strategies. In the last decade, most of the cultivated genotypes had a narrow genetic base. Also, different cotton breeders and growers have attributed yield reduction and fiber quality deterioration to less genotype diversity (Farooq et al., 2017; Rathinavel, 2018; Akter et al., 2019; Jarwar et al., 2019). So, more information about genetic diversity is the password to a good breeding program.

The successful breeding program depends on more knowledge about levels and types of genetic diversity which has different methods; analysis of genetic variability and identifying parental diversity to create good crosses which could produce transgressive segregation and introgression of desirable genes into the new genetic base. A better understanding of the genetic diversity between varieties or germplasm may be useful for planning crop breeding programs such as; how to create new crosses in assigning lines to specific heterotic groups (Rathinavel, 2018; Akter et al., 2019).

The Principal component analysis (PCA) is a multivariate statistical technique attempt to simplify and analyze the inter-relationship among a large set of variables to a small set of variables or components. The plant breeders used PCA to partition total variation to its components and extract all the important components and highlight their contribution toward the total variability. Breeders have

widely used principal component analysis, which helps in the exploration of promising genotypes and to speed up cotton breeding programs (Jarwar et al., 2019; Rathinavel, 2019).

Cotton yield is a quantitative trait controlled by many genes and affected by environmental factors so, yield improvement needs a better understanding of crop nature, genotype behavior, and association between different agronomic traits with a yield to overcome yield-limiting factors (Latif et al 2015 and Farooq et al., 2017). The correlation coefficient between different traits is a principal endeavor to investigate the relationship between these traits which could help the breeder to take a selection decision. Path analysis is a handy technique that helps plant breeders to estimate the contribution of multiple traits in total variation by partitioning total phenotypic or genotypic correlation into direct and indirect effects (Latif et al. 2015).

The current study aimed to assess genetic variation within and between thirty cotton genotypes belonging to G. barbadense L., using univariate analysis (ANOVA) and multivariate statistical techniques such as principle components analysis, cluster analysis, and path analysis based on ten quantitative yield and fiber quality traits.

Results and Discussions

Quantitative traits are considered the most important traits used to identify a particular plant variety. These traits are genetically controlled by many genes and affected by many environmental factors. Thirty cotton genotypes were assessed for variation in yield and fiber quality traits. The mean squares for all the quantitative traits showed highly significant genotypic differences ($P < 0.01$) (Table 1). These results indicate a presence of genetic variability among these genotypes across the studied ten traits. The current study suggests that the PCV was higher than the GCV for all traits. Phenotypic and Genotypic coefficient of variation ranged from 1.292 to 39.055 and from 1.147 to 38.866 for uniformity ratio and fiber fineness, respectively. Environmental effect on any trait is indicated by the differences between the genotypic and phenotypic coefficients of variation. In this study, the environmental effect was very small. These results indicated that the observed variation was not only controlled by genotype but also influenced by environmental effects on these traits. Also, selection based on these traits could be effective for future crossing programs. Broad sense heritability (h2b) of the ten studied traits was moderate for all traits except fiber strength and fiber fineness was more than 95% (Table 1). The higher estimates of broad sense heritability are useful for selecting the superior parents based on their phenotypic performance which has a higher ability for transferring to its offspring. So, these traits would be more effective and efficient in future hybridization breeding programs (Shakeel et al., 2015).

The basic descriptive statistics for the ten quantitative traits among thirty cotton genotypes such as minimum, maximum, range of means, standard deviation, standard error, and coefficient of variation (CV %) are given in Table 2. The thirty cotton genotypes are classified into two categories long and extra-long staple. The long staple genotypes are characterized by high yield and good fiber quality; like Giza 94 and Giza 97 which had high lint % (40%) coupled with fiber strength 10.26 and 10.55, fiber finesses (4), and fiber length 34.2 and 33.9 respectively. While most of the extra-long staple genotypes had high fiber quality and low or moderate yield traits, especially for lint %. This may be due to the negative linkage between these traits but the cotton breeder succeed to break this linkage and produce Giza 96 which is characterized by lint % (39.2 %), fiber finesses 3.99, fiber strength 11.8, and fiber length 36.1.

The highest variation was found for seed cotton yield, lint yield, lint%, and fiber length. Relatively, low variation was noticed for boll weight, fiber fineness, fiber strength, and uniformity ratio. The observed variability among cotton genotypes reflects the inheritance system and the environmental effects in which they were grown. The coefficient of variation (CV %) was found in the range of 1.20% for uniformity ratio to 12.34% for lint yield indicating a good control of experimental conditions (Table 2). This variability may be due to genetics, geographical distribution, or pedigree. Similar results were found by Abd El-Moghny et al., 2015 for twenty cotton genotypes belonging to G. barbadense. Latif et al. 2015 reported that the cotton breeder should be based on genetics rather than geographical distribution to select the best parents for the crossing program.

These outputs could be used for preceding a further analysis. The multivariate statistical technique was used like; principal component analysis to partition total variation to its components which could provide an opportunity to use suitable cotton genotypes for cotton breeding program improvement (Farooq et al., 2017). As well as, cluster analysis to classify these genotypes into different clusters based on dissimilarity coefficient.

Table 1: Analysis of variance, phenotypic and genotypic variance and broad sense heritability for yield components and fiber quality traits

SOV	df	Mean squares									
		Yield components							Fibre quality		
		BW g	SCY/P g	LY/P g	L%	SI g	LI	FF	FS	FL mm	UR %
Replications	2	0.018	56.9	14.110	0.615	0.137	0.145	0.110	0.292	0.873	1.180
Genotypes	29	0.157**	475.36**	175.29**	15.46**	0.74**	1.43**	6.66**	6.47**	9.21**	3.24**
Error	58	0.021	62.020	11.870	0.728	0.109	0.070	0.022	0.091	0.472	0.275
PCV		8.087	8.063	12.661	6.486	5.603	12.351	39.055	13.521	5.217	1.296
GCV		6.715	6.695	11.473	6.053	4.560	11.495	38.866	13.242	4.839	1.147
h^2_{bs}		0.689	0.690	0.821	0.871	0.662	0.866	0.990	0.959	0.860	0.782

* and ** significant at 5% and 1% levels of probability, respectively

Principal component analysis

Principal component analysis (PCA) is a powerful tool and the most basic multivariate data reduction statistical technique which indicates the genetic variation of the germplasm. PCA measures the importance and contribution of each component to total variance (Rathinavel, 2019). Generally, the sum of the Eigenvalue is equal to the number of variables. The Eigenvalue is often used to determine the number of major principal components to be explained. While Eigenvectors indicate a correlation between principle components and studied traits. The Eigenvalue, variability (%), and cumulative (%) are presented in Table 3.

Out of ten principal components, three PCs were depicted with an Eigenvalue more than unity and exhibited a very high correlation between them. The three principal components explained 50.5%, 18.1%, and 12.9% of the variation, respectively with 81.5% of the total cumulative variance among thirty cotton genotypes assessed for yield components and fiber quality traits. However, the remaining seven principle components contributed only 18.5% towards the total variation. These three PCs are responsible for a high magnitude of variance in the population. The genotypes loading on PC1 showed maximum positive loadings for all the studied traits except, fiber length, fiber strength, and uniformity ratio. All the studied traits had maximum positive loadings on PC2 except lint index and fiber finesses. While, the genotypes loading on PC3 showed a negative sign for all the studied traits except boll weight, seed cotton yield, and lint yield (Table 3). Shakeel et al., 2015 and Farooq et al., 2017 found positive loading for yield traits and fiber quality except for fiber fineness and fiber strength on PC1. So, principle components analysis is a good tool to confirm the extent of variation for traits among genetic materials which could be used to improve yield and increase intra-varietal hybridization efficiency. These results are following Farooq et al., 2017 and Rathinavel, 2018.

Table 2: Distractive statistics for yield components and fiber quality traits across thirty cotton genotypes

Genotypes		Yield components						Fiber quality			
		BW g	SCY g	LY g	L%	SI g	LI g	FF	FS	FL mm	UR %
Giza 36	G1	3.05	167.93	59.03	35.15	9.80	5.31	3.89	11.46	35.51	87.02
Giza 45	G2	2.98	164.08	55.26	33.68	9.57	4.85	3.02	11.38	37.63	87.82
Giza 68	G3	3.03	166.53	55.93	33.58	10.73	5.43	4.10	11.65	36.94	87.23
Giza 71	G4	2.98	163.90	58.27	35.55	9.53	5.26	3.65	11.59	37.39	87.60
Giza 74	G5	2.98	163.90	58.27	35.55	10.13	5.59	3.65	11.19	37.01	87.60
Giza 75	G6	3.27	179.67	67.65	37.65	9.63	5.82	4.28	10.09	33.48	86.03
Giza 76	G7	2.86	157.30	57.80	36.75	9.90	5.76	3.56	11.68	35.65	86.01
Giza 84	G8	2.66	146.21	56.29	38.50	10.23	5.70	3.88	11.11	36.43	89.38
Giza 85	G9	3.29	180.89	66.01	36.49	9.73	5.59	3.99	10.47	31.35	85.48

Giza 86	G10	3.19	175.54	67.76	38.60	9.73	6.14	4.03	10.56	33.87	83.86
Giza 87	G11	3.20	176.00	59.95	34.06	10.27	5.30	3.58	11.66	37.47	87.56
Giza 88	G12	3.17	174.17	58.81	33.77	10.13	5.16	3.44	11.74	37.11	87.31
Giza 89	G13	3.34	183.64	65.54	35.69	9.53	5.41	4.66	10.64	32.51	87.08
Giza 92	G14	3.31	182.11	66.53	36.53	10.20	5.87	3.99	11.64	37.01	87.19
Giza 93	G15	3.33	182.88	63.10	34.51	9.53	5.02	3.63	11.24	35.18	86.79
Giza 94	G16	3.72	204.60	82.58	40.36	10.43	7.24	4.06	10.26	34.21	85.74
Giza 96	G17	3.37	185.17	72.50	39.16	11.13	5.88	3.99	11.84	36.10	87.12
Giza 97	G18	3.57	214.00	86.11	40.24	10.40	7.00	4.09	10.55	33.95	85.55
6022	G19	3.06	168.06	64.29	38.26	9.80	6.07	4.33	10.44	34.29	85.56
10229	G20	2.95	162.10	57.27	35.33	11.33	6.20	3.99	10.95	34.43	85.78
24202	G21	3.36	184.56	61.46	33.30	10.20	5.09	3.07	11.92	37.12	87.28
Suvin	G22	2.73	150.33	51.99	34.58	10.00	6.44	3.64	10.60	34.25	86.25
OZ1	G23	3.10	170.50	62.92	36.90	9.63	5.64	4.08	10.29	35.58	86.66
CB58	G24	3.12	171.60	60.96	35.52	9.67	5.33	3.70	11.03	33.27	87.36
PS1	G25	3.46	190.06	75.26	39.60	9.93	6.52	4.66	10.56	35.61	86.12
PHL	G26	3.33	182.97	72.37	39.56	10.40	6.81	3.68	11.00	35.28	86.23
PE	G27	3.16	173.56	62.76	36.16	10.07	5.70	3.77	10.61	34.25	86.13
PHL	G28	3.22	176.92	68.93	38.96	10.67	6.82	4.20	10.47	34.57	86.58
PS6	G29	3.28	180.28	62.76	34.81	10.47	5.59	3.64	11.34	35.60	87.99
PS7	G30	3.27	179.97	69.29	38.50	11.13	7.62	3.82	10.91	33.38	85.82

Mean	3.18	175.31	64.33	36.62	10.13	5.87	3.83	11.02	35.26	86.67
Min	2.66	146.21	51.99	33.30	9.53	4.85	2.76	10.09	30.87	83.86
Max	3.72	214.00	86.11	40.96	11.33	7.62	4.66	11.92	37.63	89.38
Rang	1.06	67.79	34.12	7.65	1.80	2.77	1.90	1.82	6.77	5.52
Variance	0.05	195.43	63.02	4.82	0.25	0.48	0.18	0.28	2.90	1.08
SD	0.23	13.98	7.94	2.19	0.50	0.69	0.42	0.53	1.70	1.04
SE	0.04	2.55	1.45	0.40	0.09	0.13	0.08	0.10	0.31	0.19
CV%	7.20	7.97	12.34	5.99	4.93	11.79	11.06	4.78	4.83	1.20

Biplot

The principal component biplot showed that variables were superimposed on the plot as vectors; the relative length on the vector represented the relative proportion of the variability in each variable (Figure 1). The straight line connecting the marked point of any attribute and the base point of a biplot graph is mentioned as a "trait vector" and the cosine angle between parameters illustrated the relationship among all variables. The variables having vector trait <90°presented positive correlation while variables with vector trait >90° showed negative correlation and at a right angle (90°) presented independent behavior (Latif et al. 2015). The outcome of the biplot graph showed a positive correlation within most studied yield traits which showed acute angle. Fiber quality traits showed a positive correlation between fiber length, uniformity ratio, and fiber strength. Latif et al. 2015 had a positive association between five yield traits from the biplot graph among sixty Upland cotton genotypes.

Table 3: Eigenvalues, the proportion of variability for yield components and fiber quality traits that contributed to the principal components

Statistical Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigenvalue	5.055	1.809	1.287	0.890	0.465	0.234	0.144	0.088	0.028	0.000
Proportion %	0.505	0.181	0.129	0.089	0.047	0.023	0.014	0.009	0.003	0.000
Cumulative	0.505	0.686	0.815	0.904	0.951	0.974	0.988	0.997	1.000	1.000
Traits	Eigenvectors loading by various traits									
Boll weight	0.321	0.388	0.355	0.124	0.139	0.128	-0.080	-0.081	0.744	0.059
Seed cotton yield	0.331	0.389	0.334	0.122	0.076	0.100	-0.059	-0.066	-0.527	-0.558
Lint yield	0.398	0.295	0.093	-0.115	-0.146	-0.051	0.120	0.113	-0.349	0.748
Lint %	0.357	0.073	-0.255	-0.411	-0.376	-0.225	0.414	0.315	0.214	-0.354
Seed index	0.103	0.299	-0.686	0.298	0.395	0.284	0.007	0.322	-0.010	0.000
Lint index	0.353	-0.013	-0.450	0.102	-0.297	-0.123	-0.326	-0.673	0.021	-0.014
Fiber fineness	0.290	-0.222	-0.024	-0.500	0.727	-0.249	-0.067	-0.146	-0.032	0.003
Fiber strength	-0.320	0.410	-0.056	0.212	0.177	-0.589	0.462	-0.301	0.000	0.014
Fiber length	-0.298	0.443	-0.068	-0.329	-0.092	-0.313	-0.658	0.248	0.014	-0.023

Uniformity ratio %	-0.300	0.321	-0.092	-0.534	-0.018	0.564	0.217	-0.384	-0.018	0.013

In the biplot graph, the distance between the bases of the graph to traits represents the natural diversity of the studied genotypes (Figure 1). All the studied traits represented a high variability in the desirable direction. A similar result of the high amount of variability in yield components was reported by Shakeel et al., 2015 and Rathinavel, 2018. The highest variability in each trait reflected high genetic diversity between thirty cotton genotypes, which may be useful to cotton breeders.

Genotype by trait analysis

The genotype-by-traits (GT) biplot analysis has been used to estimate nature relationships between traits, evaluate genotypes for multiple traits and investigate the superior genotypes in cretin traits. The evaluation and notification of outclassing genotypes for different traits were carried out by using a biplot as presented in Figure 1. Many of the long-staple cotton genotypes are found near to yield traits. On the other hand, Most of the extra long staple genotypes especially Giza 93 was located to fiber length, fiber strength, and uniformity ratio. These results indicated that these genotypes are more related to these traits and could transmit them to their progeny. Also, these results conformed to the phenotypic mean performance (Table 2). These results are consistent with cotton breeder goals, as focusing on fiber quality characteristics for extra-long staple program more than the yield. On the contrary, breeding for the long-staple category focuses on yield components and maintaining fiber quality for this category. So, the cotton breeder could use these genotypes as a good donor for these traits as a useful hybridization strategy to produce commercial verities (Latif et al. 2015 and Rathinavel, 2018 and 2019).

Score plot

A score plot emanated out of the principal component analysis of the thirty cotton genotypes depicted that the genotypes close to each other and near to the origin point showed less breeding value and were less diversified. On the other hand, the genotypes clogged at the vertex of the polygon that is farthest from the origin point showed more diversified and had high breeding value (Rathinavel, 2018 and 2019). The score plot classified the thirty cotton genotypes into eleven clusters depending on their phenotypic mean performance as illustrated in Figure 2. Five clusters had unique genotypes; Giza 45, Giza 86, Giza 96, 24202, and Suvin plus anther cluster has only two genotypes belonging to the long staple category (Giza 94 and Giza 97) were located on the polygon vertex are useful for the future cotton improvement program. While three genotypes; 10229, OZ1, and PE were to be near the origin point.

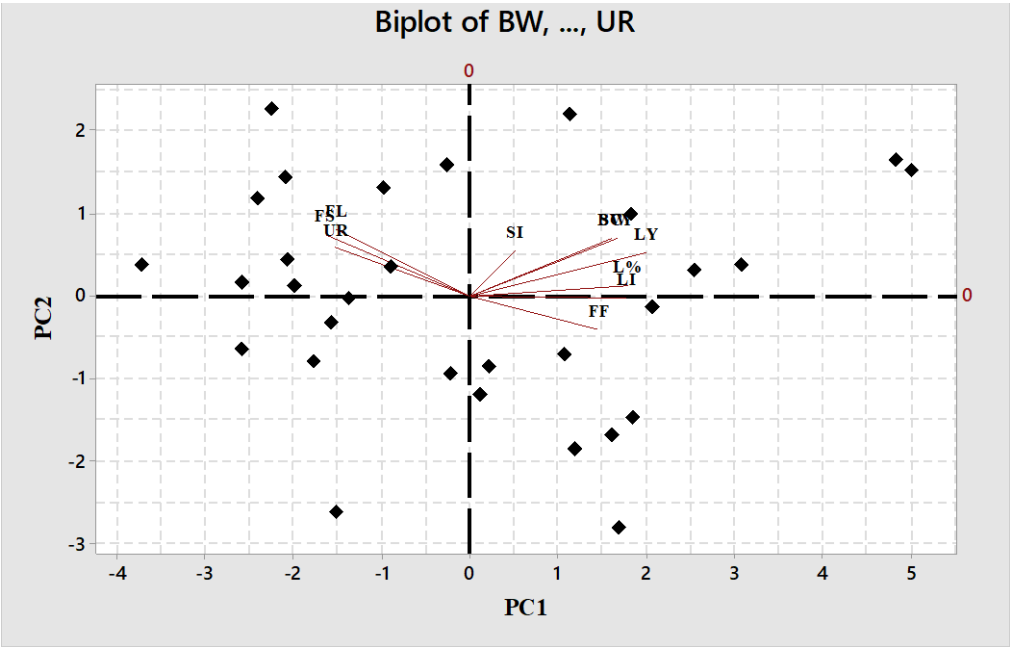

***Figure 1**: Principal Component's Biplot of ten quantitative traits among thirty cotton genotypes*

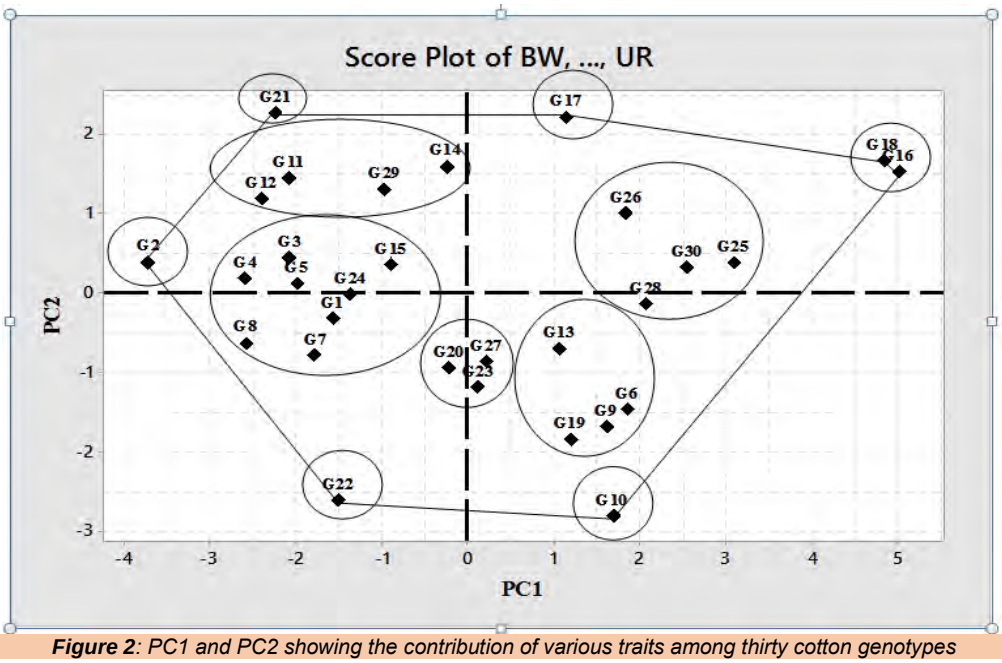

***Figure 2**: PC1 and PC2 showing the contribution of various traits among thirty cotton genotypes*

Two clusters had four genotypes for each one consisting of most of the long staple categories, (Giza 75, Giza 85, Giza 89, and 6022) and (PS1, PHL, PHY, and PS7). Also, two clusters had extra-long staples one cluster had four genotypes (Giza 87, Giza 88, Giza 92, and PS6), and the other consisted of eight genotypes (Giza 36, Giza 68, Giza 71, Giza 74, Giza 84, Giza 93 and CB58). The genotypes Giza 94, Giza 97, and Giza 96 lay in the positive region, while Suvin lay in the negative region. Rathinavel, 2018 reported that the genotypes in the positive ordination may be utilized for heterosis breeding programs.

Cluster analysis

Cluster analysis provides more information about the interrelationship between genotypes and gives a graphical assessment of genetic variability. Cluster analysis based on Agglomerative hierarchical clustering performed on the Euclidean distance matrix utilizing the average linkage (within groups) method to investigate genetic distance and diversity between the thirty cotton genotypes among ten quantitative traits. Cluster analysis sequestrated thirty cotton genotypes into eleven major clusters based on dissimilarity coefficient with contributed studied traits as shown in Figure 3. Five clusters had unique genotypes; Giza 84, Giza 86, 10229, Giza 96, and Suvin. Two clusters had extra-long staple genotypes; (Giza 68, Giza 87, Giza 88, Giza 92, 24202, and PS6) and (Giza 36, Giza 45, Giza 71, Giza 74, Giza 76, Giza 93, and CB58). While, three clusters consisted of long staple genotypes; (Giza 85 and PS1), (Giza 75, Giza 89, 6022, OZ1 and PE), (Giza 94 and Giza 97), and (PHL, PHY, and PS7).

The two analyses PCA and cluster analysis classified thirty cotton genotypes according to their phenotypic performance expression into different clusters. These results investigate a wide range of genetic distances among these genotypes which reflected the presence of genetic variation between the studied traits and provide an opportunity to use intraspecific hybridization for cotton improvement. So, the cotton breeder should be depending on genetics or mean expression rather than geographical distribution (Latif et al. 2015).

Genotypes grouped in the same cluster (intra-cluster) are expected to be genetically similar to genotypes grouped in different clusters (inter-cluster) (Table 4). The highest inter-cluster distance was observed between clusters 2, 3 followed by clusters 3, 8, and 8, and 11 respectively. While the lowest genetic distance occurred between clusters 4, 5 followed by clusters 1, 7, and clusters 6, and 9. On the other hand, the diversity within clusters was lower than between clusters. This indicated that the genotypes had lower dissimilarity within the cluster and higher divergence between clusters. So, hybridization between clusters will be more useful to give better progenies than within clusters, because their genetic make-up is almost different (Abd El-Moghny et al., 2015). The cluster analysis partly confirmed the findings of the score plot obtained from principle components analysis with few differences. This demonstrated that significant variation exists in this study and the obtained data for

this study were accurate, precise, and reliable. The breeder could use one of these two methods to design a crossing map to achieve the breeding program targets.

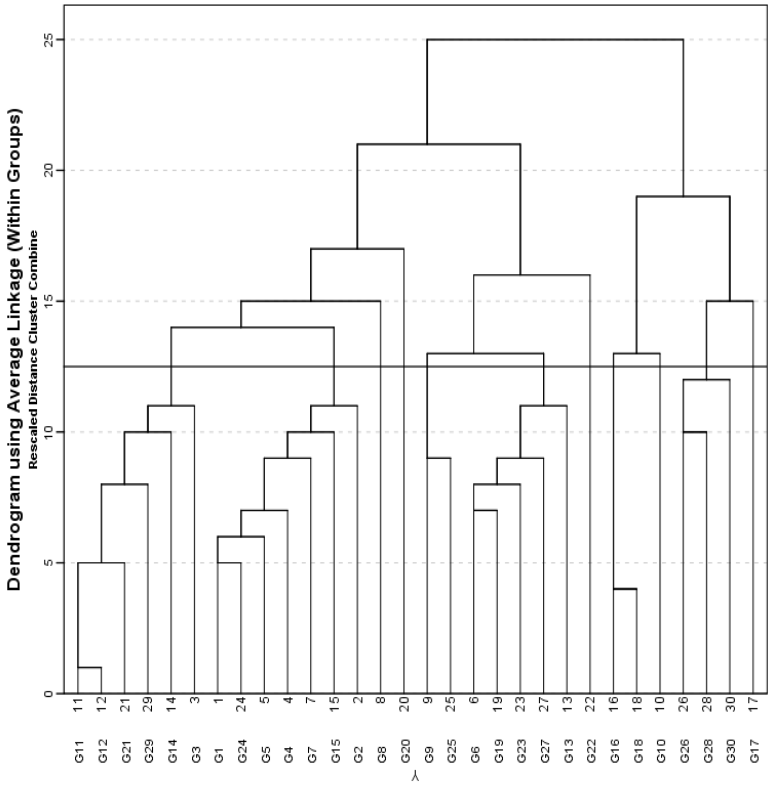

***Figure 3**: Dendrogram of thirty cotton genotypes based on yield components and fiber quality traits*

Path analysis

Path analysis partitioned the correlation into undeviating and deviating effects of cotton variables. The estimation of direct and indirect effects of different yield components on seed cotton yield per plant was computed through path analysis at the phenotypic level depending on phenotypic correlation as presented in Table 5. The phenotypical path diagram for seed cotton yield/plant is given in Figure 4. Seed cotton yield is the end product of all yield components and could be enhanced by exploiting the positive influence of these components (Rathinavel, 2019). The path analysis indicated that lint yield/plant (1.295) has a high direct effect on seed cotton yield per plant followed by boll weight (0.134). While the direct effects of all other component traits on seed cotton yield were low and negative except for the lint index. The positive indirect effect of boll weight, lint %, lint index, and seed index with seed cotton yield per plant was high at 1.119, 1.042, 0.264, and 0.862 respectively through lint yield per plant. While, the indirect effect of lint yield and lint % was 0.116 and 0.100, respectively through boll weight. The indirect effects of all the other traits can be negligible.

Table 4: Average intra- (Diagonal values) and inter- (Above diagonal values) cluster divergence of eleven clusters for thirty cotton genotypes using ten quantitative traits

Distances between Final Cluster Centers											
Clusters	1	2	3	4	5	6	7	8	9	10	11
1	2.53	16.20	57.00	9.03	9.64	18.82	6.30	19.58	18.07	26.77	47.97
2		0.00	72.47	25.11	24.28	34.07	11.07	8.14	34.00	41.97	63.24
3			0.00	48.41	48.24	38.43	61.53	74.24	39.95	30.78	9.72
4				2.16	5.71	11.02	14.74	28.33	9.07	18.93	39.63
5					3.60	9.86	13.46	27.02	11.38	18.02	39.10
6						3.28	23.17	36.34	6.83	8.53	29.33
7							2.73	14.28	23.55	30.97	52.29
8								0.00	37.20	43.49	64.76
9									2.24	12.74	31.60
10										3.21	21.41
11											0.00

Therefore it is suggested that the cotton breeder should used direct selection for lint yield and boll weight and indirect selection for boll weight, lint %, lint index and seed index will help to increase seed cotton yield per plant.

Table 5: Phenotypic path analysis for yield components attributing traits among thirty cotton genotypes

Traits	Boll weight (g)	Lint yield (g)	Lint (%)	Seed index (g)	Lint index (g)
Boll weight	0.134	1.119	-0.268	0.001	-0.010
Lint yield	0.116	1.295	-0.494	0.001	-0.018
Lint %	0.100	1.042	-0.614	0.001	-0.022
Seed index	0.017	0.264	-0.132	0.004	-0.015
Lint index	0.048	0.852	-0.481	0.002	-0.028

Conclusion

Finally, greater distance revealed greater diversity among genotypes and provided significant information about the genotypic performance. Genetic variation not only depends on the geographical distribution of genotypes but this may be due to other components like genetic flow, environmental variability, natural and non-natural selection, and interchange of hereditary materials (Latif et al., 2015). Choosing parents for crossing programs is a very important step to obtaining the best combination. So, the parental lines should be based on genetics or have higher mean performance rather than geographical distribution to be used in the crossing program.

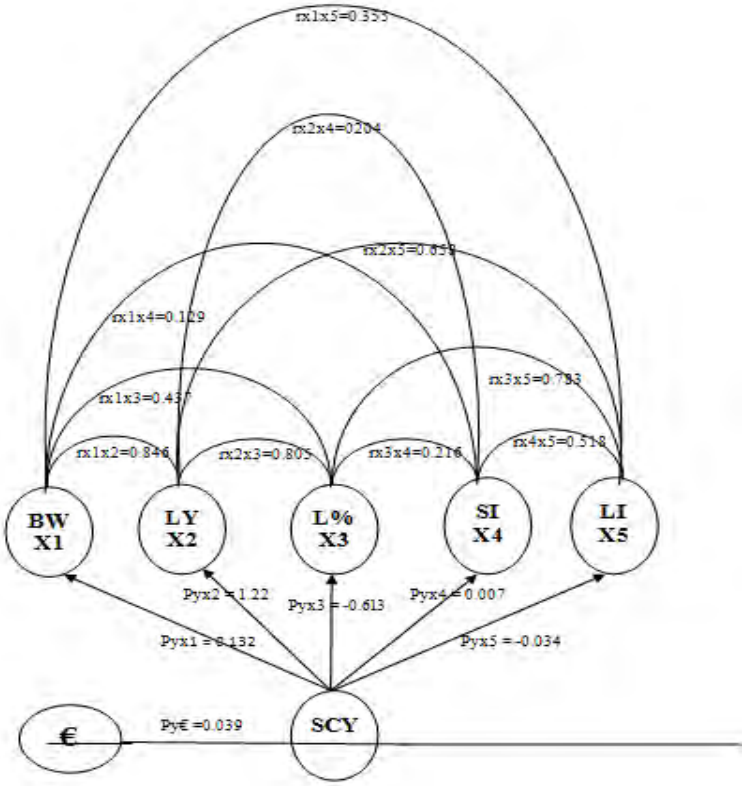

Figure 4: *Phenotypical path diagram for seed cotton yield per plant with other yield components*

Materials and Methods

The experimental material for the present study consisted of thirty cotton genotypes belonging to Gossypium barbadense L. The selfed seeds of these genotypes were kindly supported by the Cotton Breeding Department; Cotton Research Institute, Agriculture Research Center, Giza, Egypt. These genotypes were evaluated during the crop seasons of 2018 - 2019 at Sakha Experimental Station; Agriculture Research Center, Kafr El-Sheikh government; Egypt.

The experimental design was randomized complete block fashion (RCBD) with three replications. Each replicate consisted of four rows for each genotype. The row was 4.0 m long; the distance between rows was 0.7 m and within plants 0.4 m to insure 10 plants per row. Hills were thinned to keep a constant stand of one plant per hill at the seedlings stage. Normal agronomic, cultural practices (irrigation, weeding, hoeing, and fertilizer applications), and plant protection measures were adopted as and when required.

At harvest, the inner eight individual plants of the thirty cotton genotypes were harvested from each row in each replicate and ginned to estimate yield components traits; boll weight (BW) in grams as the average weight of five

bolls/plant, seed cotton yield per plant (SCY/P) in grams, lint yield per plant (LY/P) in grams, lint percentage (L%), seed index (SI) and lint index (LI) in grams. Also, fiber quality characters fiber length (FL), fiber strength (FS), fiber fineness (FF), and uniformity ratio (UR%) were estimated at Cotton Technology Laboratory, Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

The average data of all the studied ten quantitative traits were subjected to basic descriptive statistics and determining variability through the analysis of variance (ANOVA) as outlined by Gomez and Gomez, 1984. Principal component analysis (PCA) based on ten quantitative traits was computed to investigate the relative importance of different traits in capturing the genetic variation. The standardized values were used to perform PCA employing Minitab software version 18. A score plot was used for the visual assessment of components or factors that explain most of the data variability. Cluster analysis using multivariate analysis based on yield, its components, and fiber quality traits using averaged linkage (within groups) was calculated as outlined by Johnson and Wichern 1998. The dissimilarity coefficient and dendrogram were done by using SPSS software. The inter and intra-cluster distance among different clusters was calculated according to Singh and Chaudhary (1985). Path coefficient analysis was carried out to decipher the direct and indirect effects of yield attributing traits on seed cotton yield as described by Dewey and Lu, 1959.

References

1. Abd El-Moghny, A. M.; Mariz, S. Max and Reham, H. A. Gibely. 2015. Nature of genetic divergence among some cotton genotypes. J. Cotton Sci. 19:368–374.
2. Akter, T.; A. K. M. A. Islam; M. G. Rasul; S. Kundu; Khalequzzaman and J. U. Ahmed. 2019. Evaluation of genetic diversity in short duration cotton (Gossypium hirsutum L.). J. of Cotton Research, 2(1): 2-6.
3. Dewey, D. R. and K. H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agron. J. 51(9): 515-518.
4. Gomez, K. A. and A. A. Gomez. 1983. Statistical Procedures for Agricultural Research. 2nd Ed John Wiley and sons, NewYork, USA.
5. Farooq, J.; M. Rizwan; I. Sharif; S. Saleem; S. M. Chohan and R. A. Kainth. 2017. Genetic diversity studies in some advanced lines of Gossypium hirsutum L. for yield and quality related attributes using cluster and principle component analysis. AAB Bioflux, 2017, 9(3): 111-118.
6. Jarwar, A., H.; x. Wang; M. S. Iqbal; Z. Sarfraz; L. Wang; Q. Ma and F. Shuli. 2019. Genetic divergence on the basis of principal component, correlation and cluster analysis of yield and quality traits in cotton cultivars. Pak. J. Bot., 51(3): 1143-1148.
7. Johnson, R. A. and D. W. Wichern. 1998. Applied Multivariate Statistical Analysis. 4th Ed., Prentice-Hall, New York.
8. Latif, A.; M. Bilal; S. B. Hussain and F. Ahmad. 2015. Estimation of genetic divergence, association, direct and indirect effects of yield with other attributes in cotton (Gossypium hirsutum L.) using biplot correlation and path coefficient analysis. Tropical Plant Research J. 2(2): 120–126.
9. Rathinavel, K. 2018. Principal component analysis with quantitative traits in extant cotton varieties (Gossypium hirsutum L.) and parental lines for diversity. Curr. Agri. Res. J., Vol. 6(1), 54-64.
10. Rathinavel, K. 2019. Agro-morphological characterization and genetic diversity analysis of cotton germplasm (Gossypium hirsutum L.). Int. J. Curr. Microbiol. App. Sci., 8(2): 2039-2057.
11. Shakeel, A.; I. Talib; M. Rashid; A. Saeed; K. Ziaf and M. F. Saleem. 2015. Genetic diversity among upland cotton genotypes for quality and yield related traits. Pak. J. Agri. Sci., Vol. 52(1), 73-77.
12. Singh, R. K. and B. D. Chaudhary. 1985. Biometrical Method in Quantitative Genetics Analysis. Kalyani Publishers, New Delhi.

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Development and characterization of *Gossypium hirsutum* × *Gossypium arboreum* hybrids and backcross derivatives for response to some biotic stresses

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Abstract

Background: *G. arboreum, an A-genome cultivated diploid cotton species belonging to secondary gene pool is resistant to dreaded cotton leaf curl disease, leafhopper and whitefly. With these considerations, the present investigation was initiated to transfer resistance to these biotic stresses in upland cotton, the predominantly cultivated cotton species worldwide.*

Methods: *A set of five upland cotton genotypes were used as female parents in crosses with desi cotton (G. arboreum) genotypes. A total of 12392 flowers of upland cotton were pollinated. Hybridity of the F_1 plants was confirmed at the morphological, cytological, and molecular levels. For mitotic slide preparation in the F_1 plants, young roots were induced in branches through air layering. Besides, response of the cotton test material to CLCuD and jassid was recorded. Trichome density on the young and mature leaves was recorded. Cotton specific SSR markers were used for tagging of introgressed genes conferring leafhopper tolerance.*

Results: *Variable phenotypic expression was observed among the F_1 hybrids (G. hirsutum × G. arboreum). A little fertility to complete sterility was recorded in interspecific hybrids. Of the three cytologically analyzed F_1 plants, one was found to possess 2n=3x=39 chromosomes, whereas two plants registered somatic chromosome number of 2n=4x=52. Hybrid status of the F_1 plants was confirmed using cotton specific microsatellite markers. The upland cotton and desi cotton parents were found to be susceptible and resistant to CLCuD, respectively. G. arboreum genotypes were observed to be tolerant to jassid. Two genes either singly or in combination were found to govern tolerance to jassid. Two SSR markers, namely NAU 922 (chromosome A5) and BNL 1705 (chromosome A11) were associated with leafhopper tolerance. Significant negative correlation of leaf trichome density with jassid population was observed. Variable number of chromosomes was observed in the BC_1F_1 plants.*

Conclusion: *Segregants with resistance to jassid have been recovered which will be useful for the development of upland cotton cultivars for sustainable cotton production. Besides, the study will help in mapping the introgressed genes for their subsequent transfer in elite backgrounds through marker assisted selection.*

Keywords: *Alien introgression, Wide hybridization, Resistance breeding, Cotton leaf curl disease, Leaf hopper*

Background

Of the eight genera in the tribe *Gossypieae*, *Gossypium* is the largest and most widely distributed genus with more than 50 species. Eight genome groups *viz*., A, B, C, D, E, F, G, and K have been documented in this genus. Based on certain considerations, *Gossypium* species have been divided into primary, secondary, and tertiary gene pools. The primary gene pool includes all the allotetraploid (AD) *Gossypium* species. The A, D, B, and F - genome species are part of the secondary gene pool, whereas C, E, G, and K-genome species are included in the tertiary gene pool. Two diploid [*G. arboreum* (A_2) and *G. herbaceum* (A_1)] and two tetraploid (*G. hirsutum* and *G. barbadense*) cotton species are cultivated. Upland cotton is the dominating cotton species accounting for greater than 98 per cent of the cotton area

worldwide (Kranthi 2019), whereas *G. arboreum* and *G. herbaceum* (known as Old world or Asiatic cottons) are planted on less than one per cent of the global cotton acreage. India is perhaps the only country in the world to grow all the four cultivated cotton species and their compatible interspecific hybrids. About 70 years ago, of the total cotton area in the country, area under *G. arboreum* and *G. herbaceum* was 65% and 32%, respectively, which in 2010 declined to 4% and 5%, respectively (www.cicr.org.in). Presently, *G. arboreum* is cultivated on about 1 - 3% of the cotton area in India (Kranthi 2019).

G. arboreum possesses several desirable characteristics such as tolerance to several insect-pests, diseases and abiotic stresses. Cotton leaf curl disease (CLCuD) is a serious threat to Upland cotton cultivation in North Indian cotton growing states, Pakistan, and China. According to Briddon and Markham (2000), losses to Pakistan economy between 1992 and 1997 due to CLCuD have been US $ 5 billion. *G. arboreum* is known to be resistant to this malady. Cultivation of transgenic Bt cotton has provided relief from the bollworm menace in India. The *hirsutum* Bt hybrids grown in the country are highly vulnerable to sap-sucking insects (Kranthi and Stone 2020). Therefore, their large scale cultivation has shifted the pest profile to sap sucking insect pests such as whitefly, leafhopper etc. (Farooq et al. 2014). This fact is illustrated by the devastation of cotton by whitefly during 2015 in the Indian Punjab leading to one of the lowest productivity of 197 kg lint/ha (Kumar et al. 2020). Jassid is among the most economically important sucking pests of cotton after whitefly (Ghelani et al. 2014). The pest has potential of causing 25-45 % loss in seed cotton yield and may have a negative impact on fibre quality (Kalyan et al. 2017). With these considerations, the present research work was initiated with the objectives to transfer resistance to CLCuD, jassid and other pests from *G. arboreum* to *G. hirsutum*.

Materials and Methods

Development of plant material

A set of five upland cotton genotypes namely F 846, LH 900, LH 1556, LH 2107, and LH 2108 were used as female parents in crosses with *desi* cotton (*G. arboreum*) genotypes *viz*., LD 491, LD 902, LD 909, and LD 949. All the cotton genotypes used in the present study except LH 2107, LD 902, and LD 909 have been released for commercial cultivation. The plant material was planted in the crossing block of cotton field experimental area, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (Punjab), India. Upland cotton lines were emasculated in the afternoon one day prior to anthesis at the candle stage. The emasculated flower bud was then covered with a small straw pipe to prevent pollination by undesirable pollen. One day prior to pollination, flowers of the male (*G. arboreum*) parents were tied at the pedicel whereas, the other end of the thread was tied at the one-third distance from tip of flower to prevent opening of flower and hence ensuring purity of pollen. The emasculated buds were pollinated the next day between 8 and 11 am. The crossed buds were again covered with the straw pipe. The pollinated flowers were administered with growth regulators for three successive days starting from day of pollination. The growth regulators consisted of a mixture of 50 ppm gibberellic acid (GA3) and 100 ppm naphthalene acetic acid (NAA). The mixture was prepared fresh and applied at base of pedicel with the help of cotton buds. Hybridization was carried out from mid-August through first week of October. A total of 12392 flowers of American cotton genotypes were pollinated to obtain four true interspecific cotton hybrids. The interspecific F_1 hybrid (*G. hirsutum* acc. LH 2107/ *G. arboreum* cv. LD 491) was backcrossed as pollen parent with LH 2107 to develop BC_1F_1 (*G. hirsutum* acc. LH 2107/ *G. arboreum* cv. LD 491/*G. hirsutum* acc. LH 2107) population.

Induction and fixation of roots

For chromosome count in the F_1 hybrids and selected backcross derivatives, young roots were induced in branches through air layering. About 1" bark was removed from the branch of pencil thickness. IBA @ 100 ppm or 200 ppm was applied on the bark less portion and moist moss placed on a polythene sheet was wrapped around it. Depending on the season, it took two to five weeks before the roots were visible. Root tips were harvested in the morning in 2mM hydroxyquinoline to arrest metaphase and later on fixed in the fixative (alcohol: acetic acid in 3: l). After washing root tips with citrate buffer, these were incubated in the enzyme mixture containing cellulase and pectinase. Slides were checked under a phase-contrast microscope for even metaphase spreads and then frozen on a metal plate. Slides were allowed to air-dry followed by DAPI staining for visualization under fluorescent microscope. For parental lines, root tips for the cytological analysis were collected after germinating the seeds.

Characterization of interspecific hybrids and backcross derivatives

Parental lines, interspecific F_1 hybrids, and backcross derivatives were characterized at various levels. Hybridity of the F_1 plants was confirmed at the morphological, cytological, and molecular levels using cotton specific microsatellite markers. Besides, response of the experimental plant material to cotton leaf curl disease (CLCuD) and jassid was recorded. Jassid injury grade (JIG) was recorded as per the guidelines of ICAR-All India Coordinated Research Project on Cotton (2020-21) (www.cicr.org.in/aicrp-2021.htm): the entire foliage free of curling and yellowing (JIG I); curling of few leaves in the lower portion of plant + marginal yellowing (JIG II); curling of leaves almost all over the plant (JIG III); and extreme curling, bronzing and drying of leaves (JIG IV).

Results and Discussion

Production of interspecific hybrids and backcross derivatives

Many pre- and post- zygotic barriers are known to interfere with the successful production of wide-hybrids. Extensive fertilization and embryological studies in direct and reciprocal crosses involving *G. hirsutum* and *G. arboreum* revealed that endosperm and embryo abortion occurred in both. *G. hirsutum* and *G. arboreum* are sexually incompatible species and it is not easy to develop F_1 hybrids between them through direct hybridization due to post-zygotic barriers. In the present investigation, *G. hirsutum* genotypes namely F 846, LH 900, LH 1556, LH 2107, and LH 2108 were used as seed parents in crosses with *desi* cotton (*G. arboreum*) genotypes *viz*., LD 491, LD 902, LD 909, and LD 949. A total of 12,392 flowers of upland cotton genotypes were pollinated and 621 seeds were obtained. About 40 plants were obtained, of which four were observed to be true interspecific hybrids (Fig.1). The parentage of *G. hirsutum* × *G. arboreum* F_1 hybrids is given in Table 1 below. Due to their partial pollen fertility, two F_1 plants (F_1- 3 and F_1- 4) were used as male parents for the development of first generation backcross derivatives. A total of 2319 seeds were obtained, of which 211 (9.1%) seeds germinated.

Table 1 Pedigree of the interspecific F_1 hybrids

G. hirsutum (seed parent)	*G. arboreum* (pollen parent)	Designation of F_1 plant
F 846	LD 949	F_1- 1
LH 2108	LD 949	F_1- 2
LH 2107	LD 949	F_1- 3
LH 2107	LD 491	F_1- 4

Characterization of F_1 hybrids

Some of the morphological traits of parents and their respective F_1 hybrids are presented in Table 2. Variable phenotypic expression was observed among the F_1 hybrids. They manifested either dominance or intermediate expression for various morphological traits. All the female parents *viz*. LH 2107, LH 2108, and F 846; and one of the male parents LD 491 have green stems, whereas, LD 949 possesses intense red stem coloration . Stems of F_1-1 and F_1-2 were observed to be red, which is an indication of a successful cross as the character was inherited from male parent. Similar results of stem coloration in the interspecific hybrids between *G. hirsutum* × *G. arboreum* have been reported by Tahir et al. (2011) and Chen et al. (2015). Interestingly, F_1-3 having the same red stemmed male parent (LD 949) as those of F_1-1 and F_1-2 was found to possess green stem like its *hirsutum* parent. This plant is actually tetraploid with 2n=52. All the F_1 hybrids recorded the presence of petal spot, which is consistent with the findings of Tahir et al. (2011) and Chen et al. (2015). The presence of petal spot in the male parent as well as F_1 hybrid is another morphological evidence for hybridity confirmation. F_1-1 and F_1-2 recorded intermediate leaf shape, whereas, other two F_1 plants (F_1-3 and F_1-4) were more similar to their female parent (LH 2107). All the parental lines except LD 949 were found to possess nectary on the mid-rib of lower surface of the leaves.

a b c d

Fig 1 Interspecific hybrids between G. hirsutum × G. arboreum a) F_1-1, b) F_1-2, c) F_1-3, d) F_1-4

Table 2 Morphological characterization of parental species and their interspecific hybrids

Character	GH cv. F 846	GA cv. LD 949	F_1-1	GH cv. LH 2108	GA cv. LD 949	F_1-2	GH Acc. LH 2107	GA cv. LD 949	F_1-3	GH Acc. LH 2107	GA cv. LD 491	F_1-4
Stem colouration	Green	Red	Red	Green	Red	Red	Green	Red	Green	Green	Green	Green
Leaf shape	Palmate	Digitate	Intermediate	Palmate	Digitate	Intermediate	Palmate	Digitate	Palmate	Palmate	Digitate	Palmate
Leaf lobe number	3-5	5-6	3-5	3-5	5-6	3-5	3-5	5-6	3-4	3-5	5-6	3-4
Midrib nectary	Present	Present	Present	Present	Absent	Present	Present	Absent	Present	Present	Absent	Present
Trichome density (ML)	44.33	82.78	83.89	44.22	82.78	80.33	43.67	82.78	85.67	43.67	83.89	85.11
Trichome density (YL)	87.67	178.78	136.89	85.67	178.78	130.11	86.33	178.78	113.56	86.33	177.78	108.0
Flower petal spotting	Absent	Present	Present	Absent	Present	Present	Absent	Present	Present	Absent	Present	Present
Average anther No.	117.2	105.1	111.2	122.8	105.1	101.3	116.4	105.1	120.8	116.4	113.5	101.3
Pollen fertility (%)	96.7	80.1	0	93.7	80.1	0	94.5	80.1	40.5	94.5	87.6	17.8
Average pollen size (µm)	121.07	91.5	79.4	119.4	91.5	82.2	122.3	91.5	103.1	122.3	89.6	91.4

Character	GH cv. F 846	GA cv. LD 949	F_1-1	GH cv. LH 2108	GA cv. LD 949	F_1-2	GH Acc. LH 2107	GA cv. LD 949	F_1-3	GH Acc. LH 2107	GA cv. LD 491	F_1-4
CLCuD symptoms	Present	Absent	Absent	Present	Absent	Absent	Present	Absent	Present	Present	Absent	Present

GH: G. hirsutum, GA : G. arboreum, ML: Mature leaf, and YL: Young leaf

Same observation was reported by Singh and Pathak (2017) for LD 949 and LD 491. Presence of midrib nectary in *G. hirsutum* genotypes has also been reported by Tahir et al. (2011); Kaur et al. (2016) etc. Nectaries were found in all the four F_1 hybids thus, manifesting their dominance over their absence. According to pollen viability test by acetocarmine staining, parental lines were observed to have high pollen fertility, but a little fertility to complete sterility was recorded in interspecific hybrids. In the triploid interspecific hybrid between *G. hirsutum* × *G. arboreum*, 2.38% pollen viability was reported by Ahmad et al. (2011), whereas, complete pollen sterility was observed in the triploid F_1 hybrids in the present study. Similar results of pollen sterility of the interspecific hybrids between *G. hirsutum* and *G. arboreum* have been reported by Chen et al. (2015). The tetraploid hybrids namely, F_1-3 and F_1-4 in the present investigation recorded an average pollen fertility of 17.8% and 40.5%, respectively. This is in contrast to the findings of Ahmad et al. (2011) who reported pollen fertility of tetraploid interspecific hybrids to be merely 1.90%.

Cytological characterization of interspecific cotton hybrids and backcross derivatives

Cytological characterization of F_1 hybrids were carried out by mitotic analysis. *G. hirsutum* genotypes namely, LH 2108 and LH 2107 were found to be tetraploids with 52 chromosomes each, whereas, *G. arboreum* cv. LD 949 and LD 491 recorded 26 chromosomes each (Fig. 2a). These observations on chromosome numbers in Upland and *desi* cottons are consistent with the findings of Webber (1935); Beasley (1940); Ahmad et al. (2011); Tahir et al. (2011); Newaskar et al. (2013) and Montes et al. (2017). A triploid hybrid with somatic chromosome number 39 is expected from a cross between a tetraploid and diploid cotton species. Tahir et al. (2011); Ahmad et al. (2011), and Chen et al. (2015) obtained putative triploid (2n=3x=39) hybrid from a cross between *G. hirsutum* and *G. arboreum*. In this present study, F_1-2 was found to possess 2n=3x=39 chromosome at metaphase.

Interestingly, F_1-3 and F_1-4 both registered somatic chromosome number of 2n=4x=52 at metaphase (Fig. 2a). One probable reason can be the formation of unreduced (2n=26) gametes from the diploid male parent *G. arboreum* (AA) and fertilization with normal female gamete (n=26) of *G. hirsutum* (AADD) resulting into F_1 plants with 52 somatic chromosome number. Montes et al. (2017) obtained an interspecific hybrid of *G. herbaceum* × *G. hirsutum*, having somatic chromosome number of 52 (AAAD) resulted from a fertilization of unreduced female gamete (2n) of *herbaceum* with normal pollen of *hirsutum*. Chromosome count of three randomly taken BC_1F_1 plants was conducted. Two of these plants (numbered 4-61 and 4-168) were observed to have 52 chromosomes, whereas, plant 4-198 had 47 chromosomes. (Fig. 2b)

Molecular characterization of F_1 hybrids

Cotton specific SSR markers were used to provide another line of evidence supporting the hybrid status of *G. hirsutum* × *G. arboreum* F_1s. Two SSR primers namely, BNL1064 and BNL1438 were employed for the hybridity confirmation of the interspecific F_1s (Fig. 3). BNL1438 amplified a single locus in both the parental species, whereas, BNL1064 amplified single locus in *G. arboreum* genotypes and three loci in each of the *G. hirsutum* genotypes. Both of these primers unequivocally confirmed the hybrid status of the interspecific *G. hrsutum* × *G. arboreum* F1s. Two polymorphic SSR primer pairs (BNL1317 and BNL2634) were employed to confirm the hybrid status of putative interspecific F_1s obtained from *G. hirsutum* and *G. arboreum* crosses by Tahir et al. (2011). Similarly, seven polymorphic SSR markers (BNL1064, BNL1438, BNL1679, BNL2960, BNL3590, BNL3888, and NAU933) were used to confirm the hybrid status of putative hybrids between *G. hirsutum* and *G. arboreum* (Vij et al. 2016).

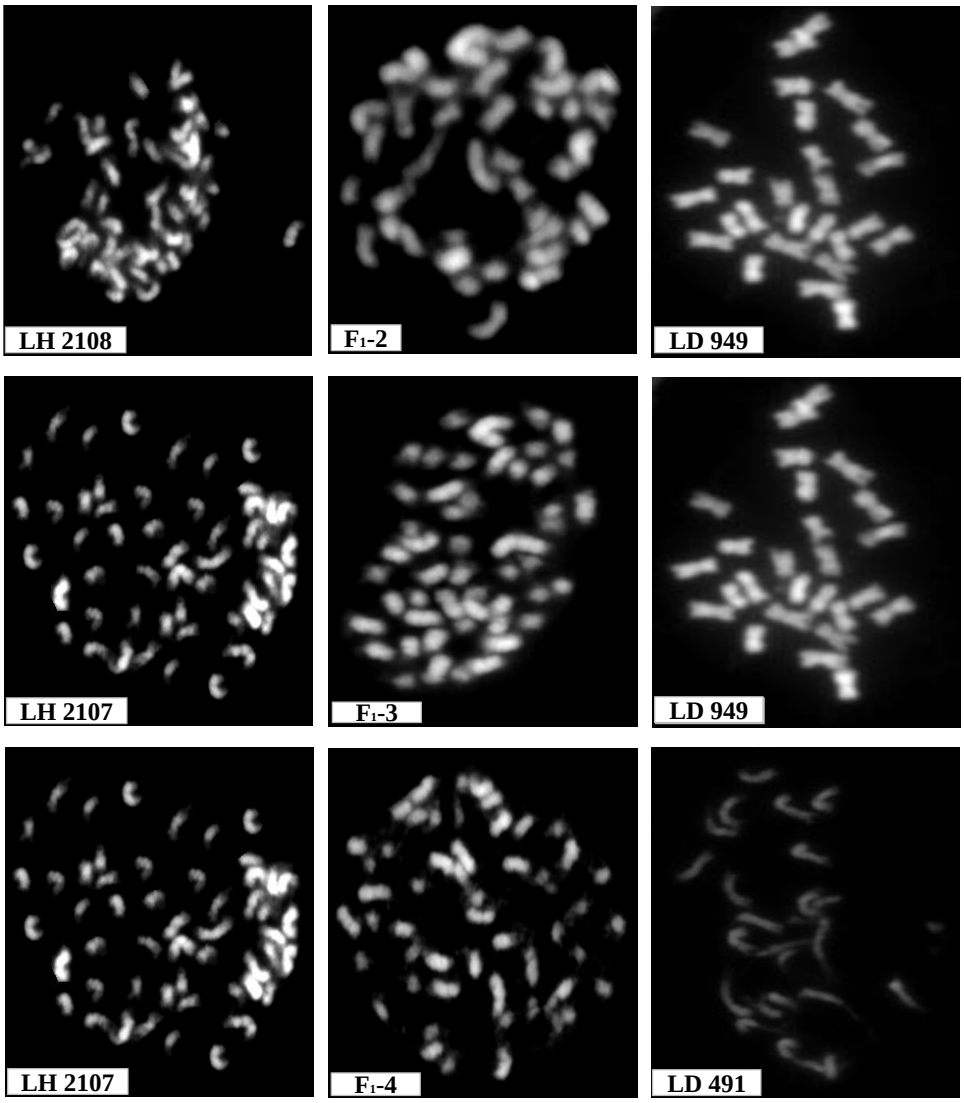
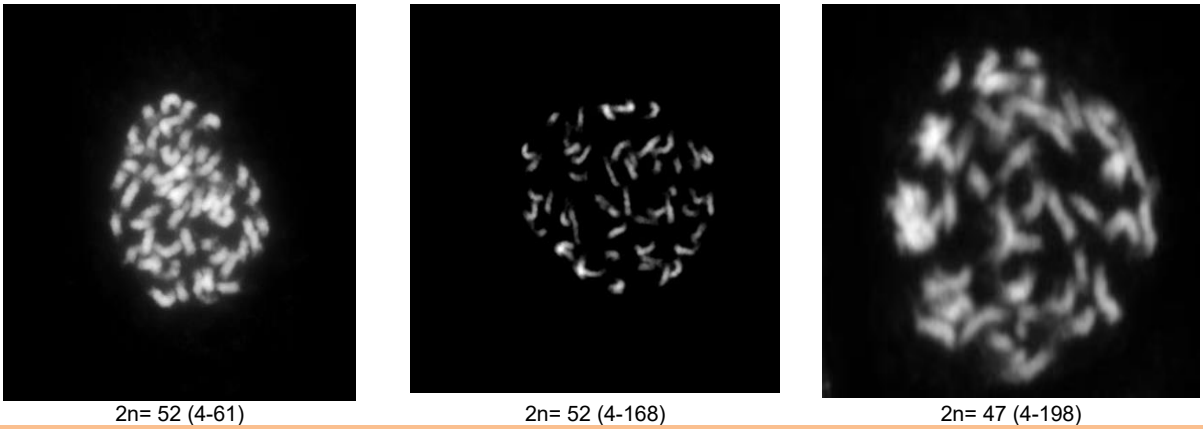


Fig. 2a *Comparison of chromosome number among the parental species and their respective hybrids*



2n= 52 (4-61) 2n= 52 (4-168) 2n= 47 (4-198)

Fig. 2b Chromosome count in selected BC_1F_1 plants

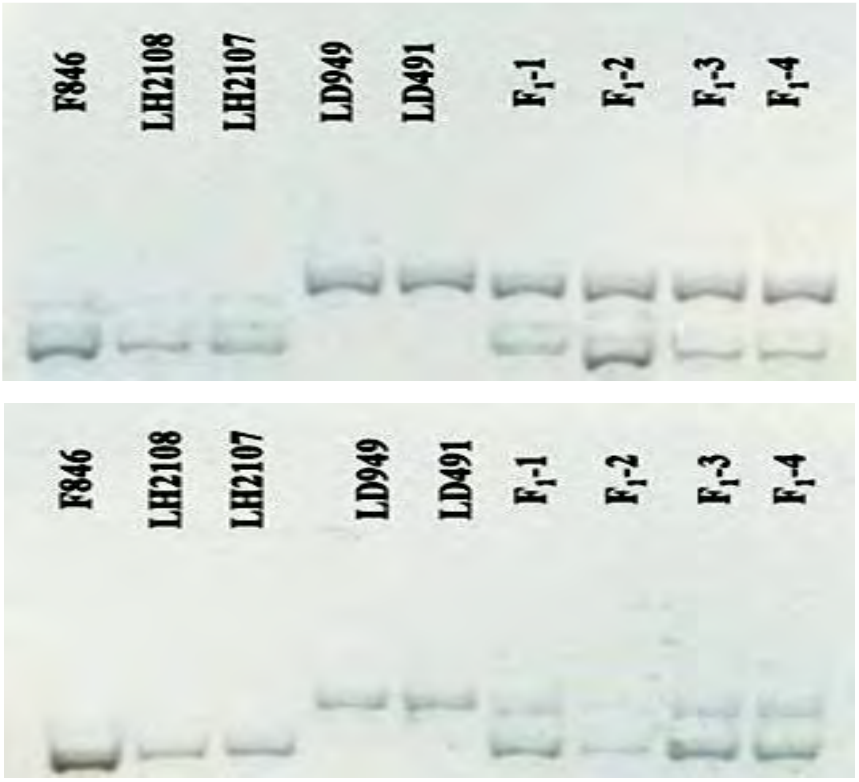


Fig. 3 Hybridity confirmation of interspecific F_1 hybrids through SSR markers (BNL1064 - Top and BNL1438 - Below)

Reaction of cotton test genotypes cotton leaf curl disease

Presently, cotton cultivation suffers from various biotic and abiotic stresses (Rahman et al. 2012; Rahman 2016). Cotton leaf curl disease is the most serious concern that significantly reduces the seed cotton yield (Monga et al. 2008, Nazeer et al. 2014a). Phenotypic reaction to CLCuD in parents along with their respective F_1s was observed. Female *hirsutum* parents were found to possess clear symptoms, whereas, male parents (*G. arboreum*) were symptomless. Similar reports about the susceptibility of *G. hirsutum* and resistance of *G. arboreum* are available (Tahir et al. 2011; Iqbal et al. 2015) Several reports are available where interspecific *G. hirsutum* × *G. arboreum* hybrids were found to be resistant to leaf curl virus (Tahir et al. 2011, Ahmad et al. 2011). In the present study, all four interspecific hybrids were found to be CLCuD susceptible and exhibited typical symptoms of the disease during 2017 (Pathak et al. 2017). However, in the next season (2018) symptoms disappeared from F_1-1 and F_1-2, whereas, F_1-3 and F_1-4 retained symptoms of the disease (Table 2). In an earlier study by Nazeer *et al* (2014) the F_1 hybrids were observed to be completely resistant to CLCuD, whereas, the level of resistance decreased in the successive backcross generations.

In the present investigation CLCuD reaction of the test genotypes was confirmed at the molecular level through the amplification of viral DNA using virus specific primers. Virus specific bands were amplified in all the *G. hirsutum* parents namely, F 846, LH 2108, and LH 2107; whereas, no virus specific band was observed in *G. arboreum* parental lines (LD 949 and LD 491). Two of the interspecific hybrids namely, F_1-1 and F_1-2 did not show the amplification of virus specific bands, whereas, amplification of the same were found in the other hybrids (F_1-3 and F_1-4) (Fig. 4). Amplified viral DNA of the susceptible genotypes (female parents, F_1-3, F_1-4) was of approximately 500 bp each which resembled the results reported by Deng et al. (1994); Maruthi et al. (2006); and Mahesh et al. (2010).

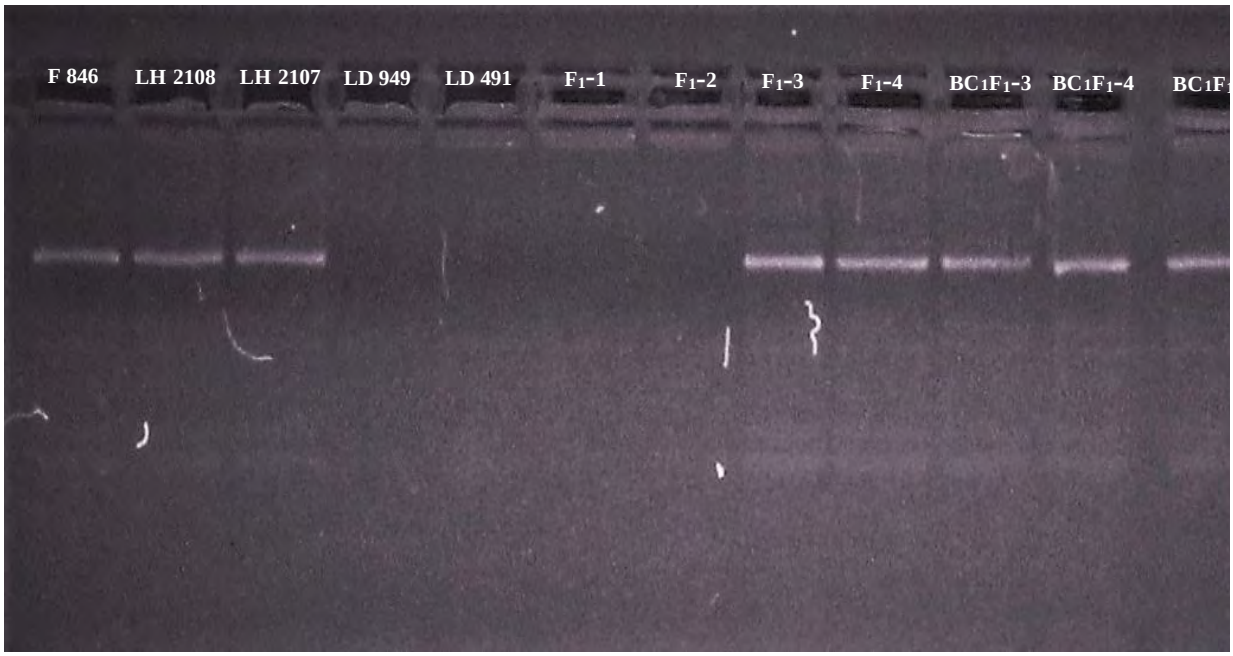


Fig. 4 *PCR amplification of virus specific bands in parents (G. hirsutumcv. F 846, cv. LH 2108, Acc. LH 2107, G. arboreumcv. LD 949, cv. LD 491), F_1 plants and first generation back cross derivatives; NC: Negative control*

Reaction of backcross derivatives to jassid

A total of 293 plants were assessed for leafhopper response in BC_1F_1 generation. The plants exhibiting JIG I and II were categorized as leafhopper tolerant whereas, plants showing JIG III and IV were considered susceptible. No insecticide treatment was given to the cotton seeds before sowing. Similarly, no chemical control was undertaken for leafhopper control throughout the crop season. The number of leafhopper tolerant and susceptible plants was counted in backcross generations and the χ^2 test was used to determinethe number of genes governing leafhopper tolerance. Data on nymph population were recorded on three leaves per plant, one each in the top, middle and lower canopy. Nymphal population was recorded thrice till 90 days after sowing (DAS) and data recorded during peak infestation were used for analysis. Data on leaf trichome density were recorded on parental lines and individual plants of the backcross population following Wright *et al*. (1999).

Of the 293 BC_1F_1 plants, 218 plants were found to be tolerant to leafhopper whereas, 75 plants were observed to be susceptible. Chi square analysis revealed that the observed and expected numbers did not differ significantly for digenic control. Thus, it is apparent that two dominant genes, either singly or in combination governed tolerance to leafhopper. Similar duplicate gene action has been observed in F_2 inter-varietal upland cotton population segregating for leafhopper resistance in 15:1 ratio (Pushpam and Raveendran 2005). Two gene control of leafhopper resistance has also been reported by Sikka and Singh (1953) in cotton. Simple monogenic dominant genetic control for leafhopper resistance has been demonstrated by Painter (1958) and Sikka and Singh (1953). On the other hand, Roy *et al*. (2017) observed inhibitory gene action (13 susceptible: 3 resistant) for resistance to leafhopper in an F_2 population derived from *G. hirsutum* × *G. barbadense* cross. The foregoing discussion reveals that tolerance to leafhopper in cotton is controlled by major genes but the gene action varies with different genetic backgrounds.

A set of 304 cotton- specific SSR markers was used to document polymorphism between the parental lines, of which 99 (32.6%) were observed to be polymorphic. Bulked Segregant Analysis (BSA) was conducted to identify SSR markers associated with leafhopper tolerance. Accordingly, DNA of 10 BC_1F_1 plants showing Jassid Injury Grade I was pooled to constitute 'tolerant' bulk in Population I. Similarly, 'susceptible' bulk was created by pooling DNA of an equal number of BC_1F_1 plants exhibiting JIG IV symptoms. The 99 polymorphic SSR markers were genotyped on the leafhopper 'tolerant' and 'susceptible' bulks so as to

identify the uncommon markers between the two bulks. Two SSR markers, namely NAU 922 (chromosome A5) and BNL 1705 (chromosome A11) were associated with leafhopper tolerance.

Various studies have explored the contribution of trichomes towards tolerance/susceptibility to insect pests in cotton. Higher leaf trichome density has been shown to influence feeding and reproduction of jassid (Murugesan and Kavitha 2010; Kanher *et al*. 2016). In the present investigation, leaf trichome density was assessed on one young and one mature leaf of each of 200 BC_1F_1 individuals in Population I and five plants of each parental line. Trichome density on the young and mature leaves was found to be 194 and 82.8, respectively, in LD 491. LH 2107 registered trichome density of 91 and 49 on young and mature leaves, respectively. Higher trichome density was present on young leaves in parents as well as in the backcross population compared to that of mature leaves. Similar findings on trichome density have been reported by Wright *et al* (1999); Nawab *et al*. (2011); Turley and Vaughn (2012); Grover *et al*. (2016); Suthar *et al*. (2021) in cotton. A higher number of nymphs was recorded on American cotton parent LH 2107 (6.9) in comparison to *desi* cotton parent LD 491 (2.6). Nymph count ranged from 2.1 to 8.1 with a mean value of 5.12 in the backcross population. A significant negative correlation (r = -0.39*) between nymph count and overall leaf trichome density was observed. Similarly, a significant negative association of nymph count with young (r = -0.36*) and mature leaf trichome density (r = -0.37*) was recorded in the backcross population in the present investigation. Thus, it is evident that higher trichome density on the cotton leaves is unfavourable to leafhopper incidence. Similar reports of the negative association of trichome density with the jassid population are available in cotton (Ashfaq *et al*. 2010; Murugesan and Kavitha 2010; Khalil *et al*. 2017; Khan *et al*. 2017). The results reported here set the stage for mapping and use of these genes conferring leafhopper tolerance in breeding new upland cotton cultivars.

Conclusions

The interspecific derivatives developed in the present investigation will serve as the base material for transferring leaf hopper resistance in upland cotton. Besides, the interspecific hybrids will be useful to study genetic and epigenetic events occurring in the nascent polyploids.

Funding

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References

1. Ahmad S, Mahmood K, Hanif M, et al. Introgression of cotton leaf curl virus-resistant genes from Asiatic cotton (*Gossypium arboreum*) into upland cotton (*G. hirsutum*). Genet Mol Res. 2011;10:2404-14.
2. Ashfaq M, Ane MN, Zia K, et al. The correlation of abiotic factors and physicomorphic characteristics of (*Bacillus thuringiensis*) Bt transgenic cotton with whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) and jassid, *Amrasca devastans* (Homoptera: Jassidae) populations. African J Agri Res. 2010;5:3102-7.
3. Beasley JO. The origin of American tetraploid *Gossypium* species. Am Nat. 1940;74:285-6.
4. Briddon RW, Markham PG. Cotton leaf curl virus disease. Virus Res. 2000;71:151-59.
5. Chen Y, Wang Y, Zhao T, et al. A new synthetic amphiploid (AADDAA) between *Gossypium hirsutum* and *G. arboreum* lays the foundation for transferring resistances to *Verticillium* and drought. PLoS One. 2015;10:e0128981.
6. Deng D, McGrath PF, Robinson DJ, et al. Detection and differentiation of whitefly transmitted gemini viruses in plants and vector insects by the polymerase chain reaction with degenerate primers. Ann Appl Biol. 1994;125:327-36.
7. Eigenbrode SD, Espelie KE. Effect of epicuticular lipid on insect herbivores. Annu Rev Entomol.1995;l40:171-94.
8. Farooq J, Farooq A, Riaz M, et al. Cotton leaf curl virus disease a principle cause of decline in cotton productivity in Pakistan (a mini review). Can J Plant Prot. 2014;2:9-16.
9. Ghelani MK, Kabaria BB, Chhodavadia SK. Field efficacy of various insecticides against major sucking pests of Bt cotton. J Biopestic. 2014;7:27.
10. Grover G, Kaur B, Pathak D, et al. Genetic variation for leaf trichome density and its association with sucking insect-pests incidence in Asiatic cotton. Indian J Genet. 2016;76:365-68.
11. Iqbal MA, Ammad A, Zafar Y. Characterization of indigenous *Gossypium arboreum* L. Genotypes for various fiber quality traits. Pak J Bot. 2015;47:2347-54.
12. Kalyan RK, Saini DP, Meena BM, et al. Evaluation of new molecules against jassids and whiteflies of Bt cotton. J Entomol Zoo Stud. 2017;5:236-40.

13. Kanher FM, Syed TS, Abro GH, et al. Some physio-morphological leaf characters of gamma irradiated cotton lines to resistance against Jassid (*Amrasca devastans* Dist.). J Entomol Zool Stud. 2016;4:80–5.
14. Kaur H, Pathak D, Rathore P. Development and characterization of an interspecific *Gossypium hirsutum* × *Gossypium armourianum* hybrid. Appl Biol Res. 2016;18:146-54.
15. Khalil H, Raza ABM, Afzal M, et al. Effects of plant morphology on the incidence of sucking insect pests complex in few genotypes of cotton. J Saudi Soc Agric Sci. 2017;16(4):344-9.
16. Khan MA, Shahid AA, Rao AQ, et al. Defense strategies of cotton against whitefly transmitted CLCuV and Begomoviruses. Adv Life Sci. 2015;2:58-66.
17. Khan MA, Shahid AA, Rao AQ, et al. Role of epicuticular waxes in the susceptibility of cotton leaf curl virus (CLCuV). Afr J Biotech. 2011;10:17868-74.
18. Khan MM, Ali A, Nadeem I, et al. Morphological and biochemical Bt cotton plant trait induced resistance against cotton jassid *Amrasca biguttula biguttula* (Ishida) and cotton whitefly *Bemisia tabaci* (Genn.). J Innov Bio Res. 2017;1:36-45.
19. Kranthi KR, Stone GD. Long-term impacts of Bt cotton in India. Nat. Plants. 2020;6:188-96.
20. Kranthi KR. ICAC Cotton Data Book 2020. International Cotton Advisory Committee, Washington DC, USA. 2019; 6
21. KumarV, Kular JS, Kumar R, et al. Integrated whitefly [*Bemisia tabaci* (Gennadius)] management in Bt-cotton in North India: An agroecosystem-wide community-based approach. Curr Sci. 2020;119:618–24.
22. Mahesh YS, Shankarappa KS, Rangaswamy KT, et al. Detection and characterisation of a begomovirus associated with leaf curl disease of ornamental croton (*Codiaeum variegatum*). J Hort Sci Biotechnol. 2010;85:101-5.
23. Maruthi MN, Rekha AR, Alam SN, et al. A novel begomovirus with distinct genomic and phenotypic features infects tomato in Bangladesh. Pl Pathol. 2006;55:290.
24. Monga D, Kumar M, Chander S, et al. Identification of cotton leaf curl virus disease (CLCuD) resistant lines. J Cott Res and Dev. 2008;22:234-7.
25. Montes E, Coriton O, Eber F, et al. Assessment of gene flow between *Gossypium hirsutum* and *G. herbaceum*: evidence of unreduced gametes in the diploid progenitor. G3. 2017;7:2185-93.
26. Murugesan N, Kavitha A. Host plant resistance in cotton accessions to the leafhopper, *Amrasca devastans* (Distant). J Biopestic. 2010;3(3):526-33.
27. Nawab NN, Khan IA, Khan AA, et al. Characterization and inheritance of cotton leaf pubescence. Pak J Bot. 2011;43:649-58.
28. Nazeer W, Tipu AL, Ahmad S, et al. Evaluation of cotton leaf curl virus resistance in BC_1, BC_2, and BC_3 progenies from an interspecific cross between *Gossypium arboreum* and *Gossypium hirsutum*. PloS one. 2014;9:e111861.
29. Newaskar GS, ChimoteVP, Mehetre SS, et al. Interspecific hybridization in *Gossypium* L.: characterization of progenies with different ploidy confirmed multigenomic backgrounds. Plant Breed. 2013;132:211-6.
30. Painter RH. Resistance of plants to insects. Annu Rev Entomol. 1958;3(1):267-90.
31. Pathak D, Pooja, Rathore P, et al. Interspecific hybrids between *Gossypium hirsutum* × *Gossypium arboreum* are susceptible to cotton leaf curl disease. Proc Indo-US Symp.2017;85. Punjab Agricultural University, Ludhiana (Abstr), India.
32. Pushpam R, Raveendran TS. Artificial screening and inheritance studies on resistance to jassid (*Amrasca devastans*) in *Gossypium hirsutum* L. Indian J Genet 2005;65(03):199-201.
33. Radhakrishnan G, Malathi VG, Varma A. Biological characterization of an isolate of cotton leaf curl Rajasthan virus from Northern India and identification of sources of resistance. Indian Phytopath. 2004; 57:174-80.
34. Rahman M, Shaheen T, Tabbasam N, et al. Cotton genetic resources: A review. Agron Sustain Dev. 2012;32:419-32.
35. Rahman M. Cotton improvement for economically stressed economies. ICAC Recorder2016*;* 1:12-3.
36. Rahmat Z, Ahmed S, Zia ZU, et al. Genetic monitoring of introgressed alleles from *Gossypium arboreum* L. into *G. hirsutum* L. using SSR markers: a potential approach for bringing new alleles under cultivation. Pak J Bot.2019;51:479-86.
37. Roy U, Patil RS, Katageri IS, et al. Genetic studies on resistance to jassid (*Amrasca biguttula biguttula*) in segregating F_2 population of interspecific (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) Cotton. Int J Curr Microbiol App Sci. 2017;6(4): 2683-6.
38. Sikka SM, A Singh. Inheritance of jassid (*Empoasca devastans* Dist) resistance in some upland American cottons. Indian Cott Grow Rev. 1953;7:113-7.
39. Singh L, Pathak D. Genetic variation for some morphological characters in desi cotton (*Gossypium arboreum* L.). App Biol Res. 2017;19:232-6.
40. Suthar T, Gupta N, Pathak D, et al. Morpho-anatomical characterization of interspecific derivatives of *Gossypium hirsutum* L.× *G. armourianum* Kearney cross for whitefly tolerance. Phytoparasitica. 2021;50(2):1-19.
41. Tahir MS, Noor UI. Development of an interspecific hybrid (Triploid) by crossing *Gossypium hirsutum* and *G. arboreum*. Cytologia. 2011;76:193-9.
42. Turley RB, Vaughn KC. Molecular biology and physiology differential expression of trichomes on the leaves of Upland cotton (*Gossypium hirsutum* L.). J Cotton Sci. 2012;16:53-71.
43. Vij S, Pathak D, Kaur N, et al. Development and molecular confirmation of interspecific hybrids between *Gossypium hirsutum* and *Gossypium arboreum*. Agric Res J. 2016;53:169-72.
44. Webber JM. Interspecific hybridization in *Gossypium* and the meiotic behavior of F_1 plants. J Agric Res.1935;51:1047-70.
45. Wright RJ, Thaxton PM, El-Zik KM, et al. Molecular mapping of genes affecting pubescence of cotton. J Hered. 1999;90(1):215-9.

6

Epicuticular Wax Content in the Hairy Leaves of Cotton (*Gossypium Barbadense*) As a Mechanical Barrier against Some Insect Pests

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Abstract

***Background**: In recent times, the pest scenario has completely changed since the introduction of Bt cotton in India. The earlier called secondary pests such as leafhoppers (*Amrasca biguttula biguttula*) and aphids (*Aphis gossypii *(Glover) become the primary pests and become quite serious from the seedling stage to the boll bearing stage and their heavy infestation at times reduces the crop yield to a greater extent. The study of epicuticular wax with plant hairiness is of significant importance in cotton, especially in India where insect pests are growing into a very serious menace. Among the four cultivated species of cotton, the Egyptian cotton* Gossypium barbadense *is highly susceptible to sucking pests. Hence, there is an urgent need to identify some germplasm accessions that have inbuilt natural resistance /tolerance for the sucking pests in* G. barbadense *cotton.*

***Methods**: The impact of epicuticular wax content on damage levels of cotton pests was studied under field conditions at ICAR-Central Institute of Cotton Research, Regional Station, experimental farm, Coimbatore, India during the 2015-16 and 2016-17 crop seasons. The wax content estimation was carried out in 8 germplasm accessions of Gossypium barbadense with Suvin as a check variety.*

***Results**: The insect pests showed the least preference for cotton bolls with high epicuticular wax. The accession EC-18 had the maximum amount of wax (343 µg/cm2) followed by HAG-02 (305 µg/cm2). The eight germplasm accessions were also scored for disease index (DI %), seed cotton yield (SCY), number of sympodia, number of bolls/plant (NB), and plant height (Pht) for the two crop seasons. A significant negative correlation between wax content and disease index percentage was found during both crop seasons. Wax content was found positively correlated with seed cotton yield (SCY), number of sympods (NS), and number of bolls (NB). These results indicated that wax content was involved in resisting sucking pests and it also had a positive effect on plant growth and yield potential. There was a strong negative relationship between epicuticular wax content and the level of plants infested by sucking pests.*

***Conclusions**: Four germplasm accessions (EC-18, EC-2/13, ICB-67, and ICB-124) were not showing any symptoms of pest infestation during both seasons. These cotton genotypes contained relatively higher wax content compared to other accessions.*

***Keywords**: Gossypium barbadense, germplasm, epicuticular wax, aphids, leafhoppers, infestation*

Background

Cotton is an important commercial crop and plays a vital role in the Indian economy, both in terms of providing employment directly or indirectly to about 60 million people and in terms of production of wealth and earning foreign exchange for the country. In recent times, the pest scenario has completely changed since the introduction of Bt cotton in India. The earlier called secondary pests such as leafhoppers (Amrasca biguttula biguttula) aphids (Aphis gossypii (Glover) become the primary pests and become quite serious from the seedling stage to the boll bearing stage and their heavy infestation at times reduces the crop yield to a greater extent (Dhawan et al. 2008, Murugesan and Kavitha 2010). To have a productive crop we need to spray (insecticides/fungicides) at least 6-8 times per crop season. Waxes are the waterproofing component of the plant cuticle and are therefore essential for life in an aerial environment. Glossy crop phenotypes are often less susceptible to insect herbivores than are normal phenotypes (Eigenbrode and Espelie 1995). Insects interact with the surfaces of coatings (wax)

when they land, crawl or climb on them many insects are considered pests because they pose a serious threat to agriculture (Muller C 2018). Epicuticular wax covers plant aerial organs to protect them from various biotic and abiotic stresses (Kosma et al. 2010, Muhammed Saeed et al. 2019) and this wax mediates other ecological functions including host plant resistance against pathogens (Reina-Pinto and Yephremov, 2009)) and herbivores (Eigenbrode et al., 1991b; Muller, 2008). In addition, waxes may protect plants from bacterial and fungal pathogens and play a role in the plant to insect interactions (Muhammed Saeed et al. 2019). The study of epicuticular wax with plant hairiness is of significant importance in cotton, especially in India where insect pests are growing into a very serious menace. Among the four cultivated species of cotton, the Egyptian cotton Gossypium barbadense is highly susceptible to sucking pests starting from 30 days of sowing up to harvest (Dhamayanthi et al. (2020). The costs of chemical control growing the ineffectiveness of these pesticides against insects have necessitated finding out an effective alternative method. Possible strategies required to tackle insect pests include insecticides, insect-repellent, and low insect adhesion coatings Mayee et al. (2004). The former however is harmful to the environment and alternative strategies are preferred. Hence, there is an urgent need to identify some germplasm accessions that have inbuilt natural resistance /tolerance for the sucking pests in G. barbadense cotton. Keeping in view of the present pest scenario, the experiment was designed to assess the wax content in the hairy germplasm accession of G. barbadense and estimate the sucking pest resistance/tolerance, and also find out the role of wax content in controlling the sucking pest infestation in cotton.

Results

The results reveal that mean squares from ANOVA referring to the effect of G. barbadense germplasm lines in the percentage of disease Index (DI), wax content (WC), plant height (Pht), number sympodia (NS), number of bolls (NS) and seed cotton yield (SCY) for the 2016 and 2017 crop season were significant ($p = 0.05$). Based on the data, the accessions had significant differences concerning studied traits (Table 2). For the percentage of disease index, the differences in values across seasons and genotype × environment were not significant. These results suggested that the behavior of the germplasm lines was consistent concerning jassids and aphids resistance and susceptibility during 2016 and 2017. However, great variation in the disease index percentage was observed among genotypes and it was reflected in a high coefficient of variation (109.4%) for disease index percentage. This indicates that germplasm lines under study possess different genetic potentials to tolerate sucking pests. Some of the accession was resistant to sucking pests during both 2016 and 2017, so the minimum values for disease index percentage were zero during both years. For each accession, wax content differed by 35-40 µg/cm-2 in both seasons. Genotype × environment interactions for wax content were also significant ($p = 0.001$). This indicates that the wax content for each accession is greatly influenced by environmental conditions. Several environmental factors (such as drought, temperature, and pathogen attack) influence the quantity of cuticular wax in plants (Jeffree, 2006; Kosma et al., 2010; Yeats & Rose, 2013; Xue et al., 2017).

Table 2. Mean squares for traits of germplasm accessions of *G. barbadense* for two seasons (2016 & 2017).

SoV	DI%	WC	Pht	NB	NS	SCY
Replication	252 NS	1 NS	359 NS	69NS	332NS	822NS
Genotypes	180*	6321***	676***	198***	668***	1713***
Season	133NS	299***	4682***	1289***	1013***	4728***
G x E	104 NS	1057***	542***	196***	233***	1397***
Error	109.4	4	192	66	167	611
R^2	0.51	1.00	0.59	0.62	0.54	0.70
CV %	0.183	5.2	6.7	23.5	22.6	34.2

SoV= Source of variation; R2 = Model sum of squares / Total sum of squares; CV = Coefficient of variation. NS, non-significant; *, **,***, significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively

Data recording for morphological and yield-related traits

Morphological data such as plant height (cm), number of sympodia per plant, number of bolls per plant, and seed cotton yield (kg/ha) were recorded at maturity for all eight germplasm accessions during the seasons (2016 & 2017).

Tolerant /susceptible germplasm lines against sucking pest infestation

The germplasm accessions under study exhibited consistent behavior concerning sucking pest tolerance/susceptibility during the 2016 and 2017 seasons (Table-3).

Table-3: Screening hairy lines for resistance against leafhopper (*Amrasca biguttula biguttula*) and aphids (*Aphis gossypii. Glover*)) in *G. barbadense* cotton

Cotton hairy lines	Mean population of jassids (per 3 leaves in cotton)		Mean population of Aphids (per 3 leaves in cotton)		Leafhopper resistance index	Resistant category
	45 DAS	90 DAS	45 DAS	90 DAS		
EC-2/13	3.7 (2.03)	4.6 (2.25)	12.1(3.45)	9.6(3.17)	2.4	T
ICB-67	3.6 (2.01)	2.5 (1.6)	11.3(3.4)	28.1(5.27)	2.4	T
ICB-85	4.3 (2.16)	6.7 (2.67)	12.3(3.38)	7.1(2.74)	2.6	S
ICB-124	5.7 (2.49)	2.9 (1.83)	17.3(4.2)	18.3(4.32)	2.7	T
EC-18	5.5 (2.43)	3.3 (1.93)	11.9(3.5)	11.0(3.37)	2.5	T
ICB-105	4.9 (2.29)	4.1 (2.13)	10.9(3.36)	11.3(3.37)	3.1	S
ICB-281	2.1 (1.51)	3.3 (1.94)	9.3(3.13)	15.1(3.91)	2.4	S
ICB-264	5.3 (2.39)	4.7 (2.27)	4.7(2.26)	12.5(3.6)	2.8	S
Suvin	0.576	0.555	14.5(3.86)	16.9(4.14)	-	-
SE(m)	0.194	0.187	1.126	1.258	-	-
SE(d)	0.274	0.26	0.422	0.423	-	-
C.V.	14.17	11.92	0.597	0.599	-	-
			11.565	14.302	-	-

Values in parentheses are transformed values √x + 0.5; DAS = Days after Sowing;T=Tolerant: S=Susceptible

Accessions viz, EC-2/13, ICB-124, ICB-67, and EC-18 were found to be tolerant /highly tolerant to infestation during both the 2016 and 2017 seasons. It is interesting to note that the difference between the amount of wax content in these cotton accessions was ≤ 35µg/cm2. Among these germplasm accession EC-2/13, ICB-67, ICB-124, and EC-18 appeared tolerant and did not show any symptoms of infestation during both 2016 and 2017 (Table 3). All these tolerant cotton accessions possessed relatively higher wax content. All these 6 exotic accessions possessed excessive hairs all over the leaves of germplasm lines (Pilose). The hirsute germplasm accessions viz., ICB-85, ICB-105, ICB264, and ICB-281are have comparatively less wax content and fewer leaf hairs ranging from 189-225 µg/cm-2 and all these lines are less tolerant to leafhoppers and aphids than the other accessions and the susceptible check variety Suvin. It reveals that a minimum threshold quantity of wax content (30-35µg/cm2) is required to offer resistance to sucking pest infestation.

Table 4. Correlation coefficients among traits (%DI, disease index; WC, wax content, µg/cm2; SCY, seed cotton yield/plant, g; NB, number of bolls/plant; Pht, plant height, cm) of cotton genotypes.

Trait	Crop seasons (years)	DI (%)	WC	SCY	NS	NB	Pht
DI (%)	Seasons	1					
	2016						
	2017						
WC	Seasons	-0.30***	1				
	2016	-0.36***					
	2017	-0.23*					
Pht	Seasons	-0.37***	0.21**	1			
	2016	-0.43***	0.32***				
	2017	-0.34***	0.06 NS	0.95***			
NS	Seasons	-0.34***	0.23***	0.95***	1		
	2016	-0.45***	0.33***	0.95***			
	2017	-0.28**	0.11NS	0.15*			
NB	Seasons	-0.38***	0.12NS	0.21*	0.12*	1	
	2016	-0.43***	0.11NS	0.25**	0.15*		
	2017	-0.40***	0.14NS	0.13*	0.21**		
SCY	Seasons	-0.37***	0.20**	0.22**	0.23**	0.06*	1

2016	-0.43***	0.32***	0.15*	0.17*	0.13*
2017	-0.34***	0.05 NS	0.21*	0.23**	021**

NS non significant * ** *** Significant $p \leq 0.01$ and $p \leq 0.001$, respectively

There was a significant negative correlation between the percentage disease index and wax content during 2016 ($p \leq 0.001$) and 2017 ($p \leq 0.05$) (Table-4). Similarly, disease index percentage had a negative correlation with seed cotton yield, number of sympodia, number of bolls, and plant height during both seasons. Wax content showed a positive correlation with seed cotton yield and the number of bolls. This correlation was highly significant in two seasons (2015-16 & 2016-17). Wax content had also a positive correlation with Pht, but it was non-significant.

Discussions

Epicuticular waxes play a very important role in hindering the attachment of insects to the leaves (Reina-Pinto and Yephremov, 2009) and herbivores (Eigenbrode et al., 1991b; Muller, 2008). A plant cuticle is a protecting film covering the epidermis of leaves, young shoots, and other aerial plant organs without a periderm. It consists of lipid and a hydrocarbon polymer impregnated with wax and is synthesized exclusively by the epidermal cells (Smith G R 1999). The cuticle is the first layer of defense for protecting plants from environmental adversities (Muller C 2018). The cuticle consists of a cutin/cutan matrix to which the cuticular waxes are either embedded (intra-cuticular waxes) or presents on the outer layer of epicuticular waxes (Smith R.G.1999, Yeats & Rose, 2013). These cuticular waxes are reported to protect plants against various biotic and abiotic stresses (Shepherd & Griffiths, 2006; Yeats & Rose (2013), Xue et al. (2017) and Rami Horowitz A, and Isaac Ishaaya (2004). A study based on the reduced susceptibility of glossy plants can help elucidate the role of plant surface lipids in insect-plant interactions (Eigenbrode and Espelie 1995).

Experiments carried out in the present study indicated that a significant negative correlation was found between wax content and disease index percentage. This showed that cuticular wax had a role in conferring resistance to cotton plants against sucking pests such as leafhoppers and aphids infestation. These sucking pests never prefer the leaves which have more hairs with high wax content for feeding that rendering these germplasm accessions resistant to sucking pest infestation (Kumar et al.2015). The findings of our research support a new indirect role of wax in giving tolerance against sucking pests in G. barbadense cotton germplasm. In earlier studies, it was mentioned that cuticular wax has a role in giving resistance to the insect Hessian fly attacks. Kosma et al. (2010) reported that resistant plants to Hessian fly attack produced more waxes and cutin than susceptible plants. In the case of sucking pests, the role of cuticular wax will open new horizons for research endeavors in near future. Four G. barbadense germplasm (EC-2/13, ICB-67, ICB-124, and EC-18) which did not show any signs of sucking pest infestation during both the 2016 and 2017 crop seasons possess the highest level of tolerance against sucking pests. Smith R G (1999) suggested that the aphids incident were less in the plant population where the wax deposition is reportedly more. In this study, cotton germplasm accession with ≤ 25 µg/cm2 wax content was found less tolerant or susceptible to sucking pests (leafhoppers and aphids). The findings are matched with the suggestions rendered by (Muhamed Saeed et al. 2018). Further, it is suggested that the quantity of wax content is critical for resistance to sucking pests and may be used as a useful indicator for screening tolerance or susceptibility of cotton germplasm lines. Sucking pest infestation is mainly influenced by environmental prevailing in the Coimbatore area during crop seasons (Kumar et.al.2015). As a result of the timely application of insecticides for sucking pest control, cotton germplasm lines were free from jassids and aphids. So, Environment × Genotype interaction with disease index percentage was non-significant. There was a significant temperature difference during the 2016 and 2017 cotton growing seasons in the experimental area (Coimbatore). Temperature influenced wax content, So E x G interaction with wax content was highly significant. The difference in wax content of each cotton genotype with temperature fluctuation was proportional to the amount of wax present in it (Fig. 1), so each cotton accession showed its susceptibility/tolerance to sucking pest infestation according to its inherent potential of wax content.

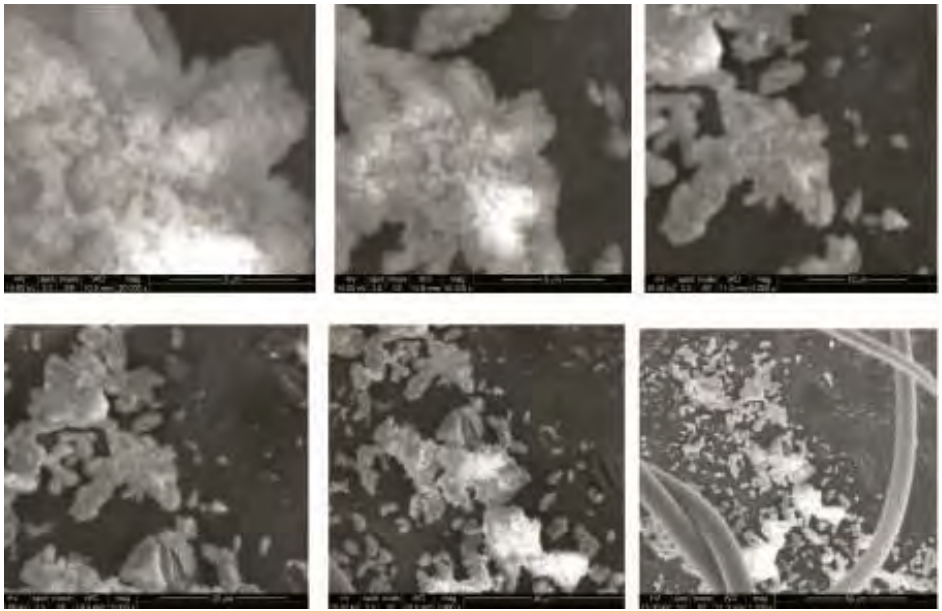

Fig. 1: *Scanning Electron Microscopic studies on wax content of G. barbadense germplasm accession*

Conclusion

The present study reveals that to resist sucking pests, the germplasm lines must possess a minimum threshold quantity of wax content, otherwise, they would be rendered susceptible to sucking pests. It implied that epicuticular wax rendered a mechanical barrier in controlling sucking pests in G. barbadense cotton. Classical breeding efforts to produce waxy genotypes of barbadense cotton are in progress at ICAR-Central Institute for Cotton Research Regional Station, Coimbatore. Because genetical studies reports that the waxy trait is determined by single genes and breeding these traits in to genotypes/varieties should be straightforward. Chemical manipulation of leaf surface wax to emulate the glossy trait has the potential to control pests. The door is now open for genetic engineering of crops with epicuticular waxy phenotypes designed to limit pest damage (Eigenhrode and Espelie 1995). Single gene mutations affecting leaf surface wax have been produced and characterized in several crops as well as in Arabidopsis thaliana. To harness this potential trait effort has to be taken by the cotton breeders/entomologist to have high-yielding sucking pest-resistant genotypes in cotton irrespective of the species.

Methods

The experiments and field trials were conducted at ICAR - Central Institute for Cotton Research, Regional Station Research farm, Coimbatore during the 2015-16 and 2016-17 crop seasons. Eight germplasm accession of Gossypium barbadense, L. (EC-2/13, EC-18, ICB-67, ICB-85, ICB-105, ICB-124, ICB-264, ICB-281 and Suvin (check) to find out the tolerance level for sucking pest infestation (Table 1). Plant material was sown in two crop seasons July 2016 and July 2017 following randomized block design (RBD) with three replications. A standard agricultural package of practices was followed for growing these germplasm accessions. Data for sucking pests (leafhopper and aphids) were scored at weekly intervals. Data were scored by counting several insect pests from randomly selected 15 leaves from 15 plants of each plot. Leaves were selected as one leaf from the upper portion of one plant, the second leaf from the middle portion of 2nd plant, and the third leaf from the lower portion of 3rd plant. This process was repeated in the same sequence, till the 15th plant. Data were scored from 5 randomly selected plants on each plot. To calculate percentage infestation, a total number of bolls and squares/flowers, and infested bolls and squares/flowers were counted. During the crop seasons (2016 and 2017) experimental area was sprayed six times to control insect pests. Special care was taken to control both leafhoppers and aphids and when its population crossed the threshold of 5 adults per cotton leaf.

Methodology of screening for leaf hoppers

In each plant, three leaves - one each from the top, middle, and bottom strata were observed, and the mean population per three leaves was worked out at 45 & 90 DAS.

Table-1 Cotton genotypes identified for the experiment

Code Nos	Variety/ Accessions	Origin	Hairiness Stage	Wax content (µg/cm-2)
EC-2/13	HAGR-25-25-02	Exotic	Pilose	305 µg/cm-2
EC-18	EC-617860	Exotic	Pilose	343 µg/cm-2
ICB-124	ERB-4488	Indigenous	Pilose	316 µg/cm -2g
ICB-85	1/5W	Exotic	Hirsute	132 µg/cm -2g
ICB-105	87/1	Exotic	Hirsute	119µg/cm -2g
ICB-67	EC-141729	Exotic	Pilose	225 µg/cm-2
ICB-281	EC-13758	Exotic	Hirsute	107µg/cm-2
ICB-264	26-1-3	Exotic	Hirsute	189µg/cm-2
Suvin	Sujatha x St.Vincent	CICR, Regional Station, Coimbatore	Glabrescent	-

Leafhopper Resistance Index (LHRI)

The entries were classified into different categories based on the leafhopper resistance index (LHRI) (Nageswararao, 1973b).

$$LHRI = \frac{(G_1 xP_1)+(G_2 XP_2)+(G_3 XP_3)+(G_4 XP_4)}{P_1+P_2+P_3+P_4}$$

Where, G - Leafhopper Injury Grade,
P - The plant population under the grade for each category

Hopper burn assessment was rated by adopting the 1-4 Grade Scale:

Grade 1: Leaves free from crinkling and curling, yellowing, bronzing, and drying
Grade 2: Crinkling, curling, slight yellowing in a few leaves on the lower portion of the plant
Grade 3: Crinkling, curling, yellowing, browning, and bronzing in the middle and lower portion and plant growth hampered
Grade 4: Extreme crinkling, curling, yellowing, browning, bronzing, and drying of leaves, defoliation, and stunted growth

After indexing, the entries were categorized as highly resistant (1.0 - 1.5), resistant (1.51 - 2.0), moderately resistant (2.01 - 2.5), susceptible (2.51 - 3.0) and highly susceptible (3.01-4.0) detailed below following Pandi (1997).

Aphid Resistance Index (ARI)

Leaf cupping/crumpling due to aphid infestation was rated by adopting the 1-4 Grade Scale:

Grade 1: Entire plant free from cupping/crumpling.
Grade 2: Cupping/crumpling of a few leaves on the upper portion of the plant.
Grade 3: Cupping of leaves upper leaves and aphids all over the plant.
Grade 4: Extreme cupping, sickness/ sooty mold.

The entries were classified into different categories based on the aphid resistance index (ARI)

$$ARI = \frac{(G_1 xP_1)+(G_2 XP_2)+(G_3 XP_3)+(G_4 XP_4)}{P_1+P_2+P_3+P_4}$$

Where, G - Leaf cupping/crumpling Grade,
P - The plant population under the grade for each category

After indexing, the entries were categorized as highly resistant (1.0 - 1.5), resistant (1.51 - 2.0), moderately resistant (2.01 - 2.5), susceptible (2.51 - 3.0) and highly susceptible (3.01-4.0) detailed below following Pandi (1997).

Extraction of cuticular wax and quantification of wax content

Leaf samples of (25 days-old) l from each accession of germplasm were collected on November 17, 2016, and November 17, 2017. Cuticular wax was extracted from these leaves following the method of Bondada et al. (1996). Leaves were immersed in pre-weighed Petri plates having chloroform (10mL) for 10-15 seconds. Afterward, leaves were removed and after complete evaporation of chloroform, Petri plates were weighed again. Wax content was calculated by subtracting the initial weight from the final weight of Petri plates and expressed as µg/cm2 of leaf area.

Sucking pests infestation scoring.

All agricultural practices were uniformly applied to the experimental field area so that a constant level of Jassids and Aphids were present to spread among the cotton accessions. Sucking pest infestation (disease index) was scored following the method described by Pandi et al. (2010). This disease rating scale has been used in different plant species to calculate the % of disease incidence in different plant diseases (Muller C. 2018.).

Data analysis

Data were analyzed by statistical software GenSat for descriptive statistics, Pearson's correlation coefficients, and analysis of variance (ANOVA) estimates. Pearson's correlation coefficients were estimated at probability p = 0.05. ANOVA was performed according to RBD with two factors (genotypes and years)

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References

1. Bondada B R, Oosterhuis D M, Murphy J B, Kim K S, 1996. Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (Gossypium hirsutum L.) leaf, bract and boll. Environ Bot 36:61-69.
2. Dhamayanthi K. P. M, Rameash K, Manivannan A, Annie Sheeba and. Abirami S. 2020. Studies on leaf hairiness and sucking pest resistance in Egyptian cotton (Gossypium barbadense, L.,) J Ento & Zoology Studies. 8(1): 591-594.
3. Dhawan, A.K, Sher, P.S. Jindal V and Aggarwal. N. 2008. Changing scenario of cotton insect pests and their management strategies. 81 - 99 PP. In Punjab. Dept. Plant Breeding, Genetics and Biotechnology, Punjab Agril. Univ., Ludhiana. 109 PP.
4. Eigenbrode, S. D., Stoner, K. A., Shelton, A. M., and Kain, W. C. (1991b). Characteristics of glossy leaf waxes associated with resistance to diamondback moth (Lepidoptera: Plutellidae) in Brassica oleracea. J. Econ. Entomol. 84, 1609–1618. doi: 10.1093/jee/84.5.1609.
5. Eigenbrode, S. D and Espelie, K. E. (1995). Effects of plant epicuticular lipids on insect herbivores. Annu. Rev. Entomol. 40, 171–194.
6. Jeffree CE, 2006. The fine structure of the plant cuticle. In: Biology of the plant cuticle, Riederer M, Müller C (Eds.). Annual Plant Reviews Series, 3, 11-125. Blackwell Publication Ltd, Oxford.
7. Kumar B. D., Sridevi D., Babu T. R. and Radhakrishna K. V. (2015). Determination of trichome density in different dual toxin transgenic Bt cotton (Gossypium spp.) hybrids (BG-II) and non-Bt. J. Res. PJTSAU, 43(3): 28-29.
8. Kosma DK, Nemacheck JA, Jenks MA, Williams CE, 2010. Changes in properties of wheat leaf cuticle during interactions with Hessian fly. Plant J 63: 31-43.
9. Mayee, C.D., Gautam, H.C. and Barik, A. 2004. Cotton scenario in India vis-a-vis world and future need. In : Recent Advances in Cotton Research and Development (Chauhan, M.S. and Sain, R.K., Eds.) Haryana Agricultural University and Cotton Research and Development Association, CCSHAU, Hisar. PP 245-253.
10. Muhammed Saeed, Xianliang Song, Sun Xuezhen and Muhammad Riaz. 2019 Leaf cuticular wax content is involved in cotton leaf curl virus disease resistance in cotton (Gossypium hirsutum L.) Spanish Journal of Agricultural Research :6 (4), e0705. DOI. 10.5424/sjar/2018164-13085.
11. Muller, C. (2008). "Resistance at the plant cuticle," in Induced Plant Resistance to Herbivory, ed. A. Schaller (Dordrecht: Springer-Verlag), 107–129].
12. Muller, C. (2018). Plant–Insect Interactions on Cuticular Surfaces in 'Biology of the Plant Cuticle'. Annual Plant Reviews book series, Vol- 23.
13. Murugesan N and Kavitha A.2010. Host plant resistance in cotton accessions to the leaf hopper Amrasca devastans (Distant), Journal of Biopesticides, 3(3): 526 - 533 (2010).
14. Nagerwararao, P. 1973a. An index for resistance in cottonhopper burn assessment. Madras AgriculturalJournal, 60 (4) : 264 – 266
15. Pandi R. 1997. Ecology and management of Leafhopper, Amrasca devastans (Distant) Thesis. Tamil Nadu Agrl. Univ., Killikulam.

16. Patel R.K and Radadia G.G. 2018. Screening of Cotton Varieties/Genotypes against Jassid, Amrasca biguttula biguttula (Ishida) under Rainfed Conditions. Int.J. Curr. Microbiol. App.Sci (2018) 7(12): 1099-1110.
17. Rami Horowitz A. and Isaac Ishaaya 2004. Insect Pest Management: Field and Protected Crops. Book chapters Pp 149-217.
18. Reina-Pinto JJ, Alexander Yephremov 2009.Surface lipids and plant defensesurface lipids and plant defenses. Plant Physiology and Biochemistry 47(6):540-9.
19. Smith RG, 1999. Wax glands, wax production and the functional significance of wax use in three aphid species (Homoptera: Aphididae). J Nat His 33: 513-530.
20. Xue D, Zhang X, Lu X, Chen G, Chen ZH, 2017. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Front Plant Sci 8: 621.
21. Yeats TH, Rose JKC 2013.The formation and function of plant cuticles. Plant Physiol. 163:5-20.

7

Genotype × Environment Interaction and Sensitivity to Planting Date of Seed Cotton Yield in Senegal

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Abstract

***Background**: In West Africa, climate change should affect temperatures and rainfall patterns. Therefore, good resilience to emerging climate risks will require a judicious choice of cultivars. However, the evaluation of the performance of varieties through multilocal and multi-annual trials should not be limited to the significance or not of genotype x environment interactions (GxE). This study aims to analyze GxE and assess the relationships between agronomic performance and stability in seed cotton yield of eight contrasting genotypes under rainfed cultivation in Senegal. Field experiments were conducted in 2018 and 2019 using a two-factor split-plot design with three replicates in three experimental stations in Senegal (Koussanar, Vélingara, and Kédougou).*

***Results**: The combined analysis of variance indicated significant effects for genotypes, environments, and GxE. Average seed cotton yields varied from 1,225 kg ha-1 (CS 50) to 938 kg ha-1 (Allen 51-106) across genotypes and from 1,687 kg ha-1 (KG19P1) to 328 kg ha-1 (KO18P2) across environments. GxE was well described by the method of Analysis of Additive Effects and Multiplicative Interactions (AMMI2) biplot. As a result, the best seed cotton yields were obtained in environments with good rainfall. In addition, we found that high yield susceptibility to poor rainfall was generally associated with high performance for all the genotypes evaluated, except for the cultivar IRMA Q302 which gave an interesting average performance in all environments. Cultivars with high yield potential (CS50, Stam 129A, IRMA Q302) perform well under normal rainfall. Cultivars IRMA Q302 and Allen 51-106 are the most stable but Allen 51-106 gave the lowest yield on average.*

***Conclusions:** The cultivar IRMA Q302, because of its stability and good yield in all environments, can be recommended for extension in the Senegalese cotton basin.*

***Keywords**: Gossypium hirsutum L, Seed cotton yield, Genotype x Environment interaction, Planting date, Sensitivity, AMMI, Yield stability, Sub-Saharan Africa.*

Background

Cotton cultivation in West Africa is mainly carried out by smallholders under rainfed conditions in small areas (Bagayoko, 2013). This production system is generally manual and not input-intensive (Fok, 2006) but labor-intensive (CNUCED, 2016). In Senegal, cotton is the second most important cash crop after groundnut (Diouf et al., 2019). However, farmers have huge difficulties in planting at the recommended periods due to irregular rains and labor constraints (Ndour, 2018): late planting is very frequent. In recent years, production, cotton areas, and yields have been decreasing significantly (Diouf et al., 2017). As a result, in 2018-2019 the area was 21,735 ha for a national production of 15,122 metric tons, or an average yield of 696 kg ha-1 (SODEFITEX, 2019). West Africa is expected to face an increase in temperature and a reduction in rainfall (Guan et al., 2017). Such climate should have a negative impact on rainfed cotton production in West Africa (ITC, 2011). In addition, there is no cultivar adapted to all environments of the Senegalese cotton basin (Ndour, 2018). Therefore, to sustainably boost cotton production, the introduction of cultivars adapted to Senegalese agro-climatic conditions is a priority. An optimal combination of morphological and physiological characteristics (traits) or their genetic determinants that give a plant material satisfactory suitability for a given environment, production, and use method is called an ideotype (Debaeke and Quilot-Turion, 2014). Ideotypes should be identified. The performance of cultivars is generally assessed through multilocal and multiannual

trials (Lacape, 1998). This method often encounters difficulties because genotype x environment interactions (GxE) are not detailed, and the analysis is bounded to a test of significance (Ndiaye et al., 2019). Several approaches are possible to analyze GxE (Debaeke and Quilot-Turion, 2014). One of them, the AMMI (Additive Main effects and Multiplicative Interaction), is an effective tool for graphically describing GxE (Kaya et al., 2009). AMMI uses an analysis of variance (ANOVA) followed by principal component analysis (PCA) (Crossa et al., 1990). The ANOVA will investigate the main effects of genotypes and environments and the PCA focuses on the non-additive part of the model (GxE).

The objectives of this study were (i) to decipher seed cotton yield GxE of 8 cotton genotypes in 12 environments in Senegal, (ii) to identify genotypes with high seed cotton yield potential, and (iii) to identify the most stable genotypes.

This study could: (i) contribute to the judicious choice of resilient cultivars for late planting and (ii) assist the breeding program of the Institut Sénégalais de Recherches Agricoles (ISRA) by designing ideotypes adapted to groups of environments in the Senegalese cotton basin through simulation.

Results

Climatic data

In the 12 environments, the minimum temperatures ranged from 20.3°C to 26.4°C (Table 1). These temperatures are higher than the base temperature of cotton (13°C; Crétenet and Gourlot, 2016). Cumulative rainfall varied from 349.2 to 1268.2 mm. In the KO18P1, KO18P2, KO19P1, KO19P2, and VL18P2 environments, low cumulative rainfalls were observed (411.7, 382, 468.8, 349.2, and 656.2 mm, respectively). Such rainfall can generate hydric stress. In Sub-saharan Africa, even though the water requirements of the cotton plant vary greatly according to the intensity of sunshine, humidity, runoff, and irregularity of rainfall, the minimum required average annual rainfall is 700 mm (Sément, 1986).

Average Performance of Genotypes, Environments and GxE Effects

The results of the combined analysis of variance for seed cotton yield of the 8 genotypes in environments are reported in Table 2. Significant differences were observed between genotypes ($P < 0.001$) and between environments ($P < 0.001$). Interactions were marginally significant ($P < 0.1$) so the differences between genotypes depended on the environment. The average seed cotton yield of the genotypes ranged from 1,225 kg ha-1 (CS 50) to 938 kg ha-1 (Allen 51-106) (Table 3). Only the IRMA Q302 genotype obtained relatively good seed cotton yields in all environments. Four genotypes had average seed cotton yield greater than the general average (1,099 kg ha-1): CS 50, Stam 129A, IRMA Q302, and IRMA L484. Their average seed cotton yield were 1,225 kg ha-1, 1,152 kg ha-1, 1,137 kg ha-1 and 1,124 kg ha-1, respectively. On the other hand, the three least performing genotypes were Allen 51-106, BUJA, and TAMCOT CAMD-S-75-C with average seed cotton yield of 938 kg ha-1, 1,029 kg ha-1, and 1,091 kg ha-1, respectively. The average seed cotton yield in the different environments (Table 3) varied from 328 kg ha-1 (KO18P2) to 1,687 kg ha-1 (KG19P1). Six of the twelve environments had yields higher than the overall average (1,099 kg ha-1). These were KG19P1 (1,687 kg ha-1), KG18P1 (1,620 kg ha-1), KO19P1 (1,488 kg ha-1), VL19P1 (1,464 kg ha-1), VL19P2 (1,283 kg ha-1) and VL18P1 (1,228 kg ha-1).

AMMI analysis of the variance of seed cotton yield of the 8 genotypes across the 12 environments revealed that 79.6%, 2.7%, and 10.0% of the total sum of the squares of the differences were attributed to environment, genotype and GxE, respectively. Thus, the decomposition of the GxE according to the AMMI model shows that the first two main components of the interaction (IPC1 and IPC2) explain 67.2% of the sum of the squares of GxE for seed cotton yield (Table 2).

Adaptability and phenotypic stability of genotypes

The AMMI2 biplot (Figure 1) provides a summary of the GxE in which we have: i) the first axis which opposes early planting dates to late planting dates and ii) the second axis where both genotypes and environments are less well distributed. The KG18P1, KG19P1, and VL19P1 environments are the most atypical in terms of varietal response because they are most distant from the mean response in the directions defined by the two axes. In the environment KO19P2 which is near the center, the varietal response is close to the mean response. On the first axis, genotypes that are located to the left most respond strongly to the planting date while genotypes that are located to the right most respond less. The environments KG18P1 and KG19P1, have a varietal response typical of early planting dates: they

favor CS50, IRMAL484, BUJA, and STAM129A the most. The second axis highlights the positive anomaly from the environment VL19P1 that favored the cultivar SIOKRA L23. In the environment, KO19P2 contrasts between varieties are similar to those observed on average in all the environments (Figure 1).

The cultivars IRMA Q302 and ALLEN 51-106 (on the right position) were among the most stable genotypes. However, IRMA Q302 had an average yield that was above the overall average and ALLEN 51-106 gave the lowest average yield. On the other hand, genotypes CS 50, Stam 129A, IRMA L484, BUJA, and SIOKRA L23 were very sensitive to late planting because of their left position far from the center. Lastly, CS 50, Stam 129A and IRMA L484 genotypes gave the best average seed cotton yields but were also the most sensitive to late planting (Figure 2).

The analysis of the average yield of the 8 genotypes compared to the yield differences obtained between early planting environments (environments with P1 code in Table 1) and late planting environments (environments with P2 code) shows that some genotypes have very high yield differences between early and late plantings (more than 840 kg ha-1 for CS 50 and Stam 129A). By contrast, these yield differences between early and late planting are less important for IRMA Q302 and ALLEN 51-106 genotypes (less than 530 kg ha-1) (Figure 2). Among these two stable genotypes, IRMA Q302 had the best average yield.

Table 1. Description and characterization of the 12 locations in two growing seasons.

Location	Coordinates	Sand (%)	Clay (%)	Silt (%)	*Soil texture	Year	Planting date	Planting code	Environment code	T_{min} (°C)	T_{max} (°C)	Rainfall (mm)
Kédougou	12°39'N, 12°7'W	19.2	33.7	47	Silty clay loam	2018	June 28	P1	KG18P1	21.6	31.8	1095.9
							July 19	P2	KG18P2	20.3	32.2	984.4
						2019	July 5	P1	KG19P1	25.2	26.2	1268.2
							July 20	P2	KG19P2	25.1	26.0	1114.6
Vélingara	13°9'N, 14°2'W	27.9	48	24.6	Clay	2018	July 9	P1	VL18P1	23.1	30.9	801.2
							July 30	P2	VL18P2	22.8	30.6	656.2
						2019	June 30	P1	VL19P1	26.4	27.4	970.4
							July 15	P2	VL19P2	26.2	27.2	835.2
Koussanar	13°55'N, 14°3'W	59.3	12.7	28.4	Sandy loam	2018	June 30	P1	KO18P1	23.2	33.2	411.7
							July 17	P2	KO18P2	21.9	33.6	382.0
						2019	July 7	P1	KO19P1	23.0	33.4	468.8
							August 19	P2	KO19P2	20.4	34.3	349.2

*Classification according to the USDA method based on data over the 0–30 cm horizon.
KD = Kédougou, KO = Koussanar, VL = Vélingara, Rainfall = total rainfall during the trial (from planting to harvest).

Table 2: Summary of the combined analysis of variance of seed cotton yield and decomposition of GxE according to AMMI

Source	DF	SS	MS	P-value	TSS Explained (%)	GxE SS (%)
Environments	11	55029243	5002658	$5.0.10^{-10}$	79.6%	
Block	24	5305275	221053	$7.6.10^{-06}$	7.7%	
Genotypes	7	1866676	266668	0.00073	2.7%	
GxE	77	6924770	89932	0.093	10.0%	100.0%
IPC1	17	2878124	169301	0.0022		41.6%
IPC2	15	1775623	118375	0.057		25.6%

IPC3	13	1259950	96919	0.17	18.2%
IPC4	11	682463	62042	0.55	9.9%
IPC5	9	183176	20353	0.98	2.6%
IPC6	7	79216	11317	0.99	1.1%
IPC7	5	66218	13244	0.97	1.0%
Residuals	168	11766281	70037		

Note: DF: Degree of Freedom, SS: Sum of Square, MS: Mean of Square, TSS: total sum of square, GxE: Genotype x Environment interaction.

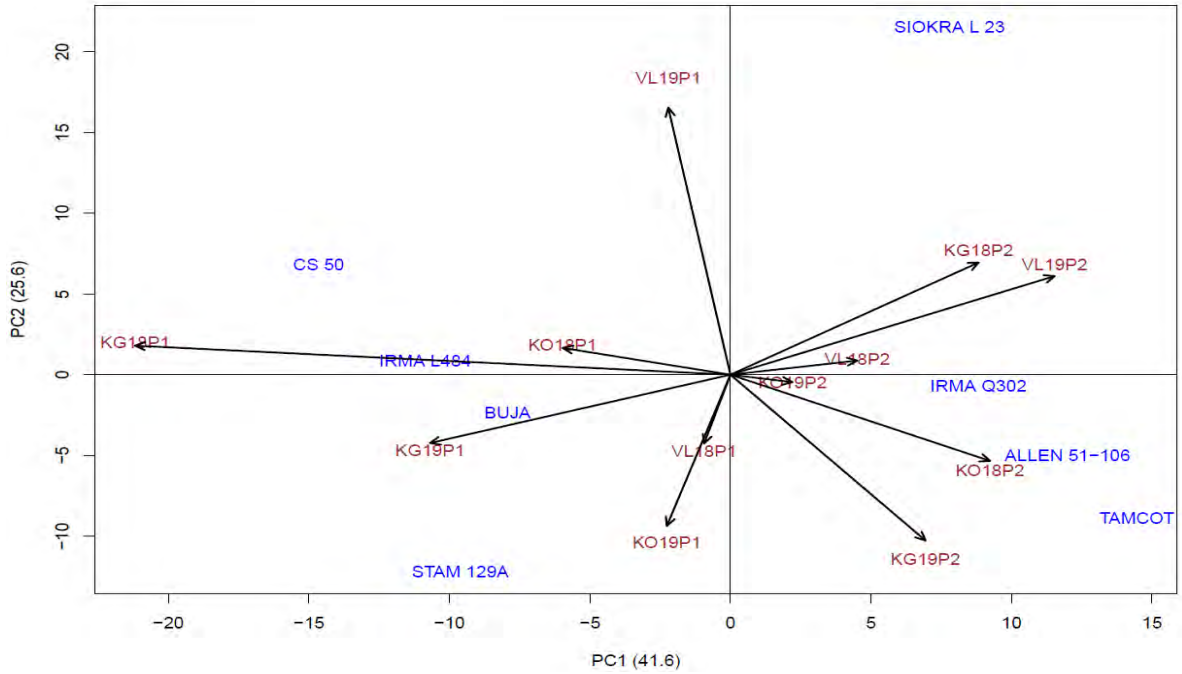

Figure 1. Polygon view of Additive main effects and multiplicative interaction 2 (AMMI2 biplot). Genotypes names are written in blue while environment codes in red. PC1 and PC2 captured 67.2% of the GxE.

Table 3. Seed cotton yield (kg ha^{-1}) of 8 genotypes (Cultivars) tested in 12 environments. For the description of the environments, please refer to Table 1.

Cultivar	Environment												Genotypic mean
	KO18P2	VL18P2	KO19P2	KG18P2	KO18P1	KG19P2	VL18P1	VL19P2	VL19P1	KO19P1	KG18P1	KG19P1	
ALLEN 51-106	313	254	382	931	772	1111	899	1322	1117	1458	1332	1361	938
BUJA	165	343	569	752	1145	986	1143	1110	1314	1417	1681	1722	1029
TAMCOT CAMD-S-75-C	577	472	694	975	1047	1111	1229	1306	1263	1583	1129	1701	1091
SIOKRA L 23	264	491	639	1006	1140	903	1272	1476	1924	1208	1401	1458	1099
IRMA L484	349	405	611	914	1123	1000	1106	1125	1533	1625	1937	1764	1124
IRMA Q302	366	489	653	1091	853	1375	1315	1457	1445	1389	1619	1597	1137
STAM 129A	312	424	653	575	1194	1292	1638	1113	1450	1597	1759	1819	1152
CS 50	274	423	653	1123	1207	986	1226	1354	1663	1625	2098	2069	1225
Environmental Index	328	413	607	921	1060	1095	1228	1283	1464	1488	1620	1687	1099

Note: The environments are rank from low to high average seed cotton yield across cultivars (environmental index). The cultivars are ranked from low to high average seed cotton yield across environments (genotypic mean).

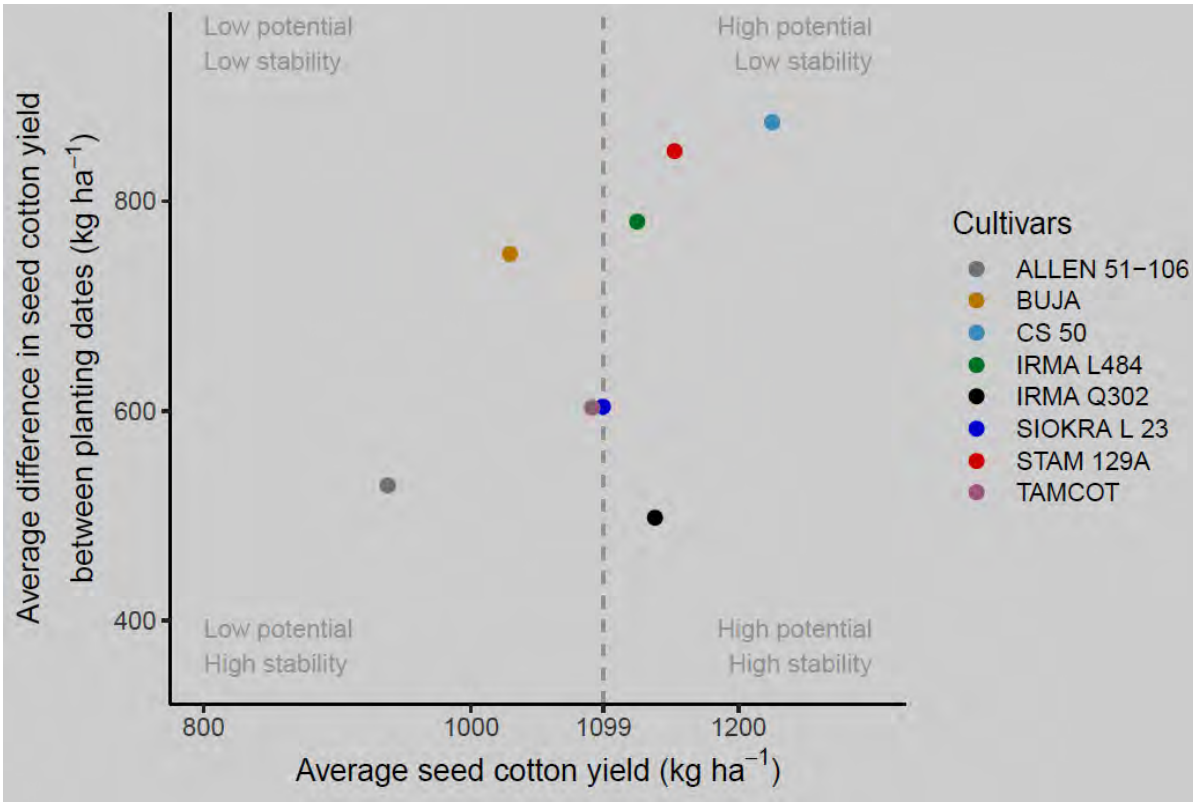

Figure 2. The average difference in seed cotton yield between planting dates as a function of cultivar average seed cotton yield: Resilience to suboptimal planting date. Ideal cultivars with high average yield and high resilience to suboptimal planting dates are located at the bottom right. The vertical dashed-line is the average seed cotton yield across all environments and cultivars.

Discussions

The combined analysis of variance showed different seed cotton yield performance due mainly to the environments (79.6% of the total sums of squares were attributed to environmental fluctuations). This indicates that the environments are contrasted, resulting in the variation in seed cotton yield performance. These results corroborate those of Pretorius et al. (2015). Overall, the best yields were obtained with early plantings (P1), which proves that late plantings (P2) cause significant losses in seed cotton yield, as other researchers have found (Khan et al., 2017; Taner et al., 2006). The lowest seed cotton yield obtained in the KO18P2 environment is due to the late planting, the low rainfall, and the nature of the soil, which is sandy type. Other parameters such as irregular rainfall distribution and periods of drought during the season have also contributed to this drop in yield as the water requirements of cotton vary according to the weather (Sément, 1986).

In this study, the KG18P1 and KG19P1 environments favored the CS 50, IRMA L484, BUJA, and Stam 129A genotypes. This adaptation could be explained by the good agro-pedoclimatic conditions of these environments with abundant rainfall (greater than 950 mm) and good soil type (silty clay). The cultivar CS 50, with its large differences in seed cotton yield between early and late planting, has been confirmed to be a late maturing cultivar with irregular performance in dry areas in Australia as demonstrated by Stiller et al. (2005). Cultivar Stam 129A, cultivated all over the entire Senegalese cotton basin, confirms its sensitivity to the water deficit previously observed (Gnofam et al., 2014). This is the reason why the differences in seed cotton yield between early and late planting for cultivar Stam 129A are huge. However, for the cultivar SIOKRA L23 which was favored by the VL19P1 environment, the result is different from that found by Stiller et al. (2005) who described it as an efficient cultivar in dry areas in Australia. This could be explained by the fact that in Australia, the soil types used for cotton in dry areas have a high water retention capacity (Chan and Hodgson, 1981) while in Senegal, in dry areas, soils are generally sandy with low water retention capacity. The wet environment can explain the adaptability of the cultivar SIOKRA L23 genotype to the VL19P1 environment with a good water retention capacity because the soil is clayey. Authors such as Al Majou et al (2013) and

Cornet (1980) have shown that there is a close linear correlation between the clay content of soil and its water retention capacity. In general, the poor performance and good phenotypic stability of the cultivar ALLEN 51-106, a very ancient cultivar, were not surprising. These results corroborate those of Ndiaye et al. (2019) who found that low agronomic performance can be associated with phenotypic stability. In summary, the performance of the genotypes in the environments depended on: i) soil type, all genotypes performed well in clay or silty-clay soils, ii) rainfall, yields were better in the rainiest environments (early planting). This study showed that the cultivar IRMA Q302 was one of the most stable cultivars (along with ALLEN 51-106) among the eight tested. In addition, it had an average yield that was above the overall average. This result is similar to that of Farias et al. (2016) who demonstrated that productive genotypes with good phenotypic stability could be obtained. The cultivar IRMA Q302 was more productive and stable than IRMA L484. These results are in agreement with those of Cameroonian breeders who decided to extend IRMA Q302 to replace IRMA L484 in the driest region of the Cameroonian cotton production basin (Oumarou et al., 2014). The study shows that yield losses are lower with IRMA Q302 (498 kg ha-1, 44% of average yield of this cultivar) than with Stam 129A (848 kg ha-1, 74% of average yield of this cultivar). In addition, Senegalese farmers are often unable to complete plantations within the required time frame due to several constraints, including irregular rains and the unavailability of labor. For example, during the 2019-2020 season, cotton was cultivated on 15,839 ha in Senegal, distributed as follows: 12,466 ha of early planting (emergence before 15 July) and 3,373 ha of late planting (emergence after 15 July). Therefore, the extension of the IRMA Q302 cultivar could generate significant gains for the Senegalese cotton sector.

Conclusion

This study showed that seed cotton yields were highly dependent on genotypes, environments, and genotype x environment interaction. Different genotype responses were obtained in contrasting environments. Some genotypes showed a preference for specific environments (soil type and rainfall). CS 50 and Stam 129A had the best seed cotton yields. This study also showed that many of these genotypes do not have good phenotypic stability because their yield highly depended on the timing of the planting date. However, only the cultivar IRMA Q302 showed both good phenotypic stability and a high average yield. Overall, early plantings gave the best seed cotton yields. The cultivar CS 50, as well as the cultivar Stam 129A, have a very high seed cotton yield potential but when conditions are difficult, showed a dramatic decrease in yield. Therefore, in this study, AMMI made it possible to formulate the following recommendation: the extension of the IRMA Q302 genotype, which appears to be the best because of its acceptable yield in all environments. With IRMA Q302, the yield differential is 350 kg ha-1, farmers would have gained approximately FCFA 354 000 000 in 2020 on late planting dates and total seed cotton production in Senegal would have increased by 1,180 tons. In this study, the environment is defined as the combination of location*years*dates of planting but another approach could be based on the calculation of the water balance and the definition of the water satisfaction index from planting to harvest. A comparison of these two methods would provide further explanations for the performance of genotypes in different environments. Additional studies in highly contrasting localities would help to explain these differences in genotypic performance as a function of the environment (GxE).

Methods

Experimental sites

The trials were installed in three research stations located in the cotton-growing area of Senegal (the southern third of the country), during two growing seasons (2018 and 2019). These three stations were Koussanar, Vélingara and Kédougou. The characteristics of its different sites are listed in Table 1.

Climatic and soil data

At each location, meteorological data were recorded by automatic weather stations (iMETOS® IMT280 or ATMOS 41) within about 50 m of the plots. Climatic data summaries were reported from the planting date to the end of the growing season. Soil samples were taken and analyses were carried out in the IMAGO laboratory of the Institut de Recherche pour le Développement (IRD) in Dakar.

Experimental design

At each site, the experimental device is in Split-plot with 2 factors and 3 replicates. The two planting dates were randomly assigned to main plots in a complete block design (P1: early planting and P2: late

planting). The eight cultivars were then randomly assigned to subplots within the main plots. They were chosen for their wide a priori range of responses to drought. The characteristics of these genotypes are shown in Table 4.

Table 4: Name, geographic origin and traits of cultivars used in the study.

Cultivar name	Origin	Traits
Stam 129A	Togo	Widely cultivated in Senegal
CS 50	Australia	Drought sensitive
TAMCOT CAMD-S-75-C	USA	Long vegetative phase and short reproductive phase
BUJA	Ivory Coast	Strong leaf reduction in drought conditions
ALLEN 51-106	Chad	Short vegetative phase and long reproductive phase
IRMA L484	Cameroon	Drought tolerant
IRMA Q302	Cameroon	Drought tolerant
SIOKRA L23	Australia	Drought tolerant and okra-leaf

Each subplot consisted of six (06) rows of ten meters (10 m). The planting configuration was 0.80m between the lines and 0.25m between hills. The technical itinerary recommended by SODEFITEX was used in all sites. For fertilization, 250 kg ha-1 of NPKSBCaO granular fertilizer (14-18-18-5-1-2.5) was applied at planting and 100 kg ha-1 of Urea at 46% N between 40 and 45 days after planting were added. For pesticides, the same products were used according to a single calendar (based on planting date) to have the same application dates (in days after planting) and the same application rates. Seed cotton yields were measured on each plot on the 3 central lines and converted into kg ha-1 for statistical analysis.

Data analysis

A combined analysis of variance was performed, considering the effect of genotype and environment as fixed, according to the following statistical model:

$$Y_{ijk} = \mu + G_i + E_j + B_k(E_j) + (GE)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the measured yield on ith genotype in the jth environment and the kth block; μ is the grand mean; B_{jk} is the effect of the kth block in the jth; G_i is the effect of the ith genotype; Ej is the effect of the jth environment; $(GE)ij$ is the effect of the interaction of the ith genotype with the jth environment; and ε_{ijk} is experimental error.

Finally, the analyses on phenotypic adaptability were performed by the AMMI method according to the following statistical model:

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=n}^{n} \lambda_k \alpha_{ik} y_{jk} + r_{ij} + \varepsilon_{ij}$$

Where Y_{ij} is the mean response of genotype i in environment j; μ is the grand mean; g_i is the fixed effect of genotype i; e_j is the fixed effect of environment j.; ε_{ij} is the experimental error; the GxE is approximated by a sum of components indexed by k; For each component k, l_k is the singular value, α_{ik} is the eigenvector value for the ith genotype, y_{jk} is a value the eigenvalue for the jth environment; if singular value decomposition is not pushed to the maximum number of components, there is a residual interaction term r_{ij}.

In the AMMI2 graph, genotypes that have low scores on both ICPA1 (first main axis of interaction) or IPCA2 (second main axis of interaction), contribute little to the interaction. This indicates general adaptation. In contrast, those with a high distance from origin have strong interactions and are specifically adapted to the environment that has distant from the origin (Zobel et al., 1988).
All statistical analyses were performed using R software version 3.6.3 (2020-02-29) and the AMMI was performed with agricola package 1.3.1 (Mendiburu, 2019).

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References:

1. Al Majou, H., Bruand, A., Duval, O., Josière, O., 2013. Études des proprié té s de rétention en eau des sols argileux Plan de la pr é sentation Introduction Mat é riel et m é thodes Résultats et discussion Conclusion et perspectives.
2. Bagayoko, K., 2013. L'importance et l'avenir du coton en Afrique de l'Ouest : cas du Mali 2006, n.p.
3. Chan, K.Y., Hodgson, A.S., 1981. Moisture regimes of a cracking clay soil under furrow irrigated cotton. Aust. J. Exp. Agric. 21, 538–542. https://doi.org/10.1071/EA9810538
4. Cláudio, L., Kist, V., Otávio, T., Paula, M. De, Henrique, M., Barbosa, P., Alexandre, L., 2013. AMMI analysis to evaluate the adaptability and phenotypic stability of sugarcane genotypes. Sci. Agric. 27–32.
5. CNUCED, 2016. COTON - Un profil de produit de base par INFOCOMM.
6. Cornet, A., 1980. Observations sur la capacité de rétention , ses relations avec les valeurs de potentiel et la texture des sols sableux du Nord Sénégal. North.
7. Crétenet, M., Gourlot, J.-P., 2016. Le cotonnier.
8. Crossa, J., Gauch, H.G., Zobel, R.W., 1990. Additive Main Effects and Multiplicative Interaction Analysis of Two International Maize Cultivar Trials. Crop Sci. 30, 493–500. https://doi.org/10.2135/cropsci1990.0011183x003000030003x
9. Debaeke, P., Quilot-Turion, B., 2014. Conception d'idéotypes de plantes pour une agriculture durable.
10. Diouf, F.B.H., Sall, M., Dia, D., Gueye, M., Sy, M.R., Sarr, M., 2017. Etat des lieux de l'amélioration du cotonnier au Sénégal et perspectives de recherches 24–26.
11. Diouf, N., Mbaye, M.S., Gueye, M., Dieng, B., Bassene, C., Noba, K., 2019. La flore adventice des cultures cotonnières dans le Sénégal Oriental et en Haute Casamance. Int. J. Biol. Chem. Sci. 13, 1720. https://doi.org/10.4314/ijbcs.v13i3.41
12. Farias, F.J.C., Carvalho, L.P., Silva Filho, J.L., Teodoro, P.E., 2016. Biplot analysis of phenotypic stability in upland cotton genotypes in Mato Grosso. Genet. Mol. Res. 15, 1–10. https://doi.org/10.4238/gmr.15028009
13. Fok, M., 2006. Libéralisation, distorsion de concurrence et évolution technologique : portée et limites du succès du coton en Afrique Zone Franc. Int. Conf. Championing Agric. Successes Africa's Futur. A Parliam. Dialogue NEPAD 40.
14. Gérardeaux, E., Affholder, F., Bernoux, M., Muller, B., 2016. Relationships Between Tropical Annual Cropping Systems and Climate Change. https://doi.org/10.1007/978-94-017-7462-8
15. Gnofam, N., Tozo, K., Bonfoh, B., Akantetou, K., Kolani, L., Amouzouvi, K., 2014. Effets D ' Un Deficit Hydrique Sur Certains Parametres Morphologiques , Physiologiques. Agron. Africaine 26, 113–125. https://doi.org/10.1016/j.spinee.2005.12.002
16. Guan, K., Sultan, B., Biasutti, M., Baron, C., Lobell, D.B., 2017. Assessing climate adaptation options and uncertainties for cereal systems in West Africa. Agric. For. Meteorol. 232, 291–305. https://doi.org/10.1016/j.agrformet.2016.07.021
17. ITC, 2011. Coton et changement climatique. Impacts et options de réduction et d'adaptation. Genève, 36p
18. Kaya, Y., Palta, Ç., Taner, S., 2009. Additive main effects and multiplicative interactions analysis of grain yield performances in rice genotypes across environments. Asian J. Plant Sci. 8, 48–53. https://doi.org/10.3923/ajps.2009.48.53
19. Khan, A., Najeeb, U., Wang, L., Tan, D.K.Y., Yang, G., Munsif, F., Ali, S., Hafeez, A., 2017. Planting density and sowing date strongly influence growth and lint yield of cotton crops. F. Crop. Res. 209, 129–135. https://doi.org/10.1016/j.fcr.2017.04.019
20. Lacape, M.J., 1998. Analyse écophysigiogique de la réponse de variétés de cotonnier au déficit hydrique.
21. Mendiburu, F., 2019. agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-1. https://CRAN.R-project.org/package=agricolae
22. Ndiaye, M., Adam, M., Ganyo, K.K., Guiss, A., Ciss, N., Muller, B., 2019. Genotype-Environment Interaction : Trade-O ff s between the Agronomic Performance and Stability of Dual-Purpose Sorghum (Sorghum bicolor L . Moench) Genotypes in Senegal.
23. Ndour, A., 2018. Estimation de la production cotonnière dans un contexte de chagement climatique: cas du bassin cotonnier du Sénégal.
24. OCDE, 2005. Importance économique et sociale du coton en Afrique de l'Ouest 3–3. https://doi.org/10.1787/185426ad-fr
25. Oumarou, P., Graveleau, A., MemenaA, O., Dessauw, D., Klassou, C., 2014. SÉLECTION COTONNIÈRE RAPPORT ANNUEL COMPLET CAMPAGNE 2013-2014.
26. Pretorius, M.M., Allemann, J., Smith, M.F., 2015. Use of the AMMI model to analyse cultivar-environment interaction in cotton under irrigation in South Africa 2, 76–80.
27. Sément, G., 1986. Le cotonnier en Afrique tropicale.
28. SODEFITEX, 2018. Production coton Sénégal.
29. Stiller, W.N., Read, J.J., Constable, G.A., Reid, P.E., 2005. Selection for water use efficiency traits in a cotton breeding program: Cultivar differences. Crop Sci. 45, 1107–1113. https://doi.org/10.2135/cropsci2004.0545
30. Taner, B., Volcan, S., Aydin, U., 2006. The effect of sowing date plant on cotton yield.pdf.
31. Zobel, R.W., Wright, M.J., Gauch, H.G., 1988. Statistical Analysis of a Yield Trial. Agron. J. 80, 388–393. https://doi.org/10.2134/agronj1988.00021962008000030002x

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Genotypes by Environment Interaction Effect and Biplot Analysis (Gge) for Seed Cotton Yield in Some Egyptian Cotton Genotypes (*Gossypium Barbadense L*)

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Abstract

Seed cotton yield is a trait governed by multiple genes that cause changes in the performance of genotypes depending on the cultivation environment, therefore plant breeders always test their genotypes across diverse environments to assess the consistency of superior genotypes for wide adaption. Twelve Egyptian cotton genotypes (G) were studied across three various environments € representing Middle and Upper Egypt during the three seasons (S) 2016, 2017, and 2018. Genotypes (G) across environments € over years (Y) revealed significant differences in seed cotton yield. Significant values of mean squares due to genotypes environment interaction (G × E) and genotypes x year interaction (G × Y) for (SCY K/f). Moreover, the interaction effects due to (Y × E × G) were also significant for seed cotton yield (SCY K/f), indicating that (SCY K/f) of cotton genotypes is mostly affected by environment and years. Environment significance is explained by 73.3% (7.2%, 32.3%, and 35.9%) for the year, environment, and their interactions, respectively of the total sum squares due to G, E, and (G × E) interaction however partitioning of variance components for environment revealed that both predictable € and unpredictable (Y) components were an important source of variation. The environment €, genotype (G), and (G × E) interaction effects explained about 43, 9% (32.3%, 4.4%, and 7.2%), respectively, of the total sum squares variance components. According to ideal genotype Biplot analysis, genotypes G1, G5, and G7 are more stable and had a high yielding ability compared with the grand mean performance of other genotypes thus genotypes are identified as ideal genotypes for seed cotton yield (SCY K/f).

***Keywords**: Cotton, GGE-biplot, multilocation varietal experiment, Egypt, environment adaptation*

Background

Egyptian cotton germplasm has a narrow genetic base and little variation is available for the development of high-yielding cotton cultivars. Cotton genotype performance depends on genotype (G), environment (E) components, and interactions (GE) between them, however, genotype-environment (GE) interaction becomes more important and challenging when the ranking of breeding lines changes in different environments (Baker and lean 1988 and Ali et al. 2017). Genotype environment (GE) interaction complicates the process of selection of genotypes with superior performance, for this reason, the Plant breeders evaluate genotype's performance at various environment traits because the interaction components provide the basic information related to adaptability of cotton genotypes. Numerous methods have been developed to reveal patterns of Genotype environment (GE) interaction, such as joint regression (Finlay and Wilkinson 1963, Eberhart and Russell 1966, and Perkins and Jinks 1968). Stable expression of different attributes of cotton genotypes in different environments is very difficult to attain (Kerby et al .2000).

GGE Biplot analysis is believed to be very effective in explaining patterns of genotype-environment (GE) interaction and usually is the first choice of plant breeders to identify best performing genotypes for targeted environments (Yan et al., 2007). The GGE Biplot analysis is the graphical approach to assess genotypes' main effects integrated with genotype by environment interaction (GE) for evaluation of genotypes under diverse environments (Yan and Hoiland 2010). GGE Biplot has very useful features such as visually assessing the discrimination ability of the genotypes to different environments, the relationship among the genotypes and environments, and the ideal environment and genotype (Yan, 2001).

In the current study, cotton genotypes collected from the Egyptian cotton breeding program, Cotton Research Institute were used to assess the relative performance of genotypes across different

environments using GGE Biplot analysis to identify the ideal genotypes which have stable yielding for lint cotton yield in Middle and Upper Egypt regions for the future breeding program.

Results and Discussion

The present investigation included the evaluation of 12 long-staple cotton genotypes belonging to Egyptian cotton (Gossypium barbadense. L) at the three different locations representing Middle and Upper Egypt in the three seasons of 2016, 2017, and 2018 to study genotypes' performance and stability under different environments. The mean performance of 12 Egyptian cotton genotypes for seed cotton yield (SCY K/f) showed in Table (2). Genotypes G2, G5, G8, and G9 recorded significant seed cotton yield (SCY K/f) compared with the grand mean performance for all cotton genotypes. On the other hand, the genotypes G1 and G7 gave insignificant higher seed cotton yields.

Combined analysis of variance Table (2), revealed significant mean squares of years (Y), environments (E), and years × environments interactions (Y × E) for seed cotton yield (SCY K/f), possibly due to environmental condition change across various environments over years. Significant values of mean squares due to genotypes (G) and (G × E) interaction and (G × Y) interaction indicated that differential genotype expression across environments depends on the reaction of genotype to changing environmental conditions across locations and years. The second order interaction (Y × E × G) was significant for seed cotton yield (SCY K/f), indicating that seed cotton yield of cotton genotypes is mostly affected by environment and years. Significant environmental effects explained about 73.3% (7.2%, 32.3%, and 35.9%) for year, environment, and their interactions, respectively of the total sum squares due to G, E, and (G × E) interaction Table (2), However partitioning of variance components for environment revealed that both predictable (E) and unpredictable (Y) components were an important source of variation. The environment (E), genotype (G), and (G × E) interaction effects explained about 43, 9% (32.3%, 4.4%, and 7.2%) respectively, of the total sum squares variance components. Significant differences in all sources of variation could help cotton breeders for selecting stable genotypes. The present data were in agreement with Killi and Harem (2006) and Satish and Chabra (2009). Campbell et al. (2012) and Gul et al (2016). They reported that the effect of genotypes × environments interaction (G × E) was significant for seed cotton yield. These results indicated that the cotton crop as well as other crop varieties showed differential responses when grown in different locations and years.

Table 2: Mean performance of 12 Egyptian cotton genotypes for seed cotton yield (SCY K/f) for three seasons at three locations.

Code	Genotypes	2016	2017	2018	G. Means
G1	[(G91 × G90)] ×G85	10.21	11.27	9.09	10.19
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	10.06	11.41	9.46	10.31
G3	[(G91 × G90)] × [(G85 × G83)]	9.80	10.53	9.28	9.87
G4	[(G91 × G90)] × [(G83 × G80) × G89]	10.31	10.97	8.67	9.98
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	10.33	11.34	9.66	10.44
G6	[(G91 × G90)] × Karshink	10.19	10.30	9.20	9.90
G7	[(G83 × G80) × Dandara] × [(G90 × Aust)]	10.86	10.25	9.49	10.20
G8	[(G91 × G90)] × G80	10.44	11.49	9.37	10.43
G9	(Giza 90 × CB 58)	10.64	11.48	9.24	10.45
G10	[(G83 × G80) × G89] ×Aust	10.69	9.72	8.61	9.67
G11	Giza 95	10.21	8.69	9.72	9.54
G12	Giza 90	9.28	9.89	7.91	9.03
Grand means		10.25	10.61	9.14	10.00
LSD 0.05		0.31	0.21	0.21	0.27

Table 3 : Analysis of variance for seed cotton yield (SCY) trait across different environments.

SOV	df	SS	MS	GE%
R	5	1707695.142	341539.028	
Y	2	9869070.559	4934535.279**	7.2
E	2	44324540.32	22162270.16**	32.3
Y x E	4	49347043.28	12336760.82**	35.9

G	11	6417546.92	583413.356**	4.4
Y x G	22	5923804.293	269263.832*	4.3
E x G	22	9937044.978	451683.863**	7.2
Y x E x G	44	11570455.75	262964.903*	8.4
Er	535	69081958.53	129125.156	

Seed cotton yield (SCY K\f) and stability of 12 Egyptian cotton genotypes were assessed from the coordination of the middle environment (CAE), (Fig.1). Genotypes on the extreme right on the CAE ordinate axis indicate a relatively stable. The stability of these genotypes depends on their distance from AE abscissa. Genotypes G1, G3, G5, and G7 were the best stable genotypes compared with other genotypes. On the other hand, the genotypes G2, G4, G6, and G8 showed the absolute length of the projection of a genotype stable. In contrast, genotype G11was the most unstable genotype. From the above results, it could be concluded that the genotypes G1, G5, and G7 are stable and had a high yielding ability compared with the grand mean performance and be incorporated as breeding materials in the future breeding program to produce stable and high-yielding cultivars. These results are in agreement with those obtained by Farias et al (2016), Saide (2016), Baker (2017), and Imtiaz et al (2017).

The ideal genotype can be used as a benchmark for selection, genotypes that are far away from the ideal genotype can be rejected in early breeding cycles, while genotypes that are close to it can be considered in further tests (Yan et al 2009). An ideal genotype should have a mean seed cotton yield (SCY) that is consistently high in the overall environments of interest.

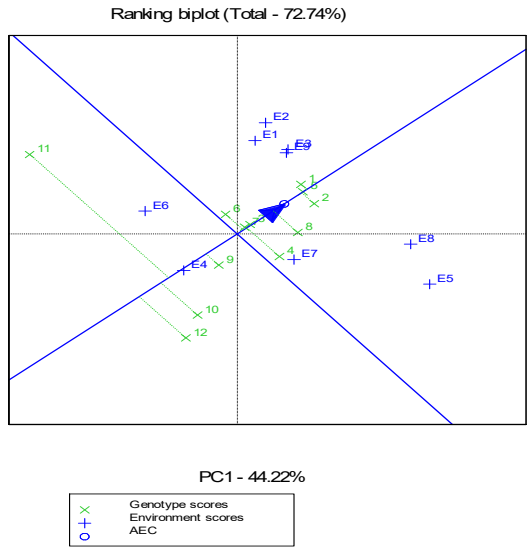

Fig 1. The" Mean vs. Stability" view of the GGE Biplot ranking for seed cotton yield of 12 genotypes across 3 environments in Middle and Upper Egypt over three seasons.

This ideal genotype is graphically defined by the longest vector in PC1 and PC2 without projections and represented by an arrow in the center of concentric circles (Yan and Rajcan, 2002). Although this genotype is more of a representative model, it is used as a reference for assessing genotypes. Thus, the genotypes G2 and G5 which fell into the center of concentric circles were ideal genotypes in terms of higher yield ability and stability, compared with the genotype of the rest genotypes. In addition, genotype G1 and genotypes G8 and G3 located on the second and third concentric circles, respectively, are the closest ideal in terms of high seed cotton yield and phenotypic stability (Fig. 2). The genotypes G9, G10, G11, and G12 were undesirable genotypes because they were at distant from the first concentric circle. Baker (2017) used Biplot analysis of phenotypic stability in some Egyptian cotton genotypes and they found that the genotype G8 {(G91× G90) × [(G80 × G83) × Dendera]} was ideal genotype and had high cotton productivity and phenotypic stability.

The ideal environment for seed cotton yield (SCY) and stability of the genotype has been shown in Fig (3). The environment located in the first concentric circle in Biplot termed as ideal environment and environments located close to the ideal present study, E3 (Sohage 2016) and E9 (Sohage 2018) are

located in the first concentric circle followed by E1 (Bani-Souf 2016) and E2 (El-Fayuom 2016) environments which are close to the ideal environments as desirable environments (Fig 3); therefore, it should be regarded as the most suitable to select widely adapted genotypes.

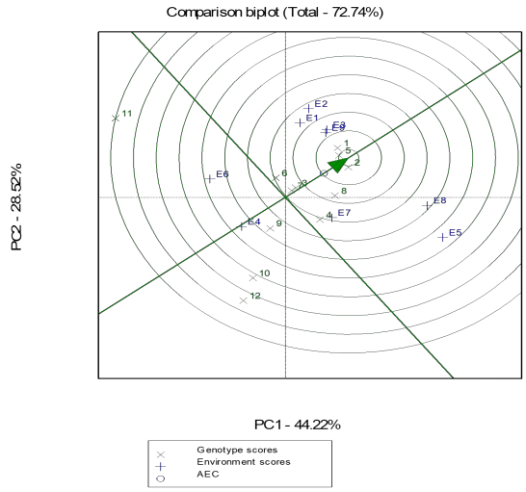

Fig 2. Classification of the genotypes from the GGE Biplotof seed cotton yield (SCY) in different environments

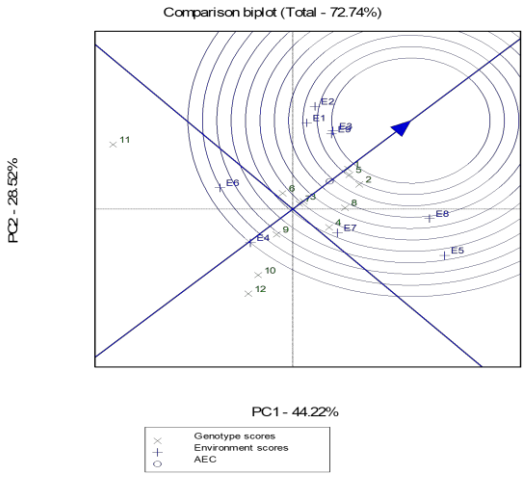

Fig 3.Visualization of the ideal environment using GGE Biplotfor seed cotton yield (SCY).

According to the Biplot analysis in Fig. (4), the corner genotypes that are the most responsive can be visually determined. These corner genotypes were G1, G5, G2, G4, G8, G11, and G12. In this figure, locations are divided into six rays divided by the Biplot into sectors. The first sector contains four environments, E1, E2, E3, and E9 with Genotypes G1, G5, and G2 as the most favorable. The second sector represents E8 and E5 with the genotypes G8 and G4 as the most favorable. The two other corner genotypes G11 and G12 were the poorest yieldings (Fig.4). They were located far away from all of the test locations, reflecting the fact that they yielded poorly in each environment. The genotypes within the polygon nearer to the plot origin (for example G3 and G7 for E1, E2, E3, and E9) are less responsive than vertex genotypes (Yan et al 2000).

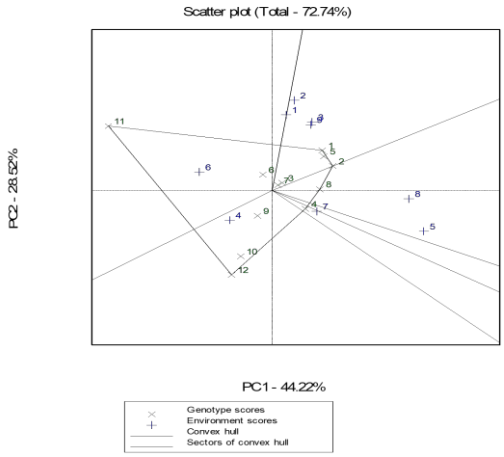

Fig. 4. GGE Biplot identification of winning genotypes and their related mega-environment.

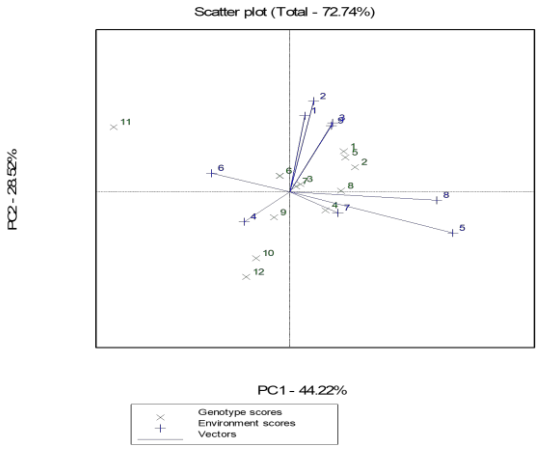

Fig. 5. GGE Biplot for the evaluation of the relationship among nine environments.

Figure (5) represents the evaluation and relationship among different environments over three seasons. Environment having vector smaller angles are closely related. Positive correlations were found between E1 (Bani-Souif 2016), E2 (El-Fayuom 2016), and E3 (Sohage 2018) in a location as the angle between them. On the other hand, the genotypes groups G1, G2, G3, G5, G7, and G8 with the environments E3, E9, and E8 are close to each other in the graph area representing the specific adaptation of genotypes to the environments and specific interactions were observed between them. The results indicated that the environmental effect is minimal in the variation of seed cotton yield.

The mean performance of the fiber quality for all cotton genotypes under different environments over seasons is presented in Table (4). Micronaire values (Mic) of all genotypes ranged from 3.9 to 4.1 units

with a general mean of 4.0 units. The highest value for (Mic) was recorded by the genotypes G1, G3, G4, G8, and G9 which was (4.1 units). The lowest (Mic) value was recorded by the genotype G12 (3.9 units). The genotypes G5 and G2 recorded the highest and lowest Upper half mean length (UHM), it was (30.9 and 29.2 mm), respectively. Concerning the uniformity ratio (UR %) of 12 genotypes in Table (4), ranging from 83.1 to 84.2% of the genotypes G1, G6, G7, G10, G11, and G12 exceeded the genotypes' general means. The general mean performance of maturity ratio for the genotypes was 0.92, on the other hand, the highest and lowest value for maturity ratio was recorded in genotypes G1 (0.94), G5 (0.91), and G11 (0.91), respectively. Fiber strength (F.St gm/tex) Table (4), revealed that fiber strength (gm/tex) for the genotypes ranged from (36.5 to 38.0 gm/tex). The highest value and lowest value of fiber strength were recorded for the genotypes G3 and G7, it was (39.0 and 36.5 gm/tex). Yarn strength (Y.St) trait exhibited wide variation which ranged from (1840 to 1940) for the genotypes G7 and G11, respectively. The same genotypes recorded the highest and lowest values of yarn strength.

Table 4: Means of fiber quality of 12 Egyptian cotton genotypes at three environments over seasons

Code	Genotypes	Mic	UHM (mm)	UR%	Mature	F.St (gm/tex)	Y. St
G1	[(G91 × G90)] ×G85	4.1	29.9	84.1	0.94	37.8	1900
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	4.0	29.2	83.8	0.92	36.8	1915
G3	[(G91 × G90)] × [(G85 × G83)]	4.1	30.2	83.2	0.92	38.0	1905
G4	[(G91 × G90)] × [(G83 × G80) × G89]	4.1	30.3	83.3	0.93	37.4	1905
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	4.0	30.9	83.5	0.91	37.7	1890
G6	[(G91 × G90)] × Karshink	4.0	30.6	84.1	0.92	37.1	1850
G7	[(G83 × G80)×Dandara]×[(G90 × Aust)]	4.0	30.0	84.2	0.92	36.5	1840
G8	[(G91 × G90)] × G80	4.1	30.4	83.8	0.93	37.6	1915
G9	(Giza 90 × CB 58)	4.1	29.9	83.1	0.93	36.5	1895
G10	[(G83 × G80) × G89] ×Aust	4.0	29.7	84.0	0.92	37.1	1870
G11	Giza 95	4.0	30.2	84.1	0.91	36.9	1940
G12	Giza 90	3.9	30.0	84.2	0.92	36.7	1915
	Means	4.0	30.1	83.8	0.92	37.2	1895

The fiber quality of all cotton genotypes under study had a suitable fiber quality for Egyptian long staple cotton grown in Middle and Upper Egypt.

Conclusion

Based on genotype-environment interaction and GGE Biplot analysis, the genotypes G1 [(G91 × G90)] × G85, G5 [(G90 × Aust)] × [G83 × (G75 × 5844)] and G7 [(G83 × G80) × Dandara] × [(G90 × Aust)] were declared as best performers and ideal genotypes concerning stability and producing maximum seed cotton yield in all environments. Therefore, the genotypes G1, G5, and G7 could be used in breeding programs as a promising material in future breeding programs to produce stable and high-yielding cultivars.

Material and Methods

Ten Egyptian cotton genotypes and two cotton cultivars, Table (1) were grown in 2016, 2017, and 2018 seasons at three different locations representing Middle and Upper Egypt regions, i.e. Beni–soufe (E1), El-Fayoum (E2), and Sohag (E3). The experimental design in all locations was a randomized complete block design with six replicates. Each experimental plot consisted of five rows, 4m long, 60 cm in width and 30 cm between hills within a row. The hills were thinned to two plants. Cultural practices were applied as recommended for growing cotton. The middle three rows of each plot were hand harvested to determine seed cotton yield (SCY K/f).

Fifty open bolls were picked from the two outer rows per plot to determine fiber properties, i.e. yarn strength (Y.St unit), fiber uniformity ratio (UR %), Upper half mean length (UHM mm), and Micronaire reading (Mic). The lint cotton samples were tested at Cotton Technology Laboratory, Cotton Research Institute, ARC. High Volume Instrumentation (HVI) was used for the determination of fiber traits.Seed cotton yield (SCY K/f) data for genotypes under study at different locations was analyzed using analysis of variance to determine the effects of environment (E), genotype (G) and their interaction (GE).

Table 1. Code and pedigree of 24 cotton genotypes, their parents and their origins.

Code	Genotypes
G1	[(G91 × G90)] ×G85
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]
G3	[(G91 × G90)] × [(G85 × G83)]
G4	[(G91 × G90)] × [(G83 × G80) × G89]
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]
G6	[(G91 × G90)] × Karshink
G7	[(G83 × G80) × Dandara] × [(G90 × Aust)]
G8	[(G91 × G90)] × G80
G9	(Giza 90 × CB 58)
G10	[(G83 × G80) × G89] ×Aust
G11	Giza 95
G12	Giza 90

A combined analysis of variance was computed for genotypes, locations, seasons and their interaction according to Snedecor and Cochran (1982) for each location. GGE Biplot analysis (Yan, 2001) was used to interpret the genotype by environment interaction (GE) using Gen-stat14th ed. (Gen-Stat 2011). Only variables with significant effects of G and GE were appropriate for analysis using GGE Biplot (Blanche et al 2006).

Table 5: Mean effect of the interaction between cotton genotypes and environments over three seasons

2016 Code	Genotypes	E1	E2	E3	Means
G1	[(G91 × G90)] ×G85	11.68	10.38	8.58	10.21
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	11.37	10.04	8.78	10.06
G3	[(G91 × G90)] × [(G85 × G83)]	10.20	10.49	8.70	9.80
G4	[(G91 × G90)] × [(G83 × G80) × G89]	10.14	10.62	10.17	10.31
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	11.10	10.29	9.61	10.33
G6	[(G91 × G90)] × Karshink	10.74	10.32	9.51	10.19
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	10.83	11.83	9.93	10.86
G8	[(G91 × G90)] × G80	11.04	10.74	9.53	10.44
G9	(Giza 90 × CB 58)	10.78	11.23	9.91	10.64
G10	[(G83 × G80) × G89] ×Aust	9.98	12.23	9.85	10.69
G11	Giza 95	11.45	11.27	7.90	10.21
G12	Giza 90	8.96	10.75	8.13	9.28
	Grand Means	10.86	10.85	9.21	10,25
2017					
G1	[(G91 × G90)] ×G85	8.40	13.02	12.40	11.27
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	7.55	13.96	12.73	11.41
G3	[(G91 × G90)] × [(G85 × G83)]	7.48	13.34	10.77	10.53
G4	[(G91 × G90)] × [(G83 × G80) × G89]	7.70	13.01	12.19	10.97
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	8.22	13.61	12.19	11.34
G6	[(G91 × G90)] × Karshink	7.50	12.63	10.76	10.30
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	7.19	12.78	10.79	10.25
G8	[(G91 × G90)] × G80	7.78	13.91	12.79	11.49
G9	(Giza 90 × CB 58)	7.50	14.69	12.26	11.48
G10	[(G83 × G80) × G89] ×Aust	5.66	13.12	10.38	9.72
G11	Giza 95	8.20	9.16	8.72	8.69
G12	Giza 90	5.85	12.57	11.24	9.89
	Grand Means	7.42	12.98	11.44	10.61
2018					
G1	[(G91 × G90)] ×G85	8.66	8.41	10.19	9.09
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	9.14	9.17	10.07	9.46
G3	[(G91 × G90)] × [(G85 × G83)]	8.59	8.88	10.36	9.28
G4	[(G91 × G90)] × [(G83 × G80) × G89]	7.71	8.82	9.47	8.67
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	9.62	9.56	9.79	9.66
G6	[(G91 × G90)] × Karshink	8.28	9.65	9.67	9.20
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	9.10	9.47	9,89	9.49
G8	[(G91 × G90)] × G80	8.69	10.31	9.10	9.37
G9	(Giza 90 × CB 58)	8.62	10.22	8.88	9.24
G10	[(G83 × G80) × G89] ×Aust	7.70	9.33	8.79	8.61
G11	Giza 95	8.39	11.40	9.38	9.72
G12	Giza 90	6.63	9.23	7.87	7.91
	Grand Means	8.43	9.54	8.63	9.14

E1 . Bani-Souf environment E2 . El-Fayuom environment E3 . Sohag environment

Table 6: Fiber quality means of 12 Egyptian cotton genotypes in three seasons at three locations.

Code	Genotypes	Mic	UHM	UR%	Mature	F.St (gm/tex)	Y.St
		2016					
G1	[(G91 × G90)] ×G85	4.0	30.0	84.8	0.94	37.2	2060
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	3.8	28.7	83.4	0.93	36.1	1980
G3	[(G91 × G90)] × [(G85 × G83)]	3.8	29.9	82.5	0.91	38.0	2050
G4	[(G91 × G90)] × [(G83 × G80) × G89]	3.8	30.0	83.3	0.92	36.9	2070
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	4.0	30.5	82.9	0.92	36.5	2040
G6	[(G91 × G90)] × Karshink	3.8	30.8	84.3	0.92	36.4	1900
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	3.8	30.1	85.1	0.91	35.9	1920
G8	[(G91 × G90)] × G80	4.0	30.4	84.1	0.93	37.2	2125
G9	(Giza 90 × CB 58)	3.9	29.8	83.3	0.92	36.2	2035
G10	[(G83 × G80) × G89] ×Aust	3.8	30.0	84.1	0.90	36.1	2055
G11	Giza 95	3.9	30.5	84.9	0.92	36.9	2035
G12	Giza 90	3.8	30.0	84.2	0.92	35.1	2045
	Means	3.9	30.1	83.9	0.92	36.5	2025
		2017					
G1	[(G91 × G90)] ×G85	4.1	29.1	83.4	0.93	37.0	1695
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	4.1	28.6	83.8	0.92	35.8	1855
G3	[(G91 × G90)] × [(G85 × G83)]	4.1	29.9	82.6	0.93	37.3	1775
G4	[(G91 × G90)] × [(G83 × G80) × G89]	4.0	29.8	82.1	0.92	35.7	1800
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	3.9	31.2	83.3	0.90	37.3	1785
G6	[(G91 × G90)] × Karshink	4.1	30.1	83.7	0.94	35.8	1745
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	3.9	29.2	83.5	0.90	35.0	1760
G8	[(G91 × G90)] × G80	4.0	30.4	83.0	0.92	36.5	1780
G9	(Giza 90 × CB 58)	4.0	28.9	83.0	0.92	35.8	1785
G10	[(G83 × G80) × G89] ×Aust	4.1	28.1	83.8	0.92	36.3	1695
G11	Giza 95	3.9	29.4	83.2	0.90	35.2	1935
G12	Giza 90	4.0	29.4	84.2	0.91	36.2	1820
	Means	4.0	29.5	83.3	0.92	36.2	1785
		2018					
G1	[(G91 × G90)] ×G85	4.3	30.5	84.2	0.94	39.1	1947
G2	[(G91 × G90)] × [G83 × (G75 × 5844)]	4.2	30.4	84.2	0.92	38.5	1907
G3	[(G91 × G90)] × [(G85 × G83)]	4.3	30.9	84.4	0.92	38.6	1893
G4	[(G91 × G90)] × [(G83 × G80) × G89]	4.4	31.1	84.5	0.95	39.6	1840
G5	[(G90 × Aust)] × [G83 × (G75 × 5844)]	4.2	31.0	84.4	0.92	39.3	1847
G6	[(G91 × G90)] × Karshink	4.2	31.0	84.2	0.91	39.2	1900
G7	[(G83×G80) × Dandara] × [(G90×Aust)]	4.3	30.6	84.1	0.95	38.7	1833
G8	[(G91 × G90)] × G80	4.3	30.5	84.4	0.94	39.0	1847
G9	(Giza 90 × CB 58)	4.4	31.0	82.9	0.96	37.5	1860
G10	[(G83 × G80) × G89] ×Aust	4.2	31.1	84.2	0.93	39.0	1860
G11	Giza 95	4.2	30.6	84.1	0.91	38.7	1847
G12	Giza 90	4.0	30.5	84.1	0.94	38.7	1873
	Means	4.3	30.8	84.1	0.93	38.8	2940

References

1. Ali, I.; N. U. Khan; F. Mohammad; M. A. Iqbal; A. Abbas Farhatullah; Z. Bibi; S. Ali; I. A. Khalil,; S. Ahmad and M. Ur Rahman (2017). Genotype by environment and GGE-biplot analysis for seed cotton yield in Upland cotton. Pak. J. Bot., 49: 2273-2283.
2. A.S.T.M. (1986). American society for testing materials, D-4605. U.S.A.
3. Baker, H.C. and J. Leon, (1988). Stability analysis in plant breeding. Plant Breed., 101: 11-23.
4. Baker, Kh. M. A (2017). Graphical analysis of multi-environment yield trials using Biplot method to study performance stability for some Egyptian cotton genotypes.Egypt. J. Plant Breed. 21(2):245 – 260 (2017)
5. Blanche, S.B.; G.O. Myers; J.Z. Zumba; D. Caldwell and J. Hayes (2006). Stability comparisons between conventional and near-isogenic transgenic cotton cultivars. J. Cotton Sci. 10: 17–28.
6. Campbell, B.T; P. W. Chee; E. Lubbers; D. T. Bowman; W. R. Meredith; J. Johnson; D. Fraser; W. Bridges and D. C. Jones(2012). Dissecting genotypes × environment interactions and trait correlations present in the pee dee cotton germplasm collection following seventy years of plant breeding. Crop Sci 52 (2):690-699.
7. Eberhart, S.A. and W.A.Russell (1966). Stability parameters for comparing varieties. Crop.Sci. 9: 36-40.
8. Farias F.J.C.; L.P. Carvalho; J.L. Silva Filho and P.E. Teodoro (2016). Biplot analysis of phenotypic stability in upland cotton genotypes in Mato Grosso. Genetics and Molecular Research, 15 (2): 1-10.
9. Imtiza. A; N. U. Khan; F. Mohammad; M. A. Iqbal; A. Abbas, F. Tullah; Z. Bibi; S, Ali; I. A. Khalil; S. Ahmad and M. U. Rahman (2017). Genotype by environment and GGE-Biplot analysis for seed cotton yield in Upland cotton. Pak. J. Bot., 49 (6): 2273-2283, 2017

10. GenStat (2011). Genstat Procedure Library Release PL22.1. 14th Edition, VSN International Ltd., Hemel Hempstead.
11. Gul, S. ; N.U. Khan,R.Gul; M.Baloch; A. Latif and I.A. Khan. (2016). Genotype by environment and phenotypic adaptability studies for yield and fiber variables in Upland cotton. J, Anim. Plant Sci., 26(3): 776-786.
12. Kerby. T. A.; J. Burgess; M. Bates; D. Albers and K. Lege. (2000). Partitioning variety and environmental contribution to variation in yield, plant growth and fiber quality. Proceed. Beltwide cotton conference, San Antonio, USA, Jam 4-8, 1: 528-532.
13. Killi, F. and Harem, E. (2006). Genotype × environment interaction and stability analysis of cotton yield in Aegean region of Turkey. Journal of Environmental Biology. 27(2): 427-430.
14. Perkins. J. M. and J. L. Jinks (1968). Environmental and genotype-environmental interactions and physical measures of the environment. Heredity 25: 29-40.
15. Satish, Y.; P.P. Jain and B.S. Chabra (2009). Stability analysis for yield and its component traits in American cotton (Gossypium hirsutum, L.). J. Cotton Res. Dev., 23(2): 175-182.
16. Said, S.R.N. (2016). Stability of yield and yield components for some Egyptian cotton genotypes. Egypt. J. Plant Breed. 20 (3): 541-552).
17. Snedecor, G.W. and W.G. Cochran (1982). Statistical methods. The Iowa State Univ. Press. Ames. Iowa, USA.8 th Ed.
18. Yan, W K; Rajcan I. 2002. Biplot analysis of test Sites and trait relations of soybean in Ontario. Crop Science, 42, 11-20.
19. Yan, W.; L.A. Hant; S. Qinglai and Z. Szalvincs (2000). Cultivar Evaluation and Mega environment Investigation Based on the GGE Biplot. Crop Sci. 40: 597-605.
20. Yan, W. (2001). GGE biplot–A windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron. J. 93: 1111-1118.
21. Yan, W.; M. S. Kang; S. Woods and P. L. Cornelius (2007). GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci. 47: 643-655.
22. Yang, R. C.; Crossa J; Cornelius P L; Burgueño J. 2009. Biplot analysis of genotype × environment interaction: proceed with caution. Crop Science, 49, 1564-1576.
23. Yan, W. and J. B. Holland (2010). A heritability-adjusted GGE biplot for test environment evaluation. Euphytica, 171: 355-369.

Heterosis and Combining Ability for Phenological, Yield and Fiber Traits of Cotton (*Gossypium Hirsutum L.*) Genotypes

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Abstract

The seeds of four lines, three testers, and their twelve F1 hybrids were sown in a randomized complete block design (RCBD) with three replications at Botanical Garden, Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam during Kharif 2019. The F1 hybrids were raised for the estimation of heterotic effects, GCA and SCA for days to 1st squaring, days to 1st boll formation Bolls opened at 90 days after sowing, Sympodial branches plant-1, Bolls plant-1, Boll weight (g), Seed cotton yield plant-1 (g), 100-seed weight (g), Ginning outturn (%) and staple length (mm). Genotypes, parents, crosses, parents v/s crosses, lines, testers, and line x testers were significant for days to 1st squaring, days to 1st boll formation Bolls opened at 90 days after sowing, sympodial branches plant-1, bolls plant-1, boll weight, seed cotton yield plant-1, 100 seed weight, ginning out turn and staple length. Lines and testers were non-significant for boll opened at 90days after sowing and parents for boll weight, 100 seed weight, and staple length was also non-significant. Line CRIS-134, Sindh-1, and tester FH-901 gave a higher mean performance for all the traits. Among the hybrids, Mehran x FH-901, Sindh-1 x CIM-602, CRIS-134 x CIM-602, CRIS-342 x Koonj and CRIS-342 x CIM-602 gave a desirable mean performance and heterotic effect for all the traits. CRIS-134, Sindh-1, CRIS-342, and tester CIM-602 gave desirable GCA effects for most of the traits studied. CRIS-342 x Koonj produced maximum SCA effects for bolls plant-1. Mehran x FH-901 recorded higher SCA effects for seed cotton yield plant-1 and 100-seed weight and CRIS-342 x CIM-602 for lint percent and CRIS-342 x FH-901 for staple length. It is suggested that these lines and testers should be utilized in the breeding program for the improvement of these traits.

***Keywords**: Phenological, Yield, Fiber traits, Line x tester analysis, Heterosis and combining ability, Upland cotton*

Background

Cotton (Gossypium hirsutum L.) is one of the most important fiber crops in our country. Being a cash crop it plays a vital role in the economy of the country for earning foreign exchange. It is grown in many countries of the world such as the USA, China, India, Pakistan, Uzbekistan, Australia, and Africa (Bilwal et al., 2018). It requires a long growing season, plenty of sunshine and water during the period of its growth, and dry weather for harvest. The cotton crop has a share of 1.0 percent of GDP and donates 5.5 percent of cost accumulation to Agriculture. It is the sixth largest basis of vegetable oil and be able to increase the economy of any country by making edible oil for human consumption, feed for animal, and for local consumption in textile industries. Several peoples get profit from cotton in clothing manufacturing, textile industries, making edible industries, and dairy products (Ali et al., 2011). According to the economic survey of Pakistan, 2019, cotton is considered the lifeline of the economy of Pakistan because it has a 0.8 percent share of GDP and contributes 4.5 percent to agriculture value addition. The production remained moderate at 9.861 million bales, a decrease of 17.5 percent over the last year's production of 11.946 million bales and 31.5 percent against the target of 14.4 million bales. This below-expectation performance of the crop was largely due to contraction in the cultivated area on account of less economic incentive to the farmers by 12.1 percent to 2,373 thousand hectares compared to last year's area of 2,700 thousand hectares (Economic Survey of Pakistan, 2019). Cotton

breeders selected genotypes for development through different breeding methods and breeding techniques. However, the adverse connotation among yield and fiber strength and efforts to increase fiber quality through conventional breeding methods are the foremost problems faced by the cotton breeder. For the development of potential hybrids in cotton, it is essential to achieve economic heterosis by means of genetic variance of good combining ability of parents, which can lead to higher production and productivity (Anonymous, 2017). Gene action is the most essential criterion for selection in cotton crops. However, heterosis and combining ability are some of the essential statistical approaches for improving the yield and fiber quality traits of cotton (Sajjad et al., 2015). Heterosis studies achieve the crosses with high genetic potential while associated with their better parents for different traits. While, heterosis is simply beneficial when the performance of a new combination is better than its parents (Adsare et al., 2017). However, heterosis breeding is used for the improvement of economic yield and fiber traits in cotton (Monicashree et al., 2017; Gohil et al., 2017). It is also known as hybrid vigour the superiority of F1 hybrid over mid-parent or better parent in one or more characters in such a way the increased growth, fertility, and yield of hybrid over both the parents is known as heterosis or hybrid vigour.

Combining ability may be defined as the performance or ability of parents to combine each other during hybridization to transmit desirable even undesirable characters into their progenies is knowns as combining ability. It provides a chance for the cotton breeder the selection of better parent as it reveals the mode of inheritance for several plant traits and helps the plant breeder evaluate parental combination. Sprague and Tatum (1942) used the term GCA and SCA to estimate the average show of line, tester, and hybrid combination (Bolek et al., 2010). Anjum et al. (2018) reported that significant mean squares owing to both male and female parents determine the general combining ability (GCA) designated the main effect of additive variances promoting the studied parameters. They reported that the occurrence of dominant gene action signifies the possibility of hybrid crop development. Bilwal et al. (2018) and Deosarkar et al. (2019) also described that the highest heterotic effect was recorded in the hybrids over the standard check for seed cotton yield plant-1 and yield attributing traits. The existing information in cotton showed the importance of both additive and non-additive and the main importance of SCA. This indicated a higher chance of producing F1 hybrids for bolls plant-1, boll weight, seed cotton yield, and lint yield (Jatoi et al., 2011). Keeping in view the need for a higher yield of cotton to face the ever-increasing human population, the present study was designed to generate information about the heterosis of parents and combining ability in various plant characteristics of cotton. It will provide a guideline to cotton breeders for the development of hybrid cotton and segregating populations for selecting transgressive segregates.

Results and Discussions

Cotton (*Gossypium hirsutum L.)* is one of the most important fiber crops in the world as well as in Pakistan. It is a cash crop of Pakistan and plays a vital role in the economy of the country for earning foreign exchange. Cotton requires a long growing season, plenty of sunshine and water during the period of its growth, and dry weather for harvest (Rasheed et al., 2019). The results are described here and discussed in light of other cotton breeders with our findings in different sub-headings here as under

Analysis of variance

The mean square of analysis of variance revealed that genotypes, parents, crosses, parents v/s crosses, lines, testers, and line x testers were significant for most of the traits like days to 1st squaring, days to 1st boll formation Bolls opened at 90 days after sowing, sympodial branches plant-1, bolls plant-1, boll weight, seed cotton yield plant-1, 100 seed weight, ginning out turn and staple length (Table 1). Among them, lines and testers were non-significant for boll opened at 90days after sowing, and parents for boll weight, 100 seed weight, and staple length were also non-significant. However, the GCA variances were greater than SCA variances for most of the traits which indicated the preponderance of additive variances for controlling the traits. Makhdoom et al. (2019) reported that genotypes were significant for studied traits. The significant GCA and SCA variances indicated that both additive and non-additive variance were important for controlling the trait however the magnitude of non-additive variance was greater than the additive for most of the traits. Other researchers like Anjum et al., 2018

Rasheed et al., 2019, Bandhavi et al., 2019 and also reported significant mean squares of genotypes, lines, testers, and line x testers for various studied traits.

Mean performance

The mean performance (Table 2) showed that among the lines CRIS-134 gave minimum days to 1st squaring, staple length, bigger bolls, and more lint percent. Sindh-1 recorded more bolls opening at 90days after sowing and more sympodial branches plant-1, higher seed index, CRIS-342 set more number of bolls plant and higher seed cotton yield plant-1. Whereas in testers, Koonj counted less number of days for setting 1st squaring, minimum days for 1st boll formation and maximum boll weight, 100-seed weight, and FH-901 recorded maximum bolls plant-1 and seed cotton yield plant-1 and staple length. It is suggested that these lines and testers should be utilized in a hybridization program for the further improvement of these traits. Among the F1 hybrids, Mehran x FH-901 showed minimum days to 1st squaring and days to 1st boll formation, Sindh-1 x CIM-602 gave maximum boll opening at 90days after sowing, CRIS-134 x CIM-602 gave more number of boll plant-1, seed cotton yield plant-1 and second scorer for staple length. While CRIS-342 x Koonj and CRIS-342 x CIM-602 both hybrids recorded more sympodial branches plant-1. Jatoi et al. (2011), Muhammad et al. (2014), and Bandhavi et al. (2017) reported similar types of mean performance for lines, testers, and their F1 hybrids for earliness, yield and fiber traits.

Heterosis

Heterotic effects of F1 hybrids for yield and yield contributing traits are presented in Tables 3 to 5 the results are described here as under.

The top three scorers for negative heterosis were CRIS-134 x FH-901, Mehran x FH-901 and CRIS-134 x CIM-602 they recorded (-6.49,-16.37 & -10.55%) relative heterosis and (-12.19, -17.07 & -17.83%) heterobeltiosis respectively for days to 1st squaring. The maximum heterotic effects ranged from 13.32 to 18.42% (Table 5) among the hybrid, the F1 hybrid Sindh-1 x Koonj gave maximum (18.42%) relative heterosis followed by CRIS-342 x Koonj (16.23%) for the trait. While CRIS-342 x Koonj also gave the highest (13.32%) heterobeltiosis followed by sindh-1 x Koonj (12.50%) for days to 1st squaring. The hybrid CRIS-342 x CIM-602 and Mehran x CIM-602 gave minimum (0.39 & 2.32%) relative and heterobeltiosis for the trait respectively.

Table 1. Mean squares from ANOVA for phenological, yield and fiber traits of lines, testers and line x tester of upland cotton genotypes

Source of variation	D.F.	Days to 1st squaring	Days to 1st boll formation	Bolls opened at 90 days after sowing	Sympodial branches plant^{-1}	Bolls plant^{-1}	Boll weight	Seed cotton yield plant^{-1}	100 Seed weight	Ginning outturn	Staple length
Replication	02	0.85	0.21	14.75	5.69	3.00	0.08	452.72	0.13	1.41	0.38
Genotypes	18	37.40**	26.75**	14.53**	23.23**	58.45**	0.38**	1777.78**	3.95**	12.02**	4.05**
Parents (P)	6	20.76**	20.60**	23.80**	24.19**	80.88**	0.10$^{n.s}$	646.73**	0.02$^{n.s}$	17.56**	0.93$^{n.s}$
Crosses (C)	11	46.17**	31.94**	7.90**	9.48**	41.28**	0.19**	847.51**	2.04**	3.66**	3.09**
P vs C	1	40.81**	6.55**	31.74**	168.72**	112.84**	3.57**	18796.87**	48.59**	70.65**	33.28**
Lines (L)	3	71.88**	62.70**	6.91$^{n.s}$	19.07**	113.65**	0.04**	884.19**	2.09**	3.89**	2.10**
Testers (T)	2	13.88**	15.86**	5.44 $^{n.s}$	2.33**	42.75**	0.04**	512.96**	0.66**	4.00**	1.45**
L x T	6	44.08**	20.89**	9.22 $^{n.s}$	7.07**	4.60**	0.31**	940.69**	2.48**	3.44**	4.13**
Error	36	0.74	0.93	5.37	0.77	1.25	0.01	67.27	0.03	1.16	0.63

** = Significant at 1% probability level n.s. = non-significant

Table 2. Mean performance of lines, testers and F1 hybrids for phenological, yield and fiber traits of cotton genotypes

Lines and Testers	Days to 1st squaring	Days to 1st boll formation	Bolls opened at 90 days after sowing	Sympodial branches plant^{-1}	Bolls plant^{-1}	Boll weight	Seed cotton yield plant^{-1}	100 Seed weight	Ginning outturn	Staple length
CRIS-134	36.00	45.00	5.66	24.00	51.66	2.99	154.57	5.92	37.79	26.50
Mehran	40.33	46.66	6.00	22.00	55.00	2.86	157.53	5.88	37.74	27.16
Sindh-1	36.00	42.66	13.00	25.33	50.00	2.48	124.21	5.89	35.57	27.33
CRIS-342	38.00	45.33	8.60	21.40	58.00	2.84	164.77	5.69	34.95	28.00
FH-901	41.00	49.00	10.46	17.13	62.33	2.58	161.38	5.73	34.08	28.16
Koonj	40.00	48.00	5.46	19.00	46.66	2.91	135.77	5.95	39.62	27.66
CIM-602	43.00	50.33	9.00	20.00	54.33	2.73	148.66	5.84	32.70	27.33
Average	39.19	46.41	8.31	21.26	53.99	2.77	149.55	5.84	36.06	27.44
F_1 hybrids										
CRIS-134 x FH-901	36.00	44.00	8.66	24.66	56.00	3.00	168.03	6.43	38.50	28.50
Mehran x FH-901	34.67	41.66	9.66	24.00	55.00	3.50	192.46	7.26	38.50	28.60
Sindh-1 x FH-901	40.00	45.66	11.00	24.33	59.00	3.46	204.50	8.10	36.83	29.00
CRIS-342 x FH-901	40.00	48.00	11.33	21.00	51.00	3.90	198.86	9.13	38.00	28.00
CRIS-134 x Koonj	41.66	48.00	9.00	25.00	51.00	3.10	158.16	6.40	41.00	28.50
Mehran x Koonj	43.00	48.00	7.00	23.00	54.00	3.06	165.60	7.23	39.00	29.16
Sindh-1 x Koonj	45.00	50.00	10.33	25.00	57.66	3.33	192.23	8.20	38.50	28.16
CRIS-342 x Koonj	45.33	50.66	8.00	27.00	60.33	3.13	189.03	8.56	38.16	28.83
CRIS-134 x CIM-602	35.33	42.66	10.00	26.33	61.66	3.46	213.76	8.46	37.16	31.00
Mehran x CIM-602	44.00	51.00	12.00	26.66	56.00	3.13	175.56	7.70	37.16	31.16
Sindh-1 x CIM-602	45.66	51.33	12.33	24.00	60.33	3.20	193.10	7.73	38.50	29.00
CRIS-342 x CIM-602	40.66	48.00	9.00	27.00	61.00	3.20	195.13	7.90	39.16	28.50
Average	40.94	47.41	9.85	24.83	56.91	3.28	187.20	7.75	38.37	29.03
LSD at 5%	1.43	1.59	3.83	1.45	1.85	0.21	13.58	0.30	1.79	1.32

Table 3. Heterotic effects of F1 hybrids for days to 1st squaring, days to 1st boll formation, bolls opened at 90days after sowing and sympodial branches plant-1

F_1 hybrids	days to 1st squaring		Days to 1st boll formation		Bolls opened at 90 days after sowing		Sympodial branches plant-1	
	M.P*	B.P**	M.P*	B.P**	M.P*	B.P**	M.P*	B.P**
CRIS-134 x FH-901	-6.49	-12.19	-6.38	-10.20	7.44	-17.20	19.94	2.75
Mehran x FH-901	-16.37	-17.07	-12.89	-14.97	17.37	-7.64	22.69	9.09
Sindh-1 x FH-901	3.89	-2.43	-0.37	-6.81	-6.22	-15.38	14.60	-3.94
CRIS-342 x FH-901	1.26	-2.43	1.78	-2.04	18.88	8.31	9.03	-1.86
CRIS-134 x Koonj	9.65	4.17	3.22	0.00	61.87	59.01	16.27	4.16
Mehran x Koonj	7.07	6.62	1.41	0.00	22.16	16.66	12.19	4.54
Sindh-1 x Koonj	18.42	12.50	10.33	4.16	11.91	-20.53	12.81	-1.30
CRIS-342 x Koonj	16.23	13.32	8.57	5.54	13.79	-6.97	33.66	26.16
CRIS-134 x CIM-602	-10.55	-17.83	-10.49	-15.23	36.42	11.11	19.68	9.70
Mehran x CIM-602	5.61	2.32	5.17	1.33	60.00	33.33	26.95	21.18
Sindh-1 x CIM-602	15.62	6.20	10.41	0.01	12.09	-5.15	5.91	-5.25
CRIS-342 x CIM-602	0.39	-5.44	0.35	-4.62	2.27	0.00	30.43	26.16

*M.P.= Mid parent heterosis, **B.P.= Better parent heterosis

The relative heterosis and heterobeltiosis of F1 hybrids for days to 1st boll formation is summarized in Table 3. The relative heterosis for days to 1st boll formation varied from 10.41 to -12.89%, while heterobeltiosis ranged from 5.54 to -15.23%. Positive heterosis was observed in six crosses. Among the hybrids CRIS-134 x CIM-602, Mehran x FH-901 and CRIS-134 x FH-901 gave higher negative (-10.49, -12.89 & -6.38%) mid parent heterosis and (-15.23, -14.97 & -10.20%) batter parent heterosis for the trait days to 1st boll formation respectively. Whereas Sindh-1 x CIM-602 and CRIS-342 x Koonj produced maximum (10.41 & 5.54%) mid and better parent heterosis for the trait respectively and CRIS-342 x CIM-602 and Sindh-1 x CIM-602 gave minimum positive (0.35 & 0.01) for mid and better parent heterosis for the trait respectively. It is suggested that the above hybrids should be utilized for hybrid crop development in cotton.

The relative heterosis and heterobeltiosis of F1 hybrids for bolls that opened 90 days after sowing is depicted in Table 3. The positive relative heterosis for bolls opened at 90 days after sowing varied from 2.27 to 61.87%, while positive heterobeltiosis ranged from 0.00 to 59.01%. Among the hybrids, CRIS-134 x Koonj exhibited maximum (61.87%) relative heterosis while decreased heterotic effects were observed in Sindh-1 x FH-901 (-6.22%). Maximum (59.01%) heterobeltiosis was recorded in CRIS-134 x Koonj. However, minimum (8.31%) effects of heterobeltiosis were observed in the cross CRIS-342 x FH-901 for the trait bolls opened 90 days after sowing. It is suggested that for phenological traits negative heterosis is desirable for the development of short-duration cotton genotypes similar results were obtained by Jatoi et al. (2011) and Ashok Kumar et al. (2013) who also recorded negative heterosis for phenological traits.

The heterotic effects for sympodial branches plant-1 is presented in Table 3 which exhibited that all the hybrids expressed positive relative and heterobeltiosis effects except one. The maximum (33.66 & 26.16%) mid and better parent heterosis was given by CRIS-342 x Koonj followed by CRIS-342 x CIM-602 (30.43 & 26.16%) for sympodial branches plant-1 respectively. Nonetheless, Sindh-1 x CIM-602 and CRIS-134 x FH-901 exhibited minimum (5.91 & 2.75%) mid-parent and better-parent heterosis respectively for the trait.

The information for heterotic effects of F1 hybrids regarding the number of bolls plant-1 is presented in (Table 4) which indicated that the relative positive heterosis for bolls plant-1 was observed in six crosses which varied from (2.45 to 19.30%) mid parent and 1.81 to 15.32% for better parent heterosis. The maximum (19.30 & 15.32%) mid and better parent heterosis was given by Sindh-1 x Koonj followed by CRIS-134 x CIM-602 (16.36 & 13.49%) mid and better parent heterosis for the trait respectively. Whereas Mehran x CIM-602 displayed minimum (2.45 & 1.81%) mid-parent and better-parent heterosis respectively for bolls plant-1.

Furthermore, the information for heterotic effects of twelve F1 hybrids regarding the boll weight presented in Table 4 shows the relative heterosis for boll weight, Positive heterosis was observed in all crosses. Among the F1 hybrids, CRIS-342 x FH-901 exhibited maximum (43.91 & 37.32%) for mid-parent and better-parent heterosis. while Mehran x Koonj and CRIS-134 x FH-901 produced minimum (6.25 & 0.33%) mid-parent and better parent heterosis for the trait respectively. Similarly other researchers like Chaudhary et al. (2015), Soomro et al. (2016), Chhavikant et al. (2017) and Prakash et al. (2017) and showed the similar result of relative heterosis and heterobeltiosis of F1 hybrids for yield and fiber traits of cotton.

Table 4. Heterotic effects of F1 hybrids for bolls plant-1, boll weight, Seed cotton yield plant-1 and 100 Seed weight

F_1 hybrids	Bolls plant^{-1}		Boll weight		Seed cotton yield plant^{-1}		100 Seed weight	
	M.P*	B.P**	M.P*	B.P**	M.P*	B.P**	M.P*	B.P**
CRIS-134 x FH-901	-1.73	-10.15	7.91	0.33	6.36	4.12	10.48	8.61
Mehran x FH-901	-6.23	-11.75	28.67	22.37	20.70	19.25	25.17	23.46
Sindh-1 x FH-901	5.05	-5.34	36.75	34.10	43.21	26.71	39.41	37.52
CRIS-342 x FH-901	-15.22	-18.17	43.91	37.32	21.94	20.68	59.89	59.33
CRIS-134 x Koonj	3.74	-1.27	5.08	3.67	8.94	2.32	7.92	7.56
Mehran x Koonj	6.23	-1.81	6.25	5.15	12.92	5.12	22.33	21.51
Sindh-1 x Koonj	19.30	15.32	23.79	14.43	47.88	41.58	38.51	37.81
CRIS-342 x Koonj	15.28	4.01	9.05	7.56	25.79	14.72	47.07	43.86
CRIS-134 x CIM-602	16.36	13.49	20.97	15.71	40.99	38.29	43.87	42.90
Mehran x CIM-602	2.45	1.81	12.18	9.44	14.67	11.44	31.39	30.95
Sindh-1 x CIM-602	15.66	11.04	23.07	17.21	41.53	29.89	31.91	31.23
CRIS-342 x CIM-602	8.61	5.17	15.10	12.67	24.51	18.42	37.15	35.27

*M.P.= Mid parent heterosis, **B.P.= Better parent heterosis

Heterotic effect of F1 hybrids over their mid and better parent for seed cotton yield plant-1 (Table 4), the result shows that all twelve hybrids exhibited considerable amount of both relative heterosis and heterobeltiosis. The relative heterosis varied from 6.36 to 47.88% and 2.32 to 41.58% heterobeltiosis for seed cotton yield. The maximum (47.88 & 41.58%) mid and better parent heterosis was given by Sindh-1 x Koonj followed by Sindh-1 x Fh-901 and CRIS-134 x CIM-602 (43.21 & 38.29%) mid and better parent heterosis respectively, while CRIS-134 x FH-901 and CRIS-134 x Koonj produced minimum (6.36 & 2.32%) mid and better parent heterosis respectively for the trait. Similarly Patel et al. (2012), Soomro et al. (2016), Chinchane et al. (2018) and Khokhar et al. (2018) also obtained similar results for high relative heterosis and heterobeltiosis for yield contributing traits and fiber quality parameters of cotton.

The heterotic effect of F1 hybrids over their mid and better parent for 100 seed weight is presented in Table 4. The result shows that all twelve hybrids exhibited a considerable amount of heterosis. The relative heterosis varied from 7.92 to 59.89% while heterobeltiosis effects ranged from 7.56 to 59.33%. The maximum (59.89 & 59.33%) mid and better parent was given by CRIS-342 x FH-901. while minimum (7.92 & 7.56%) mid and better parent heterosis found in CRIS-134 x Koonj for seed index respectively.

In the calculation regarding the heterotic effect of F1 hybrids over their mid and better parent for ginning outturn (Table 5) the effects of relative heterosis varied from 0.82 to 15.75 % while heterobeltiosis effects ranged from 12.04 to -3.68. It was also observed that CRIS-342 x CIM-602 produced maximum (15.75 & 12.04%) mid and better parent heterosis for ginning outturn and Mehran x Koonj and CRIS-134 x FH-901 produced minimum (0.82 & 1.87%) mid and better parent heterosis for the trait respectively.

Table 5. Heterotic effects of F1 hybrids for ginning outturn and staple length

F_1 hybrids	Ginning outturn		Staple length	
	M.P*	B.P**	M.P*	B.P**
CRIS-134 x FH-901	7.15	1.87	4.28	1.20
Mehran x FH-901	7.21	2.01	3.39	1.56
Sindh-1 x FH-901	5.77	3.54	4.54	2.98
CRIS-342 x FH-901	10.11	8.72	-0.28	-0.56
CRIS-134 x Koonj	5.94	3.48	5.24	3.03
Mehran x Koonj	0.82	-1.56	6.38	5.42
Sindh-1 x Koonj	2.42	-2.82	2.43	1.80
CRIS-342 x Koonj	2.36	-3.68	3.59	2.96
CRIS-134 x CIM-602	5.44	-1.66	15.19	13.42
Mehran x CIM-602	5.50	-1.53	14.39	14.01
Sindh-1 x CIM-602	12.80	8.23	6.11	6.11
CRIS-342 x CIM-602	15.78	12.04	3.03	1.78

*M.P.= Mid parent heterosis, **B.P.= Better parent heterosis

The heterotic effect of F1 hybrids over their mid and better parent for staple length in cotton are presented in Table 5. The result shows that all hybrids exhibited a considerable amount of both positive relative heterosis and heterobeltiosis except one. The relative heterosis varied from 15.19% to -0.28 while heterobeltiosis effects ranged from 14.01 to -0.56%. The CRIS-134 x CIM-602 and Mehran x CIM-602 produced maximum (15.19 & 14.01%) mid and better parent heterosis for staple length respectively. Nonetheless, Sindh-1 x Koonj and CRIS-342 x FH-901 gave a minimum (2.43 & 1.20) mid and better parent for the trait. Chinchane et al. (2018) and Khokhar et al. (2018) also obtained similar results for high relative heterosis and heterobeltiosis for fiber quality parameters of cotton.

Combining ability effects

The combining ability effects of lines, testers, and line x tester for various quantitative traits are summarized in Tables 6 &7, the results are described here as under

Among the parents, the maximum negative general combining ability (GCA) effects (-4.06) were recorded in the line CRIS-134 and (-1.19) in the tester CIM-602 for days to 1st squaring (Table 6). However negative specific combining ability (SCA) effects (-5.36) were observed in the hybrid Sindh-1 x CIM-602 (Table 16). While maximum positive GCA effects (2.50) were recorded in the line CRIS-342 and (0.89) in the tester Koonj. While minimum positive GCA effects (0.61) were recorded in the line Mehran and (0.31) in the tester FH-901 However, CRIS-134 x CIM-602 followed by Sindh-1 x FH-901 produced maximum (4.31 & 2.81) SCA effects respectively. However minimum (0.22) positive SCA effect was given by Mehran x Koonj among the hybrids.

The negative GCA effects (-3.61) were recorded in the line CRIS-134 and (-1.30) in the tester CIM-602 for days to 1st boll formation (Table 6). The GCA effects for days to 1st boll formation showed that the maximum positive GCA effects (2.73) were recorded in the line CRIS-342 and (0.87) in the tester FH-901 among the parents. However negative specific combining ability effects (-3.70) were observed in the hybrid Sindh-1 x CIM-602 (Table 16). The minimum (0.28) positive GCA effects were recorded in the line Sindh-1 and (0.45) in the tester Koonj. The higher (3.19 & 2.22) SCA effects were given by CRIS-134 x CIM-602 and Sindh-1 x Koonj and minimum (0.02) SCA effects were recorded in CRIS-342 x FH-901 for days to 1st boll formation (Table 7).

The GCA effects for bolls opened at 90 days after sowing, among the parents, maximum positive (1.25) GCA effects were recorded in the line CRIS-342 and (0.72) in the tester FH-901. While minimum GCA effects (-0.09) were recorded in the line CRIS-134 and (-0.11) in the tester Koonj for bolls opened at 90 days after sowing (Table 6). However, the hybrid CRIS-134 x CIM-602 produced maximum (1.84) SCA effects followed by Mehran x FH-901 (1.50), However negative specific combining ability (-1.83) effects were observed in the hybrid CRIS-134 x FH-901 (Table 7).

The data regarding GCA effects for sympodial branches plant-1 showed that among the parents, the maximum positive (1.28) GCA effects were recorded in the line Sindh-1 and (0.33) in the tester CIM-602 while minimum (0.28) positive GCA effects were recorded in the line Sindh-1 and (0.45) in the tester Koonj for sympodial branches plant-1 (Table 6). However, the hybrid Mehran x Koonj produced maximum (1.83) SCA effects followed by CRIS-342 x FH-901(1.25), However negative specific combining ability (-2.05) effects were observed in the hybrid CRIS-342 x Koonj (Table 7).

The results regarding GCA effects for bolls plant-1 showed that among the parents, the maximum (2.97) positive GCA effects were recorded in the line Sindh-1 and (2.00) in the tester CIM-602 while minimum positive GCA effects (2.20) was recorded in the line CRIS-342 for bolls plant-1 (Table 6). However, the reduced and negative (-4.91) GCA effects were observed by Mehran. Among the hybrid CRIS-342 x Koonj produced maximum (1.47) SCA effects followed by CRIS-134 x FH-901 (1.09), However negative specific combining ability (SCA) effects (-1.41) were observed in the hybrid CRIS-134 x Koonj (Table 7).

The data regarding GCA effects for boll weight showed that among the parents, the line Mehran and CRIS-134 gave maximum (0.06 & 0.03) GCA effects respectively, and (0.06) was noted in the Tester FH-901. The minimum (0.02) positive GCA effects were recorded in the line Sindh-1 and (0.01) in the Tester CIM-602 for boll weight (Table 6). However, the reduced and negative GCA (-0.09) effects were observed by CRIS-342. Among the hybrid CRIS-342 x Koonj produced maximum (1.06) SCA effects. However, negative specific combining ability (-0.38) effects were observed in the hybrid CRIS-134 x FH-901 (Table 7).

Furthermore, the GCA effects for seed cotton yield plant-1 showed that among the parents, the line Sindh-1 gave higher (11.14) GCA effects and (7.55) in the Tester CIM-602. While minimum (0.73) positive GCA effects were recorded in the line CRIS-342 for seed cotton yield plant-1 (Table 6). However, the reduced and negative (-12.98) GCA effects were observed by Mehran. The hybrid Mehran x FH-901 produced maximum (28.18) SCA effects for seed cotton yield. However, negative (-16.77) SCA effects were observed in the hybrid CRIS-134 x FH-901 (Table 7).

Table 6. General combining ability (GCA) effects for phenological, yield and fiber traits of four female lines and three testers of cotton genotypes

Female lines	Days to 1st squaring	Days to 1st boll formation	Bolls opened at 90 days after sowing	Sympodial branches plant-1	Bolls plant-1
CRIS-134	-4.06	-3.61	-0.09	-0.50	-0.25
Mehran	0.61	0.62	-0.75	-1.83	-4.91
Sindh-1	0.94	0.28	-0.41	1.28	2.97
CRIS-342	2.50	2.73	1.25	1.05	2.20
S.E (gi)	0.40	0.44	1.09	0.41	0.51
Testers/Pollinators					
FH-901	0.31	0.87	0.72	-0.49	-1.74
Koonj	0.89	0.45	-0.11	0.17	-0.24
CIM-602	-1.19	-1.30	-0.61	0.33	2.00
S.E (gi)	0.34	0.38	0.94	0.34	0.44
Female lines	Boll weight	Seed cotton yield plant-1	100 Seed weight	Ginning outturn	Staple length
CRIS-134	0.03	1.13	-0.5	-0.43	-0.33
Mehran	0.06	-12.98	-0.17	0.96	-0.53
Sindh-1	0.02	11.14	0.65	-0.42	0.30
CRIS-342	-0.09	0.73	0.01	-0.09	0.52
S.E (gi)	1.48	3.86	2.58	0.50	0.37
Testers/Pollinators					
FH-901	0.06	-3.52	0.10	-0.32	-0.08
Koonj	-0.05	-4.00	-0.26	0.67	-0.30
CIM-602	0.01	7.55	0.16	-0.32	0.38
S.E (gi)	1.28	3.34	2.23	0.43	0.31

The information regarding GCA effects for 100 seed weight exhibited that among the parents, the line Sindh-1 gave maximum (0.65) GCA effects as compared to others and (0.16) in the Testers CIM-602 While minimum positive GCA effects (0.01) were recorded in the line CRIS-342 and (0.10) in the Tester FH-901 for 100 seed weight (Table 6). However, the reduced and negative (-0.17) GCA effects were observed by Mehran. The hybrid Mehran x FH-901 gave maximum (1.45) SCA effects. However, negative (-3.40) SCA effects were observed in the hybrid Mehran x Koonj (Table 7).

The results regarding GCA effects for ginning outturn exhibited that parents, the line Mehran gave maximum (0.96) GCA effects and (0.67) in the Testers Koonj for ginning outturn (Table 6). However the minimum negative (-0.43) GCA effects were observed by CRIS-134. Among the hybrids, CRIS-342 x CIM-602 produced maximum (1.22) SCA effects. However, negative (-0.78) SCA effects were observed in the CRIS-342 x FH-901 (Table 7).

The GCA effects for staple length indicated that among the parents, the line CRIS-342 showed maximum (0.52) GCA effects and (0.38) in the Testers CIM-602 while minimum (0.30) positive GCA effects were recorded in the line Sindh-1 for staple length (Table 6). However the minimum (-0.53) negative GCA effects were found in Mehran. The F1 hybrid like CRIS-342 x FH-901 recorded maximum (1.69) SCA effects for staple length. However, negative (-1.43) SCA effects were found in the hybrid CRIS-342 x CIM-602 (Table 7). From the present results, it is concluded that line Sindh-1, CRIS-134, and tester CIM-602 should be utilized in the hybridization program for the improvement of these traits. Similarly, Bandhavi et al. (2017), Anjum et al. (2018), Rasheed et al. (2019) and Makhdoom et al. (2019) exhibited significant GCA effects for most of the traits studied and observe the best combiner among two lines. Similarly, other researchers like Usharani et al. (2016), Kumar et al. (2017), Deosarkar et al. (2019), and Kumar et al. (2019) also reported high SCA effects similar results were obtained for traits studied.

Table 7. Specific combining ability (SCA) effects of F1 hybrids for phenological, yield and fiber traits of twelve hybrids of cotton derived from crosses of four female lines with three testers

F_1 hybrids/Crosses	Days to 1st squaring	Days to 1st boll formation	Bolls opened at 90 days after sowing	Sympodial branches plant^{-1}	Bolls plant^{-1}
CRIS-134 x FH-901	-1.19	-0.64	-1.83	0.83	1.09
Mehran x FH-901	-1.86	-0.87	1.50	-1.50	0.75
Sindh-1 x FH-901	2.81	1.47	0.17	-0.61	-0.47
CRIS-342 x FH-901	0.25	0.02	0.17	1.25	-1.36
CRIS-134 x Koonj	-3.11	-2.56	0.00	-0.5	-1.41
Mehran x Koonj	0.22	-0.45	0.00	1.83	-0.75
Sindh-1 x Koonj	2.56	2.22	-1.33	0.72	0.70
CRIS-342 x Koonj	1.33	1.22	1.33	-2.05	1.47
CRIS-134 x CIM-602	4.31	3.19	1.84	-0.33	0.34
Mehran x CIM-602	2.64	1.30	-1.50	-0.33	0.00
Sindh-1 x CIM-602	-5.36	-3.70	1.17	-0.11	0.22
CRIS-342 x CIM-602	-1.59	-0.81	-1.50	0.79	-0.11
S.E (si)	0.70	0.78	1.89	0.71	0.91
F_1 hybrids/Crosses	Boll weight	Seed cotton yield plant^{-1}	100 Seed weight	Ginning outturn	Staple length
CRIS-134 x FH-901	-0.38	-16.77	-0.83	0.89	-0.12
Mehran x FH-901	0.49	28.18	1.45	-1.00	-0.47
Sindh-1 x FH-901	-0.04	-2.58	-0.31	0.89	-1.09
CRIS-342 x FH-901	-0.06	-8.84	0.17	-0.78	1.69
CRIS-134 x Koonj	0.24	8.14	-2.22	-2.11	0.20
Mehran x Koonj	0.81	-12.04	-3.40	1.00	0.25
Sindh-1 x Koonj	0.88	-5.30	-2.07	-0.45	-0.20
CRIS-342 x Koonj	1.06	9.18	-2.26	-0.44	-0.25
CRIS-134 x CIM-602	0.13	8.62	0.68	-0.78	-0.08
Mehran x CIM-602	-0.30	-16.16	-0.51	0.00	0.23
Sindh-1 x CIM-602	0.14	7.87	-0.11	-0.45	1.29
CRIS-342 x CIM-602	0.01	-0.35	-0.03	1.22	-1.43
S.E (si)	2.58	6.69	0.14	0.87	0.64

Conclusions

Genotypes, parents, crosses, parents v/s crosses, lines, testers, and line x testers were significant for days to 1st squaring, days to 1st boll formation, bolls opened at 90 days after sowing, sympodial branches plant-1, bolls plant-1, boll weight, seed cotton yield plant-1, 100 seed weight, ginning out turn and staple length. Line CRIS-134, Sindh-1, CRIS-342, and tester CIM-602 gave desirable mean values and GCA effects for most of the traits studied. Sindh-1 x CIM-602 gave a negative SCA effect for days to 1st squaring and days to 1st boll formation and CRIS-134 x CIM-602 gave maximum positive SCA effects for boll opening at 90days after sowing.CRIS-342 x Koonj produced maximum SCA effects for bolls plant-1, Mehran x FH-901 recorded higher SCA effects for seed cotton yield plant-1 and 100-seed weight and CRIS-342 x CIM-602 for lint percent and CRIS-342 x FH-901 for staple length.

Materials and Methods

The experiment was conducted at Botanical Garden, Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, during Kharif 2019, in Randomized Complete Block Design with three repeats and nineteen genotypes were studied Four Lines Such as CRIS-134, Mehran, Sindh-1, CRIS-342 and three testers like FH-901, Koonj and CIM-602 were crossed in line x tester mating design and developed twelve F_1 hybrids for days to 1st squaring, days to 1st boll formation, bolls opened at 90 days after sowing, sympodial branches plant^{-1}, bolls plant^{-1}, boll weight (g), seed cotton yield plant^{-1} (g), 100-Seed weight (g), ginning out turn (%) and staple length (mm). The collected data were subjected to the statistical analysis of variance as adopted by Gomez and Gomez (1984). Whereas heterosis by Fehr, (1987) and combining ability were estimated according to Kempthorne (1957) and adopted by Singh and Choudhry (1984).

References

1. Adsare, A.D., A.N. Salve and N.P. Patil. 2017. Heterosis studies for quantitative traits in interspecific hybrids of cotton (Gossypium hirsutum L. × Gossypium barbadense L.). J. Phytol., 09:11-14.
2. Ali, B., M.S. Iqbal, K.M.N. Shah, G. Shabbir and N. M. Cheema. 2011. Genetic analysis for various traits in (Gossypium hirsutum L.) Pak. J. Agri. Res., 24(4): 8-13.
3. Anjum, R., M. J. Baloch, G. M. Baloch and Q. Chachar. 2018. Combining ability estimates for yield and fibre quality traits in Bt and non-Bt upland cotton genotypes. Pure and App Bio., 7(1): 389-399.
4. Anonymous. 2017. All India Coordinated Cotton Improvement Project. Annual Report 2016-2017, CICR Regional Station, Coimbatore.
5. Ashokkumar, K., K. S. Kumar and R. Ravikesavan. 2013. Heterosis studies for fibre quality of upland cotton in line x tester design. African J. Agri. Res., 8 (48), pp. 6359-6365.
6. Bandhavi, R.D., H. V. Kalpande, V. N. Chinchane and A. Kumar. 2017. Combining ability studies for yield and yield contributing traits in desi cotton (Gossypium arboreum L.). Int. J. Pure App. Bio. Sci., 5(6): 1482-1487.
7. Bandhavi, R.D., H.V. Kalpande, R. Khan and S. Panda. 2019. Combining ability studies for fiber quality traits in desi cotton (Gossypium arboreum L.) Int. J. Curr. Micro bio. App. Sci., 8(3): 415-421.
8. Bilwal, B.B., K.V. Vadodariya, G.R. Lahane and B.K. Kumar. 2018. Heterosis study for seed cotton yield and its yield attributing traits in upland cotton (Gossypium hirsutum L.). J. Pharmacognosy and Phytochemistry, 7(1): 1963-1967.
9. Bolek, Y., H. Cokkizgin and A. Bardak. 2010. Combining ability and heterosis for fiber quality traits in cotton. Plant Breeding and Science, 62: 3-16.
10. Chaudhary, M. H., M. A. Abid, Shoaib ur Rehman, J. Ashraf, W. Malik, Fakhar uz Zaman Khan, M. Bilal, G. Qanmber and F. Ahmad. 2015. Exploitation of Heterosis in F1 and F2 generations of cotton (G. hirsutum L.) Basic Res. J. of Agri. Sci., 4 (8) pp. 234-238.
11. Chhavikant, K., S. Nirania, A. Kumar and S.R Pundir. 2017. Heterosis studies for seed cotton yield and other traits in upland cotton (Gossypium hirsutum L.) J. of Pharmacognosy and Phytochemistry, 6(6): 583-586.
12. Chinchane, V.N., K. R. Thombre and N. E. Jayewar. 2018. Heterosis studies for fibre quality traits in desi cotton (Gossypium arboreum L.) Int. J. Curr. Microbiol. App. Sci., 6: 983-989.
13. Deosarkar D., J. Deshmukh, V. Deshmukh. 2019. Combining ability analysis for yield and fiber quality traits in upland cotton (Gossypium hirsutum L.). J. of Cotton Res. and Development, 28: 18-23.
14. Economic Survey of Pakistan, 2019. Pakistan Economic Survey, 2019. Satellite Based Crop Monitoring System Ministry of National Food Security And Research Bulletin, 2019, Volume Viii, Issue 6, Serial No. 90.
15. Fehr, W.R. 1987. Principles of cultivar development. Theory and Techniques. Macmilian Pub. Com. Inc. New York, Pp-115-119.

16. Gohil, S.B., M.B. Parmar and D.J. Chaudhary. 2017. Study of heterosis in interspecific hybrids of cotton (Gossypium hirsutum L. × Gossypium barbadense L.). J. Pharm. Phytochem., 6(4): 804-810.
17. Gomez, K.A. and A. A. Gomez. 1984. Statistics for Agricultural Research (Second Edition). John Willey and Sons, New York.
18. Jatoi, W.A., M. J. Baloch, F.V. Nasreen, and S.A. Panhwar. 2011. Combining ability estimates from line x tester analysis for yield and yield components in upland cotton genotypes. J. Agri. Res., 49(2): 165-172.
19. Kempthorne, O., 1957. An introduction to genetic statistics. John Wiley and Sons, Inc: New York.
20. Khokhar, E.S., A. Shakeel, M. A. Maqbool, M. K. Abuzar, S. Zareen, S. S. Aamir and M. Asadullah. 2018. Studying combining ability and heterosis in different cotton (Gossypium hirsutum L.) genotypes for yield and yield contributing traits. Pak. J. Agri. Res., 31(1): 55-68.
21. Kumar K.S., K. Ashok Kumar and R. Ravikesavan. 2019. Genetic effects of combining ability studies for yield and fiber quality traits in diallel crosses of upland cotton (Gossypium hirsutum L.). African J. Biotech., 13:119-126.
22. Kumar, S.K., J. M. Nidagundi and A. C. Hosamani. 2017. Genetic analysis for yield and its component traits in upland Cotton (Gossypium hirsutum L.) Int. J. Agri. Sci. Res., 7(2): 469-476.
23. Makhdoom, K., N. U. Khan, S. U. Khan, S. Gul, Z. Bibi, R. Gul, S. Ali, N. Ali, S. M. Khan. 2019. Genetic effects assessment through line × tester combining ability for development of promising hybrids based on quantitative traits in (Gossypium hirsutum L.) Tarim Bilimleri Dergisi J. Agri. Sci., 25, 47-61.
24. Monicashree, C., P. A. Balu and M. Gunasekaran. 2017. Combining ability and heterosis studies on yield and fibre quality traits in upland Cotton (Gossypium hirsutum L.) Int. J. Curr. Microbiol. App. Sci., 6(8): 912-927.
25. Muhammad, M.Y., T, S. Mari, S. Laghari, Z. A. Soomro and S. Abro. 2014. Estimation of heterosis and heterobeltiosis in F1 hybrids of upland cotton. J. Bio, Agri. and Healthcare ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) 4 (11):230-245
26. Patel, N.A., B.N. Patel, J. P. Bhatt and J.A. Patel. 2012. Heterosis and combining ability for seed cotton yield and component traits in inter specific cotton hybrids (Gossypium hirsutum L. x Gossypium barbadense L.) Madras Agric. J., 99(10-12): 649-656.
27. Prakash, G. and S. L. Korekar. 2017. Assessment of heterosis in Bt cotton (Gossypium hirsutum L.) hybrid under contrasting planting densities. J. Global Bio. sci., 6(8): 5193-5200.
28. Rasheed, M.S., R. Afzal, H. M. U. Waqas, S. Zia, A. Muneer, A. Manan, U. F. Soomro and J. Johar. 2019. Estimation of combining ability for yield and fiber quality traits in (Gossypium hirsutum L.) The Int. J. Biol. Res., 2019, 2, 118-142.
29. Sajjad, M., S. Malook, A. Murtaza, I. Bashir, M.K. Shahbaz, M. Ali, and M. Sarfaraz. 2015. Gene action study for yield and yield stability related traits in (Gossypium hirsutum L.) J. Life Sci., 12(5):1-11.
30. Singh, R. K. and B.D. Choudhry. 1984. Biometrical methods in quantitative genetic analysis. Haryana Agric. Uni. Hissar, India, pp. 191-200.
31. Soomro, A.W., F. H. Panhwar, A. R. Channa, M. Z. Ahsan, M. S. Majeedano and K. B. Sial. 2016. Study of heterosis and heterobeltiosis in upland cotton. Int. J. Biol. Biotech., 13(1): 111-114.
32. Sprague, G.F. and L.A Tatum. 1942. General versus specific combing ability in single cross of corn. J. Agronomy, 34(10): 923-932.
33. Usharani C.V., S.M. Manjula and S.S. Patil. 2016. Estimating combining ability through Line × Tester analysis in upland cotton. Res. Environ. Life Sci., 9(5) 628-63.

10

High Density Cotton Population Improves Productivity of Cotton and Tolerance against Cotton Leaf Curl Virus Under Semi-Arid Subtropical Conditions

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Abstract

***Background**: The cotton leaf curl virus (CLCuV) is a serious threat to cotton production all over the world. Early-grown cotton genotypes normally escaped this problem, but due to the late harvesting of wheat crops in the cotton-wheat cropping system, it severely affects late-sown cotton.*

***Results**: To address this problem a two-year experiment was conducted at the Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur, Pakistan. The experiment includes a commercially grown cotton genotype IUB-13 grown at two spacings i.e. normal spacing (30cm plan to plant distance) and high-density population (15 cm plant to plant distance). In both cases, the row-to-row distance was kept constant i.e. 75 cm. From the experiment, data were collected for CLCuV infestation, seed cotton yield (SCY), number of bolls per plant (NB), boll weight (BW), above ground fresh biomass (AGFB), harvest index (HI), ginning out turn (GOT) and fiber quality traits (fiber length (FL) and fiber fineness (FF). Significant reduction in the above-mentioned traits was observed mainly attributed to higher CLCuV infestation.*

***Conclusion**: It was evident from the results that yield losses due to CLCuV were effectively compensated by increasing the plant densities (reducing the plant spacing) by improving crop productivity.*

***Keywords**: CLCuV, Gossypium hirsutum L., plant spacing*

Background

Cotton (Gossypium hirsutum L.) is the most important cash and fiber crop that plays an important role in the economy of Pakistan because cotton not only provides the raw material to the entire textile industry but also earns valuable foreign exchanges for the country. Cotton yield is greatly affected by environmental conditions and applied cultural practices. Many biotic and abiotic factors affect cotton productivity. Among biotic factors, the cotton leaf curl virus (CLCuV) is on the most devastating factors severely affecting cotton yield. Significant losses in the yield of cotton are caused by CLCuV in Pakistan. CLCuV was reported for the first time in Pakistan in 1967 near Multan (Ghazanfar et al. 2007). At that time disease was of minor importance and it did not get much attention. In 1988, the disease appeared in an epidemic form near Multan and damaged the crop on about sixty thousand hectares with a loss of 0.3 billion bales in production (Mahmood, 1999). Since, 1988, the geographic spread of CLCuV disease has increased tremendously, and more than 7.7 million bales of cotton have been lost due to CLCuV disease from 1988 to 2002 (Akhtar et al., 2005). Since then field losses have become a constant phenomenon every year (Mahmood, 1999).

The CLCuV-affected plants showed stunted growth, less number of balls, reduction in ball size, and deterioration in fiber quality in upland cotton (Tanveer and Mirza 1996). There are two possible options to solve the problem of CLCuV menace.

a) To develop genetically resistant varieties to CLCuV;
b) Management practices to minimize losses, due to CLCuV infestation.

However, as far as the first option is concerned right now no cotton genotype is reported that is 100% CLCuV resistant. So, the second option, which is to manage the losses, matters a lot for cotton growers. Among the management practices, plant population is the most important factor in managing the cotton crop and its yield. There are many reports available that signify the role of higher plant density with

narrow plant spacing in enhancing seed cotton yield (James et al., 2004; Nadeem et al., 2010). Planting time has a significant effect on seed cotton yield and its components. The cause of the reduction in seed cotton yield in late planting time is mainly attributed to higher CLCuV infestation. Therefore, the major objective of this study was to find out the impact of narrow plant spacing as a management tool to compensate for yield losses due to CLCuV, especially in the context of late planting.

Results and Discussion

Analyses of variance showed that all traits were significantly affected by sowing date and plant spacing. Plant density plays an important role in crop development and production. Maximum above-ground fresh biomass (1235g) was observed in early sown cotton with narrow spacing followed by late sown with narrow spacing (911g) but it was statistically at par with early sown with normal spacing. Yang et al. (2014) reported that high plant density leads to better growth and development of cotton plants due to improvement in the microenvironment. They further explained that high plant density moderates maximum temperature which leads to better growth and development of the crop. Mao et al., (2014) that increased plant density improved crop light utilization, especially during the reproductive phase which leads to higher yield. As there were a greater number of plants per unit area, the number of bolls per unit area was also maximum in early sown cotton with narrow spacing leading it to the highest seed cotton yield per unit area. It is worth mentioning that early sown cotton with normal spacing has statistically at par yield with late sown cotton with narrow spacing and the same was observed for the number of bolls per unit area (Figure 1).

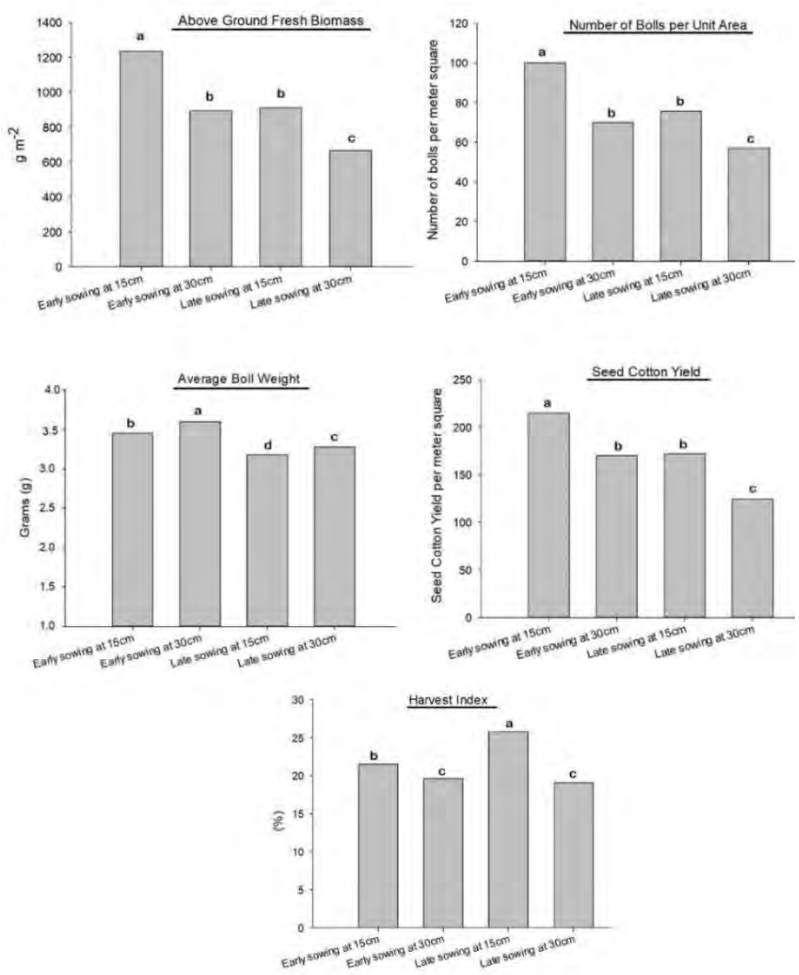

Figure 1. Effect of sowing date and plant density on different yield contributing traits of cotton

As with improved light utilization during the reproductive phase, plants produced more biomass and more biomass converted into reproductive parts, which lead to a higher number of bolls, boll weight, and seed cotton yield per unit area (Yang et al., 2014; Mao et al., 2014; Dai et al., 2015). Dai et al. (2015) reported that cotton yield can be stable at various plant densities by manipulating the number of bolls and boll weight under intensive management. In our case, even under late sown, when crop yield decreases with normal spacing, it becomes at par with early sown cotton, when plant density was increased with narrow spacing due to a greater number of bolls and with nearly the same average boll weight (Figure 1).

Table 2: Mean values of different traits under different sowing dates as observed in the experiment

Sowing date	Spacing	AGFB/m^2 (g)	NB/ m^2	SCY/ m^2 (g)	BW (g)	HI	GOT (%)	FL (mm)	FF	CLCuV severity index (%)
30 April	15cm	1235a	100a	215a	3.45b	21.5b	38.8a	28.6a	4.98a	17b
	30cm	895b	70b	170b	3.6a	19.1c	39.0a	28.5a	4.96a	20b
30 May	15cm	911.4b	75.51b	172.16b	3.18d	25.88a	39.8a	29.4a	4.61a	78a
	30cm	665.525c	57.1c	124.47c	3.28c	19.70c	40.2a	29.5a	4.65a	80a

Means showing different letter in same column are significantly different at 5% probability level

Boll weight was maximum with early sown with normal spacing followed by early sown with narrow spacing. It was further reduced in late sown cotton and a minimum was observed in late sown with narrow spacing (Figure 1). Early sown cotton received a relatively lower temperature during early development that late sown cotton, which gives it more time to accumulate the required degree days for boll development. More time for development ultimately leads to higher boll weight (Iqbal et al., 2017; Iqbal et al., 2018). But in late sown, this boll weight is compensated by a greater number of bolls per unit area (Mao et al., 2014; Dai et al., 2015).

Harvest index was maximum in early sown with normal spacing followed by early sown narrow spacing. But early sown narrow spacing was at par with late sown narrow spacing (Figure 1). Narrow spacing has more harvest index due to improvement in the efficiency of light utilization and better partitioning of dry matter into reproductive parts (Dong et al., 2010; Dai et al., 2015).

Maximum GOT was observed in early sown cotton with normal spacing while all others have statistically similar GOT. Fiber length and fiber fineness remained the same in all treatments (Figure 2). As GOT and fiber traits are predominantly controlled by genetic factors, and we used the same genotype for all treatments, therefore, GOT and fiber traits remained statistically similar under all conditions.

Disease severity index was minimum in early sown with narrow spacing followed by early sown with normal spacing. Disease severity was maximum in late sown cotton i.e. 78-80% due to the existence of favorable conditions (Figure 3). CLCuV is mainly associated with late sown cotton as early cotton completes its early development before the onset of favorable conditions of CLCuV i.e. late July and August. But in late sown cotton, the plant is still under initial growth and development and is severely attacked by the disease (Iqbal and Khan, 2010; Ali et al., 2014; Nawaz et al., 2019). Ali et al., 2015 reported that delayed planting of cotton increases the chances of CLCuV infestation due to favorable environmental conditions for whitefly which is the carrier of the virus and it reduces substantial yields. Under Narrow plant spacing, there is more number of plants and a favoured environment due to high plant density, which up to a great extent compensates for virus losses (Dong et al., 2010; Dai et al., 2015).

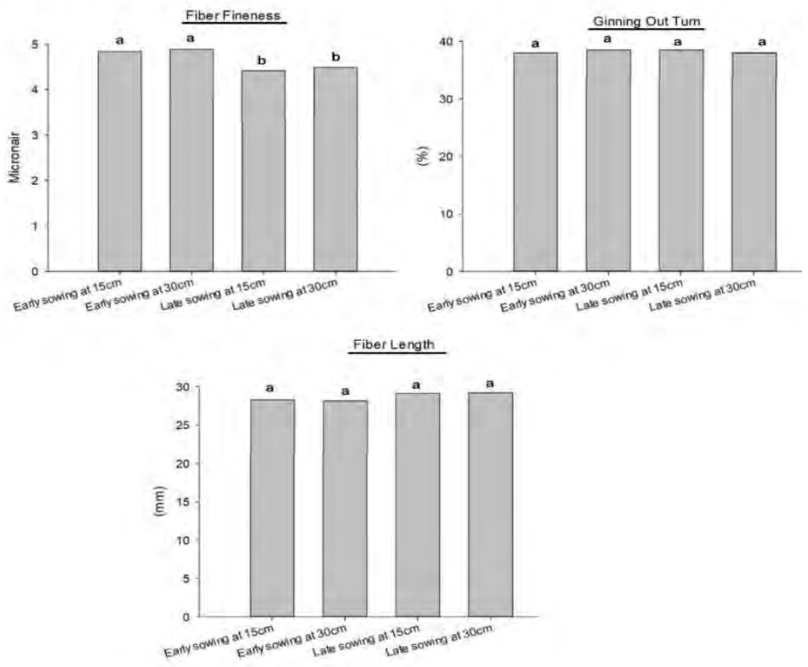

Figure 2. Effect of sowing dates and plant density on GOT and fiber-related traits of cotton

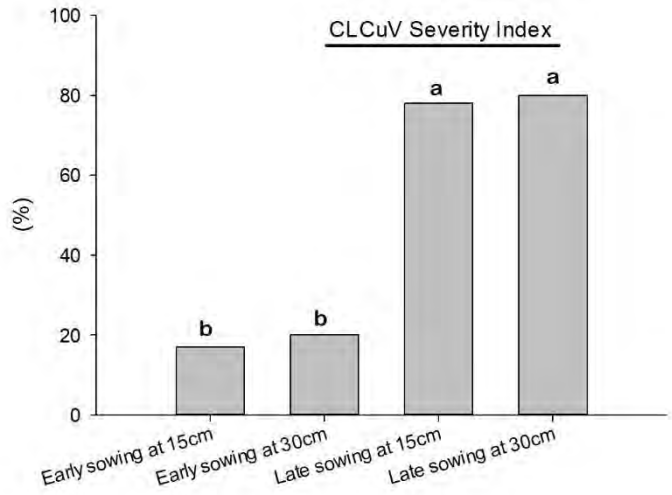

Figure 3. Effect of sowing date and plant density on CLCuV severity index of cotton

Conclusion

It is concluded from the above discussion the losses of CLCuV can be minimized by early sowing of cotton or by reducing the plant spacing. Due to the late harvesting of the wheat crop, normal cotton cultivation is delayed which increases the chances of CLCuV infestation. Therefore, to minimize these losses narrow plant spacing (15cm) for cotton cultivation is recommended. The results of this study may be very helpful in improving the cotton yield in the cotton-wheat cropping system.

Materials and Methods

Experimental site and genotype details

Field experiments were conducted in 2018 and 2019 on the experimental farm of the Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur (29° 24' N latitude, 71° 41' E longitude, 214 m above sea level) Pakistan. The cotton genotype included in the study was an approved cotton cultivar IUB- 13 which was developed by the Department of Plant Breeding & Genetics, The Islamia University of Bahawalpur (IUB), Pakistan.

Experimental design and crop management
Cotton variety IUB-13 was planted on 30th April 2018 under randomized complete block design (RCBD) with three replications. For the control experiment plant to plant and row-to-row spacings were 30 and 75cm respectively while for the narrow spacing plant to plant distance was maintained at 15 cm and row to distance was the same as control. For late sown, the same experiment was again sown on 30th May 2018 under previous sowing arrangements. Standard agronomic practices (e.g., hoeing weeding, pesticide application, and irrigation) were carried out according to the crop requirement. The same experiment was repeated in 2019 to authenticate the results of the first year.

CLCuV rating, sampling, and processing
CLCuV rating was determined following the disease scale described by Akhtar et al., (2010) and Farooq et al., (2011), and the rating was computed in the third week of august. The disease scale is given in Table 1.From both populations, 15 plants were selected randomly, and data were collected for the following parameters.

Table 1. Rating scale for cotton leaf curl virus disease (CLCuV) symptoms.

Symptoms	Disease rating	Disease index (%)	Disease reaction
No symptom	0	0	Immune
Thickening of only secondary and tertiary veins	1	0.1–10	Highly tolerant
Thickening of tertiary veins, secondary and primary veins	2	10–30	Tolerant
Vein thickening, leaf curl or enation or both	3	30–50	Susceptible
Stunting alone with, vein thickening leaf curl/enation	4	>50	Highly susceptible

Number of Bolls per plant (NB)
The number of effective matured bolls per plant for both picks was recorded.

Seed cotton yield (SCY)
The matured bolls were obtained at two various picking times and separately seed cotton was obtained for the individual plant in Kraft paper bags and weighed average seed cotton yield/plant in grams.

Boll weight (BW)
To calculate the BW, SCY was divided by BP.

Above ground fresh biomass (AGFB)
At crop maturity, the individual plant was removed from the soil and fresh plant weight was measured in grams after the separation of roots.

Harvest Index (HI)
The harvest index is the ratio of the harvested product (lint and seed) to the above-ground plant biomass calculated by the following formula.
Harvest index (HI) = Seed cotton yield/Above ground fresh biomass × 100

Disease severity index
The disease severity index was calculated by the formula

$$\text{Disease severity Index} = \frac{\text{Number of diseased plants per unit area}}{\text{Number of healthy plants per unit area}} \times 100$$

Lint Percentage/Ginning Out-Turn (GOT)
Lint percentage or ginning out-turns (GOT) is the weight of lint that can be obtained from a given weight of seed cotton and is expressed as a percentage. Samples of seed cotton were weighed and ginned separately with a single roller electrical gin in the laboratory. Lint was weighed and GOT was calculated as a percentage of lint.

Fibre Traits

For fiber trait analysis, the ginned samples were re-conditioned by placing samples in a blow room (65% humidity and 18-20°C temperature) using a humidifier. High Volume Instrument (HVI-900-SA; Zellweger Ltd., Switzerland), was used to analyze staple length (SL) (mm), and fiber fineness (FF) (micronaire).

Statistical analyses

All collected data were subjected to analyses of variance using statistical software Statistix 8.1. Significant means were then compared by using Tukey's test. As the year effect was not significant, therefore, data are presented as the average of two years.

References

1. Akhtar K.P., Haider S., Khan M.K.R., Ahmad M., Sarwar N., Murtaza M.A., Aslam M., 2010 Evaluation of Gossypium species for resistance to leaf curl burewala virus. Ann Appl Biol 157:135–147.
2. Akhtar, K. P., M. Aslam, M. A. Haq, F. F. Jamil, A. I. Khan, and M. T. Elahi, 2005. Resistance to cotton leaf curl virus (CLCuV) in a mutant cotton line. Jour. Cotton Sci. 9: 175-181.
3. Ali, H., Hussain, G.S., Hussain, S., Shahzad, A.N., Ahmad, S., Javeed, H.M.R. and Sarwar, N., 2014. Early sowing reduces cotton leaf curl virus occurrence and improves cotton productivity. Cercetari Agronomice in Moldova, 47(4), pp.71-81.
4. Dai, J., Li, W., Tang, W., Zhang, D., Li, Z., Lu, H., Eneji, A.E. and Dong, H., 2015. Manipulation of dry matter accumulation and partitioning with plant density in relation to yield stability of cotton under intensive management. Field Crops Research, 180: 207-215.
5. Dong, H., Kong, X., Li, W., Tang, W. and Zhang, D., 2010. Effects of plant density and nitrogen and potassium fertilization on cotton yield and uptake of major nutrients in two fields with varying fertility. Field Crops Research, 119(1), pp.106-113.
6. Farooq A., Farooq J., Mahmood A., Shakeel A., Rehman A., Batool A., Riaz M., Shahid M. T. H., Mehboob S., 2011 An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity. Aus J Crop Sci 5: 1823-1831.
7. Ghazanfar, M.U., Sahi, S.T., Ilyas, M.B. and Randhawa, M.A., 2007. Influence of sowing dates on CLCuV incidence in some cotton varieties. Pak J Phytopathol, 19(2), pp.177-180.
8. Iqbal, M. and Khan, M.A., 2010. Management of cotton leaf curl virus by planting time and plant spacing. Advances in Agriculture & Botanics, 2(1), pp.25-33.
9. Iqbal, M., Ul-Allah, S., Naeem, M., Hussain, M., Ijaz, M., Wasaya, A. and Ahmad, M.Q., 2018. Reproductive Development and Seed Cotton Yield of Gossypium hirsutum as affected by Genotype and Planting Time. International Journal of Agri and Biol, 20(7), pp.1591-1596
10. Iqbal, M., Ul-Allah, S., Naeem, M., Ijaz, M., Sattar, A. and Sher, A., 2017. Response of cotton genotypes to water and heat stress: from field to genes. Euphytica, 213(6), p.131.
11. James JM, Wright DL, Wiatrak PJ, Vargas MA (2004). Effect of row width and nitrogen on cotton morphology and canopy microclimate. Crop Sci. 44: 707-710.
12. Mao, L., Zhang, L., Zhao, X., Liu, S., van der Werf, W., Zhang, S., Spiertz, H. and Li, Z., 2014. Crop growth, light utilization and yield of relay intercropped cotton as affected by plant density and a plant growth regulator. Field Crops Research, 155, pp.67-76.
13. Nadeem MA, Ali A, Tahir M, Naeem M, Chadhar AR, Ahmad S (2010). Effect of nitrogen levels and plant spacing on growth and yield of cotton. Pak. J. Life Soc. Sci. 8(2): 121-124.
14. Nawaz, B., Naeem, M., Malik, T.A., Muhae-Ud-Din, G., Ahmad, Q. & Sattar, S. (2019). A Review about Cotton Leaf Curl Viral Disease and Its Control Strategies in Pakistan. International Journal of Innovative Approaches in Agricultural Research, 3(1), 132-147.
15. Yang, G.Z., Luo, X.J., Nie, Y.C. and Zhang, X.L., 2014. Effects of plant density on yield and canopy micro environment in hybrid cotton. Journal of Integrative Agriculture,1 3(10), pp.2154-2163.

Multi-Locational Evaluation of Medium-Staple Cotton Genotypes for Seed-Cotton Yield under the Middleveld Agro-Ecological Zone of Zimbabwe

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Abstract

Background: *The Zimbabwe national cotton breeding program has the mandate to develop superior cotton (Gossypium Hirsutum) varieties with good field performance and high fiber properties. Cotton productivity in Zimbabwe has remained very low, with a national average seed cotton yield record of 650kg/ha (AMA Report, 2019) compared to the potential 2000kg/ha. Though this is a result of many biotic and abiotic factors, field experiments laid in a Randomized Complete Block Design were conducted on ten genotypes (seven test genotypes and three check varieties) from 2012 to 2019 across 13 diverse locations in Zimbabwe to evaluate cotton yield performance, stability and adaptability by Analysis of Variance (ANOVA) and Genotype and Genotype by Environment Interaction (GGE) Biplot methods.*

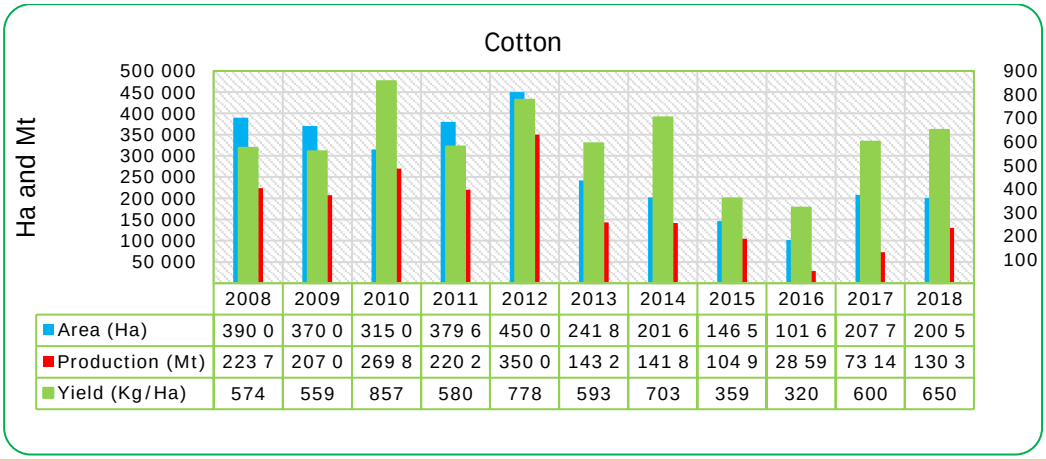
Results: *The Analysis of Variance indicated significant (P<.001) effects of Genotype (G), Environment (E), and their Interaction (GE). The highest percentage of variation was explained by E/G/GE (60.34%) while G/E+GE together explained the rest of the variation (<40%). Joint effects of G and GE were partitioned using the GGE biplot analysis explaining a total of 59.08% (PC1 = 36.96% and PC2 =22.12%) of the GGE sum of squares. The biplot analysis revealed that the candidate 917-05-7 was the ideal and stable genotype. The candidate variety 917-05-7 significantly (P< .001) showed superior yield performance over checks CRI-MS1 and CRI-MS2 recording 5% and 5.5% yield increases respectively. Candidate 917-05-7 recorded a higher earliness index (78.11%) over checks CRI-MS1 and CRI-MS2 (77 and 76% respectively) thus indicating potential attributes for good cotton production with more pick-able bolls earlier than the current commercial varieties.*

Conclusion: *Candidate 917-05-7 has been identified as the ideal genotype in terms of high yielding potential, and stability hence will be promoted for commercial release and used as a breeding parent for future breeding programs.*

Keywords: *Cotton, Gossypium Hirsutum, GGE, Genotype, Environment, Stability, Yield, Adaptability*

Background

Cotton (*Gossypium Hirsutum*) is predominantly a smallholder crop and represents a crucial source of income for millions of farmers and their families in more than 20 countries across all regions of Sub-Saharan Africa (Travella, 2017). Despite its economic potential, cotton variety development in Zimbabwe has been very low leaving farmers to continuously cultivate obsolete varieties. Currently, cotton production in Zimbabwe is very low, with a national average seed cotton yield of 650kg/ha.



	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Area (Ha)	390 0	370 0	315 0	379 6	450 0	241 8	201 6	146 5	101 6	207 7	200 5
Production (Mt)	223 7	207 0	269 8	220 2	350 0	143 2	141 8	104 9	28 59	73 14	130 3
Yield (Kg/Ha)	574	559	857	580	778	593	703	359	320	600	650

Figure 1: Cotton production trend (average yield, production, and area under production) for the period 2008 -2018 (Source: AMA 2019).

The cotton genotype selection and recommendations by breeders have been slowed down due to the effects of genotype by environment interaction (GEI). This complicates the identification of superior cotton genotypes, thus making the selection and recommendation of new genotypes for different environments difficult and expensive. Some multi-locational field experiments from the year 2012 to 2019 growing season were conducted aiming to evaluate the relative response of cotton genotypes across different environments and identify varieties with good adaptation and stability (Baafi and Safo-Kantanka, 2008) through the use of GGE biplot analysis. Multilocational Yield Trials (MYT) are important to evaluate the relationship between genotypes and environments for selected traits by use of a genotype by genotype by environment (GGE) biplot that allows visual assessment of genotype by environment interaction (GEI) pattern (Yan et al., 2000; Yan and Hunt, 2001). GGE is the most recent approach proposed by Yan et al. (2000) and has shown extensive usefulness as a more comprehensive tool in quantitative genetics and plant breeding (Yan et al., 2001; Yan and Rajcan, 2002). This tool for analysis of GEI is increasingly being used in GEI studies in plant breeding research (Butran et al., 2004). The objectives of this study were (i) to identify the genotype and environmental components that are associated with the GxE interaction across diverse environments, looking at the percentage source of variation and joint effects of G and GE as partitioned by Principal Components (PC) in the total sum of squares, (ii) to identify the ideal genotype(s) based on high yielding potential and stability across test-locations, (iii) to identify the mega, representative and ideal environment for testing genotypes and (iv) to identify which variety won were of the given test-locations.

Results

Across seasons and environments, a general combined analysis of variance (ANOVA) was conducted and the results indicated that variance in the measured yield and yield-related traits was due to the presence of genotype by environment interaction (GEI) (P<0.001) except for boll weights. The highest percentage of variation was explained by E/G/GE (60.34%) while G/E+GE together explained the rest of the variation (<40%) (Table 3). Joint effects of G and GE were partitioned using the GGE biplot analysis explaining a total of 59.08% (PC1 = 36.96% and PC2 =22.12%) of the GGE sum of squares (Table 3). The effect of GxE interaction on the parameters invited the need for further analysis using the GGE biplot analysis to be able to identify genotypes that are stable and adaptable. Overall seed cotton yield mean for the candidates was 1663kg/ha, whilst candidates recorded 1755kg/ha (Table 4) which was 5% and 5.5% yield gain over checks CRI-MS1 and CRI-MS2 respectively (Figure 4).

Table 3: Summary of the general analysis of variance for grain yield (kg/ha) showing the level of significance for the genotype, environment and GEI of advanced cotton genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.	Exp% ss
Genotype (G)	9	5618628	624292	4.93	<.0.001	14.06
Environment (E)	11	22707644	20643313	163.18	<.0.001	25.6
Genotype x Environment (GEI)	99	15502745	156593	1.24	0.005	60.34
Residual	234	29433635	42169			
Total	353	27763145	786491			

** DF= Degrees of freedom; SS= sums of square; MS= means square.

Table 4:Overall Field performance of the genotype 917-05-7 against three commercial check cultivars during the 7 seasons (2012-2019)

Genotype name	Seed cotton yield (kg ha^{-1})	Boll weight (g)	Earliness Index (%)	Gin out Turn (%)	Lint Yield (kg ha^{-1})	100 seed weight (g)
917-05-7	1755^{d}	6.4	78.11	41.83	743.9	10.88
CRI-MS-1	1677^{bcd}	6.3	77.04	42.12	717.9	11.40
CRI-MS-2	1659^{bcd}	6.3	76.13	41.54	709.1	10.96
SZ9314	1737^{cd}	6.3	78.58	41.92	744.7	11.06
Grand Mean	1663	6.4	77.4	41.8	709.8	11.12
F-Pro (G)	***	***	**	ns	ns	***
F-pr (G x E)	**	ns	***	***	***	***
Av. SED	60.34	0.1163	1.278	1.249	27.6	0.081
CV %	21.2	10.91	9.98	17.78	21.26	4.31

-Sig - Significance level, LSD Least Significant Differences, CV% Coefficient of Variation, SE Standard Error of Differences, *** significantly different at < 0.001, ** - significantly different at < 0.01, * - significantly different at < 0.05, NS – Not significantly different.

NB: The Grand mean, F-pr, LSD and CV% values displayed above are for the whole trial (all the genotypes).

Genotype Stability Analysis (GEI) for total seed cotton yield for cotton genotypes across seasons and environments

Which-won-where and mega-environments (ME)

The GGE scatter plot (Figure 3) showed dissected pentagon into sectors with winning genotypes located at the vertex of the polygon. The biplot revealed that candidates 917-05-7 (G5) and TN96-05-9 were the winning genotypes in six environments (Chisumbanje Exp, Umguza, Muzarabani, Matikwa, Panmure, and Save Valley) which fell under that sector/ mega-environment 1. The biplot revealed the existence of three Mega-environments (ME), with ME1 comprised of Chisumbanje Exp, Umguza, Muzarabani, Matikwa, Panmure, and Save Valley, ME2 comprised of Chitekete, CC Mollen, and CRI where CRI-MS1 was the winner whilst ME3 consisted of Wozhele, Kuwirirana, and Shamva where SZ9314 was the winner.

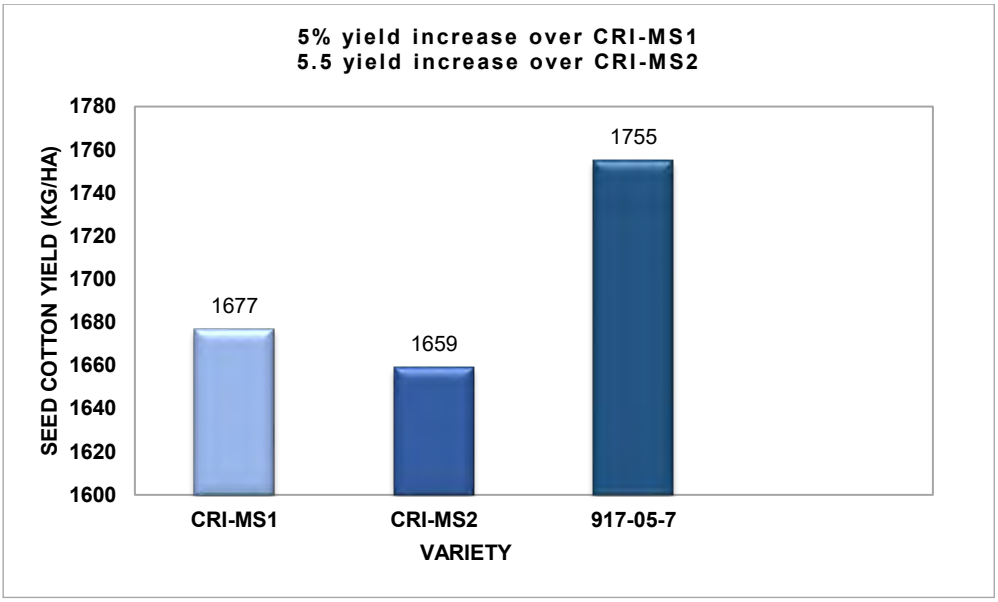


Figure 2. Overall seed cotton yield performance of 917-05-7 and percentage increase over check varieties over five seasons

Genotype Ranking based on mean performance and stability

Genotype by genotype-by-environment (GGE) interactions biplot analysis revealed that candidate 917-05-7 was high yielding and stable thus located on the far right and a short projected perpendicular line to the environmental axis whilst candidate TN96-05-9 was more stable and above average in terms of yield performance (Figure 4). Candidate 912-05-1 was moderately yielding thus above average and very stable. So candidates 917-05-7, TN96-05-9 and 912-05-1 are selected as good varieties which are

high yielding and stable compared to the check varieties CRI-MS1 and SZ9314 which were around average yield performance and highly unstable.

Ideal Genotype and environment

The GGE analysis positioned the candidate genotype 917-05-7 in the first concentric ring (Figure 5), identifying it as the ideal genotype. This also reveals that the genotype is high yielding and moderately stable compared to check varieties which are positioned in the 11th concentric ring thus low yielding and unstable. Some good varieties closer to the ideal genotypes were shown, and these included TN96-05-9, 912-05-1 and GN 96 (b)-05-8. The biplot displayed Umguza as the most ideal environment (Figure 5) identified by its location in the second concentric circle. However, Umguza showed poor discriminating ability as compared to Save Valley which had the best discriminating ability thus giving more information about the performance of tested genotypes. This indicates that the GEI greatly influenced the effect of Umguza site on the performance of the test genotypes. Good environments such as Matikwa, Save Valley and Chisumbanje Experiment were displayed.

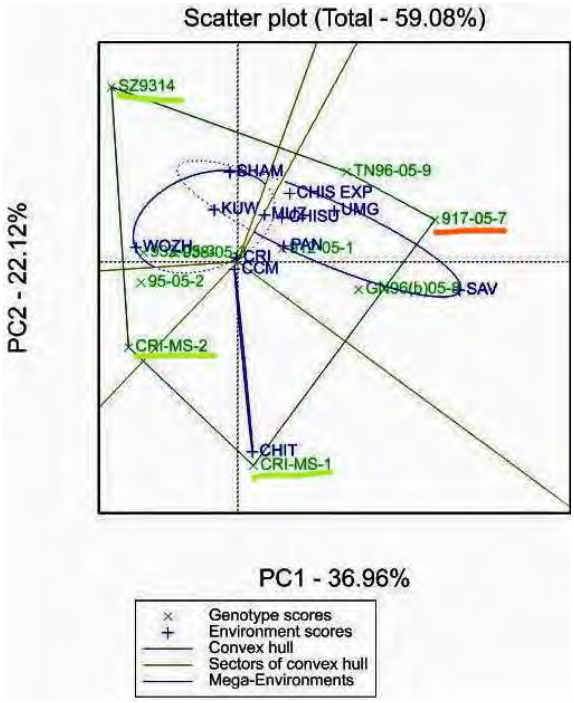


Figure 3. Best performing genotypes (Which-won-where) and mega-environments

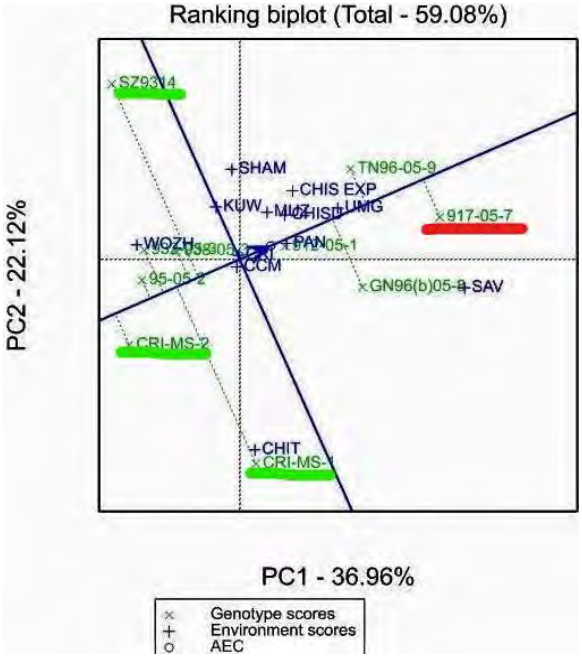


Figure 4. Ranking biplot showing the high yielding and stable test genotypes

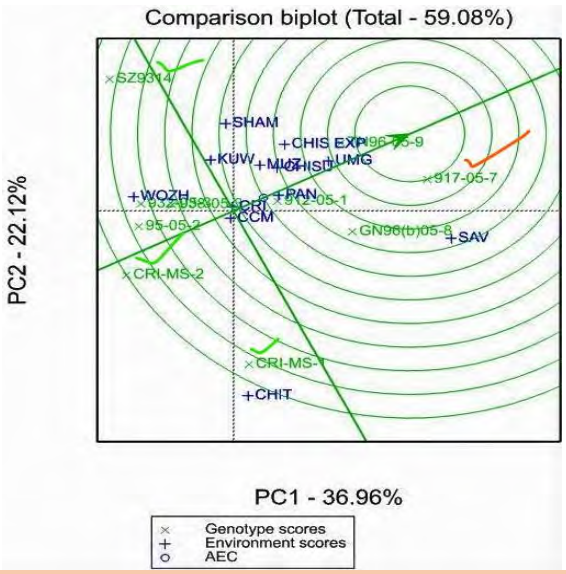


Figure 5. Comparison biplot showing the ideal genotype and ideal environment

Discussions and Conclusion

The general analysis of variance recorded candidate genotype 917-05-7 as the highest yielding (1755kg/ha) over the check varieties (<1680kg/ha) across seasons and environments by recording >5% yield advantage more than the commercial check varieties. The GGE biplot analysis was successful in giving more information about the test genotypes and test environments which could not be synthesized by analysis of variance (ANOVA) only. Biplot analysis revealed good varieties based not only on high yielding but stability, which is very important when yield experiments are done in many different environments. Candidate variety 917-05-7 was the ideal variety based on high mean yield performance and stability, and candidate variety TN96-05-9 was a good variety that was moderately yielding and very stable. Other candidate genotypes such as 912-05-1 and GN 96 (b)-05-8 were presented as good varieties with high and moderate stability respectively. The biplot analysis gave more information about the relationship between environments and genotypes, whereby high mean performing environments with high discriminating ability was revealed, the identified sites were Umguza and Save Valley respectively. Umguza recorded a high mean yield but was not well discriminating whilst Save Valley was moderately high yielding and high discriminating compared to the other environments. Good environments such as Matikwa, Save Valley and Chisumbanje Experiment were displayed in the biplot graphs. Three mega environments (ME) were shown through the GGE biplot analysis, and candidate varieties 917-05-7 and TN96-05-9 were the winning genotypes for ME1 which consisted of six environments out of twelve. The information revealed on mega-environments was relevant to the general information about the experimental sites where all the Lowveld institutes fell in one ME. This means no much differences were realized in terms of their effect on the variety performances and the sites are similar. This study was relevant in context of making good progress in selecting the best ideal and stable varieties under multi-locational variety trials. The study with reference to Zimbabwe's national cotton variety development programme, enabled the breeder to efficiently select and recommend superior genotypes for further evaluations and subsequent release (Yan et al., 2001; Yan and Rajcan, 2002). The use of GGE biplot analysis in the study showed extensive expediency in quantitative genetics and plant breeding hence the researcher implies this as a more comprehensive and relevant tool in multilocational research trials. Henceforth, the study results have identified genotypes 917-05-7, TN96-05-9, 912-05-1 and GN 96 (b)-05-8 as the superior candidates for commercial release. The genotypes are also recommended for use in future breeding programmes as parents.

Materials and Methods

Study Sites

The multi-locational experiments were conducted for six seasons (2011/12, 2013/14, 2015/16, 2016/17, 2017/18 and 2018/19 growing seasons) at four On-station sites (CRI, Panmure, Chisumbanje Exp, and Save Valley Exp) and eight Off-station sites (Matikwa, Shamva, Kuwirirana, Muzarabani, Wozhele, CC Mollen, Umguza & Chitekete). The sites represent the high cotton production zones, thus the Middleveld, and Lowveld. The sites are generally characterized by low average annual rainfall (<800mm) and high temperatures (>36oC). A general description of the sites encompassing longitude, altitude etc., is given in Table 1.

Table 1: Description of sites used in the multi-locational trials

Location	Code	Latitude	Longitude	Altitude	Av. Annual Rainfall	Max Temp^{0}C
Chitekete	E1	17°25' South	28° 56' East	914	450-500	45
Kadoma	E2	18°19' South	29° 53' East	1156	750-1000	38
Wozhele	E3	19°31' South	30°14' East	1345	650-790	37
Kuwirirana	E 4	21°15' South	30°48' East	1483	500-600	38
Matikwa	E5	20°48' South	32°14' East	300	450-500	40
Shamva	E6	17°32' South	31°71' East	1149	675-700	38
Muzarabani	E7	16° 23' South	31° 00' East	432	600-800	42
Panmure	E8	17°16' South	31°47' East	881	700-800	35
CC Mollen	E9	18°30' South	29° 13' East	1120	700-850	38
Save Valley	E10	21°29' South	32°51' East	466	450-500	41
Chisumbanje	E11	20°47' South	32°13' East	448	450-500	40
Umguza	E12	20° 03' South	28°34' East	1374	450-500	34

Source: Agritex planning branch, (1983): *Zimbabwe natural regions and farming areas boundaries*

Table 2: Description of Cotton genotypes used in the multi-locational trials

Genotype Name	Code	Type & breeding status	Origin
TN 96-05-9	G1	Experimental Line	Cotton Research Institute
912-05-1	G2	Experimental Line	Cotton Research Institute
S0-99-9	G3	Experimental Line	Cotton Research Institute
GN 96 (b)-05-8	G4	Experimental Line	Cotton Research Institute
917-05-7	G5	Experimental Line	Cotton Research Institute
932-05-3	G6	Experimental Line	Cotton Research Institute
938-05-3	G7	Experimental Line	Cotton Research Institute
SZ9314	G8	Commercial Check Variety	Cotton Research Institute
CRI-MS1	G9	Commercial Check Variety	Cotton Research Institute
CRI-MS2	G10	Commercial Check Variety	Cotton Research Institute

Experimental Description and Design

The experiment included ten genotypes thus seven test genotypes (TN 96-05-9, 912-05-1, S0-99-9, GN 96 (b)-05-8, 917-05-7, 932-05-3, and 938-05-3) and three check varieties (SZ9314, CRI-MS1 and CRI-MS2 (Table 3). All the test genotypes were developed by Cotton Research Institute using the Pedigree Breeding Method. The experiments were laid in a Randomized Complete Block Design replicated three times and each treatment was represented by plots measuring 32.4m2.

Trial/ Crop Management

Uniform crop management was done at all the sites and across all the various projects in the programs. Compound L (N: P: K: S = 5:18:10:8: {0.25B}) was banded at a rate of 250 kg per hectare to the planting furrows manually. Ammonium nitrate (34.5% N) was applied at a rate of 150 kg per hectare to the crop in the ninth week after crop emergence. The crop was thinned to one plant per station at 1 m inter-row by 0.3 m within a row to achieve the desired plant population of about 33 333 plants per hectare. Weeding using herbicides and hand hoeing was done to remove any weeds from the trials when necessary. Weeding at all sites was averagely done three times for the whole season. The following cotton pests were controlled using the generally recommended cotton pest scouting and control protocol developed at CRI in 1993 by the Cotton Research Institute entomology section (Annual Report, 1993). The pest control were: aphids (Aphis gosypii), red bollworms (Diaparposis castanea) and Heliothis bollworm (Helicorvepa amirgera). Pests were kept below the economic threshold levels following weekly scouting.

Field Data Collection and Analysis

Crop performance data from emergence to post-harvest (seed cotton yield, lint yield, seed weights, boll weights, seed weights, lint ratios, earliness index) was collected from all experiments. The genotype and genotype by environment (GGE) model were used to understand and structure interactions between genotypes and the environment. The model was used to identify the mega, representative and ideal environment for testing genotypes. The Principal Component Analysis (PCA) was used to explain patterns in the Genotype x Environment interaction. It was also used to identify superior genotypes and estimate the adaptability and stability of the genotypes across the sites in different years. GenStat 14th edition for windows was the statistical software for the partitioning of the variance components (general combined analysis of variance (ANOVA). Where significant difference was noted, treatment means were separated using Fischer's (1930) Least Significant Difference at $P \leq 0.05$ (Williams and Abdi, 2010).

Acknowledgments

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References

1. Agricultural Marketing Authority, 2019. Cotton production trend (average yield, production and area under production) for the period 2008 -2018
2. Agritex planning branch, (1983). Zimbabwe natural regions and farming areas boundaries as at 1 July 1983. The department of conservation and extension; Agritex
3. Butran A, Velasco P, Ordas A, Malvar RA (2004). Yield evaluation of maize cultivars across environments with different levels pink stem borer infestation. Crop Sci. 44:741-747.
4. Baafi, E. and O. Safo-Kantanka, 2008. Correlation and path coefficient analysis for earliness and yield traits in cotton (G. hirsutum L.). Asian J.
5. Cotton handbook, (2003). Cotton Growers Association. Cotton Training centre, Kadoma, Zimbabwe
6. Travella SR (2017). Cotton in Africa. Retrieved from http://www.vib.be/en/about-vib/Documents/vib_fact_CottonAfrican_EN_2017_0901_LR_FINAL.pdf
7. Williams Lynne J. and Herv`e Abdi, (2010). Fisher's Least Significant Difference (LSD) Test. In Neil Salkind (Ed.), Encyclopaedia of Research Design. Thousand Oaks, CA: Sage. 2010.
8. Yan WK, Hunt LA, Sheng Q, Szlavnics Z (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci. 40:597-605.
9. Yan WK (2001). GGE biplot-A windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron. J. 93:1111-1118
10. Yan W, Hunt LA (2002). Biplot Analysis of Multi-Environment Trial Data In. Quantitative Genetics, Genomics and Plant Breeding. Kang, M. S., (Ed.), CABI Publishing, New York. pp. 289-303.
11. Yan W, Kang MS (2003). GGE biplot analysis: A graphical tool for Breeders, Geneticist and Agronomist. CRC press LLC, Boca Roton, Florida, USA.

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Physical and Chemical Mutagenic Treatments for Cotton Improvement

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Abstract

In a genetic improvement program using the technique of induced mutations, to achieve a population of mutagenized cotton plants and to carry out a subsequent selection of mutants of interest with success, it is important to make previous dosimetry tests for the plant material to be improved. The objective was to evaluate the effect of different physical and chemical mutagenic agents applied in different doses on seeds of an elite variety of cotton "Guazuncho 3 INTA". The treatments were X-Ray (RX), Sodium Azide (SA), Ethyl Methane Sulfonate (EMS), and a combination of SA+RX, with four doses each. Germination percentage (GP), germination velocity index (GVI), survival (Sv), and morphological characteristics such as height, misshapen plants, and chlorophyll deficiencies were evaluated. The control treatment showed the highest yield for all the evaluated variables. EMS was the agent that produced the most damage, both in germination and growth, as well as in morphological variables. The RX did not affect GP, GVI, and Sv, but they produced significant damage to adult plants through malformations and chlorophyll deficiencies. SA delayed germination, but did not significantly affect the number of germinated seeds and caused slight morphological damage. The combined treatment was mainly influenced by SA and the behavior for different variables measured was similar to SA. For this cotton variety, intermediate doses are the best options to start a genetic improvement program with the technique of induced mutations.

***Keywords**: Cotton, Genetic improvement, Induced mutations, Dosimetry*

Background

In the Northern region of Santa Fe Argentina, cotton (Gossypium hirsutum L.) is a crop of great economic importance, making it the third province with the largest area planted in the country (Scarpin et al., 2019). It has regional importance due to the added value it generates and the labor it occupies, but it also has a share in foreign trade and a relationship with the industrial sector (Paytas and Ploschuk, 2013).

Currently, in Argentina, there are few cotton genotypes adapted to the different agroecological conditions, and it makes necessary to advance in local investigations that involve new genetic materials in response to the environment, its growth and development limitations, and adjusted agronomic management practices. For this reason, the development of improved varieties of cotton is one of the fundamental objectives prioritized by the cotton team of the EEA INTA Reconquista and demanded by the organization's cotton sector of the province of Santa Fe.

One of the techniques chosen by the research group to contribute to the improvement in cotton cultivation is the technique of induced mutations or mutagenesis (TIM), which has been used successfully on countless occasions to obtain new genetic variability in crop plants. (Hussain et al., 1982; Auld et al., 2000). Today there are thousands of registered cultivars in the world obtained by this methodology. Included in this list are crops of great economic importance, such as the main cereals, and oilseeds, as well as numerous species of vegetables and industrial crops.

Mutations are the primary origin of genetic variability, and therefore some control over their frequency can be considered a valuable tool for the improvement of cultivated plants. (Prina et al., 2010). For mutation induction, chemical, physical mutagens or a combination of both can be used.

Determining the most appropriate dose of mutagen is the most important factor, since determining the optimal dose increases the probability of producing useful mutants. (Aslam et al., 2013) Therefore, to achieve a successful mutagenized plant population that allows the selection of mutants of interest to be carried out, it is of the highest importance to perform previous dosimetry tests for the plant material with which you will work. The objective of the present work was to evaluate the effect of different physical and chemical mutagenic agents applied in different doses on seeds of an elite variety of cotton "Guazuncho 3 INTA".

Results and Discussions

Results showed that 15 days after planting there were differences in GP between the different treatments. As a general rule, the control had the highest GP to a greater or lesser extent compared to all the treatments and their respective doses. The greatest effect on germination was observed in the treatment with EMS, producing a reduction of germinated seedlings more abruptly than in the other treatments as the dose was increased. This result was expected because EMS produces a delay in germination (Prina, 1989).

Figure 1 shows germination percentage (GP), that is, seedlings that have emerged with fully expanded cotyledons, for each of the treatments with their respective doses. To the RX treatment, there were no significant differences in the percentage of germination between the different doses, with a relatively low coefficient of determination (R^2 = 0.16). This effect is to be expected since ionizing radiation only affects cell division and not the elongation of the predetermined cells, therefore it does not affect germination so much (Prina, 1989). In treatments with SA, GP was similar between the different doses, and in some cases (2 and 4 mM) reached the same level as the control. Something very similar was observed in the combined treatments; GP in the different doses was similar and in turn, the doses 2 mM + 100 Gy and 4 mM + 100 Gy were almost like control, tending to decrease the GP as the dose increased with an R^2=0.99.

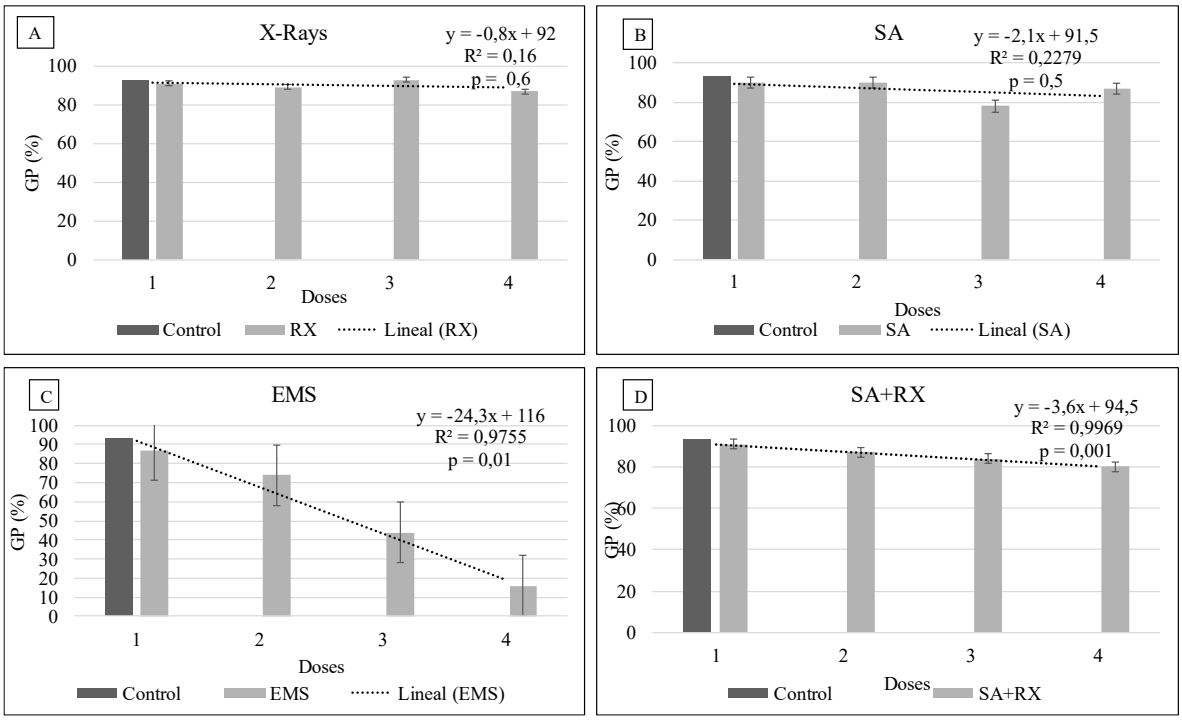

Figure 1 Germination percentage (GP) 15 days after sowing for each mutagenic agent (RX, SA, EMS, SA + RX) with their respective doses: A) RX (1) 200, 2) 300, 3) 400, 4) 500 gray), B) SA (1) 2, 2) 4, 3) 8, 4) 16 mM), C) EMS (1) 0.1, 2) 0.2, 3) 0.3, 4) 0.4%) and D) SA + RX (1) 2, 2) 4, 3) 8, 4) 16 mM + 100Gy, adding to each graph the control treatment.

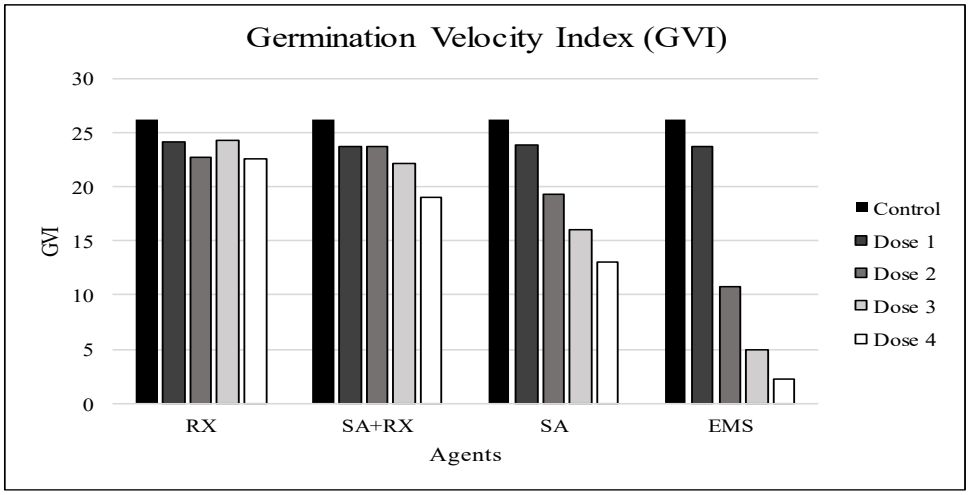

Figure 2 Germination Velocity Index (GVI) for each treatment with their respective doses

Another variable measured was the germination velocity index (GVI) which can be seen in Figure 2. These indices did not show a significant difference between doses of RX treatment, although it can be seen that the indices of all doses were below the control. EMS treatment showed a clear tendency to decrease the GVI as the dose was increased. This could be mainly influenced by the number of plants germinated, as shown in Figure 1 because the main effect of EMS is on GP. In the SA treatments, it could also be observed how the GVI decreased as the dose of the mutagen increased. Comparing this analysis with figure 1 of GP, we can define that the SA treatment did not affect the number of sprouted plants; however, this germination was delayed by the mutagenic agent to a greater degree the higher the dose applied. In the combined treatments a similar behavior to the SA treatment could be appreciated, but with less effect; establishing that SA is the agent that is acting mainly on GVI.

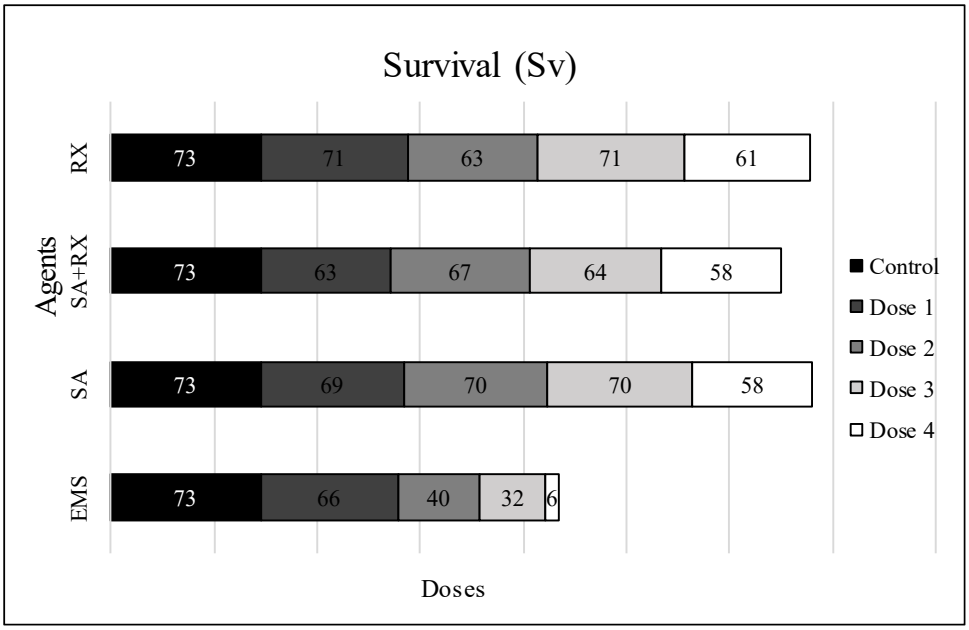

***Figure 3** Plant survival, measured up to the second leaf fully expanded on the total of germinated plants for each of the doses of the different mutagenic agents.*

Figure 3 shows the survival results (Sv) of the plants that have managed to germinate. Treatment that caused the most damage to the survival rate was EMS and a dose-dependent decrease in survival was observed. RX treatments had the least effect on survival rate relative to the control. Whereas SA and SA+RX treatments have shown a similar response, with a small decrease in the percentage of live plants in the combination treatment. Table 1 shows that the greatest significant effects were observed

with the highest dose of EMS; Furthermore, it shows that the treatment with 4 mM SA also produced a significant difference.

Table 1 Average heights of each dose for each treatment, their differences and their significance.

Agents	Doses	Height (cm)		
Control		62.47	ns	
RX	200 Gy	61.93	ns	P value 0.15
	300 Gy	59.4	ns	LSD 9.59
	400 Gy	68.14	ns	CV 14.77
	500 Gy	63.67	ns	
SA	2mM	63.13	ns	
	4mM	66.73	*	P value 0.03
	8mM	64.19	ns	LSD 5.57
	16mM	60.13	ns	CV 8.82
EMS	0.10%	60.4	ns	
	0.20%	60.54	ns	P value 0.0001
	0.30%	50.23	**	LSD 7.98
	0.40%	40.67	***	CV 16.44
SA+RX	2mM+100 Gy	60.07	ns	
	4mM+100 Gy	66.27	ns	P value 0.08
	8mM + 100 Gy	63.73	ns	LSD 6.02
	16mM + 100 Gy	62.75	ns	CV 9.4

ns = not significant, * = p <0.05; ** = p <0.01; *** = p <0.001. LSD = Minimal significant difference.
CV = coefficient of variation.

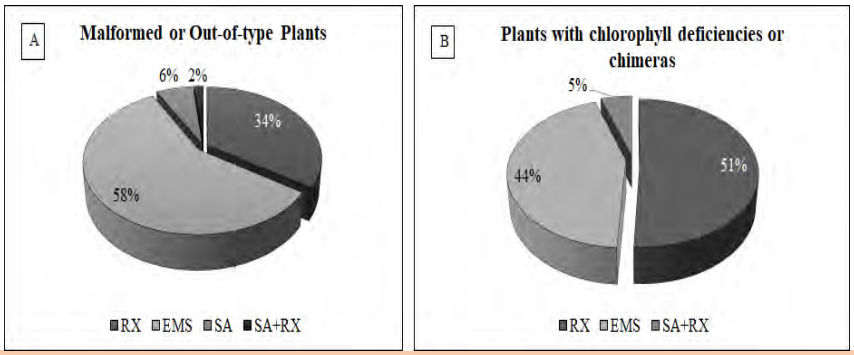

Figure 4 Percentage of malformed (A) and chlorophyll-deficient (B) plants over the total plants for each mutagenic agent.

Figure 4 shows the number of plants in which malformations and chlorophyll deficiencies or chimeras were observed, according to the treatments. It was observed that treatments with EMS and RX were the ones that originated the highest number of malformed plants and with the presence of chimeras. These results showed that intermediate doses were the optimal doses; because if we increase the dose considerably we will obtain a higher rate of mutations, but at the same time the survival rate is low and we would not obtain a sufficient number of individuals in the population to carry out an efficient selection. On the other hand, a decrease in the dose would produce a greater number of individuals in the population, but in turn a lower rate of mutations.

Conclusion

Considering the genetic material evaluated, (Gossypium hirsutum L.) variety Guazuncho 3 INTA, we can conclude that the control treatment showed the highest values for all the evaluated variables. For this test, it was concluded that the mutagenic agent that produced more damage in germination and growth variables, as well as in morphological variables was EMS. The RX does not affect germination (GP, GVI), but it was possible to observe the presence of damage in adult plants through malformations and chlorophyll deficiencies. This result indicates that ionizing radiation has no effect on cell elongation, but it does have an effect on cell division. It is concluded that the combined treatment was mainly influenced by SA and that its behavior in the different variables was similar to SA in the different doses. Furthermore, it can be said that SA delays germination at first, but does not significantly affect the seeds germinated number. It was also observed that it affected very slightly the morphological variables. It is

also important to note that the mutagenic effect of SA depends largely on the pH of the treatment solution (Gruszka et al., 2012). Therefore, the effect of SA could be evaluated in next studies using a phosphate buffer with a lower pH. In a genetic improvement program using the mutagenesis technique, to use a mutagen and induce the desired variability, the determination of the dose to be used is undoubtedly the most important factor in creating a population of mutagenized cotton plants and a subsequent effective selection.

Materials and Methods

Plant material: Cotton seeds of the variety Guazuncho 3 cv elite from INTA were collected in the EEA Reconquista. This variety has good yield and quality characteristics, and is early, with a high percentage of ginning, intermediate vigor, low number of vegetative branches, medium lobed leaves, and low tolerance to abiotic stress. (Bonacic Kresic et al., 2004)

Mutagenic treatments: The treatments were carried out at the "Ewald A. Favret" Genetics Institute (IGEAF) of the CNIA-INTA with the group of Induced Mutations in Cultivated Plants. Physical and chemical mutagens and combined treatments of both were used over a total of 100 seeds, for each agent with 4 different doses. The treatments were the following:

Agent	X-Rays (RX)				Sodium Azide (SA)			
	1	2	3	4	1	2	3	4
	200 Gy	300 Gy	400 Gy	500 Gy	2mM	4mM	8mM	16mM
Agent	Ethyl methane sulfonate (EMS)				Combined (SA+RX)			
	1	2	3	4	1	2	3	4
Dose	0.10%	0.20%	0.30%	0.40%	2mM + 100 Gy	4mM + 100 Gy	8mM + 100 Gy	16mM + 100 Gy

For the treatment of RX, dry seeds were irradiated with an X-Rays generator equipment, brand/model: PHILIPS MG 160, of 160 kV max. and 30 mA max. For SA the different concentrations were prepared in a phosphate buffer at pH 6. Then, 100 seeds were added to each of the solutions and placed in an agitator at 18 °C and 165 rpm for 18 hours. The seeds were removed, successively washed and rinsed and left to dry on absorbent paper to remove surface moisture. For EMS treatments, the reagent was diluted in water at different concentrations. 100 seeds were placed in each dilution and shaken for 18 hours at 18 °C and 165 rpm. The seeds were removed, successively washed and rinsed, and left to dry on absorbent paper to remove surface moisture. For the combined treatments the SA treatments were first carried out in the same way as the procedure described above and then the same dried seeds were subjected to a 100 Gy X-ray dose. Finally, 100 seeds of the same variety were used without being subjected to any kind of agent as a control treatment.

Sowing: The seeds were sown in the EEA INTA Reconquista in trays of 0.5 x 0.35 m with inert substrate previously disinfected in the autoclave. The trays were placed in a growth chamber at 30° C and 10 days after sowing, 15 representative plants were taken at random and transplanted into 5-liter pots (2.5 kg soil/pot) under semi-controlled conditions in a greenhouse. The pots were arranged in a completely randomized design.

Dosimetry evaluations of the different treatments in the mutagenized generation 1 (M1)
Germination trays: for each treatment the number of plants that emerged was evaluated 15 days after sowing, to determine germination percentage (GP). Germination velocity index (GVI) was also determined and calculated using the equation described by Nakagawa (1994). Finally, survival (Sv) was evaluated through the number of plants that emerged and continued their development until the second leaf was completely expanded.

Pots: In the plants that were taken to pots we evaluated morphological variables such as plants height at the end of the phenological cycle of plants, and also malformed or out-of-type plants, and the presence of chimeras, which are variables that indicate the damage of the mutagen and/or effect of the treatment. To observe differences between treatments and doses in the height data, an ANAVA was carried out and for comparison of average values, the Tukey test was used through the software InfoStat (Di Rienzo et al., 2011).

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References

1. Aslam, U., Ali Khan, A., Naseer Cheema, H. M., Imtiaz, F., & Malik, W. (2013). Kill curve analysis and response of ethyl methanesulfonate and γ-rays in diploid and tetraploid cotton. International Journal of Agriculture & Biology, 15(1).
2. Auld, D. L., Bechere, E., Ethridge, M. D., Becker, W. D., Hequet, E., & Cantrell, R. G. (2000). Registration of TTU 202-1107-B and TTU 271-2155-C mutant germplasm lines of upland cotton with improved fiber quality. Crop science, 40(6), 1835-1835.
3. Bonacic Kresic, I., Ibalo, S., Erazzu, L. E., Royo, O. M., Fariña Nuñez, J. R., Ortiz, R., Poisson J. A., Montenegro, A. (2004). GUAZUNCHO 3 INTA. https://inta.gob.ar/variedades/guazuncho-3-inta
4. Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., González, L., Tablada, M., & Robledo, Y. C. (2011). InfoStat versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www. infostat. com. ar, 8, 195-199.
5. Gruszka, D., Szarejko, I., & Maluszynski, M. (2012). Sodium azide as a mutagen. Plant mutation breeding and biotechnology, 159-166.
6. Hussein, H. A. S., Al-Enani, A., & El-Moghazi, M. (1982). Histological and morphological characteristics of a glandless cotton mutant induced with sodium azide. Egyptian journal of genetics and cytology.
7. Nakagawa, J. (1994). Seed vigour tests based on seedling characteristics. Vieira, RD; Carvalho, NM Vigor tests on seeds. FUNEP, Jaboticabal, SP, Brazil. (in Portuguese), 49-85.
8. Paytas, M. J., & Ploschuk, E. L. (2013). Cultivos Industriales. Algodón.
9. Prina, A. R. (1989). Consideraciones sobre la aplicación eficiente de la mutagénesis inducida en fitomejoramiento. Mendeliana, 9(1), 27.
10. Prina, A., Landau, A., Pacheco, M. G., & Hopp, E. H. (2010). Mutagénesis, TILLING y EcoTILLING.Biotecnología y Mejoramiento vegetal II.
11. Scarpín, G., Dileo, P., Winkler, H.M., Paytas, M. (2019). Evaluación de variedades comerciales y pre-comerciales. Publicación anual APPA, Campaña 2018/19.

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QTL Mapping of Fiber Quality and Yield-Related Traits in an Intra-Specific Upland Cotton Using GBS

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Abstract

Fiber quality and yield improvement are crucial for cotton domestication and breeding. With the transformation in spinning techniques and multiplicity needs, the development of cotton fiber quality and yield is of great importance. A genetic map of 5178 Single Nucleotide Polymorphism (SNP) markers were generated using 277 F2:3 population, from an intra-specific cross between two upland cotton accessions, CCRI35 a high fiber quality as female and Nan Dan Ba Di Da Hua (NH), with good yield properties as male parent. The map spanned 4768.098 cM with an average distance of 0.92 cM. A total of 110 Quantitative Traits Loci (QTLs) were identified for 11 traits, but only 30 QTLs were consistent in at least two environments. The highest percentage of phenotypic variance explained by a single QTL was 15.45%. Two major cluster regions were found, cluster 1 (chromosome17-D03) and cluster 2 (chromosome26-D12). Five candidate genes were identified in the two QTL cluster regions. Based on GO functional annotation, all the genes were highly correlated with fiber development, with functions such as protein kinase and phosphorylation. The five genes were associated with various fiber traits as follows: Gh_D03G0889 linked to qFM-D03_cb, Gh_D12G0093, Gh_D12G0410, Gh_D12G0435 associated with qFS-D12_cb and Gh_D12G0969 linked to qFY-D12_cb. Further structural annotation and fine mapping is needed to determine the specific role played by the five identified genes in fiber quality and yield-related pathway.

***Keywords**: QTL; fiber quality; upland cotton; intra-specific; yield traits; GO; GBS*

Background

Cotton is one of the most important natural fibers and oil crops in the world. Its annual global market value was estimated to be $630.6 billion in 2011 (Wang et al., 2012). Cotton fiber is the primary raw material in the textile industry (Liang et al., 2013). The advancements in techniques and diversified methods of spinning have made cotton fiber quality and related yield traits of paramount significance in the breeding and production of cotton (Kohel et al. 2001). Fiber quality is determined by several factors such as fiber strength, fiber length, fiber micronaire, and fiber color, while yield is mainly determined by lint quantity (Lokhande and Reddy, 2014). However, lint yield and fiber quality are negatively correlated (Zhang et al, 2003; Shen et al., 2007), which has long been a critical issue in cotton breeding (Yu et al, 2013). Recently, Shang et al. (2015) identified 20 QTLs for fiber quality-related traits, however, four QTLs were validated. Moreover, five fiber quality traits were linked to 59 QTLs in an earlier report across five environments (Tan et al., 2014). So far, few numbers of QTLs have been employed in marker-assisted selection (MAS) which is one of the enhanced breeding methods (Collard and Mackill, 2008).

In all the identified and documented QTLs related to fiber and yield traits, most of them have been localized in a wide range of genomic regions and are often not stable across a wide genetic background (Jamshed et al., 2016). Therefore, a dense interspecific map was generated, which included 2316 loci on the 26 cotton chromosomes to reduce and enhance accuracy in the mapping (Yu et al., 2011). However, these maps developed from interspecific hybridization have limited use in breeding due to limitations in controlling defective genes (Liang et al., 2013; Zhang et al., 2003).

To overcome the inefficiency of maps developed from interspecific hybridization, it is therefore imperative to generate molecular maps based on an intraspecific population due to their ability to reduce the wide genome gap (Liang et al., 2013). The employment of molecular marker techniques in cotton breeding through MAS and more advanced approaches such as genomic selection (GS) (Meuwissen et al., 2001) would help break the bottleneck and, in turn, development of genetically advantaged genotypes. A small part of a DNA can be archived by reducing the complexity of the genome by restriction enzymes, such as genotyping-by-sequencing (GBS), the reduced-representation libraries (RRLs), restriction-site-associated DNA sequencing (RAD-seq), and next-generation sequencing (NGS) (Davey et al., 2011).

The next-generation sequencing (NGS) of crop plant genomes has transformed the field of plant breeding. In the recent past, a lot of data generated has facilitated the discovery and use of a large scale of single nucleotide polymorphisms (SNPs) in different genomes (Huang et al., 2012; Xu et al., 2012). One of which was, genotype by sequencing (GBS), which holds the potential to narrow down the genotyping gap between references of large interest and mapping or breeding populations of local or specific interest (Spindel et al., 2013). GBS protocol techniques with their sample multiplicity have kept molecular research costs low while their output has diverse applications in many research areas, ranging from gene discovery to genomic-assisted breeding (Thomson, et al., 2012). The ability to generate large amounts of unbiased markers in an inexpensive method has enabled GBS to become a more attractive approach to genotype and to construct high-resolution genetic maps, genomic selection, and facilitated QTL mapping (Poland and Rife, 2012).
Mapping of QTLs has become an important technique to facilitate quantitative trait research and has been largely used in crops to map several beneficial agronomic traits including fiber quality and related yield traits.

In this investigation, a genetic map of 5178 SNP markers was generated using a 277 F2:3 intraspecific population developed from two tetraploid upland cotton accessions, mainly cultivated in China. CCRI35 with good fiber quality as a female parent and Nan Dan Ba Di Da Hua (NH) is known for high yield fiber as a male parent. The map generated was employed to analyze QTLs related to fiber quality and yield-related traits using a QTL cartographer (Wang and Zeng, 2007). This study aimed to identify QTLs related to fiber quality, and yield component traits, localize their position within the cotton genome, and identify the genes tightly linked to those QTLs. The findings of this research could provide valuable insights for breeders to develop cultivars with both traits, yield and quality fiber and enhance selection in cotton breeding.

Results

Phenotypic Variation between the Two Parents

In the determination of phenotypic variation of the 11 measured traits, Boll weight (BW), lint percentage (LP), fiber reflectance (FR), fiber yellowness (FY), spinning consistency index (SCI), and mature index (MI) were not used in the analysis of the phenotypic variation between the parental lines due to the huge missing data throughout the phenotyping periods. The five traits used were fiber length (FL), fiber uniformity (FU), fiber strength (FS), fiber micronaire (FM), and fiber elongation (FE). FL, FU, and FS showed significant differences between the parental lines. All traits were higher in CCRI35 than in NH with an exemption of FE which was higher in NH. In addition, no significant difference was noted between the two parental lines for fiber micronaire (FM) and fiber elongation (FE), Figure 1. However, there was a wide range of phenotypic variation among the $F_{2:3}$ population, for all the measured traits; BW, LP, FL, FU, FM, FS, FE, FR, FY, SCI, and MI. Across the three environments, 2014, 2015, and 2016 all the traits showed normal segregation with normalized distribution patterns (Figure 2).

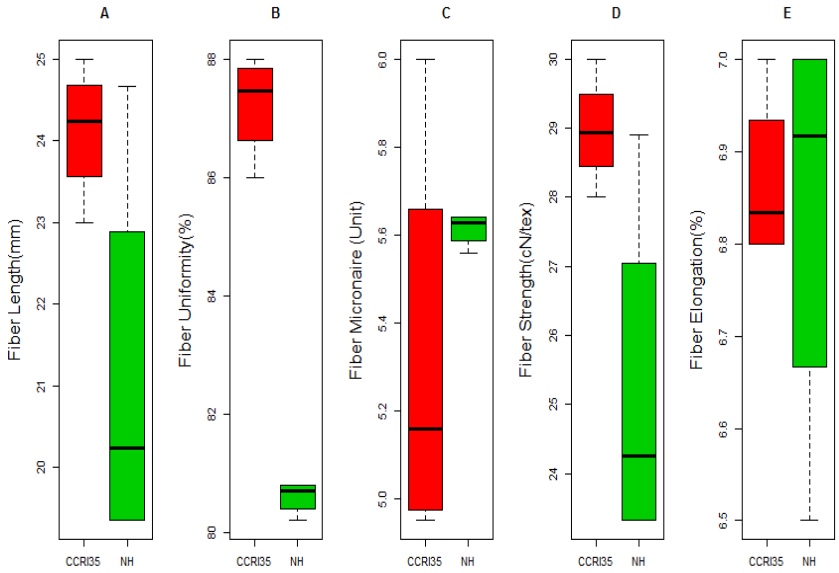

Figure 1. Phenotypic analysis of the two parents for fiber quality and yield-related traits; (A) Fiber Length (mm); (B) Fiber Uniformity (%); (C) Fiber Micronaire (Unit), (D) Fiber Strength (cN/tex); (E) Fiber Elongation (%). CCRI35: female parent, NH: male parent.

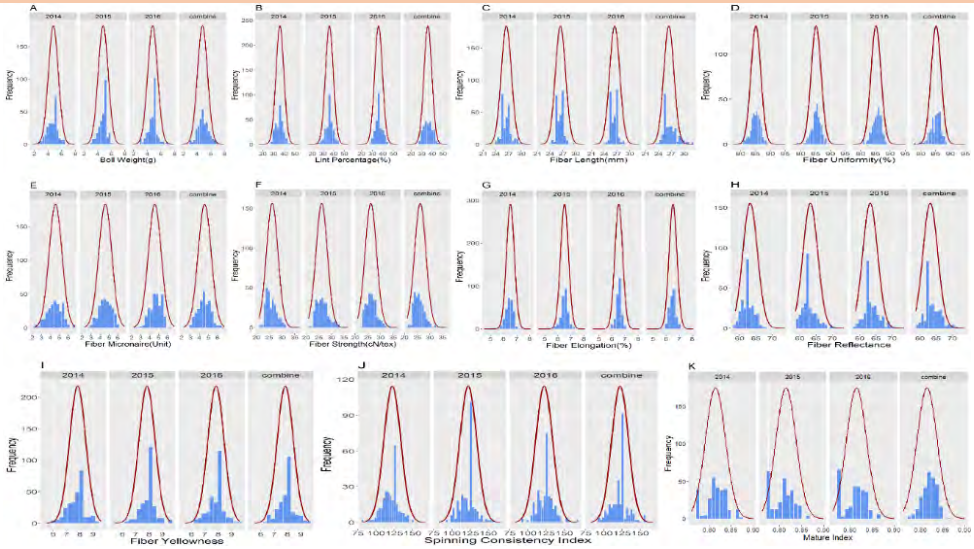

Figure 2. Frequency distribution of the 11 traits for fiber quality traits in F2:3, (A) Boll Weight (g); (B) Lint Percentage (%); (C) Fiber Length (mm); (D) Fiber Uniformity (%); (E) Fiber Micronaire (Unit); (F) Fiber Strength (cN/tex); (G) Fiber Elongation (%); (H) Fiber Reflectance; (I) Fiber Yellowness; (J) Spinning Consistency Index; (K) Mature Index.

Correlation Analysis

To determine the correlations among different traits, a Pearson's correlation coefficient on yield-related and fiber quality traits was done using the "Performance Analytics" package with the Chart correlation function in R software version 3.4.2 (Team, 2008). Significant and positive correlations were noted between BW with FL, FU, FM, FS, FE, FR, and MI; LP with FM and MI; FL with FU, FS, FE, FR, and SCI; FU with FS, FE, and SCI; FM with MI; FS with FE and SCI; FE with SCI and finally FR with SCI. Negative correlations were observed between LP with FR and SCI; FL with FM; FM with FR and SCI; FY with SCI and finally SCI with MI (Figure 3). However, no significant correlation was noted between the other traits.

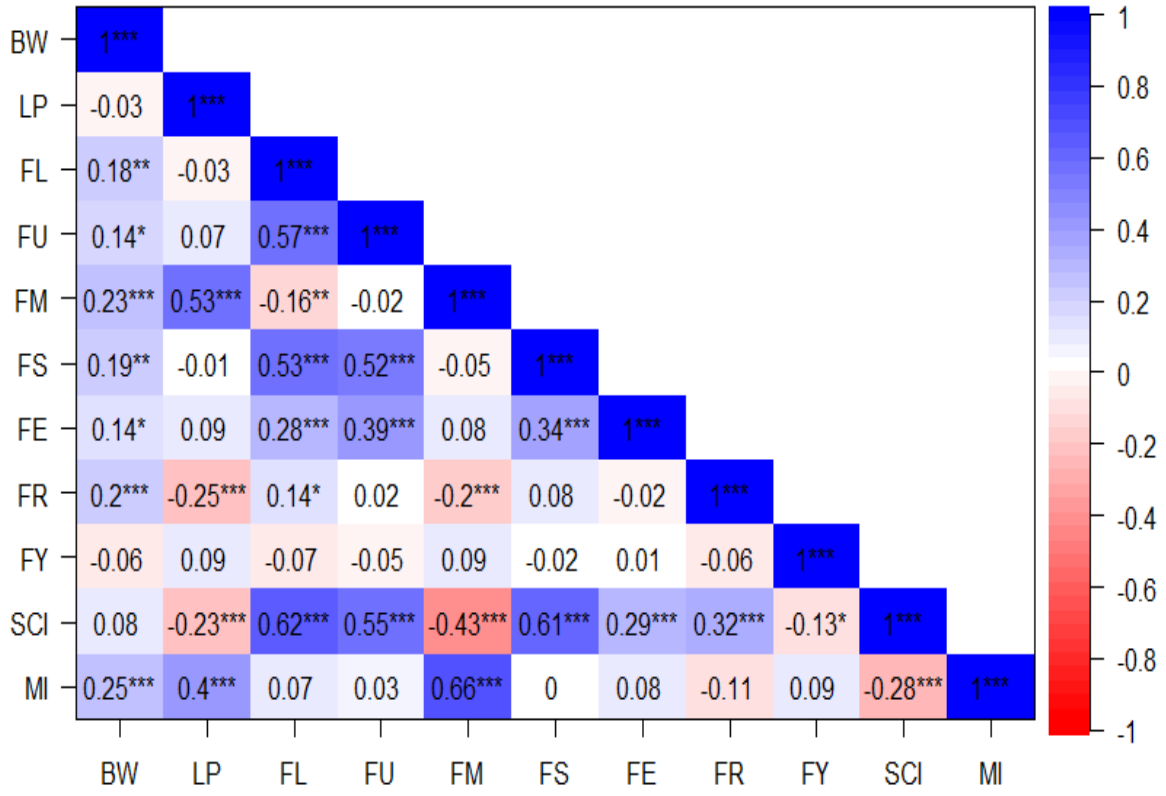

*Figure 3. Pearson's correlation of the 11 traits for the $F_{2:3}$ in three environments. *, **, ***: significant levels of 0.5, 0.01 and 0.001 respectively. BW: Boll weight; LP: lint percentage; FL: fiber length; FU: fiber uniformity; FM: fiber micronaire; FS: fiber strength; FE: fiber elongation; FR: fiber reflectance; FY: fiber yellowness; SCI: spinning consistency index; MI: mature index. For the units, see in Figures 1 and 2.*

ANOVA, Broad Sense Heritability and Phenotypic Analysis of Fiber Quality for the two Parents and the $F_{2:3}$ Population

The ANOVA result revealed significant differences between the genotypes, environment, and their interactions for all the traits (Table 1). The broad sense heritability was much higher for the fiber quality traits as opposed to yield-related traits. The highest broad sense heritability was observed with fiber micronaire (FM), with 92.4% while the lowest broad sense heritability was observed in fiber elongation (FE) with 61.8%.

Table 1. ANOVA, broad sense heritability and phenotypic analysis of fiber quality and yield related traits for the two parents and the $F_{2:3}$ population.

Trait	Source	DF	SS	MS	F	Pr > F	H_b (%)	P1	P2	P1 − P2	$F_{2:3}$					
											Mean	SD	Max	Min	Skew	Kurt
BW	e	3	327.4	109.1	1.9×10^{10}	<0.0001	67.5	-	-		4.77	0.73	7.9	1.8	−0.16	0.94
	g	276	728.6	2.6	4.59×10^{08}	<0.0001										
	g*e	828	710.5	0.9	1.49×10^{08}	<0.0001										
LP	e	3	1856.7	619	9.5×10^{10}	<0.0001	82.1	-	-		35.97	3.71	54	16.08	0.09	1.2
	g	276	28,244	102.3	1.57×10^{10}	<0.0001										
	g*e	828	15,200.7	18.4	2.82×10^{09}	<0.0001										
FL	e	3	1561.4	521	9.23×10^{24}	<0.0001	63.6	24.12	21.12	3	26.46	1.2	32.2	21.55	0.94	0.55
	g	276	1530.1	5.5	9.83×10^{22}	<0.0001										
	g*e	828	1670.7	2	3.58×10^{22}	<0.0001										
FU	e	3	1200.6	400	9×10^{23}	<0.0001	77.1	87.23	80.6	6.63	85.14	1.69	89.3	77.8	−0.75	0.12
	g	276	4844.2	17.6	3.95×10^{22}	<0.0001										
	g*e	828	3321.3	4	9.02×10^{21}	<0.0001										
FM	e	3	12.700	4	2.19×10^{25}	<0.0001	92.4	5.61	5.32	0.29	4.57	0.72	6.75	2.2	−0.19	-0.15
	g	276	1406.5	5.1	2.63×10^{25}	<0.0001										
	g*e	828	320.3	0.4	1.99×10^{24}	<0.0001										
FS	e	3	2038.7	680	3.37×10^{25}	<0.0001	76.8	28.97	25.18	3.79	26.19	2.27	36.5	21	0.76	0.75
	g	276	8851.8	32.1	1.59×10^{24}	<0.0001										
	g*e	828	6155.4	7.4	3.69×10^{23}	<0.0001										
FE	e	3	10.800	4	2.15×10^{24}	<0.0001	61.8	6.83	6.87	-0.04	6.51	0.3	8.1	4.5	−1.02	0.02
	g	276	137.1	0.5	2.96×10^{23}	<0.0001										
	g*e	828	157.5	0.2	1.13×10^{23}	<0.0001										
FR	e	3	335.500	112	5.58×10^{22}	<0.0001	84.8	-	-		63.23	2.27	73.6	58.7	0.9	1.22
	g	276	11,577.9	41.9	2.09×10^{22}	<0.0001										
	g*e	828	5282.3	6.4	3.18×10^{21}	<0.0001										
FY	e	3	38.2	12.7	8.03×10^{23}	<0.0001	86.3	-	-		7.83	0.61	9.6	5.5	−0.32	1.19
	g	276	845.6	3.1	1.93×10^{23}	<0.0001										
	g*e	828	348	0.4	2.65×10^{22}	<0.0001										
SCI	e	3	24,493.6	8164.5	1.39×10^{25}	<0.0001	86.6	-	-		122.26	11.57	167	82	0.09	0.97
	g	276	299,967	1086.8	1.85×10^{24}	<0.0001										
	g*e	828	120,302.4	145.3	2.48×10^{23}	<0.0001										
MI	e	3	0.5	0.2	6.73×10^{25}	<0.0001	66.7	-	-		0.81	0.02	0.87	0.75	−0.19	−0.79
	g	276	0.7	0	1.18×10^{24}	<0.0001										
	g*e	828	0.5	0	2.77×10^{23}	<0.0001										

P1 = CCRI35: parental female with good fiber quality traits; P2 = NH: good yield fiber; DF: degree of freedom; SS: sum square; MS: mean square; F: F value; H_b (%): Broad sense heritability percentage; SD: Standard deviation; Min: Minimum; Max: Maximum; Skew: Skewness; Kurt: Kurtosis, (<0.0001): means significant at level $p < 0.001$.

GBS Genotyping, SNP Detection, and Annotation

The genotypic data for the entire population was developed by use of the genotyping by sequencing (GBS) technique. Fifteen (15) individuals of each of the parents were sequenced and mapped on to the reference genome, which we obtained from the cotton research institute (available online: http://mascotton.njau.edu.cn). We obtained a total of 20,542,731 and 20,244,825 reads for CCRI35 and NH, respectively. An average of 80,372 and 112,128 SNPs were eventually identified for the female parent (CCRI35) and the male parent (NH), respectively, with an enzyme digestion efficiency of 99%. In genotyping the $F_{2:3}$ population, the enzyme efficiency was slightly lower compared to its efficiency in the parents, with an efficiency of 98.9%. The overall mapped reads for the population and the two parents were 1,507,193,217, with an average of 4,909,424.16 mapped reads per individual which correspond to nearly 180.889 Gb of clean bases. The clean reads obtained were equivalent to 80.42-fold haploid genome coverage of raw paired-end Illumina reads by sequencing whole genome shotgun (WGS) libraries of homozygous cv. “TM-1” compared to Li et al. (2015) in their study which generated a total of 445.7 Gb of clean reads translating to about 181-fold haploid genome coverage of raw paired-end Illumina reads by sequencing whole genome shotgun (WGS) libraries of homozygous cv. “TM-1” with fragment lengths ranging from 250 bp to 40 kb. The average GC content of the sequences was 38.25%, with a Q20 score of 94.66%. The parental lines were genotypes such as AC and AA, in which the female parent CCRI35 was heterozygous while the male parent (NH) was homozygous. The total resulting SNPs markers were 103,381 markers which were used to carry out further analysis. We assessed the distribution of the alleles across the $F_{2:3}$ population, and those markers which had a coverage threshold of 75% were filtered out, eventually, 34,090 markers were used. Markers with significant distortion ($p < 0.001$) were filtered and 6405 markers were retained to determine bin markers.

Construction of the Linkage Maps

In the construction of the linkage groups, we used 6405 markers (Table S1), and phenotypic data of the $F_{2:3}$ population developed from an intra-specific cross of two tetraploid upland cottons were utilized for developing the intra-specific linkage map. A total of 5178 GBS markers were used for mapping the $F_{2:3}$ population, all the distorted markers were filtered out, and the linkage groups were generated by the use of Join Map 4.0 (Stam et al., 1995). Twenty-six (26) LGs were generated from 5178 markers (Figures 4A and S1, Tables 2 and S2). Markers in linkage groups were ordered, rippled, and re-ordered according to pairwise recombination fractions, LOD scores (Logarithm of Odds), and linkage group length (Figure 4B). The 26 LGs were designated as A01 to A13 for A_t sub-genome and D01 to D13 for D_t sub-genome. The map generated had a map distance of 4768.098 cM, higher than the most current upland cotton linkage map with a map distance of 4450 cM (Rong et al., 2004). The average distance between adjacent markers was 0.92 cM, and the marker distances were narrowed in the map generated compared to earlier maps with 1.7 cM between adjacent markers (Rong et al., 2004). The At sub-genome spanned 2611.43 cM, with a total of 3313 markers in the 13 linkage groups, with an average distance of 0.79 cM, while in D_t sub-genome, thirteen linkage groups comprised 1865 markers spanning a distance of 2156.67 cM, with an average of 1.156 cM. The maximum gap between adjacent loci was 26.598 cM and 30.082 cM in A_t and D_t respectively, affirming the genome lengths between A_t and D_t (Rong et al., 2004) (Table 2). Chromosomes; A02, D02, A01, A05, A03, D01, and A10 exhibited higher marker loci with higher recombination frequency compared to the rest of the chromosomes such as D06 and D13 (Figure 4A, B). The chromosome with the highest marker loci was chromosome A02, 705 loci with a map distance of 346.314 cM and an average distance of 0.49 cM, while the lowest marker loci was detected in chromosome D06 with only 16 markers, and a total length of 79.084 cM (Figure 4B).

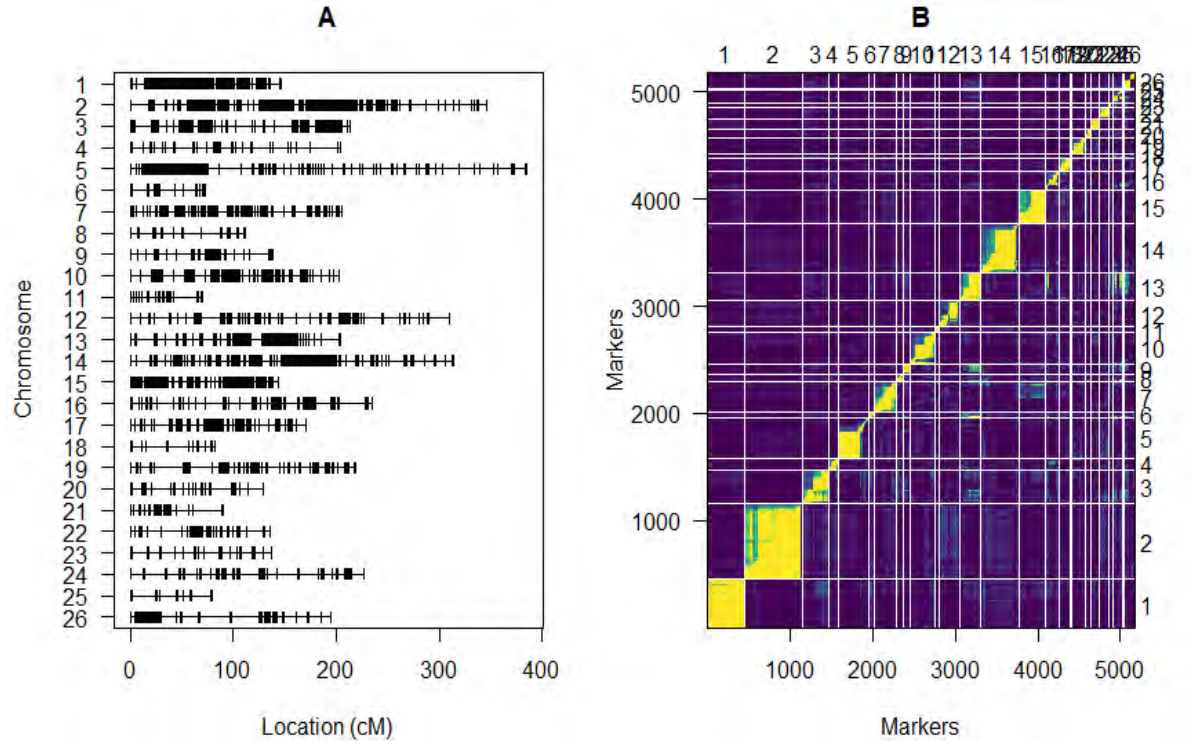

Figure 4. (A) Genetic linkage map constructed using the $F_{2:3}$ Population; (B) Plot of estimated recombination fractions of all markers used in the $F_{2:3}$ population. X and Y axis are the markers and Z is the linkage groups (LGs).

Table 2. Genomic distributions of SNPs markers.

Group	Marker Number	Map Length (cM)	Av Distance (cM)	Max Gap (cM)	<10 cM	>10 cM	Ratio
A01(c1)	448	146.704	0.33	8.505	447	0	1
A02(c2)	705	346.314	0.49	17.848	699	5	0.99
A03(c3)	323	213.937	0.66	17.145	319	3	0.99
A04(c4)	106	203.891	1.92	26.598	99	6	0.93
A05(c5)	378	385.092	1.02	21.198	365	12	0.97
A06(c6)	58	73.063	1.26	15.032	54	3	0.93
A07(c7)	279	205.892	0.74	11.622	276	2	0.99
A08(c8)	69	112.137	1.63	18.894	65	3	0.94
A09(c9)	98	138.501	1.41	19.234	95	2	0.97
A10(c10)	292	202.134	0.69	10.551	287	4	0.98
A11(c11)	51	70.548	1.38	23.241	49	1	0.96
A12(c12)	244	309.608	1.27	19.593	236	7	0.97
A13(c13)	262	203.61	0.78	17.425	256	5	0.98
Subtotal A_t	3313	2611.43	0.79	26.598	3247	53	0.98
D01(c15)	319	144.092	0.45	6.351	318	0	1
D02(c14)	454	313.268	0.69	14.541	450	3	0.99
D03(c17)	133	170.555	1.28	14.993	131	1	0.98
D04(c22)	114	136.228	1.19	20.275	110	3	0.96
D05(c19)	153	218.788	1.43	27.062	148	4	0.97
D06(c25)	16	79.084	4.94	22.389	12	3	0.75
D07(c16)	169	235.366	1.39	26.041	161	7	0.95
D08(c24)	118	226.688	1.92	20.878	109	8	0.92
D09(c23)	40	136.744	3.42	14.48	33	6	0.83
D10(c20)	80	129.051	1.61	20.539	76	3	0.95

D11(c21)	98	89.782	0.92	27.564	95	2	0.97
D12(c26)	143	194.735	1.36	30.082	135	7	0.94
D13(c18)	28	82.286	2.94	20.917	25	2	0.89
Subtotal D_t	1865	2156.67	1.156	30.082	1803	49	0.97
TOTAL (A_t + D_t)	5178	4768.1	0.92	30.082	5050	102	0.98

Ratio: number of markers less than (<) 10 cM divided by the total number of markers within the chromosome. Av: Average; Max: Maximum.

Identification of Consistent and Clustering QTLs for Yield Related and Fiber Quality Traits

Thirty (30) QTLs were consistent among all the 110 QTLs identified for 11 traits in at least two environments (Table 3 and Figure S1). The 30 consistent QTLs were located on 16 chromosomes; A02 (2), A03 (1), A05 (2), A09 (3), A10 (2), A12 (1), D01 (1), D02 (1), D03 (4), D04 (1), D05 (2), D08 (2), D10 (2), D11 (1), D12 (4), and D13 (1). The distribution of the QTLs within the identified chromosomes, exhibited multiple positions as illustrated in Figure S1 and Tables S2 and 3. Of the 30 detected QTLs, 11 were localized on A_t sub-genome while the remaining 19 were mapped on the D_t sub-genome. The contributions of the parents toward the QTLs: 19 QTLs were linked to the good fiber quality parent (CCRI35) while only 11 QTLs were contributed by the high yield fiber parent (NH). Only16 chromosomes out of 26 were found to harbor consistent QTLs for ten traits except MI (Mature Index) for yield-related and fiber quality (Tables S2 and 3).

Four types of gene actions were revealed by the genetic effects of which one gene exhibited dominant effects (De), four partial dominances (PD), 20 over dominances (OD), and five additive effects (Ae). OD was observed for most of the traits in response to yield-related and fiber-quality traits.

The highest percentage of phenotypic variance explained by a single QTL was 15.45%. The highest percentage of phenotypic variance was noted in lint percentage (LP), with a range of 10.03–15.46%. The distribution of the QTLs within the identified chromosomes exhibited multiple positions in some chromosomes; A02, A03, A09, A10, D01, D03, D05, D08, D12, and D13 as illustrated in Table S2, Table 3, and Figure S1. Moreover, a total of two important clusters with more than three traits per region, with high broad sense heritability and a high percentage of phenotypic variation were identified as D03 (c17) and D12 (c26), which we designated as cluster 1 and cluster 2 (Table 3, Fig 5 and 6).

The Gene Ontology Enrichment Analysis Based on QTL Clusters

Based on phenotype variation and QTL frequency, D_t-sub genome of the whole tetraploid chromosomes harbored the highest number of stable QTLs with the highest level of phenotypic variation. In place of this, chromosome 17 (D03) and chromosome 26 (D12) had two clusters with four QTLS in each. Within the two cluster regions, we were able to mine the putative genes which could be having a role in fiber and yield-related traits. In cluster 1 (Chr17, D03), 136 genes were obtained, in which 14 were found to be highly expressed based on the RNA sequence while in cluster 2 (Chr26, D12), a total of 1280 genes were mined, out of which 153 were highly expressed at various stages of fiber development, 5 DPA, 10 DPA, 20 DPA, and 25 DPA.

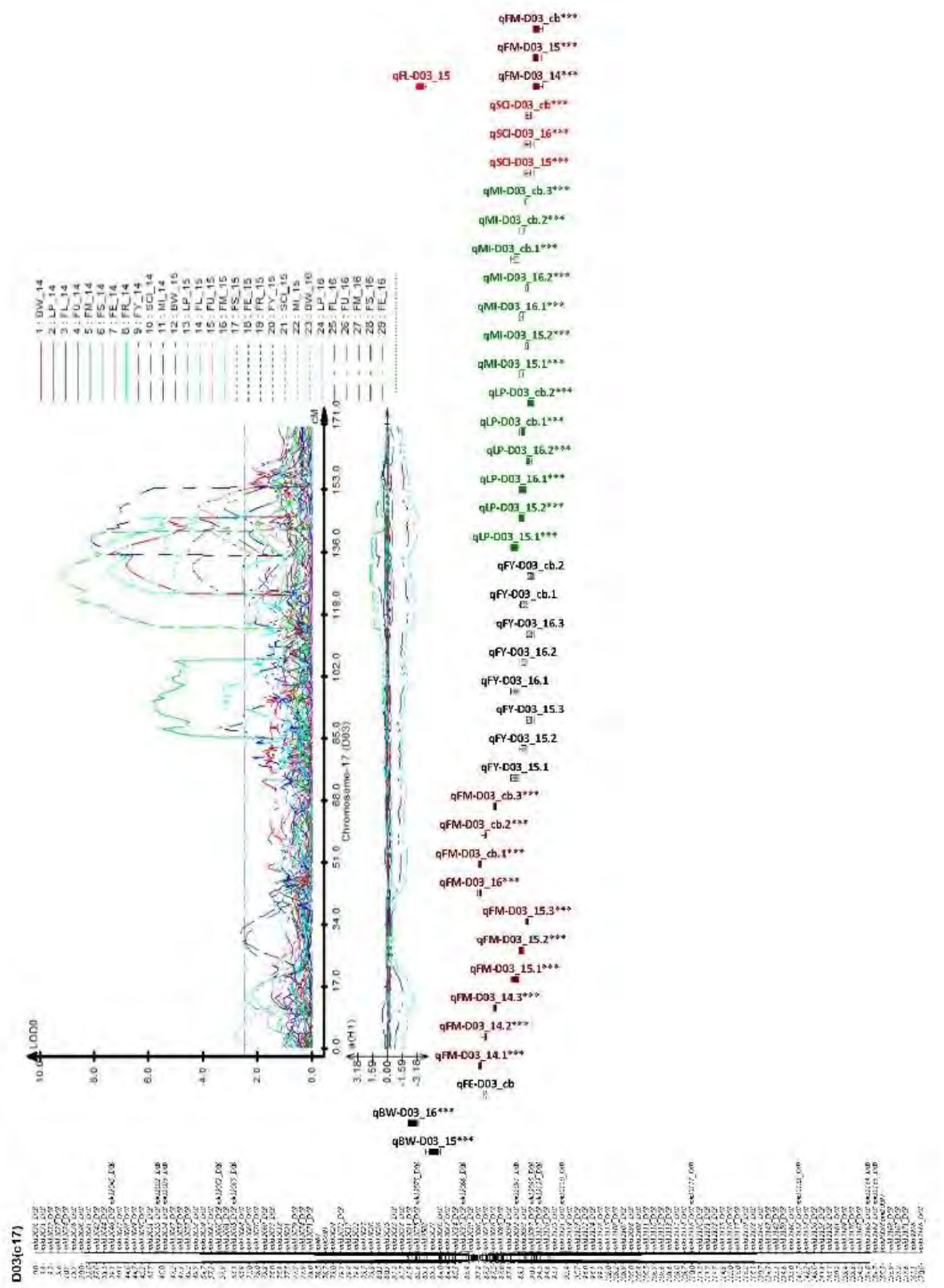

*Figure 5. Clustered QTLs identified in D03 (c17) of yield-related and fiber quality traits. Bars and lines on the right-hand side of the linkage groups show the QTL likelihood intervals. Map distances in centiMorgan (cM) are indicated on the left-hand side of the linkage groups. For trait meanings, see Figure 1 or Figure 2, *** asterisk means the QTL is consistent.*

Moreover, in order to identify the set of the most robust candidate genes for yield-related traits and fiber quality; we mainly focused on the 153 highly expressed genes as obtained from “TM-1”_RNA-seq data (available online: http://mascotton.njau.edu.cn). Out of 153 highly expressed genes, five showed high level of expression across the various stages of fiber development, and therefore, the five genes could be the potential candidate genes with greater roles in the regulation of various fiber traits Table S3. Furthermore, all the five genes were localized in different positions of the genome: one gene (*Gh_D03G0889)* was located in cluster 1 (D03 (Chr 17)) within the marker mk12119_D03 (30,535,745 bp) to marker mk12123_D03 (30,566,883 bp), the trait localized in this region was fiber micronaire (FM); while the other three genes: *Gh_D12G0093*, *Gh_D12G0410*, and *Gh_D12G0435* were localized in cluster 2 (D12 (c26)) within the marker mk19853 (101,319 bp) to marker mk17913_D12 (13,479,261 bp), the trait localized in the genome region was fiber strength (FS). Finally, the fifth gene, *Gh_D12G0969* was also mapped in cluster 2 (D12 (Chr 26)), from marker mk1009 (18,989 bp) to marker mk17992_D12 (37,732,030 bp), the trait localized in that area was fiber yellowness (FY). Based on the expression profile and GO functional annotation, these five genes were therefore found to be the most robust and possibly the putative candidate genes for fiber quality and yield-related traits (Table S3, Figures 7 and 8).

Based on GO enrichment analysis, the five highly up-regulated genes were as follows: *Gh_D03G0889* was mainly involved in molecular function and biological processes, such as up-regulation of translational elongation (GO: 0003746), poly-A RNA binding (GO: 0003723), ribosome receptor activity (GO: 0043022), hypusine anabolism (GO: 0008612), translation elongation factor (GO: 0003746), regulation of translation elongation (GO: 0045901) and regulation of translation termination (GO: 0045905). The second gene, *Gh_D12G0093* was involved only in molecular function, protein amino acid binding (GO: 0005515). The third gene, *Gh_D12G0410* was involved in all the GO functional annotation, in biological process, it was mainly involved in translation elongation (GO: 0006414), molecular function, it was mainly involved in translation elongation factor activity (GO: 0003746) and protein binding (GO: 0005515) while in a cellular component, it was found to be involved in eukaryotic translation elongation factor 1 complex (GO: 0005853). The fourth gene, *Gh_D12G0435,* had no functional annotation, however, it was found to function in nucleoside diphosphate kinase activity and the last gene, *Gh_D12G0969,* functions both in biological process and molecular function, nucleoside diphosphate kinase activity (GO: 0004550), nucleoside diphosphate phosphorylation (GO: 0006165), GTP biosynthetic process (GO: 0006183), UTP biosynthetic process (GO: 0006228), CTP biosynthetic process (GO: 0006241) and ATP binding (GO: 0005524). With gene action analysis, the five putative and robust genes with a direct role in fiber development in cotton were all contributed by the female parent, CCRI35, known for its superior fiber quality (Table S3 and Figures 7 and 8). The five genes had similar sequences based on phylogenetic tree analysis; the same was affirmed by their expression profile and all from D_t-sub genome. High-quality fiber attributes are highly linked to the D-genome of the diploid cotton such as *G. barbadense*, and tetraploid cotton originated from the polyploidization of the A and D genomes of the diploid cotton.

Table 3. Consistent QTLs for fiber quality and yield related traits identified in this study.

Trait	QTL	Chr	Start Marker	End Marker	Start Marker (bp)	End Marker (bp)	Start Marker (cM)	End Marker (cM)	Position (cM)	LOD	Ae	De	|d/a|	GA	R^2 (%)	DPE
FS	qFS-A02_15	A02	mk1761_A02	mk1778_A02	80,488,799	81,766,125	0	17.848	5.01	3.761129	−0.0052	1.5095	290.28846	OD	0.5295	NH
	qFS-A02_cb	A02	mk1761_A02	mk1778_A02	80,488,799	81,766,125	0	17.848	7.01	2.903366	0.0292	0.5368	18.383562	OD	0.0421	CCRI35
	qFS-A02_cb	A02	mk1020_A02	mk1022_A02	827,449	909,242	337.304	346.314	337.11	5.507058	0.2417	0.1944	0.8043029	A	5.6762	CCRI35
SCI	qSCI-A02_15	A02	mk1761_A02	mk1778_A02	80,488,799	8,176,6125	0	17.848	1.01	3.268187	−0.7383	9.0221	12.2201	OD	1.2775	NH
	qSCI-A02_cb	A02	mk1761_A02	mk1778_A02	80,488,799	81,766,125	0	17.848	1.01	2.740499	−0.4142	5.1274	12.379044	OD	0.9695	NH
	qSCI-A02_cb	A02	mk1018_A02	mk1019_A02	822,030	827,340	334.259	337.053	336.31	3.27253	0.9943	4.7528	4.7800463	OD	0.277	CCRI35
FL	qFL-A03_14	A03	mk1922_A03	mk1927_A03	1,863,137	1,863,215	194.163	194.228	194.21	2.54506	0.1746	0.5337	3.056701	OD	0.4959	CCRI35
	qFL-A03_15	A03	mk1989_A03	mk2007_A03	2,881,061	2,936,448	168.853	169.141	168.91	2.599349	0.276	0.1484	0.5376812	PD	3.0929	CCRI35
	qFL-A03_cb	A03	mk11099	mk2084_A03	31,386	666,6259	94.16	102.466	102.21	3.565689	0.148	0.3166	2.1391892	OD	1.445	CCRI35
	qFL-A03_cb	A03	mk2085_A03	mk2087_A03	6,666,473	6,736,164	130.379	130.946	130.41	2.723127	0.1219	0.2515	2.0631665	OD	1.3067	CCRI35
FM	qFM-A05_15	A05	mk2943_A05	mk2952_A05	21,550,988	23,173,778	195.463	206.855	197.51	2.927253	0.1377	−0.2204	1.600581	OD	4.6742	CCRI35
	qFM-A05_cb	A05	mk2943_A05	mk2952_A05	21,550,988	23,173,778	195.463	206.855	197.51	3.040174	0.0684	−0.0932	1.3625731	OD	5.0485	CCRI35
LP	qLP-A05_14	A05	mk2943_A05	mk2952_A05	21,550,988	23,173,778	195.463	206.855	195.51	4.76873	0.9516	−0.8514	0.8947037	D	11.2688	CCRI35
	qLP-A05_cb	A05	mk2943_A05	mk2952_A05	21,550,988	23,173,778	195.463	206.855	195.51	2.673181	0.4032	−1.1877	2.9456845	OD	3.4428	CCRI35
BW	qBW-A09_15	A09	mk6774_A09	mk6775_A09	60,948,395	62,054,979	7.252	15.619	15.61	2.599349	0.1034	0.335	3.2398453	OD	0.2668	CCRI35
	qBW-A09_15	A09	mk6764_A09	mk6772_A09	59,295,756	59,503,467	25.198	26.005	25.21	2.744843	0.0794	0.3894	4.9042821	OD	0.0173	CCRI35
	qBW-A09_cb	A09	mk6764_A09	mk6772_A09	59,295,756	59,503,467	25.198	26.005	25.21	2.831705	0.004	0.2838	70.95	OD	0.6913	CCRI35
FU	qFU-A09_16	A09	mk6410_A09	mk6462_A09	4,242,475	7,339,105	115.638	134.872	117.71	2.62975	−0.0221	0.6762	30.597285	OD	0.1419	NH
	qFU-A09_cb	A09	mk8762	mk6732_A09	13,222	55,126,525	35.486	44.202	40.51	2.586319	−0.0343	0.6945	20.247813	OD	1.3251	NH
SCI	qSCI-A09_15	A09	mk18838	mk6517_A09	64,093	33,530,295	79.726	79.934	79.91	4.60152	2.2915	9.0716	3.9588043	OD	0.5063	CCRI35
	qSCI-A09_15	A09	mk6528_A09	mk6531_A09	37,420,628	37,694,390	87.803	91.571	88.81	3.400651	2.6194	7.2082	2.7518516	OD	1.1267	CCRI35
	qSCI-A09_cb	A09	mk6491_A09	mk6493_A09	15,408,937	17,834,574	73.597	73.647	73.61	2.875136	3.6441	4.9294	1.3527071	OD	0.804	CCRI35
	qSCI-A09_cb	A09	mk18838	mk6517_A09	64,093	33,530,295	79.726	79.934	79.91	3.413681	0.7693	5.3779	6.9906408	OD	0.0048	CCRI35
	qSCI-A09_cb	A09	mk6528_A09	mk6531_A09	37,420,628	37,694,390	87.803	91.571	88.81	2.686211	1.0069	4.6458	4.6139637	OD	0.1298	CCRI35
FM	qFM-A10_15	A10	mk7018_A10	mk7020_A10	15,617,318	15,617,342	165.83	165.941	165.91	2.421281	0.0767	−0.322	4.1981747	OD	2.1861	CCRI35
	qFM-A10_15	A10	mk11965	mk6991_A10	8,852	12,815,805	171.292	171.66	171.31	3.339848	0.0919	−0.3583	3.898803	OD	3.1176	CCRI35
	qFM-A10_cb	A10	mk18875	mk18876	355	389	58.679	59.519	58.71	2.912052	0.0306	−0.1769	5.7810458	OD	2.1098	CCRI35
	qFM-A10_cb	A10	mk11965	mk6991_A10	8,852	12,815,805	171.292	171.66	171.31	2.779587	0.0402	−0.1538	3.8258706	OD	2.621	CCRI35
FS	qFS-A10_16	A10	mk19550	mk7479_A10	62,129	69,679,150	93.736	94.208	93.81	2.877307	−0.4206	0.0644	0.1531146	A	7.1603	NH
	qFS-A10_16	A10	MulMa189-m_A10	mk7438_A10	65,789,277	67,378,405	106.543	109.182	106.81	3.170467	0.0048	0.3517	73.270833	OD	0.4704	CCRI35
	qFS-A10_cb	A10	mk6982_A10	mk6986_A10	1,111,5569	12,645,781	177.095	185.191	177.11	3.676439	−0.1987	0.1727	0.8691495	D	6.206	NH
FE	qFE-A12_14	A12	mk9173_A12	mk9187_A12	79,355,806	81,262,301	23.53	38.305	31.51	2.54506	−0.0177	0.1178	6.6553672	OD	3.0701	NH
	qFE-A12_16	A12	mk8958_A12	mk8961_A12	59,776,633	6,102,5182	161.739	173.999	163.81	2.838219	0.0006	0.0458	76.333333	OD	0.0468	CCRI35
FE	qFE-D01_15	D01	mk10708_D01	mk10809_D01	42,734,090	44,178,280	105.061	106.117	106.11	2.551574	−0.0001	0.1905	1905	OD	0.4005	NH
	qFE-D01_cb	D01	mk10832_D01	MulMa266-m_D01	46,916,080	51,199,148	77.706	82.287	79.71	4.141151	0.0444	0.0955	2.1509009	OD	0.7434	CCRI35

	qFE-D01_cb	D01	mk10708_D01	mk10709_D01	42,734,090	42,734,155	106.1	106.117	106.11	2.998914	0.0009	0.0961	106.77778	OD	0.4425	CCRI35
SCI	qSCI-D02_15	D02	mk11587_D02	mk11605_D02	51,060,053	5,122,5737	120.178	120.621	120.61	2.690554	1.4485	−6.7494	4.6595789	OD	2.84	CCRI35
	qSCI-D02_cb	D02	mk11595_D02	mk11603_D02	51,118,850	51,193,929	115.939	116.818	116.01	2.521173	0.2862	−4.485	15.67086	OD	1.8379	CCRI35
BW	qBW-D03_15	D03	mk12041_D03	mk12042_D03	2,290,601	2,894,288	22.573	37.566	30.61	2.644951	−0.0899	0.4957	5.5139043	OD	2.9343	NH
	qBW-D03_16	D03	mk12031_D03	mk12032_D03	1,002,704	1,037,917	3.775	9.172	3.81	2.870793	0.0006	0.4782	797	OD	0.7748	CCRI35
FM	qFM-D03_15	D03	mk12142_D03	mk12159_D03	36,697,656	3,861,6587	125.137	134.584	130.21	7.52443	0.2667	0.1477	0.5538058	PD	8.7707	CCRI35
	qFM-D03_15	D03	mk12152_D03	mk12159_D03	37,665,167	38,616,587	134.584	141.665	136.01	7.685125	0.2661	0.0664	0.2495303	PD	10.0325	CCRI35
	qFM-D03_15	D03	mk12153_D03	mk12158_D03	37,668,262	37,938,158	143.823	145.337	144.81	6.304017	0.2329	0.0922	0.3958781	PD	7.4939	CCRI35
	qFM-D03_cb	D03	mk12085_D03	mk12086_D03	25,573,334	25,700,132	87.285	87.516	87.31	4.95114	0.0975	0.0212	0.2174359	PD	6.3845	CCRI35
	qFM-D03_cb	D03	mk12119_D03	mk12123_D03	30,535,745	30,566,883	95.393	95.724	95.41	6.644951	0.1133	0.0204	0.180053	A	8.5806	CCRI35
	qFM-D03_cb	D03	mk12108_D03	mk12115_D03	2,763,8133	29,511,299	101.991	103.218	101.31	4.827362	0.095	0.061	0.6421053	PD	5.0024	CCRI35
FY	qFY-D03_15	D03	mk12142_D03	mk12159_D03	36,697,656	38,616,587	125.137	134.584	130.21	2.610206	0.1828	−0.0298	0.1630197	A	4.7288	CCRI35
	qFY-D03_15	D03	mk12154_D03	mk12155_D03	37,676,414	37,682,981	141.665	142.221	141.71	3.806732	0.184	−0.1844	1.0021739	D	6.9294	CCRI35
	qFY-D03_15	D03	mk12158_D03	mk12161_D03	37,938,158	39,407,242	145.337	150.198	148.31	4.210641	0.2038	−0.2276	1.1167812	D	8.0817	CCRI35
	qFY-D03_cb	D03	mk12109_D03	mk12111_D03	27,707,667	29,136,194	105.804	106.491	105.81	2.571118	0.0879	−0.0931	1.0591581	D	4.7115	CCRI35
LP	qLP-D03_15	D03	mk12142_D03	mk12159_D03	36,697,656	38,616,587	125.137	134.584	129.21	9.233442	1.7674	−0.2922	0.1653276	A	15.4584	CCRI35
	qLP-D03_15	D03	mk12152_D03	mk12160_D03	37,665,167	38,832,736	134.986	141.665	138.01	8.214984	1.6425	−0.399	0.2429224	PD	13.9321	CCRI35
	qLP-D03_16	D03	mk12152_D03	mk12160_D03	37,665,167	38,832,736	134.986	141.665	138.01	5.439739	1.4767	−0.9737	0.6593756	PD	9.8343	CCRI35
	qLP-D03_16	D03	mk12153_D03	mk12158_D03	37,668,262	37,938,158	143.823	145.337	144.81	4.621064	1.2806	−1.0766	0.8406997	D	7.9495	CCRI35
	qLP-D03_cb	D03	mk12152_D03	mk12160_D03	37,665,167	38,832,736	134.986	141.665	139.01	8.169381	1.2356	−0.1607	0.1300583	A	13.3867	CCRI35
	qLP-D03_cb	D03	mk12158_D03	mk12161_D03	37,938,158	39,407,242	145.337	150.198	147.31	7.090119	1.1317	−0.3152	0.278519	PD	12.0557	CCRI35
FR	qFR-D04_15	D04	MulMa448_D04	MulMa451_D04	49,867,798	50,187,192	4.736	8.337	5.01	2.660152	−0.1841	−1.5832	8.5996741	OD	0.0079	NH
	qFR-D04_cb	D04	MulMa448_D04	MulMa451_D04	49,867,798	50,187,192	4.736	8.337	5.01	2.781759	−0.227	−0.8557	3.7696035	OD	0.6158	NH
FM	qFM-D05_15	D05	mk12822_D05	mk12824_D05	30,214,244	30,216,527	152.95	153.434	153.01	5.061889	0.2063	0.008	0.0387785	A	6.8075	CCRI35
	qFM-D05_cb	D05	MulMa463-m_D05	mk12861_D05	30,373,354	31,354,896	144.668	148.809	146.71	4.021716	0.08	−0.1214	1.5175	OD	6.6096	CCRI35
	qFM-D05_cb	D05	mk12822_D05	mk12824_D05	30,214,244	30,216,527	152.95	153.434	153.41	4.627579	0.0886	−0.0879	0.9920993	D	7.3098	CCRI35
SCI	qSCI-D05_15	D05	MulMa463-m_D05	mk12861_D05	30,373,354	31,354,896	144.668	148.809	144.71	2.566775	−2.7495	3.5799	1.3020185	OD	4.5444	NH
	qSCI-D05_cb	D05	MulMa463-m_D05	mk12861_D05	30,373,354	31,354,896	144.668	148.809	144.71	2.579805	−1.778	1.3725	0.7719348	PD	4.7561	NH
FR	qFR-D08_15	D08	mk15992_D08	mk15995_D08	56,628,640	56,628,844	181.945	182.082	182.01	2.655809	0.587	−0.2932	0.4994889	PD	4.6002	CCRI35
	qFR-D08_cb	D08	mk15992_D08	mk15995_D08	56,628,640	56,628,844	181.945	182.082	182.01	3.441911	0.408	−0.2977	0.7296569	PD	6.2705	CCRI35
FL	qFL-D08_14	D08	MulMa514_D08	mk16004_D08	54,937,781	58,533,805	187.587	196.342	196.31	3.583062	0.3322	−0.0152	0.0457556	A	5.8804	CCRI35
	qFL-D08_cb	D08	MulMa514_D08	mk16004_D08	54,937,781	5,853,3805	187.587	196.342	196.31	3.639522	0.1799	0.0892	0.495831	PD	4.9405	CCRI35
	qFL-D08_cb	D08	mk16017_D08	mk16020_D08	59,691,087	59,698,388	208.553	208.76	208.61	3.255157	0.1595	0.1876	1.1761755	D	3.2928	CCRI35
FE	qFE-D10_14	D10	mk17141_D10	MulMa593-m_D10	56,817,432	56,887,380	102.161	106.55	106.21	2.523344	−0.0328	0.082	2.5	OD	3.7527	NH
	qFE-D10_15	D10	MulMa575-m_D10	MulMa581_D10	24,793,863	24,918,141	1.604	2.047	1.91	2.959826	0.0751	0.0306	0.4074567	PD	3.9966	CCRI35
FL	qFL-D10_14	D10	mk17141_D10	MulMa593-m_D10	56,817,432	56,887,380	102.161	106.55	105.21	3.411509	−0.1394	0.7384	5.2969871	OD	3.6287	NH
	qFL-D10_cb	D10	mk2492	MulMa366-m	519	33,037	78.155	98.694	83.21	2.614549	−0.045	0.464	10.311111	OD	2.0017	NH

FL	qFL-D11_16	D11	mk17462_D11	mk17463_D11	15,695,804	15,711,598	88.706	88.935	88.71	2.831705	0.0011	0.1765	160.45455	OD	0.8561	CCRI35
	qFL-D11_cb	D11	mk17464_D11	mk17514_D11	15,711,711	21,298,890	61.142	88.706	82.21	3.079262	−0.0461	0.5185	11.247289	OD	2.2603	NH
FS	qFS-D12_14	D12	mk18221_D12	MulMa605_D12	50,554,371	5,129,3378	148.99	160.984	153.01	3.270358	0.0077	0.8261	107.28571	OD	0.6079	CCRI35
	qFS-D12_15	D12	mk17994_D12	mk17997_D12	3,798,8313	3,8143,957	65.608	66.056	65.61	3.743757	0.3487	−0.7277	2.0868942	OD	5.7325	CCRI35
	qFS-D12_cb	D12	mk19853	mk17913_D12	101,319	13,479,261	16.392	16.946	16.41	4.049946	0.1807	−0.3037	1.6806862	OD	6.3446	CCRI35
FY	qFY-D12_15	D12	mk17995_D12	mk18057_D12	38,058,755	41,722,495	66.861	96.943	70.91	2.677524	0.047	−0.4721	10.044681	OD	2.0175	CCRI35
	qFY-D12_cb	D12	mk1009	mk17992_D12	18,989	37,732,030	66.348	66.861	66.41	3.252986	0.0211	−0.2739	12.981043	OD	1.9523	CCRI35
SCI	qSCI-D12_14	D12	mk18202_D12	mk18207_D12	48,411,387	48,718,111	132.168	133.627	133.61	3.14658	1.3176	−0.8128	0.6168792	PD	6.2241	CCRI35
	qSCI-D12_15	D12	mk19857	mk17916_D12	117,142	15,801,265	23.692	24.245	23.71	3.072747	2.636	4.6094	1.7486343	OD	1.4248	CCRI35
FL	qFL-D12_14	D12	mk18210_D12	mk18214_D12	48,923,084	49133419	135.788	138.113	135.81	2.851249	0.281	−0.216	0.7686833	PD	5.2886	CCRI35
	qFL-D12_cb	D12	mk18221_D12	MulMa605_D12	50,554,371	51,293,378	148.99	160.984	158.01	2.896851	0.1755	0.1739	0.9908832	D	3.2967	CCRI35
	qFL-D12_cb	D12	MulMa604-m_D12	mk18232_D12	51,286,859	52,905,207	161.79	172.395	165.81	2.773073	0.1653	0.1375	0.8318209	D	3.0451	CCRI35
FL	qFL-D13_14	D13	mk18377_D13	MulMa619_D13	4,171,037	34,392,983	36.052	56.969	46.11	3.237785	0.1336	−0.7846	5.8727545	OD	3.0376	CCRI35
	qFL-D13_15	D13	mk18378_D13	mk18379_D13	4,310,490	4,329,364	64.756	65.409	64.81	2.529859	0.2624	0.2724	1.0381098	D	2.1912	CCRI35
	qFL-D13_cb	D13	mk18516_D13	mk18533_D13	41,759,681	47,859,575	2.023	10.429	9.01	3.051031	0.17	−0.0572	0.3364706	PD	5.3187	CCRI35
	qFL-D13_cb	D13	mk20382	mk18378_D13	141	4,310,490	65.409	72.523	65.41	3.072747	0.1705	−0.0658	0.3859238	PD	5.4653	CCRI35

LOD: logarithm of odds; 0 < Ae (additive effect) < 0.20; 0.21 < PD (partial dominance) < 0.80; 0.81 < De (dominance effect) < 1.20; OD (over dominance) > 1.20; |d/a| = De/Ae; GA: gene action; DPE: direction of phenotypic explanation. For traits meaning see Figure 1 or Figure 2.

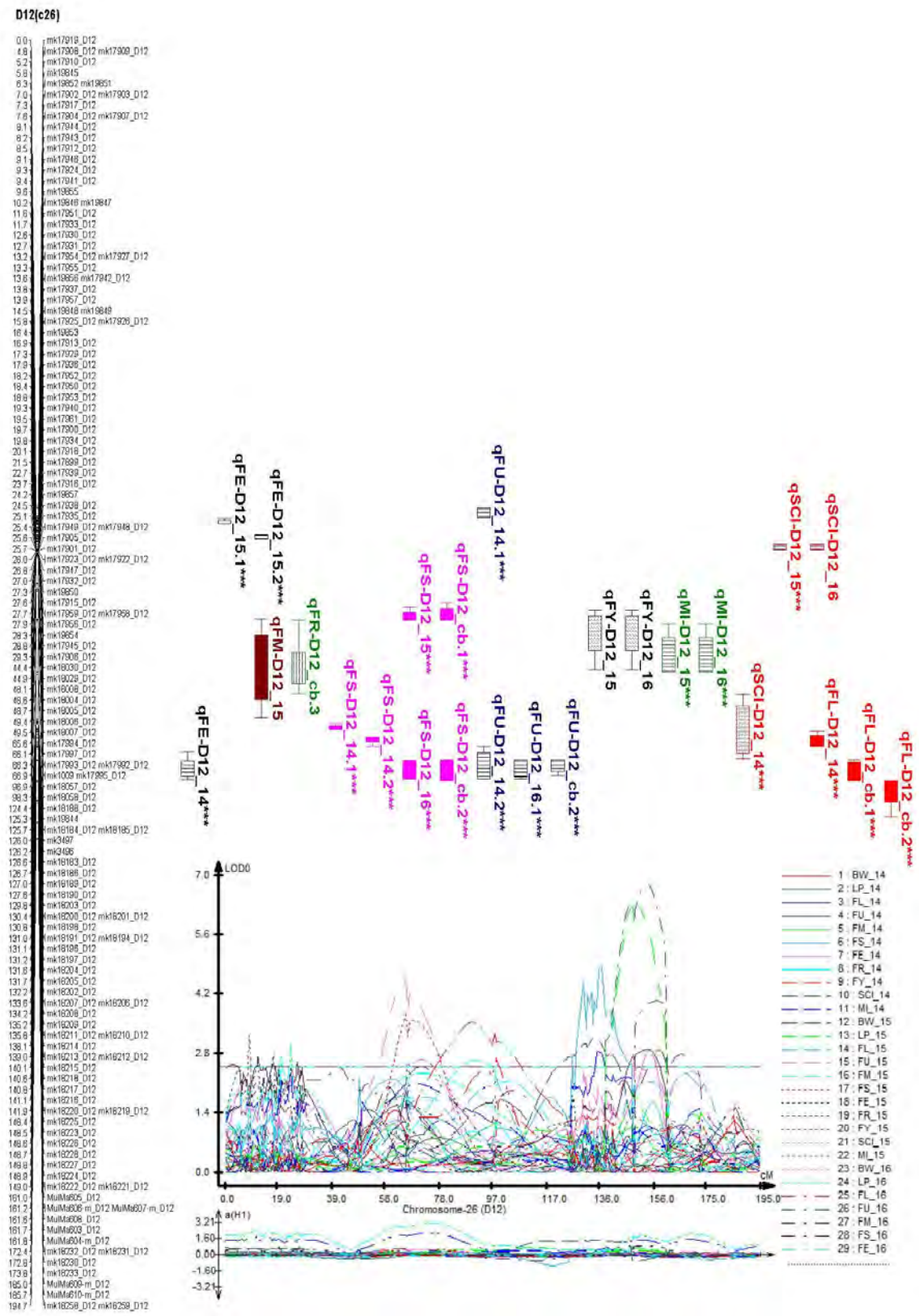

*Figure 6. Clustered QTLs identified in D12 (c26) of yield-related and fiber quality traits. Bars and lines on the right-hand side of the linkage groups show the QTL likelihood intervals. Map distances in centiMorgan (cM) are indicated on the left-hand side of the linkage groups. For trait meanings, see Figure 1 or Figure 2, *** asterisk means the QTL is consistent.*

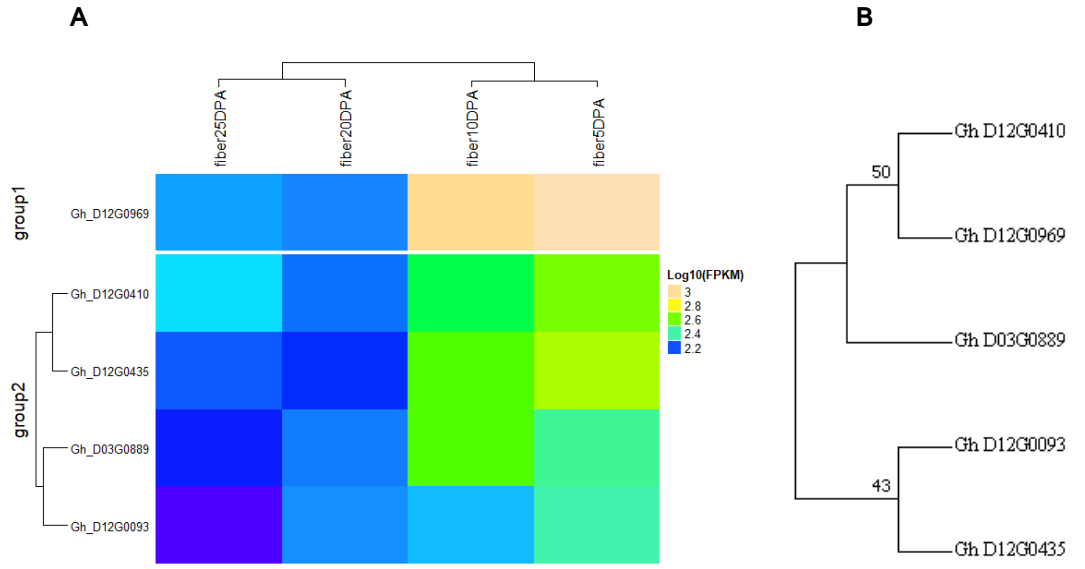

Figure 7. (A) Candidate genes involved in yield and/or fiber quality traits in this study; (B) phylogenetic tree analysis of the five involved genes in yield related and fiber quality traits of upland cotton.

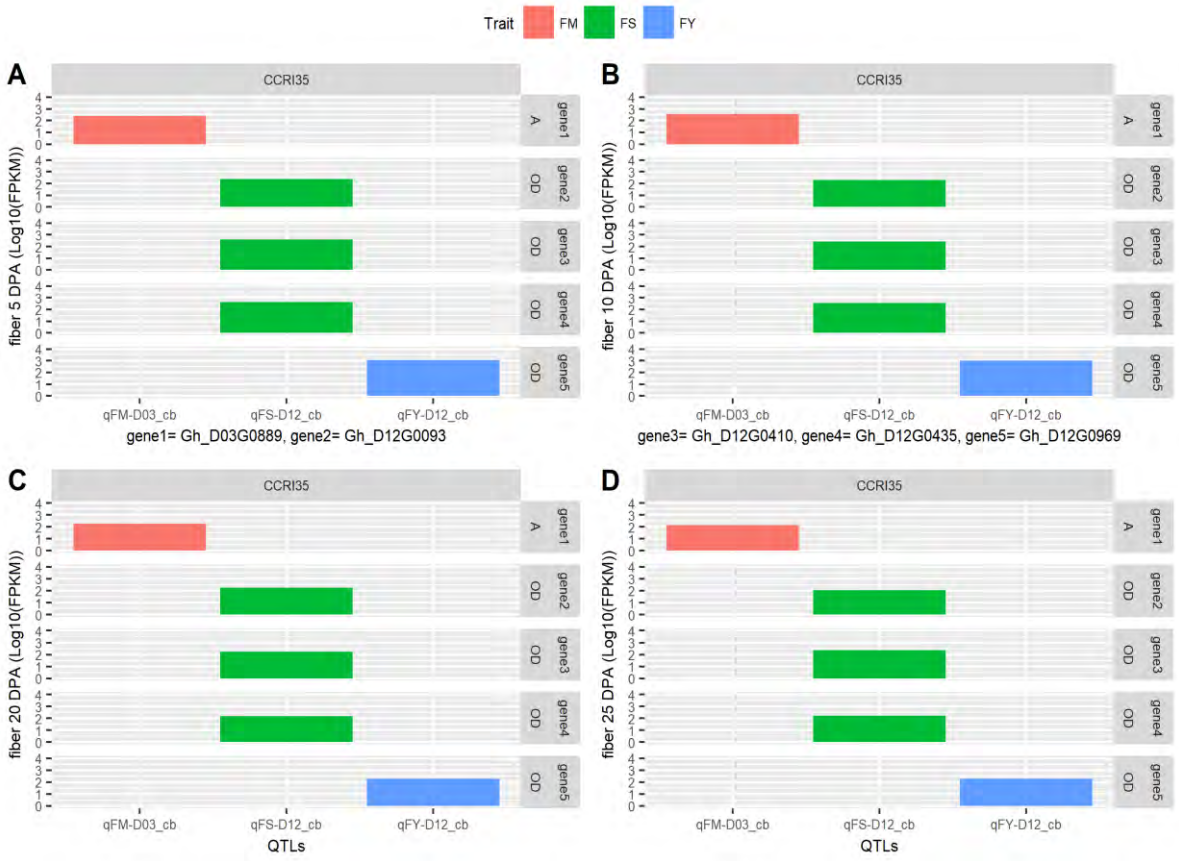

Figure 8. The five genes with highest expression in different stages of fiber development. x axis: QTLs for fiber Quality; CCRI35: good fiber quality parental line; y axis: fiber DPA (Log10(FPKM)); FM: fiber micronaire (%); FS: fiber strength (cN/tex); FY: fiber yellowness; Gh_D03 or Gh_D12 are genes identified with high expression and involved in fiber development; cb: combine analysis; 0 <Ae (additive effect) <0.20; OD (over dominance) > 1.20; (A–D) are respectively fiber expression at 5, 10, 20 and 25 DPA. For trait meanings, see Fig 1 or Fig 2.

Discussions

The determination of stable QTLs for superior agronomical traits and the construction of a high-resolution map is crucial for MAS. Several intra-specific genetic maps have been generated and used for QTL detection related to fiber and yield components (Liang et al., 2013). Even though these maps have been used, they are limited in scope and accuracy due to huge marker intervals and narrow genome coverage. The greatest impediment in the construction of a high-resolution map in intraspecific crosses is due to low rate of polymorphism within *G. hirsutum* and the presence of fixed homozygous genetic blocks (Jamshed et al., 2016; Fang et al., 2014). Therefore, there is a need to find additional markers to fill in the gaps in the genetic map (Jamshed et al., 2016). In this current research, a genetic map consisting of 5178 SNP markers obtained through the GBS technique was developed using a 277 $F_{2:3}$ population derived from an intra-specific cross. In addition, the contrasting difference between the two parental lines used in this investigation could be explained based on inherent genetic characteristics. The male parent is known for superior agronomic traits such as early flowering and the ability to generate a high percentage of fruits of large size, while the female parent is known for superior fiber traits. Fiber length (FL), fiber uniformity (FU), fiber micronaire (FM), and fiber strength (FS) showed significant differences between the two parental lines. These traits were attributed to CCRI35, except FE which was linked to NH. There was no significant difference noted between the two parents for FE and FM. This result confirmed the good quality fiber trait of the female parent, CCRI35, compared to the male, NH.

In addition, there was a wide range of phenotypic variation among the $F_{2:3}$ population, with respect to the following measured traits: BW, LP, FL, FU, FM, FS, FE, FR, FY, SCI, and MI. In the three environments, all traits exhibited normal segregation patterns, with equal distribution. The low absolute values for skewness and kurtosis showed that these traits had a normal distribution. In addition, in the $F_{2:3}$ population, the maximum phenotypic data values in all the variables were much higher than in CCRI35, the parent known for superior fiber traits, fiber length (FL), fiber uniformity (FU), fiber micronaire (FM), fiber strength (FS), and fiber elongation (FE). This finding showed that all traits were transgressively segregated in the $F_{2:3}$ population. Previous research reported that transgression was the difference observed between the mapping parents of upland cotton (Jamshed et al, 2016; Wang et al., 2015; Oluoh et al., 2016; Lacape et al., 2010).

Furthermore, positive correlations were noted between the following traits: boll weight (BW) with fiber length (FL), fiber uniformity (FU), fiber micronaire (FM), fiber strength (FS), fiber elongation (FE), fiber reflectance (FR), and mature index (MI); lint percentage (LP) with FM, FL with FU, FS, FE, and spinning consistency index (SCI); FU with FS, FE, and SCI; FM with MI; FS with FE, and SCI; FE with SCI and finally FR with SCI. However negative correlations were observed in the following traits: LP with FR, and SCI; FL with FM; FM with FR, and SCI; finally, SCI with MI. This result is consistent with those from Jamshed et al. (2016) who showed that positive correlations were observed between fiber elongation (FE), fiber length (FL), fiber strength (FS), and fiber uniformity (FU), with a significance level of 0.01. Moreover, FL and FS were both negatively correlated with fiber micronaire (FM). In this study, the correlations between FM and FS were found to be negative but were not significant, which does not agree with previous findings. This deviation could be attributed to the population background used in this study.

It is known that broad sense heritability with a high percentage is more useful and very easy to manipulate in MAS. Therefore, the extent of transmission of traits from the parents to the descendants or offspring was determined by the level of heritability, hence traits with a high broad sense of heritability could be easier to manipulate (Saba et al., 2001). The broad sense heritability was high for LP (82.65%), FM (91.68%), FR ((86.08%), FY (88.89%), SCI (87.47%), and moderate for BW (68.92%), FL (61.66%), FU (76.42%), FS (76.54%), FE (60.58%), and MI (76.61%). The lowest broad sense heritability was noted for fiber elongation (FE), 60.58%. Similar findings were observed by Jamshed et al. (2016) who found that fiber elongation had the lowest broad sense heritability (27%), whereas other fiber traits were higher, ranging from 80% (FU) to 93% (FL) (Jamshed et al., 2016; Lacape et al., 2010).

A total map distance of 4768.098 cM was generated, higher than the most current linkage map with a map distance of 4450 cM of the cotton genome (Rong et al. 2004). This is the densest intra-specific map developed in upland cotton. This map could be helpful for further studies in MAS, especially in fine mapping. The average distance of the adjacent markers was 0.92 cM. A_t sub-genome spanned 2611.43 cM, and consisted of 3313 markers with 13 LGs. The average marker distance in A_t sub-genome was

0.79 cM with a maximum gap of 26.598 cM of the adjacent markers. In D_t sub-genome, 13 LGs were assigned which comprised 1865 markers spanning 2156.67 cM, with an average of 1.156 cM. The maximum gap was 30.082 cM between adjacent loci. Due to the nature of the upland cotton genome, mapping QTLs not only for fiber as in this research but for other agronomic traits has been difficult. This is because of the narrow genetic background, which resulted in low diversity of alleles with a significant role in fiber quality traits between two given varieties (Shao et al., 2014). Therefore, only a few QTLs could be mapped based on two parent crossing populations, which has been verified by previous reports (Kohel et al., 2001; Shen et al., 2007; Lin et al., 2005; Shen et al. 2005); Zhang et al. 2005; Zhang et al. 2009; Wang et al., 2006; He et al., 2007; Rong et al., 2007; Ma et al. 2008; Sun et al., 2012). In this current study, a total of 110 QTLs were identified for 11 traits, but only 30 QTLs were consistent in at least two environments. The 30 consistent QTLs were located on 16 chromosomes; A02, A03, A05, A09, A10, A12, D01, D02, D03, D04, D05, D08, D10, D11, D12, and D13 with 2, 1, 2, 3, 2, 1, 1, 1, 4, 1, 2, 2, 2, 1, 4, and 1 QTL respectively. Of the 30 detected QTLs, 11 were located on A_t sub-genome while the remaining 19 were located on the D_t sub-genome. This finding is consistent with previous reports in which 58 QTLs were found on the A_t sub-genome, whereas 107 QTLs were localized on the D_t sub-genome (Jamshed et al., 2016). Fifty-eight QTLs were located on the A_t sub-genome (Chr01–Chr13), and 73 QTLs on the D_t sub-genome (Fang et al., 2014). These QTLs explained from 2.03 to 16.85% of phenotypic variation, with an average of 6.26% explained in all five fiber quality traits (Zhang et al. 2016).

Most of the QTLs distributed in the cotton genome revealed the complexity of the cotton genome and the arduousness of QTL mapping in cotton. Therefore, comparing our QTLs with other QTLs mapped from previous studies could be of great help in determining the reliability of the QTLs detected (Wang et al, 2016). Up to now, 4268 QTLs from 140 publications on cotton have been documented in the collected Cotton QTL Database (available online: http://www2.cottonqtldb.org:8081/index). In this study, the GBS-SNP markers are unique and thus lack a common identity with the SSR-based markers. However, five QTL clusters in this investigation were found to have a common bearing to those documented by Said et al. (2013), which have been known as one of the strongest references in QTL mapping in recent years. The five QTL clusters were: cluster A07 was identical to c7-cluster-Gh × Gb-4:55–79 cM; cluster A08 had an approximate position of 4.81–110.81 cM, which was similar to c8-cluster-Gh-2:21–31 cM; cluster D01, had an approximate position of 2.21–139.31 cM, similar to c15-cluster-Gh-3:49–68 cM; cluster D02, had an approximate position of 0.01–206.11 cM, similar to c14-cluster-Gh-2:76–91 cM and lastly cluster D08, had an approximate position of 100.71–208.61 cM, nearby to c24-cluster-Gh-2:41–62 cM. The high correlation of the QTLs detected in this study to the previous finding, provides the opportunity for the utilization of these QTLs in MAS to improve the fiber quality of Upland cotton.

Gene Ontology enrichment analysis revealed five genes with very high expression and was linked to three fiber quality traits, FM, FS, and FY. Interestingly, the five genes took their alleles from the parental line known for superior fiber quality CCRI35. This result supported our study. Our findings provide an opportunity for the improvement of fiber quality especially fiber color (FY: fiber yellowness). Cotton fiber development occurs through various stages, namely fiber initiation, elongation, secondary cell wall formation, and maturation (Tiwari et al., 1995). Cotton fiber development is controlled by a multi-complex of gene interactions rather than a single gene effect (Guan et al., 2011; Pu et al., 2008). *GhD12G0969* was mainly found to have a functional role in phosphorylation; phosphorylation is a process mediated by protein kinases to activate critical cellular pathways such as metabolism, cell division, and cell differentiation during initiation stages in cotton fiber development (Ma et al., 2014). In addition, *Gh_D12G0435* was found to be involved in kinase activity; protein kinase activity plays an important role in signal transduction through the phosphorylation process during cotton fiber development (Chaudhary et al., 2008). Therefore, the five highly up-regulated genes could be the key genes with major functional roles in fiber development and turn superior quality as evident in the CCRI35, female parent.

Conclusions

A genetic linkage map comprising of 5178 SNP markers, obtained by the GBS genotyping method, was generated using a 277 $F_{2:3}$ population derived from an intra-specific cross of two tetraploid upland cottons. The map constructed in this study is the highest dense genetic map ever developed from an intra-specific population of the tetraploid upland cotton. The average distance of 0.92 cM was observed between adjacent markers. A total of 110 QTLs were obtained for 11 traits, however, only 30 QTLs were consistent in more than one environment. In addition, we identified 1709 genes that were found

in the two main hot spot regions, named clusters 1 and 2, with four QTLs in each. Out of the 1709 genes, 153 genes exhibited higher expression levels while the rest showed lower expression levels in all stages of fiber development. We further identified five key genes: *Gh_D03G0889*, *Gh_D12G0093*, *Gh_D12G0969*, *Gh_D12G0410*, and *Gh_D12G0435* to be the candidate genes involved in fiber development. This research provides the very first foundation in which future molecular work can be done, such as cloning of the identified genes and/or saturation of the genes to boost the current elite cultivated cotton cultivars.

Methods

Plant Materials, Growth Conditions and Trait Data Collection

The accessions used in this research were, Nan Dan Ba Di Da Hua in Chinese annotation, but for simplicity, we abbreviated the name as (NH), the male parent; it has moderate fiber quality traits but high yielding in fiber (Zhen et al., 2014; Gong et al., 2017). The female parent was Zhong35, also with the Chinese name, which was then abbreviated as CCRI35; it is known for high fiber quality traits but with moderate yield (Tan et al., 2014). The parental lines and 277 $F_{2:3}$ population were evaluated for fiber quality traits and yield components in Anyang research station (36°100′ N, 114°350′ E), Henan province, Yellow River. The field experiment was carried out during summer periods in three consecutive years, 2014, 2015 and 2016. The experimental layout adopted, was a complete randomized block design (CRBD) with three replicates. The plot sizes were 5 m long with row spacing of 0.75 m. Fiber quality and yield component traits were collected following the laid down scheme as described by Said et al. (2013). Fully opened bolls in each sampled plant were collected within the middle region of the plant, 25 bolls were collected from each line for fiber quality and yielded component determination. The balls were ginned for the determination of lint percentage (LP), fiber length (FL), fiber uniformity (FU), fiber micronaire (FM), fiber elongation (FE), fiber strength (FS), fiber reflectance (FR), fiber yellowness (Socquet-Juglard et al., 2013), spanning consistency index (SCI) and mature index (MI) by the HVI 900 fiber testing system, which was done in our cotton fiber quality testing unit, cotton research institute, Anyang, China. The test conditions were set with a temperature of 20 °C and relative humidity of 65%.

Sample Collection, Library Preparation, Sequencing and SNP Genotyping

DNA Extraction, Quantification and Quality Determination

Fresh leaf samples were obtained from each line, together with the two parents, and immediately frozen in liquid nitrogen then stored under −80 °C before DNA extraction. DNA of the $F_{2:3}$ populations of 277 individuals and 10 samples for individual parents was extracted by the CTAB method as described by Paterson et al. (1993). Each sample was then crushed separately in liquid nitrogen to fine powder, then immediately added to CTAB solution. For every 100 mg of ground tissues, we added 500 µL of CTAB Buffer. The samples were then shaken for 15 min and then centrifuged. The centrifuged mixture was then put in a water bath at 60 °C for 30 min. Then, samples were centrifuged for 5 min at 12,000 revolutions per minute (rpm). After centrifuging, the supernatant was transferred to a new tube. Then, 5 µL of RNase solution was added to digest RNA and then incubated for 20 min at 32 °C. An equal amount in volume of chloroform/isoamyl alcohol (24:1) was added and then shaken for 5 s before centrifuging the samples for 1 min to separate the phases. We pippeted the aqueous upper phase to a new tube; the method was then redone until the upper phase was clear. The upper clear phase was then pipetted into a new tube. DNA samples were later precipitated by adding 70% volume of ice-cold isopropanol and incubated for 15 min at −20 °C. The condensed DNA samples were then centrifuged at 12,000 rpm for 10 min. The supernatant was then decanted and subsequently washed with 500 µL ice-cold 70% ethanol twice then absolute alcohol. DNAs were later dissolved in 20 µL TE buffer (10 mM Tris, pH 8, 1 mM EDTA) (Krizman et al., 2006). The degradation and contamination of DNA were checked through 1% agarose gels. The purity of DNA was determined by using a Nano Photometer® spectrophotometer (IMPLEN, Westlake Village, CA, USA). The ratio of absorbance at 260 and 280 nm was used to assess the purity of DNA. The DNA samples with a ratio of ~1.8 were then qualified as pure (Wlfinger and Chomczynski, 2006). The concentration of DNA was done by using the Qubit® DNA Assay Kit in Qubit® 2.0 Fluorimeter (Life Technologies, Carlsbad, CA, USA). The Qubit® dsDNA HS (High Sensitivity) Assay Kits make DNA quantitation easy and accurate. The kits contain concentrated assay reagents, dilution buffer, and prediluted DNA standards. The reagents were mixed with the buffer solution and then added to 1–20 µL of each DNA sample.

The concentrations were read using the Qubit® Fluorometer (Life Technologies, Carlsbad, CA, USA); only the DNA samples with a concentration range of 10 pg/μL to 100 ng/μL were finally used (available online: https://tools.thermofisher.com/content/sfs/manuals/Qubit_dsDNA_HS_Assay_UG.pdf).

GBS Library Preparation, Sequencing and SNP Genotyping

GBS is a low-cost and efficient method of large-scale genotyping, which is based on high-throughput sequencing but with a reduced-representation library (RRL). The following were step-by-step processes in the GBS technique; firstly, we carried out a GBS pre-design experiment to test the accuracy of the GBS protocol and the quality of the output data. The enzymes and sizes of restriction fragments were examined by using training data. Three basic criteria were followed: (a) the suitability of the number of tags to the project needs; (b) the homogenous distribution of the enzymatic tags throughout the examined sequences; (c) elimination of redundant tags (repeated tags must be avoided). This was to ensure the effectiveness and accuracy of data obtained from GBS reads; 50 bp was the selection criterion to ensure sequence depth uniformity.

Secondly, we constructed the GBS library using the pre-designed scheme. The genomic DNA of the $F_{2:3}$ population was incubated at 37 °C with MseI Restriction Enzyme obtained from New England BioLabs (Ipswich, MA, USA), NEB, T4 DNA ligase, and ATP. MseI Y adapter N containing a barcode. Restriction-ligation reactions were activated at 65 °C, followed by digestion for additional restriction enzyme NlaIII at a temperature of 37 °C. The samples were then purified by using Agencourt AMPure XP (Beckman, Brea, CA, USA). Then carried out polymerization chain reaction (PCR) using the purified samples, Phusion Master Mix universal primer, and index primer were used to add an index, complete i5 and i7 sequence. The Agencourt AMPure XP (Beckman) was used to purify the PCR products, which were pooled and then ran through 2% agarose gels. Fragments of 375–400 bp (with indexes and adaptors) in size were obtained by using a Gel Extraction Kit (Qiagen, Hilden, Germany). The isolated fragment products were then purified using Agencourt AMPure XP (Beckman), and finally diluted for sequencing.

GBS analysis was strictly carried out as outlined by Elshire et al. (2011); integrating 3 of 96-well plates across 288 barcodes for library preparation and sequencing. For SNP calling, the raw sequence data for the 277 $F_{2:3}$ population together with the F_1 generation was processed through the TASSEL 3.0 Genotype By Sequencing (GBS) pipeline (Glaubitz et al., 2014) using the Gossypium_hirsutum_v1.1.fa as the reference genome (Paten and Haussler, 2014) which was obtained from Cotton research institute (available online: http://mascotton.njau.edu.cn/info/1054/1118.htm), for alignment and the Burrows-Wheeler Aligner (BWA) mem (Li, 2009) with default parameters. The output consisted of variant call format (VCF) file version 4.1 (Danecek et al., 2011) including single nucleotide polymorphisms (SNPs) present in at least 40% of the progeny and with a minor allele frequency (MAF) 0.1. Subsequently, the data in variant call format (VCF) was filtered using VCF tools version.1.12a (Danecek et al., 2011) and TASSEL (Bradbury et al., 2007) versions 3.0 and 4.0. A total of 93,384 single nucleotide polymorphisms (SNPs) were identified in 277 $F_{2:3}$ populations by TASSEL 3.0, then a custom filtering process was applied for alignment. The filtering was based on maintaining sites with a minimum read depth of 6% and 75% completeness by site across progeny and by progeny across sites. Results were obtained as a TASSEL hapmap file.

Finally, using a custom Perl script marker heterozygous in the F_1generations and with a co-dominant segregation ratio of 1:2:1 among the $F_{2:3}$ population were identified using a chi-squared (χ^2) goodness-of-fit test at $\alpha \leq 0.01$. These were reconverted and imported in JoinMap® 4.1 for linkage group generation. A total of 26 LGs were obtained, and each linkage group was assigned to its corresponding chromosome by using BLASTN-search (available online: https://blast.ncbi.nlm.nih.gov/Blast.cgi), for the marker sequence.

Data Analysis and Linkage Map Construction

Analysis of variance (ANOVA) was performed by using field phenotype data of the three consecutive seasons 2014, 2015, and 2016, and the combined analysis (CB). A mixed procedure was used; the genotypes and the environments were fixed as factors to detect heritability (IBM, 1999). A post hoc test (Turkey's) to compare means was done (IBM, 1999). The broad-sense heritability percentage, H_b (%), was calculated for each trait using the formula described by Singh et al., 2017).

$$H = \sigma^2G/\sigma^2G + (\sigma^2e/r)$$

With σ^2G is the genotypic variance; σ^2e: phenotypic variance and r: replication.

Most of the data were analyzed using R software version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) (Team, 2008). Markers were ordered, rippled, and re-ordered according to pairwise recombination fractions, LOD scores and linkage group length (Broman et al., 2003). Linkage group analyses were conducted using Join Map 4.0 (Stam et al. 1995) with a recombination frequency of 0.40 and a logarithm of odds (LOD) score of 2.5 for the $F_{2:3}$ population. The Kosambi mapping function was employed in the conversion of the recombination frequencies to map distances. Each data point represented the mean of three replications. Fiber quality and yield-related traits such as boll weight (BW), lint percentage (LP), fiber length (FL), fiber uniformity (FU), fiber micronaire (FM), fiber strength (FS), fiber elongation (FE), fiber reflectance (FR), fiber yellowness (FY), spinning consistency index (SCI) and mature index (MI) were used to conduct QTL analysis. The quantitative trait loci (QTLs) were detected using composite interval mapping (CIM) (Rodriguez da Silv et al., 2016) by WinQTL Cartographer version 2.5 (Wang and Zeng, 2007). In the CIM mapping method, version 6, forward–reverse regression method with 1 cM walking speed, a probability into and out of the model of $p = 0.01$ and window size set at 10 cM. The LOD (Podliesna et al., 2017) threshold value was determined by 1000 permutation tests for all traits and was used to declare the significant QTLs with a significance level of $p = 0.05$. In addition, QTLs with LOD threshold of 2.5 in more than one environment were considered as common QTLs based on the explanation by Lander and Kruglyak (Lander and Kruglyak, 1995).

QTL nomenclature was done based on the description by Liang et al. (2013). The proportion of observed phenotypic variance explained by each QTL was estimated by the coefficient of determination R^2 (%) as a percentage. The additive and dominance effects from QTL cartographer results were used to calculate genetic effects (|d/a|). The results were used to classify the QTL as additive effect (Ae) (0–0.20), partial dominant (PD) (0.21–0.80), dominant effect (De) (0.81–1.20) and over dominant (OD) >1.20 according to Stuber et al. (1987) (Stuber et al., 1987). The graphic presentation of the linkage group and QTLs marked were created by R software version 3.4.2 (Team, 2008) and Map Chart 2.2 (Voorrips, 2002), respectively

Gene Mining and Expression Analysis

In this study, only segments of linkage groups associated with significantly detected QTLs were presented. The detected consistent QTLs were used to identify the crucial candidate genes for fiber yield and fiber quality-related traits. The genes identified were searched through the available resources (Zhu et al., 2017) (available online: https://cottonfgd.org). The physical position of the GBS-SNP markers flanking major QTLs for fiber quality and yield-related traits was used to find the gene located in each QTL region. The function of the identified genes was determined through gene annotation. Furthermore, the expression profile of the candidate genes was analyzed by mapping it in the “TM-1”_RNA-seq transcriptome data of cotton (available online: https://cottonfgd.org). The expression values for each gene mined were used to generate the heat map using an R-software script (Team, 2008).

The Gene Ontology Enrichment Analysis BaseD on QTL Clusters

To determine the functions of the identified genes, we carried out gene ontology enrichment analysis through the online software, Blast2GO (available online: https://www.blast2go.com/). Gene ontology describes the genes in three functional annotations, namely cellular component (CC, biological process (BP), and molecular functions (MF); three functions provide information on the possible roles played by the genes in the plant; of interest were the genes responsible or fiber qualities and yield-related traits. The choice of genes used for GO analysis was based on the genes mined from the two clusters, clusters 1 (D03) and 2 (D12), which had a high percentage of phenotypic variation (PV) and heritability (Hb).

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References

- Bradbury PJ, Z.Z., Kroon DE, et al TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 2007. 23: p. 2633–2635

- Broman, K.W.W., H.; Sen, S.; Churchill, G.A. , R/QTL: QTL mapping in experimental crosses. *Bioinformatics*, 2003. 19: p. 889–890.
- Chaudhary, B., Ran Hovav, Ryan Rapp, Neetu Verma, Joshua A. Udall, and Jonathan F. Wendel, Global Analysis of Gene Expression in Cotton Fibers from Wild and Domesticated Gossypium Barbadense. *Evolution and Development* 2008. 10 (5): p. 567–82.
- Collard, B.C.Y. and D.J. Mackill, Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2008. 363(1491): p. 557-572.
- Danecek P, A.A., Abecasis G, Albers CA, Banks E, DePristo MA, et al. , The variant call format and VCFtools. *Bioinformatics*, 2011. 27(15): 2156–8.
- Davey, J.W.,P.A. Hohenlohe,P.D. Etter,J.Q. Boone,J.M. Catchen and M.L. Blaxter, Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, 2011. 12: p. 499–510
- Elshire RJ, G.J., Sun Q, et al A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 2011. 6.
- Fang, D.D.,J.N. Jenkins,D.D. Deng,J.C. McCarty,P. Li and J. Wu, Quantitative trait loci analysis of fiber quality traits using a random-mated recombinant inbred population in Upland cotton (Gossypium hirsutum L.). *BMC Genomics*, 2014. 15(1): p. 397.
- Glaubitz JC, C.T., Lu F, Harriman J, Elshire RJ, Sun Q, et al, TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. *PLoS One*, 2014. 9(2): e90346.
- Gong, W.,F. Xu,J. Sun,Z. Peng,S. He,Z. Pan et al., iTRAQ-Based Comparative Proteomic Analysis of Seedling Leaves of Two Upland Cotton Genotypes Differing in Salt Tolerance. *Frontiers in Plant Science*, 2017. 8.
- Guan, X., Jinsuk J. Lee, Mingxiong Pang, Xiaoli Shi, David M. Stelly, and Z. Jeffrey Chen, Activation of Arabidopsis Seed Hair Development by Cotton Fiber-Related Genes. *PLoS ONE* 2011. 6 (7).
- He DH,Lin ZX,Zhang XL,Nie YC,Guo XP,Zhang YX et al., QTL mapping for economic traits based on a dense genetic map of cotton with PCR-based markers using the interspecific cross of Gossypium hirsutum 9 Gossypium barbadense. *Euphytica* 2007. 153:181–197.
- Huang, X.,N. Kurata,X. Wei,Z.-X. Wang,A. Wang,Q. Zhao et al., A map of rice genome variation reveals the origin of cultivated rice. *Nature*, 2012. 490(7421): p. 497-501.
- IBM, C., SPSS Base 10.0 for Windows User's Guide. SPSS Inc., Chicago IL. 1999.
- Jamshed, M.,F. Jia,J. Gong,K.K. Palanga,Y. Shi,J. Li et al., Identification of stable quantitative trait loci (QTLs) for fiber quality traits across multiple environments in Gossypium hirsutum recombinant inbred line population. *BMC Genomics*, 2016. 17: p. 197.
- Kohel, R.J.,J. Yu and Y.H. Park, Molecular mapping and characterization of traits controlling fiber quality in cotton. *Euphytica*, 2001. 121: p. 163–172.
- Krizman M, J.J., Baricevic D, et al Robust CTAB-activated charcoal protocol for plant DNA extraction. . *Acta Agriculturae Slovenica*, 2006. 87: p. 427–433.
- Lacape, J.-M.,D. Llewellyn,J. Jacobs,T. Arioli,D. Becker,S. Calhoun et al., Meta-analysis of cotton fiber quality QTLs across diverse environments in a Gossypium hirsutum x G. barbadense RIL population. *BMC Plant Biology*, 2010. 10(1): p. 132.
- Lander, E. and L. Kruglyak, Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics*, 1995. 1: p. 1241–1247.
- Li H, D.R., Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009. 25: p. 1754–1760.
- Li, F.,G. Fan,C. Lu,G. Xiao,C. Zou,R.J. Kohel et al., Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. *Nat Biotechnol*, 2015. 33(5): p. 524-30.
- Liang, Q.,C. Hu,H. Hua,Z. Li and J. Hua, Construction of a linkage map and QTL mapping for fiber quality traits in upland cotton (Gossypium hirsutum L.). *Chinese Science Bulletin*, 2013. 58(26): p. 3233-3243.
- Lin, Z.,D. He,X. Zhang,Y. Nie,X. Guo,C. Feng et al., Linkage map construction and mapping QTL for cotton fibre quality using SRAP, SSR and RAPD. *Plant Breeding*, 2005. 124(2): p. 180-187.
- Lokhande, S. and K.R. Reddy, Quantifying Temperature Effects on Cotton Reproductive Efficiency and Fiber Quality. *Agronomy Journal*, 2014. 106(4): p. 1275.
- Ma Xuexia,Yezhang Ding,Baoliang Zhou,Wangzhen Guo,Yanhui Lv,Xiefei Zhu et al., QTL mapping in A-genome diploid Asiatic cotton and their congruence analysis with AD-genome tetraploid cotton in genus Gossypium. *Journal of Genetics and Genomics*, 2008. 35: p. 751-762.
- Ma, Q.,M. Wu,W. Pei,H. Li,X. Li,J. Zhang et al., Quantitative phosphoproteomic profiling of fiber differentiation and initiation in a fiberless mutant of cotton. *BMC Genomics*, 2014. 15(1): p. 466.
- Marlon Jocimar Rodrigues da Silva.,Tamires Esther Ferreira.,Sofia Domiciano.,Ana Paula.,Maia Paiva.,Marco Antonio Tecchio. et al., Phenology, yield and fruit quality of four persimmon (Diospyros kaki L.) cultivars in So Paulos Midwest countryside, Brazil. *African Journal of Agricultural Research*, 2016. 11(52): p. 5171-5177.
- Meuwissen, T.H.E.,B.J. Hayes and M.E. Goddard, Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 2001. 157: p. 1819–1829
- Oluoch, G.,J. Zheng,X. Wang,M.K.R. Khan,Z. Zhou,X. Cai et al., QTL mapping for salt tolerance at seedling stage in the interspecific cross of Gossypium tomentosum with Gossypium hirsutum. *Euphytica*, 2016. 209(1): p. 223-235.
- Paten B, N.A., Haussler D Mapping to a Reference Genome Structure. . *ArXiv e-prints* 2014: p. 1–26.

- Paterson, A.H.,C. Brubaker and J.F. Wendel, A rapid method for extraction of cotton (Gossypium spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Mol Biol Rep*, 1993 11 p. 122–127.
- Podliesna, S.,C.R. Bezzina and E.M. Lodder, Complex Genetics of Cardiovascular Traits in Mice: F2-Mapping of QTLs and Their Underlying Genes. *Methods in Molecular Biology*, 2017. 1488: p. 431-454.
- Poland, J.A. and T.W. Rife, Genotyping-by-sequencing for plant breeding and genetics. *Plant Genome* 2012. 5: p. 92–102
- Pu, L.,Q. Li,X. Fan,W. Yang and Y. Xue, The R2R3 MYB Transcription Factor GhMYB109 Is Required for Cotton Fiber Development. *Genetics*, 2008. 180(2): p. 811-820.
- Rong J,Feltus FA,Waghmare VN,Pierce GJ,Chee PW,Draye X et al., Meta-analysis of polyploidy cotton QTL shows unequal contributions of subgenomes to a complex network of genes and gene clusters implicated in lint fiber development. *Genetics*, 2007. 176:2577–2588.
- Rong, J.,C. Abbey and J.E. Bowers, A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (Gossypium). *Genetics and Molecular Biology*, 2004. 166: p. 389–417.
- Saba, J.,M. Moghaddam,K. Ghassemi and M.R. Nishabouri, Genetic properties of drought resistance indices. *Journal of Agricultural Science and Technology*, 2001. 3: p. 43–49.
- Said Joseph I Z.L., Xianlong Zhang, Mingzhou Song and Jinfa Zhang, A comprehensive meta QTL analysis for fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton. *BMC Genomics* 2013. 14: p. 776.
- Shang, L.,Q. Liang,Y. Wang,X. Wang,K. Wang,A. Abduweli et al., Identification of stable QTLs controlling fiber traits properties in multi-environment using recombinant inbred lines in Upland cotton (Gossypium hirsutum L.). *Euphytica*, 2015. 205(3): p. pp 877–888.
- Shao, Q.,F. Zhang,S. Tang,Y. Liu,X. Fang,D. Liu et al., Identifying QTL for fiber quality traits with three upland cotton (Gossypium hirsutum L.) populations. *Euphytica*, 2014. 198(1): p. 43-58.
- Shen, X.,W. Guo and Q. Lu, Genetic mapping of quantitative trait loci for fiber quality and yield trait by RIL approach in upland cotton. *Euphytica*, 2007. 155: p. 371–380.
- Shen, X.L.,W.Z. Guo,X.F. Zhu,Y.L. Yuan,J.Z. Yu,R.J. Kohel et al., Molecular mapping of QTLs for fiber qualities in three diverse lines in Upland cotton using SSR markers. *Molecular Breeding*, 2005. 15(2): p. 169-181.
- Singh, U.M.,S. Yadav,S. Dixit,P.J. Ramayya,M.N. Devi,K.A. Raman et al., QTL Hotspots for Early Vigor and Related Traits under Dry Direct-Seeded System in Rice (Oryza sativa L.). *Frontiers in Plant Science*, 2017.
- Socquet-Juglard, D.,D. Christen,G. Devenes,C. Gessler,B. Duffy and A. Patocchi, Mapping Architectural, Phenological, and Fruit Quality QTLs in Apricot. *Plant Molecular Biology Reporter*, 2013. 31(2): p. 387-397.
- Spindel, J.,M. Wright,C. Chen,J. Cobb,J. Gage,S. Harrington et al., Bridging the genotyping gap: using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. *Theoretical and Applied Genetics*, 2013. 126(11): p. 2699-716.
- Stam, P., van Ooijen, J.W. JoinMapTM Version 2.0: Software for the Calculation of Genetic Linkage Maps; Plant Research International B.V. and Kyazma B.V.: Wageningen, The Netherlands, 1995. *Wageningen: CPRO-DLO*, 1995.
- Stuber, C.,M.D. Edwards and J.F. Wendel, Molecular marker facilitated investigations of quantitative trait loci in maize.II. Factors influencing yield and its component traits. *Crop Science*, 1987. 27: p. 639–648.
- Sun, F.-D.,J.-H. Zhang,S.-F. Wang,W.-K. Gong,Y.-Z. Shi,A.-Y. Liu et al., QTL mapping for fiber quality traits across multiple generations and environments in upland cotton. *Molecular Breeding*, 2012. 30(1): p. 569-582.
- Tan, Z.,X. Fang,S. Tang,J. Zhang,D. Liu,Z. Teng et al., Genetic map and QTL controlling fiber quality traits in upland cotton (Gossypium hirsutum L.). *Euphytica*, 2014. 203(3): p. 615-628.
- Team, R.D.C., R: A language and environment for statistical computing. R Foundation for Statistical Computing. *Vienna, Austria*, 2008.
- Thomson, M., Zhao, K., ,M. Wright, McNally, K., ,J. Rey, Tung, C.W., Reyn-,A. olds, Scheffler B, Eizenga G,,K.H. McClung A, Ismail A,,M.C. de Ocampo M et al., High-throughput single nucleotide polymorphism genotyping for breeding applications in rice using the BeadXpress platform. *Molecular Breeding*, 2012. 29(4): p. 875–886.
- Tiwari, S.C., and T A Wilkins, Cotton (Gossypium-Hirsutum) Seed Trichomes Expand Via Diffuse Growing Mechanism. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 1995. 73 (5): p. 746–57.
- Voorrips, R.E., MapChart: Software for the graphical presentation of linkage maps and QTLs. . *The Journal of Heredity* 2002. 93 (1): p. 77-78.
- Wang BH,Guo WZ,Zhu XF,Wu YT,Huang NT and Z. TZ, QTL mapping of fiber quality in an elite hybrid derived-RIL population of upland cotton. *Euphytica*, 2006 152:367–378.
- Wang, H.,C. Huang,W. Zhao,B. Dai,C. Shen,B. Zhang et al., Identification of QTL for Fiber Quality and Yield Traits Using Two Immortalized Backcross Populations in Upland Cotton. *Plos One*, 2016. 11(12): p. e0166970.
- Wang, K.,H. Wang,C. Huang,H. Guo,X. Li,W. Zhao et al., QTL Mapping for Fiber and Yield Traits in Upland Cotton under Multiple Environments. *Plos One*, 2015. 10(6): p. e0130742.
- Wang, K.,Z. Wang,F. Li,W. Ye,J. Wang,G. Song et al., The draft genome of a diploid cotton Gossypium raimondii. *Nature Genetics*, 2012. 44(10): p. 1098-1103.
- Wang, S. and Z.B. Zeng, Department of Statistics. North Carolina State University, Raleigh, NC.Windows QTL Cartographer 2.5. 2007.
- Wilfinger WW, M.K., Chomczynski P 260/280 and 260/230 Ratios NanoDrop ® ND-1000 and ND-8000 8-Sample Spectrophotometers. *BioTechniques* 1997. 22: p. 474–481.

- Xu, X.,X. Liu,S. Ge,J.D. Jensen,Hu,X. Li et al., Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nature Biotechnology*, 2012. 30(1): p. 105–111.
- Yu, J.,Zhang K,Li S,Yu S,Zhai H,Wu M et al., Mapping quantitative trait loci for lint yield and fiber quality across environments in a Gossypium hirsutum × Gossypium barbadense backcross inbred line population. *Theoretical and Applied Genetics*, 2013. 126: p. 275–287.
- Yu, Y.,D. Yuan and S. Liang, Genome structure of cotton revealed by a genome-wide SSR genetic map constructed from a BC1 population between Gossypium hirsutum and G. barbadense. *BMC Genomics*, 2011. 12: 15.
- Zhang, S.W.,X.F. Zhu,L.C. Feng,X. Gao,B. Yang,T.Z. Zhang et al., Mapping of fiber quality QTLs reveals useful variation and footprints of cotton domestication using introgression lines. *Scientific Reports*, 2016. 6: p. 31954.
- Zhang, Z.-S.,M.-C. Hu,J. Zhang,D.-J. Liu,J. Zheng,K. Zhang et al., Construction of a comprehensive PCR-based marker linkage map and QTL mapping for fiber quality traits in upland cotton (Gossypium hirsutum L.). *Molecular Breeding*, 2009. 24(1): p. 49-61.
- Zhang, Z.S.,X.B. Li and Y.H. Xiao, Combining ability and heterosis between high strength lines and transgenic Bt (Bacillus thuringiensis) bollworm-resistant lines in upland cotton (Gossypium hirsutum L.). *Agricultural Sciences in China*, 2003. 2: p. 13–18.
- Zhang, Z.-S.,Y.-H. Xiao,M. Luo,X.-B. Li,X.-Y. Luo,L. Hou et al., Construction of a genetic linkage map and QTL analysis of fiber-related traits in upland cotton (Gossypium hirsutum L.). *Euphytica*, 2005. 144(1-2): p. 91-99.
- Zhen, P.,Shoupu He,G. Wenfang,S. Junling,P. Zhaoe,X. Feifei et al., Comprehensive analysis of differentially expressed genes and transcriptional regulation induced by salt stress in two contrasting cotton genotypes. *BMC Genomics* 2014. 15: p. 760.
- Zhu, T.,C. Liang,Z. Meng,G. Sun,Z. Meng,S. Guo et al., CottonFGD: an integrated functional genomics database for cotton. *BMC Plant Biology*, 2017. 17(1): p. 101.

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Studies on Selection Efficiency, Performance Stability and Plant Type in Newly Developed Genotypes of Cotton (*Gossypium hirsutum L.*)

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Abstract

Background: *The development of new productive varieties is a constant endeavor of a plant breeder. Planned crosses were executed focusing on high seed cotton yield in the final derived varieties. The present exercise spanned eight years from 2009-10 till 2017-18 and will culminate in the release of new varieties. Apart from yield, an exercise was made to assess the selection efficiency achieved by picking the two best individuals in all the segregating generations and tracking their progeny performance in the stabilized generations later. Secondly, plant type was assessed as there is an increasing need for compact genotypes suited for high-density planting and robust genotypes for intensive cultivation. Thirdly, the yield stability of 44 genotypes derived from the planned crosses was also evaluated over three years. Overall, a consolidated effort was made to address three facets of a practical cotton breeding exercise spread over eight years aiming at the successful identification of productive genotypes suited to varying situations.*

Results: *The study has given satisfying results. It was realized that just two individual plant selections in each cross starting from F3 till F5 followed by yield evaluation trials in later generations was quite efficient in giving high-yielding genotypes thus proving practically rewarding. There was a mean improvement of 58 percent in the IPS 1 selection set as compared to 20.8 percent in the IPS 2 selection set over years. However, the second plant selection set showed a higher mean yield compared to the IPS 1 set proving that recombination in the segregating generations can throw up productive genotypes from relatively less productive ones picked in the F2. Nineteen high-yielding genotypes were identified against a medium-low-yielding check. Most high-yielding stable genotypes were highly robust types suited to rich environments. One compact genotype, L9T4-8-1 was stable with a high yield suited to all environments which can be adopted for high-density planting also.*

Conclusions: *The study proved that resources can be judiciously used while not compromising on the results underlining the importance of judging a cross for its future worth. The environment has a huge role in deciding the usability of a genotype and hence identifying plant types for different growing situations is a must. Compact plant types in high-density planting become highly amenable to machine harvesting in cotton.*

Keywords: *Selection efficiency, Compact plant type, Stability, Cotton, Gossypium hirsutum L.*

Background

The development of new productive cotton varieties is a constant endeavor of a plant breeder. New variability is usually created through planned crosses having carefully chosen parents focusing on high seed cotton yield in the final derived varieties. During the process of selection in the segregating generations, there will always be a trade-off between resources and breeding priorities. It is common practice to choose many individual plants in the early segregating generations. But is the high number justified? Can we reduce the number and still be able to isolate good genotypes? If we do, what will be the consequences? Answers to the above could only be found by embarking upon a systematic breeding program that was planned. Carefully chosen parents were used in producing hybrids which were evaluated and 9 of them were continued for isolating varieties. Only two individual plant selections were practiced in F3 and onwards till F5 in every progeny. Thus, 22 progeny pairs were making up 44 genotypes which were evaluated over 3 years to assess their stability. In case superior genotypes were identified, it would prove the rationale of stringent selection in making plant breeding efficient.

With the climate variations bringing a change in the growing conditions of cotton, it is imperative to identify plant types that suit these environments. A resource-poor rainfed area may need a compact plant type suited for high-density planting and robust genotypes may suit well for intensive cultivation.

Compact types are highly amenable to machine picking which could be the next important intervention in cotton cultivation. Hence, there is a need to characterize the cotton plant type. Any new variety to be successful across a wide area needs to be assessed for its stability. Testing the yield stability of the 44 genotypes derived from the planned crosses over three years was a natural corollary. Overall, a consolidated effort was made to address three facets of a practical cotton breeding exercise spread over eight years aiming at successful identification of productive genotypes suited to varying situations.

Results

The results of the comparison of IPS 1 with IPS 2 of all the 22 pairs are given in Table 1. The mean seed cotton yield over years fell drastically owing to the harsh climate caused by less rainfall and late sowing. Significant differences among the 22 progeny pairs were evident pointing toward effective genetic material generation. The heritability and genetic advance over the mean were very high, making selection effective. The progeny pairs were evaluated during 2015-16, 2016-17, and 2017-18. Results are presented in Table 2. Over three years, nearly 59.1 % of the progeny pairs were consistent in yield. Six (27.3 %) showed an increase of IPS 1 over 2 and seven (31.8 %) showed better performance of IPS 2 over 1. Nine pairs were inconsistent. The average increase of yield in the IPS 1 set over the IPS 2 set was more (58.0 %) than in IPS 2 set over IPS 1 set (20.8 %). Among the two consistent IPS sets, two progenies became progressively more productive over the three years. These increases point to the increased efficiency achieved in simple yield-based selection. Also, it showed that the increase within IPS 1 set was better than in IPS 2 set over 3 years. But, the yield levels of the consistent IPS 2 set were more than in the consistent IPS 1 set across the 13 genotypes. This was despite the harsh climate during both the second and third years when average yields fell by over 150 percent over three years. There was an increase of 4.56 percent in the IPS 1 set over the IPS 2 set across the 3 years. The consistency in the performance of the 13 progenies points to the accumulation of desirable genes leading to produce new varieties. Interestingly, no correlation existed between IPS 1 and 2 sets during 2015-16 and 2016-17. However, there was a positive significant correlation during 2017-18. Again, considering the correlation over three years, there was no correlation between the yields of the two IPS sets.

The 44 above said derived genotypes were evaluated for yield performance over three years. The correlation between all combinations of years gave positive and significant results. Since performance stability is key for the release of a variety, evaluation done over three years was subjected to stability analysis. The results are shown in Table 3. Of the 44 genotypes, 19 showed high to very high yield levels ranging from 1988 kg/ha to 1292 kg/ha. The rest 25 had medium to low yields. Thirty-seven genotypes were better than the check variety, ARBH-813 (1002 kg/ha). Genotypes L3T2-2-2 (1988 kg/ha) and L6T4-3-2 (1666 kg/ha) showed very high yield levels. But both were suited to rich environments and had highly robust plant types. The most rewarding success of the entire exercise is the performance of the genotype L9T4-8-1 (1445 kg/ha). This is a compact genotype that can be planted in high density to further increase productivity.

Table 1-A comparative performance of the two IPS sets over years

Seed cotton yield (kg/ha) of Set 1 (IPS 1)					Seed cotton yield (kg/ha) of Set 2 (IPS 2)				
Entry	2015-16	2016-17	2017-18	Mean	Entry	2015-16	2016-17	2017-18	Mean
L5T3-4-1	1838	1069	733	1213	L5T3-4-2	1691	1259	1009	1320
L9T4-3-1	1573	1137	665	1125	L9T4-3-2	724	550	643	639
L6T4-8-1	1679	962	779	1140	L6T4-8-2	600	809	329	579
L5T3-6-1	1298	1005	722	1008	L5T3-6-2	1846	1216	926	1330
L9T4-8-1	2274	1259	801	1445	L9T4-8-2	1734	968	926	1209
L1T1-8-1	1729	947	643	1106	L1T1-8-2	1761	1286	703	1250
L7T2-4-1	1466	831	495	931	L7T2-4-2	1245	1148	616	1003
L7T1-4-1	1722	1169	522	1138	L7T1-4-2	1623	962	816	1134
L9T4-7-1	2326	1026	639	1330	L9T4-7-2	1235	767	507	836
ARBH-813	1439	788	491	906	ARBH-813	1173	1243	590	1002
L2T1-8-1	1876	1211	386	1158	L2T1-8-2	1905	1391	593	1296
L7T2-5-1	2135	1216	635	1329	L7T2-5-2	978	1142	533	884
L5T3-10-1	2081	1185	609	1292	L5T3-10-2	1954	841	711	1169
L6T4-2-1	2009	1466	684	1386	L6T4-2-2	1467	1238	677	1127
L7T1-6-1	1645	1174	699	1173	L7T1-6-2	2117	1312	484	1304
L7T2-1-1	2355	2285	567	1736	L7T2-1-2	1704	1343	454	1167

L8T4-10-1	1831	1180	735	1249	L8T4-10-2	1904	1417	900	1407
L8T4-1-1	2021	1397	665	1361	L8T4-1-2	2183	957	782	1307
L1T1-7-1	1464	1507	692	1221	L1T1-7-2	1962	1275	571	1269
L3T2-6-1	1953	1592	813	1453	L3T2-6-2	1962	1645	843	1483
L6T4-3-1	2225	1201	824	1417	L6T4-3-2	2340	1634	1024	1666
L3T2-2-1	2152	1296	926	1458	L3T2-2-2	2758	2158	1047	1988
Mean	**1868**	**1223**	**669**	**1253**	Mean	**1676**	**1207**	**713**	**1199**

Correlation between Set I and Set II over years			Mean of 3 years
2015-16	2016-17	2017-18	0.35 ns
0.26 ns	0.25 ns	0.50*	

Table 2- Per cent increase between IPS in the consistent genotypes

Genotype	Per cent increase of IPS 1 over IPS 2				Mean Seed Cotton Yield (kg/ha)		
	2015-16	2016-17	2017-18	Mean	IPS 1	IPS2	
L9T4-3-1	117.2	106.7	3.5	75.8	1125	L9T4-3-2	639
L6T4-8-1	179.5	18.9	136.8	111.7	1140	L6T4-8-2	579
L9T4-7-1	88.4	33.8	26.1	49.4	1330	L9T4-7-2	836
L7T2-5-1	118.2	6.5	19.1	48.0	1329	L7T2-5-2	884
L6T4-2-1	37.0	18.4	1.1	18.8	1386	L6T4-2-2	1127
L7T2-1-1	38.2	70.1	25.0	44.5	1736	L7T2-1-2	1167
Mean	96.4	42.4	35.3	58.0	1341		872

Genotype	Per cent increase of IPS 2 over IPS 1				Mean SCY (kg/ha)		
	2015-16	2016-17	2017-18	Mean	IPS2	IPS 1	
L5T3-6-2	42.3	21.0	28.3	30.5	1330	L5T3-6-1	1008
L1T1-8-2	1.8	35.8	9.4	15.7	1250	L1T1-8-1	1106
L2T1-8-2	1.5	14.9	53.9	23.4	1296	L2T1-8-1	1158
L8T4-10-2	4.0	20.1	22.4	15.5	1407	L8T4-10-1	1249
L3T2-6-2	0.4	3.3	3.7	2.5	1483	L3T2-6-1	1453
L6T4-3-2	5.2	36.1	24.3	21.9	1666	L6T4-3-1	1417
L3T2-2-2	28.2	66.5	13.1	35.9	1988	L3T2-2-1	1418
Mean	11.9	28.2	22.2	20.8	1489		1258

It was suited to all environments and had high yield. Another genotype, L7T1-6-2 (1304 kg/ha) was also suited to all environments, had high yield and was robust in nature. There were 10 unstable varieties, though one genotype L7T2-1-1, had very high yield of 1736 kg/ha. Most good yielders were in the high yield to very high yield range and were mostly robust in plant type and were suited to rich environments. Low yielders were in general suited to poor environments.

Table 3- Stability analysis and Plant type characterization

Genotype	2015-16	2016-17	2017-18	Mean Seed Cotton Yield (kg/ha)	bi	S^2di	Yield type	Plant Type
L3T2-2-2	2758	2158	1047	1988	1.58*	38540.9	Very High	HR
L7T2-1-1	2355	2285	567	1736	1.64	468385.7*		C
L6T4-3-2	2340	1634	1024	1666	1.22*	-13675.3		HR
L3T2-6-2	1962	1645	843	1483	1.03*	30719.3	High	HR
L3T2-2-1	2152	1296	926	1458	1.14*	19326.8		C
L3T2-6-1	1953	1592	813	1453	1.05*	19959.5		HR
L9T4-8-1	2274	1259	801	1445	1.37	29543.1		**C****
L6T4-3-1	2225	1201	824	1417	1.30*	46630.6*		C
L8T4-10-2	1904	1417	900	1407	0.93*	-13586.8		HR
L6T4-2-1	2009	1466	684	1386	1.22*	-1246		C
L8T4-1-1	2021	1397	665	1361	1.25*	-10502.3		R
L9T4-7-1	2326	1026	639	1330	1.57*	109420.8*		SC
L5T3-6-2	1846	1216	926	1330	0.85*	2097.6		SC
L7T2-5-1	2135	1216	635	1329	1.39*	67.1		R
L5T3-4-2	1691	1259	1009	1320	0.63*	-9846		C
L8T4-1-2	2183	957	782	1307	1.31*	155478.9*		HR
L7T1-6-2	2117	1312	484	1304	1.51	-13328		**R**
L2T1-8-2	1905	1391	593	1296	1.21*	3196.5		C
L5T3-10-1	2081	1185	609	1292	1.36*	-1591.1		C
L1T1-7-2	1962	1275	571	1269	1.29*	-13615.9	Medium	HR
L1T1-8-2	1761	1286	703	1250	0.98*	-10940.7		R

L8T4-10-1	1831	1180	735	1249	1.01*	-9235.7		R
L1T1-7-1	1464	1507	692	1221	0.71	115435.4*		R
L5T3-4-1	1838	1069	733	1213	1.03*	12584.53		C
L9T4-8-2	1734	968	926	1209	0.75	67394.6*		R
L7T1-6-1	1645	1174	699	1173	0.87*	-14029.8		TC
L5T3-10-2	1954	841	711	1169	1.16*	134464.1*		SC
L7T2-1-2	1704	1343	454	1167	1.15*	39245.3		R
L2T1-8-1	1876	1211	386	1158	1.38*	-7167		SC
L6T4-8-1	1679	962	779	1140	0.84*	28458.6		R
L7T1-4-1	1722	1169	522	1138	1.11*	-11360.7		C
L7T1-4-2	1623	962	816	1134	0.75*	25986.2		R
L6T4-2-2	1467	1238	677	1127	0.73*	6951		R
L9T4-3-1	1573	1137	665	1125	0.84*	-13546.4		C
L1T1-8-1	1729	947	643	1106	1.01*	18761		R
L5T3-6-1	1298	1005	722	1008	0.53*	-14191.1		R
L7T2-4-2	1245	1148	616	1003	0.58*	20156		C
ARBH-813	1173	1243	590	1002	0.53	77150.6*		C
L7T2-4-1	1466	831	495	931	0.90*	-1977.5		R
ARBH-813	1439	788	491	906	0.88	3482.1		C
L7T2-5-2	978	1142	533	884	0.40	88728.2*		R
L9T4-7-2	1235	767	507	836	0.68*	-8493.2	Low	HR
L9T4-3-2	724	550	643	639	0.08	-2544.7		CS
L6T4-8-2	600	809	329	579	0.24	66706.9*		C
Mean	**1772**	**1215**	**691**		SC- Super Compact		TC-Tall Compact	
CV	15.5	9.5	15.2		C- Compact		R- Robust	
CD5%	141	233	212		CS-Compact Spreading		HR- Highly Robust	

The study yielded genotypes with different combinations of traits and plant type categorization was also done. Plant type was categorized into 6 classes. Accordingly, the distribution of the 44 genotypes with their average yield level in parentheses was, four (1246 kg/ha) in Super-compact, fifteen (1221 kg/ha) in Compact, one (639 kg/ha) in Compact-spreading, one (1173 kg/ha) in Tall-compact, fifteen (1161 kg/ha) in Robust and eight (1426 kg/ha) in Highly-robust classes. As a group, the super compacts were better than the robust types and only marginally less than the highly robust class.

Table 4 The distribution of 44 genotypes in to plant type classes and the mean yield of the six different classes

Plant height (cm)	Plant Diameter (cm)		
	41-57	58-74	75-91
77-98 99-119	Super Compact Tall Compact	Compact Robust	Compact Spreading Highly Robust
The 44 genotypes spread across plant type classes			
Number of genotypes	4 1	15 15	1 8
Seed Cotton Yield (kg/ha)			
Group yield	1246 1173	1221 1161	639 1426

Discussions

The present plant breeding exercise aimed at the development of productive cotton varieties. Planned crosses between carefully chosen parents were done. This is the beginning of new variability being created. The IPS would then begin in F2 and continue till F5 before the identical looking fairly stabilized plants are bulked and genotype evaluation starts in F6. Unlike in other selection programs, fewer IPS were planned, in fact just two IPS per progeny in a generation. It is here that the novelty of this study becomes evident. Since the best two plants were chosen, the chances of identifying a better genotype in the end increase. The observed differences in the yield between the IPS sets 1 and 2 could be attributed to the good genes being collected over years of selection in a genotype, preferentially. Selection from F2 on visual and yield basis was adopted. The results, over 3 years, showed consistent superiority of IPS 1 over IPS 2, vice-versa and at the same time there were erratically behaving genotypes considered as inconsistent. In the end, a superior genotype might have come from either IPS in the two consistent sets. It can be surmised

that two good IPS per progeny in each generation can reduce cost of breeding without compromising on efficiency.

It is the fixation of the genes which determines the final expression. Environment also plays a role in the differential expression. Hence, stability analysis was carried out in the newly developed genotypes. There were all types of genotypes ranging from unstable but high yielding to stable but low yielding. Promising genotypes were then identified to suit the requirements of a place. If intensive cultivation needs to be done with high input cost then it is the robust to highly robust genotypes which can be grown. Since, the rainfed area in India is huge, the niche requirement would be a compact plant variety which can be grown in high density situations and harvested mechanically thereby saving a lot in the cost of cultivation.

Plant type characterization is a key component in ideotype breeding. A simple method which depends on relative expression of plant type in genotypes was developed earlier to characterize the cotton plant and is now being used successfully to designate genotypes to the different plant type classes. The identification of high yielding compact and highly robust genotypes in this exercise which began with only two IPS per progeny is hugely commendable. There were no parallel studies found in this direction and hence the paucity of literature.

Conclusions

The study proved that resources can be judiciously used while not compromising on the results underlining the importance of judging a cross for its future worth. Further, as few as two IPS in the segregating generations could lead to the development of productive genotypes. The environment has a huge role in deciding the usability of a genotype and hence identifying plant types for different growing situations is a must. The method to characterize the plant type in cotton is quite simple and fairly standardized. Compact plant types in high-density planting become highly amenable to machine harvesting in cotton and this can be the future of cotton cultivation. An additional attraction is the availability of the Cry 1Ac Bt. Gene can be introgressed into such compact Gossypium hirsutum L. varieties which can pose a challenge to the hybrids. The multipronged breeding approach followed here has been successful and can serve as a model.

Methods

Selection following the creation of new variability is an integral part of crop improvement which ends in the choice of the best performing genotype. Individual plant selection (IPS) is resorted to in cotton in the early generations of the segregating generations. The promising Gossypium hirsutum L. genotypes viz., CPD-813, 8-1-2, L-761, R-221, Sahana, 1-2-1, SC-81, and DC-12 IPS were involved in producing forty hybrids in a line X tester set up in 2009-10 and were evaluated in 2010-11. Nine promising F1s, based on performance and morphological traits, especially root and shoot parameters, were selected and advanced to the F2 generation in 2011-12. The objective was to screen and develop genetic material for the rainfed situation. Parameters like root: shoot ratio, high biomass, and high productivity were considered.

The F2 generation of the nine hybrids was assessed and the IPS was made in each population. The number of F2 plants chosen per cross was different ranging from 6 to 16. These F2 plants were taken to F3 generation in 2012-13. To assess the efficiency of the method of selection, in the F3 generation, 2 promising high-yielding IPS per progeny of the nine crosses were made. The same was done till F5 and similar looking four plants were bulked to serve the seed requirement for a replicated evaluation in F6. The 22 pairs were evaluated at ARS Dharwad during 2015-16. Such pairs numbered 22, including a pair of the check ARBH-813. The 2 IPS were chosen based on yield per plant and general vigour. IPS 1 was relatively superior to IPS 2 in all the progenies. Across the hybrids, there were 44 genotypes that were evaluated in 2015-16 (F6), 2016-17 (F7), and 2017-18 (F8) in replicated design with 4-row plots per genotype for stability analysis of yield as per Eberhart and Russell (1966). The IPS 1 of all 22 genotypes is called Set 1 and the IPS 2 of all is called Set 2. A comparison between the sets was also

made across the three years. The performance of each progeny pair vis-a-vis each other over the three years was also tracked. If the IPS 1 was always better than IPS 2 or vice-versa over years, the pair was considered a consistent performer. Differential performance of the two IPS over years was considered inconsistent. The rationale behind such comparison was to see if the genetic recombinations were capable of giving highly productive genotypes in either IPS of the 22 progenies and also to visualize the effect of environment on such recombinants over years. In the end, a superior genotype may have come from either IPS even though IPS 1 was superior to IPS 2 when the journey began. Stability analysis of the 44 genotypes was carried out to ensure the stable performance of a genotype along with high yield which is the practical outcome of such an elaborate experiment.

In 2015-16, the genotypes were also characterized for the plant type as delineated by Rajesh et al., (2016). Plant height and plant diameter were considered to group the genotypes into 6 classes viz., Super-compact, Compact, Compact-spreading, Tall-compact, Robust, and Highly-robust (Table 4). Plant diameter was calculated based on the sympodial length at 50% plant height and the angle it made with the main stem. The categories of plant diameter and plant height were based on the Index Score method of Singh and Chaudhary (1985) where the range of the traits was equally divided into 3 and 2 categories, respectively. This was done to identify genotypes suitable for resource-poor rainfed areas as well as for rich environments. A compact genotype can be planted in high density under rainfed conditions which can be mechanically harvested making cotton variety cultivation more remunerative. For intensive cultivation, robust types can be used.

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References

- Rajesh S. Patil, Khadi B.M., Katageri I.S., Vamadevaiah H.M and Patil S.S., 2016,
- Breeding for productive compact cotton varieties and a method to categorise the plant type in Gossypium hirsutum L. World Cotton Research Conference-6, GOIANIA-GOIAS, BRAZIL MAY, 2-6, 2016, Book of abstracts
- Eberhert, S. A. and Russel, W. L., 1966, Stability parameters for comparing varieties. Crop Sci., 6: 36-40.
- Singh, R. K. and Chaudhary, B. D., 1985, Biometrical methods in quantitative genetic analysis. 39-68. Kalyani Publishers, New Delhi.

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The Characterization of Anatomical Indices of Seed Coat Formation of Diploid and Tetraploid Representatives of the Genus *Gossypium L*

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Abstract

As of today ripening of cotton breeds and infestation of its fiber is one of the global problems on a world scale, causing great harm to the branches of economics. 'At present, in the world, the most widely used is textile fiber. At present its market share is 56 percent, fiber infestation problem is one of the main'. In agriculture of our country during the years of independence wide-ranging reforms are held, meanwhile, special attention was paid to the gaining of early ripening and less infested breeds of the cotton plant. On the ground of realized program measures in this area, certain results are obtained, including the use of some representatives not by a long shot of the genetic potential of a genus, having useful economic valuable features, as well as the preservation of the global gene pool of cotton for its further use in genetic and breeding works.

Keywords: *Genus Gossypium, seed anatomy, earliness, pest resistance, inter-specific crossing*

Background

One of the actual problems is the determination of predisposition to fiber defect-formation based on anatomical traits of species Gossypium L. genus and their introduction into practice. Determination of the anatomical features of generative organs of Gossypium L. genus for determination of fiber contamination and their use in practice is as follows: classification of systematics and phylogeny of species of Gossypium L. genus, the study of the structural features of the development of the generative organs with the help of anatomical methods, identification of biometrical readings, rate of growth and development integumental layers; identification of diagnostically significant features in the structure of the seed coat.

Foreign scientists Patil Pravin, Malik Surendra, Carlos B. Armijo, Kevin D., et al., Frank E. Groves, Freddie M.Bourland., J. Clif Boykin, and Sam Ray., carried out research in the field of micro- and macro morphology of seeds as well as layered surface structure of a peel of a mature seed from various representatives of cotton, it was found that the successful use of LM/SEM technique assists in solution of taxonomy problems as well as genetic resources management of a genus. In particular, it was determined the dependence of seed and gin spindle diameter, as well as the number of turns and the effect of these parameters on fiber infections.

The aim of the study is the determination of signs of formation and rate of development of the seed coat of breeds for clarification of the systematic position and evolution of the Gossypium L. genus and the possibility of practical use of individual representatives in selection.

The objects and methods of the study are wild subspecies of cultivated species (2n = 26), cultivars and breed-samples created on the basis of their subtropical subspecies: G. herbaceum (subsp. africanum, subsp. pseudoarboreum, subsp. frutescens, A-738 (90 days), A-833 (105 days), A-739 (135 days), A-184 (149 days)); G. arboreum (subsp. obtusifolium, subsp. perenne, subsp. neglectum, subsp. nanking, A-352 (85 days), A-361 (115 days), A-2802 (147 days), A-2845 (161 days)).

The experiments were carried out in 2005-2015. Seed material was obtained from the collection of cotton gene pool of Laboratory of Systematic and introduction of cotton IG & EBP Uzbek Academy of Sciences. We studied 50 plants of each breed and breed sample grown on allotment areas of the

laboratory. Temporal fixation was performed for anatomical analysis (in 50% of ethanol) of ovules uneven-aged ovaries (1, 2, 3, 4-week-old and mature seeds). Anatomical investigations were performed according to accepted methods of R. P. Barikina et al. (2000). At conduction of research microscopes Avicon Tex and Leica ES3.

Cultivated representatives of *G. herbaceum*

Active development and growth of ovule integuments begin from fertilization moment, and differentiation into separate layers marked by us in all 2 weeks-old age (from blossoming). Each integument - outer (OI) and inner (II) consists of 3 outer layers- (OE) and inner (IE) layer epidermis and parenchyma (P) located between them in all studied representatives (see Fig.1). Breed samples of G. herbaceum species, possessing the general plan of the structure of seed peel differ from each other in terms of quantitative readings of structural signs, rate, and duration of growth of integumental layers, the percentage of tissues in the ovules of different ages in all stages of development. The epidermal layers of the outer integument (OEOI, IEII) and internal (IEII) are monostichous. OEOI cells increase in size, their walls are thickened and lignified. A parenchymal layer of the outer integument (POI) 3-4 or 4-6-rows depending on the sample, a great number of rows (6) in late-ripening breed-sample A-184. The numbers of rows in the parenchyma in the process of development of integuments in this layer do not change. The cells of all layers of the outer integument are developing at unequal speeds and thus reach a maximum value at different times. Cells of parenchymal layers differ with a higher rate of development. In mature peel, they become elongated in the tangential direction of the form in all studied representatives. The walls of the cells of all integumental layers are thickened and lignified. The outer integument at the initial stage of development is thicker in late breed-sample A-184, and the peel of the mature seeds in early-ripening breed-sample A-738.

Age-specific alterations more intensively occur in the inner integument, whose thickness is always greater than the outside. The outer epidermis (OEII) – is represented by a single-row palisade layer, in which cells in the development process rapidly increased in the first 2-3 weeks in the radial direction and reach the greatest height at the time of maturity. In early-ripening and middle-maturing breeds they increase by 10-12 times, and the late-maturing A-184 15.5 times. The growth rate of the cell layer is higher, in early-ripening breed samples A-738, A-833, and the height (thickness) in the late-ripening breed-sample A-184.

A parenchymal polystichous layer of inner integuments differs in thickness during all development time as compared to the other layers and the most intense cell growth up to 2 weeks of age. The maximum thickness of this layer reaches all samples in 3 weeks of age. In the early ripening breed sample, A-738 in this age parenchymal layer is thicker than late ripening breed-sample A-184, in the peel of the mature seeds- in the late-ripening. The number of parenchyma cells' rows in the development process is changed. In the integuments of 2 weeks-old, they form 15-20 rows at ripening (A-738, A-833) and 18-21- at late ripening (A-739, A-184) representatives. By the time of maturity, the number of rows is reduced to 3-4 times due to starting with a 3-week-old process of degradation of the lower layers of cells, adjacent to the inner epidermis. As a result, the thickness of the parenchymal layer decreased to 14 times in precocious breed-sample A-738 and 8.5 in late-ripening ones. The destruction and reduction processes of parenchymal layer are more active in early ripening (A-738, A-833) and middle ripening (A-739) breed-samples. Cells of this layer are different from others by the high speed of up to two weeks of age and especially in late-ripening A-184, and subsequently from precocious breed-samples A-738.

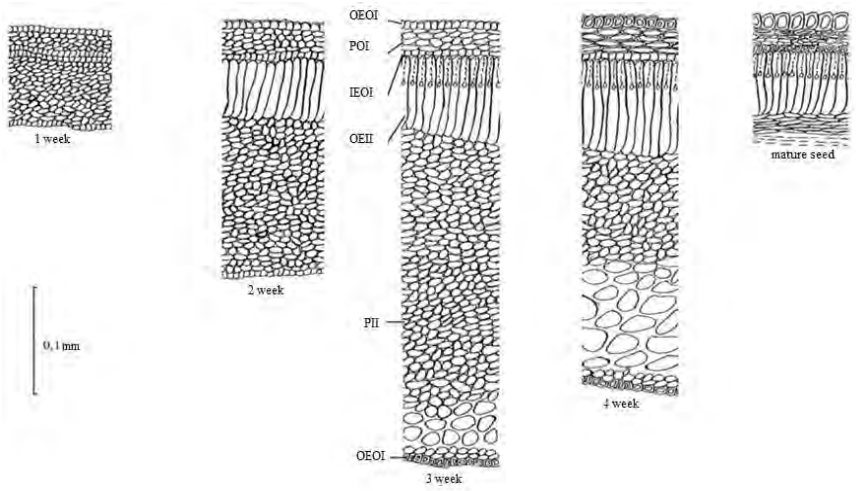

Fig. 1. A structure of integuments of uneven-aged ovules and ripe seed peel of A-738 (G. herbaceum L.)

In general, early ripening breed sample A-738 significantly differs from the mid-season A-739 by the large thickness of all integumental layers in the peel of the mature seed and has a lower palisade indicator. Late-ripening breed sample A-184 differs from other with the greatest thickness of the peel and its palisade parenchymal layers (see Table. 1) and a high rate of development in the early stage (from one to two weeks old). Cells of a monostichous layer of the inner epidermis II breed sample differ little on quantitative indicators of their height in the development process and as compared to cells of other layers have the lowest indicators of growth rate.

Wild-growing representatives of *G. herbaceum*.

Comparative analysis of wild growing representatives and breed samples of G. herbaceum species revealed significant differences in quantitative readings of analyzed traits, both within each group and between them (see Table. 1). Cells of the outer epidermis of the outer integument reach the maximum height in the representatives of the two groups to 3-4 weeks (see Fig.2). In the peel of mature seeds, a height of cells of this layer varies from 33.5 to 51.5 micrometer in wild growing, from 22.5 to 44.5 micrometer for samples. The parenchyma of the outer integument (POI) in wild-growing subspecies consists of 3-4 rows of cells, in breed samples — 4-6. The greatest height of this layer reaches to 2 weeks (subsp .africanum, subsp. pseudoarboreum), 3 weeks in subsp. frutescens and increases in the first two 1.3-1.8 times, the latter is 2.3 times. In breed samples to the 2-week (A-184), 3-week (A-738, A-739), and 4-weekly (A-833), its thickness is increased to 1.3; 1.1-1.2 and 1.7 times, respectively. Readings of the thickness of this layer are higher in breed samples compared to the wild-growing species.

Wild-growing representatives differ as well, by fewer readings of the height of cells of the inner epidermis of the outer integument (OEOI). The cells of this layer have the lowest rates of growth rate, especially in the wild-growing ones. The inner integument (II) palisade layer (OEII) is different from others in a high rate of its thickness in the peel of mature seeds, especially wild species, except subsp. frutescens, but this indicator is higher in cultivars. Multi-row parenchymal layers are characterized by the greatest thickness and the speed of development, especially up to two weeks. In all wild subspecies the maximal quantity of rows and thus the height of this layer is marked at 2 weeks (15-20) in the breed-samples in 3-week (17-20 rows). Readings of the thickness is higher in wild representatives - subsp.africanum, subsp. pseudoarboreum, in subsp. frutescens is considerably less. The destruction process leads to a significant reduction in the number of rows and consequently the layer thickness and the overall thickness of the integuments. In the wild representatives in the mature seeds are 4-5 rows, in cultivars - 5-8. The remaining parenchymal layer is considerably thicker and occupies a larger percentage of the thickness of the peel of wild growing except for subsp. frutescens (see Table. 1). The cells of the inner epidermis of the inner integument (IEII) are larger in cultivars than those of the wild-growing ones. In the peel ripe seeds, this layer is absent at all.

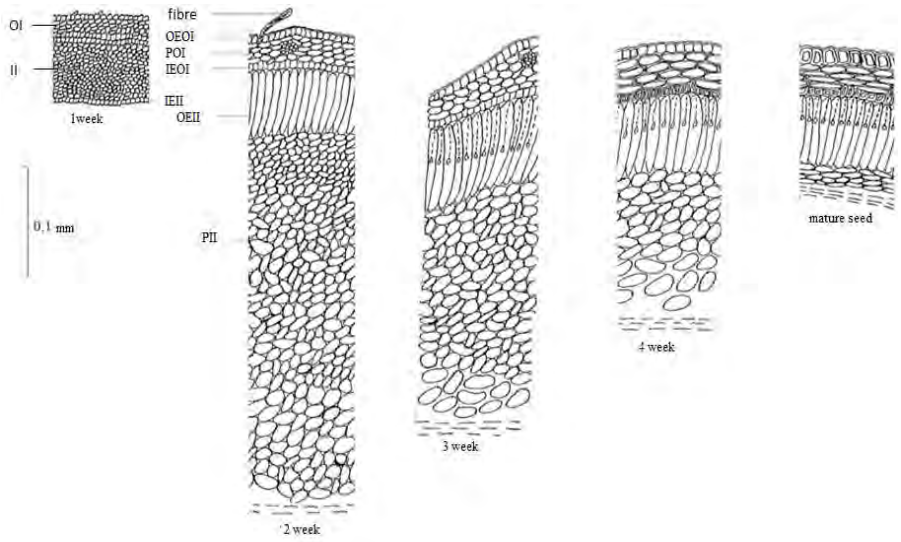

Fig. 2. A structure of integuments of uneven-aged ovules and ripe seed peel of subsp.frutescens (G.herbaceum L.)

Cultivated growing representatives of *G. arboreum.*

The results are stated of the study of breed samples of different origins and degrees of earliness: A-352 (85 days), A-361 (105 days), A-2802 (147 days) and A-2845 (151 days) and representatives of the diploid wild Indo-Chinese species G. arboreum. Among the samples of G. arboreum species higher rates of the thickness of the outer integument and parenchymal layer of the inner integument ovules 1-week-old in early ripening breed-sample A-352 (see Fig. 3). With increasing age of ovules differences are getting smaller, but at the time of maturity manifest themselves more clearly and distinctly different from each other in the total thickness of the peel of ripe seeds, the height of the outer epidermal cells of the outer integument, palisade and parenchyma layers (see the Tab. 1). In early ripening breed-samples A-352, A-361 marked the high altitude of the palisade layer and lower of parenchymal one. Late ripening A-2845 has a thin peel of the mature seed, the outer epidermis OE and the lower height of the palisade layer.

The speed of cell development of the palisade layer is higher than in early ripening breed samples A-352, A-361. The parenchymal layer of the inner integument is multi-row and at an early stage of development consists of 5-10 rows – in early ripening and late-ripening 10-13. The growth speed of these cells is different and does not depend on the earliness of breed samples, but the process destruction is more active in early ripening ones. The height of cells of the inner epidermis IE in the development process is not significantly altered. Active growth of these cells in height occurs within the first 2 weeks in early ripening and 3 week- in late-ripening breed-samples. A layer of these cells is missed in the mature peel.

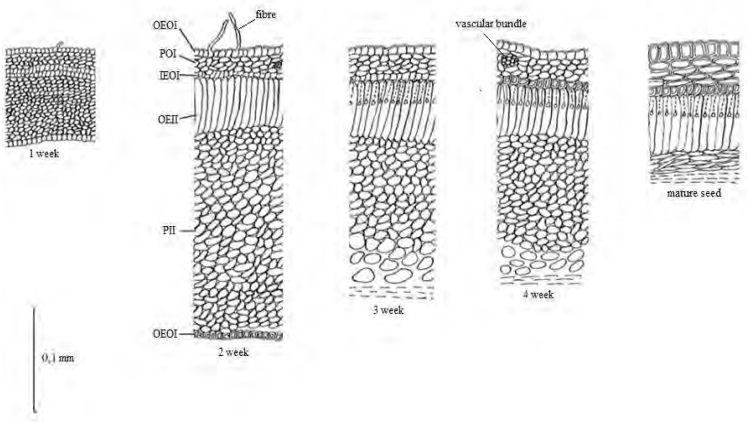

Fig. 3. A structure of integuments of uneven-aged ovules and ripe seed peel of A-361 (G .arboreum)

Wild growing representatives of *G. arboreum*.

Growth and development of integumental layers the cultural breed samples and wild-growing species of G. arboreum subspecies proceed similarly. They differ from each other by biometric indicators of analyzed signs, the growth rate and development of the separate layers, the ratio of tissues. In the peel of mature seed indicators of the overall thickness of integuments, OI, its epidermal layer (OEOI), the height of the palisade and parenchymal layers OI higher in wild growing subspecies (Fig. 4 and Table. 1).

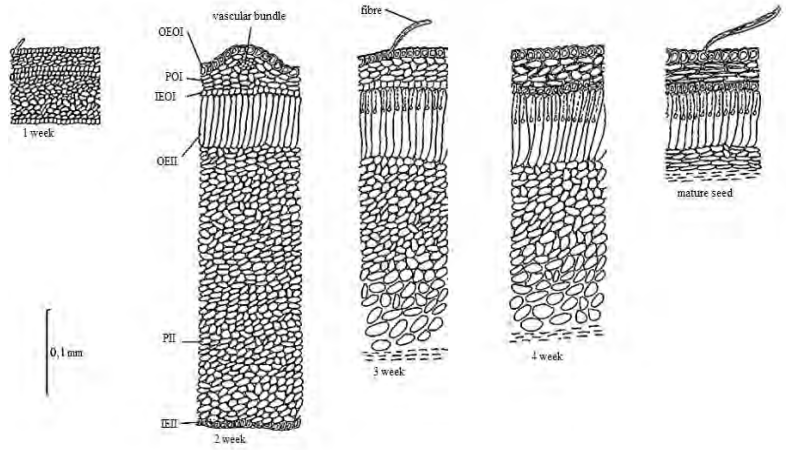

Fig. 4. A structure of integuments of uneven-aged ovules and ripe seed peel of subsp. obtusifolium (G. arboreum)

Table 1. Readings of structural signs of integuments of ripe seed peel of diploid representatives of *Gossypium* L, genus *n* = 10

Representatives	Total thickness of seed peel, µm	OI		OEOI, µm	IEOI, µm	OEII		PII	
		µm	%			µm	%	µm	%
				G. herbaceum L.					
subsp.africanum	502,8 ± 37,8*	96,0 ± 8,5*	19,0	51,5 ± 2,4*	12,5 ± 1,2	236,0 ± 22,6*	48,0	170,8 ± 15,2*	34,0
subsp. pseudoarboreum	473,5 ± 36,8	111,5 ± 10,2	23,5	33,5 ± 2,9	11,7 ± 0,4	213,8 ± 19,6	45,1	148,2 ± 12,9	31,3
subsp. frutescens	284,0 ± 45,1	68,7 ± 6,3	24,2	34,3 ± 2,8	13,3 ± 1,3	175,5 ± 15,9	61,7	39,8 ± 2,9	14,1
A-738 (90 days)	364,9 ± 10,3*	104,9 ± 5,5*	28,7	44,5 ± 2,7	17,6 ± 1,1*	199,2 ± 2,9*	54,6	60,8 ± 8,7*	16,7
A-833 (105 days)	327,8 ± 6,4	91,7 ± 2,9	27,9	37,3 ± 1,8	22,2 ± 1,0	187,8 ± 4,1	57,0	48,4 ± 3,8	15,0
A-739 (135 days)	276,8 ± 3,8	57,5 ± 1,9	20,8	22,5 ± 1,3	15,6 ± 0,7	172,6 ± 2,6	62,4	46,7 ± 2,6	16,9
A-184 (149 days)	379,5 ± 10,7	81,7 ± 2,7	21,5	43,1 ± 1,4	15,1 ± 0,5	216,1 ± 4,0	57,0	81,8 ± 7,9	21,5
				G.arboreum L.					
subsp. obtusifolium	415,1 ± 37,5	97,4 ± 9,1	23,5	42,1 ± 3,6*	14,0 ± 1,1*	243,6 ± 22,6	58,7	74,1 ± 6,9*	17,9
subsp. perenne	378,5 ± 34,3	84,4 ± 8,1	22,3	39,0 ± 3,5	12,5 ± 1,2	223,1 ± 18,4	59,0	71,0 ± 6,5	18,7
subsp. neglectum	450,9 ± 21,9	95,2 ± 9,1	21,1	36,7 ± 2,3	10,9 ± 1,0	209,8 ± 20,3	46,5	145,9 ± 11,8	32,4
subsp. nanking	413,6 ± 38,4	87,5 ± 6,3	21,1	51,3 ± 4,2	12,0 ± 1,0	227,0 ± 20,7	54,9	99,1 ± 10,2	24,0
A-352 (85 days)	303,4 ± 15,2	62,3 ± 5,0	20,5	25,4 ± 2,1	12,4 ± 0,8	198,0 ± 14,7	65,3	43,1 ± 4,4*	14,2
A-361 (115 days)	321,1 ± 28,5	64,9 ± 5,0	20,2	32,1 ± 2,5	10,2 ± 1,3	216,0 ± 20,3	67,3	40,2 ± 3,1	12,5
A-2802 (147 days)	314,0 ± 7,2	61,1 ± 4,0	19,5	29,9 ± 1,6	12,2 ± 1,2	194,9 ± 6,5	62,1	57,7 ± 3,5	18,4
A-2845 (161 days)	300,3 ± 10,1	60,6 ± 4,0	20,1	24,8 ± 1,5	11,7 ± 1,0	185,0 ± 7,2	61,6	54,7 ± 4,7	18,2

Note: ∑- total peel thickness

Underlined representatives compared with each other on studied marks

*- existence of significant differences between readings of the compared representatives.

Conclusions

1. The integuments of uneven-aged ovules and a peel of ripe seed in all studied representatives have a common plan of structure that shows the phylogenetic relationship and monophyletic origin of species of *Gossypium L*. genus; differences of quantitative readings of structural signs, different speed, and duration of growth of integumental layers indicate membership of a particular taxonomic group.
2. Common marks of formation and development of the seed peel for wild species and cultivars of different ploidy are differentiation of structure of one-week ovules on integumental layers; active growth and development in the first two weeks; the different growth rate of integumental cell layers; large thickness of the inner integument cells compared with an external, due to the palisade layer, reaching the highest value to ripening; Multiserial of parenchymal layer II and high-rate of growth of its cells in the first few weeks; destruction of inner rows of parenchymal layer, resulting in a significant reduction in its thickness and its number of rows; different levels of thickening of the walls of integumental layers.
3. Diagnostically significant marks in the structure of a peel of wild-growing diploid species are: the peel thickness, external integuments, crystal-bearing cells (IEOI), and the palisade and parenchyma layers of the inner integument. The thickness of a peel of ripe seed correlates with the duration of the period of germination in representatives of *G .herbaceum L.* species.
4. Firmness (strength) of the seed peel due to cell size and wall thickness of the outer epidermis, the height of palisade layer, ratio of mechanical parenchymal tissues, the degree of pigmentation of integumental layers, clarification and lignification of cell walls.
5. The thickness of the mature seed peel is not always an indicator of early ripening breeds and more dependent on the rate and duration of cell growth of integumental layers.
6. It was specified a degree of evolutionary advancement and systematic position of individual taxa.
7. The readings of primitiveness are small-seediness, high thickness and density of the peel, conservativeness of structure, expressed in general plan of the structure of the seed peel.

References

1. Patil, Pravin; Malik, Surendra; Sutar, Shrikant; Yadav, S. R.; John, Joseph; Bhat, K. V Taxonomic importance of seed macro- and micro-morphology in Abelmoschus Medik. (Malvaceae) Nordic Journal of Botany.Exploring the botanical biodiversity on Earth. – 2015. – N 5. – P. 114-123.
2. Carlos B. Armijo, Kevin D. Baker, Sidney E. Hughs, Edward M. Barnes, and Marvis N. Harvesting and Seed Cotton Cleaning of a Cotton Cultivar with a Fragile Seed Coat. The Journal of Cotton Science. – 2009. – №13. – P. 158–165 http://journal.cotton.org, The Cotton Foundation 2009
3. Clif Boykin, and Sam Ray The Effects of Seed Cotton Cleaning on Seed Coat Fragments.The Journal of Cotton Science. – 2010. – №14. – P. 91-98. http://journal.cotton.org, © The Cotton Foundation 2010
4. Frank E. Groves, and Freddie M. Bourland Estimating Seed Surface Area of Cottonseed The Journal of Cotton Science – 2010. – №14. – P. 91-98. http://journal.cotton.org, © The Cotton Foundation 2010.
5. Barykina R.P., Veselova T.D., Devyatov A.G., Dzhalilova H.Kh., Ilyina G.M., Chubatova N.V. Fundamentals of microtechnical research of botany. Reference guide. -. Moscow, 2000. -129 p.

Development, Production and Commercialization of Transgenic Extra Long Staple (ELS) Public sector Cotton Hybrid DCH-32 BG-II Bt in India

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Abstract

Background: *DCH-32 is the world's first extra long staple (ELS) interspecific cotton hybrid (G.hirsutum x G.barbedense) developed and released in Karnataka, India by UAS Dharwad in 1981. This is considered a landmark in heterosis breeding. The hybrid is very popular in India among cotton-growing farmers of Karnataka, Maharashtra, Tamilnadu, Andhra Pradesh, Madhya Pradesh & Gujarat states for its high seed cotton yield, superior fiber properties, and premium price. Hybrid gives @ 20-22 q/ac under irrigated conditions and 15-16 q/acre under assured rainfed conditions. The hybrid fiber qualities can be compared with superior varieties like Suvin, Egyptian Giza 70, Peruvian Pima, and the U.S.A. Pima & Isreal Pima. The ELS cotton production has come down drastically well short of local consumption i.e less than 4 lakh bales. This has compelled India to import ELS cotton. It is, therefore, imperative that domestic cultivation and production of ELS cotton to meet the growing demand of the consuming industry has to be increased by the Mission Mode approach for ELS development. The major threat to sustainable cotton production is bollworm infestation (60-75%). The main production constraint is the lack of availability of ELS hybrids with a Bt background to overcome bollworm infestation. So the most popular public sector ELS cotton hybrid DCH-32 was converted to BG-II transgenic under RKVY project funding and commercialized as DCH-32 BG-II Bt. With the Bt conversion, all agronomic and fiber characters of the hybrid DCH-32 Bt remained the same with the additional advantage of bollworm resistance through BGII technology. The development, seed production, and commercialization challenges of the DCH-32 BG-II Bt hybrid are discussed.*

Results: *Trait introgression was carried out by crossing donor parent of event MON 15985 carrying bollgard II genes with recurrent DS-28 parent. Backcrossing up to BC5 generation and further advancing to BC5 F2:3 generation resulted in the selection of four near isogenic lines of DS-28 BG-II Bt confirmed for homozygosity for cry1Ac and cry 2 Ab genes with high Cry protein expression. The average Cry protein expression across the different plant tissues over the selected plants was 3.57 (µg/g) for Cry 1Ac and 516.89 (µg/g) for Cry2Ab proteins across different plant tissues. The DUS character's observations on the DS-28 BG-II Bt line indicated high recovery of the recurrent genome. Further interspecific crossing of transgenic DS-28 BG-II Bt with SBYF425 line belonging to G.barbadense (DS-28BG-II Bt x SBYF-425) resulted in the development of DCH-32 BG-II Bt ELS hybrid. The hybrid evaluation under yield performance trials across the locations and over the years showed that the DCH-32 BG-II Bt hybrid performance was better than the check variety MRC-7189 BG-II Bt (Bahubali) and significantly superior over non-Bt DCH-32. The Cry protein expression was high in leaves followed by boll rind and squares. For Cry 1Ac the range was 1.75 to 6.02 µg/g and for Cry 1C it was 160.53 to 473.33 µg/g over the tissues and season. The fiber characters were superior to the check MRC-7189 BG-II Bt while similar fiber traits were similar to the non-Bt DCH-32 hybrid. Commercial seed production was done on farmers' fields. The average seed yield obtained was 4 quintals/ acre. Commercialization of the hybrid was challenging as the Bt version of the DCH-32 from the public sector is new and competition from private sector hybrids which have already occupied the market space under the ELS category.*

Conclusion: *The result of the project of development of transgenic, public sector, textile industry preferred ELS Bt cotton hybrid DCH-32 BG-II Bt is accomplished without compromising on yield and fiber properties. There is high scope for large-scale quality seed production and sustainable seed supply to meet the demands of the Indian farmer*

and textile industry. The Bt version of the hybrid helps the farmer to reduce the cost on crop protection chemicals and earn profit with the premium price fixed for ELS cotton.

Keywords: Cotton, Transgenic, ELS, DCH-32 BG-II Bt hybrid, Seed Production, Commercialization

Introduction

The Indian cotton production scenario changed phenomenally with the adoption of commercially successful transgenic Bollgard technology (Bt technology) in cotton hybrids conferring resistance to Bollworm insect pests. Transgenic cotton effectively reduces the cost of cotton production and increases the productivity and economic benefits for the farmers (Naseem and Qaim, 2016) The estimates of adoption of Bt technology in India is to the tune of 93% with variations in cotton growing states (DOES, MoA, GOI, 2019-20)). However, The majority of cotton hybrids commercialized belong to H x H type i,e cross between *G.hirsutum x G.hirsutum* lines that fall in the medium to long fiber staple length category. Whereas, availability and production constraints of very few ELS hybrids like DCH-32 and TCH-213 (*G.hirutum x G.barbadense*) and variety Suvin (*G.barbadense*) that meet international ELS specifications with more than 34 mm fiber length for higher spin counts has affected the ELS cotton hybrids production environment in India. The area under ELS cotton has come down drastically over the years with a mere production of 5 to 6 lakh bales (1 bale =170kg) while the requirement is 20 lakh bales (Cotton and Products Annual 2020).

DCH-32 is the world's first extra long staple (ELS) interspecific cotton hybrid (G.hirsutum x G.barbedense) developed and released in Karnataka. Hybrid is in great demand by textile industries. So the development of ELS cotton hybrids with a Bt background can be a solution to overcome bollworm pest attacks which in turn help in increasing productivity and adoption by the farmer. Considering this efforts were made to develop a transgenic version of the most popular ELS hybrid in India "DCH-32 hybrid" with Bt technology. The DCH-32 hybrid fiber qualities can be compared with superior varieties like Suvin, Egyptian Giza 70, Peruvian Pima, and the U.S.A. Pima & Israel Pima. The DCH-32 BG-II Bt was developed under the RKVY-funded project " Development and Introduction of Bt cotton hybrids through Public sector agencies in Karnataka through KSSC in coordination with State Agricultural Universities" and approved and notified for cultivation through the Indian transgenic regulatory body Genetic Engineering Approval Committee (GEAC).

Material & Methods

Transgenic donor seeds with approved event MON 15985 with BG-II genes *cry1 Ac and cry 2 Ab* were obtained under MOU with M/s Mahyco Monsanto Biotech (India) Pvt. Ltd. (MMB) for trait introgression with DS-28 female parent of DCH-32 hybrid procured from University of Agricultural Sciences (UAS) Dharwad, India. The back cross method of breeding was followed for trait introgression. The recurrent parent was backcrossed in each back cross F1 generation based on qualitative ELISA. ELISA test was made using "DesiGen" ELISA kit supplied by MMB for testing the expression of Cry 1Ac and Cry 2 Ab proteins as per the standard protocol. Plants showing expression for both the proteins in each back cross generation were considered for crossing with recurrent parent and continued up to BC5F1. The BC5 F2 generation raised from BC5F1 containing both the cry genes were subjected to homozygosity test and quantitative ELISA and the plants homozygous for both the genes *cry 1Ac and cry 2 Ab* with high Cry protein expression were selected for seed multiplication and hybrid development. The hybrid was evaluated under the EBAM trial (Event-Based Approval Mechanism). The data on cry protein expression, Insect bioassay, DUS, agronomic parameters, and fiber properties were generated with support from UAS, Dharwad. The fiber properties were generated using an HVI machine. Commercial hybrid seed production was made on farmers' fields following seed production and certification standards.

Results

Development and characterization of transgenic DCH-32 cotton hybrid

The seeds carrying transgenic event MON15985 with a double gene construct *cry1 Ac and cry 2 Ab* was used as donor parent for trait introgression into DS-28 *G.hirsutum* female parental line of interspecific DCH-32 hybrid. The line was tested negative for the presence of any

intended and unintended events to start introgression. The conventional back cross program was used for trait introgression with DS-28 as a recurrent parent. At each stage of backcrossing, the plants tested positive for ELISA for both cry proteins were selected and used for the next stage of backcrossing. The backcrossing continued up to five generations to achieve maximum recovery of recurrent parent genomes. The BC5F1 plants that tested positive for qualitative ELISA were advanced to BC5F2 generation. The plants that tested positive for qualitative ELISA were further subjected to homozygosity tests for both genes. A total of thirteen plants homozygous for both the genes were selected at BC5F2 (Table-1) and further based on high cry protein expression (Cry1Ac > 3 µg/g and Cry 2Ab >400 µg/g) and high per se yield performance (>450g seed cotton yield/plant) four plants were selected and finally P-55 and P-58 plants were used for seed multiplication and hybrid development (Table-2) based on DUS characters compared to recurrent parent DS-28.

Table.1 Details of plant selections in each generation of backcrossing.

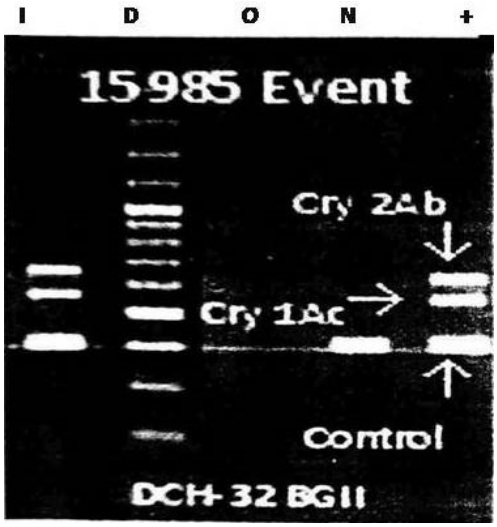
Sl. No.	Generation	Total no of plants	BG-II positive plants (Qualitative ELISA)	# of plants selected based on homozygosity for *cry 1 Ac* and *cry 2 Ab* genes
2	F1 (DS-28 X Bt Donor)	24	24	-
3	BC1F1	190	41	-
4	BC2F1	480	74	-
5	BC3F1	62	20	-
6	BC4F1	382	132	-
7	BC5F1	474	79	-
8	BC5F2	359	156	13

Table.2 Cry proteins expression (µg/g) across different plant tissues and seed cotton yield per plant (g) of selected plants at BC5F2:3 generation of the cross DS-28 x Donor Bt line under field evaluation

Plant ID	Cry 1 Ac (µg/g)				Cry 2 Ab (µg/g)				seed cotton yield (g/ plant)
	Leaf 60 DAS	Square 90 DAS	Boll 110 DAS	Average	Leaf 60 DAS	Square 90 DAS	Boll 110 DAS	Average	
P 52	5.56	2.63	2.80	3.66	584.5	651.4	404.5	546.8	485
P 55	5.00	3.42	3.50	3.97	583.9	577.8	425.9	529.2	485
P 58	4.85	2.56	3.16	3.52	613.4	659.7	408.7	560.6	745
P 67	3.26	2.47	3.58	3.10	424.3	487.1	381.4	430.9	675
Average	4.67	2.77	3.26	3.57	551.53	594.02	405.11	516.89	

P-55 and P-58 plants of transgenic DS-28 BG-II lines were used to develop interspecific hybrid DCH-32 BG-II Bt by crossing with *G.barbadense* line SBYF-425 (DS-28 BG-II Bt x SBYF-425). The DUS characters of the Bt hybrid were compared to Non-Bt DCH-32 (Table.3) and the event confirmation was made with molecular characterisation (Fig.1). The Bt hybrid was evaluated for yield, fiber, Bt proteins expression at various stages of crop growth and Insect pests infestation.

Fig.1 . Confirmation of MON15985 event through molecular characterization



I = DCH-32 BG-II; D = DNA Marker ; 0= No DNA Control; N= Negative; + = Positive

Table 3: DUS characters of DCH-32 Bt and Non Bt DCH-32 Hybrid

Sl. No.	Characters	DCH-32 BGII Bt	DCH-32 Non Bt
1	Hypocotyl pigmentation	Slight pigmentation	Slight pigmentation
2	Leaf colour	Green	Dark green
3	Leaf hairiness	Glaborous, only in shoot tips sparcely hairy	Glaborous
4	Leaf appearance	Flat, medium lobed	Flat, medium lobed
5	Leaf gossypol glands	Present	Present
6	Leaf nectaries	Present	Present
7	Leaf petiole pigmentation	Slightly pigmented	Slightly pigmented
8	Leaf shape	Palmate	Palmate
9	Stem hairiness	Glaborous with sparcely hairy at tip	Glaborous with sparcely hairy at tip
10	Stem pigmentation	Slightly pigmented	Slightly pigmented
11	Plant height (cm)	140-150	145-160
12	Plant growth habit	Spreading	Spreading
13	Bract type	Normal	Normal
14	Days to 50% flowering	70-75	68-72
15	Petal colour	Yellow	Yellow
16	Petal spot	Present	Present
17	Flower stigma	Embedded	Embedded
18	Anther filament colouration	Absent	Absent
19	Pollen colour	Yellow	Yellow
20	Male sterility	Absent	Absent
21	Boll bearing habit	Solitary	Solitary
22	Boll colour	Green	Green
23	Boll shape	Elliptic	Elliptic
24	Boll surface	Pitted	Pitted
25	Boll prominence of tip	Pointed	Pointed
26	Boll opening	Good	Good
27	Boll weight	4.0-4.2	3.7-3.9
28	Seed fuzz	Fuzzy	Fuzzy
29	Fuzz colour	White	White

30	Seed index (100 seed weight) (g)	9.5 – 10.0	8.5 - 9.0
31	Ginning %	35.1	32.3
32	Fiber colour	White	White
33	Fiber length (2.5% span length in mm)	36.0	36.0
34	Fiber strength (g/tex)	37.0	37
35	Fiber fineness (micronaire value)	3.0	2.9
36	Fiber uniformity	49	49
37	Fiber maturity (%)	84	83

Performance evaluation of DCH-32 BG-II hybrid

In the yield performance trials, the test hybrid DCH-32 BG-II Bt recorded 18.3 q/ha seed cotton yield which was significantly superior to the Non-Bt DCH-32 hybrid (12.66 q/ha) whereas the performance was at par with BG-II Bt commercial check MRC-7918 which has recorded 18.04 q/ha. No. of bolls/plant were high in Bt hybrids compared to non-Bt hybrid DCH-32. The fiber characters remained the same over the Bt hybrids and Non-Bt Hybrid with ELS qualities (Tables.4 & 5).

Table 4: Performance of DCH-32 BG-II Bt for various yield and related characters

Sl. No.	Entries	Plant height (cm)	No. of monopodia	No. of sympodia	No. of bolls /plant	Boll wt (g)	Lint index (g)	Seed index (g)	Ginning out turn (%)	Seed cotton yield (q/ha)
1	DCH-32 BGII	162.0	1.3	20.8	57.4	4.8	6.6	12.5	35.1	18.30 *
2	DCH-32 Non Bt	168.0	1.5	18.6	38.4	4.8	6.4	12.0	34.7	12.66
3	MRC-7918 BGII (Check)	165.0	1.6	21.2	56.0	4.6	6.8	12.8	34.7	18.04 *
	CD	16.5	0.4	4.2	5.8	0.8	0.7	1.2	1.5	4.15
	CV	1.5	14.1	9.2	11.6	8.5	7.5	8.1	6.3	16.2

- Significance at p=0.05

Table.5: Fiber Characters of DCH-32 BG-II Bt in comparison to Non Bt and Bt Check

Fiber characters	DCH-32 BGII	DCH-32 Non Bt	MRC-7918BGII (Check)
Upper Half Mean Length (mm)	36.0	35.9	35.0
Uniformity Index	86.0	87.0	86.0
Micronaire	2.9	2.9	2.7
Bundle strength (g/tex)	37.5	37.9	36.6

Table 6. : Cry protein Expression in µg/g across the tissues in DCH-32 BG-II Bt and Check

Hybrid	Tissue	Cry 1 Ac (µg/g)				Cry 2 Ab (µg/g)			
		Season mean	SD	Season high	Season low	Season mean	SD	Season high	Season low
DCH-32 BGII Bt	Terminal leaf	4.59	0.96	6.02	3.42	260.02	39.7	299.2	193.69
	Square	2.53	0.54	3.39	2.04	269.84	74	473.33	277.89
	Boll epicarp	3.66	1.49	5.51	1.86	304.67	38.3	358.68	274.92
MRC-7918 BGII Bt (Check)	Terminal leaf	4.09	1.27	5.86	2.36	198.09	24.1	219.88	160.53
	Square	2.24	0.38	2.78	1.75	290.09	41.8	357.83	249.81
	Boll epicarp	3.11	0.12	3.24	2.95	249.98	11.3	261.01	234.52

The Cry proteins expression was studied at different dates after sowing (Table.6). The highest season means for Cry 1 Ac protein was 4.59 µg/g in terminal leaf tissue while the lowest expression was 2.24 µg/g in the square. For Cry 2 Ab expression it was high in boll epicarp with 249.98 µg/g while the lowest was in terminal leaf with 198.09 µg/g. In both test hybrid and check the pattern of Cry proteins expression was similar across the tissues. The insect bioassay for American bollworm resistance was conducted for DCH-32 BGII, DCH-32 Non-Bt and Bahubali BGII using leaves. Data is presented in Table 7. It was carried out in 15 replications per genotype with 15 neonatal larvae per replication. DCH-32 BGII recorded 85.3 percent corrected mortality while Bahubali BGII with 83.4 percent which was on par with DCH-32 BGII. The pink bollworm damage incidence was observed on 50 days old matured green bolls. The incidence was recorded at 10.0 and 10.3 percent respectively in DCH-32 BGII and Bahubali BGII as against 65.90 in non-Bt DCH-32. In DCH-32 BGII and Bahubali BGII hybrids, the infestation observed was as just a single seed in a locule with dead larvae. But in the case of DCH-32 Non-Bt, the infestation was noticed as complete damage of 1-2 locules or even more (Table.8). The sucking pest incidence was high in non-Bt DCH-32 with severe Jassid injury as compared to both the Bt hybrids and whitefly infestation was below an economic threshold level in all the entries (Table.9).

Further with the approval from the regulatory body a large-scale foundation and hybrid seed production were made on farmers' fields with the objective of commercialization of public-bred ELS cotton hybrid DCH-32 BG-II Bt.

Table.7. Insect bioassay data for bollworm incidence

Sl. No.	Genotypes	% Mortality	Corrected mortality (%)
1	DCH-32 BGII Bt	92.00	85.34
2	DCH-32 Non Bt	06.66	00.00
3	Bahubali BGII	90.60	83.94
	CV (%)		8.19
	C.D (p=0.05)		0.04

Table.8 . Incidence of Pink Boll worm

Genotypes	No. of bolls observed	No. of bolls infested	Percent infestation
DCH-32 BGII Bt	975	98	10.0
DCH-32 Non Bt	692	456	65.9
Bahubali BGII	887	92	10.4

Table.9. Reaction to sucking pests

Sl. No.	Entry	Jassids/3 leaves	Jassids injury grade	Whitefly/3 leaves	Reaction
1	DCH-32 BGII Bt	4.49	II (low)	2.56	BETL
2	DCH-32 Non Bt	5.4	IV (High)	2.98	BETL
3	Bahubali BGII	4.75	II (low)	2.63	BETL
	CD @ 5 %	0.55		0.15	
	CV %	7.64		7.26	

BETL= Below Economic threshold level

Discussion

The world's first interspecific Extra-long staple cotton DCH-32 is in great demand by the Indian textile Industry. With the introduction of Bt cotton, the non-Bt DCH-32 lost its area to medium to long staple high-yielding hybrids which are tolerant to bollworm infestation. In India, the estimated production of 4.0 lakh bales of ELS cotton consists of mainly DCH32 and MCU5 (superfine) which are non-Bt varieties. The estimated requirement of ELS cotton in India is 20 lakh bales to meet the demand it was suggested to increase the acreage and productivity by introducing of Bt varieties of ELS cottons (Arindum Basu & Chellamani, 2007, Khadi & Gopalkrishnan, 2007). In that direction, under the mission mode approach with the funding under Rashtriya Krishi Vikasa Yojana (RKVY), the Government of Karnataka gave thrust for developing transgenic ELS cotton with successfully used approved BG-II event in India MON15985 focusing on DCH-32 a well established ELS cotton hybrid of the public sector.

Trait introgression was done with donor parent with approved event MON 15985 with BG-II genes *cry1 Ac and cry 2 Ab* using the conventional back cross method of breeding (Fehr,1987; Zhang et al 2011a). Back crosses up to BC5 generation with high selection pressure were practiced to break the bad linkage from the donor parent and to achieve higher recovery of recurrent parent genome (Zhang et.al. 2014b). In each back cross F1 to recurrent parent results in hemizygous transgene and homozygous null in a 1:1 ratio. ELISA test was employed to detect transgenic plants at each stage of backcrossing (Zhang, 2015) Maintaining a high population size with good selection pressure helped to recover the recurrent genome. At BC5 F2:3 generation field evaluation of four selected plants for DUS characters indicated the maximum recovery of the recurrent genome and further zygosity test and quantitative ELISA for BG-II genes acted as foreground selection for the trait to finalize the plants for hybrid development. Depending on high heterosis and high Cry protein expression plant numbers P-55 and P-58 (DS-28 BG-II Bt) were selected for transgenic hybrid development DCH-32 BG-II Bt and further for breeder seed increase.

The transgenic hybrid DCH-32 BG-II Bt was characterized for the event confirmation of MON 15985 through molecular characterization and further quantified the Cry protein expression under field conditions along with Bt check hybrid MRC-7918 BG-II Bt for comparison over the crop season. Adequate expression of Cry protein is very important for sustainable insect pest control. It was observed that the Cry proteins expression was high in terminal leaf and low in squares in both the test and check hybrids and over the growing season there will be a decline in expression. Similarly, Kranti et. Al. (2005); A K Singh, et. al (2016) reported high Cry protein expression in green plant parts. The high expression event will allow for effective resistance

management against lepidopteran insect pests, particularly *Helicoverpa armigera*, using a high dosage strategy. Insect bioassay showed up to 85% bollworm mortality on both the test and check BG-II hybrids similarly up to 93% insect mortality was reported by Siddiqui et. al.(2019). Very high pink bollworm damage incidence was observed on non-Bt DCH-32 hybrid while both the Bt hybrids showed 10% incidence. Maturing seeds and flowers are the most vulnerable parts for bollworm attack especially pink bollworm, which imposes high selection pressure for the development of resistance in pink bollworm due to the very low expression of Cry1Ac as already reported by Fabrick *et al*., (2014) in US and India.

Under agronomic yield performance trials both the test hybrid DCH-32 BG-II Bt and check hybrid recorded significantly higher seed cotton yield over the Non-Bt DCH-32 hybrid. Yield contributing trait no of bolls/plant was high in Bt hybrids compared to non-Bt hybrid DCH-32 because of boll retention due to lower damage to fruiting bodies due to bollworm tolerance of BG-II Bt genes. Similar observations were made by Bheemanna et.al (2008) and Omkarmurthy et.al (2016).

Conclusion

DCH-32 is the most important textile industry preferred ELS cotton in India. The non availability of Bt version of the hybrid resulted in reduced acreage of production. Considering the huge demand from the textile industry the Government of Karnataka has decided to develop Bt version of DCH-32 hybrid with bollgard II technology under project mode. Thus the BG-II technology was introduced to develop DCH-32 BG-II cotton hybrid which need to be popularized by bringing it under sustainable seed input supply chain and premium pricing so as to help the farmer and textile industry. This helps the farmer to reduce the cost on crop protection chemicals and earn profit with premium price fixed for ELS cotton.

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References

1. Amarjeet Kumar Singh, Kumar Paritosh, et.al. , 2016, High Expression of Cry1Ac Protein in Cotton (*Gossypium hirsutum*) by Combining Independent Transgenic Events that Target the Protein to Cytoplasm and Plastids PLoS One. 2016; 11(7): e0158603.
2. Arindam Basu & K.P. Chellamani (2007) Present status and Future Requirement of Extra Long Staple (ELS) Cotton in India. Fiber 2 Fashion.com.
3. Bheemanna M, Patil BV, Hanchinal SG, et.al. 2008. Comparative performance and economics of Bollgard-II cotton under irrigated condition. Journal of Cotton
4. Research and Development **22**: 118-121.
5. Fehr, W.R. 1987. Principles of cultivar development: Theory and technique. Macmillan, New York.
6. Hamid Anees Siddiqui, Muhammad Asif, et.al , 2019, Development and evaluation of double gene transgenic cotton lines expressing Cry toxins for protection against chewing insect pests. Sci Rep. 2019; 9: 11774.
7. India Cotton and Products Annual-2020 Gain Report No. IN 2020-0011.
8. K. R. Kranthi, S. Naidu, et.al . 2005, Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera (Hübner)(Noctuidae: Lepidoptera). *Curr. Sci*. 291–298.
9. Khadi B.M and Gopalkrishnan, 2007, Extra Long Staple Cotton Scenario in India and World. Model training Course on Cultivation of long staple cotton (ELS), December 2015,22-27, Central Institute for Cotton Research, Regional Station, Coimbatore.
10. Jeffrey A. Fabrick, Jeyakumar Ponnuraj et.al (2014) Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton inIndia. *PloSOne*. 2014;**9**:e97900. oi: 10.1371/journal.pone.0097900.
11. Naseem, A., Qaim, M. (2016). Genetically modified crops and agricultural development. *Am. J. Agr. Econ.* 98, 1561–1564.
12. S. G. Onkaramurthy , K. Basavana Goud, et,al , 2016, Field performance of second generation (BG-II) Bt cotton genotypes against bollworm complex under rainfed conditions Journal of Phytopathology and Pest Management 3(1): 12-20, 2016 pISSN:2356-8577 eISSN: 2356-6507

13. Zhang, J.F., et.al. 2011a. Registration of 'Acala 1517– 09R' cotton. J. Plant Regist. 5:164–169. doi:10.3198/jpr2010.05.0268crc.

14. Zhang, J.F., et.al. 2014a. Genetic variation and comparative analysis of thrips resistance in glandless and glanded cotton under field conditions. Euphytica 199:373–383. doi:10.1007/s10681-014-1137-x

15. Zhang J , 2015 ,Transgenic cotton breeding In book: Cotton (pp.1-25) Edition: 2ndChapter: 10

16. Publisher: Agronomy Monograph 57. ASA, CSSA, and SSSA Editors: Fang D. D. and R. G. Percy.

The Inheritance of Agro-morphological and Fiber Quality Traits in Cotton (*Gossypium hirsutum* L.) Line X Tester Hybrids

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Abstract

Background: *Turkiye is an important cotton producer and also Which has developed textiles. While Turkiye was a cotton seed importer country, now risen to a position where Turkiye can export varieties developed by both the public and private sectors. We have developed a variety with this project and we continue to develop varieties with our promising lines.*

Results: *This study determined the general combining ability (GCA) of parents and the specific combining ability of their hybrid combinations. Furthermore, the hybrid vigor of F_1 was evaluated by investigating the genetic structure of the lines and testers. The study was conducted according to randomized complete block design with three replications at Nazilli Cotton Research Institute in 2015 and 2016. A total of 28 F_1 hybrids (4 lines and 7 testers) developed from the line × tester method were used in the study. Investigated traits in the created populations were significantly influenced by the non-additive gene effect. The heterosis values for plant height, monopodial branches, number of bolls, and radiation were positive, while the days to opening boll, and yellowness were negative. The best parents for opening bolls were 'Gloria', 'TMN170' and 'UA48', while the best parents for monopodial branches were 'UA48', 'ZN108', and 'ADN712'. Similarly, 'İPEK607' and 'GLORIA' proved best parents for plant height, while 'TMN199', 'STV-468', 'GW2357' and 'TMD139' proved best parents for the number of bolls. In the same way, 'GLORİA', and 'ADN712' remained best for radiation, whereas 'UA48', and 'İPEK607' remained superior parents for yellowness. These parents had the best overall combining abilities for respective traits.*

Conclusion: *The most promising hybrid combinations with the highest special combining abilities were 'GLORİA' × 'ADN712' and 'FLASH' × 'ZN1018' for days to opening boll, 'Gloria' × 'İPEK 607' for monopodial branches, 'GLORİA' × 'UA48' for plant height, 'STV-468' × 'İPEK 607' and 'TMN199' × 'TMN139' for the number of bolls, 'TMN199' × 'İPEK607', and 'GLORİA' × 'UA48' for radiation, and 'GLORİA' × 'UA48' for yellowness.*

Keywords: *Cotton, line × tester, quality, variety, seed yield*

Introduction

Cotton is the most important fiber source for Turkey as well as other countries in the world. It is the most significant material for textile, ginning, oil, feed, and paper industries. Approximately 86% of world cotton production comes from 8 countries, including Turkey (Anonymous, 2018). Turkey has an important place in cotton consumption due to the developing textile industry in the country. Turkey ranks 4th in cotton consumption after China, India, and Pakistan (Anonymous, 2018).

Turkey grows non-GMO cotton and is regarded as the most productive cotton-producing country after Australia. The Turkish textile industry not only meets the country's needs but contributes 11.4% of Turkey's export income (TUIK, 2019). Due to this importance to the Turkish economy, cotton is one of the most important agricultural products for Turkish agriculture, the textile industry, commercial life, and socio-economic structure.

It is necessary to develop varieties with high yields and superior fiber quality for the textile sector. For this purpose, cotton breeding studies should be accelerated and intensively done to develop new varieties that are productive and possess high fiber quality to fulfill the demands of producers and the

textile industry. Crossbreeding in cotton is very important. The estimation of the genetic structure of the studied trait of the population created is directly related to the selection of suitable parents and hybrid combinations (Boyacı, 2011).

Community awareness has rapidly increased on the products produced from cotton. The quality of the products is affected by many features of the fiber. These circumstances forced breeders to develop new cultivars with more productivity and high fiber quality. Furthermore, determining the gene effects of parents and hybrids on the desired traits could ease the breeding efforts. Line × Tester is a statistical method that can fulfill this requirement. It was first used by Kempthorne in 1957. Hybrid potency is a method that compares the traits of the hybrids with their parents in the early generation. It is known as heterosis and heterobeltiosis. While heterosis is a method of determining how a hybrid is closer to which of the parents, heterobeltiosis is a method of determining the status of the hybrid versus the superior parent.

The studies on this subject have reported that several traits are managed by non-additive gene effects. Boyacı (2011) reported that fiber fineness had negative heterosis, boll seed weight and earliness characteristics showed negative heterosis, while both positive and negative heterosis could be seen in other characteristics. In addition, many researchers determined that non-additive genes, in other words, dominant, epistatic, or dominant × epistatic genes were effective in the management plant height, monopodial branches, number of bolls, radiations, and yellowness traits in Line × Tester populations (Ünay, 1993; Kalsy et al. 1982; Ahmad et al. 2000; Boyacı,2011; Çoban et al. 2013; Sajjad et al. 2016; ve Ali et al. 2016; Temiz, 2003; Boyacı, 2011; Başal et al. 2009; Ünay, 1993; Başal et al. 2009; Ünay, 1993; Kanoktip, 1987, Ahmad et al 2005; Kaini et al 2007, Saravanan et al 2010 and Sajjad et al 2016).

The aim of this study was to determine the general adaptive abilities (GAA) of the parents and the special adaptive abilities (SAA) of the hybrids. Genetic influence affecting traits in populations as well as the hybrid powers of the crossed lines (Heterosis and Heterobeltiosis) were tested to create new gene pools and assist the breeding studies in the future by Line × Tester Analysis Method among the cotton varieties developed.

Materials and Methods

The study was carried out under Nazilli-Aydin province (37° 54′ 45″ N, 28° 19′ 14″ E, altitude:64 m) climatic conditions during 2015-2016 years. The ‘Gloria’, ‘TMN 199’, ‘TMN 170’ and ‘ST 468’ cotton varieties were used as homozygous lines, whereas ‘GW 2357’, ‘ZN 1018’, ‘GW 2357’, ‘UA 48’, ‘ADN 712’, ‘İpek 607’ and ‘TMN 139’ were used as testers in the study.

The crosses were made under the Line × Tester Analysis Method and 28 hybrid combinations were obtained in 2015. The hybrid combinations and their 11 parents were planted at the trial site of Nazilli Cotton Research Institute on 11 May 2016. The experiment was carried out according to the randomized complete blocks design with 3 replications. Parents and hybrids were planted in 1 row with a length of 12 m, inter-row spacing of 0.70 m, and intra-row spacing of 0.10 m. The experimental soil is rich in phosphorus (3.5 ppm) and potassium (350 ppm), poor in organic matter (1.4%), medium calcareous (20.98%), alkaline (pH: 7.95), and saline (0.036%). In 2016, 90 kg ha^{-1} pure nitrogen (46% urea) was applied, followed by the first irrigation (4 furrow irrigations in total). Harvesting was performed by hand twice on 8 October 2016 and 1 November 2016.

The highest temperature in the region was 38.2 °C in August and the lowest temperature was 7.1 °C in November. In the long-term data, the highest average temperature was observed in July (28.4 °C), the lowest average temperature in December (8.2 °C), and the maximum temperature in July (36.1 °C).

Radiation (Rd), yellowness (+b), plant height (Ph-cm), monopodial branches, boll number, and cotton boll opening days were investigated. Fiber properties were determined by USTER HVI 1000. Values obtained were analyzed according to the Line × Tester Method with the statistical package program (TARPOPGEN) developed by Özcan and Açıkgöz (1999) following Kempthorne (1957) and Sing et al. (1982), and the means were compared with the LSD (0.05) (Least significant difference test). Heterosis (Chiang and Smith, 1967) and heterobeltiosis (Fonseca and Patterson, 1968) values were determined with the following formulas.

Heterosis(%) Ht = (F1-MP)/MP) ×100,MeanParents = (Line parent + Tester parent)/2 (1)
Heterobeltiosis (%) Htb = (F1-Better Parent)/Better Parent) ×100 (2)

Results and Discussion

The differences between genotypes were very significant (p>0.01). Both parents and crosses significantly contributed to variation; however, parents were more important (Table 1). This result stems from the significance of the parents GAA and the hybrids' SAA values. However, parents showed negative GAA in terms of yellowness indicating that the GAA/SAA ratio was negative and other all examined traits pointed out dominant genes were effective.

Table 1. Variance analysis according to line × tester analysis

		PH	MB	BC	FBOD	R	Y
Source of variation	Degree of freedom				F vlaue		
Replications	2	18.986**	2.243	10.731**	0.005	1.364	0.180
Genotypes	38	2.866**	2.205**	4.883**	11.084**	5.693**	1.785*
Parents (P)	10	5.381**	1.372	1.154	6.201**	10.478**	2.330*
P vs C	1	3.327	28.223**	51.064**	8.241**	0.440	7.245**
Crosses (C)	27	1.917*	1.550	4.554**	12.998**	4.116**	1.381
Lines	3	4.687**	1.683	15.275**	13.622**	13.974**	2.565
Tester	6	1.134	1.472	2.649*	26.700**	7.673**	2.004
Lines × Tester	18	1.717	1.554	3.402**	8.326**	1.287	0.976
Error	76						
Total	116						
□² GUY		0.103	0.000	0.144	0.703	0.108	0.001
□²ÖUY		5.524	0.022	4.516	16.535	0.911	-0.001
□² GUY/□² ÖUY		0.018	0.000	0.03	0.04	0.118	-1
Maternal effect		27.16%	12.06	37.27%	11.64%	37.72	20.63%
Paternal effect		13.14%	21.10	20.92%	45.64%	41.43	32.25%
Interaction		59.69%	66.83	41.81%	42.70%	20.84	47.11%

Table2. Mean values of observed traits

Variation	PH (cm)		MB		BC	
Females	Mean	GCA	Mean	GCA	Mean	GCA
TMN199	104.90 a	-0.707	2.17 abc	0.096**	17.46 a	0.723**
FLASH	102.80 ab	0.221	2.33 ab	-0.123**	16.43 abc	-1.630
GLORIA	106.63 a	2.964**	2.17 ac	-0.032	17.56 a	-1.634
STV-468	89.33 d	-2.479**	2.33 ab	0.058	17.50 a	2.541**
Males						
ADN712	97.47 bc	-2.132**	1.83 bc	0.093	16.16 abc	-0.499
GW2357	103.20 ab	1.601**	2.37 a	0.060	15.05 abc	1.243**
İPEK- 607	106.6 a	0.010	1.93 abc	-0.174**	15.40 abc	-0.740**
TMD139	102.7 ab	1.410**	1.93 abc	0.143**	17.76 a	1.860**
TMN170	96.6 c	1.285	2.03 abc	0.051	13.40 c	-0.808**
UA48	92.0 cd	-1.099	1.70 c	-0.024	14.23 bc	-1.007**
ZN1018	90.7 d	-1.074	1.80 c	-0.149**	16.80 ab	-0.049
Ort	99.3		2.1		16.1	
CV (%)	3.39		15.1		11.38	
LSD (0.05)	5.72		0.4		3.13	
Hybrids ♀+♂	Mean	SCA	Mean	SCA	Mean	SCA
TMN199×ADN712	94.0 c-h	-0.735	2.43 a-d	-0.188	18.13 d-h	-1.739
TMN 99×GW2357	100.9 a-f	2.432	2.50 a-d	-0.088	24.46 ab	2.852**
TMN199×İPEK607	98.96 a-g	2.090	2.27 c-e	-0.088	16.56 f-h	-3.064**
TMN199×TMD139	98.57 a-h	0.290	2.97 a	0.295**	25.2 abc	2.269**
TMN199×TMN170	99.60 a-g	1.449	2.87 a-b	0.287**	17.63 d-h	-1.930
TMN199×UA48	90.27 h	-5.501**	2.53 a-d	0.029	19.96 c-g	0.602
TMN199×ZN1018	95.77 b-h	-0.026	2.13 d-e	-0.246**	20.63 b-f	0.311
FLASH×ADN712	97.47 b-h	1.804	2.47 a-d	0.064	19.56 c-g	2.046**
FLASH×GW2357	102.33 abc	2.937	2.37 b-e	-0.002	18.26 d-h	-0.995
FLASH×İPEK607	96.03 b-h	-1.771	2.40 a-e	0.264**	17.86 d-h	0.588
FLASH×TMD139	101.57 a-e	2.362	2.37 b-e	-0.086	16.73 e-h	-3.145**
FLASH×TMN170	96.67 b-h	-2.413	2.13 d-e	-0.227**	18.43 d-h	1.222
FLASH×UA48	92.27 g-h	-4.430**	2.37 b-e	0.081	17.70 d-h	0.688
FLASH×ZN1018	98.23 a-h	1.512	2.07 d-e	-0.094	17.56 d-h	-0.404
GLORİA×ADN712	93.50 d-h	-4.906**	2.87 a-b	0.374**	20.60 b-f	3.084**
GLORİA×GW2357	101.97 a-d	-0.173	2.53 a-d	0.074	19.93 c-g	0.676
GLORİA×İPEK607	102.13 abc	1.586	1.83 e	-0.393**	15.10 h	-2.174
GLORİA×TMD139	98.03 a-h	-3.914	2.57 a-d	0.024	20.80 b-e	0.926
GLORİA×TMN170	102.90 ab	1.077	2.40 a-e	-0.051	16.33 g-h	-0.873
GLORİA×UA48	106.10 a	6.661**	2.43 a-d	0.057	16.73 e-h	-0.274**
GLORİA×ZN1018	99.13 a-g	-0.331	2.17 d-e	-0.085	16.60 f-h	-1.366
STV 468×ADN712	96.80 b-h	3.837	2.33 b-e	-0.250**	18.30 d-h	-3.391**
STV-468×GW2357	91.50 g-h	-5.196**	2.57 a-d	0.017	20.90 b-d	-2.533**
STV-468×İPEK 607	93.20 e-h	-1.905	2.53 a-d	0.217	26.10 a	4.650**
STV-468×TMD139	97.77 a-h	1.262	2.40 a-d	-0.233	23.30 abc	-0.750
STV-468×TMN170	96.27 b-h	-0.113	2.53 a-e	-0.008	22.96 abc	1.581
STV-468×UA48	97.27 b-h	3.270	2.20 b-e	-0.167	20.16 c-g	-1.016
STV-468×ZN1018	92.87 f-h	-1.155	2.77 a-c	0.425**	23.60 abc	1.459
Means	97.5		2.4		19.6	
CV (%)	5.32		14.4		12.92	
LSD (0.05)	8.52		0.56**		4.14	

In addition, it is clearly understood from the GAA/SAA ratio is less than one indicating that dominant genes are effective for all traits (Kanoktip 1987; Ahmad et al. 2005; Kaini et al. 2007; Saravanan et al. 2010; Sajjad at al. 2016; White et al.; Kohel 1996, Ünay 1993, Kalsy et al. 1982).

General Combining Ability of Parents (GCA)

Regarding parents' GCA, 'TMN 199' variety was positive for monopodial branch, boll count, negative to radiation, yellowness, plant height, and first boll opening time. The 'Flash' variety was positive to radiation, yellowness, the first boll opening time, and plant height, whereas negative to

monopodial branch, and boll count. The 'Gloria' variety was positive for radiation, first boll opening time, and plant height, while negative for monopodial branches, and boll count. Similarly, 'ST 468' was positive

to yellowness, monopodial branch, boll count, and first boll opening time, whereas negative to plant height, and radiation.

On the other hand, the 'ADN 712' variety from the tester contributed positively to yellowness, first boll opening time, and monopodial branch, and negatively to radiation, plant height, and boll count. The 'GW 2357' variety was positive for radiation, first boll opening time, plant height, and monopodial branches. Similarly, the 'İPEK 607' variety was positive to all traits except the monopodial branch and boll count. Likewise, the 'TMD 139' variety was positive for yellowness, plant height, first boll opening time, and monopodial branch, while negative for the first boll opening time radiation. The 'TMN 170' variety was positive for yellowness, plant height, and monopodial branches, whereas negative for radiation, boll count, and first boll opening time. Likewise, 'UA48' was positive for radiation, and the first boll opening time, while negative for yellowness, plant height, monopodial branches, and boll count. In the same way, 'ZN1018' contributed positively to yellowness, and negatively to the rest of the traits. These results indicated that crucial new genotypes can be developed if 'Gloria', 'İPEK 607', 'ADN712', and 'UA48' varieties are crossed with high radiation and low yellowness cultivar.

Specific Combining Ability of Hybrids (SCA)

The 10 hybrids displayed negative SCA for radiation, 10 for yellowness, 8 for first boll opening time, 14 for plant height, 15 for monopodial branch, and 14 for the number of bolls. It has been reported that non-additive gene effects manage radiation (Başal 2009; Ünay, 1993), yellowness (Başal 2009; Ünay, 1993), monopodial branches (Temiz 2003; Boyacı 2011; Çoban and Ünay 2013), and plant height (Kanoktip 1987, Ahmad et al. 2000, Kaini et al. 2007, Saravanan et al. 2010; Sajjad et al. 2016). Verhalen et al. (1971) reported that non-additive gene effects control first boll opening. It has been reported by several researchers that the number of bolls is managed by non-additive genes (White and Kohel 1996; Ünay 1993, Kalsy and Vithal 1982; Ahmad et al. 2005; Boyacı 2011; Çoban and Ünay 2013; Sajjad et al. 2016; Ali et al. 2016b). Among the traits examined, 'GLORIA' × 'TMN170' was significant for radiation, 'STV468' × 'ZN1018' for yellowness, 'TMN199' × 'TMN170', 'FLASH' × 'ZN1018', and 'STV468' × 'GW2357' for first boll opening time, 'FLASH' × 'TMN170', and 'GLORIA' × 'TMN170' for the number of bolls, 'TMN199' × 'TMD139', 'FLASH' × 'ADN712', and 'STV468' × 'İPEK607' for plant height. In this research, the hybrids with the highest special adaptability for radiation was 'STV468' × 'ZN1018' whereas 'STV468' × 'İPEK607', had the highest special adaptability for boll count.

Mean performance of parents and F_1 crosses

The mean values of the examined traits of the parents (line and tester) are given in Table 3. Radiation ranged from 77.2 to 72.2, yellowness from 8.3 to 7.4, first boll opening time from 118 to 101, plant height from 106 to 90, monopodial branch from 2.97 to 1.83, and number of bolls ranged from 26.10 to 15.10.

On the other hand, radiation of hybrids of these parents ranged from 77.2 ('TMN' × 'İPEK 607') to 72.2 ('TMN199' × 'TMN170'), yellowness from 8.3 ('STV468' x 'TMN170') to 7.3 ('GLORIA' x 'UA48'), first boll opening time from 118 day ('GLORIA' x 'ADN712') to 101 days ('TMN199' × 'TMD139'), plant height from 106 cm ('GLORIA' x 'UA48') to 90.27 cm ('TMN199' × 'UA48'), and monopodial branches from 2.97 ('TMN199' x 'TMD139') to 1.83 ('GLORIA' x 'İPEK607').

Among the lines, 'GLORIA' variety had the highest radiation (78.4) and number of bolls (17.56). Among the testers, 'ADN712' variety had the highest radiation, 'UA48' had the highest yellowness (7.5), and 'İPEK607' had the highest plant height (106.6).

Table3. Mean values of observed traits

Variation	FBOT (day)		Radiation		Yellowness	
Females	Mean	GCA	Mean	GCA	Mean	GCA
TMN199	113.33 ab	-3.024*	75.1 d	-0.385	8.0 b-d	-0.117**
FLASH	109.00 bc	1.262*	75.6 c-d	0.006	8.6 a	0.145**
GLORIA	115.33 ab	1.548*	78.4 a	1.454	7.8 b-d	-0.121**
STV-468	115.00 ab	0.214	70.7 f	-1.075	8.3 a-b	0.093
Males						
ADN712	114.00 ab	4.762*	78.2 a-b	-0.154	7.9 b-d	0.010
GW2357	114.00 ab	0.262	76.6 b-d	0.163	8.3 a-b	-0.024
İPEK 607	114.0 ab	4.095*	77.1 a-c	1.938	7.6 c-d	0.026
TMD139	112.00 ac	-4.071*	73.1 e	-0.387	8.1 a-d	0.051
TMN170	105.66 c	-0.988*	73.4 e	-1.237	8.2 a-c	0.235**
UA48	106.00 c	1.512*	76.3 c-d	0.555	7.5 d	-0.315
ZN1018	116.60 a	-5.571*	72.8 e	-0.879	7.7 b-d	0.018
Ort	111.7		75.2		8.0	
CV (%)	3.6		1.19		2.4	
LSD (0.05)	6.8		1.6*		0.6*	
Hybrids ♀+♂	Mean	SCA	Mean	SCA	Mean	SCA
TMN199×ADN712	114 c-e	1.524	75.3 b-h	0.801	7.8 a-e	0.100
TMN 99×GW2357	109 f-g	1.024	74.8 d-ı	-0.015	7.7 a-e	0.067
TMN199×İPEK607	112 e	0.190	**77.2 a-c**	0.610	**7.6 b-e**	-0.117
TMN199×TMD139	101 ı	-2.643*	75.1 c-h	0.901	7.7 a-e	0.025
TMN199×TMN170	108 g-h	1.274	72.2 j	-1.182*	7.6 b-e	-0.325*
TMN199×UA48	109 f-g	-0.226	74.5 e-ı	-0.674	7.4 d-e	0.092
TMN199×ZN1018	101 ı	-1.143	73.3 h-j	-0.440	7.8 a-e	0.158
FLASH×ADN712	118 a	1.238	74.9 d-ı	0.011	8.1 a-d	0.138
FLASH×GW2357	117 a-b	4.738*	75.4 b-h	0.194	8.2 a-c	0.271
FLASH×İPEK607	116 a-c	-0.095	76.7 a-e	-0.248	8.1 a-d	0.121
FLASH×TMD139	113 d-e	5.071*	74.2 f-j	-0.423	8.1 a-d	0.030
FLASH×TMN170	106 h	-5.012*	73.7 g-j	-0.039	7.9 a-e	-0.220
FLASH×UA48	115 b-d	1.488	75.7 b-g	0.102	7.6 b-e	-0.037
FLASH×ZN1018	99 ı	-7.429*	74.5 e-ı	0.402	7.6 b-e	-0.304*
GLORİA×ADN712	118 a	0.952	76.5 a-e	0.196	7.6 b-e	-0.029
GLORİA×GW2357	109 f-g	-3.548*	77.1 a-d	0.413	7.5 c-e	-0.095
GLORİA×İPEK607	117 a-b	0.619	77.5 a-b	-0.862	7.9 a-e	0.188
GLORİA×TMD139	101 ı	-7.214*	75.7 b-g	-0.404	7.4 d-e	-0.270
GLORİA×TMN170	116 a-c	4.702*	76.3 a-f	1.080*	8.2 a-b	0.313
GLORİA×UA48	116 a-c	2.202	78.0 a	0.955	7.3 e	-0.037
GLORİA×ZN1018	109 f-g	2.286	74.2 f-j	-1.379*	7.6 b-e	-0.070
STV 468×ADN712	112 e	-3.714*	72.8 ı-j	-1.008	7.7 b-e	-0.210
STV-468×GW2357	109 f-g	-2.214	73.5 g-j	-0.592	7.6 b-e	-0.243**
STV-468×İPEK 607	114 b-e	-0.714	76.4 a-f	0.500	7.7 a-e	-0.193
STV-468×TMD139	111 e-f	4.786*	73.5 g-j	-0.075	8.1 a-c	0.215
STV-468×TMN170	109 f-g	-0.964	72.8 ı-j	0.142	8.3 a	0.232
STV-468×UA48	109 f-g	-3.464*	74.1 f-j	-0.383	7.5 c-e	-0.018
STV-468×ZN1018	111 e-f	6.286*	74.5 e-j	1.417*	8.1 a-c	0.215
Means	110.6		75.01		7.7	
CV (%)	1.5		1,73		5.14	
LSD (0.05)	2.8*				0.6*	

Among hybrids, 'GLORIA' x 'UA48' had the highest radiation (78), and lowest yellowness (7.3), whereas 'STV468' × 'İPEK607', 'TMN199' × 'TMD139', 'STV468' × 'ZN1018' had the highest number of bolls (26,10, 25,2, 23,6). The highest plant height was noted for 'GLORIA' × 'İPEK607', and 'FLASH' x 'GW2357' (102.1 cm, and 101.97cm). The highest boll opening time was recorded for 'GLORIA' x 'ADN712', and 'GLORIA' x 'İPEK607' (118, 117). The lowest monopodial branches were observed from 'GLORIA' × 'İPEK607'.

Hybrid vigor (Heterosis and Heterobeltiosis)

Heterosis values of hybrids ranged between 3.23% ('FLASH' x 'TMD139') and 97.18% ('FLASH' x 'ZN1018') for radiation, -64.85% ('TMN199' × 'GW2357') and 76.92% ('FLASH' x 'ZN1018') for yellowness, -12% ('FLASH' x 'ZN1018') and 4% ('FLASH' x 'İPEK 607') for first boll opening time, -

8.38% ('GLORIA' x 'ADN712') and -8.29% ('FLASH' x 'İPEK607') for plant height, -10.57% ('GLORIA' x 'İPEK607') and 44.72% ('TMN199' × 'TMD139') for monopodial branches, 5.71% ('FLASH' x 'ZN1018') and 58.66% ('STV468' × 'İPEK607') for number of bolls.

The heterobeltiosis values of the hybrids were observed between -3.75% ('TMN199' x 'ADN712') and –6.95% ('STV468' × 'ADN712') for radiation, -7.31 ('FLASH' x 'TMD139') and -12.31% ('FLASH' x 'UA48') for yellowness, -4.4% ('TMN199' × 'GW2357') and -14.7% ('FLASH' x 'ZN1018') for first boll opening time, -9.94% ('FLASH' x 'İPEK607') and 13.95% ('TMN199' × 'UA48') for plant height, 36.92% ('TMN199' × 'TMD139') and -5.71 ('STV468' × 'UA48') for monopodial branches, 49,14% ('STV468' × 'İPEK607'), 31.14% ('STV468' × 'TMD139') for number of bolls.

An average of 4.7% heterosis and -1.8% heterobeltiosis were determined for radiation values in all hybrid populations. This shows that there are heterotic effects in terms of the studied traits (El-Feki et al., 1999). An average of -0.9% heterosis and -1.1% heterobeltiosis were determined for yellowness in all hybrid populations. This shows that there are heterotic effects for this trait (El-Feki et al., 1999). An average of 2.8% heterosis and -5.5% heterobeltiosis were determined in terms of plant height values in all hybrid populations. These heterotic effects for plant height have been reported in earlier syıdies (Kanoktip 1987, Kalsy and Vithal 1982, El-Feki 1995). An average of 21.6% heterosis and 13.3% heterobeltiosis were determined for the number of boll in all hybrid populations. Heterotic effects for the number of bolls per plant have been reported earlier (Kumar et all. 1974, Gülyaşar 1987, El-Feki et all. 1995; Karademir 2005). An average of -3.3% heterosis and -3.7 % heterobeltiosis was recorded for the first boll opening time in all hybrid populations.

Table. 4. Heterosis and heterobeltiosis of cross combinations for plant height, number of monopodial branches, and boll number

	BB (cm)		MB		BC	
Hybrids♀+♂	Ht (%)	Hb (%)	Ht (%)	Hb (%)	Ht (%)	Hb (%)
TMN199×ADN712	-7.10	-10.39**	21.67**	12.31	7.82	3.81
TMN 99×GW2357	-2.04	-3.81	10.57**	5.63	-8.47	40.07**
TMN199×İPEK607	-6.43	-7.19	10.57	4.62	0.81	-5.15
TMN199×TMD139	-5.07	-6.04	44.72**	36.92**	43.04**	41.83**
TMN199×TMN170	-1.17	-5.05	36.51**	32.31	14.25	0.95
TMN199×UA48	-8.33*	-13.95**	31.03**	16.92	25.97*	14.31
TMN199×ZN1018	-2.08	-8.71	7.56	-1.54	20.42	18.12
FLASH×ADN712	-2.66	-5.19	18.40**	5.71	20.04	19.06
FLASH×GW2357	-0.65	-0.84	0.71	0.000	16.04	11.15
FLASH×İPEK607	-8.29*	-9.94**	12.50**	2.86	12.25	8.72
FLASH×TMD139	-1.18	-1.20	10.94**	1.43**	-2.14	-5.81
FLASH×TMN170	-3.07	-5.97	-2.29**	-8.57	23.57*	12.17
FLASH×UA48	-5.29	-10.25**	17.63**	1.43	15.43	7.70
FLASH×ZN1018	-0.44	-4.44	77.14**	-11.43	5.71**	4.56
GLORİA×ADN712	-8.38*	-12.32**	43.33**	32.31**	22.13	17.26
GLORİA×GW2357	-2.81	-4.38	11.76**	7.04	22.22	13.47
GLORİA×İPEK607	-4.22	-4.22	-10.57**	-15.38	-8.39	-14.04
GLORİA×TMD139	-6.37	-8.07	25.20**	18.46	17.73	17.07
GLORİA×TMN170	1.23	-3.50	14.29**	10.77	5.48	-7.02
GLORİA×UA48	6.81	-0.50	25.86**	12.31	5.24	-4.74
GLORİA×ZN1018	0.47	-7.03	9.24**	0.00	-3.39	-5.50
STV 468×ADN712	3.64	-0.68	12.00**	0.00	8.71	4.571
STV-468×GW2357	-4.95	-11.34**	9.22**	8.45**	28.42*	19.42
STV-468×İPEK 607	-4.88	-12.60**	18.75**	8.57**	58.66**	49.14**
STV-468×TMD139	1.79	-4.87	12.50**	2.86**	32.13**	31.14**
STV-468×TMN170	3.51	-0.41	16.03**	8.57**	48.62**	31.21**
STV-468×UA48	7.26	5.69	9.09**	-5.71**	27.10	15.23
STV-468×ZN1018	3.17	2.39	33.87**	18.57**	37.60**	34.85**
Ortalama	2.8	-5.5	18.0	7.3	21.6	13.3

Table 5. Heterosis and heterobeltiosis of cross combinations for first boll opening time, radiation, and yellowness

	FBOT (day)		Radiation		Yellowness	
Hybrids♀+♂	Ht (%)	Hb (%)	Ht (%)	Hb (%)	Ht (%)	Hb (%)
TMN199×ADN712	0.3	0.0	-1,80	-3,75**	-2,30	-2,50
TMN 99×GW2357	-4.1*	-4.4**	34,61**	-2,35	-64,85**	-7,57*
TMN199×İPEK607	-1.5	-1.8	1,45	0,17	-2,56	-5,00
TMN199×TMD139	10.4**	-10.9**	1,46	0,04	-3,12	-3,32
TMN199×TMN170	-1.4	-4.7**	-2,74	-3,86**	-6,37	-7,69*
TMN199×UA48	-0.6	-3.8	-1,56	-2,32	-3,66	-6,67
TMN199×ZN1018	11.9**	-12.9**	-0,88	-2,40	-0,21	-1,67
FLASH×ADN712	5.8**	3.5	-2,64	-4,26**	-2,61	-6,54
FLASH×GW2357	4.9**	2.6	-0,94	-1,57	-3,72	-5,38
FLASH×İPEK607	4.0*	1.8	0,50	-0,43	-0,41	-6,54
FLASH×TMD139	2.3	0.9	3,23**	1,45	-3,79	-7,31*
FLASH×TMN170	-1.2	-2.8	-0,38	-1,85	-5,72	-8,08*
FLASH×UA48	7.0*	5.5**	-0,35	-0,79	-5,98	-12,31**
FLASH×ZN1018	12.0**	-14.7**	97,18**	-1,41	76,92**	-11,54**
GLORİA×ADN712	2.9	2.3	-2,28	-2,38	-2,95	-3,77
GLORİA×GW2357	-4.9*	-5.5**	-0,56	-1,70	-6,58	-9,56**
GLORİA×İPEK607	2.0	1.4	-0,21	-1,06	2,38	0,85
GLORİA×TMD139	11.1**	-12.4**	-0,02	-3,44*	-5,88	-7,05
GLORİA×TMN170	5.0*	0.6	0,57	-2,64	2,49	0,00
GLORİA×UA48	4.8**	0.6	0,84	-0,51	-4,35	-6,38
GLORİA×ZN1018	-5.8*	-6.0**	-1,83	-5,31	-2,14	-2,55
STV 468×ADN712	0.4	0.0	-2,26	-6,95**	-5,52	-7,60*
STV-468×GW2357	-7.4*	-7.8**	-0,18	-4,00	-8,58*	-8,76*
STV-468×İPEK 607	0.4	0.0	3,38*	-0,87	-2,93	-7,20
STV-468×TMD139	-1.6	-2.9	2,25	0,64	-0,20	-2,00
STV-468×TMN170	-1.2	-5.2*	1,11	-0,73	1,01	0,40
STV-468×UA48	-1.4	-5.2*	0,84	-2,84*	-4,42	-9,20*
STV-468×ZN1018	-3.3	-3.7	3,78*	2,29	1,04	-2,40
Means	-3.3	-3.7	4,7	-1.8	-0,9	-1,1

Conclusion

This study determined the general combining ability (GCA) of the parents and the specific combining ability of their hybrid combinations. The study also evaluated the hybrid vigor of F_1 by investigating the genetic structure of the lines and testers. The values of heterosis for plant height, monopodia, number of bolls, and radiation were positive, while the days to opening boll, and yellowness were negative. The 'Gloria', 'TMN170' and 'UA48' proved best parents for days to opening boll, 'UA48', 'ZN108' and 'ADN712' for monopodial branches, 'İPEK607' and 'GLORIA' for plant height, 'TMN199', 'STV-468', 'GW2357' and 'TMD139' for the number of bolls, 'GLORİA', 'ADN712' for radiation, 'UA48', and 'İPEK607' for yellowness with the best overall combining abilities for respective traits. The most promising hybrid combinations having special combining ability were 'TMN 199' x 'İPEK 607', 'GLORIA' x 'UA48', 'GLORIA' × 'GW2357', 'FLASH' x 'İPEK607' for radiation, 'GLORIA' x 'UA48', 'GLORIA' × 'TMD139', 'GLORIA' × 'GW2357' for yellowness, 'TMN199' × 'TMD139', 'TMN199' × 'ZN1018', 'GLORIA' x 'TMD139' for first boll opening time, 'STV468' × 'İPEK607', 'TMN199' × 'TMD139', 'STV468' × 'ZN1018' for the number of bolls, 'GLORIA' 'İPEK607', 'GLORIA' x 'UA48', 'FLASH' x 'GW2357' for plant height.

References

1. Anonymous2018. 2017 Cotton Report. Ministry of Custom and Trade, General Directorate of Cooperatives March 2018.http://koop.gtb.gov.tr/data/2014 %20 Pamuk%20 Raporu.pdf, accessed 10.05.2018.
2. Ahmad, R. D., Malik, A. J., Hassan, G. and Subhan, M., 2005. Estimation of combining ability for seed cotton yield and its components in inter- varietal crosses of cotton. Gomal University Journal of Research, 2(1): 1-6.
3. Ali, I., Shakeel, A., Saeed, A., Hussain, M., Irshad, A., Mahmood, M., Zia, Z., Malik, W., Aziz, M. and Hussain, M., 2016b. The most basic selection criteria for improving yield and quality of upland cotton. Turkish Journal of Field Crops, 21(2): 261-268.
4. Başal, H., Unay. A., Canavar. O. and Yavaş, I. 2009. Combining ability for fiber quality parameters and within-boll yield components in intraspecific and interspecific cotton populations. Spanish Journal of Agricultural Research, 7(2): 364-74.

5. Boyaci, K., 2011. A research on the ınherıtance of agronomıcal and technologıcal propertıes of some cotton (*Gossypium* ssp.) in line x tester hybrids. Unıversity of Çukurova Institute of Institute of Natural and Applied Sciences Phd Thesis, Adana 137s.
6. El-Feki., T.A., Abdel-Razik, F.B., Gharab, M.A.M. and Emam, G.M.L., 1995. Heterosis and combining ability in top crosses of cotton. Beltwide Cotton Conferences, s.588-589.
7. Falconer, D.S., 1981. Introduction to Quantitative Genetics, Ed. 2 Longmans Green, London/New York.
8. Gülyaşar, F., 1987. The Researh on the inheritance of important agricultural and technological traits in the population formed by hybridization of regional standard cotton varieties (*G. hirsutum* L.) and some pest-resistant varieties (*G.hirsutum* L.) in Çukurova. . Unıversity of Çukurova Institute of Institute of Natural and Applied Sciences Phd Thesis, Adana, 110s.
9. Henderson, C.R. 1952. Specific and general combining ability. Heterosis, John W. Gowen. Iowa State Collage Pres, Ames, Iowa, USA
10. Kalsy, H.S. and VITHAL, B.M., 1982. Inheritance of some quantitative characters in upland cotton *Gossypium hirsutum* L). Cott. Trop. Fib. Abst., 7(10): 155.
11. Kanoktip, K., 1987. Study on the inheritance of certain agronomic characteristics in cotton. Field Crops Abs. Abs. No: 92-073564.
12. Karademir, Ç., 2004 Determination of superior parent and hybrid combinations in drought stress resistant of cotton breeding. Unıversity of Çukurova Institute of Institute of Natural and Applied Sciences Phd Thesis, Adana, 118s.
13. Kaushik. L.S., Singh. D.P. and Paroda, R.S., 1984. Line x tester analysis for fixed effect model in cotton (*Gossypium hirsutum* L.). Theor. Appl Genet, 68:487-491.
14. Kaynak, M.A., 1996. Genetic analysis of some cotton (Gossypium hirsutum L.) cultivars with different morphological and physiological characteristics. TUBITAK Turkish Journal of Agriculture and Forestry Vol:20 Supplementary Issue.
15. Kempthorne, O., 1957. An Introduction to Genetic Statistics. John Wiley and Sons. Inc., Newyork, U.S.A.
16. Kiani, G., Nematzadeh, G., Kazemitabar, S. K. and Alishah, O.I., 2007. Combining ability in cotton cultivars for agronomic traits. International Journal of Agriculture and Biology, 9 (3):521-522.
17. Kumar. P., Pathak. R.S. and Singh. R.K., 1974. Heterosis and combining ability in upland cotton (*Gossypium hirsutum* L.). Indian Journal of Agricultural Sciences, 44 (3): 145-150
18. Sajjad, M., Malook, S., Murtaza, A., Bashir, I., Shabaz, M.K. and ALI, M., Sarfraz, M., 2016. Gene action study for yield and yield stability related traits in *Gossypium hirsutum* L.: An overview. Life Science Journal, 12 (5): 1-11.
19. Saravanan, N.A., Ravikesaven, R. and Raveendran, T.S, 2010. Combining analysis for yield and fibre quality parameters in intraspecific hybrids of *Gossypium hirsutum* L. Electronic Journal of Plant Breeding 1:856-863.
20. Sprague, G.F. and Tatum, L.A., 1942. General and specific combining ability in single crosses of corn. J. Am. Society Agronomy, 34: 923 -932.
21. Unrau, J., 1947. Heterosis in Relation to Sunflower Breeding. Scientific Agriculture, 27(9): 414-427.
22. Ünay, A., 1993. Research on earliness and inheritance of some agricultural traits in cotton (*G. hirsutum* L.). Unıversıty of Trakya Institute of Natural and Applied Sciences PhD Thesis, Edirne, 114s.
23. Verhalen, L.M., Morrison, W.C., Al-Rawi, B.A., Fun, K.C. and Murray, Y.J.C., 1971. Diallel analysis of several agronomic traits in upland Cotton *(Gossypium hirsutum* L.*)* Crop Sci., 11: 92-96
24. White, T.G. and KoheL, R.J., 1966. A diallel analysis of agronomics characters in selected lines of cotton. Crop Science, 6: 254-257.
25. Temiz, M., 2003 The research inheritance of some fiber quality properties of some cotton genotypes (*Gossypium hirsutum* l.) in line x tester hybrids. Unıversity of Çukurova Institute of Institute of Natural and Applied Sciences Phd Thesis, Adana, 111s
26. Tuik, 2020. Turkish Statistical Institute, Public and Private Sector seed production data. https://www.tuik.gov.tr/tr/.
27. Zengel, M., 2003. A study on the genetic structure of agricultural and fiber characteristics in the F1 generation of *Gossypium hirsutum* L. x *Gossypium hirsutum* L. and *Gossypium hirsutum* L. x *Gossypium barbadense* L. cotton hybrids. Unıversity of Çukurova Institute of Institute of Natural and Applied Sciences PhD Thesis, Adana, 59s.

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AMMI analysis for GE interaction in cotton (*Gossypium hirsutum*)

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Abstract

Background: *AMMI analysis has evolved into an important statistical tool in plant breeding to test the adaptability of cotton genotypes in multi-environment. The objective of this study was to determine the genotype × environment interaction of four introduced and two local genotypes across the five environments in Bangladesh. The experiment was conducted in a randomized complete block design with three replications during the Kharif 2020-2021 growing season.*

Results: *The AMMI analysis revealed that the seed cotton yields of the studied genotypes were significantly influenced by the genotype × environment interaction.*

Keywords: *AMMI, Cotton, genotype, environment*

Introduction

Multi-environment trials are usually conducted to evaluate the genotype-environment interaction to release new genotypes in targeted environments (Ceccarelli, 1996; Kaya *et al*., 2006; Karimizadeh*et al*., 2012; andMitrovic*et al*., 2012;).The differential response of a genotype or cultivar for a given trait across environments is defined as the genotype × environment interaction (G × E) that helps in the selection process to recommend a genotype for a target environment (Gauch, 2006). Genotype X environment (GE) interactions are of major concern to plant breeders for developing improved cultivars. A genotype is considered as promising if it performs well across a range of environments. GE interaction can be exploited by selecting location-specific superior genotypes or by choosing widely adapted and stable genotypes over different locations (Lakew*et al.,* 2017). There are two widely used approaches of grain yield stability analysis for multi-location trial data such as AMMI (Gauch*et al.,* 2008) and GGE-Biplot (Yan, 2002).

The AMMI model is a popular approach to studying GE interaction. The main effects of the environment and genotype with principal components analysis of GE interactions are combined through AMMI ANOVA (Sharifi*et al*., 2017). The present study was conducted to compare the performance and stability of 4 introduced cotton genotypes with 2 cotton varieties in Bangladesh for recommending the farmers' wider or specific cultivation in different cotton growing areas.

Materials and methods

The experiments were conducted at five cotton research centers across Bangladesh viz., Sreepur, Gazipur (E1); Jagdishpur, Jashore (E2); Mahigonj, Rangpur (E3); Sadarpur, Dinajpur (E4); and Balaghata, Bandarban (E5). The biophysical characteristics of the test environment is given in Table 1.Four cotton genotypes viz. Turkish-1 (G1), Turkish-2 (G2), Turkish-3 (G3), Turkish-4 (G4), introduced from the Cotton Research Institute of Turkey, and two local varieties viz. CDB-Tula-M1 and CB-15 were used as test materials. The experiments in all locations were designed in a Complete Randomized Block Design (CRBD) with three replications per environment under rain feed conditions. Sowing was done manually in rows. The experimental plot consisted of four ridges, 12 m long and 70cm apart. The other agriculturalpractices were applied as recommended by the Cotton Development Board, Bangladesh. Seed cotton yield

was determined by harvesting the busted boll of the two middle lines. The seed cotton yield data were subjected to AMMI analysis by PBTools, version 1.4. 2014. Biometrics and Breeding Informatics, PBGB Division,International Rice Research Institute, Los Baños, Laguna.

Table 1. The biophysical characteristics of the test environments

Environment	Month	Longitude	Latitude	Attitude (m)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)	Humidity (%)
	Jul				33.68	27.53	130.05	82.57
	Aug				33.88	27.44	98.66	81.74
E1	Sep	90.4202724	23.9999405	14	33.57	27.01	115.46	81.15
	Nov				32.43	24.82	74.53	77.36
	Oct				30.21	21.04	17.44	67.15
	Dec				27.28	17.7	5.29	60.76
	Jul				32.58	26.86	142.52	77.15
	Aug				32.23	26.3	154.44	79.76
E2	Sep	89.1801225	23.1777682	10.89	31.79	25.6	109.1	80.74
	Nov				30.71	23.22	75.51	75.06
	Oct				29.2	19.53	38.57	60.08
	Dec				26.8	16.45	3.23	49.6
	Jul				32.55	26.45	387.58	81.29
	Aug				33.22	26.77	286.41	78.54
E3	Sep	89.275227	25.7438916	33.66	32.25	25.42	330.85	80.77
	Nov				30.92	22.22	77.06	76.44
	Oct				28.55	18.47	4.85	66.39
	Dec				25.92	15.96	2.94	59.06
	Jul				34.92	28.38	415.83	87.22
	Aug				35.64	28.72	307.29	84.27
E4	Sep	88.6437649	25.6221009	40.09	34.6	27.27	354.97	86.66
	Nov				33.17	23.84	82.68	82.01
	Oct				30.63	19.82	5.2	71.23
	Dec				27.81	17.12	3.15	63.37
	Jul				30.29	26.55	449.38	86.47
	Aug				30.27	26.4	321.64	86.62
E5	Sep	92.2187476	22.1935628	22.08	30.5	26.32	219.66	85.5
	Nov				29.87	24.87	171.11	83.21
	Oct				28	21.02	28.77	77.45
	Dec				25.41	17.81	2.03	72.16

Results and discussion

Combined analysis of variance

Table 2 presents the combined analysis of variance. Genotype (G), environment (E) and genotype × environment interaction (GEI) were highly significant (P<0.001) for seed cotton yield. The factors explained showed that seed cotton yield was affected by genotype (10.07%), environment (64.26%) and their interaction (19.47%).

Table 2. Combined analysis of variance of seed cotton yield for 6 cotton genotypes evaluated at five environments

Source	DF	SS	MS	F	P	SS%
Genotype (G)	5	5870181	1174036	19.5	0.0000	10.07
Environment (E)	4	3.75E+07	9362052	155.48	0.0000	64.26

G x E interaction (GEI)	20	1.14E+07	567514	9.42	0.0000	19.47
Error	60	3612926	60215			
Total	89	5.83E+07				

AMMI analysis of variance

The AMMI analysis of variance for seed cotton yield is presented in Table 3. IPCs 1 to 3 jointly accounted for 95.8% of the entire variation among the genotypes.

Table 3. AMMI analysis for seed cotton yield

	Variance (%)	Acum. Variance (%)	Df	SS	MS	F	P
PC1	55.8	55.8	8	4219561	527445	9.62E+18	0
PC2	33.9	89.7	6	2561790	426965	7.79E+18	0
PC3	6.1	95.8	4	462425	115606	2.11E+18	0
PC4	4.3	100.1	2	323074	161537	2.95E+18	0

AMMI biplot display

The AMMI biplots are graphs where aspects of both genotypes and environments are plotted on the same axes so that interrelationships can be visualized. There are two basic AMMI biplot, the AMMI 1 biplot, where the main effects of seed cotton yield (genotype mean and environment mean) and IPCA1scores for both genotypes and environments are plotted against each other. On the other hand, the second biplot is AMMI 2 where scores for IPCA1 and IPCA2 are plotted. In the AMMI 1 biplot, the usual interpretation of biplot is that the displacements along the abscissa indicate differences in main (additive) effects, whereas displacements along the ordinate indicate differences in interaction effects. Genotypes that group together have similar adaptation while environments that group together influences the genotypes in the same way (Kempton, 1984). The AMMI 1 biplot gave a model fit 55.8% (Fig. 1). Among the genotypes, G3 and G6 exhibited high yield with a positive IPCA1 score. These two genotypes (G3 and G6) were adapted to the environment E5. Genotype G5 showed a negative IPCA1 score with over average yield and was adapted to E1 and E3. Other genotypes showed below-average yield and negative IPCA1 score.

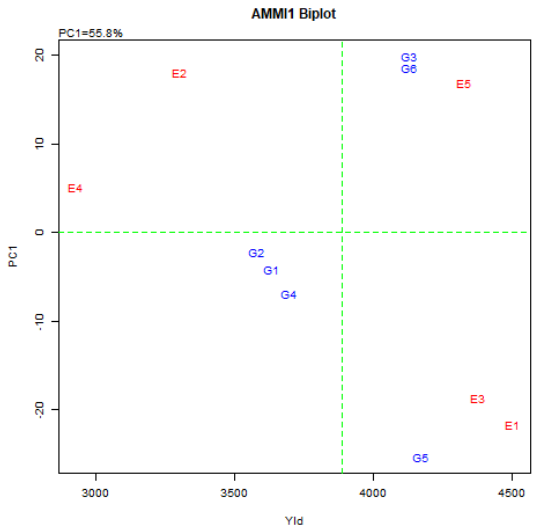

Figure 1. AMMI 1 Biplot for seed cotton yield of six cotton genotypes (G) and five environments (E)

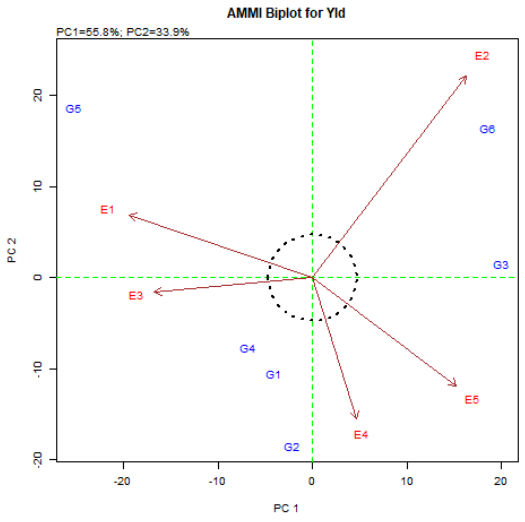

Figure 2. AMMI II biplot for cotton yield showing the interaction of PC1 and PC2 scores of six cotton genotypes (G) and five environments (E)

In AMMI 2 biplot (Figure 2), the environmental scores are joined to the origin by side lines called vectors. Environments with short vectors did not exert strong interaction effects while those environments that have long vectors located away from the origin exert strong interaction effects. The vector length in the AMMI model can be used to determine the discriminative ability of environments for genotypes (Li *et al,* 2003). The environments E1, E2, E3, E4 and E5 had longer vectors. Thus, they were the best discriminative environments for investigated

genotypes (Yan and Hunt, 2001). The acute angle between vectors of E1 and E3 environments indicated that these two environments were similar for yield determination. Yet, environments with obtuse angles were different, i.e., E2 and E4.

Conclusion

Our study revealed that none of the studied genotype is suitable for all environments. All of the studied genotypes are highly influenced by the Genotype × Environment interaction. The G3 and G6 genotypes were adapted to E5 environment while the G5 genotype were adapted to E1 and E3 environments.

References:

1. Ceccarelli, S. 1996. Adaptation to low or high input cultivation. Euphytica, 92: 203-214
2. Gauch, H.G., Jr, Piepho, H.P., Annicchiarico, P. 2008. Statistical analysis of yield trials by AMMI and GGE: Further considerations. Crop Sci., 48(3):866–889.
3. Gauch, H. G. 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Sci. 46: 1488- 1500.
4. Karimizadeh, R, Mohammadi, M., Sabaghnia, N., Shefazadeh, M. K. 2012. Using Huehn's Nonparametric Stability Statistics to investigate Genotype × Environment Interaction. Not Bot HortiAgrob. 40: 195-200.
5. Kaya, Y., Akcura, M., Taner, S. 2006. GGE biplot analysis of multi-environment yield trials in bread wheat. Turk. J. Agric. 30: 325- 337.
6. Kempton, R. A. 1984.The use of biplots in interpreting variety by environment interactions. Journal of Agricultural Science, 103: 123-135.
7. Lakew, T., Dessie, A., Tariku, S., Abebe, D. 2017. Evaluation of performance and yield stability analysis based on AMMI and GGE models in introduced upland rice genotypes tested across Northwest Ethiopia. International Journal of Research Studies in Agricultural Sciences, 3(2):17–24.
8. Li, B., J. Yan, Z. He and Z. Li (2003). Analyzing site discrimination in crop regional
9. yield trials by AMMI model. Zuo wu xue bao 30(6): 593-596.
10. Mitrovic, B, D Stanisavljevi, S Treski, M Stojakovic, M Ivanovic, G Bekabac and M Rajkovic. 2012. Evaluation of experimental maize hybrids tested in multi-location trials using AMMI and GGE biplot analysis. Turkish J. Field Crops. 17(1): 35-40.
11. Sharifi, P., Aminpanah, H., Erfani, R., Mohaddesi, A., Abbasian, A. 2017.Evaluation of genotype× environment interaction in rice based on AMMI model in Iran. Rice Sci., 24(3):173–180.
12. Yan, W., Hunt, L.A. 2002. Biplot analysis of diallel data. Crop Sci., 42(1):21–30.
13. Yan, W., Hunt, L.A. 2001. Interpretation of genotype× environment interaction for winter wheat yield in Ontario. Crop Sci. 41(1) 19-25.

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Gossypium diploid species genetics: Inheritance and linkage of bracteoles

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Abstracts

*The first report on bracteole arrangement as a phenotypic marker described major genes and interactive relationships in diploid species G. herbaceum and G. arboreum cotton (2n = 26). The population of three families (IC-371437 x PA - 785, IC-371437 x PA - 740, IC-371437 x PA - 812) and reciprocal crosses were studied for bracteoles, colour and boll shape. Further linkage was confirmed in IC-371362 x PA - 812. Genotype IC-371362 has red pigmented bracteoles, colour and boll shape considered as contrasting traits. The information on bracteoles (Flared vs Closed) confirmed two new genes one single dominant and one duplicate gene. The heterogeneity and χ^2 test were non-significant indicating action of a single dominant gene. The P value ranged from 0.8 to 0.3 indicating rich agreement between families for 3:1 ratio controlled by a single gene. The reciprocal crosses were tested for duplicate gene action and showed non – significant χ^2 and heterogeneity test with a good fit of 15:1 ratio indicated duplicate gene action where genes of both genotypes act independently. The independent assortment of two traits viz. Flared bracteoles with dark green coloured bolls [FFGG] and closed bracteoles with light green coloured bolls [ffgg] were studied. Families of direct crosses showed good fit for 9:3:3:1 ratio with non-significant χ^2 and heterogeneity test assorted independently. However, reciprocal crosses showed the deviation of the calculated ratio. Linkage and crossing-over values were estimated. Linkage values ranged from 90.04% to 91.14% and crossing-over values ranged from 8.86 % to 9.96 %. The gene order was decided as GFR and calculated distances between **G** and **F** and between **F** and **R**. The distance between gene **G** and **F** was 18.94cM and the distance between gene **F** and **R** was 7.76cM. The bracteoles gene is important as a phenotypic marker because bracteoles appear in the initial flower bud induction stage helping to maintain the genetic purity of the parents. The study concluded that G. herbaceum is genotypically dissimilar with G. arboreum for bracteoles traits and is considered to be a phenotypic marker for further study.*

Key words: *Cotton, Gossypium, Herbaceum, Arboreum, Bracteoles, Inheritance, Duplicate gene action, Linkage distance, Genetic ratio, Gene order.*

Introduction

India is the third richest country in the world for its total holdings and diversity of cotton genetic resources. The germplasm of *desi* cotton is conserved in India. The stable phenotypic trait has importance to differentiate Gossypium species that justify the conservation and maintenance of vast germplasm. Diploid species have differentiable bracteoles arrangement like flare away from bolls and closed bracteoles. Deviation in bracteoles arrangement is either due to segregation, linkage, epistasis or gene action that differentiates parents and germplasm. Phenotypic traits alter only after introgression and heterosis breeding helping to the genetic improvement of the species. Bracteole arrangement, a phenotypic trait was kept constant and studied the genetics, inheritance, and linkage of bracteoles in *Gossypium* diploid species. This help to maintain genetically pure parents, germplasm, and selections. The first report described major gene action, linkage distance, gene order, and interactive relationships in both the diploid species *G. herbaceum* and *G. arboreum* cotton for bracteoles arrangement, colour and boll shape.

Materials and Methods

Selection of parents

A single seed of each 582 germplasm of *G. herbaceum race Wightianum* was raised in paper tubes using the paper tubes nursery technique in 2015 crop season. Fifteen days old seedlings were transplanted in the main research farm of ICAR – Central Institute for Cotton Research, Nagpur (Latitude : 21.037N; Longitude : 79.056E; Annual rainfall: 960mm – 1100mm; pH = 6.2 – 6.7; Soil: Calcareous to Black cotton soil) Maharashtra state, India. The trial was conducted in augmented design with two replications and germplasm was evaluated. Promising homogeneous parental lines having flared away bracteoles were selected for the introgression breeding program.

Crossing Program

The crossing block was raised during the 2016 crop season with the spacing of 90cm x 45cm. Three *G. arboreum lines* (PA - 740, PA - 785 and PA – 812) having closed bracteoles with long fiber lengths (28.5mm to 29.8mm) were raised along with *G. herbaceum* parents (IC - 371437 and IC - 371362) with flared bracteoles [Fig. A & B] and initiated a crossing program. Genotype IC – 371362 has a red pigmented plant type with red colour, round shape bolls and flared bracteoles used as contrasting characters for linkage study [Fig.C]. The crossing technique was followed using the artificial crossing method refers to hand emasculation and pollination. The crossing work was started after one week of flower initiation. The flower buds of proper stage having flared bracteoles were selected for emasculation. Well develop having cream colour flower buds (candle shape) that are likely to open the next day were chosen for emasculation. Anthers of selected buds were gently removed with the help of nail of the thumb (emasculation). The emasculated buds were covered with a red colour tissue paper bag to prevent natural outcrossing. Emasculated buds were pollinated the next day morning at 8 - 11 AM because the stigma is receptive during this period. Candle stage flower buds [Fig.D] of tagged male parents about to open were selected and kept in brown paper bags for exposure of sunlight. From 8.30 AM – 10.00, AM pollen was in the active stage for dispersion. Liberated pollen was touched to the hand and confirmed the yellow colour pollen dispersion. The flower of the male parent touched the emasculated female buds and the pollination process was completed. After pollination red tissue paper bags were replaced by white tissue paper bags for identification. A label with the date of pollination and parents was tied on the pedicel of crossed bud for identification of crossed bolls. The crossed buds remain covered till the boll formation, development and busting stage after pollination. Recommended package of practices was followed for crop management. Fertilizer @ 50:50:80 NPK kg/ha was applied in split doses after 30 and 60 days after sowing.

Collection of F1, F2, F3 and F4 population seeds

Crossed F_1 bolls were collected and F_1 seeds were kept ready for the next crop season. In 2017 crop season F_1 plants of three families (IC - 371437 x PA - 785, IC - 371437 x PA - 740 and IC- 371437 x PA - 812) and reciprocals (PA - 785 x IC - 371437; PA - 740 x IC - 371437 and PA - 812 x IC - 371437 were raised in the main field. Each plot consist 60 plants (6 rows x 10 plants / row) with spacing of 90cm x 45cm in three replications. The F_2 population of three families was raised in the field in 2018 cop season and observed a segregated population for bracteoles arrangement (flared [FF], closed [ff] and segregated population [Ff] ; Fig. E & F). In the 2019 and 2020 crop season, F_3 and F_4 populations were raised in the field with three families and observed the bracteoles arrangement respectively. In F_2 segregated population of 144 plants of each cross (48 plants x 3 families) with parental lines (P_1 + P_2) were raised [Fig. G, H & I].. Further F_3 segregated population of 576 plants of each cross (12 x 8 rows x 6 families + P_1 and P_2 parental lines) were raised and field evaluation was carried out.

Data collection

Morphological and yield contributing traits were recorded during crop growth and the yield component *viz*., seed cotton yield per plant were measured at maturity and harvesting. The present study is confined to inheritance, gene action and linkage for bracteoles arrangement, boll colour and shape. Observations were recorded on the total number of plants, total number of bolls per plant, flared, closed and segregated bracteoles, red pigmented and green coloured bolls, and round and oblong shape bolls in parents and segregated population. The F_3 and F_4 population were advanced for further study.

Data analysis

Inheritance, gene action and genetic ratios were compared of bracteoles arrangement, boll colour and shape in F_2 segregated population subjected to standard χ^2 test procedure (Cochran, 1942, 1954). Maximum likelihood tables were used to calculate recombination values (Allard, 1956). The sampling distribution of χ^2 worked out to determine the probability (P). The P value was compared with degree of freedom, P = 1.00 for $\chi^2 = 0$. As χ^2 increases, P value diminishes (Fisher, 1922). Recombination values are denoted by p and probabilities by P. The P value of 0.05 to 0.02 indicated poor agreement between cross and segregated trait or families. To use probability table for χ^2, the degree of freedom was calculated for independence with product of rows and columns minus one [d. f. = (r-1) x (c-1)]. The χ^2 that corresponds to specific values of P was observed using the χ^2 table (Fisher, 1923, 1954). The data was statically analyzed using OPSTAT software (Sheoran *et al*., 1998) and tested at 5% and 1% significance levels (P < 0.05 and P < 0.01) respectively.

The formula for χ^2 test

$$\chi^2 \text{ test} = \sum \frac{(O - E)^2}{E}$$

O = Observed in any one group of the experimental distribution
E = Expected theoretically calculated number for the same group.

Heterogeneity χ^2 test

The most valuable property of χ^2 is its additive characters provide test of homogeneity which enables one to combine the result of a set of experiments to determine the probability of the whole set of experiments (Mather,1951). When several genetic families are combined to obtain a composite χ^2 value, a serious lack of consistency may be masked in the numerical ratios of the separate families (Kirk and Immer, 1928). The heterogeneity test worked out whether the percentage of variation across studies is due to P value rather than chance (Harris, 1912).

Estimation of linkage

The deviation from classical ratios of reciprocal crosses of two alleles in bracteoles and boll colour were observed in the population. Hence the linkage study was carried out and linkage and crossing overvalue were calculated (Morgan, 1911). Linkage and crossing over values were calculated from F_2 data using additive method. Crossing over value (%) = 100 - linkage value. Linkage value is a square root of p2 values expressed in percentage (Bridges and Olbrycht, 1926).

$$\text{Linkage value } (p^2) = \frac{\text{Total number of recombinant progenies}}{\text{Total number of progenies}}$$

$$\text{Linkage value } (p^2) = \frac{E - M}{N}$$

Where,
E = ∑ of parental classes
M = ∑ of recombinant classes
N = Total number of progenies

Results and Discussion

Inheritance: Bracteole shape and arrangement

Inherited traits of homogeneous plants for bracteoles showed from the initiation of square buds in a plant after 60 – 65 days after sowing. The growth of bracteoles enlarged the square buds development and growth. The population of bracteoles (flared, closed and segregated) was grouped and total number of bolls per plants per cross were studied. The F_1 was heterozygous [Ff] with flared bracteoles in the direct and closed bracteoles in the reciprocal crosses.

Test for single dominant gene effect (3:1) in direct crosses

Three families were tested for single dominant gene effect. In direct crosses IC-371437 x PA – 785, IC-371437 x PA - 740 and IC-371437 x PA - 812 total 2296, 2041 and 2435 bolls per family showed good fit of 3:1 with a non-significant χ^2 test respectively. Sum of 3 families χ^2 test was 1.90 which is non-significant. The heterogeneity between the 3 families were non-significant (χ^2 value = 1.67 for 2 degree of freedom; Non significant at 1%) indicating action of a single dominant gene. The P value ranged from 0.8 to 0.3 indicated rich agreement between families for 3:1 ratio controlled by single gene (Table 1).

Table 1. Inheritance and test for single dominant gene effect for bracteoles trait in direct crosses (*G. herbaceum* x *G. arboreum*)

Families	F_1 [Ff]	F_2 population of bracteoles: Flared bracteoles [FF] Observed	Flared bracteoles [FF] Expected	χ^2 test	Closed bracteoles [ff] Observed	Closed bracteoles [ff] Expected	χ^2 test
Direct crosses (3:1)							
IC-371437 x PA – 785	Flared	1734	1722	0.08 NS	562	574	0.25 NS
IC-371437 x PA – 740	Flared	1515	1530.75	0.16NS	526	510.25	0.48NS
IC-371437 x PA – 812	Flared	1847	1826.25	0.23NS	588	608.75	0.70NS
Total χ^2 value				**0.47NS**			**1.43NS**
Composite value		5096	5079	**0.056NS**	1676	1693	**0.170NS**
Sum of 3 families χ^2							1.90
Sum of 3 families χ^2 composite							0.226

Item	Degree of freedom	χ^2	P
Deviation (T - 1)	1	0.226	0.5 - 0.3
Heterogeneity (F-1)	2	1.674	0.5 - 0.8
Total	3	1.90	0.5 - 0.6

T = traits; F = Families ; NS = Non significant ; ** = Significant

Test of reciprocal crosses for single dominant gene effect (3:1)

Similarly, test of reciprocal crosses for a single dominant gene effect (3:1) was carried out in three families PA – 785 x IC-371437, PA – 740 x IC-371437 and PA - 812 x IC-371437. The Sum of 3 families χ^2 test was highly significant (1340.93**). The sum of 3 families χ^2 composite (1340.52**) was also highly significant showed not good fit of 3:1 ratio. The heterogeneity between the 3 families were very low when compared with the value of χ^2 composite for 2 degree of freedom indicating the action of some other gene action which hided the effect. The P value ranged from 0.05 to 0.02 indicating poor agreement between families for 3:1 ratio at both 5% and 1% level of significance indicating the action of a single gene obscured by the action of modifiers (Table 2).

Table 2. Test for single dominant gene effect for bracteoles trait in reciprocal crosses (*G. arboreum* x *G. herbaceum*)

Families	F_1	F_2 population of bracteoles: Flared bracteoles [FF] Observed	Flared bracteoles [FF] Expected	χ^2 test	Closed bracteoles [ff] Observed	Closed bracteoles [ff] Expected	χ^2 test
Reciprocal crosses (3:1)							
PA -785 x IC-71437	Closed	215	824.50	450.56**	3083	2473.50	150.18**
PA -740 x IC-71437	Closed	112	480.00	282.13**	1808	1440.00	94.04**
PA – 812 x IC-71437	Closed	109	465.50	273.02**	1753	1396.50	91.00**
Total χ^2 value		-	-	**1005.71****	--	--	**335.22****
Composite value		436	1770	1005.39**	6644	5310	335.13**
Sum of 3 families χ^2							1340.93**
Sum of 3 families χ^2 composite							1340.52**

Item	Degree of freedom	χ^2	P
Deviation (T - 1)	1	1340.52	0.02 - 0.03
Heterogeneity (F-1)	2	0.41	0.01 - 0.02
Total	3	1340.93	0.05 - 0.02

T = Traits; F = Families ; NS = Non significant ; * P = 0.05; **P = 0.01

Test of reciprocal crosses for duplicate gene effect (15:1)

Considering the other factor's interaction of two pairs of alleles the reciprocal crosses of three families were tested for duplicate gene effect (15:1). The χ^2 test was non – significant for three families with a good fit of 15: 1 ratio indicating duplicate gene action. Sum of 3 families χ^2 test was 1.46 which is non-significant (**P = 0.01). The heterogeneity between the 3 families was non-significant (χ^2 value = 1.35 for 2 degrees of freedom). The P value ranged from 0.5 to 0.3 indicating rich agreement between families for a 15:1 ratio for duplicate genes action where genes of both genotypes act independently (Table 3). The study showed that genes were interacting with each other to produce novel phenotypes that did not exhibit a dominance relationship found in Mendelian ratios. The study also indicated that a gene does not completely mask another gene (epistasis), but modified the effect of the second gene (closed bracteoles). Such types of independent genes produce the same effect and act independently (CICR, Annual Report, 2018 -19).

Table 3. Duplicate gene action due 15:1 ratio for bracteoles trait in G. arboreum x G. herbaceum

Families	F_1	F_2 population of bracteoles					
		Closed		χ^2 test	Flared		χ^2 test
		Observed	Expected		Observed	Expected	
Reciprocal crosses							
PA -785 x IC-371437	Closed	3083	3091.80	0.025NS	215	206.12	0.38 NS
PA -740 x IC-371437	Closed	1808	1808.00	0.035 NS	112	120.00	0.53NS
PA – 812 x IC-371437	Closed	1753	1745.55	0.031 NS	109	116.37	0.46 NS
Total χ^2 value				0.091NS			1.37NS
Composite value		6644	6637.35	0.007NS	436.00	442.49	0.095NS
Sum of 3 families χ^2							1.46
Sum of 3 families χ^2 composite							0.102
Item		Degree of freedom		χ^2		P	
Deviation (T-1)		1		0.102		0.07 - 0.06	
Heterogenity (F-1)		2		1.674		0.005 - 0.004	
Total		3		1.90		0.3 - 0.2	

T = traits ; F =Families ; NS = Non significant ; ** = Significant

Independent assortment for two alleles for bracteoles and boll colour trait in direct crosses (9:3:3:1)

Further study was extended for an independent assortment of two traits *viz.* Flared bracteoles with dark green coloured bolls [FFGG] and Closed bracteoles with light green (parrot) coloured bolls [ffgg] and compared for 9:3:3:1 ratio. The F_1 was flared bracteoles with dark green coloured bolls [FfGg]. The F_2 population of three families was segregated in flared bracteoles with dark green coloured bolls [FFGG], flared bracteoles with light green coloured bolls [FFgg], closed bracteoles with dark green coloured bolls [ffGG] and closed bracteoles with light green coloured bolls [ffgg] with good for 9:3:3:1 ratio with non-significant χ^2 test indicated both traits assorted independently. The heterogeneity test was non-significant when the composite population was compared. The data afford a good fit to the calculated 9:3:3:1 ratio indicating that two genes assert independently without any changes in bracteoles arrangement and boll colour. The breeding behaviour of bracteoles and boll colour in F_2 segregated population are given in Table 4 and 5.

Deviation from classical ratios of reciprocal crosses of two alleles in bracteoles and boll colour

The independent assortment of two traits in reciprocal crosses in the flared bracteoles and dark green coloured bolls [FFGG] and closed bracteoles with light green coloured bolls [ffgg] were compared for 9:3:3:1 ratio. The F_1 was closed bracteoles with dark green coloured bolls [FfGg]. The segregated F_2 population of three families does not show a good fit for 9:3:3:1 ratio. The χ^2 test was highly significant. The heterogeneity test was highly significant when the composite population was compared (Table 6).

The families of three reciprocal crosses of two alleles in bracteoles and boll colour showed the deviation of the calculated ratio from the theoretical ratio and are highly significant. The data does not afford a good fit to calculated 9:3:3:3:1 ratios. Parental values are higher in number

while recombinant are low. The lower the recombination frequency more the closely the genes are physically linked (Table 6).

Table 4. Inheritance of bracteoles and boll colour

Segregated F_2 population			Breeding behavior
Phenotype	Genotype	Ratio	
Flared Bracteoles	FFGG	1	1 Flared bracteole with dark green coloured bolls (FFGG)
	FFGg	2	3 Flared dark green (FFGG) : 1 Flared light green (FFgg)
	FfGG	2	3 Flared dark green (FFGG) : 1 Closed dark green (ffGG)
	FfGg	4	9 Flared dark green (FfGg) : 3 Flared dark green (Ffgg) : 3 closed light green (ffGG): 1 Closed light green (ffgg)
Dark green coloured bolls	FFgg	1	1 Flared bracteole with light green coloured bolls(FFgg)
	Ffgg	2	3 Flared light green (FFgg) : 1 Closed light green (ffgg)
Light green coloured bolls	ffGG	1	1 Closed bracteole with dark green coloured bolls (ffGG)
	ffGg	2	3 Closed dark green (ffGG) : 1 Closed light green (ffgg)
Closed bracteoles	Ffgg	1	1 Closed bracteole with light green coloured bolls (ffgg)
Possible combinations		16	

Table 5. Inheritance and independent assortment of two alleles in bracteoles and boll colour

Families	No. of bolls observed [No. of bolls expected] F_2 segregated population (9:3:3:1)				Total bolls	χ^2 test	P value
	Flared bracteoles with dark green coloured bolls	Flared bracteoles with light green coloured bolls	Closed bracteoles with dark green coloured bolls	Closed bracteoles with light green coloured bolls			
Symbol	FFGG	Ffgg	ffGG	ffgg			
IC-371437 x PA -785	1300 [1291.50]	434 [430.50]	422 [430.50]	140 [143.50]			
χ^2 test	0.055NS	0.028NS	0.167NS	0.085NS	2296	0.335NS	0.5 - 0.7
IC-371437 x PA -740	1137 [1148.04]	378 [382.68]	394 [382.68]	132 [127.56]			
χ^2 test	0.106NS	0.057NS	0.334NS	0.154NS	2041	0.651NS	0.3 - 0.5
IC-371437 x PA - 812	1388 [1369.62]	459 [456.56]	440 [456.56]	148 [152.18]			
χ^2 test	0.24NS	0.013NS	0.60NS	0.11NS	2435	0.969NS	0.3 – 0.6
Composite	3825 [3809.25]	1271 [1269.75]	1256 [1269.75]	420 [423.35]			
χ^2 test	0.06NS	0.002NS	0.14NS	0.02NS	6672	0.222NS	0.3 – 0.5
Total χ^2 value						1.959NS	
Heterogeneity test							
		Degree of freedom		χ^2		P	
Deviation (T - 1)			3	0.222		0.5 - 0.7	
Heterogeneity (T - 1) (F- 1)			6	1.728		0.3- 0.5	
Pooled (C – 1)			3	1.959		0.3 - 0.7	

C = Classes; T = Traits; Bold letter denoted expected frequency; Based on χ^2 values for 9:3:3:1; NS = Non significant ; ** = Significant

Estimation of linkage from F2 data of two alleles in bracteoles and boll colour

Further study was extended and estimated linkage (cM) and crossing over value using the additive method for reciprocal crosses. Among the three families, the linkage value ranged from 90.04% to 91.14%. Crossing over values ranged from 8.86 % to 9.96 % (Table 7).

Table 6. Deviation from classical ratios (9:3:3:1) in reciprocal crosses of two alleles in bracteoles and boll colour

Families	No. of bolls observed [No. of bolls expected] F_2 segregated population (9:3:3:1)				Total bolls	χ^2 test	P value
	Flared bracteoles with dark green coloured bolls	Flared bracteoles with light green coloured bolls	Closed bracteoles with dark green coloured bolls	Closed bracteoles with light green coloured bolls			
Symbol	FFGG	Ffgg	ffGG	ffgg			
PA -785 x IC-371437	1424 [1855.08]	96 [618.36]	183 [618.36]	1595 [206.12]			
χ^2 test	100.17**	441.26**	306.51**	9358.56**	3298	10206.50**	0.05 - 0.07
PA -740 x IC-371437	739 [1080]	76 [360]	102 [360]	1003 [120]			
χ^2 test	107.66**	224.04**	184.90**	6497.40**	1920	7014.00**	0.03 - 0.05
PA - 812 x IC-371437	714 [1047.33]	72 [349.11]	104 [349.11]	972 [116.37]			
χ^2 test	106.08**	219.75**	172.09**	6291.16**	1862	6789.28**	0.03 – 0.05
Composite	2877 [3982.41]	244 [1327.47]	389 [1327.47]	3570 [422.49]			
χ^2 test	306.83**	884.31**	663.46**	23448.64**	7080	24009.78**	0.03 – 0.07
Total χ^2 value						25303.24**	
Heterogeneity test							
	Degree of freedom		χ^2		P		
Deviation (T - 1)	3		24009.78		0.05 - 0.07		
Heterogeneity (T - 1) (F- 1)	6		1293.46		0.03 - 0.05		
Pooled (C – 1)	3		25303.24**		0.03 - 0.07		

C = Classes; T = Traits; Bold letter denoted expected frequency; Based on χ^2 values for 9:3:3:1; NS = Non significant ; ** = Significant

Table 7. Estimation of linkage from F_2 data of two alleles in bracteoles and boll colour

F_2 segregated population	PA -785 x IC-371437	PA -740 x IC-371437	PA - 812 x IC-371437
Flared bracteoles with dark green coloured bolls [FFGG]	1424	739	714
Flared bracteoles with light green coloured bolls [Ffgg]	96	76	72
Closed bracteoles with dark green coloured bolls [ffGG]	183	102	104
Closed bracteoles with light green coloured bolls [ffgg]	1595	1003	972
p^2 value	0.8308	0.8145	0.8109
Linkage value	91.14%	90.25%	90.04%
Crossing over value	8.86 %	9.75%	9.96%

Deriving linkage distance and gene order

In two-point crosses data were analysed for three families indicated deviation from classical ratio and linkage is present (Table 6). To confirm the linkage, contrasting characters were studied and added third gene. This helps to have several different types of crossing-over products. Phenotypic traits like Red coloured (gg), Round shape boll (RR) with Flared red bracteoles (FF) crossed with Green coloured (GG), Oblong shape boll (rr) with closed green bracteoles (ff). The F_1 was Red coloured, Round shape boll with Flared red bracteoles (GgRrFf). The genotype of *G. herbaceum* (IC – 371362) has red plant type with red coloured round shape bolls and flared bracteoles. Whereas genotype of *G. arboreum* (PA - 812) has a green plant type with green-coloured oblong shape bolls and closed bracteoles (Table 8). The

test cross was performed with F_1 and expected 1:1: 1:1: 1:1: 1:1 ratio. The progenies were deviated from the calculated ratio from theoretical ratio as highly significant. The data does not afford a good fit to calculated 9:3:3:1 ratio and over represented in numbers. In this group there was an excess of light green plants and a shortage of the other genotypes. The recombinant configuration was lesser in number (Table 8). The distance between genes was calculated the corresponding percentage to the degree of genetic linkage expressed in a units of a centimorgan (cM). The distance between gene G and F = 18.94cM and the Distance between gene F and R = 7.76cM.

The genetic study of the various bracteole characters in interspecific crosses between *G. hirsutum* and *G. tomentosum* showed that the two species differ by at least several pairs of genes for each character. Considerable dominance was also reported by one parent or the other for each character. Various degrees of genetic association is also explained between the different bracteoles characters along with four marker gene segregated independently of the genes controlling the bracteoles characters. The present investigation confirmed and agreed with the findings of Murry and Yin (1964).

The presence of duplicate linkage of glandless and Nectarless genes in Upland cotton G. hirsutum was reported. The linkage value for glandless gl_2 in linkage group V in the **A** genome was 32.23 ± 1.40% cross over and the linkage value for gl_1 in linkage in the D genome was 38.27 ± 1.40 cross-over was explained. The presence of duplicate gene action explored in the present study is conformity with the findings (Holder *et al.*, 1968).

A genetic study of number of involucre bract teeth in cotton indicated that two parents differed by at least three pairs of genes which acted additively. The present study are in opinion that parents differed by at least two pairs of genes confirmed the findings (Wilson, 1985).

Table – 8. Confirmation of linkage and crossing over in IC – 371362 x PA – 812

P1	P2
G. herbaceum	*G. arboreum*
IC – 371362	PA – 812
Red pigmented coloured bolls [gg]	Green coloured bolls [GG]
Round shape bolls [RR]	Oblong shape bolls [rr]
Flared bracteoles [FF]	Closed bracteoles [ff]
ggRRFF x GGrrff	
(Red pigmented coloured, Round shape bolls, Flared red bracteoles)	
(GgRrFf)	
F_1 x Test cross	

Table – 9. Confirmation of linkage and crossing over in IC – 371362 x PA – 812

Genotypes	Observed Plants	Type of gametes	Phenotypic traits
GRF	788	Parental	Red coloured, round shape bolls, flared bracteoles
Grf	741	Parental	Green coloured, oblong shape bolls, closed bracteoles
GrF	66	Single cross over between G & F	Red coloured, oblong shape bolls, flared bracteoles [Oblong x flared]
gRf	44	Single cross over between R & f	green coloured, round shape bolls, closed bracteoles [Round x Green]
GRf	11	Double cross over between R & f	Green coloured, round shape bolls, closed bracteoles [Flared x Closed]
grF	36	Double cross over between r & F	Green coloured, oblong shape bolls, flared bracteoles [Flared x Closed]
Grf	144	Single cross over between G & f	Red coloured, oblong shape bolls, closed bracteoles [Red x oblong x closed]
gRF	192	Single cross over between g & F	Green coloured, round shape bolls, flared bracteoles [Green x Round x Flared]
Total	2022		

The genotypes found most frequently are the parental genotypes has high frequency of genotypes (Table 9). The double crossover gametes has lowest frequency (GRf = 11 and grF = 36).The **F** gene must be in the middle because the recessive **f** allele is now on the same chromosome as G and R alleles and the dominant **F** allele is on the same chromosome as the recessive **g** and **r** alleles. Hence decided gene order GFR and calculated distances between **G** and **F** and between F and R. The linkage distance was calculated as followed and expressed in cM.

$$\text{Distance between gene G and F} = \frac{100 \times [144 + 192 + 36 + 11]}{2022}$$

Distance between gene G and F = 18.94cM

$$\text{Distance between gene F and R} = \frac{100 \times [66 + 44 + 36 + 11]}{2022}$$

Distance between gene F and R = 7.76cM

Linkage map was drawn as

G F R
I----------I----------I
18.94cM 7.76cM
Colour Bracteoles Boll shape

The double cross over gives indication of interference that gives specific recombination rates in two adjacent chromosomal intervals. The rate of double crossovers was estimated as the product of the single cross overs. The recombination rates between gene G and F = 18.94/100 = 0.189. The recombination rates between gene F and R = 7.76/100 = 0.077. Therefore, 0.014 or 1.45 % [100 x (0.189 x 0.077)] double recombinants were expected with 2022 sample size. The closer genes are to each other on a chromosome, the closer they are physically. This physical closeness makes the likelihood of recombination much lower since they are so close. When genes are perfectly linked, they have a recombination frequency of 0. When genes are unlinked, they have a recombination frequency of 0.5, which means 50 percent of offspring are recombinants and the other 50 percent are parental types. A crossover frequency between 0 percent and 50 percent indicates that the genes are on the same chromosome and crossing over occurs some of the time. In the present study cross over frequency is 1.45 percent which is very low varies between 0 percent and 50 percent indicates that the genes are on the same chromosome and crossing over occurs some of the time.

Conclusion

The segregation in one pair of alleles is independent of the segregation in any other pair of alleles if there is no linkage and interaction. The separation in one pair of allele is entirely unaffected by the separation in the other pair of alleles but every gamete necessarily contains a gene for bracteoles, shape and colour of bolls. The bracteoles gene is important us as a phenotypic marker because bracteoles appear in the initial stage of flower bud called squaring. It also has complete penetrance and the bracteoles expression proves interesting in a developmental study. The evidence indicated that bracteoles arrangement is dominant and the trait is inherited as a single dominant gene in direct crosses. The reciprocal crosses have duplicate gene action as three families population deviated from classical ratios. The study proved the presence of linkage for the traits studied. It is also interesting as a variant chlorophyll deficient genes present in red pigmented coloured plant type with red colour round bolls and bracteoles mostly observed in *G. herbaceum* (IC - 371362) has immense importance as a contrasting trait for the linkage study and withstand during terminal drought stress. Red pigmented genotypes have the mechanism of synthesized chlorophyll and plant showed a more intermediated greenish appearance in segregated populations. The crosses developed between red pigmented and chlorophyll content green coloured plant types showed more variation from generation to generation probably due to polygenic traits linked and controlled by polygenes. Study indicated that one parent did not contain all the dominant alleles and the other all of the recessive alleles. Hence *G. herbaceum* is genotypically dissimilar to *G. arboreum*

for bracteoles traits and is considered to be a phenotypic marker for further study. Cotton is not only an important economic plant but also an excellent system for the study of genomic organization. Further confirmation of bracteoles trait through sequencing and genomic research is highly necessary.

Fig. A. DSC01400JPG. Green flared bracteoles of G. herbaceum (IC- 371437)

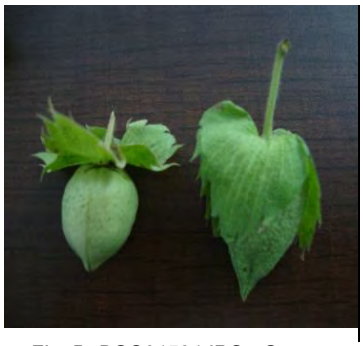

Fig. B. DSC01591JPG. Green flared and closed bracteoles

Fig. C. DSC01418JPG. Red pigmented flared bracteoles of G. herbaceum (IC- 371362)

Fig. D. DSC01387JPG. Candle shape flower bud for hand emasculation of G. herbaceum

Fig. E. DSC01426JPG. Segregated population Red pigmented flared bracteoles of IC- 371362 x Green closed bracteoles of PA – 812

Fig. F. DSC01687JPG. Segregated population red pigmented flared bracteoles and boll colour and shape

Fig. G. DSC01965JPG. Green plant type with flared bracteoles of G. herbaceum (IC- 371437)

Fig. H. DSC01982JPG. Red pigmented plant type with flared bracteoles of G. herbaceum (IC- 371362)

Fig. I. DSC0IMG 20171115-110416JPG. Green plant type with closed bracteoles of G. arboreum (PA - 812)

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References

1. Allard, R.W.1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia., 24: 235 – 278.
2. Bridges, C. B. and T. M. Olbrycht. 1926. The multiple stock 'Xple' and its use. Genetics, 11:41.
3. CICR, Annual Report, 2018-19. ICAR – Central Institute for Cotton Research, India. PP.142.
4. Cochran, W.G. 1954. Some methods for strengthening the common Chi square tests. Biometrics. 10 (4): 417 – 451.
5. Cochran, W.G.1942. The Chi square correction for continuity. Ia. State College Jour. Sci., 16 (4): 421 – 436.
6. Fisher, R.A. 1922. On the interpretation of Chi square from contingency tables and the calculation of P. Jour. Roy. Stat. Soc. Vol. LXXXV, Part I.
7. Fisher, R.A. 1923. Statistical test of agreement between observation and hypothesis, Economics, 3, 139 -147.
8. Fisher, R.A. 1954. Statistical method for research workers. 12th ed. Heffner, New York., pp.78- 113.
9. Harris, J. A. 1912. A simple test of goodness of fit of Mendelian ratios. Amer. Nat., 46: 741 – 745.
10. Holder. D.G., Jenkins and F. G. Maxwell .1968. Duplicate linkage of glandless and Nectarless genes in Upland cotton *G. hirsutum* L. Crop Science., 8(5): 577-580
11. Kirk, L.E., and Emmer, F.R. 1928. Application of goodness of fit tests to Mendelian frequencies. Sci. Agr., 8: 745 - 750.
12. Mather, K.1951. The measurement of linkage in heredity. 2nd Ed. Methuen, London, pp. 1-12.
13. Morgan, T.H. 1911. Random segregation verses coupling in Mendelian inheritance. Science 34: 384.
14. Murry J. C. and S. C. Yin. 1964. Genetic study of the bracteole differences of *G. hirsutum* and *G. tomentosum*. Bulletin of the Torrey Botanical Club. 91(8):491-501. http://doi org/10.2307/2483916.
15. Sheoran, O.P, Tonk, D.S., Kaushik, L.S., Hasija, R.C. and R.S. Pannu. 1998. Statistical software package for agricultural research workers. http.//14.139.232.166 > OPSTAT.
16. Wilson. F. D. 1985. A genetic study of number of involucre bract teeth in cotton.1985. https://doi.org/10.2135/Cropsci. 0011183X002500010009X. Crop Sci. 25(1): 32-34.

Exploiting Morphophysiological Traits for Yield Improvement in Upland Cotton Under Salt Stress

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Abstract:

The increased land salinization threatens land productivity, so it is crucial to understand crop response against salinity stress to improve food security and reduce economic losses. The current research was performed to investigate the genetic variability among 24 cotton genotypes (comprising eight lines and their 16 hybrids) by exposing them with 15 dSm-1 salt stress. The general linear model (GLM) effect test revealed significant effects of salinity for the genotypes and characters except for lint percentage and fiber strength. The genotype × treatment effects were also significant for all studied traits (name??), while non-significant effects were observed for SNB,K+/Na+, K+, POD and CAT. A notable reduction for all studied traits has been observed excel for fiber fineness, superoxidase (SOD), catalase (CAT), peroxidase (POD), carotenoid contents, and hydrogen peroxide (H2O2), which were increased under saline conditions. The hierarchical clustering and principal component analysis (PCA) classified 24 genotypes into 3 and 2 groups across normal and salt stress conditions, respectively. Salt-tolerant genotypes based on studied traits were assembled in clusters 3 and 2 under normal and respective saline environments, respectively. Based on multivariate analyses, hybrids viz. MS-71× KAHKASHAN, followedby MS-71× CRS-2007 and NS-131× CRS-2007, performed well under normal as well as saline conditions. Moreover, biochemical and agronomical traits in PCA validate that the MS-71× KAHKASHAN was the most desirable genotype under both conditions. It was demonstrated that relatively better hybrid performance under normal and 15 dSm-1 salt stress conditions supports the hybrid adaptability under salinity stress environment. The outcome of the present studies would assist breeders in developing salt-tolerant cotton varieties under climate change scenarios.

Keywords: Salt Tolerance; Productivity Enhancement; Fiber quality; Biochemical Traits; Multivariate Analysis; Variety development

Introduction

Cotton is an important cash crop and a major source of natural fiber, edible oil and biofuel for the sustainability of many people across developing countries (ZAFAR et al. 2020b). Generally, cotton is a salttolerant (moderately) crop with a threshold salinity level upto 7.7 $dS.m^{-1}$ (Zafar et al. 2021c). However, it is believed that salinity impedes cotton growth by employing osmotic stress (Sai ram and Tyagi 2004), ionic toxicity (Flowers and Colmer 2008) and nutrient deficiency (Munns 2010), which disturb the normal physiochemical and other processes leading to poor plant growth, ultimately resulting in a significant decline in yield (Ambede et al. 2012; Munns and Tester 2008).

At the reproductive stages of cotton, salt stress results in decreased fruit-bearing positions (Anagholi et al. 2005), the number of bolls, and boll weight which brings down the yield with inferior fiber quality (Farooq et al. 2020). Salinity has complicated and detrimental impacts on plants' growth and physiology. The higher salinity level, excessive soluble Na^+ and Cl^- increased soil solution osmotic potential, which prevents roots from absorbing water and causes deficit stress in cotton (Ma et al. 2021). The stomata of cotton plants closed to reduce the transpiration rate to maintain water balance. Salinity decreases photosynthesis by restricting CO2 diffusion into the leaves (Abdelraheem et al. 2019). In cotton, salinity is the cause of enhanced oxidative stress resulting from increased reactive oxygen species (ROS) (Czégény et al. 2014). ROS production damages the plasma membrane and the oxidation of carbohydrates, proteins, lipids and even DNA (Farooq et al. 2021). ROS are highly reactive and, without any protective mechanism, can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Zafar et al. 2022). Salt-tolerant plants, besides being able to regulate the ion and water movements, should also have a better antioxidative system for effectively removing ROS (Farooq et al. 2021). For mitigation of these increased oxidative stress resulting from ROS, plants produce antioxidant enzymes like peroxidases (POD), catalase (CAT), superoxidase (SOD) and non-enzymatic antioxidant carotenoids, tocopherol, flavenoids, and ascorbate which are responsible for enhancing plant salt tolerance (Manan et al. 2022; Shokat et al. 2020). The higher levels of POD, CAT, SOD, TSP and carotenoids in plants are mainly related to salt tolerance (Zafar et al. 2021c). To sustain crops across such stressful situations, an ample understanding of crop responses against salinity stress and identifying genotypes that can tolerate stress is necessary. Moreover, the adverse impacts of salinity compel the plant breeders to develop cotton genotypes with higher yields under salt stress. Assessment of significant genetic variation in the breeding material is necessary for developing stress-resilient cotton genotypes.

Developing salt-resilient germplasm through conventional breeding using morphological, physiological and biochemical markers could be cost-effective (Singh et al. 2018; Zafar et al. 2020a). The last two decades have seen a stagnant improvement in crop productivity (Figure S1), while climate change is continuously causing significant losses in productivity.

In conventional breeding programs focusing on cotton improvement, univariate statistical techniques have been extensively used based on the analysis of variance (ANOVA) proposed by steel *et al*. (Steel 1997). The mean comparisons deal with the variability among test accessions and select any desirable trait or genotype with the desired characteristics (Rahman et al. 2013).

However, there were limitations in these methods, especially in the complex nature of traits and stresses and consideration of multiple factors simultaneously to select the best suitable material with desirable characteristics (Ali et al. 2011). Hence, the advancement of different biometrical models, including leaner regression models (Least Square, GLM, MLM) and multivariate analysis techniques, including correlation, PCA and cluster analysis, has made a pace for more sophisticated and efficient selection procedures with the highest reliability as compared to previously used methods(Yeater et al. 2015). In this perspective, understanding the complexity related to biotic and abiotic stresses can be better accomplished through multivariate analysis, and it allows to address the difficult questions to be conceived through univariate approaches (Zafar et al. 2021a). Multivariate statistics examined how various variables correlate and explained how their association relates to the studied problem. Multivariate analysis is the most important technique to assess the nature and degree of genetic divergence in the germplasm (Myint et al. 2019). Different statistical analyses like cluster and principal component analysis are used to evaluate yield traits in any breeding program (Lupwayi et al. 2011;) Zafar et al. 2021b).

The development of salt-resilient cotton genotypes can alleviate the yield losses due to salt stress conditions (Zafar et al. 2020a). In contrast, these genotypes could be cultivated across marginal saline lands to meet the future increased demands of fiber. Numerous studies reported the salinity impacts at germination and vegetative phases, but the complex interaction of the yield, fiber and biochemical traits at maturity stages has remained unexplored. It was hypothesized that cotton could tolerate salt stress, which could be exploited to develop high-yielding genotypes. Therefore, this study was conducted to develop salt-tolerant cotton cultivars using different morphological and physicochemical characteristics. The outcomes of this research will be helpful in future breeding programs for developing high-yielding cotton cultivars under salt stress.

Materials and Methods

During the first year, eight cotton genotypes were selected from a screening experiment of 32 cotton genotypes developed by various research institutes in Pakistan. These genotypes were selected as parents regarding their SCY under 15 dSm-1 salt stress conditions. The selected parental material was crossed in line × tester mating fashion in the following season to obtain the subsequent F1 hybrids. The four salt-tolerant genotypes (MS-71, FH-114, IUB-65, NS-131) were used as lines and four salt susceptible genotypes (Kahkashan, CRS- 2007, FH-312, CIM-573) as testers. In the next growing season, the 24 genotypes (Table S1), including (8 lines and their 16F1 hybrids), were grown in containers (containing normal soils, 1.6 dSm-1) with three replications under RCBD. The experiment was conducted in the research area of the Plant Breeding Department, Univ. of Agriculture Faisalabad, Pakistan. Salt stress was applied through the water at the appropriate emergence of all genotypes. The direct mixing of sodium chloride in soil may hamper seed germination in containers. To overcome this problem, seeds were sown in normal soil and at desired emergence of seedlings, the salt stress was given by water application where salt was dissolved in the water based on the already known electrical conductivity (EC) of water. The EC was developed up to 15 dS m^{-1} by calculating the amount of salt required to be dissolved in the water. At two weeks old seedlings, the first dose of salt was applied to raise the salinity stress level to 7.5 dS m^{-1}. The $seco^{nd}$ dose of salt stress was given to four older plants to increase the salinity level to 15 dS m^{-1} in the containers. The salt stress was increased over two phases to prevent the seedlings from being over shocked by salt stress and give them the best chance of surviving it. When desired salinity level was achieved, the containers were irrigated with the same regular water used to irrigate the plants in control conditions and standard agricultural procedures were followed following the weather.

Metrological data have been added as Table S2 and S3. In containers, the salinity level (15 dSm^{-1}) was maintained by using US Regional Laboratory method (Richards 2012). At maturity (140 days of growing), the data were recorded for the following traits like plant height (cm), boll weight (g), number of bolls per plant (NBP), seed cotton yield (g), lint percentage (%), seed index (SI), lint index (LI), lint mass per boll (LMB), seed mass per boll (SMB), fiber strength, fiber length, and fiber fineness from five selected plants from each replication of both controlled and saline treatment.

Fiber quality characters

All the collected seed cotton samples were weighed, and ginning was performed using a single roller ginning machine. Seed and lint samples after ginning were weighed separately and lint percentage was calculated by dividing the sample's lint weight by the sample's total weight (seed cotton weight), represented in percentage. A representative lint sample has been prepared and sent to analyze fiber fineness, strength, and length with the help of high-volume instruments (HVI-900, USTER, USA) at the Fiber and Textile Technology Department UAF- Pakistan.

Biochemical analysis

The sodium and potassium analysis took fresh green leaves at noon when plants reached vegetative maturity. These leaves samples were dried in hot air and ground down and then the leaves were gone through nitric acid and sulfuric acid digestion using a 2:1 molar ratio using a hot plate. After digestion, the material was cooled down at room temperature and readings were taken by a flame photometer (410 Flame Photometer). The K+/Na+ was estimated by dividing potassium concentration by sodium concentration.

A hydrogen peroxide (H2O2) measurement was made using Bernt and Bergmeyer's method (Allen et al. 1983). The amount of H2O2 was determined by obtaining 0.5 g of leaf samples from each control and treatment group and homogenizing the sample using liquid nitrogen. The powder was then dissolved in 1.5 ml of 100mM potassium phosphate buffer (pH 6.8). Then, suspensions were centrifuged (Refrigerated SIGMA 2-16KI centrifuge, UK) at 18,000× g for 20 min at 40°C. The enzymatic reaction was started with 0.25 ml of supernatant and 1.25 ml of peroxidase reagent made up of 83 mM potassium phosphate buffer (pH 7.0), 0.005 percent (w/v) O-dianizidine, and 40 µg peroxidase/ml at 30°C. After 10 minutes, the reaction was stopped by adding 0.25 ml of 1 N perchloric acid and centrifuging the mixture for 5 minutes at 5,000× g. The absorbance was calculated at 436nm using a spectrophotometer (NanoDrop™ 8000

Spectrophotometer from Thermo Fisher Scientific, Sweden), and the amount of H_2O_2 was determined using an extinction coefficient of 39.4 mM^{-1} cm^{-1} (Zhang et al. 2014). SOD was measured in terms of units of the enzyme that inhibited the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture contained potassium phosphate buffer (pH 5), 800 µl of distilled water, 100 µl of enzyme extract, 200 µl of methionine, 200 µl of Triton X, and 100 µl of NBT. The resulting solution was then exposed to UV light for 15 minutes and then 100 µl of riboflavin was added. On a spectrophotometer, values at 560 nm absorbance were taken. POD values were computed using the quantity of guaiacol-oxidizing enzyme units. The enzymes extracted for SOD were also used to determine POD activity. In an Eppendorf tube, the reaction mixture contained 800 µl of potassium phosphate buffer (Ph 5), 100µl of H_2O_2 (40 mM), 100µl of guaiacol (20 mM), and 100µl of enzyme extract and the readings were taken using a spectrophotometer at a 470 nm wavelength (Liu et al. 2009). By centrifuging and vortex of leaf tissue extracted in phosphate buffer (pH 4), total soluble proteins (TSP) were calculated. A 40 µl aliquot was blended from the same enzyme extract with 160 µl of Bradford reagent. The mixture was added to an ELISA plate and readings from a spectrophotometer at 595 nm absorbance were taken (Bradford 1976). CAT was observed as the amount of H_2O_2 consumed by catalase and changed into H_2O and O_2. An absorbance readings were taken at 240 nm (Liu et al. 2009).

Chlorophyll Contents and Carotenoids Assay

0.5g of cotton leaf sample was crushed in 8–10 mL of 80% acetone (*v*/*v*) and then homogenization was carried out through filter paper and absorbance of the final solution was taken at 645 and 663 nm (Arnon 1949). The chlorophyll a, chlorophyll b and carotenoids were quantified as under.

$$\text{Chlorophyll a} \left(\frac{\mu g}{g}\text{FW}\right) = [12.7\,(\text{OD } 663) - 2.69\,(\text{OD } 645)] \times \frac{v}{1000 \times w}$$

$$\text{Chlorophyll b} \left(\frac{\mu g}{g}\text{FW}\right) = [22.9\,(\text{OD } 665) - 4.48\,(\text{OD } 663)] \times \frac{V}{1000 \times w}$$

$$\text{Carotenoids} \left(\frac{\mu g}{g}\text{FW}\right) = \frac{A^{car}}{Em} \times 1000$$

$$A^{car} = \text{OD } 480 + 0.114\,(\text{OD } 663) - 0.638\,(\text{OD } 645)$$

where, W = weight of leaf sample, V = volume of sample, Em =2500

Statistical Analysis

The collected data were subjected to a Generalized Linear Model (GLM) for all the variables under study, considering the replication genotypes and treatments. A generalized linear effect test was carried out to find the possible significant linear effects among replications and estimates of genotypes, treatments, and their complex interactions. The summary of estimated effects has been presented for comparison. After the estimates have been found to have sufficient variation existing through estimate tests and the replications shown non-significant estimates, the data have been pooled for replications. The mean data for genotypes and treatments were subjected to multivariate analyses, including correlation, PCA, and cluster analyses. Pairwise correlations have been calculated using the person approach using SAS-JMP Pro 16 (SAS Institute Inc., Cary, NC, USA, 1989–2011). The means from the genotype by treatment analysis were used for correlation-based principal component analysis using the default function for standardization/ scaling by JMP software: SAS-JMP Pro 16 (SAS Institute Inc., Cary, NC, USA, 1989–2011). Cluster analysis was performed using the Hierarchical clustering approach with two-way clustering to generate a tree diagram based on Euclidean distances by Ward's method.

Results

From screening experiments, eight cotton genotypes, including four salt-tolerant (MS-71, FH-114, IUB-65, NS-131) as lines and four testers viz. Kahkashan, CRS-2007, FH-312, and CIM-573 (salt susceptible genotypes) were selected based on analysis of mean methods (ANOM)- decision chart. Figure 1 showed that the mean SCY of four salt-tolerant genotypes was lying above the UDL under both saline and normal environment. In contrast, four salt-sensitive genotypes revealed a significant decline in SCY in saline conditions compared to normal conditions.

Figure 1. ANOM-decision chart with decision limits 38.72 to 50.84 for seed cotton yields across normal and salt stress (α > 0.05%). It provides a graphical test for simultaneously comparing the mean performance of these 32 cotton genotypes across normal and salt stress. Red-colored heads represent a significant deviation from the mean, either above the upper decision level (UDL) or below the lower decision level (LDL).

The impacts of salinity on physio-morphological and fiber quality parameters of parents and their hybrids were observed by GLM ANOVA. The ANOVA was constructed based on generalized linear model (GLM) effect tests. All parents and their hybrids revealed significant differences in morphological, biochemical, and agronomic traits (Table 1).

Table 1. Generalized Linear Model effect test ANOVA for genotypes under study across normal and salt stress conditions.

Source		Genotype	Treatment	Genotype × Treatment
Nparm		23	1	23
DFNum		23	1	23
PH	F Ratio Prob > F	33.2517 <.0001*	375.9295 <.0001*	10.3561 <.0001*
NBP	F Ratio Prob > F	7.7883 <.0001*	98.0490 <.0001*	1.7076 0.0382*
BW	F Ratio Prob > F	7.7105 <.0001*	97.0698 <.0001*	1.6906 0.0413*
SCY	F Ratio Prob > F	13.3863 <.0001*	649.6384 <.0001*	10.7088 <.0001*
LP	F Ratio Prob > F	4.2805 <.0001*	0.9911 0.322	2.9322 <.0001*
SI	F Ratio Prob > F	14.1159 <.0001*	51.5221 <.0001*	2.4387 0.0014*
LI	F Ratio Prob > F	2.7871 0.0003*	2.1185 0.1488	2.2401 0.0035*
SNB	F Ratio Prob > F	1.4242 0.1200	3.5785 0.0616	0.7609 0.7616
SMB	F Ratio Prob > F	11.1426 <.0001*	55.8777 <.0001*	1.0563 <.0035*
LMB	F Ratio Prob > F	11.7764 <.0001*	49.9426 <.0001*	1.0876 0.3732*
SV	F Ratio Prob > F	3.3181 <.0001*	7.2662 0.0083*	2.4122 0.0016*
FF	F Ratio Prob > F	9.8892 <.0001*	159.2814 <.0001*	1.7660 0.0299*
FS	F Ratio Prob > F	9.3028 <.0001*	1.2313 0.2700	3.8693 <.0001*
FL	F Ratio Prob > F	6.7657 <.0001*	42.9454 <.0001*	2.6955 0.0004*
K^+/Na^+	F Ratio Prob > F	6.3672 <.0001*	238.0626 <.0001*	1.1056 0.3545
Na^+	F Ratio Prob > F	7.0031 <.0001*	435.0301 <.0001*	1.8358 <.0219*
K^+	F Ratio Prob > F	27.9945 <.0001*	102.8785 <.0001*	1.3522 0.1567
H2O2	F Ratio Prob > F	6.0763 <.0001*	256.3888 <.0001*	2.5443 0.0008*

SOD	F Ratio Prob > F	4.8637 <.0001*	3085.477 <.0001*	4.1453 <.0001*
POD	F Ratio Prob > F	4.9580 <.0001*	1.019 0.031*	0.332 0.998
CAT	F Ratio Prob > F	9.4090 <.0001*	137.8633 <.0001*	0.7787 0.7487
TSP	F Ratio Prob > F	13.869 <.0001*	21.225 <.0001*	1.904 0.0162*
Chla	F Ratio Prob > F	9.9930 <.0001*	170.7653 <.0001*	6.3405 <.0001*
Chlb	F Ratio Prob > F	3.6000 <.0001*	156.3892 <.0001*	2.4787 <.0001*
Car.	F Ratio Prob > F	7.5131 <.0001*	3459.639 <.0001*	4.7220 <.0001*

*Significance ($p < 0.05$); PH = Plant Height (cm), NBP= number of boll per plant, BW= Boll weight (g), SCY= Seed cotton yield (g), LP= lint (%), LI= Lint index (g), SI= seed index (g), SNB= seed number per boll, SMB=seed mass per boll (g), LMB= lint mass per boll (g), SV= seed volume (cm^2), FS= Fiber strength (g/tex), FL=Fiber length (mm), FF= Fiber fineness (ug inch−1), Na^+, sodium concentration ($mg\ g^{-1}$); K+, potassium concentration ($mg\ g^{-1}$); K+/Na+, potassium to sodium ratio, H2O2 = Hydrogen peroxide (µmol/g), CAT = Catalase (U mg-1 protein), POD = Peroxidase (U mg-1 protein), SOD=Super-oxidase dismutase (U mg^{-1} protein), TSP = Total soluble Proteins (mg g-1 FW), Chl a&b= Chlorophyll contents a & b (mg g^{-1} FW), Caro. = Carotenoid (mg g^{-1} FW).

The treatment effects (salinity effects) were highly significant for all characters except lint% (LP) and fiber strength (FS). The genotype × treatment effects were highly significant for all traits, while non-significant effects were observed for SNB, LMB, K+/Na+, POD and CAT. All pairwise comparison using Student's t-test is presented in (figure 2 and table S4). The figure clearly showed that all traits were significantly affected by salt stress in studied genotypes except SNB. The analysis of mean methods (ANOM)-decision chart was also used to compare the performance of each genotype under normal and stress for studied traits. These charts graphically represent the variation in breeding material for studied characters across both environments (Fig S2A & B and Table S5). The response grid sliders were obtained to assess the variation of each character under normal and saline conditions. The prediction profiler graph allowed us to observe the response of all genotypes for studied characters under saline and normal conditions. It also predicts the desired genotype that performed well based on studied characters under both conditions. The response grid sliders and prediction profiler showed the reduction in plant height (PH), boll weight (g), seed cotton yield (SCY), LMB, SMB, SI, LI, fiber strength (FS), fiber length (FL), K+/Na+, and total soluble proteins (TSP) under salt stress while the fiber fineness (FF), H2O2, Na+, catalase (CAT), and POD were increased under saline conditions (Fig S3A & B). Interestingly the lint% of IUB-65× CIM-573 was increased under salt stress, while most of the genotypes revealed a reduction in lint%. The prediction profiler plot also revealed that the MS-71× KAHKASHAN exhibited higher antioxidant enzymatic activities under salt stress and performed superior for yield and fiber quality parameters in both environments (Fig S4).

Figure 2. All Pairwise comparisons for biochemical, yield, and fiber-related traits under normal and stress conditions, red Color bars represent significant comparisons whereas blue color bars indicate non-significant differences in comparison for traits under study.

Pairwise correlation

Pairwise correlation revealed a positive relationship between seed cotton yield and NBP with BW, POD, CAT, TSP, K^+/Na^+ and chlorophyll contents (a&b) under normal and salt stress conditions. Interestingly, seed cotton yield and number of bolls per plant under both conditions also revealed a significant negative correlation with H2O2, Na^+, and carotenoids (Fig 3). Under both conditions, boll weight showed a positive correlation with all of the within boll yield components and antioxidant traits. The trait seed index is negatively associated with LI, SOD, CAT, Na^+, and carotenoids under normal and salt stress conditions. Under both conditions, the trait SMB revealed a significant positive relationship with SCY, BW, LMB, K^+/Na^+ and LI and a negative association with H2O2, Na^+, and carotenoids. The positive relationship of LP was observed with SCY, BW, NBP, SOD, POD, CAT, Car, TSP, FS, FL, and FF, whereas it was negatively associated with seed index under normal

and saline conditions (Fig 3). The fiber strength and length were positively related to SCY, BW, TSP, K^+/Na^+ and chlorophyll contents (a & b) under both conditions. interestingly, FF revealed a strong positive relationship with Na+ and a negative relationship with K^+/Na^+ and Chlb. Under both conditions, K^+ showed a significant positive association with SCY, BW, TSP, and chlorophyll contents (a & b). The characters POD, CAT, and TSP were significantly and positively correlated with PH, SCY, BW and NBP under normal and saline environments. The chlorophyll contents (a & b) were also positively related with all agronomic and fiber quality traits under both conditions (Fig 3). Figure 3. Scatterplot matrix to visualize several attributes by pairwise dependencies of different traits under study across normal and heat stress conditions. The upper triangle matrix is representing correlations among biochemical, yield and fiber-related traits under normal and stress conditions. The lower triangle matrix is the revelation of bivariate density distribution with ellipses between each pair of attributes. The legends at the top right corner of the color gradient (red to blue), and the size of circles show the amount of correlation and log (p) values for the significance threshold, respectively.

Cluster analysis

The hierarchical clustering was executed to classify the 24 cotton genotypes based on their genetic potential for agronomic, fiber and biochemical characters under normal and saline conditions. This study initially considered grouping genotypes in three groups by AHMC clustering, not stating them as susceptible or tolerant genotypes by software but just grouping or clustering them. Another clustering of variables to group traits according to their trend was added along with a heatmap attached further to the two-way clustering to visualize a clear trend of all observations to extract supplementary conclusions from this tree concerning this experiment (Fig 4). The heatmap was constructed for studied traits in which blue and red boxes revealed negative and positive associations, respectively, with increasing color strength reflecting a higher coefficient. In control conditions, 24 genotypes were grouped into 3 clusters. Groups 1,2 and 3 consists of 6, 6 and 12 genotypes, respectively (Fig 4). The heat map showed that the genotypes MS-71× KAHKASHAN followed by NS-131× FH-312 exhibited higher antioxidant enzymatic activities and performed superior for yield and fiber-related parameters under a normal environment. Under salt stress conditions, 24 genotypes were classified in 2 clusters. Groups 1 and 2 contained 21, and 3 genotypes, respectively (Fig 4). Under both stressed and normal environments, various clusters were depicted in different colors. Based on studied traits, the genotypes of cluster 3 were declared as the most salt-tolerant genotypes. Depending on the pattern of clusters, the genotype MS-71× KAHKASHAN followed by NS-131× CRS-2007 and MS-71× CRS-2007 performed well in yield, physiological and fiber-related parameters under both environments. With these traits mentioned above, three better hybrid genotypes showed an increasing trend of phenotypic values under normal and stress conditions. The three hybrid genotypes got a separate cluster in the hierarchy due to a similar trend of their studied traits under stress conditions. The hybrid genotype MS-71× KAHKASHAN showed its unchanged behavior regarding superior performance based on studied traits under stress. The two-hybrid genotypes viz; NS-131× CRS-2007 and MS-71× CRS-2007 shuffled their cluster by performing better, just like the hybrid MS-71× KAHKASHAN. The physiological, morphological, and yield-related traits changed their performance under stress conditions, due to which the remaining studied genotypes shuffled their clusters in stress conditions. However, regardless of their tolerance ability, the genotypes in similar clusters got the same cluster as their fellow genotypes under stress conditions, supporting our selection criteria. Under both environments, the genotypes in obtained clusters are given in (Table S6). Figure 4. Agglomerative hierarchical clustering (AHC) to calculate the Euclidean distance matrix of 24 cotton genotypes for biochemical, yield and fiber-related traits under normal and stress conditions. The plot was constructed in JMP pro. V. 16 (SAS Institute Inc., Cary, NC, USA) using Ward's minimum variance on standardized data The horizontal axis and vertical axis represent clades formation based on the division of traits and genotypes, respectively, following the two-way clustering approach.

Principal component analysis (PCA)

The principal component analysis (PCA) was carried out to extract information regarding genotypic performance based on agronomical, biochemical and fiber quality traits under normal and saline conditions. The genetic variability among studied genotypes was explained based on the correlation among biochemical and agronomic characters and extracted clusters. The whole genetic variability was split into 19 principal components (PCs), out of which only the first 5 PCs exhibited >1 eigenvalue (Fig 5). These 5 PCs contributed 72.99% to the total diversity of cotton genotypes assessed for physiological, agronomic and fiber quality parameters under both environments. While

the rest of all PCs contributed 27.01% of the whole variation. The PC1, PC2, PC3, PC4 and PC5 revealed 33.57%, 18.13%, 9.04%, 6.39% and 5.83% variation among the understudied characters' genotypes. The characters including PH, SCY, Chlb, Chlb, PH and BW showed considerable positive factor loadings on PCI. The PC-II was characterized by H2O2, FF, CAT, Na+, BW, and POD. The PC-III was explained by variation among genotypes for LMB, SMB, SI, and LI (Table S7). Figure 5. Summary plots with (leftmost) biplot between PC1 and PC2 displaying the distribution of traits; the biplot (Center) of 24 cotton genotypes under normal and salt stress conditions; (right), scree plot showing the number of components to be considered for variability coverage through PCA.

In the biplot, variables were portrayed in vector form and the relative distance covered by variables from the origin regarding PC1 and PC2 represents the contribution of variables to the total variation of the genetic materials. The distance covered from the origin to the tip of the plot provides knowledge regarding the diversity among the genotypes. The biplot of PC1 and PC2 explained 51.7% of the total variation. The PH, SCY, Chlb, Chlb, BW, NBP, K+/Na+, and FL had long vectors and higher correlation, contributing more to this biplot variation, while LI, FS and LP showed the least variability. Interestingly, the PCA biplot revealed that these traits were strongly associated with genotypes of cluster 3. The H2O2, FF and CAT were lied towards the direction of PC2 and were highly correlated. The scatterplot matrix of PC1 and PC3 exhibited a lower variation (42.63%) than the biplot of PC2. This biplot was explained by LMB, SMB, SI, and LI and was highly correlated. The biplot of PC1 and PC4 revealed lower diversity (39.96%) compared to PC2 and PC3, respectively (Fig 6 and Table S7). The character LI, SI, FS and Chla are major contributing factors in this biplot. The biplot of PC1 and PC5 showed the least variability than other PCs mentioned above; major elaborating factors were LP, LI and SV (Fig 6 and Table S7).

The scatterplot matrix of PC2 and PC3 showed the least variation (27.18%), and LI, SV, SMB, and SI are the most discriminating factors in this biplot (Fig 6). The biplot of PC2 and PC4 was explained by LI, FS and Chla and revealed significant positive associations among themselves. The scatterplot matrix of PC2 and PC5 was characterized by LP, LI, SV and SNPB and exhibited positive relationships among themselves. The characters Chla, Chlb and FS were more discriminating factors in the biplot of PC3 and PC4. The biplot of PC4 and PC5 is explained by LP and SV and has the least variability of all other PCs (Fig 6 and Table S7). These biplots revealed that the MS-71× Kahkashan was the most superior genotype for studied traits, followed by MS-71× CRS-2007 and NS-131× CRS-2007 under normal and saline conditions. The genotypes FH-114, IUB-65, and FH-114 × CRS-2007 were not in a desirable direction and were highly susceptible genotypes under saline conditions (Fig 6).

The first three components of PCA contributed 60.75% variability to the total variation. The yield, biochemical and fiber quality parameters are depicted by factor map squared cosines (Fig 6). It is deduced that more values of squared cosines revealed a fair share of the specific variable. The PC1 covered PH, NBP, SCY, FL, K+/Na+, Na+, K+, SOD, Chla, Chlb, and Car. The traits BW, LP, SNPB, FF, FS, H2O2, POD, CAT and TSP were covered by PC2. The trait SI, LI, SMB, LMB and SV were covered by PC3 (Fig 7). The biplot of studied traits of cotton revealed three forms of groups of the traits. The on group consists of PH, NBP, SCY, FL, K+/Na+, Na+, K+, SOD, Chla, Chlb, and Car. The 2nd group comprised BW, LP, SNPB, FF, FS, H2O2, POD, CAT and TSP. The 3rd group comprised SI, LI, SMB, LMB and SV. The PC-biplot scattered genotypes to understand the genetic potential of studied genotypes against salt stress with agronomical, fiber and biochemical traits. Grouping genotypes across biplot indicated and validated the clustering results, making the decision more confident regarding classifying genotypes in three groups. The genotypes of cluster 3 revealed the maximum value of agronomical, biochemical and fiber quality traits and were considered salt tolerant.

Figure 6. Scatterplot matrix of PC1, PC2 and PC3 displays different traits and genotypes under normal and salt stress conditions. Different color dots across coordinates of scatter plots show the placement of genotypes under salinity stress, whereas different color shapes in scatterplots represent placement genotypes under normal conditions. Different color schemes represent the grouping of genotypes across stress and normal conditions.

Figure 7. Squared cosines are associated with the principal components for the studied traits under normal and salt stress conditions.

Discussion

To develop salt-tolerant cultivars, diverse genotypes are evaluated in a breeding program to increase their utility. Salt tolerance is a complex trait caused by many interrelated mechanisms of morphological and biochemical characters (Shelke et al. 2017; Zafar et al. 2020a). These characteristics are closely associated with coping with the adverse effects of soil salinity on plant growth and development. In the present study, we examined different agronomical, biochemical and fiber quality characters to assess the salt tolerance ability of 24 cotton genotypes. All genotypes behaved significantly differently for all studied characters under both conditions, revealing the presence of variation for all measured traits. It also suggests that the panel of genotypes used in this study has been selected appropriately (Kumar et al. 2021). Cotton improvement against salt stress depends on the genetic variation in studied germplasm for traits involved in salt tolerance. The studied genotypes exhibited a great magnitude of variation for salinity tolerance and performed differentially under saline conditions. Under salt stress, grid slider plots and prediction profiler showed a reduction in plant height (Zafar et al. 2020a), boll weight (Ibrahim et al. 2019), seed cotton yield (Hassan et al. 2020), K^+/Na^+, K^+, chlorophyll contents, total soluble proteins, SMB, LMB, SV, fiber length and strength. The reduction in these characteristics is due to osmotic stress (Sairam and Tyagi 2004), ionic toxicity (Flowers and Colmer 2008) and nutrient deficiency (Munns 2010), which disturb the normal biochemical processes that lead to poor plant growth and ultimately lowered the yield (Ambede et al. 2012; Munns and Tester 2008). The reduction in boll weight is due to higher sodium and chloride ions accumulations inside the cell (Akhtar et al. 2010). It is also likely that salt-induced shrinkage of compartments for storing unwanted toxic material such as vacuole may have led to reduced boll weight in the cotton genotypes (Bublitz et al. 2010). Under salt stress, the decline in seed cotton yield and lint% is associated with decreased boll weight (Sharif et al. 2019). Fiber quality traits significantly declined with increased salt stress (Zhang et al. 2013). The K^+/Na^+ ratio is a fundamental criterion for selecting salt-tolerant cotton genotypes. The salt-resistant genotypes have a higher K^+/Na^+ ratio than sensitive plants with high amounts of Na^+ inside their cells (Golldack et al. 2014). The lower K^+/Na^+ ratio is related to the high mobility of Na+ inside the cell due to cell injury and the ineffective method for excluding Na^+ outside the cell under high salt stress (Wang et al. 2013). The ANOVA results presented significant differences among genotypes regarding CAT, POD, Car, chlorophyll contents and H_2O_2 under both conditions at a $P > 0.01$ level. The hydrogen peroxide contents, CAT, POD, and fiber fineness increased under saline environments. These outcomes are consistent with the outcomes of (Farooq et al. 2020). Salt stress inhibits the photosynthesis process by higher values of ROS, which accelerates oxygen-induced cellular damage (Joseph et al. 2011). Therefore, salt tolerance depends upon a plant's capacity to regulate the antioxidant defense system, which involves various antioxidant enzymes like CAT, POD and TSP (Oueslati et al. 2010). SOD is a key antioxidant enzyme and regulates $O2^-$ and $H2O2$ concentration. The increased SOD activities were observed under salt stress in tolerant cotton genotypes. During the ROS scavenging process, POD and CAT played a vital protective role in the presence of SOD. The existence of high Peroxidase (POD) enzymes detoxifies H_2O_2 inside the cytosol and chloroplasts of the plant cells (Shu et al. 2013). Catalase is also involved in converting toxic hydrogen peroxide into water and oxygen. It was observed that the activity of POD and CAT was increased at 15 dS m^{-1} NaCl stress (Wu et al. 2014). This increase in H_2O_2 permeates the paroxysmal membrane, disrupting the balance of total soluble proteins by further increasing the cytosolic H_2O_2 concentration. Salinity in cotton plants increased the accumulation of H_2O_2 through enzymatic and non-enzymatic pathways. H_2O_2 induces the generation of other antioxidants inside the plant cell, e.g., APX. Thus, H_2O_2 is considered a signal for the plant to prepare itself against the onset of stress (Locato et al. 2008). The activities of POD and CAT are effective indicators for assessing the salt-tolerant ability of various plant species because these play a major role in the detoxification of H_2O_2 (Zhang et al. 2014). Increased POD activity enhanced photosynthetic activity, revealing the role of antioxidants' defense mechanism in mitigating salt stress. The POD and CAT were positively associated with SCY, BW, NBP, and chlorophyll contents which confirmed their role in increasing photosynthetic activities under salt stress and ultimately increase the yield under stress conditions. Correlation analysis revealed a positive relationship of SCY with chlorophyll contents, BW, NBP, POD, CAT, TSP and K^+/Na^+. So selection for these characters will increase the lint yield under both environments. ANOVA findings, genotype performance across normal and saline environments, and correlation between quantitative and biochemical traits provided confidence in moving forward with the current panel of genotypes to develop salt-tolerant cotton cultivars. Cluster analysis was done to investigate the genetic diversity among 24 cotton genotypes for studied traits, which gave directions to breeding programs (Chunthaburee et al. 2016). Hierarchical cluster

analysis showed that cultivars of cluster 2 under salt stress revealed a higher ability to tolerate salinity stress as determined by all under-studied characters. The hierarchy of parents regarding their tolerance ability remained intact under stress conditions. Almost all the designated tolerant and susceptible genotypes in a specific cluster got assigned a similar cluster with their fellow genotypes. The PCA results of the current study affirmed variations for ionic and agronomic characters in studied genotypes that could be further used in the cotton breeding program against salt stress. This approach divides the genetic variation of the breeding material into various components and exploits a particular character's efficiency in a breeding program (Mohi-Ud-Din et al. 2021). In PC-biplot, K^+/Na^+, TSP, CAT, chlorophyll contents and POD showed a positive relation with PH, NBP, BW, SCY, LP, and fiber quality traits. The CAT, TSP, Chla, Chlb and POD exhibited a positive relationship with LP, FF, FL and FS. It means these biochemical characteristics have a direct impact on agronomic characteristics. These biochemical traits will be helpful for the selection of improved cotton genotypes for yield and fiber quality traits. The PC-biplot of studied traits of cotton revealed that the genotypes of cluster 3 revealed the maximum value of agronomical, biochemical and fiber quality traits and were considered salt tolerant. Our PCA results were the following (Krishnamurthy et al. 2016; Munawar et al. 2021). In our study first two PCs covered the maximum diversity present in the studied material. It was in line with previous reports (Krishnamurthy et al. 2016; Munawar et al. 2021). Our findings revealed that the clustering of cotton genotypes was well interpreted and validated by the results derived from PCA. Altogether, it is evident that 24 cotton genotypes have notable differences under both environments for studied characters and the multivariate analyses could be practical in identifying salt-tolerant cotton genotypes. On clustering and PCA, under normal and salt-stress conditions, MS-71× KAHKASHAN followed by NS-131× CRS-2007 and MS-71× CRS-2007 were superior genotypes for most of the studied traits. These genotypes also revealed an increased TSP, CAT and POD level, positively associated with yield and fiber quality traits. In conclusion, analyzing a suite of physio-morphological characters provides a valuable paradigm evaluation of salt tolerance among studied cotton genotypes. Our findings follow the previously reported studies having similar results(Malik et al. 2020; Munawar et al. 2021). We have identified key characteristics of salt resistance and potentially useful experimental materials for future work.

Conclusion

The abrupt global climate changes mainly enlist temperature rise-related problems that adversely affect crop growth, development, and ultimate yield. Temperature rises cause the dried environment of plants that counts both aerial and underground surroundings. Underground environmental drying creates high salt content exposure to the roots, producing salinity stress. To cope with this type of abiotic stress, the first solution is always to develop potential genotypes with tolerant genetic factors using available germplasm resources. The research hypothesis was based on the concept that cotton germplasm could be screened out for its potential against salt stress, and genotypes can be brought forward for breeding programs aiming to improve cotton crops against salt stress. Salinity disturbed the normal physiological, biochemical, and molecular processes that reduced the seed cotton yield and fiber quality. The genotype MS-71× KAHKASHAN performed well under normal and saline conditions and can be effectively utilized in future breeding programs focusing on cotton improvement for salinity tolerance to enhance productivity and quality across climate change widows.

Supplementary Figures

Figure S1. Cotton productivity trend for the world from 1945/46 to 2021/22(Proj.)

Figure S2A & B. ANOM-decision chart with decision limits for studied traits across normal and salt stress ($\alpha > 0.05\%$). It provides a graphical test for simultaneously comparing the mean performance of 24 cotton genotypes across normal and salt stress. Red-colored heads represent a significant deviation from the mean, either above the upper decision level (UDL) or below the lower decision level (LDL).

Figure S3A & B. A panel of 3-dimensional response grid sliders graphs based on a GLM depicting the effect of salt stress on agronomic, biochemical and fiber quality traits of 24 cotton genotypes.

Figure S4. Prediction Profile Plot with Highest Adjusted Desirability and Factor Values for all studied agronomic, biochemical and fiber quality traits of 24 cotton genotypes under normal and salt stress conditions.

Supplementary Tables

Table S1. List of genotypes (Lines and Crosses) used in this experiment.

Table S2. Mean maximum and minimum Temperature (C)during the crop seasons 2017 and 2018

Table S3. Mean Maximum and Minimum Relative humidity, Average and Maximum monthly rainfall during the crop growing seasons 2017 and 2018

Table 4. Comparison with overall decision chart for studied traits across normal and salt stress (α > 0.05%).
Table 5. Summary statistics of t test estimates for cotton accessions under normal and salt stress condition.

Table S6. Loading Matrix for different Morphological, Fiber quality and biochemical traits across normal and salt stress conditions

Table S7. Distribution of 24 cotton genotypes in different clusters based on morph physiological and fiber quality traits under normal and salt stress conditions.

References

1. Abdelraheem, A., Esmaeili, N., O'Connell, M., Zhang, J. J. I. C., and Products. (2019). "Pro-gress and perspective on drought and salt stress tolerance in cotton." 130, 118-129.
2. Akhtar, J., Saqib, Z., Sarfraz, M., Saleem, I., and Haq, M. (2010). "Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture." *Pak. J. Bot*, 42(4), 2857-2866.
3. Ali, B., Iqbal, M. S., Shah, M. K. N., Shabbir, G., and Cheema, N. M. (2011). "Genetic analysis for various traits in Gossypium hirsutum L." *Pakistan Journal of Agricultural Research*, 24.
4. Allen, R., Farmer, K., and Sohal, R. (1983). "Effect of catalase inactivation on levels of inorganic peroxides, superoxide dismutase, glutathione, oxygen consumption and life span in adult houseflies (Musca domestica)." *Biochemical Journal*, 216(2), 503-506.
5. Ambede, J. G., Netondo, G. W., Mwai, G. N., and Musyimi, D. M. (2012). "NaCl salinity affects germination, growth, physiology, and biochemistry of bambara groundnut." *Brazilian Journal of Plant Physiology*, 24, 151-160.
6. Anagholi, A., Esmaeili, S., Soltani, V., and Khaffarian, H. (2005). "Effects of salt stress on the growth and yield of cotton at different stages of development."
7. Arnon, D. I. (1949). "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*." *Plant physiology*, 24(1), 1.
8. Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Analytical biochemistry*, 72(1-2), 248-254.
9. Bublitz, M., Poulsen, H., Morth, J. P., and Nissen, P. (2010). "In and out of the cation pumps: P-type ATPase structure revisited." *Current opinion in structural biology*, 20(4), 431- 439.
10. Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W., and Theerakulpisut, P. (2016). "Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage." *Saudi Journal of Biological Sc-ences*, 23(4), 467-477.
11. Corwin, D. L. (2021). "Climate change impacts on soil salinity in agricultural areas." *European* Journal of Soil Science, 72(2), 842-862.
12. Czégény, G., Wu, M., Dér, A., Eriksson, L. A., Strid, Å., and Hideg, É. (2014). "Hydrogen peroxide contributes to the ultraviolet-B (280–315 nm) induced oxidative stress of plant leaves through multiple pathways." *FEBS letters*, 588(14), 2255-2261.
13. Farooq, M. A., Shakeel, A., Zafar, M. M., Farooq, M., Chattha, W. S., and Husnain, T. J. J. o. N. F. (2020). "A study towards the development of salt tolerant upland cotton (Gossypium Hirsutum L.)." 1-17.
14. Farooq, M. A., Zhang, X., Zafar, M. M., Ma, W., and Zhao, J. J. F. i. P. S. (2021). "Roles of Reactive Oxygen Species and Mitochondria in Seed Germination." 12.
15. Flowers, T. J., and Colmer, T. D. (2008). "Salinity tolerance in halophytes." *New Phytologist*, 179(4), 945-963.

16. Golldack, D., Li, C., Mohan, H., and Probst, N. (2014). "Tolerance to drought and salt stress in plants: unraveling the signaling networks." *Frontiers in plant science*, 5, 151.

17. Haroon, M., Wang, X., Afzal, R., Zafar, M. M., Idrees, F., Batool, M., Khan, A. S., and Imran,603M. J. P. (2022) "Novel Plant Breeding Techniques Shake Hands with Cereals to Increase Production." 11(8), 1052.

18. Hassan, A., Ijaz, M., Sattar, A., Sher, A., Rasheed, I., Saleem, M. Z., and Hussain, I. (2020). "Abiotic stress tolerance in cotton." *Advances in Cotton Research*.

19. Hossain, M. S. (2019). "Present scenario of global salt affected soils, its management and importance of salinity research." *Int. Res. J. Biol. Sci*, 1, 1-3.

20. Ibrahim, W., Zhu, Y. M., Chen, Y., Qiu, C. W., Zhu, S., and Wu, F. (2019). "Genotypic differ-ences in leaf secondary metabolism, plant hormones and yield under alone and com- bined stress of drought and salinity in cotton genotypes." *Physiologia plantarum*, 165(2), 343-355.

21. Ivushkin, K., Bartholomeus, H., Bregt, A. K., Pulatov, A., Kempen, B., and De Sousa, L.(2019). "Global mapping of soil salinity change." *Remote sensing of environment*, 231,111260.

22. Joseph, B., Jini, D., and Sujatha, S. (2011). "Development of salt stress-tolerant plants by genemanipulation of antioxidant enzymes." *Asian Journal of Agricultural Research*, 5(1), 17-27.

23. Krishnamurthy, S., Gautam, R., Sharma, P., and Sharma, D. (2016). "Effect of different salt stresses on agro-morphological traits and utilisation of salt stress indices for reproduc-tive stage salt tolerance in rice." *Field Crops Research*, 190, 26-33.

24. Kumar, P., Nimbal, S., Sangwan, R. S., Budhlakoti, N., Singh, V., Mishra, D. C., and Choudhary, R. R. J. F. i. p. s. (2021). "Identification of novel marker–trait associationsfor lint yield contributing traits in upland cotton (Gossypium hirsutum L.) using SSRs."12, 653270.

25. Liu, D., Zou, J., Meng, Q., Zou, J., and Jiang, W. (2009). "Uptake and accumulation and oxi- dative stress in garlic (*Allium sativum* L.) under lead phytotoxicity." *Ecotoxicology*,18(1), 134-143.

26. Locato, V., Gadaleta, C., De Gara, L., and De Pinto, M. C. (2008). "Production of reactivespecies and modulation of antioxidant network in response to heat shock: a critical bal-ance for cell fate." *Plant, Cell & Environment*, 31(11), 1606-1619.

27. Lupwayi, N. Z., Clayton, G. W., O'Donovan, J. T., and Grant, C. A. J. A. J. (2011). "Soilmicrobial response to nitrogen rate and placement and barley seeding rate under no till."103(4), 1064-1071.

28. Ma, Y., Wei, Z., Liu, J., Liu, X., Liu, F. J. J. o. A., and Science, C. (2021). "Growth and physiological responses of cotton plants to salt stress." 207(3), 565-576.

29. Malik, W. A., Wang, X., Wang, X., Shu, N., Cui, R., Chen, X., Wang, D., Lu, X., Yin, Z.,Wang, J., and Ye, W. (2020). "Genome-wide expression analysis suggests glutaredoxingenes response to various stresses in cotton." *Int J Biol Macromol*, 153, 470-491.

30. Manan, A., Zafar, M. M., Ren, M., Khurshid, M., Sahar, A., Rehman, A., Firdous, H., Youlu, Y., Razzaq, A., and Shakeel, A. J. P. P. S. (2022). "Genetic analysis of biochemical, fiber yield and quality traits of upland cotton under high-temperature." 25(1), 105-119.

31. Mohi-Ud-Din, M., Hossain, M., Rohman, M., Uddin, M., Haque, M., Ahmed, J. U., Hossain, A., Hassan, M. M., and Mostofa, M. G. (2021). "Multivariate Analysis of Morpho-Physiological Traits Reveals Differential Drought Tolerance Potential of Bread WheatGenotypes at the Seedling Stage." *Plants*, 10(5), 879.

32. Munawar, W., Hameed, A., and Khan, M. K. R. (2021). "Differential Morphophysiologicaland Biochemical Responses of Cotton Genotypes Under Various Salinity Stress LevelsDuring Early Growth Stage." *Frontiers in Plant Science*, 12, 335.

33. Munns, R. (2010). "Approaches to identifying genes for salinity tolerance and the importance of timescale", *Plant stress tolerance*. Springer, pp. 25-38.

34. Munns, R., and Tester, M. (2008). "Mechanisms of salinity tolerance." *Annu. Rev. Plant Biol.*, 59, 651-681.

35. Myint, K. A., Amiruddin, M. D., Rafii, M. Y., Abd Samad, M. Y., Ramlee, S. I., Yaakub, Z., and Oladosu, Y. (2019). "Genetic diversity and selection criteria of MPOB-Senegal oil palm (Elaeis guineensis Jacq.) germplasm by quantitative traits." *Industrial Crops andProducts*, 139, 111558.

36. Oueslati, S., Karray-Bouraoui, N., Attia, H., Rabhi, M., Ksouri, R., and Lachaal, M. (2010). "Physiological and antioxidant responses of Mentha pulegium (Pennyroyal) to saltstress." *Acta Physiologiae Plantarum*, 32(2), 289-296.

37. Rahman, S. A., Iqbal, M. S., Riaz, M., Mahmood, A., Shahid, M. R., Abbas, G., and Farooq, J.(2013). "Cause and effect estimates for yield contributing and morphological traits in upland cotton (Gossypium hirsutum L.)." *J. Agric. Res*, 51(4), 393-398.

38. Ray, D. K., Mueller, N. D., West, P. C., and Foley, J. A. (2013). "Yield trends are insufficient
39. to double global crop production by 2050." *PloS one*, 8(6), e66428.

40. Razzaq, A., Ali, A., Safdar, L. B., Zafar, M. M., Rui, Y., Shakeel, A., Shaukat, A., Ashraf, M.,Gong, W., and Yuan, Y. J. J. o. f. s. (2020). "Salt stress induces physiochemical altera-tions in rice grain composition and quality." 85(1), 14-20.

41. Richards, L. A. (2012). *Diagnosis and improvement of saline and alkali soils*: Scientific Pub-
42. lishers.

43. Sairam, R., and Tyagi, A. (2004). "Physiology and molecular biology of salinity stress toler-
44. ance in plants." *Current science*, 407-421.

45. Sharif, I., Aleem, S., Farooq, J., Rizwan, M., Younas, A., Sarwar, G., and Chohan, S. M. (2019)."Salinity stress in cotton: effects, mechanism of tolerance and its management strate- gies." *Physiology and Molecular Biology of Plants*, 25(4), 807-820.

46. Shelke, D., Pandey, M., Nikalje, G., Zaware, B., Suprasanna, P., and Nikam, T. (2017). "Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes." *Plant Physiology and Biochemistry*, 118, 519-528.

47. Shokat, S., Großkinsky, D. K., Roitsch, T., and Liu, F. J. B. P. B. (2020). "Activities of leaf and spike carbohydrate-metabolic and antioxidant enzymes are linked with yield per- formance in three spring wheat genotypes grown under well-watered and drought con-ditions." 20(1), 1-19.

48. Shu, S., Yuan, L.-Y., Guo, S.-R., Sun, J., and Yuan, Y.-H. (2013). "Effects of exogenousspermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloro-plasts in Cucumis sativus L. under salt stress." *Plant Physiology and Biochemistry*, 63,209-216.

49. Singh, S., Vikram, P., Sehgal, D., Burgueño, J., Sharma, A., Singh, S. K., Sansaloni, C. P.,Joynson, R., Brabbs, T., Ortiz, C., Solis-Moya, E., Govindan, V., Gupta, N., Sidhu, H.S., Basandrai, A. K., Basandrai, D., Ledesma-Ramires, L., Suaste-Franco, M. P.,Fuentes-Dávila, G., Moreno, J. I., Sonder, K., Singh, V. K., Singh, S., Shokat, S., Arif,M. A. R., Laghari, K. A., Srivastava, P., Bhavani, S., Kumar, S., Pal, D., Jaiswal, J. P.,Kumar, U., Chaudhary, H. K., Crossa, J., Payne, T. S., Imtiaz, M., Sohu, V. S., Singh,G. P., Bains, N. S., Hall, A., and Pixley, K. V. (2018). "Harnessing genetic potential of wheat germplasm banks through impact-oriented-prebreeding for future food and nutritional security." *Sci Rep*, 8(1), 12527.

50. Steel, R. G. D. (1997). "Analysis of variance II: multiway classifications." *Principles and procedures of statistics: A biometrical approach*, 204-252.

51. Wang, M., Zheng, Q., Shen, Q., and Guo, S. (2013). "The critical role of potassium in plant stress response." *International journal of molecular sciences*, 14(4), 7370-7390.

52. Wu, Z., Peng, Y., Guo, L., and Li, C. (2014). "Root colonization of encapsulated Klebsiella oxytoca Rs-5 on cotton plants and its promoting growth performance under salinity stress." *European Journal of Soil Biology*, 60, 81-87.

53. Yeater, K. M., Duke, S. E., and Riedell, W. E. (2015). "Multivariate analysis: greater insights into complex systems." *Agronomy Journal*, 107(2), 799-810.

54. Zafar, M. M., Jia, X., Shakeel, A., Sarfraz, Z., Manan, A., Imran, A., Mo, H., Ali, A., Youlu, Y., and Razzaq, A. J. F. i. P. S. (2021a). "Unraveling Heat Tolerance in Upland Cotton(Gossypium hirsutum L.) Using Univariate and Multivariate Analysis." 12, 727835- 727835.

55. Zafar, M. M., Manan, A., Razzaq, A., Zulfqar, M., Saeed, A., Kashif, M., Khan, A. I., Sarfraz,Z., Mo, H., and Iqbal, M. S. J. A. (2021b). "Exploiting Agronomic and Biochemical Traits to Develop Heat Resilient Cotton Cultivars under Climate Change Scenarios." 11(9), 1885.

56. Zafar, M. M., Razzaq, A., Farooq, M. A., Rehman, A., Firdous, H., Shakeel, A., Mo, H., Ren,M., Ashraf, M., and Youlu, Y. (2020a). "Genetic Variation Studies of Ionic and withinBoll Yield Components in Cotton (Gossypium Hirsutum L.) Under Salt Stress." *Jour- nal of Natural Fibers*, 1-20.

57. ZAFAR, M. M., RAZZAQ, A., FAROOQ, M. A., REHMAN, A., FIRDOUS, H., SHAKEEL, A., MO, H., and REN, M. J. J. o. C. R. (2020b). "Insect resistance management inBacillus thuringiensis cotton by MGPS (multiple genes pyramiding and silencing)."3(1), 1-13.

58. Zafar, M. M., Shakeel, A., Haroon, M., Manan, A., Sahar, A., Shoukat, A., Mo, H., Farooq, M.A., and Ren, M. J. J. o. N. F. (2021c). "Effects of Salinity Stress on Some Growth,Physiological, and Biochemical Parameters in Cotton (Gossypium hirsutum L.) Germplasm." 1-33.

59. Zafar, M. M., Zhang, Y., Farooq, M. A., Ali, A., Firdous, H., Haseeb, M., Fiaz, S., Shakeel, A.,Razzaq, A., and Ren, M. J. A. (2022). "Biochemical and Associated Agronomic Traitsin Gossypium hirsutum L. under High Temperature Stress." 12(6), 1310.

60. Zhang, L., Ma, H., Chen, T., Pen, J., Yu, S., and Zhao, X. J. P. O. (2014). "Morphological andphysiological

responses of cotton (Gossypium hirsutum L.) plants to salinity." 9(11), e112807.

61. Zhang, T., Qian, N., Zhu, X., Chen, H., Wang, S., Mei, H., and Zhang, Y. (2013). "Variations and transmission of QTL alleles for yield and fiber qualities in upland cotton cultivars developed in China." *PLoS One*, 8(2), e57220.

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Genotype × Environment interaction and stability analyses of yield of 8 cotton genotypes in many locations in Senegal

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Introduction

In West Africa, due to climate change, temperature and rainfall will change (Guan et al., 2017). A good resilience to these climatic risks requires a judicious choice of variety (Gérardeaux et al., 2016). Usually, the evaluation of varieties involves assessing their yield through multilocal and multi-year trials and estimating the stability of this yield in different environments (Lacape, 1998). This approach often encounters difficulties of genotype x environment interactions (Ndiaye et al., 2018). Thus, to study and understand the interactions between this genotype and the environment is of major importance for breeding programs (Mohammadi et al., 2015).

Keywords: Cotton, Yield, genotype-environment interactions, stability, AMMI.

Materials and Methods

Eight cotton genotypes (G1-G8), chosen for their *a priori* wide range of response to drought, were tested in three rainfed research stations representative of the major rainfed cotton areas in Senegal, during two cropping seasons (2018 and 2019), resulting in 12 environments (combination of location, year and sowingdate). The three stations were Koussanar (KO18S1, KO18S2, KO19S1 and KO19S2 representing early and delayed sowingdates the 2018, and 2019 seasons), (dry area);Velingara (VL18S1, VL18S2, VL19S1 andVL19S2) (middle area); and Kedougou (KG18S1, KG18S2, KG19S1andKG19S2) (humid area). In each location, the experimental layout was a split-plot design with three replications. Plot size was 24 m^2 (3 rows, 10 m long, with 80 cmrowspacing).The same management practices were followed in all trials. The seed cotton yields were measured on a plot basis and converted to kg ha^{-1} for the statistical analyses.

Weather data were recorded on-site by automatic weather stations (iMETOS® IMT280 [Pessl Instruments GmbH] and ATMOS 41 [METER Group, Inc. US]) within 50 m from the plots. Initial soil conditions were estimated by soil samples.

In addition to yield, crop phenology, leaf area index, leaf development and soil humidity were measured in the field.

Combined analysis of variance (ANOVA) for seed cotton yield datawas performed to determine the effects of genotype (G), environment (E), and GE interaction effects. The stability was analysed graphically using the AMMI biplot for the interpretation of the GE interaction. The AMMI biplot was calculated using R studio with the Agricolae software package.

Results and Discussion

The combined Anova seed cotton yield indicated significant values for genotypes, environments and GE interactions. Therefore, genotypes performed differently in different environments. Average yields have varied from 1225Kg.ha-1 (CS 50) to 938Kg.ha-1(Allen 51-106) seed cotton for the genotypes and from 1687Kg.ha-1 (KG19S1) to 328Kg.ha-1(KO18S2) seed cotton for the environments. The AMMI analysis revealed that the best seed cotton yields were obtained in environments with good rainfall. In addition, we found that yield stability was generally associated with poor performance for all genotypes evaluated with the exception of the IRMA Q302 genotype which gave average performance in low rainfall environments.

Conclusions

Genotypes responded differently to different environments. The high yield potential varieties (CS50, Stam 129A, IRMA Q302) are adapted to normal rainfall environments. The varieties Allen 51-106 and IRMA Q302 are the most stable but Allen 51-106 gave the lowest yield in the environments. These results can be used by breeders and agronomists (choice of breeding strategies, modelling, ideotypes, etc.).

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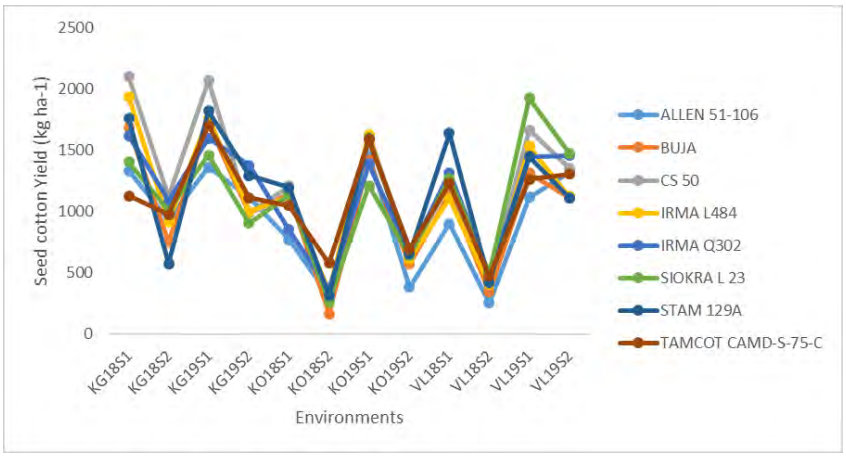

Figure1. Seed cotton yield performance of 8 genotypes tested in 2 seasons, 3 locations and 2 sowing dates.

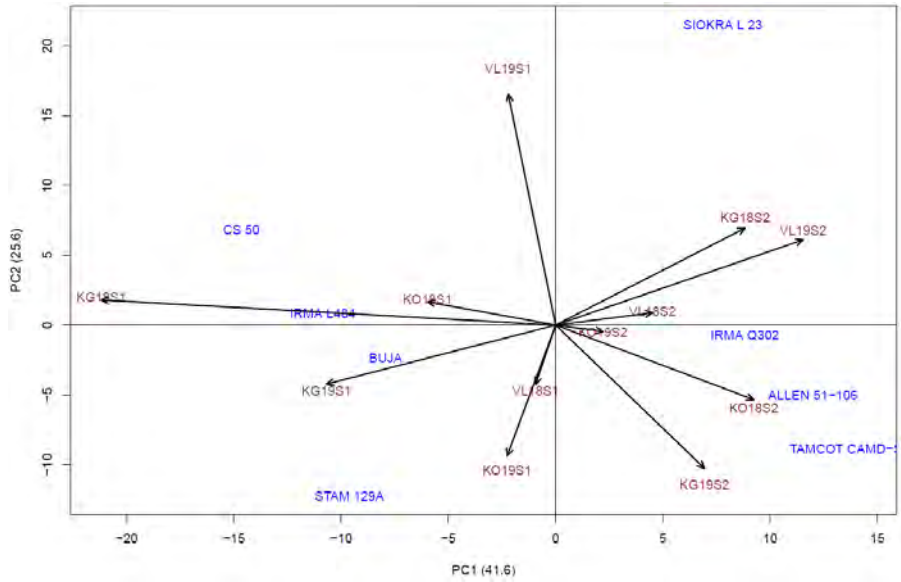

Figure 2. Polygon view of Additive main effects and multiplicative interaction 2 (AMMI2)

Table1: Summary of the combined analysis of variance and decomposition of GxE interaction according to AMMI

Source	Df	SS	MS	TSS Explained (%)
ENV	11	55029243	5002658***	79.6%
REP(ENV)	24	5305275	221053***	7.7%
GEN	7	1866676	266668***	2.7%
ENV:GEN	77	6924770	89932 .	10.0%

IPC1	17	2878124	169301.38**	41.6%
IPC2	15	1775623	118375 .	25.6%
IPC3	13	1259950	96919	18.2%
IPC4	11	682463	62042	9.9%
IPC5	9	183176	20353	2.6%
IPC6	7	79216	11317	1.1%
IPC7	5	66218	13244	1.0%
Residuals	168	11766281	70037	

*Note: df: degree of freedom, SS: Sum of Square , MS: Mean of Square, TSS = total sum of square. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

References:

1. Gérardeaux, E., Affholder, F., Bernoux, M., Muller, B., 2016. Relationships between Tropical Annual Cropping Systems and Climate Change. https://doi.org/10.1007/978-94-017-7462-8
2. Guan, K., Sultan, B., Biasutti, M., Baron, C., Lobell, D.B., 2017. Assessing climate adaptation options and uncertainties for cereal systems in West Africa. Agric. For. Meteorol. 232, 291–305. https://doi.org/10.1016/j.agrformet.2016.07.021
3. Lacape, M.J., 1998. Analyse écophysigiogique de la réponse de variétés de cotonnier au déficit hydrique.
4. Mohammadi, R., Farshadfar, E., Amri, A., 2015. Interpreting genotype × environment interactions for grain yield of rainfed durum wheat in Iran. Crop J. 3, 526–535. https://doi.org/10.1016/j.cj.2015.08.003
5. Ndiaye, M., Adam, M., Muller, B., Guisse, A., Cisse, N., 2018. Performances agronomiques et stabilité phénotypique de génotypes de Sorgho (Sorghum bicolor (L .) Moench) au Sénégal : uneétude des interactions 1, 12617–12629.

Identification of diverse interspecific lines and expression analysis of *Pectate lyase*

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Identification of diverse interspecific lines and expression analysis of *Pectate lyase*

Abstract

Background

The genome of allotetraploid cotton (AD) is the result of merger of two diploid cotton species When two genomes merged from two diploid species, it produces allotetraploid cotton with increased DNA content, which resulted rapid fibre growth and maximum expression. Interspecific hybrids also give improved fiber quality while joining two different sub genomes. Current study was designed to screen the interspecific lines originating from tri-species hybrid for better fibre traits.

Results

Three diverse lines, SL-19, SL-79 and SL-369 categorized as long fibre (34.7mm), medium long fibre (28.2 mm) and short fibre (24.5 mm) respectively were selected after screening and diversity analysis. In expression analysis, Pectate lyase revealed variable expression levels at different fibre developmental stages. Expression was maximum in SL-19 at 10 DPA fibre stage. In silico analysis also confirm the role of Pectate lyase fibre genes in fibre development. Similarly, Promotor analysis identified several stress related and light responsive cis acting elements which can contribute for fibre development.

Conclusion: *Interspecific population exhibited maximum genetic diversity and fibre gene expression which can be used in future breeding programme for the development of high-quality cotton.*

Keywords: *Field screening, GhPEL, expression analysis, in silico analysis, interspecific lines*

Background

Cotton is one of the major fibre crops worldwide, and has extensive phenotypic diversity among 54 representative species (Gallagher et al. 2017). Among these species, upland cotton (*Gossypium hirsutum* L.) is the leading fibre production crop and grown in more than 80 countries/regions of the world. Currently, *G. hirsutum* is responsible for 95% of the annual cotton production in the world (Chen et al. 2007). Because of its economic importance, such as high-yield and environmental suitability, *G. hirsutum* has attracted considerable scientific interest of plant breeders and other agricultural scientists. Domesticated upland cotton genotypes has a narrow genetic base (Abdurakhmonov et al. 2008, Tyagi et al. 2014). To broaden the genetic base through hybrid breeding programs, the genetic divergence among available germplasm is a prerequisite. Cotton breeders are interested in using diverse genotypes in hybridization that may bring variation for traits of interest, with the possibility of selection and genetic gain. Information on phenotypic and molecular diversity helps the breeders for parental selection. Numerous studies showed that there was a low level of genetic differentiation in *G. hirsutum* and that selection was extremely weak during modern genetic improvement (Fang et al. 2017, Wang et al. 2017). Therefore, the main challenge faced by researchers and breeders is how to effectively broaden the genetic base of upland cotton and improve varieties to meet the increasing demands of the textile industry. One effective strategy for broadening the genetic base is to transfer favorable genes into modern cultivars by interspecific hybridization. Changing breeding approaches from intraspecific to interspecific hybridization may be one of the key source to achieve higher yield with superior fibre traits. In present study, available resources include three

interspecific lines and three parent species carrying introgression segments from *G. arboreum* and *G. anomalum* with *G. hirsutum* background. *Gossypium arboreum* is characterized with many positive qualities for cotton improvement which the tetraploid cultivars (cultivated cotton) lack (Mehetre et al. 2003). *G. anomalum* possesses desirable characters, viz. fibre length, fibre fineness, fibre strength and maturity, cytoplasmic male sterility, narrow bracts and hairiness of leaf and biotic resistance (Margabandhu 1941, Afzal 1945, Deodikar 1949, 1950, Knight 1954, Narayanan et al. 1975, Mehetre 2010); hence found useful in providing cotton with good fibre length and fineness coupled with resistance to pest and diseases (Kalyanaraman and Santhanam 1957, Iyengar 1958). Recent new technologies covering transcriptome analysis and various *in silico* analysis will license rapid gene role finding through accurate utilization of wild species variation in cotton improvement. Comprehensive wide-ranging gene expression study is supportive to sightsee the role of genes those are up regulated, or down regulated during different cotton fibre development stages. Gene expression analysis is very influential method for identification of new and important aspects in advance research applications (Raza et al. 2016). At cellular level cotton fibre development is supported by several genes which facilitate the elongation process, for example *Pectate lyase*. Pectin substances are closely associated polysaccharides present in the plant cell wall, which are 25% responsible for of rapid elongation of cotton fibres, predicting that the pectin can impact fibre quality (Anderson and Kerr 1938, Meinert and Delmer 1977). A study examining *GhPEL* exhibited that this gene has role in cotton fibre elongation through degradation of de-esterified pectin(Zhu et al. 2012, Ahmed et al. 2018, Latif et al. 2021). However, limited research studies have been conducted on *PEL* genes in plants, and researchers have mainly focused on the function of individual genes. In cotton, most *PEL* genes are unknown. We report here the expression pattern of *Pectate lyase* on three interspecific lines after evaluation of 50 interspecific lines for long, medium and short staple lines. We assess distant hybridization with high-value interspecific germplasm for fiber quality through identification of diverse interspecific lines using field screening, expression profiling and *in silico* analysis. We recommended the operative application of these interspecific lines in cotton breeding programme.

Results

Fibre Analysis of selected lines.

Analysis of variance (Table 2) revealed that the mean squares between all interspecific lines for all fibre traits were highly significant ($p \leq 0.01$). Mean value of 50 interspecific lines for fibre traits are given in Table-2.2. Average fibre length of all genotypes was 28.222 mm with minimum value of 24.750 mm and maximum value of 34.700 mm. For fibre strength, the maximum value was 34.270 gtex^{-1} with minimum and maximum value of 22.970 and 34.270 gtex^{-1} respectively. Maximum fibre fineness in selected genotypes was 5.330 µginch^{-1} while minimum was 3.100 µginch^{-1} with an average of 4.479 µginch^{-1}. Similarly, fibre maturity percentage was maximum with value of 85.000% and minimum 66.670% (Table-3).

Table-1. ANOVA (mean squares) for fibre traits studied in present experiment

Source	DF	Fibre length	Fibre strength	Fibre fineness	Fibre Maturity
Replication	2	0.2749	0.9998	0.0242	5.6267
Genotype	49	5.8651	17.1550	0.9110	79.8951
Error	98	0.2356	1.0939	0.0424	2.0620

Principal component analysis

PCA was conducted to determine the independent effect of fibre traits under study. First two principal components (PC-1 and PC-2) extracted from all the principal components, with eigenvalue >1 and >72% contribution to the total variation among the interspecific lines (Table-4). The first principal component described more than 45.0669% of the total variation. Fibre length (0.5931), fibre strength (0.6075) and maturity percentage (0.2511) are the variables contributed by this component, among them fibre fineness has the negative influence (-0.4648). The second principal component accounted for 27.4960% of the total variation. Parameter positively correlated was maturity percentage (0.7709). SL-19, predicting a diverse position from all other lines showed its high variability was in close vicinity with fibre length and fibre strength. Similarly, SL-187, Cyto-118 and Cyto-107 were also present found near fibre length presenting their breeding value foe fibre length. SL-87, SL-35, SL-250, Cyto-111 and Cyto-124 were in close proximity with fibre maturity (Fig.-1).

Table-2 Mean values of fibre length, fibre strength, fibre fineness and fibre maturity in interspecific lines of cotton

Sr. No.	Genotypes Name	Fibre length (mm)	Fibre strength ($gtex^{-1}$)	Fibre fineness ($\mu g \cdot inch^{-1}$)	Fibre maturity (%)	Sr. No.	Genotypes Name	Fibre length (mm)	Fibre strength ($gtex^{-1}$)	Fibre fineness ($\mu g \cdot inch^{-1}$)	Fibre maturity (%)
1	SL-2	28.2	25.5	5.0	76.7	26	SL-219	28.2	25.5	5.0	75.7
2	SL-9	28.7	23.4	4.4	77.0	27	SL-232	27.2	23.4	4.4	77.0
3	SL-19	34.7	34.2	3.2	73.0	28	SL-236	28.7	26.6	4.7	73.0
4	SL-27	29.8	27.0	4.7	67.7	29	SL-241	29.8	27.0	4.7	67.3
5	SL-33	27.4	24.5	4.4	76.3	30	SL-249	27.4	24.5	4.4	75.7
6	SL-35	27.8	26.8	4.4	84.7	31	SL-250	27.8	26.8	4.4	84.3
7	SL-36	27.7	25.1	4.8	69.7	32	SL-321	27.7	25.1	4.8	69.7
8	SL-41	27.7	24.8	4.9	72.7	33	SL-352	27.7	24.8	4.9	72.7
9	SL-43	27.9	25.4	4.4	78.0	34	SL-369	24.5	25.4	4.4	77.7
10	SL-45	28.7	24.1	5.0	67.3	35	SL-159	28.7	24.1	5.0	67.3
11	SL-79	28.2	24.7	4.4	81.0	36	Cyto-110	28.2	24.7	4.4	81.0
12	SL-87	27.3	23.6	3.1	81.0	37	Cyto-111	27.3	23.6	3.1	81.0
13	SL-110	29.6	27.0	4.5	80.3	38	Cyto-112	29.6	27.0	4.5	80.7
14	SL-126	28.5	24.9	4.5	77.3	39	Cyto-113	28.5	24.9	4.5	77.3
15	SL-186	29.2	29.5	5.0	85.0	40	Cyto-114	29.2	29.5	5.0	85.0
16	SL-187	29.7	28.9	4.5	75.0	41	Cyto-107	29.7	28.9	4.5	75.0
17	SL-192	27.1	23.5	5.3	74.3	42	Cyto-108	27.1	23.5	5.3	74.3
18	SL-193	28.2	27.2	4.1	72.7	43	Cyto-109	28.2	27.2	4.1	72.7
19	SL-206	28.3	27.7	4.8	72.3	44	Cyto-120	28.3	27.7	4.8	72.0
20	SL-239	27.3	28.1	4.6	73.7	45	Cyto-115	27.3	28.1	4.6	74.3
21	SL-260	26.9	29.7	4.7	67.0	46	Cyto-116	26.9	29.7	4.7	67.0
22	SL-262	26.5	23.0	4.6	66.7	47	Cyto-117	26.5	23.0	4.6	66.7
23	SL-266	29.2	27.2	5.0	72.3	48	Cyto-118	29.2	30.0	3.6	72.3
24	SL-268	27.3	27.7	4.4	73.3	49	Cyto-122	27.3	27.7	4.4	74.0
25	SL-270	28.3	28.1	3.1	75.0	50	Cyto-124	29.7	30.6	3.1	82.7

Table-3. Means, minimums, maximums, and standard deviations for fibre traits

Variable	Minimum	Maximum	Mean	Std. deviation	F-value
Fibre length (mm)	24.570	34.700	28.222	1.3986	24.90**
Fibre strength (g tex^{-1})	22.970	34.270	26.415	2.3910	15.68**
Fibre fineness (µginch^{-1})	3.100	5.330	4.479	0.5507	21.50**
Fibre Maturity (%)	66.670	85.000	74.927	5.1606	38.75**

Table-4 PCA for fibre traits in interspecific lines

	PC-1	PC-2	PC-3	PC-4
Eigenvalue	1.8027	1.0998	0.6830	0.4145
Variability (%)	45.0669	27.4960	17.0755	10.3617
Cumulative %	45.0669	72.5629	89.6383	100.0000
Factor loadings by different traits				
Fibre length	0.5931	-0.3357	0.2724	-0.6792
Fibre strength	0.6075	-0.3231	0.0777	0.7214
Fibre fineness	-0.4648	-0.4342	0.7630	0.1147
Fibre maturity	0.2511	0.7709	0.5810	0.0712

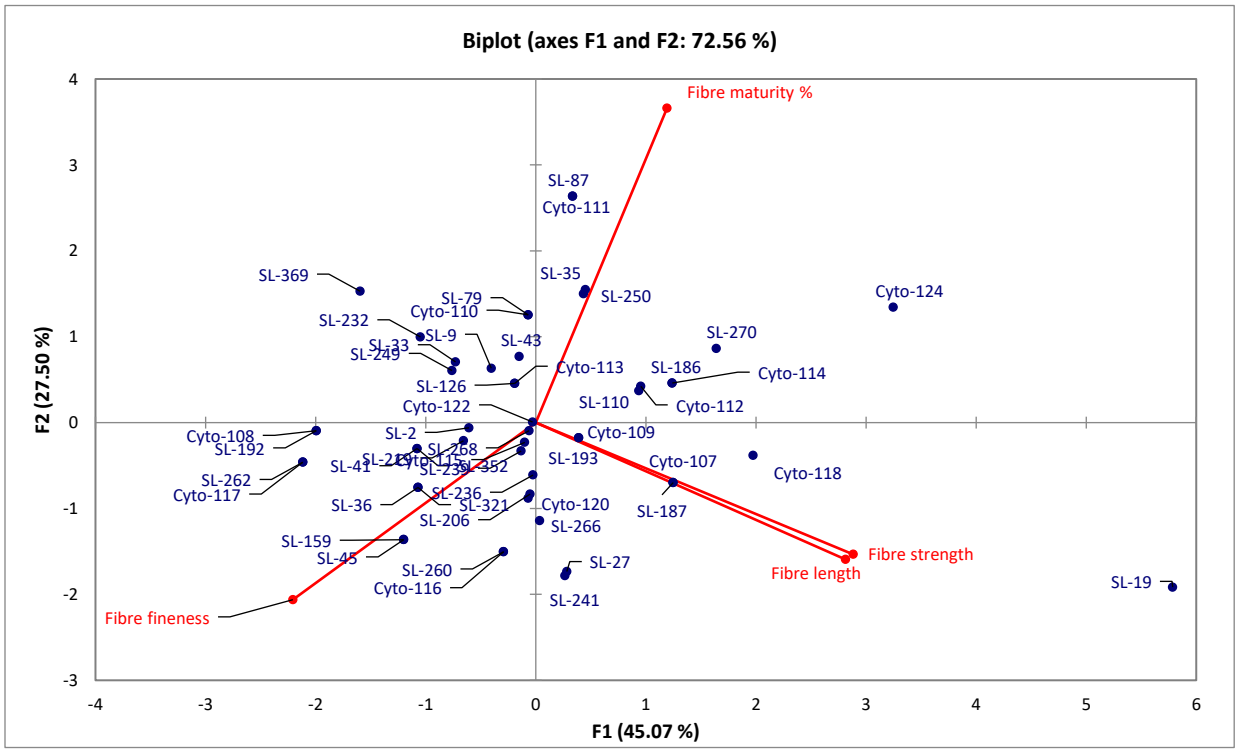


Fig. 1 Genotype × Trait biplot for fibre traits among interspecific lines of cotton.

Clustering

The cluster II was the largest followed by cluster I. Cluster analysis exhibited the different group formation (Fig.2). Cluster II genotypes showed higher values of fibre length in which SL-19 was more at distance from the origin indicating its highest variability. The cluster IV is contributed mainly by fibre strength. The cluster V is contributed by the fibre fineness.

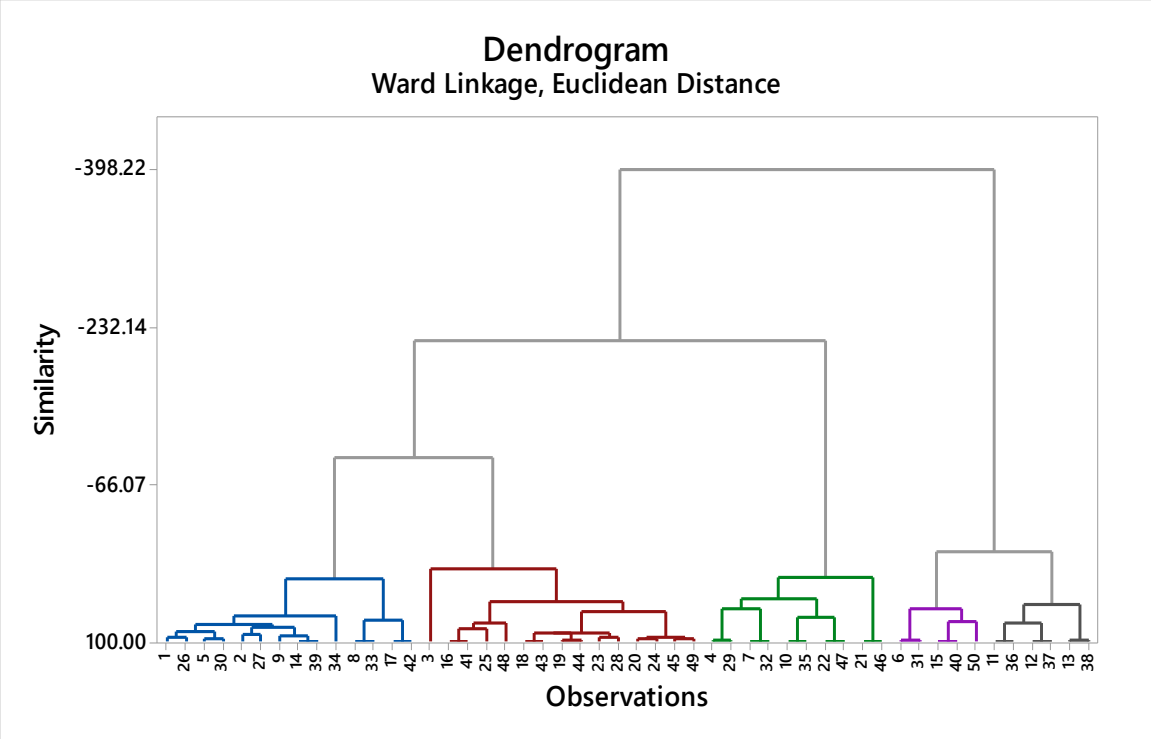
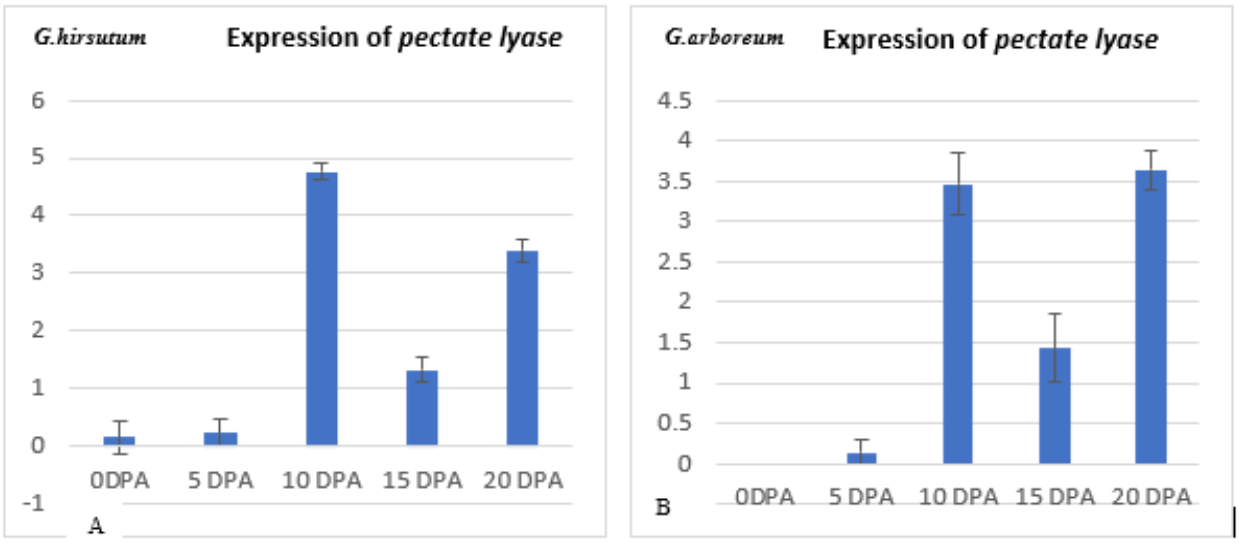


Fig. 2. Dendrogram showing the cluster analysis of fibre traits in interspecific lines of cotton.

Expression analysis of *Pectate lyase*

Expression pattern of *pectate lyase* genes was almost variable from 0-20 DPA fibre developmental stages in all genotypes. In *G. hirsutum*, *Pectate lyase* showed variable expression in different fibre stages. Expression increases in elongating fibre stage of 10 DPA and it decreases at 15 DPA stage but it began to increase at 20 DPA which is a start of maturation stage (Fig. 3A). In *Gossypium arboreum*, expression was elevated from 5 DPA till 10 DPA. It decreases at 15 DPA stage but it began to increase at 20 DPA (Fig. 3B). In *Gossypium anomalum*, expression profiling showed that its abundance at rapid elongation stage of 15 DPA (Fig. 3C). In interspecific lines, highest expression was detected at 10 DPA fibre in SL-19 and SL-79 while in SL-369 maximum expression was at 15 DPA (Fig. 3 D-F).



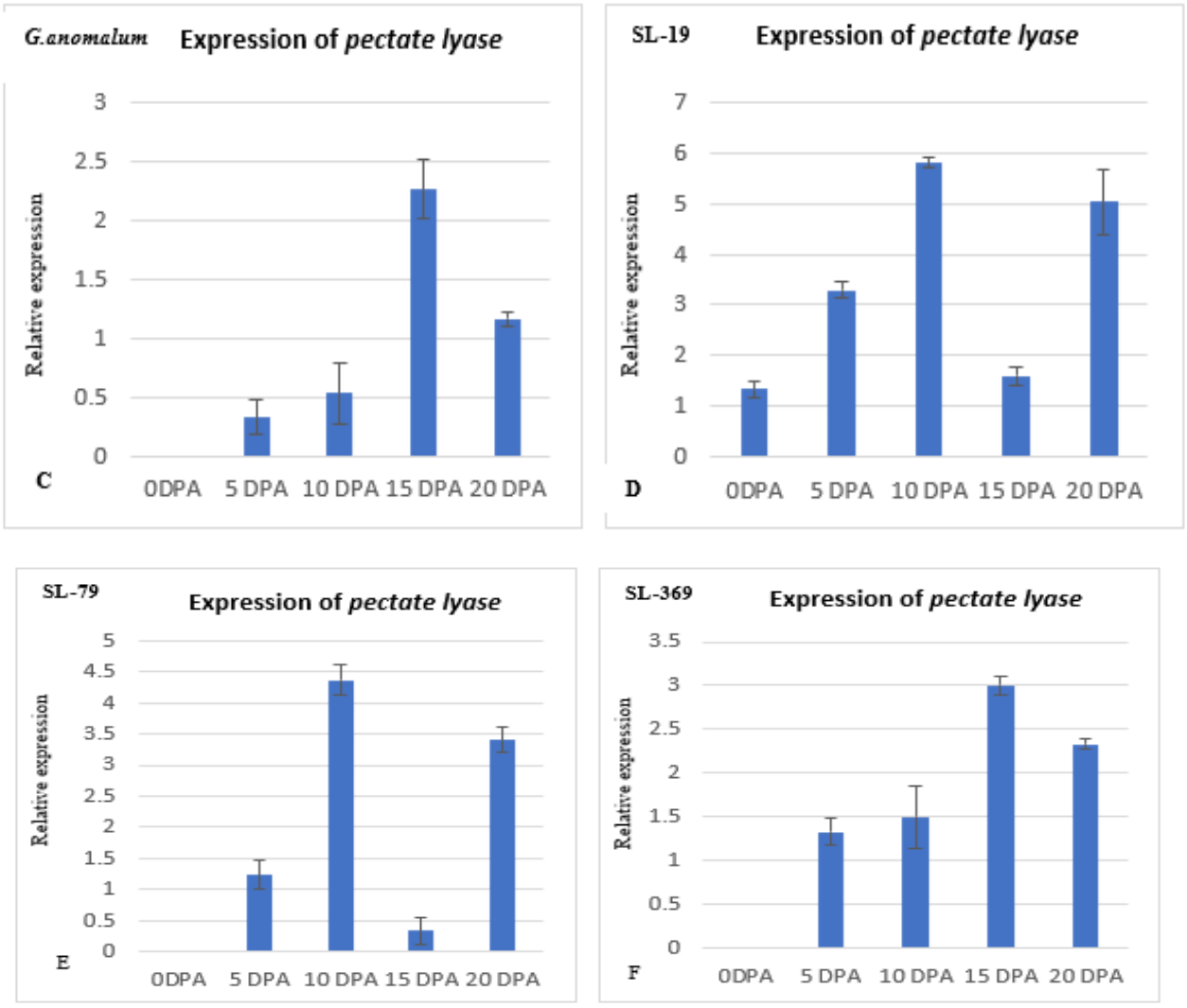


***Fig**.3. Expression profiling of Pectate lyase in Gossypium species and interspecific lines: **A** (Expression of Pectate lyase in G. arboreium), **B** (Expression of Pectate lyase in G. hirsutum), **C** (Expression of Pectate lyase in G. anomalum), **D** (Expression of Pectate lyase in SL-19) **E** (Expression of Pectate lyase in SL-79), **F** (Expression of Pectate lyase in SL-369).*

In silico analysis of *Pectate lyase*

Expasy's Protpam analysis of predicted protein showed that *Pectate lyase* was characterized as stable protein as value of instability index was 35.94 (Table-6). Subcellular localization in different organelles with the approximate values (Table-7) predicted the probability of protein localization in different organelles. Highest Extracellular values of *Pectate lyase* (2.437) showed that this is an extracellular protein. *Pectate lyase* was characterizes as extracellular membrane that's why signal peptide was present in protein coding sequence. Score values of C, S, 3Y is more than 0.45 (Table-8) that shows that peptide signal is present. Sequence analysis of cotton *Pectate lyase* promoter using PlantCare predicted many vital motifs in this region. There are few transcriptions activation related motifs along with core promoter elements like TATA and CAAT boxes. These motifs are light responsive, hormone and stress regulated cis elements.

These motifs are involved in the light, stress and hormones responsiveness. Cis-acting essential element for the abscisic acid reaction, light response elements, gibberellin-enhancer element and MYB binding site involved in drought-inducibility were present in *Arabidopsis thaliana.* For example, abscisic acid responsiveness related motifs, including ABRE, CAAT-box were abundant in promoter regions of *Pectate lyase* genes. Light responsiveness elements, including G-box and GATABOX, were also abundantly present in *Pectate lyase* promoters. (Table-9). Many regulatory elements essential for light responsive,

anaerobic induction, abscisic acid responsiveness, auxin responsiveness promoter and enhancer regions, protein binding site and MYBHv1 binding site were also present in various organism including: *Zea mays*, *Pisum sativum*, *Nicotiana tabacum*, *Hordeum vulgare* and *Brassica oleracea*.

Table-6 Physicochemical properties of *Pectate lyase*

Physicochemical properties	*Pectate lyase*
Number of amino acids	411
Total negatively amino acid charged residues (Asp + Glu)	40
Total positively amino acid charged residues (Arg + Lys)	37
Molecular weight	45109.90
Theoretical pI	6.62
Aliphatic index	78.32
Grand average of hydropathicity (GRAVY)	-0.283
Instability index (II)	35.94

Table-7.Predicted subcellular localization of *Pectate lyase*

Extracellular	2.437
Nuclear	0.678
PlasmaMembrane	0.539
Mitochondrial	0.443
Cytoplasmic	0.359
Lysosomal	0.250
Chloroplast	0.127
ER	0.067
Peroxisomal	0.034
Vacuole	0.029
Cytoskeletal	0.021
Golgi	0.015

Table-8. Signal peptide Analysis of *Pectate lyase*

Fibre gene	Measure	Position	Value	Cut Off	Signal Peptide
Pectate lyase	max.C	26	0.157		
	max.Y	26	0.325		
	max.S	18	0.847		
	Mean S	1-25	0.649		
	D	1-25	0.500	0.450	Yes

Table-9. Cis acting elements in promoter region

Site Name	*Organism*	Position	sequence	function
ABRE	*A. thaliana*	595	ACGTG	Abscisic acid responsiveness
ABRE	*A. thaliana*	895	ACGTG	Abscisic acid responsiveness
ABRE	*A. thaliana*	1225	ACGTG	Abscisic acid responsiveness
ABRE	*A. thaliana*	894	CACGTG	Abscisic acid responsiveness
CAAT-box	*A. thaliana*	258	CCAAT	Promoter and enhancer regions
CAAT-box	*A. thaliana*	279	CCAAT	Promoter and enhancer regions
CAAT-box	*A. thaliana*	779	CCAAT	Promoter and enhancer regions
CAAT-box	*A. thaliana*	919	CCAAT	Promoter and enhancer regions
CAAT-box	*A. thaliana*	936	CCAAT	Promoter and enhancer regions
CAAT-box	*A. thaliana*	1060	CCAAT	Promoter and enhancer regions

CAAT-box	*A. thaliana*	1192	CCAAT	Promoter and enhancer regions
G-box	*A. thaliana*	894	CACGTG	Light responsiveness
G-box	*A. thaliana*	892	GCCACGTGGA	Light responsiveness
GARE-motif	*B. oleracea*	995	TCTGTTG	Gibberellin-responsive element
GCN4_motif	*O. sativa*	754	TGAGTCA	Endosperm expression
GT1-motif	*A. thaliana*	337	GGTTAA	Light responsive element
MBS	*A. thaliana*	218	CAACTG	Drought-inducibility

Discussion

An effective breeding program needs comprehensive information and understanding of genetic diversity available within the germplasm. This empowers the breeders to select parental lines for creation of diverse population. Based on the results of the present investigation, a wide range of genetic diversity has been discovered in cotton interspecific lines. SL-19, predicting a distinct position from all genotype showed its high variability for fibre length and strength. SL-19 is a tri specie in background possessing three species in parentage (Anjum et al. 2015) which exhibited maximum fibre length due to polyploidy. In fact polyploidy increases DNA contents which ultimately generates larger cells (Soltis et al. 2015). When two genomes were merged from two diploid species, it produces allotetraploid cotton with increased DNA content, which resulted rapid fibre growth and enlarge size of single-celled fibres. It is identified that the nuclear DNA content increases during fibre elongation of cotton (Van't Hof 1999, Senchina et al. 2003, Wendel and Cronn 2003). Further, this nuclear augmentation of the fibre related genes is also due to polyploidization. For example, cultivated cotton has twofold more Malvaceae-specific *MIXTA* genes than *G. Raimondii* (Paterson et al. 2012, Zhang et al. 2015, Wu et al. 2018). Similarly, fibre related cellulose synthase-like and cellulose synthase genes were significantly increased in diploid cotton (Al-Ghazi et al. 2009, Haigler et al. 2012, Li et al. 2015).

The biplot analysis grouped the cotton interspecific lines on the basis of fibre traits. Genotypes such as in SL-9, SL-19, SL-87, Cyto-111, SL-369, SL-272, Cyto-117, Cyto-108, SL-459, SL-266, SL-241 were at distance from the origin representing more breeding value and more diversified (Ogunbayo et al. 2005, Rathinavel 2018).. In addition to diversity analysis, genotype-by-traits (GT) biplot analysis has been used to explore association among fibre traits, assessment of interspecific lines for fibre traits and identification of those lines which are superior in fibre quality. These identified interspecific lines could be the parental lines for cotton quality improvement breeding program (Yan and Rajcan 2002). It is also concluded that interspecific lines SL-19 was totally different from SL-369 because former lied in positive region and second lied in the negative region of the plot. Similarly, Cyto-124 lied opposite to the SL-266. These results also confirm the diversity of SL-19 with superior fibre quality trait which can be used in future breeding programme (Rathinavel 2019, Munir et al. 2020).

The dendrogram depicted five distinct clusters (Fig.2.6). The cluster-2 was the largest followed by cluster 1, 3, 5 and 6. The genotypes in cluster II showed higher values of fibre length in which SL-19 was more at distance from the origin indicating its highest variability. The cluster V is contributed mainly by fibre fineness which is in accordance with the earlier studies (Satish et al. 2009). Principal component analysis and linkage cluster analysis was used to find the similarity and differences among the lines for fibre parameters (Jian et al. 2006, Qiaoling and Zhe 2011, Rathinavel 2018, 2019, Talib et al. 2021). This will set a path for the development of superior parents and selection of heterotically potential hybrid combinations for cotton quality improvement. But due to negative correlation between yield and fibre quality, use of genetic engineering is also necessary to break this linkage by transferring identified highly expressed genes in local cultivars. Keeping in view the genetic diversity, using multivariant analysis. long staple (SL-19), short staple (SL-369) and medium staple lines (SL-79) were selected for fibre gene transcriptomic analysis. Transcriptomic analysis was also performed for *pectate lyase* genes at different fibre stage (0, 5, 10, 15 and 20 DPA) in selected three interspecific lines and three *Gossypium* species. Maximum expression was present in SL-19 and minimum transcripts were present in SL-369. Maximum transcripts were present at 10 DPA in all genotypes and species except *G. anomalum* and SL-369. SL- 369 is a short staple line while *G. anomalum* mostly contribute to fibre fineness due to which express expression at later fibre stage 20DPA. *G. anomalum* have many desirable traits, viz. jassids, mites, bollworm, rust and bacterial blight resistance, high fibre fineness, strength, maturity and cytoplasmic male sterility (Kalyanaraman and

Santhanam 1957, Iyengar 1958) thus it is of no value for fibre length or ginning percentage genes but *G. anomalum* may be useful as a source of fibre fineness and fibre strength (Silow 1941).

Bioinformatics is an imperative approach to supports analysis, such as prediction of protein sub-cellular localization (Zhao et al. 2014) and Physio chemical properties (Gasteiger et al. 2005). *Pectate lyase* was characterized as stable as value of instability index was 35.94. A protein whose instability index is less than 40 is expected as stable while a value greater than 40 indicates that the protein may be unstable. *pectate lyase* was characterized as a membrane soluble protein family exhibiting key role in plant cell wall loosening trough catalyzing lignification (Blanco-Montenegro et al. 2007, Taheri and Tarighi 2012).. *In silico* analysis also confirm the role of *pectate lyase* in fibre elongation. In plants, gene expression is regulated by transcription factors (TFs), which bind to cis-regulatory elements in promoters of target genes. Therefore, the promoter sequences of the *Pectate lyase* genes were analysed by searching the PlantCARE database. The results showed that many similar cis-acting regulatory DNA elements associated with plant tissues development processes were identified were also overrepresented in the promoter regions of *Pectate lyase*, which may play important role in plant development and stress response (Yanagisawa 2002, Pattanaik et al. 2008, Matsui et al. 2014). This indicated that *Pectate lyase* may also be involved in many stress response processes, light responsive but their functional mechanisms of such response need to be further investigated in future.

Conclusion

On the basis of these molecular analysis, it was concluded that, interspecific lines have wide range of variability for fibre traits. Cotton crop genetic improvements with limited genetic diversity among the existing cultivars can be improve by exploitation of diverse interspecific lines. Our research provided the bases for the development of high-quality cotton cultivars by utilization of SL-19 in conventional breeding programmes and *Pectate lyase* fibre gene in advanced molecular technologies.

Methods

Planting material

50 interspecific lines were screened for fibre traits in the experimental area of Central Cotton Research Institute Multan during 2018. Randomized Complete Block Design was designed with three replications. Seeds of all genotypes were planted by maintaining distance of 75 cm between two rows and distance from plant to plant was 30 cm. All cultural practices and standard plant protection measures were applied throughout the season.

Data collection

Fibre quality-related traits were tested by the HVI900 fibre testing system in Fibre section of CCRI, Multan. Data on the fibre length (mm), fibre strength ($gtex^{-1}$), micronaire value ($\mu g \cdot inch^{-1}$) and length uniformity (%) were recorded. The average of the three replicates was defined to be phenotypic information per genotype.

Biometrical analysis

The averaged data noted for fibre length, fibre strength, fibre fineness and fibre maturity was subjected to XL stat for principal component analysis (Sneath and Sokal 1973) and SPSS V20 for clustering analysis (Ogunbayo et al. 2005).

DNA/Gene Sequence retrieval and primer designing

Selected fibre gene (*Pectate lyase*) sequences were retrieved from NCBI website https://www.ncbi.nlm.nih.gov/. RT-PCR Primers were designed using PRIMER 3.0 software (Table-1).

constitutive gene primers were used as data normalizer in this assay.

Collection of fibre tissues

Three Interspecific lines SL-19, SL-79 and SL-369 along with three parent species (*G. arboreum*, *G. anomalum* and *G. hirsutum*) were used for fibre tissue collection. Cotton bolls were collected at different stages (0, 05, 10, 15 and 20 DPA). Collected bolls were rinsed with diethyl pyro carbonate (DEPC) treated water and were stored in liquid nitrogen. These frozen bolls were further used for RNA extraction.

Table-1.Primers used for Real Time PCR Assay

Gene annotation	Primer pair	Primer sequence (5'-3')	Primer length	Length (bp)	Accession No.
18S rRNA	RT18S -F	AAACGGCTACCACATCCAAG	20	153	U42827.1
	RT18S R	CCTCCAATGGATCCTCGTTA	20		
Pectate lyase	RTPEL-F	ATGGCAAGGACAATGGCAATGGCGAT	26	200	NM_001327561
	RTPEL-R	TCATCAATGGGGTTTCCGGTTCCACA	26		

Plant RNA extraction and cDNA synthesis

Using RNA reagent, RNA was extracted following Gynidium isothiocynate method (Logemann et al. 1987, Dolferus et al. 1994) . RNA quality was observed by electrophoresis and monitored under UV light. RNA samples were quantified through nanodrop (Thermo Scientific ND 2000) and concentrations was optimized prior to cDNA synthesis. Extracted RNA from fibre tissues was used for cDNA synthesis.

Real Time PCR analysis

To certify the sequence for specific gene, BLAST short (http://www.ncbi.nlm.nih.gov) was used. For expression analysis, Real Time PCR was performed by with SYBR Green Super Mix (Bio-Rad, USA) and 10 ng/µl of both set of primers.

In silico* analysis of *Pectate lyase

Sequence of *Pectate lyase* was taken from NCBI database **(**https://www.ncbi.nlm.nih.gov/) by searching accession number in all data bases. Coding sequence were identified with amino acid residues. Translation of gene sequence into amino acid sequences was done through EXPASY (https://web.expasy.org/translate/) into six reading frames.

Basic physiochemical properties and hydropathy index of protein sequences were computed through Expasy's ProtParam Proteomic server (http://web.expasy.org/protparam/). For Subcellular Location DeepLoc-1.0 (http://www.cbs.dtu.dk/services/DeepLoc) databases was used (Yu et al. 2004, Yu et al. 2006). Moreover, SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP/) (Petersen et al. 2011) was used to check existence of signal peptide. Promoter analysis was carried out at http://bioinformatics.psb.ugent.be/webtools/plantcare/html/. (Lescot 2002)

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References

1. Gallagher JP, Grover CE, Rex K, Moran M, Wendel JF: A new species of cotton from Wake Atoll, Gossypium stephensii (Malvaceae). Systematic Botany 2017, 42:115-123.
2. Chen ZJ, Scheffler BE, Dennis E, Triplett BA, Zhang T, Guo W, Chen X, Stelly DM, Rabinowicz PD, Town CD: Toward sequencing cotton (Gossypium) genomes. Plant Physiology 2007, 145:1303-1310.
3. Abdurakhmonov I, Kohel R, Yu J, Pepper A, Abdullaev A, Kushanov F, Salakhutdinov I, Buriev Z, Saha S, Scheffler B: Molecular diversity and association mapping of fiber quality traits in exotic G. hirsutum L. germplasm. Genomics 2008, 92:478-487.
4. Tyagi P, Gore MA, Bowman DT, Campbell BT, Udall JA, Kuraparthy V: Genetic diversity and population structure in the US Upland cotton (Gossypium hirsutum L.). Theoretical and Applied Genetics 2014, 127:283-295.
5. Wang M, Tu L, Lin M, Lin Z, Wang P, Yang Q, Ye Z, Shen C, Li J, Zhang L: Asymmetric subgenome selection and cis-regulatory divergence during cotton domestication. Nature genetics 2017, 49:579-587.
6. Fang L, Wang Q, Hu Y, Jia Y, Chen J, Liu B, Zhang Z, Guan X, Chen S, Zhou B: Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. Nature genetics 2017, 49:1089-1098.

7. Mehetre S, Aher A, Gawande V, Patil V, Mokate A: Induced polyploidy in Gossypium: a tool to overcome interspecific incompatibility of cultivated tetraploid and diploid cottons. Current Science 2003, 84:1510-1512.
8. Mehetre SS: Wild Gossypium anomalum: a unique source of fibre fineness and strength. Current Science 2010:58-71.
9. Margabandhu V: Preliminary Studies on Pest of Wild Cottons. In Proc 2nd Conf Cott Gr Prob India, 1941. 1941: 172-174.
10. Afzal M: Cytological investigation in some arboreum-anomalum crosses. Indian J Genet 1945, 5:82-91.
11. Deodikar G: Cytogenetic studies on crosses of Gossypium anomalum with cultivated cottons. II. Substitution and addition of anomalum chromosomes to the genome of cultivated tetraploid cottons. Indian Journal of Agricultural Science 1950, 20:399-414.
12. Deodikar G: Cytogenetic studies on crosses of G. anomalum with cultivated cottons. I (G. hirsutum× G. anomalum) doubled× G. hirsutum. Indian J Agric Sci 1949, 19:389-399.
13. Knight R: The genetics of blackarm resistance. XI. Gossypium anomalum. J Genet 1954, 52:466-472.
14. Narayanan S, Sundaram N, Peter S: note on oil content in Asiatic cottons, Gossypium anomalum and their amphiploids. Madras agricultural journal 1975.
15. Kalyanaraman S, Santhanam V: A note on the performance of some inter-specific hybrids involving wild species of Gossypium.(I). arboreum–anomalum crosses. Indian Cott Gr Rev 1957:136-140.
16. Iyengar N: Further studies in arboreum-anomalum, crosses. Indian Cott Gr Rev 1958, 12:369-375.
17. Raza S, Farooqi S, Jabeen S, Mubeen H: Gene Expression Analysis: A Review on its advanced applications and technologies. Journal of Pharmaceutical and Biological Sciences 2016, 4:24.
18. Anderson DB, Kerr T: Growth and structure of cotton fiber. Industrial & Engineering Chemistry 1938, 30:48-54.
19. Meinert MC, Delmer DP: Changes in biochemical composition of the cell wall of the cotton fiber during development. Plant Physiology 1977, 59:1088-1097.
20. Ahmed M, Shahid AA, Din SU, Akhtar S, Ahad A, Rao AQ, Bajwa KS, Khan MAU, Sarwar MB, Husnain T: An overview of genetic and hormonal control of cotton fiber development. Pak J Bot 2018, 50:433-443.
21. Zhu H, Lv J, Zhao L, Tong X, Zhou B, Zhang T, Guo W: Molecular evolution and phylogenetic analysis of genes related to cotton fibers development from wild and domesticated cotton species in Gossypium. Molecular phylogenetics and evolution 2012, 63:589-597.
22. Latif A, Ahad A, Ahmed M, Akhtar S, Yasmeen A, Shahid AA, Rao AQ, Nasir IA, Husnain T: GM Technology and Fiber Traits. In Cotton Precision Breeding. Springer; 2021: 281-300
23. Anjum Z, Hayat K, Celik S, Azhar M, Shehzad U, Ashraf F, Tariq M, Mehmood H, Azam M: Development of cotton leaf curls virus tolerance varieties through interspecific hybridization. African Journal of Agricultural Research 2015, 10:1612-1618.
24. Soltis PS, Marchant DB, Van de Peer Y, Soltis DE: Polyploidy and genome evolution in plants. Current opinion in genetics & development 2015, 35:119-125.
25. Van't Hof J: Increased nuclear DNA content in developing cotton fiber cells. American journal of botany 1999, 86:776-779.
26. Wendel JF, Cronn RC: Polyploidy and the evolutionary history of cotton. Advances in agronomy 2003, 78:139.
27. Senchina DS, Alvarez I, Cronn RC, Liu B, Rong J, Noyes RD, Paterson AH, Wing RA, Wilkins TA, Wendel JF: Rate variation among nuclear genes and the age of polyploidy in Gossypium. Molecular biology and evolution 2003, 20:633-643.
28. Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, Llewellyn D, Showmaker KC, Shu S, Udall J: Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature 2012, 492:423-427.
29. Zhang T, Hu Y, Jiang W, Fang L, Guan X, Chen J, Zhang J, Saski CA, Scheffler BE, Stelly DM: Sequencing of allotetraploid cotton (Gossypium hirsutum L. acc. TM-1) provides a resource for fiber improvement. Nature biotechnology 2015, 33:531-537.
30. Wu H, Tian Y, Wan Q, Fang L, Guan X, Chen J, Hu Y, Ye W, Zhang H, Guo W: Genetics and evolution of MIXTA genes regulating cotton lint fiber development. New Phytologist 2018, 217:883-895.
31. Li F, Fan G, Lu C, Xiao G, Zou C, Kohel RJ, Ma Z, Shang H, Ma X, Wu J: Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nature biotechnology 2015, 33:524-530.
32. Al-Ghazi Y, Bourot S, Arioli T, Dennis ES, Llewellyn DJ: Transcript profiling during fiber development identifies pathways in secondary metabolism and cell wall structure that may contribute to cotton fiber quality. Plant and cell physiology 2009, 50:1364-1381.
33. Haigler CH, Betancur L, Stiff MR, Tuttle JR: Cotton fiber: a powerful single-cell model for cell wall and cellulose research. Frontiers in Plant Science 2012, 3:104.
34. Ogunbayo S, Ojo D, Guei R, Oyelakin O, Sanni K: Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. African Journal of Biotechnology 2005, 4.
35. Rathinavel K: Principal component analysis with quantitative traits in extant cotton varieties (Gossypium hirsutum L.) and parental lines for diversity. Current Agriculture Research Journal 2018, 6:54.
36. Yan W, Rajcan I: Biplot analysis of test sites and trait relations of soybean in Ontario. Crop science 2002, 42:11-20.
37. Rathinavel K: Agro-morphological characterization and genetic diversity analysis of cotton germplasm (Gossypium hirsutum L.). Int J Curr Microbiol App Sci 2019, 8:2039-2057.
38. Munir S, Qureshi MK, Shahzad AN, Nawaz I, Anjam S, Rasul S, Zulfiqar MA: Genetic dissection of interspecific and intraspecific hybrids of cotton for morpho-yield and fiber traits using multivariate analysis. Pak J Agric Res 2020, 33:9-16.

39. Satish Y, Jain P, Chhabra B: Studies on genetic diversity in upland cotton (Gossypium hirsutum L.). Journal of Cotton Research and Development 2009, 23:18-22.
40. Jian C, Wei L, Ruili L, Feng LFW: Genetic diversity detected by cluster analysis North of Anhui major wheat cultivars. Chinese Agricultural Science Bulletin 2006, 11:031.
41. Qiaoling W, Zhe L: Principal component analysis of F2 individual selection in upland cotton (Gossypium hirsutum L.). Journal of Henan Institute of Science and Technology (Natural Sciences Edition) 2011:05.
42. Talib I, Cheema HMN, Arshad SF, Arshad HJ: DIVERSITY ESTIMATION OF FIBER RELATED TRAITS OF COTTON GERMPLASM THROUGH MULTIVARIATE ANALYSIS. Pakistan Journal of Agricultural Sciences 2021, 58.
43. Silow R: The comparative genetics ofGossypium anomalum and the cultivated Asiatic cottons. Journal of Genetics 1941, 42:259-358.
44. Zhao Y, Li X, Qi Z: Novel 2D graphic representation of protein sequence and its application. Journal of Fiber Bioengineering and Informatics 2014, 7:23-33.
45. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A: Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook 2005:571-607.
46. Taheri P, Tarighi S: The role of pathogenesis-related proteins in the tomato-Rhizoctonia solani interaction. Journal of Botany 2012, 2012.
47. Blanco-Montenegro I, De Ritis R, Chiappini M: Imaging and modelling the subsurface structure of volcanic calderas with high-resolution aeromagnetic data at Vulcano (Aeolian Islands, Italy). Bulletin of Volcanology 2007, 69:643-659.
48. Yanagisawa S: The Dof family of plant transcription factors. Trends in plant science 2002, 7:555-560.
49. Pattanaik S, Xie CH, Yuan L: The interaction domains of the plant Myc-like bHLH transcription factors can regulate the transactivation strength. Planta 2008, 227:707-715.
50. Matsui A, Mizunashi K, Tanaka M, Kaminuma E, Nguyen AH, Nakajima M, Kim J-M, Nguyen DV, Toyoda T, Seki M: tasiRNA-ARF pathway moderates floral architecture in Arabidopsis plants subjected to drought stress. BioMed research international 2014, 2014.
51. Sneath PH, Sokal RR: Numerical taxonomy. The principles and practice of numerical classification. 1973.
52. Logemann J, Schell J, Willmitzer L: Improved method for the isolation of RNA from plant tissues. Analytical biochemistry 1987, 163:16-20.
53. Dolferus R, Jacobs M, Peacock WJ, Dennis ES: Differential interactions of promoter elements in stress responses of the Arabidopsis Adh gene. Plant Physiology 1994, 105:1075-1087.
54. Yu CS, Lin CJ, Hwang JK: Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions. Protein science 2004, 13:1402-1406.
55. Yu CS, Chen YC, Lu CH, Hwang JK: Prediction of protein subcellular localization. Proteins: Structure, Function, and Bioinformatics 2006, 64:643-651.
56. Petersen TN, Brunak S, Von Heijne G, Nielsen H: SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature methods 2011, 8:785-786.
57. Lescot M: Patrice D hais, Gert Thijs, Kathleen Marchal, Yves Moreau, Yves Van de Peer, Pierre Rouz and Stephane Rombauts. Nucleic Acids Res 2002, 30:325-327.

Genetics and Pattern of Inheritance of Cotton Leaf Curl Disease Resistance Genes in Upland Cotton (*Gossypium hirsutum* L.)

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Abstract

Backgrond: Cotton Leaf Curl Disease (CLCuD) has been causing huge yield losses to the cotton crop in South Asia since its first epidemic during early 1990s. Researchers face many serious problems while screening and breeding for CLCuD resistant varieties. The absence of a reliable screening system, controversial inheritance data about the disease resistance, lack of genetic information about the resistance sources used as breeding material and reliability on small segregating populations are the major reasons in the failure of several cotton varieties that were initially released as resistant to CLCuV.
Results: In the present studies we used a highly CLCuV susceptible breeding line Stoneville-47 tagged with a herbicide resistance marker gene (Round-Up-ready cotton) to cross with newly discovered resistant accessions i.e. Mac-07 and USG13_1087 to understand the genetics of resistance and pattern of inheritance of resistance genes against CLCuD. The screening of breeding material against CLCuD, was carried out in the pots followed by grafting with susceptible scions and quantification of begomovirus and associated betasatellite by qPCR in the parental genotypes. The Chi-square test reflected a single dominant gene/cluster of tightly linked resistance genes controlling disease resistance by analyzing F_1, F_2 of direct and reciprocal crosses. However, the data from backcrosses of F_1s with the resistant parents suggested involvement of certain modifying factors/suppressors, which affect the expression of resistance gene(s). The circumvention of the suppressors of resistance from the selected progenies can only be achieved by raising larger plant populations with a clear picture of segregation of the resistant gene cluster.
Conclusions: The resistance against CLCuD in upland cotton (G. hirsutum L.) is dominant in nature and controlled by a single gene and most probably by a complex locus behaving like a single gene (gene cluster), the expression of which is altered by the presence of suppressors/modifiers. The resistance gene(s) might have differential interaction with different strains of CLCuV complex for conferring resistance. The modifying factors/genes seem locating very close to the resistance genes in the same cluster. During transferring a major gene/gene cluster, the breeder should be careful to retain, eliminate or accumulate modifying genes according to the needs of the situation.

Keywords: grafting, qPCR, CLCuD, disease resistance, genetics

1. Introduction

Cotton Leaf curl disease caused by the CLCuD-complex of begomoviruses is endemic to Pakistan and India and also other nearby localities in south Asia. Afterwards it transferred to China and the Philippines on ornamental plants, from where it has spread to infect cotton and okra in China. Losses caused are difficult to guess, however, infection at early plant stage especially in highly susceptible cultivars may result in zero harvest. Therefore, China, India and Pakistan together contributing ≥60% in the world cotton production are at prospective threat to CLCuD (Vyas et al., 2017).

Identification of some resistant sources i.e. LRA-5166, CP-15/2 etc. against this havoc and the introgression of resistant genes from these sources through conventional breeding into susceptible but otherwise high yielding varieties resulted in the development of CLCuD resistant/tolerant varieties during late 1990s, which provided a temporary relief to the cotton growers of the area. But these new

cultivars have narrow genetic base and poor resilience to different environmental conditions, and yielded less as compared to uninfected susceptible cultivars (Briddon et al., 2000; Farooq et al., 2011). In addition, the field observations indicate that most of these varieties eventually lost their resistance/tolerance against the disease within two to three years of their release.

The CLCuD is caused by a Begomovirus (family Geminiviridae) and has very distinctive disease symptoms, including upward or downward leaf curling, vein thickening and enations on the abaxial side of leaves, which frequently develop into leaf like structures (Briddon et al., 2000). Infection at early growth stages often results in shortened inter-nodal length and stunted plant growth. Yield and fiber quality are variably affected subjected to severity of symptoms which depends upon the genetic make-up of the variety, environmental conditions, plant age at the time of infection, begomovirus-satellite composition and type of infection (single or mixed) (Brown, 1992, 1994; Mansoor et al., 2003a; Brown et al., 2017).

Numerous widespread cryptic species of whitefly (*Bemisia tabaci* Gennadius) vector are involved in the transmission of CLCuD-begomoviruses (Brown, 2009; Shah et al., 2020). Characterization of whitefly-transmitted geminiviruses associated with the disease identified distinct beomoviruses that share different levels of homology with each other (Zhou *et al.,* 1998; Mansoor et al., 1999; Mansoor et al., 2003a).

Since its first occurrence in 1967, CLCuD in the Indo-Pak subcontinent has passed through four stages; pre-epidemic, epidemic, breakdown of resistance and post resistance breakdown era. Up to date available research about the disease reveals that CLCuD is related with distinct begomoviruses associated with a single predominant betasatellite species i.e., cotton leaf curl Multan betasatellite (CLCuMuB) (Zubair et al., 2017a) and alphasatellite.

Due to the development and widespread cultivation of resistant varieties developed by conventional breeding, CLCuD apparently disappeared from cotton during late 1990s. But due to the appearance of a resistance breaking strain of CLCuV during early 2000s known as Burewala strain, the resistance could not be maintained (Mansoor et al., 2003b; Mahmood et al., 2003) and all the varieties previously declared as resistant became susceptible to the new strain. The newly emerged unusual begomovirus strain, CLCuKoV-Burewala (CLCuKoVBu; previously known as Cotton leaf curl Burewala virus), is a recombinant of CLCuKoV and CLCuMuV. The new strain has also been found associated with a recombinant version of CLCuMuB (Amrao, et al., 2010).

The post-resistance breakdown era presented a sluggish shifting from CLCuKoV-Bu to a state more analogous to that of the first epidemic phase, with a little reappearance of some of the old virus species/strains in cotton (Zubair et al., 2017b). The available data represents that although other begomoviruses, and even a mastrevirus (geminivirus transmitted by leafhopper), have occasionally been reported in cotton during all the phases (Hameed et al., 2014; Zaidi et al., 2015 & 2016), the disease has always been associated with CLCuMuB which is basically required for symptoms development (Zubair, et al., 2017a).

Since the breakdown of resistance, screening of available germplasm has been carried out at massive scale to search resistance against CLCuD complex (Ahmed et al., 2013). During this scenario, when local cotton varieties were tolerant to some extent but not totally resistant, the accessions provided by the United States Department of Agriculture (USDA) and USDA Agricultural Research Service (ARS) breeding programs through ICARDA`s “Pakistan-U.S. Cotton Productivity Enhancement Program” were explored for resistant against the disease.

Out of thousands of *G. hirsutum* L. accessions provided under the project, a reasonable number of accessions were found resistant against the disease but majority of these did not produce flower due to photoperiod sensitivity. However, a few resistant accessions including Mac-07 (GVS9, ARS release number P.0063.14) and USG13_1087 (ARS release number TX-2415 607720) showed synchronized pattern of flowering with the local cultivars at CRS, Vehari.

Development of a CLCuV-resistant cultivar is the most promising control option (Akhtar et al., 2003). The knowledge about the number of genes conferring resistance and their pattern of inheritance is a pre-requisite to design the breeding strategies to develop resistant varieties. Such knowledge provides a quantitative basis for designs to recombine genes and selection for proper characters. So far, very little is known about the resistance mechanism or level of resistance against CLCuD (Mushtaq et al., 2018). The problem has been intensified by the fact that the data about inheritance is still controversial (Farooq et al., 2011; Saeed et al., 2018).

The lack of findings regarding genetics and inheritance pattern of resistant genes resulted in the failure of several cotton varieties in the field that were initially released as resistant to cotton leaf curl virus. The fact that whether several sources of resistance used in breeding contribute the same or different genes is also lacking. The problem in all these studies has been the non-availability of a reliable system for screening and assessing resistance in segregating populations.

A number of researchers have investigated to find out the genetics and inheritance pattern of genes conferring resistance against CLCuD. Most of them have reported the involvement of one or two major genes controlling the resistance. A few researchers (Haider et al., 2003a; Rahman et al., 2005) have revealed that along with the major genes, suppressor or modifying genes are also involved to affect the resistance.

Hence in the present studies, we used a highly CLCuV susceptible breeding line Stoneville-47 tagged with a herbicide resistance marker gene (Round-Up-ready cotton) to cross with newly discovered resistant accessions i.e. Mac-07 (USG13_1140) and USG13_1087 to understand the genetics of resistance against CLCuD. This information will be useful for breeders in deciding the methodology and breeding strategies to be adapted to develop the cotton genotypes for CLCuD resistance through conventional breeding and would facilitate in monitoring breakdown of resistance in the field because of the highly diverse and wide spread nature of the disease.

2. Materials and Methods

The present research work was conducted during the years 2018-20 in the greenhouse and experimental area of Cotton Research Station, Vehari, the hot spot of CLCuD.

2.1. Selection of parents

The CLCuV resistant upland cotton accessions Mac-07 (USG13_1140) and USG13_1087 provided under ICARDA`s “Pak-Us cotton productivity enhancement project 2011-15” were used as resistant sources. Whereas, transgenic *G. hirsutum* cultivar Stoneville-47 developed by Monsanto (USA) tagged with herbicide (Glyphosate) resistant gene but highly susceptible to CLCuV; was used as susceptible parent.

2.2. Screening of parents against CLCuD

Thirty plants each of the resistant and susceptible parents were grown during October, 2018 in earthen pots under greenhouse conditions where viruliferous whiteflies were maintained in abundance for the transmission of virus. The temperature of the glasshouse was kept 38-42°C during day time and 25-30ºC for night time (optimum for whitefly population) with 16 hours light period. Randomly selected 10 plants each of the resistant parents and all the 30 plants of susceptible cultivar, at 2-4 true leaf stage, were screened against foliar application of Round-Up at the recommended dose (20 ml/liter water).

2.2.1. Grafting with symptomatic scions

To ensure the virus infestation, 45-50 days old plants were graft inoculated following the “improved bottle shoot grafting method” reported by Akhtar et al. (2002) (Figure 1) with the symptomatic scions excised from the highly CLCuV susceptible variety FH-118, maintained in the greenhouse for this purpose.

2.2.2. qPCR assay to detect Begomovirus and β-satellite

After 50 days of grafting, young upper leaves from below grafting point, from graft (scion) and above graft from the visually resistant as well as susceptible parent plants and that of FH-118 (control) were collected and DNA extraction was performed using the modified CTAB method (Allen et al., 2006). The concentration of harvested genomic DNA from leaf samples and of purified plasmids were quantified by NanoDrop spectrophotometer.

For absolute quantification of the begomovirus and β-satellite, equal amount of healthy cotton genomic DNA (10ng) was spiked with plasmids carrying the full-length β-satellite as well as Cotton Leaf Curl Khokhran Virus to make standard and 5 dilutions (10 fold serial dilution) starting from 20 ng of virus (i.e 20, 2, 0.2, 0.02, 0.002 ng) were made. Whereas, for relative quantification, *SAD*1 gene (Genbank No. AJ132636) was used as an internal control to determine the relative level of virus. For β-satellite amplification, Beta qPCR F: CAAGTATATCAAGTCTGTGAACTATATCTT and Beta qPCR R: GATACTA-TCCACAAAGTCACCATCGCTAAT Primers were used. On the other hand, Begomo qPCR F: ATGTGGGATCCACTGTTAAATGAGTTCCC and Begomo qPCR R: GATTA-TATCTGCTGGTCGCTTCGACATAA Primers were used for Begomovirus DNA amplification.

Fig. 1: Improved Bottle-Shot Grafting with symptomatic scions

Amplification in both cases was performed with Eppendorf Mastercycler® ep realplex programmed for a first denaturation step of 10 min at 94 °C followed by 40 cycles of 30 Sec at 94 °C, 30 Sec at 57 °C and then 30 Sec at 72 °C and hold. A threefold analysis of these samples was conducted. The mean thresh hold cycle (Ct) of the triplicates was used for the begomovirus and satellite titers estimation. At the end of each run a melt curve analysis was performed from 57 to 95 °C, with an increment of 0.5 °C at 10 s intervals.

Standard curve for each target (virus or satellite) and for each run was calculated by linear regression analysis of the value of the Ct plotted over the amount of DNA of each of the three standard-dilutions replicates. Data was analyzed using iQ™5 Optical System Software, Version 2.1 (Bio-Rad) for each template sample separately. The analysis comprises the estimates of all parameters of the standard curves and their corresponding melt curves and also calculates the Ct values for each sample individually (Livak and Schmittgen, 2001).

2.3. Crossing of parents.

Based on above stringent screening process, only those plants of resistant parents Mac-07 and USG_13-1087 were used for crossing purpose, which showed resistance against graft inoculation and minimum titer of begomovirus and β-satellite in qPCR. On the other hand, plants of Stoneville-47 which showed resistance against Round-Up application but were susceptible to CLCuD on grafting and maximum titer of the virus and β-satellite in qPCR and field inoculation were used in crossing. Some of the flowers on each parent were self-pollinated to maintain the seed of each parent.

Crosses made were as under:

i.	Mac-07 × Stoneville-47	(A×C)	(direct cross)
ii.	USG13_1087 × Stoneville-47	(B×C)	(direct cross)
iii.	Stoneville-47 × Mac-07	(C×A)	(reciprocal cross)
iv.	Stoneville-47 × USG13_1087	(C×A)	(reciprocal cross)

At maturity, the harvested seed was preserved to grow the F_1 populations.

2.4. Raising, screening and selfing of F_1 populations

The F_0 seed from all direct as well as reciprocal crosses and parents was sown in the field during last week of May-2019 to raise F_1 population. Plants of control variety, FH-118 were also raised in between the F_1 populations to maintain and provide maximum virus inoculum throughout the growing season.

The plants of all F_1s at 2-4 true leaf stage were subjected to screening against foliar application of Round-Up. All the plants that survived were maintained adapting recommended production and plant protection practices except the control of whiteflies. Further, viruliferous whiteflies maintained under controlled conditions were released on these plants during early growth stage. At flowering, selfing was ensured by covering the flowers at bud stage. CLCuD data was recorded after 30 days interval from sowing till maturity (data not presented). At maturity, the seed cotton was picked separately from all the F_1s as well as parents and after ginning seed was stored to raise the F_2 populations. The F1 hybrids of crosses between resistant x susceptible and susceptible x resistant parents were all resistant, demonstrating the resistance was completely dominant over susceptibility and no cytoplasmic effects were observed because reciprocal crosses were all resistant.

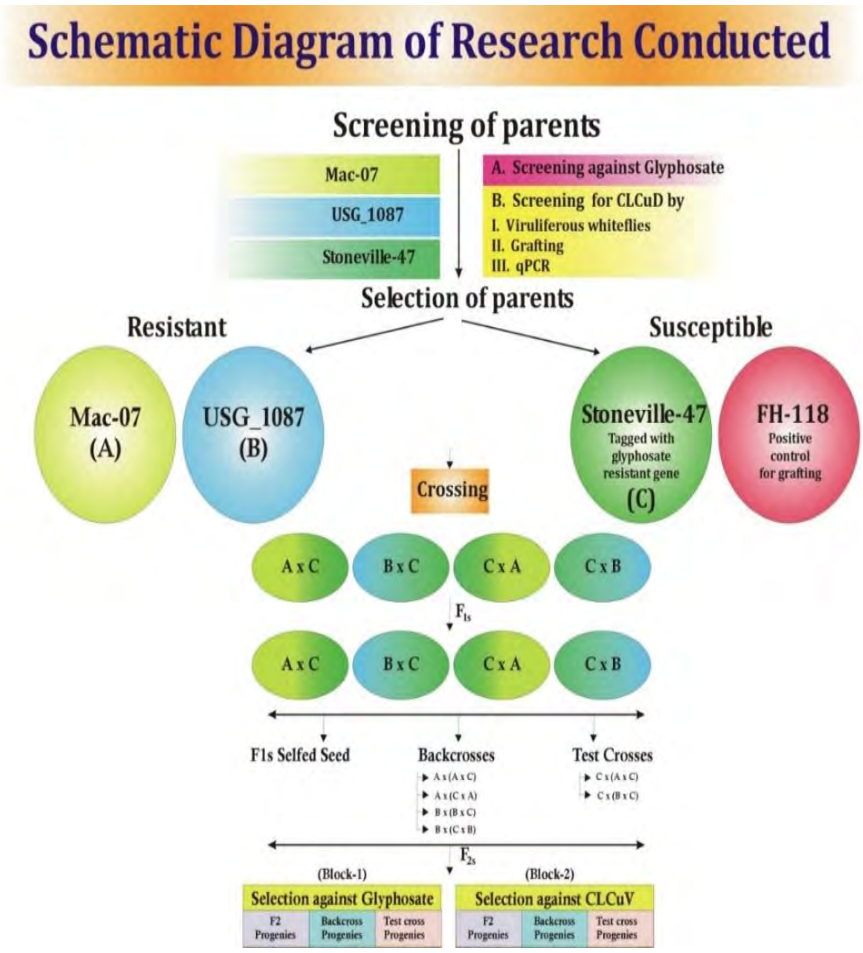

Fig. 2: Schematic diagram of research work conducted

2.5. Test crosses/Back crosses

At flowering, testcrosses were made using Stoneville-47 as female parent and F_1s of crosses Mac-07×Stoneville-47 and USG13_1087×Stoneville-47 as pollen source to observe the cytoplasmic effects, if any, imposed by the female parent on inheritance of resistance. The F_1s were also back crossed with both the CLCuV resistant parents adapting all the precautionary measures. At maturity seed cotton was picked and after ginning the seed was stored to grow the next generation.

2.6. Growing and screening of F_2 populations

During the cropping season-2020, maximum number of F_2 plants of all the direct and reciprocal crosses along with back/testcrosses was raised in two blocks of each cross combination; one for screening against Round-Up and the other for CLCuD data recording. The experimental area of CRS, Vehari (hot spot for CLCuV) was selected to conduct the trials. The sowing was done in the 1st week of June to ensure maximum availability of the virus inoculum. One row of control variety (FH-118) after every three rows of F_2s was sown as spreader line to ensure the prompt supply of virus inoculum during the whole growing season. After 15 days of germination one plant per hole was maintained by thinning.

After 45 days of sowing, first block comprising of each entry (F_2s of direct and reciprocal and back/test cross progenies) was screened against foliar application of Round-Up (avoiding the spreader line). The purpose was to check the genuineness of the plants in F_2s and back/test crosses as if they maintain 3:1 resistant to susceptible ratio in case of F_2s and 1:1 ratio in case of back cross with Stone-ville-47 against Round-Up. The data regarding the effect of Round-Up was recorded after 30 days of spray and is shown in Table 1. Data regarding CLCuV disease infestation was recorded from the 2nd block after 30 days' interval till maturity and is presented in Table 2. The data collected was analyzed using Chi-square test

Chi-square Formula: $x^2 = \sum \frac{(O-E)}{E}$

Where, O is the observed value and E is the expected value.

2.7. PCR amplification of DNA-β of CLCuV-PK

For the confirmation of resistance and susceptibility of symptomatic and asymptomatic plants, respectively, 60 days old 10 resistant and 10 susceptible plants were selected randomly from F_2 populations and back/testcrosses and DNA was extracted from the young leaves following the modified CTAB method (Allen et al., 2006). After RNase treatment and DNA quantification, a final dilution of 30ng/µl was made for PCR amplification. Universal primers designed to amplify DNA-β, based on the published nucleotide sequence of DNA-β of CLCuV-PK (Briddon et al., 2002) Beta 01 F 5`-GGTACCACTACGCTACGCAGCAGCC-3` and Beta 02 R 5`-GGTACCTACCCTCCCAGGGGTACAC-3` were used in PCR. The reaction was performed in volumes of 50µl containing 3.0µl template DNA, (10X) 5.0µl PCR buffer, 5.0µl dNTPs (0.2 mM), 3.0µl MgCl2 (50 mM), 1.0µl Primer each F and R (5 pMole) and 1µl (5 U/ µl) of Taq polymerase. Taq polymerase, together with buffer, MgCl2 and dNTPs were from MBI, Fermentas. Amplification was performed with Eppendorf 5333 Master Cycler programmed for a first denaturation step of 5 min at 94 °C followed by 40 cycles of 1 min at 94 °C, 1 min at 55°C, and 1 min at 72 °C. The reaction was kept at 72 °C for 10 min. and then held at 10 °C until the tubes were removed. Electrophoresis of PCR products was done on 1.5% agarose gel stained with ethidium bromide along with a 1kb marker. DNA-β of CLCuV-PK was identified by comparing the bands with markers as reported by (Briddon et al., 2002; Amin et al., 2006).

The schematic diagram of screening and crossing is presented in Fig 2. showing the screening of resistant and susceptible parents against CLCuV and round up resistant parents. After screening of the parents crossing was made by using direct cross and reciprocal crossing scheme. For generation advancement, F_1 was selfed.

3. Results

3.1. Screening and crossing of parents

All the 10 plants each of CLCuV resistant parents, Mac 07 and USG13_1087, were killed within 15 days of the application of Round-Up whereas, all the 30 plants of the CLCuD susceptible but Round-Up resistant parent, Stoneville-47, remained unaffected (Table 1).

Plants were designated either resistant with symptoms severity level 0 (S-0) or susceptible with severity level 1-4 (S-1 to S-4) based on complete absence of CLCuD symptoms or with minor symptoms, respectively (Figure-3).

None of the total 30 plants of each resistant source that were graft inoculated exhibited disease symptoms until maturity (Table 2). Whereas, All the 30 plants of susceptible parent, Stoneville-47, showed severe typical CLCuV disease symptoms on graft inoculation (Table 2).

In conventional end-point qPCR, no virus or betasatellite was detected from DNA samples taken from below or above the grafting point of resistant parents with zero severity rating (S-0) with both primer sets (for virus and betasatellite). On the other hand, from DNA samples extracted from infected grafts (symptomatic scions), susceptible parent and control (FH-118) with severity ratings S-1 to S-4 using the two primer pairs, both virus as well as betasatellite was amplified. DNA extracted from a healthy *Gossypium arboreum* plant raised under greenhouse conditions was used as negative control and exhibited zero amplification. For begomovirus and betasatellite the titer results are shown in bar graphs (Figs. 4a & 4b). Also for each component, the standard curves as well as melt curve analysis are figured as 5a and 5b.

Disease index (%)	Severity grade	Symptoms	Remarks
0	0	No Symptoms	Resistant
1-20	1	Thickening of only secondary and tertiary veins.	Highly tolerant (A)
21-30	2	Thickening of secondary and primary (mid rib) veins.	Tolerant (B)
31-50	3	Vein thickening (V.T), leaf curling (L.C) or enation (E) or both.	Susceptible (C)
>50	4	Stunting along with vein thickening leaf curling/enation.	Highly susceptible (D)

Fig. 3: Disease rating (symptoms rating) scale for evaluation of Cotton Leaf Curl Disease

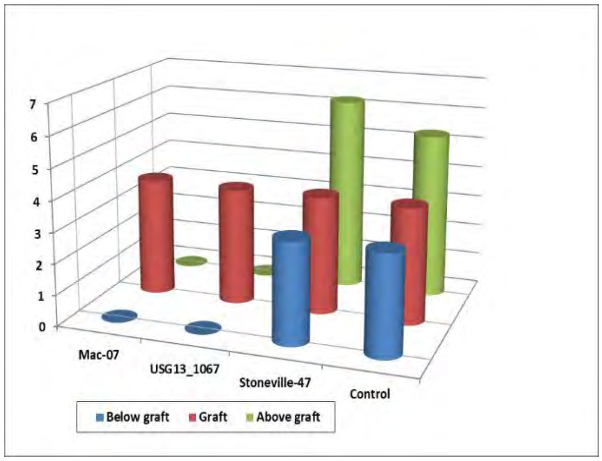

Fig. 4a: qPCR results of Begomovirus in grafted plants

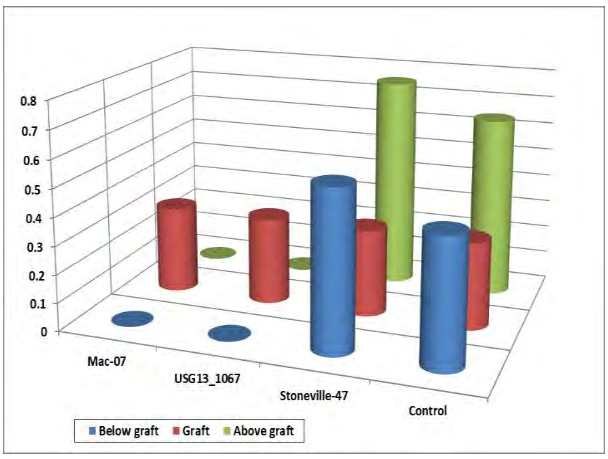

Fig. 4b: qPCR results of betasatellite in grafted plants

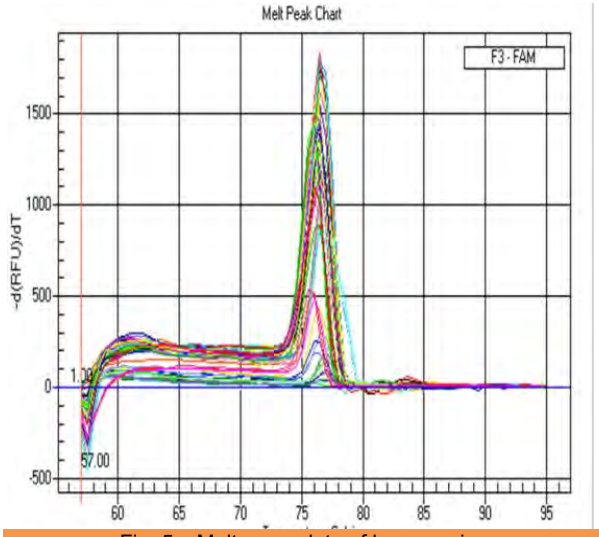

Fig. 5a: Melt curve data of begomovirus

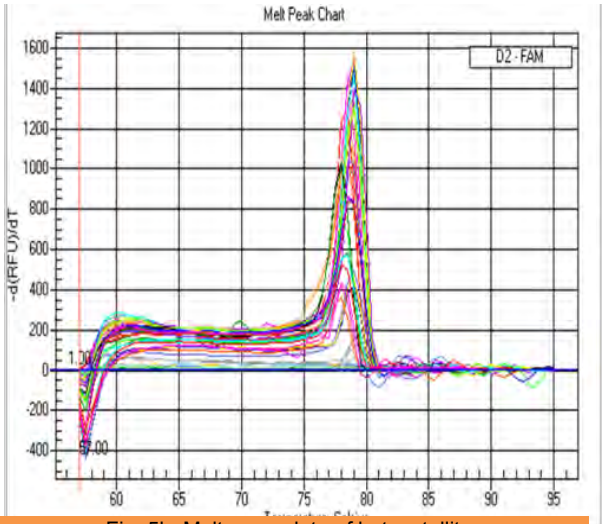

Fig. 5b: Melt curve data of betasatellite

As all the plants of both CLCuD resistant parents showed complete resistance, therefore, all the plants of both parents were crossed with susceptible Stoneville-47 to study the host plant resistance (HPR) mechanism.

Fig. 6: Results of foliar application of Round-Up after 30 days of spray on F_1 of crosses between CLCuD resistant and susceptible parents

Table: 1. Screening of cotton genotypes/crosses against foliar application of Round-Up (glyphosate)

Code	Parent/ Cross	Generation	No. of plants					Ratio	χ^2 values $[(O-E)^2/E]$
			Total	Actual (O)		Expected (E)			
				R	S	R	S		
A	Mac-07	Parent (A)	10	00	10	00	10		
B	USG13_1087	Parent (B)	10	00	10	00	10		
C	Stonrville-47	Parent (C)	30	30	00	30	00		
1	A×C (DC)	F_1	47	45	**02**†	47	00		
2	B×C (DC)	F_1	44	43	**01**†	44	00		
3	C×A (RC)	F_1	25	25	00	25	00		
4	C×B (RC)	F_1	23	23	00	23	00		
5	A×C (DC)	F_2	260	197	63	195	65	3:1	**0.0821***
6	B×C (DC)	F_2	230	180	50	173	57	3:1	**1.3043***
7	C×A (RC)	F_2	224	159	65	168	56	3:1	**1.9286***
8	C×B (RC)	F_2	245	191	54	184	61	3:1	**1.1442***
9	A×(A×C)	TC_1	248	123	125	124	124	1:1	**0.0160***
10	B×(B×C)	TC_2	184	93	91	92	92	1:1	**0.0220***
11	A×(C×A)	TC_3	228	116	112	114	114	1:1	**0.0700***
12	B×(C×B)	TC_4	206	104	102	103	103	1:1	**0.0190***
13	C×(A×C)	BC_1	198	197	01†	198	00	4:0	
14	C×(B×C)	BC_2	205	203	02†	205	00	4:0	

*Indicates that the data afford good fit to the calculated ratio at P <0.05

† Unexpected response

DC: Direct Cross; **RC**: Reciprocal Cross; **BC**: Back Cross; **TC**: Test Cross

3.2. Screening of F_1 population

Being a transgenic cultivar, resistance against Round-up (Glyphosate) in Stoneville-47 is under the control of a single dominant gene (transgene) (Wright et al., 1997). So following the Mendalian inheritance pattern, all the plants in F_1 populations of all the crosses (Direct and reciprocal) with Stoneville-47 were expected to be all resistant against Round-up application. However, out of 47 plants of the F_1 progeny of the cross between Mac-07×Stoneville-47, 45 were resistance and 2 were susceptible and killed. Whereas, in the 44 plants of F_1 progeny of the cross between USG13_1087×Stoneville-47, 43 showed resistance and one was killed within 15 days of spray (Table1).

In the F_1 population of reciprocal crosses of Stoneville with Mac-07 and USG13_1087, all the 25 and 23 plants in both crosses, respectively, were resistant against herbicide application (Table 1).

3.3. F_2 population of direct and reciprocal crosses

Data regarding response of F_2 population against Round-Up application (block-1) of both the direct crosses (A×C and B×C) were a good fit to 3:1 ratio as out of 260 total plants, 197:63 resistant to susceptible ratio; and out of 230 total plants, 180:50 resistant to susceptible ratio was observed, respectively (Table 1). The data showed a good fit to 3:1 theoretical ratio confirming that all the plants in F_2 population of above crosses were F_1 selfed progeny.

Whereas, the cotton leaf curl disease infestation data of the F_2 populations of both the direct crosses as shown in the Table 2, depicts that out of 279 total plants, 201:78 resistant to susceptible ratio; and out of 261 total plants, 188:73 resistant to susceptible ratio was recorded, respectively. These results thereby indicate that the resistance against cotton leaf curl disease is under the control of a single dominant gene because the data proved a good fit to 3:1 of resistant to susceptible ratio (Table 2). Response of F_2 populations of both the reciprocal crosses (C×A & C×B) against Round-Up application was also a good fit to 3:1 ratio as out of 224 total plants, 159:65 resistants to susceptible ratio; and out of 245 plants, 191:54 resistant to susceptible ratio was recorded, respectively (Table 1). The fitness of data to 3:1 theoretical ratio confirmed that all the plants in F_2 population of reciprocal crosses was F_1 selfed progeny.The second block of F_2s meant for CLCuD data recoding maintained a total 248 (182R: 66-S) and 266 (191R: 75-S) plants of both the above reciprocal crosses, respectively (Table 2). The analysis by Chi-square ($\chi 2$) test gave good fit results to the 3:1 (resistant:susceptible) expected ratio (Table 2) negating the cytoplasmic effects imposed by the female parent on the genetics of inheritance against Cotton Leaf Curl Disease.

3.4. Back crosses/testcrosses

In case of back crosses, the entire populations, except a few plants, in both the combinations of C× (A×C) and C× (B×C) were resistant against the herbicide application (Table 1). The testcross populations (in case of Round-Up application) in all the four combinations, A×(A×C), B× (B× C), A× (C×A) and B× (C×B), gave a good fit to the 1:1 (resistant to susceptible) theoretical ratio against response to Roud-Up application with a few exceptions (Table 1).

In testcrosses for CLCuD data recording (second block), expected 1:1 resistant to susceptible ratios were achieved (Table 2). In the first cross combination, C×(A×C), 110 plants out of 216 behaved as resistant and 106 fell prey to the cotton leaf curl disease (Table 2). Whereas, in case of second cross combination, C× (B×C), out of 244 total plants 126 were resistant and 118 were susceptible (Table 2).

Data recorded from the backcrosses in the second block meant for response against CLCuD, the expected ratios were not achieved and in each backcross population a reasonable number of plants were found to be susceptible against CLCuD (Table 2). Theoretically, all the plants in all the four backcrosses were expected to be resistant against CLCuD, but Chi Square test presented 5:1, 5:1, 2:1 and 3:1 resistant to susceptible ratios (Table 2). These results indicate that although resistance against CLCuD is under the control of a single dominant gene or complex locus (cluster of closely linked resistance genes) however, certain modifying factors may also involved which alter the expression of R gene/locus and resultantly the expected ratios were not obtained. The presence of single dominant gene suugest that CLCuD resistant gene can be transferred to any cultivar by back cross breeding.

3.5. PCR amplification of DNA-β of CLCuV-PK

PCR amplification is a much reliable technique to confirm the presence or absence of a pathogen in the host. The CLCuD betasatellite molecule is about 1.4 kb in size, pathogenesity determinant and encodes a single gene (βC1) and is responsible for cotton leaf curl disease symptoms development. PCR amplification results of β-satellite are shown in Figure 7a and 7b. The Figure 4a depicts that β-satellite

(Burewala) was not amplified from any of the DNA samples extracted from the 10 randomly selected asymptomatic (resistant) cotton plants except in the +ve control. The Figure 4b shows that a fragment of 1349 bp in size was amplified by using the specific set of primers for the amplification of DNA-β of CLCuV-PK from the DNA samples obtained from the randomly selected symptomatic cotton plants from the F_2 population as well as the +ve control. No amplification of any size was observed in the negative control. These findings shows that DNA β is essential for the development of disease symptopms.

Table: 2. Chi-square (χ^2) analyses for goodness of fit of data in theoretical segregation ratios

Category	Cross	Total Plants	Class	No. of plants		R/S ratio	χ^2 values $[(O-E)^2/E]$
				Obsd (O)	Exptd (E)		
F_2	A × C	279	Resistant	201	209.25		0.325
	(R × S)		Susceptible	78	69.75		0.976
					χ^2	3:1	1.301*
F_2	B × C	261	Resistant	188	195.75		0.307
	(R × S)		Susceptible	73	65.25		0.920
					χ^2	3:1	1.227*
F_2	C × A	248	Resistant	182	186		0.086
	(S × R)		Susceptible	66	62		0.258
					χ^2	3:1	0.344*
F_2	C × B	266	Resistant	191	199.5		0.362
	(S × R)		Susceptible	75	66.5		1.086
					χ^2	3:1	1.449*
Test cross	C × (A × C)	216	Resistant	110	108		0.037
	[*S* × (*R* × *S*)]		Susceptible	106	108		0.037
					χ^2	1:1	0.074*
Test cross	C × (B × C)	244	Resistant	126	122		0.131
	[*S* × (*R* × *S*)]		Susceptible	118	122		0.131
					χ^2	1:1	0.262*
BC_1	A × (A × C)	298	Resistant	245	298		9.42
	[*R* × (*R* × *S*)]		Susceptible	53	00		
					χ^2	5:1 †	
BC_2	A × (C × A)	214	Resistant	176	214		6.37
	[*R* × (*S* × *R*)]		Susceptible	38	00		
					χ^2	5:1 †	
BC_3	B × (B × C)	201	Resistant	135	201		21.67
	[*R* × (*R* × *S*)]		Susceptible	66	00		
					χ^2	2:1 †	
BC_4	B × (C × B)	226	Resistant	175	226		11.51
	[*R* × (*S* × *R*)]		Susceptible	51	00		
					χ^2	3:1 †	

* indicates that the data afford good fit to the calculated ratio at P <0.05
† indicates the unexpected ratios, A: Mac-07; B: USG13_1087; C: Stoneville-47, R: Resistant, S: Susceptible

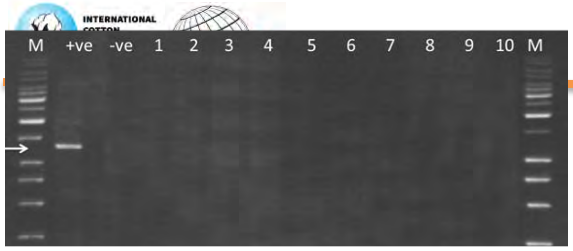

Fig. 7a: PCR amplification of DNA-β (Burewala) satellite of CLCuV-PK from the randomly selected 10 asymptomatic plants (1-10) of F2 generation. Lane M:1Kb DNA ladder (MBI, Fermentas) +ve is the positive control and -ve is the negative

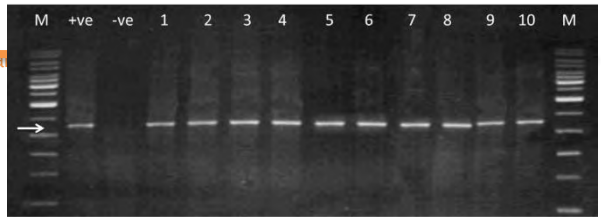

Fig. 7b: PCR amplification of DNA-β satellite (old) of CLCuV-PK from the randomly selected symptomatic plants (1-10) of F_2 generation . Lane M: 1Kb DNA ladder (MBI, Fermentas), +ve is the positive control, -ve is the negative control.

4.Discussion

4.1. Screening and crossing of parents.

Keeping in view the controversial data regarding number of genes conferring resistance against CLCuV and their pattern of inheritance, the parents were subjected to stringent and comprehensive screening against the disease by open infestation, grafting (Figure 1) as well as at molecular level to avoid false negatives; because field inoculation of the virus through whitefly vector is uneven. To identify and quantify the CLCuD causing begomoviruses and accompanying betasatellites, we subjected resistant as well as susceptible parents to qPCR. It is a recently optimized standard method to quantify the begomoviruses and related betasatellites involved in CLCuD infestation in cotton (Shafiq et al., 2017). Remarkably, begomovirus and betasatellite amplification by qPCR using specific primers pointed out that where both these were found in susceptible parents, these were almost absent in resistant genotypes (Figure 2a & 2b). Further, comparison between begomovirus and betasatellite titer in resistant and susceptible parents using qPCR, betasatellite was the most copious component in susceptible parents. To induce CLCuD in susceptible plants, betasatellite is the pathogenicity determinant and is essential for disease symptoms development (Mansoor et al*.,* 2003a; Briddon et al., 2014). The negligible titer of betasatellite in resistant parents is therefore strongly linked with the lack of CLCuD symptoms and truncated titer of begomoviruses in resistant parents (below & above graft).

The results according to Table 1 depicted that the plants of all the three selected parents passed the preliminary screening test against Glyphosate application, so were true to type as expected. Further, both the CLCuV resistant parents (Mac-07 and USG13_1087) demonstrated no disease symptoms whereas, the susceptible parent Stoneville-47 displayed severe disease symptoms under open environment and by graft inoculation (Figure 1). Transfer of virus by grafting is well documented in the literature and proven experimentally (Rahman et al*.,* 2005; Akhtar, et al*.,* 2002; Tsaballa et al*.,* 2013; Kumar et al., 2018). The qPCR amplification in case of resistant parents showed negligible titer of Begomovirus as well as betasatellite in leaves below and above the grafting point; whereas, in case of susceptible parent (Stoneville-47) and FH-118 (control) the titer was maximum (Figure 2a, 2b, 3a & 3b).

4.2. Screening and selfing of F_1 populations

In F_1s, all the plants gave expected results against Glyphosate screening except two plants in the first cross Mac-07×Stoneville-47 and one plant in second cross USG13_1087×Stoneville-47, which eliminated (Table 1). So, all the remaining F_1 plants harvested to grow F_2 populations were true F_1 hybrids. Although transgenes are dominant in nature and inherit consistently in a Mendelian pattern (Pinheiro et al*.,* 2009) but due to either uneven transmission or poor expression of the transgene, non-Mendelian segregation may be observed at a frequency of 10% to 50% in F_1 hybrids (Limanton-Grevet et al*.,* 2001; Samis et al*.,* 2002). The results also signpost that the most preferred hand emasculation method in cotton may leave some pollen intact on the female flower that may result in selfed seed and produce false positives.

Several factors are known to influence symptoms severity. These include time of infection, virus strain, plant growth stage, host's genetic make-up, environmental conditions (Akhtar et al., 2004; Kumar and Gupta, 2016; Saeed et al., 2018) and type of infection (mixed or single). Presently eight different variants of the virus are prevalent in cotton areas (Saleem et al., 2016; Biswas et al., 2020. According to Brown and co-workers (2017), at least five species and multiple variants (isolates) and strains co-evolve in cotton in south Asia.

So the adult plant stage data were considered for analysis. The F_1 hybrids of crosses between resistant × susceptible and susceptible × resistant parents were all resistant, demonstrating that resistance is dominant over susceptibility and no cytoplasmic effects were observed because reciprocal crosses (susceptible × resistant) were all resistant. These results are in agreement with the findings of Siddig (1970), Wilson and Brown (1991), Ali (1997), Randhawa (1999), Haider et al. (2003), Ahuja et al. (2007),

Rahman (2007), Pathak et al. (2009), Sangwan et al. (2015) and Shafiq et al. (2017); who have also reported that resistance against cotton leaf curl disease is under the control of dominant gene(s). Whereas, Ahuja et al., (2006) and Khan et al., (2007) have reported that extra-nuclear (cytoplasmic) effects are also involved in resistance.

However, Hutchinson et al. (1950) reported that resistance to leaf curl in *G. barbadense* is under the control of minor genes. Tarr (1951), was of the opinion that no major gene are involved in conferring resistance to the disease and resistance against virus in *G. barbadense* might be due to the cumulative effect of minor genes. However, according to Ali (1997), selecting healthy plants from susceptible genotypes in *G. hirsutum* could not develop the resistance, thereby confirming the non-involvement of minor gene effects.

4.3. F_2 population of direct and reciprocal crosses

F_2 data of both the direct and reciprocal crosses regarding response against Round-Up application (block 1) showed a good fit to 3:1 resistant to susceptible theoretical ratio (Table 1) confirming that all the plants in F_2 populations of above crosses were F_1 selfed progeny and segregated in Mendelian pattern (Pinheiro et al., 2009).

In the 2nd block, F_2 populations of both the direct as well as reciprocate crosses segregated in a 3:1 ratio of resistant to susceptible plants indicating thereby a single dominant gene governing resistance against CLCuD in both the resistant parents (Ali, 1997; Hussain et al., 2012). Test crosses of susceptible parent-C (Stoneville-47) with F_1s of both the resistant parents (A×C and B×C) segregated, as expected, in a 1:1 ratio of resistant to susceptible thus confirmed the monogenic dominant inheritance of resistance to cotton leaf curl disease. Siddig (1968), who criticized the role of minor genes and suggested that the host plant resistance against leaf curl disease in *G. barbadense* is under the control of a single gene or very closely linked genes, has also reported similar results. Haider et al., (2003a) have also demonstrated from F_1, F_2, reciprocals and back crosses that resistance to CLCuD in upland cotton *G. hirsutum L.* is under the control of a single major dominant gene, which is under the influence of several other modifying factors.

Whereas, findings contrary to the present results, have also been reported in the literature. It has been publicized that the resistance of Cedix, a highly resistant cotton cultivar to cotton leaf crumple virus (CLCrV) from El Salvador, is controlled by two dominant and supplementary genes, which must occur together in order to confer full resistance (Wilson and Brown, 1991; Randhawa, 1999). But this contradiction with the present results can be denied on the basis that cotton leaf crumple virus is a bipartite geminivirus whereas, cotton leaf curl virus has monopartite genome. According to some other researchers, duplicate dominant epistasis is involved and resistance against CLCuV is controlled by two dominant duplicate genes as F_2 ratio was modified to 15: 1 from 9:4:4:1 and the test cross ratio was modified to 3:1 instead of 1:1(Iqbal et al., 2003).

Rahman et al. (2005) based on F_3 progeny test suggested that two dominant genes at two loci acting epistatically might have conditioned the CLCuD resistance and a third gene known as suppressor gene is also involved which inhibits the expression of major genes. Hence, according to them the resistance against CLCuD is a qualitative-quantitative character. However, in case of CLCuD resistance inheritance study, one is unable to harvest seed from all the F_2 plants because the highly susceptible F_2 plants may not produce even a single boll and seed to grow an unbiased F_3 generation. Therefore, for inheritance studies of CLCuD resistance genes by conventional breeding, one has to rely on F_2 segregating population data.

In the present study, back cross ratios in both the direct (R×S) and reciprocal (S×R) F_1s with resistant parents did not agree with the theoretical ratio i.e. all resistant (Table 2). In each case, some susceptible plants were observed suggesting that some other factors are also involved in the inheritance, which modify the results (Mackay, 2014; Sackton & Hartl, 2016). Earlier, Zamir & co-workers (1994) have also reported that a major gene termed TY-1 controls resistance against TYLCV in tomato (L. chilense LA1969 source) and at least two more modifier genes are present to affect the resistance. Rahman et al. (2005) have also reported the presence of a suppressor gene affecting resistance in *G. hirsutum* against CLCuD. Whereas, Haider et al. (2003a), suggested that response in cotton against CLCuD is under the influence of some modifying factors (enhancers or suppressors) which impinge on monogenic inheritance. Modifying genes do not have any effect of their own but they may alleviate or exacerbate the severity of the disease, resulting in the variability of phenotypic outcomes (Martin and Sapienza, 1999; Weatherall, 2001; Rahit and Tarailo-Graovac, 2020).

Further, in the present study,greater number of susceptible plants were observed when F_1s (R×S or S×R) were back crossed with the resistance source USG13_1087; in this case 2:1 and 3:1 resistant to susceptible ratios were observed, respectively. Whereas, in case of backcrosses of F_1s with Mac-07, in both cases, ratio of susceptible plants was comparatively low (5:1 resistant to susceptible ratio). These results suggest that both the resistance sources have different types of R-genes conferring resistance against CLCuD in *G. hirsutum*. Earlier Randhawa (1999), Rahman et al. (2005), and Khan and Shah (2001) have reported the presence of different resistance genes in different sources of resistance in cotton.

In addition, as variation in the ratio of resistant to susceptible plants in back crosses with different resistant parents have been recorded, there might be varying number of modifying genes in both parents effecting the expression of resistance genes (Khan et al., 2007; Andersen et al., 2018; Mushtaq et al., 2018; Sun et al., 2020). A careful evaluation of published literature illustrate that interest in the genetic modifier study has substantially increased in the last decade (2010-2019: 916 records) as compared to the previous (1998-2008: 281 records) (Rahit & Tarailo-Graovac, 2020).

The resistance response exhibited by both the resistance sources under study was similar to Rx (extreme resistance) and thus dominant R-gene(s) is likely to be involved in the resistance similar to those reported against PVX (Rx 1). The resistant genes are often found in clusters (Parniske et al., 1999; Meyers et al., 2003; Mizuno et al., 2020) and thus it is likely that a single locus containing a few to several (tightly-linked) individual R-genes, which are transferred simultaneously in the experimental population grown on limited area, behave like a single gene.

Afterwards, when the plant variety is grown at large scale at farmer's field, this linkage is broken down, resistant genes segregate and different plants start showing different grades of disease symptoms depending upon the number of resistant genes they possess. This phenomenon has been widely experienced in Pakistan since the development of first CLCuD resistant varieties in 1996 (CIM-1100 and CIM-448) to onward as previously developed resistant varieties became susceptible within few years of their release. Further, disease resistance which depends on oligogenes (dominant genes) i.e., CLCuD, might no longer prevail and disappear due to recombination events among different viruses or associated satellite molecules (Mansoor et al., 2003b; Mahmood et al., 2003); or evolutionary modifications in the pathogen involved (Azhar et al., 2010).

On the other hand, the susceptible plants in F_2 populations did not exhibit similar symptoms severity level. Some earlier researchers, on the basis of symptom`s severity, have classified plants into different categories *i.e.* from 0-4 (Akhtar et al., 2010; Nazeer et al., 2014), 0-6 (Khalid and Khan, 2002; Akhtar et al., 2004), or 0-10 with zero as absence of any kind of symptoms and four, six or ten as the severe disease symptoms (severe leaf curling, development of enations and stunted growth). The symptoms variation might be due to the infection by different combinations of variants of the begomovirus (Saleem et al., 2016; Godara et al., 2017; Qadir et al., 2019; Biswas, *et al.,* 2020; Hamza, 2020) or different strains or species of the viruses (Rahman, 1997).

The presence of such types of grades is very difficult to advocate. It is known that the nature of the disease is multifarious as four major players are involved in the manifestation of the disease. This complex comprises of environment, vector (whitefly), host plant and the virus itself. According to Tarr (1951), the environment may affect host plant resistance or the virus or both. Moreover, the intensity of the disease infection fluctuates with locality and year (Siddig, 1970). Secondly, it has also been documented that environmental factors like temperature and rainfall may affect the incidence of the CLCuD El-Nour and Abu-Salih, 1966; Khan and Khan (2000); Farooq et al., 2011; Ahmed et al., 2013).

Thirdly, involvement of minor genes may also cause such types of grades (Hutchinson et al., 1950). Moreover, the susceptible varieties with faster growth rate can escape from the CLCuD better than the slow growing genotypes (Ali, 1997). Fourthly, a variety tolerant to the vector (whitefly in this case) can also escape because of less frequent visits of the viruliferous vector. The mechanism behind the different grades in the appearance of the disease symptoms might also be due to diversity in resistant genes inherited by different parents and the variation in the number and diversity among various modifying genes related to these R-genes.

4.4. PCR amplification of CLCuV betasatellite in F2 plants

The begomoviruses and the associated satellite molecules causing CLCuD, can primarily be amplified by PCR using degenerate or specific primers (Idris et al., 2011). CLCuD betasatellite amplified successfully by PCR using specific primers from all the ten randomly selected symptomatic plant samples from F_2 population. Whereas, from none of the DNA samples extracted from resistant

(asymptomatic) plants amplification was observed. These results confirmed that the plants designated as symptomatic as well as asymptomatic in F_2s were true to type and our results about the ratios of resistant to susceptible plants are authentic. In addition, Rolling Circle Amplification (RCA) technique has also been reported for the identification of various associated viruses and their recombinants (Haible et al., 2006). However, more convenient identification techniques are still not available. In future, with the advancement in genome sequencing techniques new protocols may be designed for the identification of whole virus and vector complex and monitoring of spreading of these to various other crop species (Saleem et al., 2016).

Conclusions

The resistance against CLCuD in upland cotton (*G. hirsutum* L.) is dominant in nature and controlled by a single gene and most probably by a complex locus behaving like a single gene (gene cluster), the expression of which is altered by the presence of suppressors/modifiers. The resistance gene(s) might have differential interaction with different strains of CLCuV complex for conferring resistance. Resistant parents Mac-07 and USG13_1087 might have different sources of resistance genes presenting resistance to different strains of the virus. The modifying factors/genes seem locating very close to the resistance genes in the same cluster. During transferring a major gene/gene cluster, the breeder should be careful to retain, eliminate or accumulate modifying genes according to the needs of the situation.

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GS conducted the experiment and collected, arranged and analyzed the data; IS helped in data collection and manuscript write up; JS and BS provided the experimental material and provided guidelines to conduct the research; KK helped and proof made proof reading of the manuscript.

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References

1. Ahmed A, Akhtar A, Khalid B, Shamim A. 2013. Correlation of meteorological parameters and remotely sensed normalized difference vegetation index (NDVI) with cotton leaf curl virus (CLCV) in Multan. In Journal of Physics: Conference Series. 439(1). 012044). IOP Publishing. doi:10.1088/1742-6596/439/1/012044
2. Ahuja SL, Monga D, Dhayal LS. 2007. Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum* L. under field conditions. *Journal of heredity*, 98(1), 79-83. doi:10.1093/jhered/esl049
3. Akhtar KP, Khan AI, Khan MSI. 2002. Improved bottle shoot grafting technique/ method for the transmission of cotton leaf curl virus (CLCuV). The Nucleus, 39 (1-2), 115-117.
4. Akhtar KP, Khan AI, Hussain M, Haq MA, Khan MSI. 2003. Upland cotton varietal response to cotton leaf curl virus (CLCuV). *Tropical Agriculture Research. & Extension*, 5: 29-34.
5. Akhtar KP, Hussain M, Khan AI, Haq MA, Iqbal MM. 2004. Influence of plant age, whitefly population and cultivar resistance on infection of cotton plants by cotton leaf curl virus (CLCuV) in Pakistan. *Field Crops Research*, 86, 15-21.
6. Akhtar KP, Haider S, Khan MKR, Ahmad M, Sarwar N, Murtaza MA, Aslam M. 2010. Evaluation of Gossypium species for resistance to leaf curl Burewala virus. *Annals of Applied Biology*, 157, 135-147.
7. Ali M. 1997. Breeding of cotton varieties for resistance to cotton leaf curl virus. *Pakistan Journal of Phytopathol*ogy. 9 (1), 1-7.
8. Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethyl ammonium bromide. *Nature protocols*, 1(5), 2320.
9. Amin I, Mansoor S, Amrao L, Hussain M, Irum S, Zafar Y, Bull SE, Briddon RW. 2006. Mobilization into cotton and spread of a recombinant cotton leaf curl disease satellite. *Archives of Virology*, 151, 2055-2065.
10. Amrao L, Amin I, Shahid, MS., Briddon RW Mansoor S. 2010. Cotton leaf curl disease in resistant cotton is associated with a single begomovirus that lacks an intact transcriptional activator protein. *Virus Research*. 152(1-2): 153-163.
11. Andersen EJ, Ali S, Byamukama E, Yen Y, Nepal MP. 2018. Disease resistance mechanisms in plants. *Genes*, 9(7), 339.
12. Azhar MT, Rehman MU, Aftab S, Zafar Y, Mansoor S. 2010. Utilization of natural and genetically-engineered sources in *Gossypium hirsutum* for the development of tolerance against cotton leaf curl disease and fiber characteristics. *International Journal of Agriculture and Biology*, 12, 744-748.
13. Biswas KK, Bhattacharyya UK, Palchoudhury S, Balram N, Kumar A, Arora R. 2020. Dominance of recombinant cotton leaf curl Multan-Rajasthan virus associated with cotton leaf curl disease outbreak in northwest India. *PLoS ONE*, 15(4): e0231886. https://doi.org/10.1371/journal.pone.0231886

14. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Stanley J, Markham PG. 2000). Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. *Virus Genes*, 20, 19-26.
15. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. 2002. Universal primers for the PCR-mediated amplification of DNA β. *Molecular Biotechnology*, 20(3), 315-318.
16. Briddon RW, Akbar F, Iqbal Z, Amrao L, Amin I, Saeed M. Mansoor S. 2014. Effects of genetic changes to the begomovirus/betasatellite complex causing cotton leaf curl disease in South Asia post-resistance breaking. *Virus Research*. 186, 114-119.
17. Brown JK. 1992. Virus diseases. In: Cotton diseases. Hillocks RJ, ed. Wallingford, UK: CAB International, 275-329.
18. Brown JK. 1994. Current status of *Bemisia tabaci* as a plant pest and virus vector in agro-ecosystems worldwide. *FAO Plant Protection Bulletin*, 42(1/2):3-32
19. Brown JK. 2009. Phylogenetic biology of the *Bemisia tabaci* sibling species group. In Bemisia: bionomics and management of a global pest (pp. 31-67). Springer, Dordrecht.
20. Brown JK, Ur-Rehman MZ, Avelar S, Chingandu N, Hameed U, Haider S, Ilyas M. 2017. Molecular diagnostic development for begomovirus-betasatellite complexes undergoing diversification: A case study. *Virus Research*, 241, 29-41. https://doi.org/10.1016/j.virusres.2017.04.014.
21. El-Nour E, Abu-Salih, HS. 1966. A Report, Res. Div. Min. Aric. Sudan. 1965-66, pp. 118-120.
22. Farooq A, Farooq J., Mahmood A, Batool A, Rehman A, Shakeel A, Riaz M, Shahid MTH, Mehboob S. 2011. An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity. *Australian Journal of Crop Science,* 5(13), 1823-1831
23. Fisher RA. 1930. The Genetical Theory of Natural Selection. (Clarendon Press). 1958.
24. Godara S, Paul Khurana SM, Biswas KK. 2017. Three variants of cotton leaf curl begomoviruses with their satellite molecules are associated with cotton leaf curl disease aggravation in New Delhi. *Journal of Plant Biochemistry and Biotechnology*, 26, 97-105. https://doi.org/10.1007/s13562-016-0370-x
25. Haible D, Kober S, Jeske H. 2006. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *Journal of Virological Methods*, 135(1), 9-16.
26. Haider S, Khan IA, Mansoor S. 2003a. Genetics of cotton leaf curl virus disease in upland cotton (*Gossypium hirsutum* L.). *Sarhad Journal of Agriculture*, 19 (2), 207-210.
27. Haider S, Khan IA, Mansoor S, Arshad M. 2003b. Linkage studies between bacterial blight and cotton leaf curl virus disease resistance genes in cotton (*Gossypium hirsutum* L.). *Sarhad Journal of Agriculture*, 19 (3), 359-363.
28. Haldane J. 1941. The relative importance of principal and modifying genes in determining some human diseases. *Journal of Genetics*, 41, 149-157.
29. Hameed U, Zia-Ur-Rehman M, Herrmann HW, Haider MS, Brown JK. 2014. First report of Mesta yellow vein mosaic virus, Cotton leaf curl Multan betasatellite and Cotton leaf curl Multan alphasatellite infecting cotton in Pakistan. *Plant Disease*. 140122140029006.
30. Hamza, R. 2020. Review of Cotton Leaf Curl Virus (CLCuV) along-with its strains (CLCuMuV and CLCuBuV). *Journal of Emerging Diseases and Preventive Medicine*, 2(3) 1-13.
31. Hussain M, Azhar FM, Khan AL, Ali Z. 2012. Expression of genes controlling the inheritance of resistance to cotton leaf curl virus disease (CLCuD) in *Gossypium hirsutum* L.: a quantitative analysis. *Pakistan Journal of Botany*, 44(1): 247-254.
32. Hutchinson JB, Knight RL, Pearson EO. 1950. Response of cotton to leaf-curl disease. *Journal of genetics*, 50(1), 100-111.
33. Idris AM, Shahid MS, Briddon RW, Khan AJ, Zhu J K, Brown JK. 2011. An unusual alphasatellite associated with monopartite begomoviruses attenuates symptoms and reduces betasatellite accumulation. *Journal of General Virology*, 92(3), 706-717.
34. Iqbal M, Chang MA, Mahmood A, Khumber MB, Nasir A, Ul-Hassan M. 2003. Inheritance of response to Cotton Leaf Curl Virus (CLCuV) infection in cotton. *Asian Journal of Plant Sciences*, 2(3) 261-264.
35. Khalid MR, Khan NU, 2002. Germplasm screening of Upland cotton for resistance to cotton leaf curl virus (CLCuV) in D.I. Khan. In: Proceedings of Seminar, 17-19 Dec. 2002. PARC-NARC, Islamabad.
36. Khan MA, Khan HA. 2000. Cotton leaf curl virus disease severity in relation to environmental conditions. *Pakistan Journal of Biological Sciences*, 3(10), 1688-1690.
37. Khan WS, Shah MKN. 2001. Stability of cotton cultivars under leaf curl virus epidemic in Pakistan. *Field Crops Research*, 69, 251-257.
38. Khan AI, Hussain M, Rauf S, Khan TM. 2007. Inheritance of resistance to cotton leaf curl virus in cotton (*Gossypium hirsutum* L.). *Plant Protection Science-UZPI (Czech Republic*), 43, 5-9.
39. Kumar M, Gupta A. 2016. Effect of weather variables on whitefly (Bemisia tabaci Gennadius) population in development of potato apical leaf curl virus disease. *Journal of Agrometeorology*, 18(2): 288-291.
40. Kumar S, Bharti N, Saravaiya SN 2018. Vegetable Grafting: A Surgical Approach to combat biotic and abiotic stresses-A review. *Agricultural Reviews*, 39, 1-11.
41. Limanton-Grevet A, Jullien M. 2001. Agrobacterium-mediated transformation of Asparagus officinalis L.: molecular and genetic analysis of transgenic plants. *Molecular Breeding*, 7:141-150.
42. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25(4), 402–408
43. Mackay TF 2014. Epistasis and quantitative traits: using model organisms to study gene-gene interactions. *Nature Reviews Genetics*, 15, 22-33.
44. Mahmood T, Arshad M, Gill MI, Mahmood HT, Tahir M, Hussain S. 2003. Burewala strain of cotton leaf curl virus: A threat to CLCuV cotton resistant varieties. *Asian Journal of Plant Science*, 2(13), 968-970.
45. Mansoor S, Khan SH, Bashir A, Saeed M, Zafar Y, Malik KA, Briddon RW, Stanley J, Markham PG. 1999. Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology*, 259, 190-199.
46. Mansoor S, Briddon RW, Zafar Y, Stanley J, 2003a. Geminivirus disease complexes: an emerging threat. *Trends in Plant Science*, 8(3), 128-134.
47. Mansoor S, Amin I, Iram S, Hussain M, Zafar Y, Malik KA, Briddon RW. 2003b. Breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. *Plant Pathology*, 52(6), 784-784.
48. Martin CC, Sapienza C. 1999. A role for modifier genes in genome imprinting. *Genomic Imprinting*, 251-270. https://doi.org/10.1007/978-3-540-69111-2-12

49. Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW. 2003. Genome-wide analysis of NBS-LRR–encoding genes in Arabidopsis. *The Plant Cell,* 15(4), 809-834.
50. Michelmore RW, Meyers BC. 1998. Clusters of Resistance Genes in Plants Evolve by Divergent Selection and a Birth-and-Death Process. *Genome Research*, 8(11), 1113-1130.
51. Mizuno H, Katagiri S, Kanamori H, Mukai Y, Sasaki T, Matsumoto T, Wu J. 2020. Evolutionary dynamics and impacts of chromosome regions carrying R-gene clusters in rice. *Scientific Reports*, 10(1), 1-9.
52. Mushtaq R, Shahzad K, Mansoor S, Shah ZH, Alsamadany H, Mujtaba T, Al-Zahrani Y, Alzahrani HAS, Ahmed Z, Bashir A. 2018. Exploration of cotton leaf curl virus resistance genes and their screening in *Gossypium arboreum* by targeting resistance gene analogues. *AoB Plants*, 10: ply067. doi: 10.1093/aobpla/ply067
53. Nazeer W, Tipu AL, Ahmad S, Mahmood K, Mahmood A, et al. 2014. Evaluation of Cotton Leaf Curl Virus Resistance in BC1, BC2, and BC3 Progenies from an Interspecific Cross between *Gossypium arboreum* and *Gossypium hirsutum*. *PLoS ONE*, 9(11): e111861. doi:10.1371/journal.pone.0111861
54. Parniske M, Wulff BB, Bonnema G, Thomas CM, Jones DA, Jones JD. 1999. Homologues of the Cf-9 disease resistance gene (Hcr9s) are present at multiple loci on the short arm of tomato chromosome 1. *Molecular Plant-Microbe Interactions*, 12(2), 93-102.
55. Parrella G, Moretti A, Gognalons, Lesage ML, Marchoux G, Gebre-Selassie K, Caranta C. 2004. The Am gene controlling resistance to Alfalfa mosaic virus in tomato is located in the cluster of dominant resistance genes on chromosome 6. *Phytopathology*, 94, 345-350.
56. Pathak D, Rathore P, Gumber RK. 2009. Cytoplasmic Effects in Relation to Cotton Leaf Curl Disease Resistance in *Gossypium hirsutum* L. *IUP Journal of Genetics & Evolution*, 2(4), 31-35.
57. Pinheiro PV, de Faria JC, Nogueira EOPL, Aragão FJL. 2009. Transgene inheritances and genetic similarities of near isogenic lines of genetically modified common beans. *Pesquisa Agropecuária Brasileira*, 44(9),1168-1176.
58. Qadir R, Khan ZA, Monga D, Khan JA. 2019. Diversity and recombination analysis of Cotton leaf curl Multan virus: a highly emerging begomovirus in northern India. *BMC Genomics*, 20:274, 1-13. https://doi.org/10.1186/s12864-019-5640-2
59. Rahit KM, Tarailo-Graovac M. 2020. Review: Genetic Modifiers and Rare Mendelian Disease. *Genes*, 11, 239. doi:10.3390/genes11030239
60. Rahman H, 1997. Breeding Approaches to Cotton Leaf Curl Resistance. *The ICAC Recorder*, 15(1), 11-14.
61. Rahman M, Hussaina D, Malik TA, Zafar Y. 2005. Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum*. *Plant Pathology*, 54(6), 764-772. Doi: 10.1111/j.1365-3059.2005.01280.x
62. Randhawa RH. 1999. Inheritance of resistance mechanism to cotton leaf curl virus. Proceedings of ICAC-CCRI Regional Consultation Insecticide Resistance Management in Cotton. June 28 to July 1, 1999. Multan-Pakistan, 257-260.
63. Sackton TB, Hartl DL. 2016. Genotypic context and epistasis in individuals and populations. *Cell*, 166, 279-287.
64. Saeed H, Ehetisham-ul-Haq, M, Atiq M, Kamran M, Idrees M, Ali S, Burhan M, Mohsan, M, Iqbal M, Nazir S, Saleem I, Yasin S. 2018. Prediction of cotton leaf curl virus disease and its management through resistant germplasm and bio-products. *Archives of Phytopathology and Plant Protection*, 51(3-4), 170-186.
65. Shah SHJ, Paredes-Montero JR, Malik AH, Brown JK, Qazi J. 2020. Distribution of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) mitotypes in commercial cotton fields in the Punjab province of Pakistan. *Florida Entomologist*, 103(1), 41-47.
66. Ronald, P.C. 1998. Resistance gene evolution. Current opinion in plant biology, 1, 294-298.
67. Saleem H, Nahid N, Shakir S, Ijaz S, Murtaza G, Khan AA, Mubin M., Nawaz-ul-Rehman, MS. 2016. Diversity, mutation and recombination analysis of cotton leaf curl geminiviruses. *PLoS One*, 11(3): e0151161.
68. Samis K, Bowley S, McKersie B. 2002. Pyramiding Mn-superoxide dismutase transgenes to improve persistence and biomass production in alfalfa. *Journal of Experimental Botany*, 53(372), 1343-1350.
69. Sangwan R, Siwach S, Godara A, Nimbal S. 2015. Inheritance of resistance against cotton leaf curl virus disease in *Gossypium hirsutum* L. Book of Papers, 160.
70. Shah SHJ, Paredes-Montero JR, Malik AM, Brown JK, Qazi J. 2020. Distribution of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) mitotypes in commercial cotton fields in the Punjab province of Pakistan. *Florida Entomology*, 103(1), 41-47.
71. Shafiq M, Iqbal Z, Ali I, Abbas Q, Mansoor S, Briddon RW Amin I. 2017. Real-time quantitative PCR assay for the quantification of virus and satellites causing leaf curl disease in cotton in Pakistan. *Journal of Virological Methods*, 248, 54-60. https://doi.org/10.1016/j.jviromet.2017.05.012
72. Siddig MA 1968. Genetics of resistance to cotton leaf curl in Sakel cotton. *The Journal of Agricultural Science*, 70, 99-103.
73. Siddig MA. 1970. Breeding for leaf curl resistance in Sakel cotton. Cotton Growth in the Gezira Environment, 153-158.
74. Sun H, Guo Y, Lan X, Jia J, Cai X, Zhang G, Xie J, Liang Q, Li Y, Yu G 2020. PhenoModifier: a genetic modifier database for elucidating the genetic basis of human phenotypic variation. *Nucleic Acids Research*, 48, D977-D982.
75. Tarr SJA 1951. Leaf curl disease of cotton. 1st Ed: Commonwealth Mycol. Instit. Kew. UK, pp. 20-28.
76. Tsaballa A, Athanasiadis C, Pasentsis K, Ganopoulos I, Nianiou-Obeidat I, Tsaftaris A. 2013. Molecular studies of inheritable grafting induced changes in Pepper (*Capsicum annuum*) fruit shape. *Scientia Horticulturae*, 149, 2-8.
77. van Wersch S, Li X. 2019. Stronger when together: clustering of plant NLR disease resistance genes. *Trends in plant science*, 24(8), 688-699.
78. Vyas, M., Raza, A., Ali, M.Y., Ashraf, M. A., Mansoor, S., Shahid, A. A., Brown, J. K. (2017). Knock down of whitefly gut gene expression and mortality by orally delivered gut gene-specific dsRNAs. *PloS One*, 12(1): e0168921.
79. Weatherall DJ 2001. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nature reviews genetics*, 2(4), 245-255. doi:10.1038/35066048
80. Williams EG, Auwerx J. 2015. The convergence of systems and reductionist approaches in complex trait analysis. *Cell*, 162(1), 23-32.
81. Wilson FD, Brown JK 1991. Inheritance of response to cotton leaf crumple virus infection in cotton. *Journal of Heredity*, 82, 508-509.
82. Wright S. 1988. Surfaces of selective value revisited. *The American Naturalist*, 131, 115-123.
83. Wright, Steven D., Ron N. Vargas, M. R. Jimenez Jr, M. P. Keeley, and R. Delgado. "Annual morningglory (Ipomea spp.) control in cotton." In Proc. Beltwide Cotton Conf, p. 795. 1997

84. Zaidi SSA, Iqbal Z, Amin I. Mansoor S. 2015. First report of Tomato leaf curl Gujarat virus, a bipartite begomovirus on cotton showing leaf curl symptoms in Pakistan. *Plant Disease*, 9(11), 1655.
85. Zaidi SS, Shafiq M, Amin I, Scheffler BE, Scheffler, JA, Briddon RW. Mansoor S. 2016. Frequent occurrence of Tomato leaf curl New Delhi virus in cotton leaf curl disease affected cotton in Pakistan. *PLoS ONE*, 11(5): e0155520. doi:10.1371/journal. pone.0155520
86. Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, van Oss H, Kedar N, Rabinowitch HD, Czosnek H. 1994. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, TY-1. *Theoretical and Applied Genetics*, 88, 141-146.
87. Zubair M, Zaidi SS, Shakir S, Amin I, Mansoor S. 2017a. An insight into Cotton leaf curl Multan betasatellite, the most important component of cotton leaf curl disease complex. *Viruses*, 9(10), 280.
88. Zubair M, Zaidi SS, Shakir S, Farooq M, Amin I, Scheffler JA, Scheffler BE 2017b. Multiple begomoviruses found associated with cotton leaf curl disease in Pakistan in early 1990 are back in cultivated cotton. *Scientific reports*, 7(1), 1-11.
89. Zhou X, Liu Y, Robinson DJ, Harrison BD 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. *Journal of General Virology*, 79(4), 915-923.

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Bioinformatics Analysis and Expression Profiling of *F3'h* and *F3'5'h* Genes in Cotton (*Gossypium*) Genomes

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Abstract

Background: Cotton (*Gossypium*)fiber is an elongated single-cell seed trichome that develops from the outer protective protodermal cells of the cotton seed coat. Flavonoids are a diverse group of secondary metabolites, that are commonly found in nature and their biosynthesis pathway modulates pigmentation in flowers and regulates cotton fiber development.

Results: To canvass genomic prospective of the flavonoid pathway in cotton fiber development, *in silico* analysis, was conducted. Seven *F3'H* and four *F3'5'H* peptide sequences from the Swissprot database were used as queries to search for putative homologue genes in four *Gossypium* genomes *G. raimondii* (D_5), *G. arboretum* (A_2), *G. barbadense* $(AD)_2$ and *G. hirsutum* $(AD)_1$ genome. Overall, 36 candidate genes were identified in four *Gossypium* genomes where 7 and 5 in two diploid cottons (*G. arboreum* and *G. raimondii*), while 11 and 13 were identified in two cultivated tetraploids (*G. hirsutum* and *G. barbadense*). A comprehensive annotation framework predicted that about seven genes could act to perform CYP75A (*F3'5'H*) metabolic function while the remaining 29 were like CYP75B which were predicted to perform *F3'H* activity in *Gossypium* species. Phylogenetic analysis showed that genes associated with two different isoform categories were distinctly clustered i.e., CYP75A (*F3'5'H*) and CYP75B (*F3'H*). To reveal *in silico* expression profiles of *F3'H* and *F3'5'H* candidate genes in two diploids and two tetraploid cotton genomes, RNA-seq datasets were accessed from the SRA database (NCBI) and digital expression values were determined. In addition, the expression of putative *F3'H* and *F3'5'H* gene families were compared in different vegetative and fiber tissues from two cultivated tetraploids using their standard varieties i.e., YZ1 and Pima 3-79.

Conclusion: In this study, both digital expression profiles and qRT-PCR analysis suggested that *F3'H* and *F3'5'H* candidate genes were differentially expressed in ovule and fiber tissues between the two cultivated tetraploids. This study comprehended the flavonoid-driven mechanism of fiber development in cotton.

Keywords: Cotton, *Gossypium* species, Flavonoid, Pigmentation, Fiber

Background

Flavonoids are a diverse group of secondary metabolites that are commonly found in nature and play various roles which assist plants in maintaining growth and functions (Quideau 2006; Meng et al 2015). They have been identified as biological regulators in various crop plants which provide resistance to pathogen infections (Zakaryan et al 2017). However, the most visible impact of flavonoids is their potential in regulating pigmentation in flowers, fruits, and seeds (Kumar and Pandey 2013). The flavonoid biosynthesis pathway leading to anthocyanin biosynthesis, which modulates pigmentation in flowers, is well conserved in plants (Tanaka and Brugliera 2013).

Flavonoids performed multiple biological reactions which could ultimately modulate the biosynthesis of anthocyanins, flavanols, and proanthocynadinis, and different enzymes and genes interact for flavonoids biosynthesis (Nakatsuka et al 2008). Different light conditions like intensity, quality, direction, and photoperiod effects the flavonoid biosynthesis in different fruit-bearing plants, flavonoids mainly interact with light quality. The flavonoid pathway was differentially regulated between different tissues and species and regulated the PAs composition in various plant species (Zhao et al 2010). In various plants, flavonoid biosynthetic pathway has been explored to enhance color pigmentation mechanism in flowers (Davies and Schwinn 2005). Recent research indicated that *F3'H* and *F3'5'H* were responsible for color pigmentation in *Chrysanthemum* flower (He et al 2013).

Flavonoids were regulated differentially in different cotton species (Taylor and Grotewold 2005), while flavonoids were also employed to reveal phylogenetic relationships among the *Gossypium* species. It could be plausible since flavonoid pathway genes were highly expressed in cotton fiber cells (Gou et al 2007). In fiber tissues, flavonoid genes were more preferentially expressed during the fiber elongation phase of fiber development (Rapp et al 2010). According to a recent review, 52 flavonoids of seven different classes have been identified in the cotton plant (Nix et al 2017). These flavonoids are widely distributed in the cotton plant and could regulate pigmentation and development mechanisms and the growing cotton fiber. Moreover, flavonoids were also recognized to act as biological regulators responsible for leaf reddening and modulating stress mechanisms in the cotton plant (Edreva et al 2006). Some studies have also hinted that flavonoids could also be decisively involved during anther development in cotton (Ma et al 2012).

The transcriptome datasets have hinted at the putative involvement of flavonoids in fiber development since various flavonoid biosynthesis genes were found differentially expressed in developing fiber (Naoumkina et al 2015). Similarly, comparative transcriptome and metabolome analysis revealed that flavonoids were spatially abundant in elongating fiber of *G. barbadense*, cultivated tetraploid cotton which produces high-quality fiber (Al-Ghazi et al 2009; Chaudhary et al 2009).

Flavonoids are considered crucial in modulating pigmentation in naturally colored cotton fiber and, recently many studies have explored the genetic and metabolic basis of color fiber through modern-day biotechnological tools. A study reported that upstream flavonoid biosynthesis pathway genes (*CHS*, *F3'H,* and *F3'5'H*) were differentially regulated in brown fiber (Feng et al 2013). Moreover, the critical role of flavonoids during fiber development remained obscure until a study demonstrated that silencing *F3H* suppressed fiber elongation since NAR was accumulated in developing fiber (Tan et al 2013a). Whereas, the flavonoid biosynthetic pathway mainly contributed to the synthesis of flavan-3-ols and flavan-3, 4-diols units. Similarly, another study also reported that flavonoids were majorly responsible for pigmentation in brown cotton fiber (Feng et al 2013).

Various studies demonstrated that proanthocyanidins (PAs) were found to be responsible for pigmentation in brown cotton fiber (Hua et al 2009; Li et al 2013). PAs are synthesized by oligomer chains of polyhydroxyflavan-3-ol units. Chemically, flavan-3, 4-diol, leucoanthocynadins, molecule are presumably added to initiating and terminal ends of flavan-3-ol units to form PAs polymers (He et al 2008).

A combined transcriptomic and metabolic analysis showed that proanthocyanidins (PAs) like gallocatechin and catechin were abundantly present in brown fiber because flavonoid biosynthesis pathway genes were overexpressed (Xiao et al 2014). Another study reported that *TT2-type MYB* transcription factor modulated proanthocyanidin biosynthesis by interacting with promoter elements to enhance the transcriptional activity of two downstream flavonoid biosynthesis genes *ANR* and *LAR* (Lu et al 2017). While overexpressing *GhTT2-3A* during secondary cell wall synthesis resulted in brown cotton fiber (Yan et al 2018). Although flavonoid biosynthesis-derived molecular circuitry of pigmented fiber is well established in cotton, engineering flavonoid biosynthesis pathway for the production of higher quality cotton fiber is not well exploited.

Flavonoids' metabolic process also affected fiber development and particularly played a crucial role during the fiber elongation stage. During fiber development stages, flavonoid genes were found differentially expressed between wild and cultivated cotton. Moreover, expression profiling of flavonoid genes in *G. barbadense* (Pima 3-79) and *G. hirsutum* (YZ1) at different fiber developmental stages revealed that the flavonoid metabolic pathway upstream genes like chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavonoid-3'-hydroxylase (*F3'H*) and flavonoid-3', 5'-hydroxylase (*F3'5'H*) were preferentially upregulated in YZ1 at 5 DPA as compared to 3-79 (Tan et al 2013a). Whereas, transcripts of the downstream genes like flavonoid-3-hydroxylase (*F3H*), dihydro-flavonol reductase (*DFR*), and anthocyanidins reductase (*ANR*) have more abundantly prevailed in Pima 3-79. Furthermore, functional analysis of *F3H* gene and cotton ovule culture results indicated that flavonoid naringenin (NAR) and dihydro-kaempferol (DHK) were negatively associated with fiber development (Tan et al 2013a). Color variation in cotton flower petals was also shown to be modulated by flavonoid biosynthesis. Interruptions in flavonoid biosynthesis due to silencing *F3H* (*TT6*) in cotton blocked flavonoid biosynthesis which changed flower petal color from cream to white owing to lowered anthocyanin content (Tan et al 2013b).

Results

Bioinformatics analysis of F3'H and F3'5'H genes in cotton genomes

Flavonoid NAR negatively regulated fiber development but ERI was positively associated with fiber elongation. In the flavonoid metabolic pathway, *F3'H* is involved in catalyzing NAR to ERI and *F3'5'H* catalyzes the metabolic conversion from ERI to DHT. Therefore, the identification of putative *F3'H* and *F3'5'H* candidate genes in cotton genomes could be a valuable resource.

To determine putative candidate CYP75 isoforms (A & B) genes in cotton genomes, four *F3'H* and seven *F3'5'H* peptides from the SwissProt database were used as queries to 'blastx' four *Gossypium* genomes and other plant genomes (Table 1). Thereafter, an integrated annotation framework was adopted to predict putative candidate *F3'H* and *F3'5'H* genes in cotton genomes. All these proteins primarily contained 'cytochrome P450' motif (Fig. 1). Overall, 36 candidate genes were identified in *Gossypium* genomes where 7 and 5 in two diploid cottons (*G. arboreum* and *G. raimondii*), while 11 and 13 were identified in two cultivated tetraploids (G. *hirsutum* and *G. barbadense*). All these genes were randomly distributed on different chromosomes, while some were also located on chromosome-anchored scaffolds. The genomic locations of all genes are presented in Table 2.

To determine the isoform category of these genes, transcripts and peptides were extracted from respected genome assemblies. A comprehensive annotation framework aided in validation and categorization into one of two CYP75 isoforms. The results suggested that about 7 genes were predicted to act as CYP75A (*F3'5'H*) metabolic functions while the remaining 29 were like CYP75B which were predicted to act as *F3'H* (Table 2.3). The peptide length of these proteins varied from 233 to 635 amino acids. Un-rooted leaf of all CYP75 genes from cotton and other plant genomes distinctly categorized into two isoforms types (Fig. 2A). Both isoforms were indicated as red (CYP75B) and blue (CYP75A) lines, while putative CYP75 genes identified in other plant genomes.

Overall, fewer *F3'5'H* genes were identified in four cotton genomes (Fig. 2B). The results showed that genes associated with two different isoform categories were distinctly clustered (Fig. 3). Eleven Swissprot entries were also categorized according to isoform category. i.e., CYP75A (*F3'5'H*) and CYP75B (*F3'H*). CYP75Bs were further categorized into different sub-clusters. Overall, these results hinted at the robustness of the annotation framework and structural diversity among two CYP75 isoforms conserved in *Gossypium* genomes.

Phylogenetic analysis of F3'H and F3'5'H genes in cotton genomes

To understand evolutionary footprints for two isoforms among putative *F3'H* and *F3'5'H* genes in *Gossypium* lineage, we build a neighbor-joining phylogenetic tree using 36 putative *F3'H* and *F3'5'H* genes from four cotton genomes and eleven *F3'H* and *F3'5'H* protein extracted from Swissprot database (https://www.uniprot.org/).

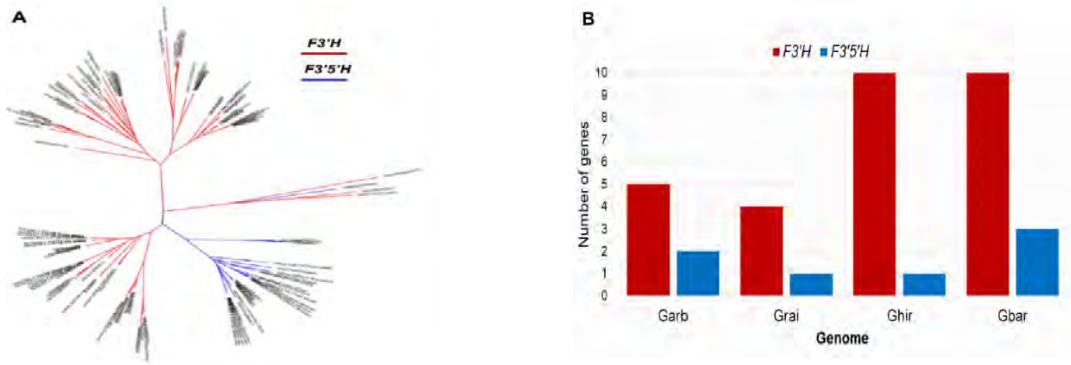

Figure 2 *Identification and phylogenetic analysis of putative CYP75 genes. (A) Un-rooted leaf tree showing orthology for two isoforms of predicted CYP75 genes among cotton and other plant genomes. (B) Bar plot showing the number of F3'H and F3'5'H genes identified in four cotton genomes [G. arboreum (Garb), G. raimondii (Grai), G. hirsutum (Ghir) and G. barbadense (Gbar)]*

Structural and motif analysis of F3'H and F3'5'H proteins

To determine structural similarities and track down evolutionary relationships between two isoforms, phylogenetic tree and motif analysis was performed for *F3'H* and *F3'5'H* family genes in two cultivated tetraploids. In the *G. hirsutum* genome, there was only one *F3'5'H* isoform (*Gh_D07G1197*) was detected which was more distant from*F3'H* in phylogenetic analysis (Fig. 4A). Similarly, motif analysis also revealed that the motif pattern of *Gh_D07G1197* was not similar to remaining F3'H and *F3'5'H* gene family members (Fig. 4B). Comparatively single shorter one intron was placed between two exons, while varied intron number and intron length was observed in putative *F3'H* genes (Fig. 4C). All these results indicated the robustness of integrated annotation framework implemented in this study. In another cultivated tetraploid (*G. barbadense*), there were three *F3'5'H* genes were predicted which were distinctly clustered from the remaining 10 in neighbor-joining analysis (Fig. 5A). Motif sequences were also distinctively organized in these proteins (*GOBAR_AA27616*, *GOBAR_AA08241,* and *GOBAR_DD30684*) (Fig. 5B).

Synteny analysis of F3'H and F3'5'H gene families

The evolutionary footprints and syntenic relationships of putative *F3'H* and *F3'5'H* genes among cultivated allotetraploid (*G. hirsutum*) and its two progenitor diploid species (*G. arboreum* and *G. raimondii*) were ascertained and visualized through circos plot (Fig. 6). As like in *G. hirsutum*, all putative *F3'5'H* genes characteristically exhibited fewer and shorter introns between exons (Fig. 5C). Overall, the two candidate genes associated to two *F3'H* and *F3'5'H* demonstrated distinct features in two tetraploid cotton genomes.

The results showed that putative *F3'H* and *F3'5'H* genes were distributed on nine different chromosomes (GhA06, GhA10, GhA11, GhA12, GhD06, GhD07, GhD10, GhD11, and GhD12) of cultivated tetraploid cotton genomes. In diploid cotton genomes, these genes were located on 4 (Ga01, Ga04, Ga06, and Ga09) and 5 (Gr01, Gr07, Gr08, Gr10, and Gr11) chromosomes. The genes of each duplicated pair associated with a distinct isoform category showed greater similarity to each other. Overall, all these genes were unevenly distributed between sub-genomes At, Dt, and diploids A_2 and D_5.

Table 1 The chromosomes and genomic locations of 36 putative F3'H and F3'5'H genes identified in four species of cotton

No.	Gene	Genome	Chromosome	Start	End
1	*Cotton_A_23720*	*G. arboreum*	Ga01	108822620	108824232
2	*Cotton_A_23722*	*G. arboreum*	Ga01	108793810	108795422
3	*Cotton_A_25162*	*G. arboreum*	Ga04	75734220	75736440
4	*Cotton_A_13353*	*G. arboreum*	Ga06	99112051	99115963
5	*Cotton_A_02976*	*G. arboreum*	Ga09	72863253	72865756
6	*Cotton_A_08518*	*G. arboreum*	Ga09	65122755	65125375
7	*Cotton_A_08519*	*G. arboreum*	Ga09	65113267	65115387
8	*Gh_A06G0689*	*G. hirsutum*	GhA06	19064603	19066823
9	*Gh_A10G0500*	*G. hirsutum*	GhA10	5439770	5441885
10	*Gh_A10G0501*	*G. hirsutum*	GhA10	5449260	5451340
11	*Gh_A11G1242*	*G. hirsutum*	GhA11	15479598	15482101
12	*Gh_A12G2650*	*G. hirsutum*	scaffold3395_A12	91821	96195
13	*Gh_D06G0790*	*G. hirsutum*	GhD06	13988132	13990221
14	*Gh_D07G1197*	*G. hirsutum*	GhD07	18211921	18213582
15	*Gh_D10G2598*	*G. hirsutum*	scaffold4479_D10	130288	132654
16	*Gh_D10G2599*	*G. hirsutum*	scaffold4479_D10	136143	138245
17	*Gh_D11G1389*	*G. hirsutum*	GhD11	13713136	13715613
18	*Gh_D12G1798*	*G. hirsutum*	GhD12	50488826	50492854
19	*GOBAR_AA08241*	*G. barbadence*	GbA07	23849201	23849899
20	*GOBAR_AA27616*	*G. barbadence*	GbA07	24010265	24011877
21	*GOBAR_AA04788*	*G. barbadence*	GbA10	6822244	6824855
22	*GOBAR_AA37751*	*G. barbadence*	GbA10	6802064	6804182

23	*GOBAR_AA25294*	*G. barbadence*	GbA11	15390211	15392718
24	*GOBAR_AA38922*	*G. barbadence*	GbA12	92739644	92745082
25	*GOBAR_DD05570*	*G. barbadence*	GbD06	14209054	14211143
26	*GOBAR_DD30684*	*G. barbadence*	GbD07	19656201	19657814
27	*GOBAR_DD25137*	*G. barbadence*	GbD10	5529716	5534459
28	*GOBAR_DD25138*	*G. barbadence*	GbD10	5537950	5540315
29	*GOBAR_DD10583*	*G. barbadence*	GbD11	12902531	12905068
30	*GOBAR_DD14889*	*G. barbadence*	GbD12	48065605	48069626
31	*GOBAR_AA00808*	*G. barbadence*	Gbscaffold_0002	256499	258719
32	*Gorai.001G134900*	*G. raimondii*	Gr01	17605306	17607373
33	*Gorai.007G151200*	*G. raimondii*	Gr07	12962565	12965526
34	*Gorai.008G198200*	*G. raimondii*	Gr08	48277406	48281826
35	*Gorai.010G088700*	*G. raimondii*	Gr10	13565665	13568062
36	*Gorai.011G060100*	*G. raimondii*	Gr11	4866114	4874265

Expression profiling F3'H and F3'5'H genes

To reveal expression profiles of *F3'H* and *F3'5'H* family genes in two diploids and two tetraploid cotton genomes, RNA-seq datasets were accessed from SRA database (NCBI) for four cotton genomes and digital expression values (FPKM) were determined by mapping counting mapped reads to respective genes. The ascertained FPKM values were plotted as a heatmap to visualize digital profiles in different vegetative (Leaf, petal, stem, stigma, and anther) and fiber tissues (ovules and fiber). The results showed that various genes showed a differential pattern in fiber tissues for two diploid cotton species. Some genes from *F3'H* category (*Gorai.008G198200*, *Gorai.007G151200*, *Cotton_A_02976,* and *Cotton_A_13353*) and CYP75A (*Gorai.001G134900*) were found to up-regulated at least one ovule and fiber tissues (Fig. 7). Similarly, both *F3'H* and *F3'5'H* genes were also differentially expressed in tissues of two cultivated cotton genomes. In *G. hirsutum*, genes like *Gh_A10G0501*, *Gh_D10G2598*, *Gh_A11G1242*, *Gh_D11G1389,* and *Gh_D10G2599* were up-regulated in 20 DPA fiber (Fig. 8A).

Table 2 Characteristics and putative functions of candidate F3'H and F3'5'H proteins detected in four cotton genomes

No.	Gene	Transcript	Peptide	Isoform	Annotation
1	*Cotton_A_23720*	1612	511	CYP75A	Flavonoid 3'5'-hydroxylase
2	*Cotton_A_23722*	1612	511	CYP75A	Flavonoid 3'5'-hydroxylase
3	*Cotton_A_25162*	2220	518	CYP75B	flavonoid 3'-monooxygenase-like
4	*Cotton_A_13353*	3912	510	CYP75B	flavonoid 3'-monooxygenase-like
5	*Cotton_A_02976*	2503	512	CYP75B	flavonoid 3'-monooxygenase-like
6	*Cotton_A_08518*	2620	511	CYP75B	flavonoid 3'-monooxygenase-like
7	*Cotton_A_08519*	2120	511	CYP75B	flavonoid 3'-monooxygenase-like
8	*Gh_A06G0689*	2220	518	CYP75B	flavonoid 3'-monooxygenase-like
9	*Gh_A10G0500*	2115	511	CYP75B	flavonoid 3'-monooxygenase-like
10	*Gh_A10G0501*	2080	502	CYP75B	flavonoid 3'-monooxygenase-like
11	*Gh_A11G1242*	2503	512	CYP75B	flavonoid 3'-monooxygenase-like
12	*Gh_A12G2650*	4374	511	CYP75B	flavonoid 3'-monooxygenase
13	*Gh_D06G0790*	2089	518	CYP75B	flavonoid 3'-monooxygenase-like
14	*Gh_D07G1197*	1661	510	CYP75A	Flavonoid 3'5'-hydroxylase
15	*Gh_D10G2598*	2366	511	CYP75B	flavonoid 3'-monooxygenase-like
16	*Gh_D10G2599*	2102	511	CYP75B	flavonoid 3'-monooxygenase-like
17	*Gh_D11G1389*	2477	512	CYP75B	flavonoid 3'-monooxygenase-like
18	*Gh_D12G1798*	4028	511	CYP75B	flavonoid 3'-monooxygenase
19	*GOBAR_AA08241*	698	233	CYP75A	Flavonoid 3'5'-hydroxylase
20	*GOBAR_AA27616*	1612	511	CYP75A	Flavonoid 3'5'-hydroxylase
21	*GOBAR_AA04788*	2611	499	CYP75B	flavonoid 3'-monooxygenase-like
22	*GOBAR_AA37751*	2118	511	CYP75B	flavonoid 3'-monooxygenase-like
23	*GOBAR_AA25294*	2507	512	CYP75B	flavonoid 3'-monooxygenase-like
24	*GOBAR_AA38922*	5438	431	CYP75B	flavonoid 3'-monooxygenase
25	*GOBAR_DD05570*	2089	489	CYP75B	flavonoid 3'-monooxygenase-like
26	*GOBAR_DD30684*	1613	510	CYP75A	Flavonoid 3'5'-hydroxylase
27	*GOBAR_DD25137*	4743	635	CYP75B	flavonoid 3'-monooxygenase-like
28	*GOBAR_DD25138*	2365	511	CYP75B	flavonoid 3'-monooxygenase-like
29	*GOBAR_DD10583*	2537	510	CYP75B	flavonoid 3'-monooxygenase-like

30	*GOBAR_DD14889*	4021	511	CYP75B	flavonoid 3'-monooxygenase
31	*GOBAR_AA00808*	2220	518	CYP75B	flavonoid 3'-monooxygenase-like
32	*Gorai.001G134900*	2067	510	CYP75A	Flavonoid 3'5'-hydroxylase
33	*Gorai.007G151200*	2961	512	CYP75B	flavonoid 3'-monooxygenase-like
34	*Gorai.008G198200*	4420	511	CYP75B	flavonoid 3'-monooxygenase
35	*Gorai.010G088700*	2397	517	CYP75B	flavonoid 3'-monooxygenase-like
36	*Gorai.011G060100*	8151	511	CYP75B	flavonoid 3'-monooxygenase-like

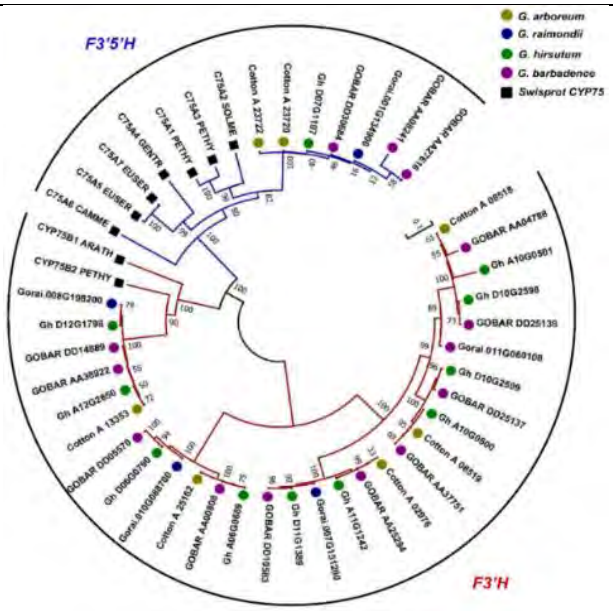

Figure 3 *Neighbor-joining phylogenetic tree of F3'H and F3'5'H protein was generated with protein sequences from four cotton genomes along with eleven F3'H and F3'5'H proteins from Swissprot.*

While the only *F3'5'H* gene (*Gh_D07G1197)* was highly expressed in 10 DPA ovules. Whereas in *G. barbadense*, 7 out of 13 genes were expressed in at least one fiber tissue (Fig. 8B). Moreover, the three putative *F3'5'H* genes (*GOBAR_AA27616*, *GOBAR_AA08241* and *GOBAR_DD30684*) were up-regulated in 0 and 10 DPA tissues. All these results showed that CYP75 gene family (both *F3'H* and *F3'5'H*) were differentially regulated during fiber development stages. Perhaps, *F3'H* and *F3'5'H* genes have a potential role in modulating fiber development in cultivated cotton.

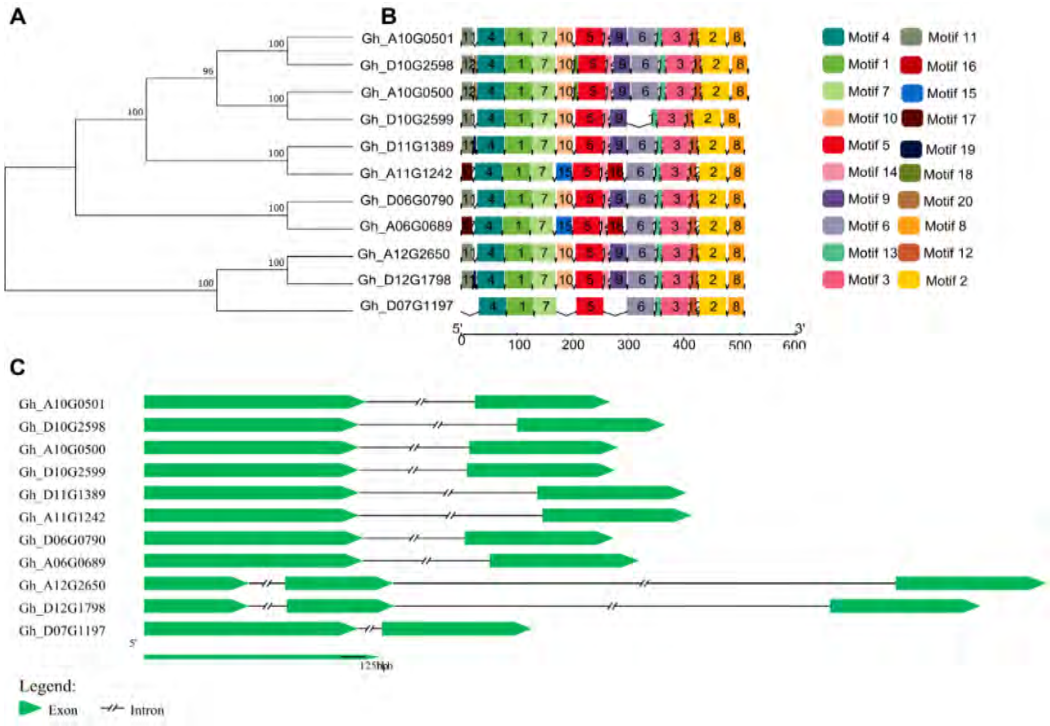

Figure 4 *Structural and motif analysis of F3'H and F3'5'H candidate genes in cultivated cotton G. hirsutum (A) Neighbor-joining phylogenetic tree was constructed among F3'H and F3'5'H genes in G. hirsutum with MEGA7. (B) Twenty distinct motifs were detected with MEME suite and represented with a different color. (C) The gene structure was proposed using a GSDS server with relative numbers and lengths for introns and exons.*

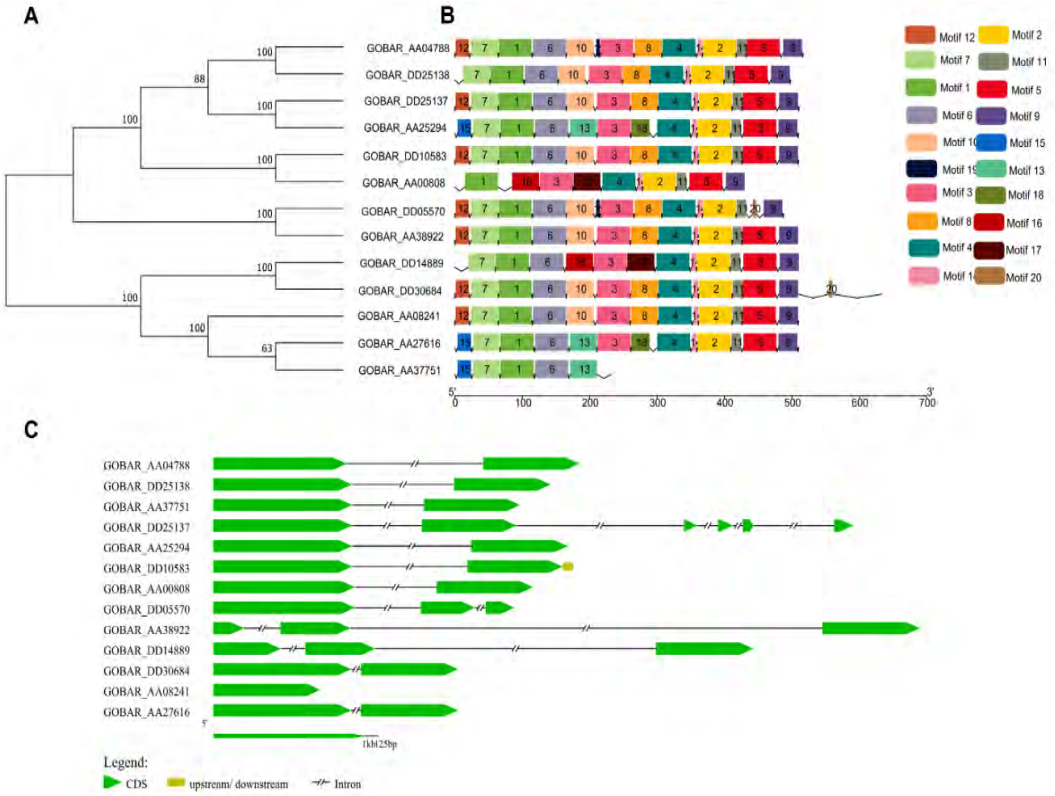

Figure 5 *Structural and motif analysis of F3'H and F3'5'H candidate genes in G. barbadense. (A) A neighbor-joining phylogenetic tree was constructed among F3'H and F3'5'H genes in G. barbadense with MEGA7. (B) Twenty*

distinct motifs were detected0 with MEME suite and represented with a different color. (C) The gene structure was proposed using a GSDS server with relative numbers and lengths for introns and exons.

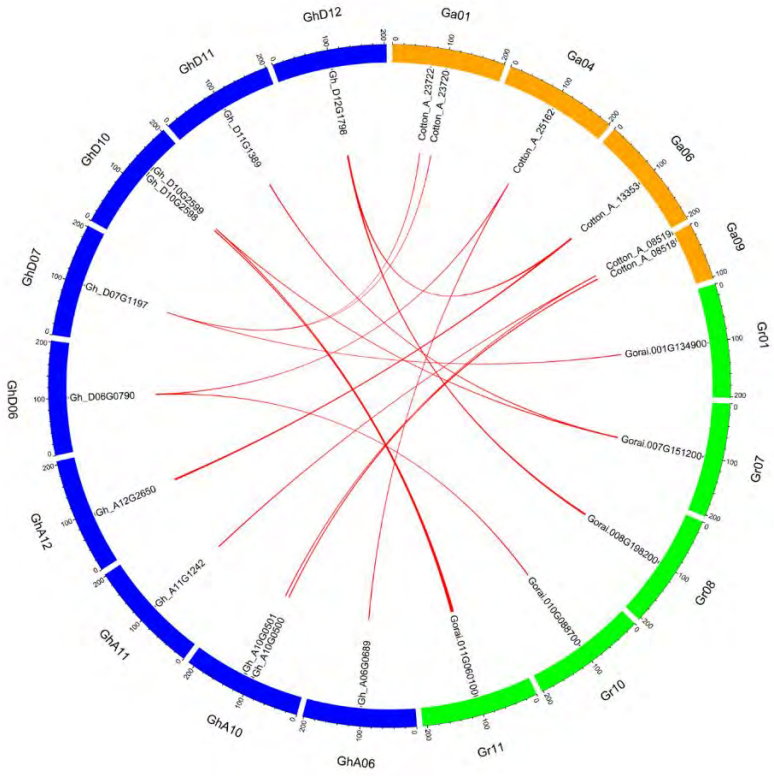

Figure 6 *Syntenic relationships among F3'H and F3'5'H genes from two progenitor cotton diploids (G. arboreum, G. raimondii) and one cultivated allotetraploid (G. hirsutum) cotton. Gene orthology relations were visualized by a circos plot*

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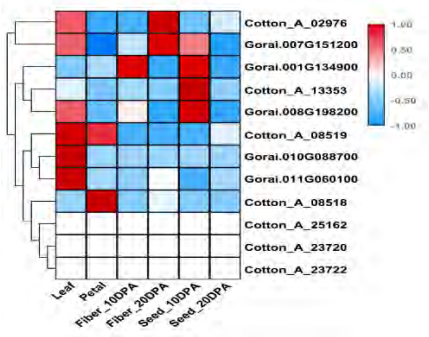

Figure 7 *Digital expression profiles of F3'H and F3'5'H genes in different tissues of two diploid cotton genomes (G. arboreum and G. raimondii). The transcriptomic data was accessed from SRA database (NCBI) and FPKM values were used to draw heatmap.*

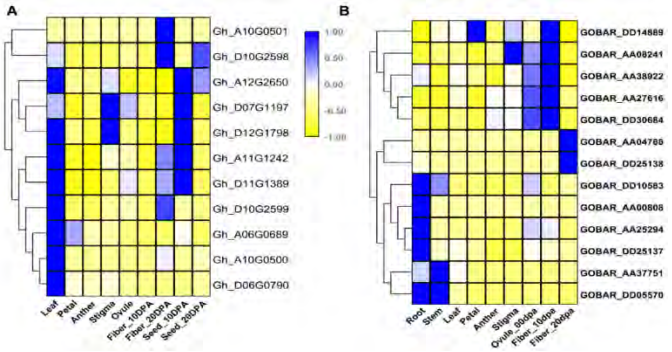

Figure 8 *Digital expression profiles of F3'H and F3'5'H genes in two cultivated tetraploid cotton genomes (G. hirsutum and G. barbadense) in different tissues. The transcriptomic data was accessed from SRA database (NCBI) and FPKM values were used to draw heatmap.*

Discussion

Cytochrome P450 is a very large and diverse protein family abundant in almost all kinds of life on earth, from simpler plants to higher animals like a human. Although the spectrum of enzymatic activity and substrate of P450 enzymes is quite diverse and it might be a prolix review of literature for clear

understanding, some general functionalities can be outlined e.g., P450 are heme-thiolate enzymes that catalyze diverse reactions based on activation or heterolytic cleavage of oxygen (Ayabe and Akashi 2006). Moreover, a conserved cysteine is required for their optimal activity which serves as a ligand to heme iron as a co-factor. *F3'H* and *F3'5'H* gene belongs to CYP75B sub-family and CYP75A of p450 proteins and is primarily involved in oxidation-reduction of secondary metabolites, and flavonoids in specific. Cotton *F3'H* and *F3'5'H* genes are speculated to be involved in fiber development by regulating flavonoid metabolic pathway.

In the era of rapid genome sequencing, the study of gene-evolving models in multiple closely related genomes could be helpful for the genetic manipulation of complex cotton plants for economic purposes, as cotton fiber is a major economic product of cotton. Evidence of consistent and prominent evolution of *F3'H* and *F3'5'H* genes can be investigated in recently sequenced diploid (D_5 and A_2) and cultivated allotetraploid AD_1 and AD_2 genomes. Moreover, deep evolutionary insights into the mechanism of gene speciation and duplication of genes involved in fiber development mechanism will provide a handful of tools that can be implemented to enhance our understanding of fiber growth stages in cotton.

The comprehensive analysis of digital expression profiles of *F3'H and F3'5'H* family genes could support us to uncover their potential and metabolic functions that might be involved in different development and growth processes in cotton. Previously, *F3H* gene expression was found related to NAR and DHK concentration in cotton (Tan et al., 2013a), NAR is a substrate for *the F3H* gene and is conveniently modulated by higher levels of its transcript. However, up-regulation of *F3H* was not found positively associated with fiber length and other fiber quality parameters. Therefore, we could speculate that there might be other genes that could further modulate higher NAR content, either completely or fractionally.

As outlined in the cotton flavonoid metabolic pathway, *F3H* and *F3'H* share a common substrate NAR and could be significantly involved in fiber development. Thus, the development of the *F3'H* transgene line will substantiate our knowledge of flavonoid metabolism which further enhances opportunities for developing elite cotton lines with superior fiber properties.

Materials and Methods

Sequence retrieval of F3'H and F3'5'H proteins

Initially, *F3'H* and *F3'5'H* gene sequences were retrieved from the Uniprot database (http://www.uniprot.org/). Four *F3'5'H* and seven *F3'H* genes were found, whose amino acid sequences were downloaded for further analysis. NCBI non-redundant protein sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was searched through "blastx" for highly similar CYP75 candidates from *G. raimondii* (Paterson et al 2012), *G. arboreum* (Li et al 2014), *G. barbadense* (Liu et al 2015) and *G. hirsutum* (Zhang et al 2015) genomes of cotton.

NCBI-BLAST 2.2.28+ package was downloaded from NCBI portal (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/LATEST) and configured to conduct custom BLAST on windows. Genome assembly, transcript, annotations, and protein databases for two diploid and two tetraploid cotton genomes were downloaded and integrated into NCBI-BLAST 2.2.28+. Protein sequences of all *F3'H* and *F3'5'H* were used as queries to search putative candidate genes in *Gossypium* lineage (expected value $\leq 10^{-5}$; identity $\geq$ 50 %) and was determined through "blastp" and "blastx".

Protein domain, motif, annotation, and GO analysis

All retrieved sequences from *Gossypium* and other plant species were submitted to the InterProScan web tool (www.ebi.ac.uk/Tools/pfa/iprscan5/) for protein domains and motif analysis. Cytochrome P450 superfamily protein domain in PFAM database (accession number PF00067) was confirmed for each entry and the sequences which did not match with PF00067 (cytochrome P450) domain were discarded. Subsequently, the remaining entries were inspected manually for PANTHER (www.pantherdb.org) and KOG (genome.jgi.doe.gov) annotations and mismatched entries were deleted. Thereafter, BastKOALA web server (http://www.kegg.jp/blastkoala/) was used to find KEGG annotations of remaining sequences i.e., gene ontology (GO terms), enzyme commission (EC), and pathways (K number) annotations specific only to *F3'H* and *F3'5'H* proteins. Finally, the functional annotations were determined through orthology-based clustering algorithms integrated into the eggNOG mapper (Huerta-Cepas et al 2016). Thus, the identification of putative *F3'H* and *F3'5'H* candidates in plant genomes requires integrating advanced in-silico-based sequence analysis strategies and up-to-date knowledge

of protein family annotation databases along with sophisticated experimental techniques, which would help to obviate false gene identification.

Sequences retrieval putative F3'H and F3'5'H candidates from other plant species

NCBI-BLAST 2.2.28+ package was also used to extract closely related *F3'H* and *F3'5'H* genes in other plant species of diverse origin e.g., poplar (*Poplus trichocarpa*) from Malvidae, cocoa (*Theobroma cocoa*) from Malvales, Brassica (*B. rappa*) from Brassicaceae, soybean (*Glycine max*) from Fabidae and *Solanum lycopersicum* were also considered for phylogenetic analysis. All retrieved sequences were curated manually and duplicates were removed immediately. All BLAST hits from four cotton genomes were retrieved and subsequently used for manual and automated annotation prediction framed work as presented in Fig.1.

Phylogenetic tree and micro synteny analysis for F3'H and F3'5'H gene family

Multiple sequence alignment (MSA) was performed using DNAMAN version 8.0 (http://www.lynnon.com/) using default parameters and gap penalties. MEGA 6.0 package was used to construct phylogenetic tree by adopting Neighbor-joining algorithms using predicted amino acid sequences. To identify genes that were paralogous or orthologous among three cotton species, all-versus-all BLASTP was performed, with e value $1e^{-5}$ comparison of all protein sequences. The gene duplication type was identified by MCScanX with the default setting (Wang et al 2012). OrthoMCL (Fischer et al 2011) was used to identify genes that were homologous among three cotton species. The relationships between orthologous and paralogous genes in and between three cotton species were plotted using the Circos program (Yu et al 2018).

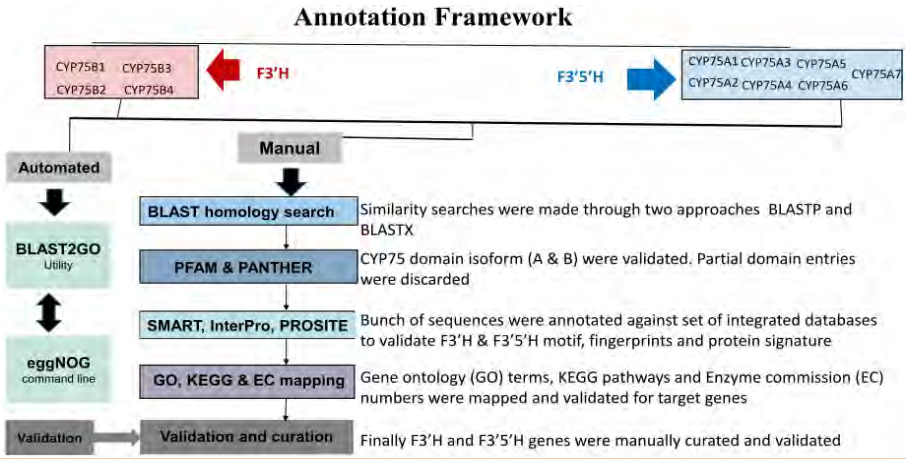

Figure 9 *Integrated annotation workflow employed for identification of CYP75Bs (F3'H) and CYP75As (F3'5'H) genes in four Gossypium species.*

Digital expression profiling of F3'H and F3'5'H candidate genes

The high-throughput RNA-sequencing data were downloaded from Short Read Archive (SRA) database under National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sra). The SRA data was downloaded for four cotton genomes (Li et al 2014; Zhang et al 2015). The cleaned RNA-seq reads were mapped to the reference cotton genome assemblies with TopHat2 (Kim et al 2013). The set of files containing mapped reads from TopHat2 was sorted and indexed using Samtools (Li et al 2009). The overlap of reads with genes was counted using HTseq-count (Anders et al 2015). The counts of genes were estimated for normalization and dispersions and were transformed into variance stabilization data with DESeq (Anders et al 2013). We produced the heat maps based on the variance stabilization transformed data for putative BR biosynthesis and signaling genes of cotton using pheatmap package (pheatmap: R package version 1.0.8, (https://CRAN.R-project.org/package=pheatmap).

References

1. Al-Ghazi Y, Bourot S, Arioli T, Dennis ES, Llewellyn DJ. Transcript profiling during fiber development identifies pathways in secondary metabolism and cell wall structure that may contribute to cotton fiber quality. *Plant Cell Physiol*, 2009, 50:1364-1381

2. Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD. Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. *Nat Protoc*, 2013, 8:1765-1786
3. Ayabe S-i, Akashi T. Cytochrome P450s in flavonoid metabolism. *Phytochem Rev*, 2006, 5:271-282
4. Chaudhary B, Hovav R, Flagel L, Mittler R, Wendel JF. Parallel expression evolution of oxidative stress-related genes in fiber from wild and domesticated diploid and polyploid cotton (*Gossypium*). *BMC Genomics*, 2009, 10:378
5. Davies KM, Schwinn KE. Molecular biology and biotechnology of flavonoid biosynthesis. In, Flavonoids: CRC Press, 2005. 154-229
6. Edreva A, Dagnon S, Gurel A, Gesheva E, Hakerlerler H. Reddening of cotton (*Gossypium hirsutum* L.) leaves: Analysis of the polyphenol complex. *Agrochemica Pisa*, 2006, 50:54
7. Feng H, Tian X, Liu Y, Li Y, Zhang X, Jones BJ, Sun Y, Sun J. Analysis of flavonoids and the flavonoid structural genes in brown fiber of upland cotton. *PLoS One*, 2013, 8:e58820
8. Fischer S, Brunk BP, Chen F, Gao X, Harb OS, Iodice JB, Shanmugam D, Roos DS, Stoeckert CJ, Jr. Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups. *Curr Protoc Bioinformatics*, 2011, Chapter 6:Unit 6.12.11-19
9. Gou JY, Wang LJ, Chen SP, Hu WL, Chen XY. Gene expression and metabolite profiles of cotton fiber during cell elongation and secondary cell wall synthesis. *Cell Res*, 2007, 17:422-434
10. He F, Pan Q-H, Shi Y, Duan C-Q. Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules*, 2008, 13:2674-2703
11. He H, Ke H, Keting H, Qiaoyan X, Silan D. Flower colour modification of *chrysanthemum* by suppression of *F3'H* and overexpression of the exogenous Senecio cruentus *F3'5'H* gene. *PLoS One*, 2013, 8:e74395
12. Hua S, Yuan S, Shamsi IH, Zhao X, Zhang X, Liu Y, Wen G, Wang X, Zhang H. A comparison of three isolines of cotton differing in fiber color for yield, quality, and photosynthesis. *Crop Sci*, 2009, 49:983
13. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucl Acids Res, 2016, 44:D286-D293
14. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol*, 2013, 14:R36
15. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *Scientific World J*, 2013, 2013:1-16
16. Li F, Fan G, Wang K, Sun F, Yuan Y, Song G, Li Q, Ma Z, Lu C, Zou C, Chen W, Liang X, Shang H, Liu W, Shi C, Xiao G, Gou C, Ye W, Poulain J, Yu S. Genome sequence of the cultivated cotton *Gossypium arboreum*, 2014.
17. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S. The Sequence Alignment/Map format and *SAMtools*. *Bioinformatics*, 2009, 25:2078-2079
18. Li YJ, Zhang XY, Wang FX, Yang CL, Liu F, Xia GX, Sun J. A comparative proteomic analysis provides insights into pigment biosynthesis in brown color fiber. *J Proteomics*, 2013, 78:374-388
19. Liu X, Zhao B, Zheng H-J, Hu Y, Lu G, Yang C-Q, Chen J-D, Chen J-J, Chen D-Y, Zhang L, Zhou Y, Wang L-J, Guo W-Z, Bai Y-L, Ruan J-X, Shangguan X-X, Mao Y-B, Shan CM, Jiang J-P, Zhu Y-Q et al. Gossypium barbadense genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites. Scientific Reports, 2015b, 5:14139
20. Lu N, Roldan M, Dixon RA. Characterization of two *TT2*-type *MYB* transcription factors regulating proanthocyanidin biosynthesis in tetraploid cotton, *Gossypium hirsutum*. *Planta*, 2017, 246:323-335
21. Ma J, Wei H, Song M, Pang C, Liu J, Wang L, Zhang J, Fan S, Yu S. Transcriptome profiling analysis reveals that flavonoid and ascorbate-glutathione cycle are important during anther development in Upland cotton. *PLoS One*, 2012, 7:e49244
22. Meng C, Zhang S, Deng YS, Wang GD, Kong FY. Overexpression of a tomato flavanone 3-hydroxylase-like protein gene improves chilling tolerance in tobacco. Plant Physiol Biochem, 2015, 96:388-400
23. Nakatsuka T, Sato K, Takahashi H, Yamamura S, Nishihara M. Cloning and characterization of the UDP-glucose: anthocyanin 5-O-glucosyltransferase gene from blue-flowered gentian. *J Exp Bot*, 2008, 59:1241-1252
24. Naoumkina M, Thyssen GN, Fang DD. RNA-seq analysis of short fiber mutants Ligon-lintless-1 (*Li1*) and - 2 (*Li2*) revealed important role of aquaporins in cotton (*Gossypium hirsutum* L.) fiber elongation. *BMC Plant Biol*, 2015, 15:65
25. Nix A, Paull C, Colgrave M. Flavonoid profile of the cotton plant, *Gossypium hirsutum*: A Review. *Plants (Basel)*, 2017, 6,43
26. Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, Llewellyn D, Showmaker KC, Shu S, Udall J, Yoo M-j, Byers R, Chen W, Doron-Faigenboim A, Duke MV, Gong L, Grimwood J, Grover C, Grupp K, Hu G et al. Repeated polyploidization
27. Quideau S. Flavonoids: Chemistry, Biochemistry and Applications. *Angewandte Chemie Inter Ed*, 2006, 45:6786-6787
28. Rapp RA, Haigler CH, Flagel L, Hovav RH, Udall JA, Wendel JF. Gene expression in developing fibres of Upland cotton (*Gossypium hirsutum* L.) was massively altered by domestication. *BMC biology*, 2010, 8:139
29. Taliercio EW, Boykin D. Analysis of gene expression in cotton fiber initials. *BMC Plant Biol*, 2007, 7:22
30. Tan J, Tu L, Deng F, Hu H, Nie Y, Zhang X. A genetic and metabolic analysis revealed that cotton fiber cell development was retarded by flavonoid naringenin. *Plant Physiol*, 2013a, 162:86-95
31. Tan J, Wang M, Tu L, Nie Y, Lin Y, Zhang X. The flavonoid pathway regulates the petal colors of cotton flower. *PLoS One*, 2013b, 8:e72364
32. Tanaka Y, Brugliera F. Flower colour and cytochromes P450. *Philos Trans R Soc Lond B Biol Sci*, 2013, 368:20120432
33. Taylor LP, Grotewold E. Flavonoids as developmental regulators. *Curr Opin Plant Biol*, 2005, 8:317-323
34. Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee T-h, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucl Acids Res*, 2012, 40:e49-e49
35. Xiao YH, Yan Q, Ding H, Luo M, Hou L, Zhang M, Yao D, Liu HS, Li X, Zhao J, Pei Y. Transcriptome and biochemical analyses revealed a detailed proanthocyanidin biosynthesis pathway in brown cotton fiber. *PLoS One*, 2014, 9:e86344

36. Yan Q, Wang Y, Li Q, Zhang Z, Ding H, Zhang Y, Liu H, Luo M, Liu D, Song W, Liu H, Yao D, Ouyang X, Li Y, Li X, Pei Y, Xiao Y. Up-regulation of *GhTT2-3A* in cotton fibres during secondary wall thickening results in brown fibres with improved quality. *Plant Biotechnol J*, 2018, 16:1735-1747
37. Yu Y, Ouyang Y, Yao W. shinyCircos: an R/Shiny application for interactive creation of Circos plot. *Bioinformatics*, 2018, 34:1229-1231
38. Zakaryan H, Arabyan E, Oo A, Zandi K. Flavonoids: promising natural compounds against viral infections. *Arch Virol*, 2017, 162:2539-2551
39. Zhang T, Hu Y, Jiang W, Fang L, Guan X, Chen J, Zhang J, Saski CA, Scheffler BE, Stelly DM, Hulse-Kemp AM, Wan Q, Liu B, Liu C, Wang S, Pan M, Wang Y, Wang D, Ye W, Chang L et al. Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement. *Nat Biotechnol*, 2015, 33:531-537
40. Zhao J, Pang Y, Dixon RA. The mysteries of proanthocyanidin transport and polymerization. *Plant Physiol*, 2010, 153:437-443

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Evaluation of Hybrid Material for Resistance to Verticillium Dahliae Klebhan with the Use of Marker Enzymes of Phytoimmunity Using Phytoimmunity Marker Enzymes

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Abstract

It is necessary to improve the method of selection of parent pairs during hybridization and qualitative assessment of interspecific hybrids at the early stages of the breeding process. The initial stage of selection should be based on test signs of resistance, which are associated with the catalytic activity of some enzymes involved in the formation of phytoimmunity against fungal infections. The earliest protective reactions of plants that form in response to damage by pathogens include the formation of reactive oxygen species, including H2O2. 48 hours after inoculation in plants, the content of H2O2 sharply increased. The object of the study is cotton hybrids of the species G.hirsutum L., created based on complex interline hybridization L-101, L-102, L-103, L-104, L-105, L-106, L-107, L-108.As a result of the conducted biochemical research, it was established:

- that at the first stage, among the F5 hybrids, 16 hybrids are of greatest interest from a breeding point, as the most resistant to V.dahliae.

- at the second stage, among the F6 hybrids, the most interesting from the breeding point of view is the hybrid F6[F4(L-101 × L-105) × L-106], as the most resistant to V.dahliae.

- at the third stage of biochemical assessment, only promising cotton families selected for resistance to V. dahliae are studied, which allows the breeder to select breeding significant families. As a result of a biochemical research in laboratory conditions, it was established that a hybrid F11[F6(L-101 x L-105) x L-106] was selected from the presented material resistant to V.dahliae.

Using the results of biochemical assessment to create new breeding material and new varieties resistant to V.dahliae, optimizes and accelerates the work of the breeder.

Keywords: *cotton, variety, enzyme, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, hybrid, resistance, Verticillium wilt.*

Background

In the world's cotton-growing countries, cotton is cultivated on the territory of 89 countries, on a total area of more than 30 million hectares, from which more than 22.4 million tons of cotton fiber are obtained. Today, there are problems in the production of high-quality cotton fiber yield. One of these problems is pathogens that cause significant damage to cotton production, with losses in the world amounting to 12-15%. In the world, considerable attention is paid to the study and control of the pathogen *Verticillium dahliae Klebahn*, which affects cotton.

The problem of breeding wilt-resistant varieties of cotton is complicated by the search for new methods and donors of resistance to the pathogen. It is necessary to improve the method of selection of parent pairs during hybridization and qualitative assessment of interspecific hybrids at the early stages of the breeding process to increase the efficiency of breeding, and speed up the process of introducing new varieties of cotton into production. The selection must be carried out based on the physiology and biochemistry of signs of resistance of the initial breeding material. The initial stage of selection should be based on test signs of resistance, which are associated with the catalytic activity of some enzymes involved in the formation of phytoimmunity against fungal infections(peroxidase, phenylalanine ammonialyase, polyphenol oxidase). The greatest interest of researchers is attracted by protective mechanisms, including the processes of lignification of cell walls and the biosynthesis of phenolic phytoalexins. These mechanisms simultaneously create a mechanical and chemical barrier to the penetration of fungal structures into the cell, preventing the spread of the pathogen.

Peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase are directly involved in the biosynthesis of lignin and phytoalexins. [1]

Glucansynthetase, peroxidase, polyphenol oxidase, play a practical role in the creation of new varieties of cotton.

Polyphenol oxidase is involved in the oxidation of polyphenols to quinones, which have antimicrobial activity and lignification of cell walls during the microbial invasion. Several studies show that polyphenol oxidase is involved in protective and hypersensitivity reactions that induce systemic resistance of plants to fungi [7]. Peroxidase is an enzyme of the oxidoreductase group. It takes an active part in the oxidation of phenols, suberization, and lignification of plant cell walls in response to infection by phytopathogens. [2] Such a resistance mechanism is associated with the induction of peroxidase activity. [6, 8, 11] Most studies of plant-pathogen interactions show that the accumulation of lignin and phenolic compounds correlates with plant resistance to disease [9, 10]. Phenylalanine ammonia lyase is a key enzyme in the mechanism of biosynthesis of phenylpraponoid compounds, the presence of which is shown in plants infected with the pathogen. Previously it is established that different plant species increase the activity of the phenylalanine ammonia lyase under biotic and abiotic stresses, including fungal infection.

The object of the study is cotton hybrids of the species *G.hirsutum* L., created based on complex interline hybridization L-101, L-102, L-103, L-104, L-105, L-106, L-107, L-108.

Results

The first step in the work was the determination of the activity of enzymes, which should be used as markers. The following enzymes were selected for research: oxidoreductase (peroxidase), polyphenol oxidase, phenylalanine ammonia lyase. In the above enzymes, optimal conditions for determining activity were studied, activity in cotton seedlings and responsiveness to the most important stressors were established and methods of identification of the best selection-significant families of different generations are worked out.

In the research were studied five hybrids (48 families) of the fifth generation, in the second stage five hybrids (21 families) of the sixth generation, and in the third stage six hybrids of the eleventh generation, that are of interest from a breeding point of view.

As a result of experimental work, a change in the enzymatic activity of seedlings of cotton seeds of various origins was established. The regularities of the biochemical adaptation of cotton hybrids to growing conditions were analyzed and generalized, and the relationship of the above forms of enzymes with various conditions of cotton growing was studied. A method for using enzymatic activity as a marker of the resistance of cotton samples to *V.dahliae* is proposed. Cotton families have been identified that should be used in breeding aimed at creating new resistant varieties for *V.dahliae*.

The activity values of the above enzymes in the parent forms determine the resistance in hybrids and breeding material. Using the developed test system allows the selection of fork-resistant breeding material at all stages of selection. Therefore, the use of biochemical approaches allows us to determine its perspective in the laboratory.

In the evaluation of wilt resistance, biochemical tests using marker enzymes of phytoimmunity - chitin-specific peroxidase - were used. As a stress load, the isolation of pathogen *V.dahliae* isolated from a naturally infected background under the field conditions of CBSPARI was used by the isolate.

During the experiment was used the method of accelerated determination of the wilt resistance of cotton. F_5-F_{11} hybrid families were tested to assess the molecular genetic diversity of the source, hybrid, and breeding material when creating new varieties using biochemical criteria for disease resistance.

As can be seen in table 1, the level of peroxidase enzyme activity in the F_5 hybrid [F_4 (L-105 × L-106) × L-105] (family 2605, 2622, 2754) in the presence of *V.dahliae* fungus increases many times.

That the level of activity of the enzyme polyphenol oxidase in seedlings of the hybrid F_5[F_4(L-105×L-106)×L-105] - family numbers 2605, 2622, 2754; F_5[F_4(L-101×L-108)×L-102] - family numbers 2904; F_5[F_4(L-105×L-106)L-106] - family numbers 2944; F_5[F_4(L-101×L-105)×L-106] - family numbers 2887, 2886, 2827; F_5[F_4 (L-105×L-108)×L-104] – family numbers 2749, 2779, 2780, 2788; F_5 [F_4 (L-103×L-106)×L-102] - family numbers 2834, 2831, 2884, in experimental versions in the presence of the fungus *V.dahliae* increases many times.

That the level of activity of the enzyme phenylalanine ammonia lyase in seedlings of the hybrid F_5[F_4(L-105×L-106)×L-105] - family numbers 2605, 2622, 2754; F_5[F_4(L-101×L-108)×L-102] - family numbers 2904; F_5[F_4(L-105×L-106) x L-106] - family numbers 2944; F_5[F_4(L-101×L-105)×L-106] - family numbers 2887, 2886, 2827; F_5[F_4 (L-105×L-108)×L-104] – family numbers 2749, 2779, 2780, 2788; F_5 [F_4 (L-103×L-106)×L-102]- family numbers 2834, 2831, 2884, in experimental versions in the presence of the fungus *V.dahliae* increases many times (Table 1).

Table 2 shows the most resistant families of F_6 hybrids to *V.dahliae*. As a result of the conducted biochemical research in laboratory conditions, it was found that families isolated from a complex interline hybrid combination of F_6[F_4-L-101 x L-105] x L-106 are resistant to *V.dahliae*. The activity of chitin-specific peroxidase in selected families exceeds the activity by more than 2 times that compared with the control, which indicates that the selected families are most resistant to damage.

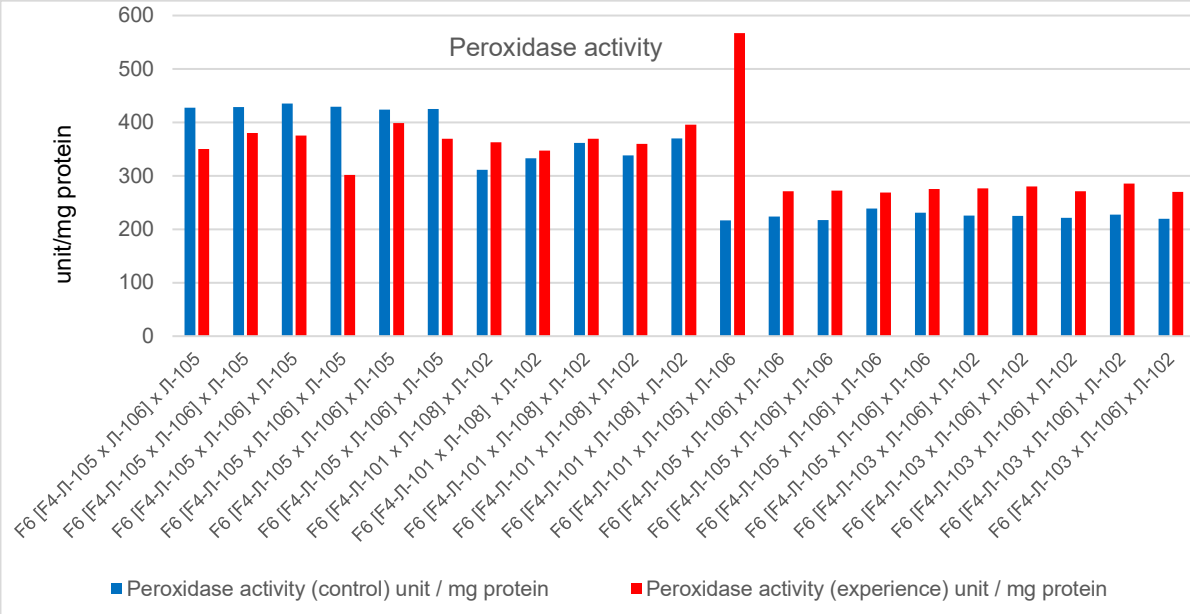

Fig. 1. *The activity of peroxidase in seedlings of the investigated samples of cotton.*

At the third stage, the study of peroxidase activity in cotton hybrids showed that when interacting with a phytopathogen in all experimental variants, with the exception of Bukhara-6, S-6524 varieties and in the hybrid F_{11} [F_6 (L-101 x L-106) x L- 105], F_{11} [F_6 (L-105 x L-106) x L-105], F_7 [L-175/276 x Namangan-102] the enzyme activity decreased 1.7–1.88 times from the control level (Fig. 2).

Table 1. *Assessment of the resistance of families of various hybrid combinations of cotton to the pathogen V. dahliae*

Sr. No	No of Family	Enzyme activity u/mg protein					
		Peroxidase		Polyphenol oxidase		Phenylalanine ammonia lyase	
		control	experience	control	experience	control	experience
		F_5[F_4(Л-105×Л-106)×Л-105]					
1	2605	490,86	2345,75	1115,51	1200,10	130,00	257,13
2	2622	1178,99	2407,60	567,50	1186,1	177,40	405,36
3	2631	3207,40	891,60	489,70	101,20	19,10	5,71
4	2656	3761,36	1492,50	3814,2	211,1	15,42	11,56
5	2684	5241,32	147,98	1228,9	284,3	20,60	13,90
6	2726	4222,83	1210,32	918,1	187,1	22,34	16,71
7	2747	137,70	153,00	237,44	232,4	52,95	63,14
8	2755	138,80	123,77	623,85	169,5	14,17	12,03
9	2754	126,82	134,33	220,64	910,5	20,00	139,4
10	2767	97,35	92,49	94,17	65,18	19,68	16,00
11	2768	104,85	100,71	217,78	130,20	0,00	0,00
12	2782	158,81	145,42	214,45	240,72	0,00	06,00
		F_5[F_4(Л-101×Л-108)×Л-102]					
13	2901	156,57	48,43	156,07	109,78	20,68	20,00
14	2904	158,1	113,98	556,70	923,50	101,2	408,0
15	2902	141,16	140,53	284,05	204,20	0,00	13,46
16	2916	153,09	121,40	278,08	108,91	11,88	12,4
17	2920	161,41	152,45	110,64	95,04	0,00	0,00
18	2922	115,8	110,96	400,25	63,2	0,00	0,00
		F_5[F_4(Л-105×Л-106)×Л-106]					
19	2923	181,93	181,18	69,08	130,00	0,83	0,75
20	2925	115,98	117,30	60,76	78,05	5,07	6,32
21	2928	122,23	120,84	63,52	70,32	8,12	2,60

22	2939	110,80	111,51	36,57	38,57	7,04	6,77
23	2943	122,26	119,55	259,38	67,60	0,00	10,05
24	2944	129,07	213,53	626,24	909,12	23,00	96,34
F_5[F_4(Л-101×Л-105)×Л-106]							
25	2887	146,04	274,58	356,0	1094,5	83,00	277,50
26	2886	139,74	251,56	341,0	1015,7	57,01	138,75
27	2827	133,01	241,71	187,0	1033,4	70,50	117,7
F_5[F_4(Л-105×Л-108)×Л-104]							
28	2749	132,30	253,86	191,0	264,25	192,3	365,0
29	2775	142,53	130,77	183,75	134,01	6,30	6,00
30	2779	142,60	251,14	167,00	285,54	79,40	276,80
31	2780	128,84	238,08	101,00	263,12	95,00	208,05
32	2785	132,88	130,98	106,20	106,06	6,62	6,30
33	2788	120,65	149,04	164,80	196,02	154,30	272,0
34	2784	130,02	116,17	125,10	100,00	5,60	5,29
35	2790	125,44	118,62	1065,0	72,14	148,6	104,0
36	2799	123,09	118,69	165,60	181,70	17,55	14,44
37	2737	128,04	113,65	148,30	105,30	7,61	0,00
38	2733	132,52	110,74	98,62	94,00	6,60	6,12
39	2714	146,39	111,16	126,74	122,20	2,83	2,33
F_5[F_4(Л-103×Л-106)×Л-102]							
40	2836	139,62	115,4	164,10	128,30	2,80	3,63
41	2839	173,12	168,23	101,60	101,50	20,30	14,00
42	2834	244,25	344,57	176,31	297,90	33,61	197,14
43	2831	189,01	525,57	232,14	444,80	21,30	155,00
44	2847	183,31	107,17	104,19	119,41	0,30	0,00
45	2874	114,43	108,40	101,10	98,90	0,00	0,00
46	2849	106,38	101,79	107,80	105,63	6,15	6,69
47	2884	166,73	325,54	160,75	308,60	65,00	120,00
48	2880	164,05	161,22	150,00	76,9	1,80	1,70

Table 2. *Peroxidase activity in seedlings of the studied families, complex hybrids of the sixth generation cotton (n = 3; M ± m)*

Sr. No.	Hybrid combinations	No. family	Protein content µg / g dry weight (Lowry)	Peroxidase activity (control) unit / mg protein	Peroxidase activity (experience) unit / mg protein
1	F_6 [F_4-Л-105 x Л-106] x Л-105	2	0,32	427,8±1.2	350,3±3.0
2	F_6 [F_4-Л-105 x Л-106] x Л-105	4	0,27	428,9±1.8	380,1±2.5
3	F_6 [F_4-Л-105 x Л-106] x Л-105	5	0,45	435,6±2.1	375,5±1.2
4	F_6 [F_4-Л-105 x Л-106] x Л-105	7	0,41	429,5±3.4	301,7±1.0
5	F_6 [F_4-Л-105 x Л-106] x Л-105	8	0,33	423,9±1.9	398,6±1.7
6	F_6 [F_4-Л-105 x Л-106] x Л-105	9	0,29	425,2±1.4	369,2±1.4
7	F_6 [F_4-Л-101 x Л-108] x Л-102	10	0,52	311,5±2.4	362,9±1.7
8	F_6 [F_4-Л-101 x Л-108] x Л-102	16	0,61	332,6±1.9	347,5±1.8
9	F_6 [F_4-Л-101 x Л-108] x Л-102	20	0,55	361,6±1.1	369,2±2.8
10	F_6 [F_4-Л-101 x Л-108] x Л-102	26	0,48	338,1±2.0	359,8±2.7
11	F_6 [F_4-Л-101 x Л-108] x Л-102	27	0,54	369,8±1.3	395,9±2.6
12	F_6 [F_4-Л-101 x Л-105] x Л-106	46	0,6	216,8±2.0	567,3±1.0
13	F_6 [F_4-Л-105 x Л-106] x Л-106	32	0,40	223,8±1.6	270,9±2.4
14	F_6 [F_4-Л-105 x Л-106] x Л-106	35	0,61	217,4±1.5	272,4±1.4
15	F_6 [F_4-Л-105 x Л-106] x Л-106	38	0,34	238,6±1.0	268,9±1.6
16	F_6 [F_4-Л-105 x Л-106] x Л-106	42	0,54	230,9±1.9	275,4±1.8
17	F_6 [F_4-Л-103 x Л-106] x Л-102	50	0,55	225,4±2.3	276,3±1.2
18	F_6 [F_4-Л-103 x Л-106] x Л-102	52	0,61	224,8±2.5	280,1±1.8
19	F_6 [F_4-Л-103 x Л-106] x Л-102	53	0,58	221,2±1.5	270,9±1.4
20	F_6 [F_4-Л-103 x Л-106] x Л-102	58	0,54	227,3±1.5	285,4±2.8
21	F_6 [F_4-Л-103 x Л-106] x Л-102	61	0,62	219,9±1.3	269,9±2.0

In hybrid F_{11} [F_6 (L-101 x L-105) x L-106], peroxidase activity was 1.3-1.6 times higher than its control, which allowed the plant to become involved in the processes that prevent the multiplication of infectious structures fungus, participating in the reactions of strengthening the cell walls when exposed to a pathogen.

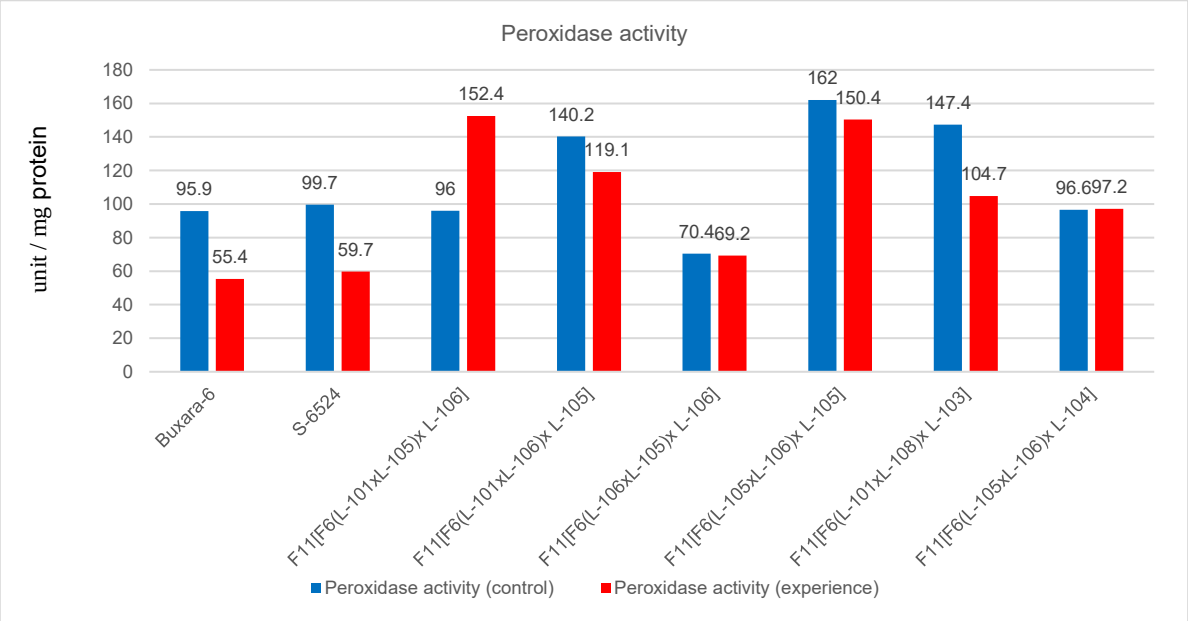

Figure 2 *-Peroxidase activity in the roots of 7-day-old seedlings of various cotton hybrids after 48 hours of exposure with conidia of the fungus V.dahliae.*

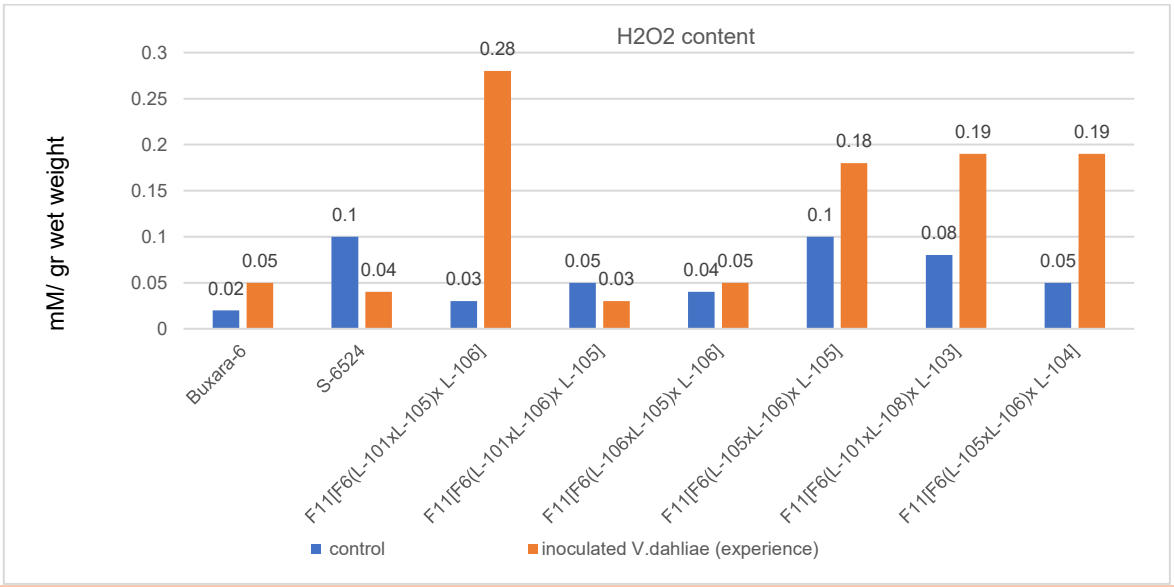

Figure 3 *The content of hydrogen peroxide in the roots of 7-day-old seedlings of various cotton hybrids after 48 hours of exposure with conidia of the fungus V.dahliae*

A decrease in enzyme activity in other hybrids may be due to inhibition of peroxidase activity due to the production of H_2O_2 in response to the spread of fungal structures in cotton tissues. It is assumed that resistance to pathogens such as *Verticillium*, depends primarily on the mechanical isolation of the pathogen during the determining phases of colonization and reaction inside and outside the vascular system [13]. Lignin forms a structural barrier that limits the spread of pathogenic fungi and prevents the diffusion of extracellular enzymes and toxins. Strengthening the cell wall can confer resistance only if lignification occurs quickly and before the penetration of the pathogen hyphae. Therefore, strengthening the cell wall by deposition of lignin and lignin-like polymers, which is preceded by the induction of the peroxidase enzyme responsible for their formation, plays an important role in the protective response of cotton seedlings against *V.dahliae*.

An important role in protective reactions is assigned to hydrogen peroxide, which possesses the properties of a signal molecule in low concentrations, and which exhibits a direct biocidal effect in high concentrations [3, 4]. In addition, H_2O_2 with the participation of peroxidases enhances cell wall strengthening by lignification [5].

The earliest protective reactions of plants that form in response to damage by pathogens include the formation of reactive oxygen species, including H_2O_2 [12]. 48 hours after inoculation in plants, the content of H_2O_2 sharply increased (Fig. 3).

Conclusion

As a result of the conducted biochemical research, it was established that

- At the first stage, among the F_5 hybrids, the following hybrids are of greatest interest from a breeding point of view: $F_5[F_4$ (L-105 × L-106) × L-105] (families numbers 1, 2, 9), F_5 [F_4(L-101 × L-108) × L-102] (family number 14), $F_5[F_4$ (L-105 × L-106) × L-106] (family number 24), $F_5[F_4$ (L-101 × L-105) × L-106] (families numbers 25, 26 and 27), $F_5[F_4$ (L-105 × L-108) × L-104] (families numbers28, 30, 31, 33, 35), $F_5[F_4$ (L-103 × L-106) × L-102] (families numbers 42, 43, 47), as the most resistant to *V.dahliae*.
- In the second stage, among the F_6 hybrids, the most interesting from the breeding point of view is the hybrid $F_6[F_4$(L-101 × L-105) × L-106], as the most resistant to *V.dahliae*.
- At the third stage of biochemical assessment, only promising cotton families selected for resistance to *V.dahliae* are studied, which allows the breeder to select breeding significant families. As a result of a biochemical research in laboratory conditions, it was established that a hybrid $F_{11}[F_6$(L-101 x L-105) x L-106] was selected from the presented material resistant to *V.dahliae*.
- Using the results of biochemical assessment to create new breeding material and new varieties resistant to *V.dahliae*, optimizes and accelerates the work of the breeder.

Methods

The research used modern methods of bioorganic chemistry and traditional breeding. The determination of chitin-specific peroxidase was carried out according to the method of N.R. Hashimova. Statistical processing of the material — following the method of O. Yu. Rebrova, included testing the hypothesis that the tabular data corresponded to the normal distribution law using the AtteStat data analysis program, v.10.9.6, which works as an add-in to the Microsoft Excel-2007 program, with using the modules "Normality Check" and "Emission Processing". Then, the average value and standard deviation σ were calculated using the “Descriptive Statistics” module. In calculating these indicators, parametric criteria were used. Variation-statistical processing of the research results was carried out using the methods of O. Rebrova and B. Dospekhov.

References

1. Allashov B., Ibragimov Sh., Ibragimov P.Sh. The study of the effectiveness of crossbreeding according to economically valuable traits in the selection of medium fiber cotton. // The state of cotton breeding and seed production and development prospects. // Materials of the International Practical Conference - Tashkent. - 2006. - P. 49-50.
2. Akhunov A.A., Golubenko Z., Hashimova N.R., Mustakimova E.Ch., Vshivkov S.O. The role of chitin-specific peroxidases in the resistance of cotton to wilt // Chemistry of Natural Compounds. - 2008. - No. 4. - P. 394–396.
3. Almagro L., Gomes Ros L.V., Belchi-Navarro S., Bru R.,Ros Barcelo A., Pedreno M.A. // J. Exp. Botany. 2009.- V. 60. - №. 2. – P.377–390.
4. Cosio C., Dunand C. // J. Exp. Botany. 2009. - V. 60.- P.391–408.
5. Curtis M.D., Rae A.L., Rusu A.G., Harrison S.J., Manners J.M. // Mol. Plant-Microbe Interactions. 1997. - V.10. - P.326–338.
6. Goncharova E.A. Strategy for diagnosing and predicting the resistance of agricultural plants to weather and climate anomalies. // Agricultural biology. - 2011. - No. 1. - P. 24–31.
7. Gechev T., Gadiev I., Van Breusegem F. et al. Hydrogen peroxide protect tobacco from oxidative stress by inducing a set of antioxidant enzymes // Cell. Mol. Life Sci. 2002. V.59.- P.708-714.
8. Hayrullin RM, Yusupova ZR, Maksimov IV. Protective reactions of wheat when infected with fungal pathogens. 1. The interaction of anionic wheat peroxidases with chitin, chitosan and teliospores Tilletiacries. // Plant Physiology 2000. Vol. 47, No. 1, P. 108–113.
9. Hayrullin P.M., Yarullina L.G., Troshina NB, Akhmetova I.E. Activation by chitoligosaccharides of oxidation of orthophenylenediamine by wheat seedlings in the presence of oxalic acid // Biochemistry. - 2001. - T. 66. - No. 6. - P. 354–358.
10. Hashimova N.R., Akhunov A.A., Pshenichnov E.A., Avtonomov V.A., Amanturdiev Sh.B. Patent "Method for determining the resistance of cotton varieties to wilt", Application No. IAP 20110549. Registered on January 20, 2015.
11. Mittler R. // Trends Plant Scie. 2002. - V. 7. -№ 9. -P.405–410.
12. Maksimov I.V., Cherepanova E.A., Khayrullin R.M. "Chitin-specific" peroxidases in plants. // Biochemistry. - 2003. - T. 68. - Vol. 1. - P. 133–138.
13. Nicholson, R. L. and Hammerschmidt R. // Annu.Rev. Phytopathol. 1992.- V.30.- P. 369.

Examining Two Sets of Introgression Lines Across multiple Environments Reveals Background Independent and Stably Expressed Quantitative Trait Loci of Fiber Quality in Cotton

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Abstract

Cotton fiber quality traits are controlled by QTLs and are susceptible to environmental influence. Fiber quality improvement is an essential goal in cotton breeding but is hindered by limited knowledge of the genetic basis of fiber quality traits. In this study, two sets of introgression lines of Gossypium hirsutum × G.barbadense were used to dissect the QTL stability of three fiber quality traits (fiber length, strength, and micronaire) across environments using 551 simple sequence repeat markers selected from our high-density genetic map. A total of 76and 120 QTLs were detected in the CCRI36 and CCRI45 backgrounds, respectively. Nine BI-QTLs were found, and 78(41.71%) of the detected QTLs were reported previously. Thirty-nine and 79QTLs were SE-QTLs in at least two environments in the CCRI36 and CCRI45 backgrounds, respectively. Forty-eight SE-QTLs, including seven BI-QTLs, was confirmed in previous reports, and 61SE-QTLs, including two BI-QTLs, were considered novel.

These results indicate that genetic background more strongly impacts fiber quality traits than environmental factors. Twenty-three clusters with BI- and/or SE- QTLs were identified,19 of which harbored favorable alleles from G. barbadense for two or three fiber quality traits. This study is the first report using two sets of introgression lines to identify fiber quality QTLs across environments in cotton, providing insights into the effect of genetic backgrounds and environments on the QTL expression of fiber quality and important information for the genetic basis underlying fiber quality traits towards QTL cloning and molecular breeding.

Keywords: *cotton, introgression lines, environments, genetic backgrounds, fiber quality, quantitative trait loci(QTLs)*

Background

Cotton is an important economic crop worldwide that produces natural fibers used in the textile industry. Fiber quality must be improved to keep pace with the development of spinning technology and cotton harvesting mechanization. However, the narrow genetic variation in Upland cotton limits the improvement of cotton varieties (Qin et al. 2008). It has been a long-term challenge for cotton breeders to improve fiber quality and yield to meet the needs of cotton producers and the textile industry. Cotton (Gossypiumspp.) contains 52 species (Li et al. 2014), including two important cultivated tetraploids species: G. hirsutum (Upland cotton), with a high fiber yield, wide adaptability, and medium fiber quality, and G. barbadense (Sea-Island, Egyptian or Pima cotton), with a low fiber yield, and narrow adaptability but high fiber quality (Lu et al. 2017; Shi et al. 2016).

Therefore, introducing desirable genes from G. barbadense into Upland cotton cultivars and mapping the quantitative trait loci (QTLs) for fiber quality traits transferred from G. barbadense using introgression lines or chromosome segment substitution lines in the Upland cotton background could facilitate improvements in the fiber quality of Upland cotton.

To transfer beneficial genes from G. barbadense into Upland cotton cultivars, we constructed two sets of CSSLs with two different Upland cotton genetic backgrounds in which Hai1 (G. barbadense) was the donor parent and CCRI36 and CCRI45 (G. hirsutum) were the recipient parents (Li et al. 2016; Li et al. 2019a; Lu et al. 2017; Ma et al. 2013; Yang et al. 2009). Some of the CSSLs were genetically evaluated, and some QTLs for yield and fiber quality were identified using secondary segregating populations derived from one, two, or four CSSLs as parents (Guoet al. 2015; Guoet al. 2018; Li et al. 2019b; Song

et al. 2017; Zhaiet al. 2016). With the rapid development of biotechnology, multiple cotton genomes have been sequenced, providing a foundation for further cotton gene identification and molecular breeding at the genome level (Hu et al. 2019; Li et al. 2015; Wang et al. 2019).

In this paper, two sets of CSSLs were evaluated and used to dissect the genetic basis of the stability of cotton fiber quality traits across multiple environments and multiple genetic backgrounds, including the identification of more genetic BI- and/or SE-QTLs for fiber quality traits, thus providing new and important stable QTLs with known genomic segments for fine gene mapping, gene cloning, and molecular breeding. To the best of our knowledge, this study represents the first report using two sets of CSSLs with different genetic backgrounds but with the same donor, parent to dissect the stability of QTLs affecting fiber-quality traits as well as to compare and analyze the influence of genetic backgrounds and environments on the expression of fiber quality QTLs in cotton.

Results

Evaluation of CSSLs and fiber quality

The Results of the descriptive statistical analysis of each trait in the different populations are shown in Table 1. The average FL, FS, and FM values of the recurrent parent were generally consistent with those of the corresponding population in all environments. The FL of the populations in all environments was slightly greater than that of the recurrent parents, except in 14XJN; the FS of the populations in the other environments was slightly higher than that of the recurrent parents, except in 10AY; and the FM value of the populations in all environments was slightly lower than that of the recurrent parents. The average FL, FS, and FM values of 36 and 45 recurrent parents were similar in all environments, with medium fiber quality. The descriptions of the statistical analysis of quality for 45Pop in 09HNA, 10HNA, 11HNA, and 11XJK follow those reported by Ma et al. (2013).

In all environments, the range and coefficient of variation of each trait in all the populations were large. For the same population in all environments, the variation in FM was the greatest among the three traits, and the variation in FS was greater than that in FL. These results indicate abundant genetic variation in the CSSL populations produced by advanced backcrossing and continuous self-crossing. The absolute skewness of all traits in all populations and environments was less than 1, thus following a normal distribution (Table 1). The correlation coefficients of each trait between different environments were significant, indicating that these materials were stable across multiple environments. Most of the correlation coefficients among environments for FL and FM were larger than 0.5, whereas those for FS were smaller than 0.5, indicating that FS was more greatly affected by the environment than FL and FM. ANOVA revealed highly significant effects of genotype (G), the environment (E), and the interaction between genotype and the environment (G × E interaction) on all three traits in the populations (Table 2). The broad-sense heritability values, calculated by partitioning the variance into genetic and G × E effects, were above 75% for all three traits. Through the evaluation and analysis of multiple environments, some of the CSSLs with excellent and stable CSSLs were screened out(Li et al. 2017; Lu et al. 2017). These materials with outstanding fiber quality can be used in cotton breeding and gene identification and cloning.

Genotype analysis

Fig.S2 and Fig.S3 show the introgressed Hai1 segments on 26 chromosomes in the 36Pop and 45Poppopulations of CSSLs, covering almost the entire genome. The maximum length of the introgressed segments from Hai1 in each individual in the 36Pop population was 488.2 cM; the minimum length was 4.5 cM, and the average length was 125.80 cM. The percent return to the background of the recurrent parent in the population was 90.5–99.8%, with an average of 97.5%. The number of introgressed Hai1 segments was generally 5–20, and the length of the introgressed Hai1 segments was mainly between 30 cM and 210 cM (Fig.1). The maximum length of the introgressed segments from Hai1 in each individual in the 45Pop population was 514.1 cM; the minimum length was 94.5 cM, and the average length was 125.80 cM. The percent return to the background of the recurrent parent in the population was 90.5–99.8%, with an average of 97.5%. The number of introgressed Hai1 segments was generally 5–20, and the length of the introgressed Hai1 segments was mainly between 150 cM and 390 cM.

Analysis of QTLs in CSSL populations in the CCRI36 background

In the CCRI36 background, a total of 76 QTLs were identified, with a phenotypic variation explained (PVE) of 2.77–13.91% for all three fiber quality traits, 39 of which were detected in at least two environments.

FL: A total of 29 QTLs for FL (FL-QTLs) were detected on 19 chromosomes in the CSSL population with the CCRI36 background, but no FL-QTL was detected on C4, C8, C9, C10, C12, C13, C18, and C25. C12 and C16 contained the most FL-QTLs. The PVE by these FL-QTLs ranged from 2.83–11.49%;26 of the 29 FL-QTLs had positive additive effects, and the Hai1 alleles increased FL.

Twenty of QTLs were detected in at least two environments: one QTL (qFL-C7-3) was detected in six environments with a PVE of 3.14–4.32%, three QTLs (qFL-C5-1, qFL-C15-1,and qFL-C20-4) were detected in five environments with a PVE of 3.05–11.49%, eight QTLs (qFL-C2-4, qFL-C3-2, qFL-C5-2, qFL-C6-3, qFL-C17-5, qFL-C19-5, qFL-C20-1,and qFL-C23-3) were detected in four environments with a PVE of 2.83–10.83%, three QTLs (qFL-C11-1, qFL-C14-6, and qFL-C16-3) were detected in three environments with a PVE of 3.10–6.11%, and five QTLs were detected in two environments. Among the 20 FL-QTLs, 18 had positive additive effects, and the Hai1 alleles increased FL.

FS: A total of 26 QTLs for FS (FS-QTLs) were detected in CSSL population with the CCRI36 background. They had PVE of 2.81–10.81%, distributed on 14 chromosomes (C2, C3, C5, C7, C11, C13, C15, C16, C17, C20, C21, C23, C25, and C26), among which C11, C16, and C20 contained the most FS-QTLs (3–5 QTLs). Twenty-three of the 26 FS-QTLs had positive additive effects, and the Hai1 alleles increased FS.

Six of 26 FS-QTLs were detected in at least two environments: two QTLs (qFS-C7-4 and qFS-C16-4) were detected in four environments with a PVE of 3.19–4.21%, four QTLs were detected in two environments. Five of the six stable QTLs had positive additive effects, and the Hai1 alleles increased FS.

FM: A total of 21 QTLs for FM (FM-QTLs) were detected in the CCRI36 background and were distributed on 11 chromosomes (C1, C3, C4, C7, C12, C16, C17, C19, C20, C23, and C25), among which C17, C19, and C25 contained the most FM-QTLs (3–4 QTLs). The PVE by these QTLs ranged from 2.77–13.91%. Seventeen of the 21 QTLs had negative additive effects, and the Hai1 alleles decreased FM.

Among the FM-QTLs, 13 were detected in at least two environments: five QTLs (qFM-C3-1, qFM-C14-1, qFM-C17-2, qFM-C17-3, and qFM-C17-4) were detected in six environments with a PVE of 4.11–13.91%, two QTLs (qFM-C19-3 and qFM-C25-6) were detected in five environments with a PVE of 2.93–5.12%, one QTLs (qFM-C1-1) was detected in three environments with a PVE of 2.61–3.91%, five QTLs were detected in two environments. Among the 13 stable QTLs, 12 had a negative additive effect, and the Hai1 alleles decreased FM.

Analysis of QTLs in CSSL populations in the CCRI45 background

In the CCRI45 background, a total of 120 QTLs were identified with a PVE ranging from 3.40–22.94% for all three fiber quality traits, 79 of which were detected in at least two environments.

FL: A total of 49 FL-QTLs were detected on 18 chromosomes in the CSSL populations with the CCRI45 background,while no FL-QTL was detected on C3, C4, C8, C9, C11, C18, C23, and C24. C12 and C14 contained the most FL-QTLs (5–6 QTLs). The PVE by these QTLs ranged from 3.12–19.95%;34 of 49 QTLs had positive additive effects, and the Hai1 alleles increased FL.

Thirty-one of 49 FL-QTLs were detected in at least two environments: five QTLs (qFL-C2-1, qFL-C2-3, qFL-C15-1, qFL-C16-2, and qFL-C21-5) were detected in eight environments with a PVE of 3.40–16.95%,five QTLs (qFL-C12-4, qFL-C17-3, qFL-C2-5, qFL-C7-3, and qFL-C21-4) were detected in5–7environments with a PVE of 3.48–15.42%, eight QTLs (qFL-C2-6, qFL-C12-1, qFL-C13-2, qFL-C13-3, qFL-C15-2, qFL-C19-2, qFL-C20-3, and qFL-C22-2) were detected in four environments with a PVE of 3.44–10.25%, six QTLs (qFL-C7-2, qFL-C10-2, qFL-C14-4, qFL-C19-1, qFL-C20-2, and qFL-C22-3) were detected in three environments with a PVE of 3.41–10.42%, and seven QTLs were detected in two environments. Among the 31 stable QTLs, 28 had positive additive effects, and the Hai1 alleles increased FL.

FS: A total of 52 FS-QTLs were detected on 20 chromosomes, with a PVE of 3.40–22.94%; and no FS-QTL was detected on C1, C3, C4, C5, C8, and C18. C15, C7, C14, and C21 contained the most FS-QTLs (4–5 QTLs). Forty-three of the 52 QTLs had positive additive effects, and the Hai1 alleles increased FS. Thirty-nine of the 54 FS-QTLs were detected in at least two environments: four QTLs (qFS-C2-1, qFS-C9-1, qFS-C20-3, and qFS-C26-1) were detected in seven environments with a PVE of 3.41–15.24%, sevenQTLs (qFS-C14-1, qFS-C22-2,qFS-C24-2, qFS-C12-1, qFS-C15-2, qFS-C16-1, and qFS-C22-1) were detected in5–6environments with a PVE of 3.55–15.55%,10 QTLs (qFS-C6-1,

qFS-C7-3, qFS-C11-6, qFS-C12-2, qFS-C15-1, qFS-C15-3, qFS-C15-4, qFS-C23-1, qFS-C24-1, andqFS-C26-2) were detected in four environments with a PVE of 3.46–12.28%, 10 QTLs (qFS-C2-2, qFS-C7-2, qFS-C7-4, qFS-C10-3, qFS-C14-4, qFS-C16-3, qFS-C17-1, qFS-C19-1, qFS-C22-3, andqFS-C25-4) were detected in three environments with a PVE of 3.40–22.94%, and eight were detected in two environments. Among the 39 stable QTLs, 37 had positive additive effects, and the Hai1 alleles increased FS.

FM: A total of 19 FM-QTLs were detected on 12 chromosomes (C2, C4, C5, C7, C11, C13, C15, C16, C17, C21, C24, and C25), in which C5 and C25 contained the most FM-QTLs (3–4 QTLs). The PVE by these QTLs ranged from 3.43–8.37%. Twelve of the 19 FM-QTLs had negative additive effects, and the Hai1 alleles decreased FM.

Among these FM-QTLs, nine were detected in at least two environments: two QTLs (qFM-C5-3 and qFM-C24-2) were detected in three environments with a PVE of 3.45–7.66%, and seven QTLs (qFM-C15-1, qFM-C16-1, qFM-C17-2, qFM-C25-1, qFM-C25-2, and qFM-C25-3) were detected in two environments with a PVE of 3.43–8.37%. Among the nine stable QTLs, six had positive additive effects, and the Hai1 alleles increased FM.

QTLs detected in both genetic backgrounds simultaneously

Among the above QTLs, nine background-independent QTLs (BI-QTLs) were simultaneously detected in both backgrounds, including four FL-QTLs, four FS-QTLs and one FM-QTLs (Table 3).

Among the four FL-QTLs, qFL-C7-3 near NAU1085 on C7 was simultaneously detected in six environments in the CCRI36 background and five environments in the CCRI45 background; qFL-C15-1 near NAU3177 on C15 was simultaneously detected in five environments in the CCRI36 background and eight environments in the CCRI45 background; qFL-C16-2 near BNL2634 on C16 was simultaneously detected in eight environments in the CCRI45 background and one environment in the CCRI36 background, and qFL-C2-6 near NAU2277 on C2 was simultaneously detected in four environments in the CCRI45 background and one environment in the CCRI36 background. The Hai1 alleles in the four FL-QTLs all increased FL.

Among the four FS-QTLs, FS-C7-4 near NAU1085 on C7 was simultaneously detected in four environments in the CCRI36 background and three environments in the CCRI45 background; qFS-C16-3 near BNL2634 on C16 was simultaneously detected in two environments in the CCRI36 background and three environments in the CCRI45 background; qFS-C17-3 near HAU0195a on C17 was simultaneously detected in two environments of each of both backgrounds; qFS-C15-3 near NAU3177 on C15 was simultaneously detected in four environments in the CCRI45 background and one environment in the CCRI36 background. The Hai1 alleles in the four FS-QTLs increased FS.

Only one FM-QTL(qFM-C17-2) near NAU2909 on C17 was detected in six environments in the CCRI36 background and two environments in the CCRI45 background.

Therefore, six QTLs (qFL-C7-3, qFL-C15-1, qFM-C17-2, qFS-C7-4, qFS-C16-3, and qFS-C17-3) were detected in multiple environments in each of both backgrounds, and three QTLs (qFL-C2-6, qFL-C16-2, and qFS-C15-3) were detected in multiple environments in one background and one environment in another background.

Fiber quality QTL clusters: The QTL clusters were defined as a QTL-rich region that contained two or more QTLs of various trait types within a common confidence region. Some of the QTLs formed clusters, which is a common and previously reported phenomenon (Lacape et al. 2010; Sun et al. 2012; Said et al. 2015; Zhai et al. 2016). A total of 23 QTL clusters were found in this paper, with at least two stable or common QTLs affecting at least two or more different traits. These clusters were distributed on 13 chromosomes (C2, C7, C10, C12, C13, C14, C15, C16, C17, C19, C20, C21, and C22) (Table 4, Table S4).

BI-and SE- QTL regions (BISERs) are those containing-and SE-QTLs affecting two or more different traits. SE-QTL regions (SERs) are those containing at least two SE-QTLs affecting at least two different traits. Twenty-three QTL clusters included six BISERs and 17 SERs. Six BISERs were involved in the control of two or three traits. BISER-C7-1was located near the NAU1085 marker (92.24 cM) on C7, which harbored two BI-QTLs (qFL-C7-3 and qFS-C7-4 with a positive additive effect) and one SE-QTL (qFM-C7-3 with a negative additive effect). BISER-C15-1 was located near the NAU3177 marker (43.61 cM) on C15, with two BI-QTLs (qFL-C15-1 and qFS-C15-3, with positive additive effects). BISER-C16-1 was located near the BNL2634 marker (65.97 cM) on C16, with two BI-QTLs (qFL-C16-2and qFS-C16-3, with positive additive effects). BISER-C17-1 was located near the NAU2909 marker (47.26 cM)

on C17, containing one BI-QTL (qFM-C17-2 with a negative additive effect) and two SE-QTLs (qFL-C17-3 and qFS-C17-1 with a positive additive effect).BISER-C17-2 was located near the HAU0195a marker (122.79 cM) on C17, containing one BI-QTL (qFS-C17-3 with a positive additive effect) and two SE-QTLs (qFL-C17-5 with a positive additive effect and qFM-C17-4 with a negative additive. BISER-C2-1 was located near the NAU2277 marker (178.82 cM) on C2, which contained one BI-QTL (qFL-C2-6 with a positive additive effect) and one SE-QTL (qFS-C2-2 with a positive additive effect). The Hai1 alleles in the three BISERs (BISER-C7-1, BISER-C17-1, and BISER-C17-2) simultaneously increased FL and FS and decreased FM, and those in the other three BISERs simultaneously increased FL and FS.

Among the 17 SERs, 15 affected both FL and FS. These 15 SERs (SER-C2-1,SER-C7-1,SER-C10-1, SER-C12-1, SER-C12-2, SER-C13-1, SER-C13-2, SER-C14-1, SER-C14-2, SER-C16-2, SER-C19-1, SER-C20-1, SER-C21-1, SER-C21-2,and SER-C22-1) were located near HAU1980b (54.78cM) on C2; near PGML01950 (53.19cM) on C7; near BNL3563 (178.11cM) on C10; near HAU1361 (84.57cM) and NAU4889 (110.86cM) on C12; near BNL1707 (8.68cM) and CGR5242 (164.25cM) on C13; near NAU3648 (40.73cM) and HAU1219a (206.88cM) on C14; near BNL3065 (176.71cM) on C16; near NAU3405 (17.39cM) on C19; near HAU1491a (139.9cM) on C20; near PGML00972 (238.32cM) and Gh132 (241.43cM) on C21; and near NAU1325 (152.64cM) on C22. The Hai1 alleles in two regions (SER-C14-2 and SER-C16-2) simultaneously decreased FL and FS, and those in the other 13SERs simultaneously increased FL and FS. The other two SERs (SER-C15-1 and SER-C16-1) affected FS and FM, and they were located near HAU1058a (26.9cM) on C15 and near CGR5149 (16.79cM) on C16. The Hai1 alleles in the two regions simultaneously increased FS and FM (Table 4).

Conclusion

This study represents the first report using two sets of CSSLs with different genetic backgrounds but with the same donor parent to dissect the stability of QTLsof fiber quality traits across multiple environments in cotton. A total of 76 and 120 QTLs were identified in the CSSLs with the CCRI36 and CCRI45 backgrounds, respectively. Among them, nine BI-QTLs were found, and 78 (41.71%) of the detected QTLs were reported previously. Thirty-nine and 79 were SE-QTLs in at least two environments in the CCRI36 and CCRI45 backgrounds, respectively. Forty-eight SE-QTLs, including seven BI-QTLs, was confirmed in previous reports and 61 SE-QTLs, including two BI-QTLs, were considered novel. Twenty-three clusters with BI- and/or SE- QTLs were identified, 19 of which harbored favorable alleles from G. barbadense for two or three fiber quality traits. In summary, these results revealed the BI- and/or SE- QTL regions, indicated that genetic background has a stronger effect on fiber quality traits than environmental factors, and provided insights into the effects of genetic background and environment on the expression of fiber quality QTLs in cotton. This study provides valuable information and new, stable QTL regions for further QTL cloning and improvement of fiber quality by MAS in cotton breeding.

Materials and Methods

Development of two sets of cotton CSSLsand multi-environment field experiments: Two sets of CSSLs were derived from two interspecific crosses with Hai1 (G. barbadense) as the donor parent and CCRI36 or CCRI45 (G. hirsutum) as the recipient parent. CCRI45 (also called CCRI221) is a late-maturing Upland cotton (G. hirsutum) cultivar, and CCRI36 is an early-maturing Upland cotton cultivar; both cultivars have high yield and were bred by the Institute of Cotton Research (ICR), the Chinese Academy of Agricultural Sciences (CAAS), Anyang, Henan Province. Hai1 is a cultivated line of G. barbadense with very high fiber quality

First, two crosses (resulting in two F1 populations) were performed, with Hai1 as the male parent and CCRI36 or CCRI45 (G. hirsutum) as the female parent. Subsequently, BC5F3-populations with the CCRI36 background were obtained by five generations of successive backcrossing (with CCRI36 as the recurrent parent) and two generations of self-pollination and MAS (Li et al.2019a; Li et al. 2016). Similarly, BC4F3- populations with the CCRI45 background were also obtained by four generations of backcrossing (with CCRI45 as the recurrent parent) and two generations of self-pollination and MAS (Yang et al. 2009; Li et al. 2016).

In 2009, 2660 CCRI36 × Hai1 BC5F3 individuals and 2328CCRI45 × Hai1 BC4F3 individuals were grown in the field in Anyang in Henan Province(Anyang Experiment Farm, ICR, CAAS). In 2010, BC5F3:4 individuals of CCRI36 × Hai1 and BC4F3:4 individuals of CCRI45 × Hai1 were planted in progeny rows. The single-row length was 5 m and the row width was 0.8 m in the 2009 Anyang (09HNA) and 2010 Anyang (10HNA) experiments.

Based on the above design, two subpopulations in each genetic background were randomly selected, including 408 CSSLs in the CCRI36 background, named 36Pop, and 332 CSSLs in the CCRI45 background, named 45Pop.

Subsequently, 36Popwas evaluated in a total of five environments as follows. In 2011, individual CSSLs in 36Pop (BC5F3:5) and their recurrent parent were grown in three environments at three different locations: Anyang in Henan Province (11HNA), Liaoyang in Liaoning Province (11LNL), and Shihezi in Xinjiang Autonomous Region (11XJS). In 2014, the same population and the recurrent parent were further evaluated in the Shihezi Experiment Farm of ICR of CAAS and the Experiment field of Cotton Research Institute of Xinjiang Academy of Agricultural and Reclamation Science in Xinjiang Autonomous Region (14XJS and 14XJN, respectively).

45 Pop wase evaluated in a total of seven environments as follows. Individual CSSLs in 45Pop (BC4F3:5) and their recurrent parent were grown in seven environments at four different locations in different years: Anyang in Henan Province in 2011 and 2015 (11HNAand 15HNA, respectively), Korla in Xinjiang Autonomous Region in 2011 (11XJK) and 2014 (14XJK), Alaer in Xinjiang Autonomous Region in 2014 (14XJA), and Zhoukou in Henan Province in 2014 (14HNZ) and 2015 (15HNZ)..

A randomized incomplete-block design with two replicates was adopted in all the environments, except in 15AY with one replicate, in which a randomized incomplete-block design with one replicate was adopted. In each environment, the recurrent parent, used as a control, was planted with 19 CSSLs at intervals in each of the environments. Single-row plots with a 5-m length and 0.8-m width were used in 11HNAand 15AY, whereas single-row plots with a 5-m length and 1-m width were used in 14HNZ, and 15HNZ. Two-row plots with a 3-m length and 0.4-m width between each row were used in 11LNL, whereas two-narrow row plots with a 3-m length and 0.2-m width between the narrow rows, a plastic membrane cover, and a wide/narrow row-spacing pattern (a 0.6-m width between two wide rows) were adopted in 11XJS, 11XJK, 14XJS, 14XJN, 14XJK, and 14XJA.

Local field management practices were carried out in each of the environments or locations.

Evaluation of phenotypic traits: Naturally opened bolls were collected from the BC5F3 individuals of CCRI36 × Hai1 and BC4F3 individuals of CCRI45 × Hai1 in 09AY, and 30 naturally opened bolls (from the middle of plants) were harvested in every plot (row) in the other environments (10HNA, 11HNA, 11LNL, 11XJS, 11XJK, 14XJN, 14XJS, 14XJA, 14XJK, 14HNA, 15HNA,15HNZ) for testing three important fiber quality parameters of fiber strength (FS, cN/Tex), fiber length (FL, mean upper-half length, mm) and fiber micronaire value (FM, an integrated fiber quality parameter of fineness and maturity, unit) using HFT9000 (Premier Evolvics Pvt. Ltd, India) instruments with HVICC Calibration at the Cotton Quality Supervision, Inspection and Testing Center, Ministry of Agriculture, Anyang, China.

Molecular markers and genotype detection: Genomic DNA was extracted from young leaves of the CSSLs and their parents using a modified cetyltrimethyl ammonium bromide (CTAB) method (Paterson 1993). The details for PCR amplification, PCR product electrophoresis, and silver staining were the same as in the report of Sun et al. (2012). Based on the genetic linkage map comprising 2292 marker loci distributed on 26 chromosomes and covering almost the whole cotton AD genome (5115.16 cM)with an average marker interval of 2.23 cM, 551 simple sequence repeat (SSR) markers with an average interval of 10 cM between two markers were selected for the screening of genotypes in two sets of CSSLs (Shi et al. 2015). Chromosome (C) 4 had the least number of markers (11 SSRs), while C11, C19, and C21 had the largest (30 SSRs). The longest genetic distance between two markers was 25.99 cM, and the shortest was 0.45 cM. The sequences of each primer used in this report can be downloaded at http://www.cottonmarker.org and were synthesized by Bioethics Engineering Co., Ltd(Shanghai).

Data analysis and QTL mapping: The phenotypic data from the CSSLs with the CCRI36 background in seven environments (09HNA, 10HNA, 11HNA, 11LNL, 11XJS, 14XJN, 14XJS) and with the CCRI45 background in nine environments (09HNA, 10HNA, 11HNA, 11XJK, 14XJA, 14XJK, 14HNZ, 15HNZ, 15HNA) were used for analysis. Descriptive statistical analysis, correlation analysis, and analysis of variance (ANOVA) were performed using SPSS 20.0 software (SPSS, Chicago, IL, America). Genotypic analysis of populations and analysis of chromosome introgressed segments calculations (including background recovery rate of the CSSLs, the number and length of introgressed segments) were performed using GGT 2.0 software developed by van Berloo (http://www.plantbreeding.wur.nl/UK/software_ggt.html) (van Berloo 2008).
QTL mapping was performed with QTL IciMapping (version 4.0), and the RSETP-LRTADD mapping method was applied with a logarithm of odds (LOD) threshold of 2.5 (Li et al. 2007; Wang et al. 2019).

The QTLs were named as follows: (q + trait abbreviation) + chromosome/linkage group + QTL number. QTLs for the same trait in different environments and populations were considered stable when their confidence intervals overlapped (Shi et al. 2015; Shi et al. 2016; Sun et al. 2012).

The resulting linkage map and QTLs were drawn using MapChart ver.2.2 software (Voorrips 2002).

References

1. Balakrishnan D, Surapaneni M, Mesapogu S, Neelamraju S (2019) Development and use of chromosome segment substitution lines as a genetic resource for crop improvement. Theoretical and Applied Genetics 132:1-25
2. Cao Z, Wang P, Zhu X, Chen H, Zhang T (2014) SSR marker-assisted improvement of fiber qualities in Gossypium hirsutum using G. barbadense introgression lines. Theor Appl Genet 127:587-594
3. Chen Y, Liu G, Ma H, Song Z, Zhang C, Zhang J, Zhang J, Wang F, Zhang J (2018) Identification of Introgressed Alleles Conferring High Fiber Quality Derived From Gossypium barbadense L. in Secondary Mapping Populations of G. hirsutum L. Front Plant Sci 9:1023
4. Fang DD, Jenkins JN, Deng DD, McCarty JC, Li P, Wu JX (2014) Quantitative trait loci analysis of fiber quality traits using a random-mated recombinant inbred population in Upland cotton (Gossypium hirsutum L.). Bmc Genomics 15
5. Guo LX, Shi YZ, Gong JW, Liu AY, Tan YN, Gong WK, Li JW, Chen TT, Shang HH, Ge Q, Lu QW, Sun J, Yuan YL (2018) Genetic analysis of the fiber quality and yield traits in G-hirsutum background using chromosome segments substitution lines (CSSLs) from Gossypium barbadense. Euphytica 214
6. Hu Y, Chen J, Fang L, Zhang Z, Ma W, Niu Y, Ju L, Deng J, Zhao T, Lian J, Baruch K, Fang D, Liu X, Ruan YL, Rahman MU, Han J, Wang K, Wang Q, Wu H, Mei G, Zang Y, Han Z, Xu C, Shen W, Yang D, Si Z, Dai F, Zou L, Huang F, Bai Y, Zhang Y, Brodt A, Ben-Hamo H, Zhu X, Zhou B, Guan X, Zhu S, Chen X, Zhang T (2019) Gossypium barbadense and Gossypium hirsutum genomes provide insights into the origin and evolution of allotetraploid cotton. Nat Genet 51:739-748
7. Jamshed M, Jia F, Gong J, Palanga KK, Shi Y, Li J, Shang H, Liu A, Chen T, Zhang Z, Cai J, Ge Q, Liu Z, Lu Q, Deng X, Tan Y, Or Rashid H, Sarfraz Z, Hassan M, Gong W, Yuan Y (2016) Identification of stable quantitative trait loci (QTLs) for fiber quality traits across multiple environments in Gossypium hirsutum recombinant inbred line population. BMC Genomics 17:197
8. Li B, Shi Y, Gong J, Li J, Liu A, Shang H, Gong W, Chen T, Ge Q, Jia C, Lei Y, Hu Y, Yuan Y (2016) Genetic Effects and Heterosis of Yield and Yield Component Traits Based on Gossypium Barbadense Chromosome Segment Substitution Lines in Two Gossypium Hirsutum Backgrounds. PLoS One 11:e0157978
9. Li F, Fan G, Lu C, Xiao G, Zou C, Kohel RJ, Ma Z, Shang H, Ma X, Wu J, Liang X, Huang G, Percy RG, Liu K, Yang W, Chen W, Du X, Shi C, Yuan Y, Ye W, Liu X, Zhang X, Liu W, Wei H, Wei S, Huang G, Zhang X, Zhu S, Zhang H, Sun F, Wang X, Liang J, Wang J, He Q, Huang L, Wang J, Cui J, Song G, Wang K, Xu X, Yu JZ, Zhu Y, Yu S (2015a) Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nat Biotechnol 33:524-530
10. Li FG, Fan GY, Lu CR, Xiao GH, Zou CS, Kohel RJ, Ma ZY, Shang HH, Ma XF, Wu JY, Liang XM, Huang G, Percy RG, Liu K, Yang WH, Chen WB, Du XM, Shi CC, Yuan YL, Ye WW, Liu X, Zhang XY, Liu WQ, Wei HL, Wei SJ, Huang GD, Zhang XL, Zhu SJ, Zhang H, Sun FM, Wang XF, Liang J, Wang JH, He Q, Huang LH, Wang J, Cui JJ, Song GL, Wang KB, Xu X, Yu JZ, Zhu YX, Yu SX (2015b) Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nat Biotechnol 33:524-U242
11. Li PT, Rashid MHO, Chen TT, Lu QW, Ge Q, Gong WK, Liu AY, Gong JW, Shang HH, Deng XY, Li JW, Li SQ, Xiao XH, Liu RX, Zhang Q, Duan L, Zou XY, Zhang Z, Jiang X, Zhang Y, Peng RH, Shi YZ, Yuan YL (2019a) Transcriptomic and biochemical analysis of upland cotton (Gossypium hirsutum) and a chromosome segment substitution line from G. hirsutum x G. barbadense in response to Verticillium dahliae infection. BMC Plant Biol 19:19
12. Li SQ, Liu AY, Kong LL, Gong JW, Li JW, Gong WK, Lu QW, Li PT, Ge Q, Shang HH, Xiao XH, Liu RX, Zhang Q, Shi YZ, Yuan YL (2019b) QTL mapping and genetic effect of chromosome segment substitution lines with excellent fiber quality from Gossypium hirsutum x Gossypium barbadense. Mol Genet Genomics. https://doi.org/10.1007/s00438-019-01566-8
13. Lu Q, Shi Y, Xiao X, Li P, Gong J, Gong W, Liu A, Shang H, Li J, Ge Q, Song W, Li S, Zhang Z, Rashid MHO, Peng R, Yuan Y, Huang J (2017) Transcriptome Analysis Suggests That Chromosome Introgression Fragments from Sea Island Cotton (Gossypium barbadense) Increase Fiber Strength in Upland Cotton (Gossypium hirsutum). G3 (Bethesda) 7:3469-3479
14. Luan M, Guo X, Zhang Y, Yao J, Chen W (2009) QTL mapping for agronomic and fibre traits using two interspecific chromosome substitution lines of Upland cotton. Plant Breeding 128:671-679
15. Qiu X, Chen K, Lv W, Ou X, Zhu Y, Xing D, Yang L, Fan F, Yang J, Xu J, Zheng T, Li Z (2017) Examining two sets of introgression lines reveals background-independent and stably expressed QTL that improve grain appearance quality in rice (Oryza sativa L.). Theor Appl Genet 130:951-967
16. Shi Y, Li W, Li A, Ge R, Zhang B, Li J, Liu G, Li J, Liu A, Shang H, Gong J, Gong W, Yang Z, Tang F, Liu Z, Zhu W, Jiang J, Yu X, Wang T, Wang W, Chen T, Wang K, Zhang Z, Yuan Y (2015) Constructing a high-density linkage map for Gossypium hirsutum x Gossypium barbadense and identifying QTLs for lint percentage. J Integr Plant Biol 57:450-467
17. Shi YZ, Zhang BC, Liu AY, Li WT, Li JW, Lu QW, Zhang Z, Li SQ, Gong WK, Shang HH, Gong JW, Chen TT, Ge Q, Wang T, Zhu HQ, Liu Z, Yuan YL (2016) Quantitative trait loci analysis of Verticillium wilt resistance in interspecific backcross populations of Gossypium hirsutum x Gossypium barbadense. Bmc Genomics 17
18. Song W, Wang M, Su W, Lu Q, Xiao X, Cai J, Zhang Z, Li S, Li P, Gong J, Gong W, Shang H, Liu A, Li J, Chen T, Ge Q, Shi Y, Yuan Y (2017a) Genetic and phenotypic effects of chromosome segments introgressed from Gossypium barbadense into Gossypium hirsutum. PLoS One 12:e0184882
19. Sun FD, Zhang JH, Wang SF, Gong WK, Shi YZ, Liu AY, Li JW, Gong JW, Shang HH, Yuan YL (2012) QTL mapping for fiber quality traits across multiple generations and environments in upland cotton. Mol Breeding 30:569-582
20. Xu Q, Zheng TQ, Hu X, Cheng LR, Xu JL, Shi YM, Li ZK (2015) Examining Two Sets of Introgression Lines in Rice (Oryza sativa L.) Reveals Favorable Alleles that Improve Grain Zn and Fe Concentrations. PLoS One 10:e0131846
21. Zhai H, Gong W, Tan Y, Liu A, Song W, Li J, Deng Z, Kong L, Gong J, Shang H, Chen T, Ge Q, Shi Y, Yuan Y (2016a) Identification of Chromosome Segment Substitution Lines of Gossypium barbadense Introgressed in G. hirsutum and Quantitative Trait Locus Mapping for Fiber Quality and Yield Traits. PLoS One 11:e0159101

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Identification and Characterization of a Zinc Finger Protein (Ghzfp) Gene Involved in Fungal Disease Resistance in *Gossypium Hirsutum*

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Abstract

***Background**: Cotton is the top renewable textile fiber in the world, supporting a multibillion-dollar industry. India is the major cotton producer and the United States is the third largest producer. However, future cotton production is, threatened by mainly fungal pathogens, etc. In this study, we have taken the field evaluated, G. hirsutum four cotton cultivars that were shown resistant to fungal disease. Among these, we aim to identify highly resistant cotton to develop a DNA molecular marker gene (GhZFP) for the introgression into susceptible high-yielding cotton cultivars and facilitate to advance the generations by using the DNA molecular marker through the molecular breeding approach.*

***Results**: We retrieved the cDNA sequences of Zinc Finger Proteins (ZFPs) and designed the DNA molecular markers coding for the fungal disease resistance from the NCBI data bank. The cotton genome was screened by using resistant DNA marker genes and found the fungal resistant genomic regions in some of the cotton high-yielding varieties and confirmed through PCR and RT-PCR analyses. The identified resistant genomic regions were cloned, sequenced and confirmed by nucleotide sequence similarity and multiple sequence alignment analyses. The resistant cotton genotype was identified among the genotypes screened through fungal bio-assays by gene over-expression studies.*

***Conclusions**: Cotton genotypes for the resistance to fungal disease were identified. These cotton genotypes could be used as the donor plants for the introgression of resistant genes into susceptible high-yielding cotton varieties through molecular breeding or genetic engineering approaches.*

***Keywords**: Gossypium hirsutum, DNA Molecular markers, Simple sequence repeats (SSRs), biotic stress resistance, fungal resistance, high yielding cotton varieties.*

Background

All the organisms sense the environmental cue in their way and responding to environmental stresses is crucial to survival. Being sessile in nature, plants are incapable of moving away from several stresses that occur due to changes in the environment and are often exposed to different stress factors in combination. Depending on the stress conditions, plants trigger a wide range of signaling responses by altering the gene expression and cellular metabolism to minimize the damage. It could be conceived that plants must have, therefore, developed efficient strategies to adjust their physiology to threatening environmental conditions. Biotic stresses, specifically, plant pathogenic fungi represent one of the most devastating problems for the growth and productivity of crop plants. Studying the mechanism that enables plants to sense and resist fungal attack, therefore, helps to understand how plants became such vital players in the biosphere. Hence, understanding the mechanism of plant stress responses and the development of stress-resistant crops are very important by using biotechnological tools.

Plant induces coordinated up-regulation of defense-related genes that contribute to disease resistance upon pathogen attack. Plant defense mechanisms involve the expression of R-gene encoded proteins that recognize, directly or indirectly, pathogen avirulence gene (Avr) products and trigger immune responses to protect themselves (Dangl and Jones 2001). If a plant possesses a resistance (R) gene product corresponding to the pathogen avirulence gene products, then the interaction is said to be incompatible and no disease symptom develops. Furthermore, if the plant does not possess a matching R- gene to the pathogen avirulence gene (Avr), then the pathogen is compatible, and the disease proceeds. Resistance and avirulence recognition event initiates a signaling cascade where Salicylic acid (SA) and Jasmonic acid (JA) are synthesized in two different pathways. Salicylic acid-mediated defenses are used to play a major role in regulating resistance against biotrophic pathogens, while

methyl jasmonate mediated defenses control resistance against necrotrophic pathogens (Glazebrook 2005). Methyl jasmonate is activated by herbivores, which signals the production of resistance associated molecules, while microbial infection induced the biochemical pathway to produce salicylic acid. Salicylic acid is an important signaling molecule to induce systemic acquired resistance leading to the production of pathogenesis-related (PR) protein (Ibeas et al. 2000) and other metabolites that contribute to defense (Durrant and Dong 2004). Jasmonic acid and salicylic acid are antagonistic; their biosynthesis suppresses each other (Thomma et al. 2001). Salicylic acid triggers plant resistance known as systemic acquired resistance (SAR), which induces hypersensitive response and localized cell death (Kombrink and Schmelzer 2001). The hypersensitive response is characterized by the collapse and death of tissue immediately surrounding the site of infection. One of the earliest events in incompatible plant-pathogen interaction is oxidative burst during which reactive oxygen species such as H2O2 and O2 molecules are produced (Sutherland 1991).

Hydrogen peroxide triggers the production of phytoalexins, pathogenesis-related proteins, and other HR-related events.

However fungal pathogen attack is a major stress that is commonly encountered by plants growing in their native environments. Upon exposure to these stresses, many genes are induced and their products either directly protect plants against stresses or further control the expression of other target genes (Kamalay & Goldberg, 1980; Skriver & Mundy, 1990; Gao et al., 2007), including those encoding transcription factors, enzymes, molecular chaperons, ion channels, and transporters, or alter the activities of their products (Maathuis et al., 2003; Sakuma et al., 2006; Cui et al., 2002; Ronald & Leung, 2002).

Zinc finger proteins are superfamily proteins that are involved in many aspects of plant growth and development. They are classified into nine types of their structural and functional properties, C2H2, C8, C6, C3HC4, C2HC, C2HC5, C4, C4HC3, and CCCH (C and H denote cysteine and histidine respectively) (Berg & Shi, 1996; Takatsuji, 1998; Moore & Ullman, 2003; Jenkins et al., 2005; Schumann et al., 2007). Zinc finger proteins are confirmed that are involved in abiotic and biotic stresses (Sakamoto et al., 2004; Ham et al., 2006; Oh et al., 2006; Ciftci-Yilmaz et al., 2007). The C2H2-type zinc finger protein plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis (Davletova et al., 2005). Over-expression of zinc finger protein gene OsISAP1 from rice (Oryza sativa) confers tolerance to salt and dehydration stress in transgenic tobacco (Nicotiana tabacum var. Xanthi) (Mukhopadhyay et al., 2004). However, few CCCH-type proteins associated with RNA metabolism have been characterized in eukaryotes. In animals, the best known CCCH-type zinc finger protein is tristetraprolin (TTP) in mice, which binds to AU-rich elements in the 3′ untranslated regions of tumour necrosis factor-α and granulocyte–macrophage colony-stimulating factor mRNAs for degradation (Carballo et al., 1998). Another protein, ZAP (Zinc-finger Antiviral Protein), isolated from Rat-2 fibroblasts was shown to inhibit retroviral RNA production (Gao et al., 2002). In plants, PEI1, an Arabidopsis Embryo-Specific Zinc Finger Protein gene 1, HUA1, a nuclear RNA binding protein that is regulating of stamen and carpel, FES1, FRIGIDA-ESSENTIAL 1, OsDOS, Oryza sativa delay of the onset of senescence and AtCPSF30, Arabidopsis thaliana gene encodes the 30-kD subunit of the cleavage and polyadenylation specificity factor were shown to be required for heart-stage embryo formation, floral reproductive organ identity, promotion of winter-annual habit, delay of leaf senescence and calmodulin-mediated RNA processing in Arabidopsis, respectively (Li & Thomas, 1998; Li et al., 2001; Kong et al., 2006). Recently, the zinc finger proteins AtSZF1, salt stress-inducible zinc finger protein 1, and AtSZF2 were revealed to be involved in the regulation of salt responses in Arabidopsis (Sun et al., 2007). To our knowledge, only two predicted CCCH-type zinc finger proteins that confer tolerance to stresses in transgenic plants have been identified.

The future sustainability of cotton production is threatened by biotic stress, specifically fungal pathogen (F. oxysporum etc.) causing the wilt in cotton by damaging the crop productivity. To improve the yield and productivity, it is an important need to develop a fungal-resistant cotton hybrid/variety. Given its susceptibility to biotic stresses, our attention has been focused on finding the resistant genes and their subsequent introgression into agronomically important cotton cultivars. Here, we present the identification and characterization of a novel zinc finger protein (GhZFP) from a resistant cotton genotype to improve the fungal resistance and provide evidence that GhZFP plays an important role in improving biotic stress resistance in cotton.

There are few reports on the source of resistance genes in cotton genotypes. However, in some cotton relatives of the Genus, Gossypium is a source of disease resistance genes and can be exploited by

cloning or by DNA molecular marker development through genomic approaches for the introgression into high-yielding cotton genotypes. In the Genus Gossypium, there are wild species at diploid and cultivated species at tetraploid that possess resistance to various biotic stresses. Tetraploid species of Gossypium, viz., G. hirsutum cv. WGCV79 etc. show high expression levels of resistance to fungal diseases (Pande and Narayana Rao 2001). These will constitute an ideal material to study the differences at molecular level involved in conferring resistance (Supplementary Fig.1). The objective of this study is to investigate the defense responses of the resistant cotton varieties, WGCV79, WGCV48, Srirama and ADB39, mainly the WGCV79 cotton genotype when challenged with fungal pathogen (F. oxysporum). Further, we have identified the resistant genes expressed upon interaction with F. oxysporum. The data presented here is elucidation of perception mechanisms and the regulation of defense responses of resistant cotton challenged by fungal pathogens to explore host genes and proteins.

Results

Isolation of genomic DNA from in vivo grown cotton seedlings

The best phenotype-based single plants of cotton (Gossypium hirsutum L. cultivars WGCV79, WGCV48, Srirama, and ADB39) were selected from the cotton field of RARS, Warangal. The seeds of the above four varieties were sown in pots filled with soil-rite (Fig. 1). Each of the varieties' cotyledons was directly collected into liquid nitrogen, and wrapped in aluminum foil for DNA isolation. The genomic DNA was isolated by using a modified CTAB method and good quality intact DNA was yielded. The DNA quality and quantity were checked by Nanodrop and subsequently by agarose gel electrophoresis before PCR analysis (Fig. 2). The cotyledons were collected as the above procedure and the total RNA was isolated from control and infected cotton leaf samples by the fungal pathogen (F. oxysporum) by using the TRI-reagent (Sigma-Aldrich, USA), following the manufacturer's instructions.

Fig 1

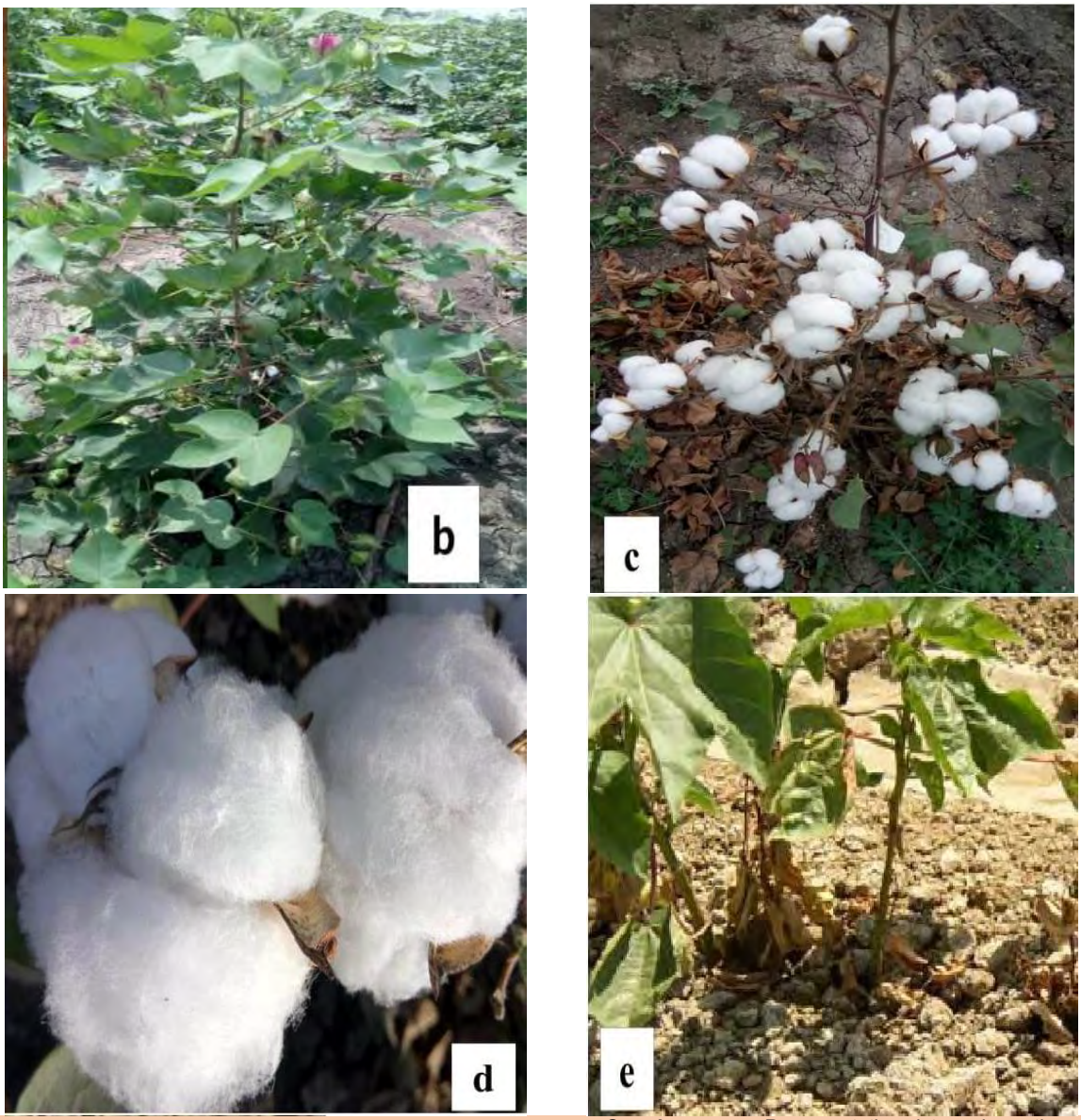

Fig. 1 *In vivo germination of cotton seedlings in the pots filled with soil-rite*

Supplementary: *Representative picture shows the reproductive and boll bursting stages of fungal disease resistant cotton crop and also the susceptible cotton plants. a,b,c,d symbols shows for the resistant cotton variety at different stages and (e) shows the susceptible cotton variety to Fusarium oxysporum infection.*

Fig. 2 *Isolation of genomic DNA from cotton leaves and DNA quantification by using 0.8% agarose gel electrophoresis*

M: Lambda DNA/EcoRI + HindIII Marker. C: C denotes DNA samples of cotton varieties

We designed the different molecular lengths of primers from the mRNA coding sequence and standardized melting temperature (Tm) and PCR amplification cycling conditions. Of which, GhZFP3, is the best DNA amplifying primer was chosen for the molecular analyses studies Further, the melting temperatures of GhZFP gene for four cotton varieties were as follows: Tm 56.5oC for WGCV48; Tm 57.1oC for WGCV79; Tm 58.4oC for Srirama and Tm 59.2oC for ADB39.

Multiple sequence alignment

The cDNA nucleotide sequence data were analyzed using BLASTN for the identification of homologs at the NCBI website (http://www.ncbi.nlm.nih.gov). Out of the BLASTN hits, we have selected the top 5 hits (in total 6 including the query protein of our interest) and downloaded their aligned sequences with our query sequence. The aligned sequences were downloaded in the FASTA format and subjected to multiple sequence alignment in TCOFFEE server. The TCOFFEE server produces HTML outputs that have color coded information about the conserved residues.

T-COFFEE, Version_11.00.d625267 (2016-01-11 15:25:41 - Revision d625267 - Build 507)
Cedric Notredame
CPU TIME:0 sec.
SCORE=964

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* BAD AVG GOOD
*Gh_001327361.1   :  98
XM_016896339.1    :  99
XM_017783701.1    :  99
XM_012596609.1    :  99
XM_016871628.1    :  99
XM_017785076.1    :  98
cons              :  96
NM_001327361.1    ACGGCCGGGGGTCCCCTTATCACTCAGCACTTTATTCTTCTCTCTCCATTAATCTTCATTGAAAGA
XM_016896339.1    ----------GTCCCCTTAGCACTCAACACTTTATTCTTCTCTCTCCATTAATCTTAGTTGAAAGA
XM_017783701.1    ----------GTCCCCTTATCACTCAACACTTTATTCTTCTCTCTCCATTAATCTTCGTTGAAAGA
XM_012596609.1    ----------GTCCCCTTATCACTCAGCACTTTATTCTTCTCTCTCCATTAATCTTCCTTGAAAGA
XM_016871628.1    ----------GTCCCCTTATCACTCAACACTTTATTCTTCTCTCTCCATTAATCTTCATTGAAAGA
XM_017785076.1    ----------------TTATCACTCAACACTTTATTCTTCTCTCTCCATTAATCTTCATTGAAAGA
cons                          *** ****** ****************************  ********
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cons              ***************** **************************************************
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cons              ************************************************************ **********
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XM_017785076.1    GCCATGGAACTTCCCCACCACGAAGCTAGGCTCCACAAATACCTCCCCTCCAATGAAGACGATGAC
cons              ********************************************** ** ************ ******
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XM_017783701.1    GGCACCGATGATCCATACGGCACCGACCATTTCCGCATGTACGAGTTCAAGGTAAGAAGGTGTACA
XM_012596609.1    GGCACCGATGATCCATACGGCACCGACCATTTCCGCATGTACGAGTTCAAGGTAAGAAGGTGTACA
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XM_017785076.1    GGTACCGAGGATCCATACGGCACCGACCATTTCCGTATATACGAGTTCAAAGTAAGAAGGTGTACA
cons              ** ***** ************************ ** ********** **********************
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XM_017785076.1    AGGAGCCGTAGCCATGACTGGACTGACTGTCCCTTCGCTCATCCAGGTGAAAAAGCCCGCCGTCGT

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XM_017783701.1  ------------------T
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XM_017785076.1  ------------------T
cons
```

***Fig.3**. Multiple sequence alignment of GhZFP with other plant species.*

PCR analysis of cotton genomic DNA for GhZFP gene template in cotton variety WGCV48

The best phenotyped, high-yielding *G. hirsutum* cv. WGCV48 cotton plants for fungal disease resistance were used for the molecular analysis. These cotton plants were taken through a single plant-based selection for molecular studies. These cotton plants were analyzed through PCR for the gene template of GhZFP in their genome. All the twenty-five cotton plants were analyzed through PCR using GhZFP molecular marker gene and found ~350bp length of DNA fragments in all the plants analyzed. In this genomic DNA analysis, we identified the house-keeping native genomic DNA region in the cotton cultivar, WGCV48 genome. Therefore, it appears that GhZFP is involved as an important regulator in plant responses to abiotic and biotic stresses.

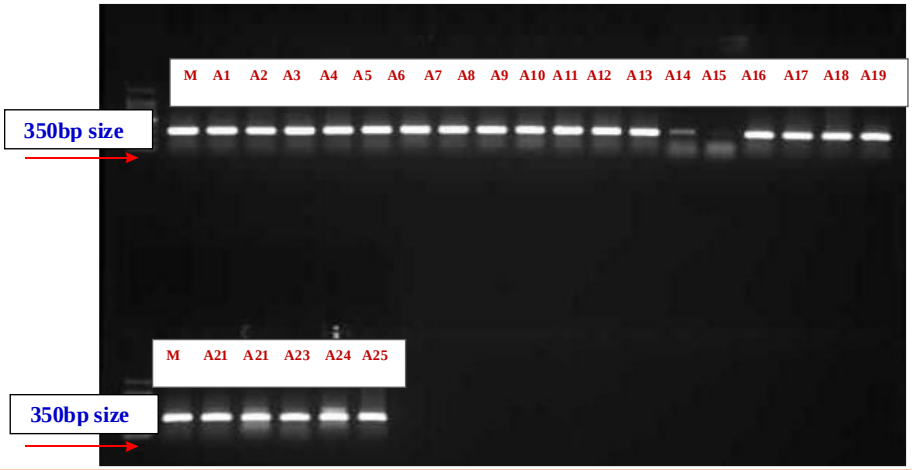

Fig. 4. Identification of genomic DNA based novel zinc finger protein (GhZFP) gene from high yielding cotton variety of WGCV48 using GhZFP DNA molecular marker gene.

M: 100bp DNA marker; DNA samples: A1 to A25 (WGCV48)

Cotton genomic DNA PCR analysis of GhZFP template in cotton variety WGCV79

We have analyzed the best phenotype based on twenty high-yielding WGCV79 cotton plants (G. hirsutum) for fungal stress resistance. All the twenty cotton plants were analyzed through PCR analysis using GhZFP molecular marker gene and found ~ 400bp length of DNA fragments in all the plants used. In this genomic DNA analysis, we identified the house-keeping native genomic DNA region in the WGCV48 genome. Further, it concluded that GhZFP is involved as an important regulator in plant responses to biotic stresses.

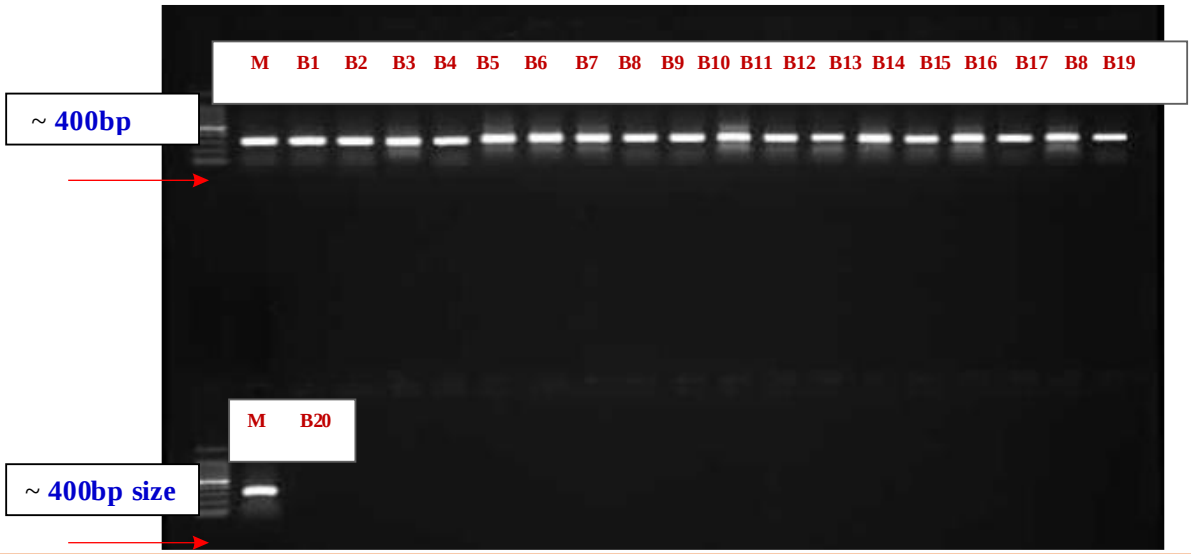

***Fig. 5**. Molecular screening of G. hirsutum cv. WGCV79 for the GhZFP gene template in it's genome by using GhZFP, DNA molecular marker gene.*

M: 100bp DNA marker; DNA samples: B1 to B20 (WGCV79)

Molecular screening of high-yielding cotton variety Srirama through PCR analysis for GhZFP template gene

We have analyzed the best phenotype based on twenty high-yielding Srirama cotton plants (G. hirsutum) for fungal stress resistance. All the twenty cotton plants were analyzed through PCR analysis using GhZFP molecular marker gene and found ~ 350bp length of DNA fragments in all the plants used. We identified the housekeeping native genomic DNA region in the Srirama cotton variety genome. Therefore, it concluded that in this genomic DNA analysis, GhZFP gene involved as an important regulator in plant responses to fungal disease resistance.

Cotton genomic DNA, PCR analysis of GhZFP template in cotton variety ADB39

We have selected the best phenotype based 14 high yielding ADB39 (G. hirsutum) cotton plants for genotyping of fungal resistance. Among the selected 14 cotton plants, 14 cotton plants were given positive results through PCR-DNA analysis for fungal resistance. In this genomic DNA analysis, we have identified the housekeeping native genomic DNA region in the ADB39 genome.

GhZFP gene - transcript expression analysis of G. hirsutum, cotton cultivars upon fungal infection.

Semi-quantitative PCR was carried out for selected gene fragments at different time points after treatment with the fungal pathogen to validate the differential gene expression. Leaf tissues of chosen resistant cotton varieties (WGCV79, WGCV 48, Srirama, and ADB39) were challenged with F. oxysporum and the samples collected at 0, 24, 48, 72 hpi as well as mock-inoculated plants. Gene-specific primers were designed from the full-length coding mRNA sequence data (NCBI) using Oligo analyzer software (IDT). First-strand cDNA was synthesized by reverse transcribing RNA (500 ng) with oligo dT (18 mer) using MMLV reverse transcriptase (Sigma-Aldrich). Subsequent PCR reactions were performed using the first strand cDNA as a template in the presence of gene-specific primers ORF-F and ORF-R. PCR reactions were standardized empirically to keep the amplification in a linear range. Actin was used as an internal control. Early upregulation of transcripts was observed during fungal pathogen infections, which was persistent till 12 hpi whereas they got upregulated at later stages also. By RT-PCR analyses, we concluded that GhZFP gene transcript levels were more upregulated in WGCV79 cotton variety compare to other cotton cultivars analyzed (Fig. 8).

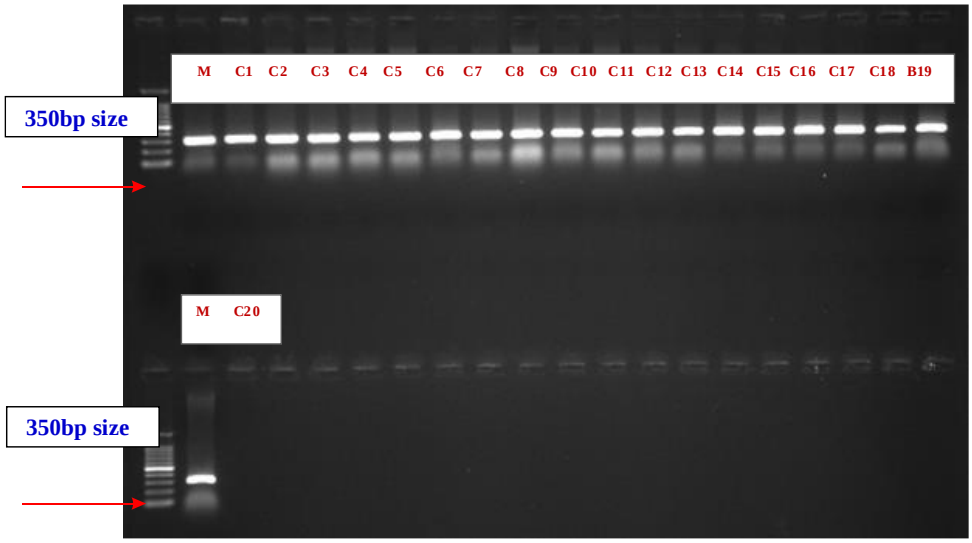

***Fig. 6**. Identification of genomic DNA based novel zinc finger protein (GhZFP) gene from cotton variety, Srirama with GhZFP, DNA molecular marker.*

M: 100bp DNA marker; DNA samples: C1 to C20 (Srirama)

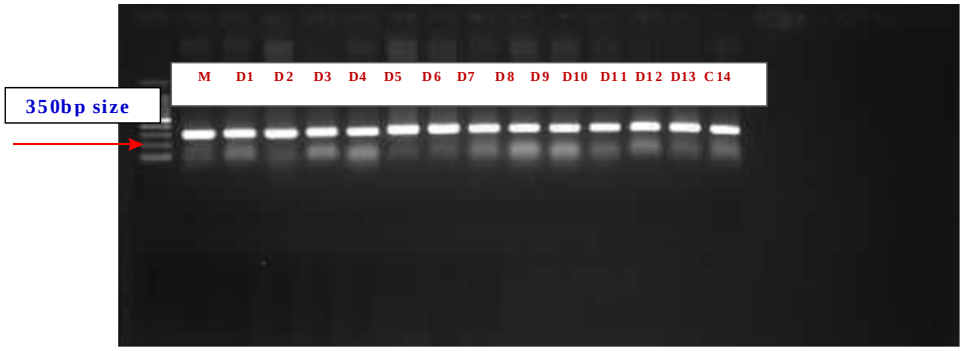

***Fig. 7.** Molecular screening of cotton variety ADB39 for GhZFP gene template through PCR analysis.*

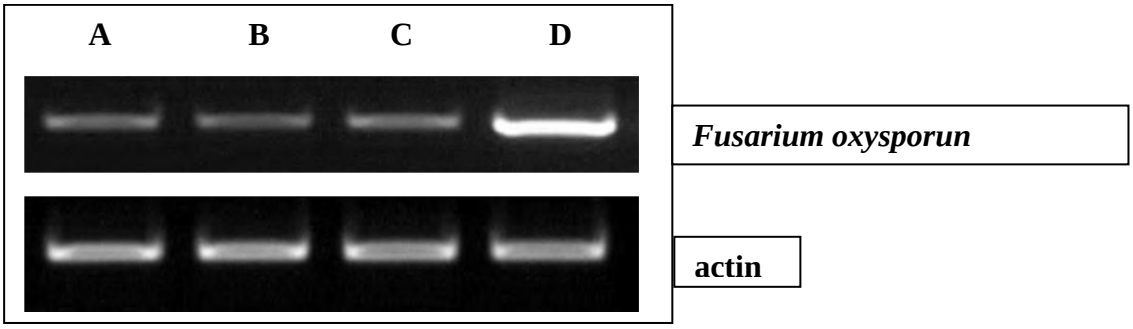

***Fig. 8.** Transcript expression analysis of GhZFP gene in four cultivars of Gossypium hirsutum was analyzed using semi-quantitative RT-PCR, during fungal pathogen treatments.*

Actin: actin gene; A: Srirama; B: ADB39: C: WGCV48; D: WGCV79

Discussions

In this study, we isolated a gene, GhZFP, encoding a novel CCCH-type zinc finger protein from a salt-induced cotton cDNA library using differential hybridization screening. By PCR-DNA analysis, we have identified the native housekeeping genomic regions in the genomes of four cotton varieties screened. And further, we have identified the best cotton genotype for fungal resistance among the four WGCV48, WGCV79, Srirama and ADB39 cotton varieties by RNA transcript and gene expression analysis. Phylogenetic analysis indicated that GhZFP belongs to a CCCH-type zinc finger protein subfamily

(SRZFP). All members of this SRZFP subfamily have several characters in common, including two typical zinc finger motifs (CX8CX5CX3H and CX5CX4CX3H) and a putative NES-like motif. In addition, they are all intronless genes that have been identified via gene structural analysis (Wang et al., 2008). The putative NES-like amino acid sequences in the SRZFP subfamily showed high similarity to those in mammalian TTP, CMG1 (mitogen-induced gene 1), IRF-3 (interferon regulatory factor 3), and E2F4 (transcription factor 4 of E2 promoter binding factor) zinc finger proteins indicating that this subfamily may act as nuclear export proteins in plants (Phillips et al., 2002; Dong et al. 2008). In view of the present results for GhZFP and the expression profiles of other SRZFP members obtained from a microarray database (Zimmermann et al., 2004; Wang et al., 2008), we propose the hypothesis that members of the SRZFP subfamily might be involved in the response to multiple stresses and may play important roles in different signal transduction pathways. To our knowledge, GhZFP is the CCCH-type zinc finger protein from the SRZFP subfamily to be identified and functionally characterized in cotton. Therefore, more studies exploring the functional diversity of the SRZFP subfamily responses to stresses in plants need to be carried out.

Biotic and abiotic stresses induce overlapping sets of genes in plants (Zhu et al., 1995; Ingram & Bartels, 1996). This suggests the hypothesis that the biotic and abiotic signal pathways may interact to activate or repress biotic and abiotic response genes in plants. Cross-coupling of transcription factors is thought to play an important role in mediating responses to various signaling events. In this study, GhZFP was induced following fungal infestation in cotton seedlings and over-expressed resulted in resistance to the fungal pathogen (F. oxysporum) (Fig. 8). These results indicate that the GhZFP gene is likely to be regulated by signaling pathways that are activated by biotic stress and SA, respectively. It will be important to determine whether GhZFP proteins can interact with other regulatory proteins that are involved in distinct signal transduction pathways. To elucidate the precise molecular mechanism of GhZFP increasing fungal disease resistance in cotton plants, two proteins – GZIRD21A and GZIPR5 – that interact with GhZFP were identified. Previous studies demonstrated that RD21A belongs to a cysteine-type proteinase family responding to water deprivation and that PR5 is one class of pathogenesis-related proteins that functions as part of the plant defense system (Koizumi et al., 1993; Stintzi et al., 1993; Wang et al., 1996). However, the roles of the RD21A and PR proteins in plant drought tolerance and disease resistance have been established, their enzymatic functions indicate that they are well suited to increase defense against pathogens (Zhu et al., 1995; Ingram & Bartels, 1996).

The present data revealed that the induced expression patterns of GZIRD21A and GZIPR5 showed a resemblance to that of GhZFP under biotic stresses. This may be a result of the increased expression of stress-inducible genes induced by the over-expression of GhZFP. The increased abundance of GhZFP transcripts probably up-regulated the transcription of several stress-inducible genes, which in turn contributed to increased tolerance/resistance under the stress conditions. Cotton plants with GhZFP gene showed resistance to fungal pathogens. Further analysis of the GhZFP binding domains demonstrated that the two zinc finger motifs and the N-terminal region consisting of amino acids 1–40 are necessary and sufficient for mediating interactions with GZIRD21A or GZIPR5, respectively. A positive domain (amino acids 186–237) and a negative domain (amino acids 41–79) for protein interaction were identified. The fact that the interaction domains for GZIRD21A and GZIPR5 are similar means that GhZFP may regulate the expression of GZIPR5 and GZIRD21A similarly or differently, and subsequently regulate biotic stress responses in plants (Ying-Hui et al. 2009). Furthermore, GhZFP may play multiple roles in different signal transduction pathways relying on different protein–protein interactions via activated or repressed responses. Recent studies suggested that some CCCH-type zinc finger proteins also act as RNA-binding proteins that interact with target RNA involved in many aspects of plant growth and development (Hall, 2005). Therefore, it might be also possible that GhZFP functions as an RNA-binding protein and regulates the RNA metabolism of stress-induced target genes by transmitting distinct sets of intracellular signals in plants. Further, it might also be possible that GhZFP functions as an RNA-binding protein and regulates the RNA metabolism of stress-induced target genes by transmitting distinct sets of intracellular signals in plants.

However, we confirmed that GhZFP is a nuclear localized protein and a comparison of the amino acid sequence of GhZFP with that of SRZFP and other members of the TTP family revealed that they show high levels of similarity in the zinc finger domains and in the putative NES peptides. In plants, the formation of the RD19 and PopP2 (Pseudomonas outer protein P2) complex was shown to be essential for the targeting of RD19 and its transfer from mobile vacuole-associated compartments to the nucleus (Bernoux et al., 2008)These findings led to the reasonable assumption that the GhZFP protein might transfer from the nucleus to the cytoplasm and facilitate the formation of higher order complexes by

interacting with GZIRD21A and GZIPR5 proteins or carry the complex into the nucleus to activate regulatory pathways under stress and thus confer tolerance to biotic and abiotic stresses in plants. In conclusion, our results strongly suggest that GhZFP acts as a novel positive regulator to confer resistance fungal pathogens in cotton plants.

Conclusions

Finally, we concluded that based on the above results, G. hirsutum cv. WGCV79 is the superior variety for fungal disease resistance and as well salinity tolerance. We identified the GhZFP is an over-expression gene for biotic stresses. Hence, it could be recommended as a donor plant for the introgression of the resistant genes into the susceptible high yielding cotton cultivars through molecular breeding approach. And also, those resistant genes could be cloned and can be transferred through genetic engineering approach into the crops of interest.

Materials and Methods

Plant Materials

The best phenotype based cotton plants resistance to fungal pathogens (Gossypium hirsutum L. cultivars WGCV79, WGCV48, Srirama and ADB39) were selected from the cotton field of Professor Jayashankar Telangana State Agricultural University, RARS, India. The seeds of the above varieties were sown in the pots filled with soil-rite.

Chemicals

All the chemicals used in the present study were procured from Sigma-Aldrich, USA; Fermentas, Germany; Takara, Clontech' New England Biolabs UK.

Genomic DNA isolation

Plant genomic DNA was isolated from the second leaf of young plants by using CTAB method (Murray and Thompson, 1980). The leaves were freshly collected, frozen in liquid nitrogen, and stored at -70 ºC. The leaf tissue (100-500 mg) was homogenized into a fine powder using liquid nitrogen. About 1.0 ml of CTAB (Cetyl/ Hexadecyltrimethyl Ammonium Bromide) extraction buffer [2% CTAB, 100 mM Tris HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl and 2% β- mercapto ethanol (β-merc)] was taken into 2.0 ml microtubes and homogenized powder was transferred to the tube, and mixed well to suspend the powder uniformly by repeated inversion of the tubes. The mixtures were incubated at 65ºC for 1 h with intermittent mixing. After incubation, 0.5 ml of Chloroform: Isoamyl alcohol (24:1) mixture was added and mixed thoroughly by repeated inversion. The two phases were separated by centrifugation at 12,000 rpm for 12 min. The upper aqueous layer was taken into a fresh 2.0 ml tube. The nucleic acid content was precipitated from the aqueous phase by mixing well an equal volume of isopropyl alcohol and incubating the tubes at -20 ºC for a minimum of 30 min. The tubes were centrifuged at 12,000 rpm for 12 min to sediment the nucleic acids. The solution was decanted completely and 1.0 ml of 75% ethanol was added and incubated for 10 min at RT. The tubes were centrifuged at 2,000 rpm for 4 min and ethanol was decanted. The pellet was air-dried and dissolved in the required volume of TE [10 mM Tris HCl and 1.0 mM EDTA (pH 8.0)] buffer.

Quantification of DNA and RNA

The quality and concentration of DNA and RNA samples were examined by agarose gel electrophoresis and Nanodrop spectrophotometer (Thermo scientific).

RNA isolation and double-stranded cDNA synthesis

The total RNA was isolated from control and infected cotton leaf samples of fungal pathogen (F. oxysporum) by using the TRI-reagent (Sigma-Aldrich, USA), following the manufacturer's instructions. To avoid DNA contamination, total RNA was treated with RNase free DNase1 (Sigma-Aldrich) according to the manufacturer's instructions. The quality of RNA was checked using the spectrophotometer (NanoDrop, Technologies Inc.) at two wavelength ratios of A260/230 and A260/280 nm. The integrity of total RNA was determined by running samples on ethidium bromide stained 1.2% agarose gel electrophoresis using Tris-boric acid EDTA (TBE) buffer. For cDNA synthesis 20 µg of total RNA was used for first strand synthesis, followed by second strand synthesis using superscriptTM double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

Consumables from Sigma-Aldrich (USA) and Invitrogen (USA) and Clontech were used for the PCR reactions. PCR reactions were performed on Eppendorf thermal cycler, Germany. PCR conditions were optimized according to the template and primer combinations.

Semi-quantitative RT-PCR analysis

Semi-quantitative PCR was carried out for selected gene fragments at different time points after treatment with the fungal pathogen to validate the differential gene expression. Leaf tissues of chosen resistant cotton varieties (WGCV79, WGCV 48, Srirama, and ADB39) were challenged with F. oxysporum and the samples were collected at 0, 24, 48, 72 hpi as well as mock inoculated plants. Gene-specific primers were designed from the full-length coding mRNA sequence data (NCBI) using Oligo analyzer software (IDT). First strand cDNA was synthesized by reverse transcribing RNA (500 ng) with oligo dT (18 mer) using MMLV reverse transcriptase (Sigma-Aldrich). Subsequent PCR reactions were performed using the first strand cDNA as the template in the presence of gene-specific primers ORF-F and ORF-R. PCR reactions were standardized empirically to keep the amplification in the linear range. For all PCR reactions Taq DNA polymerase (Sigma-Aldrich) was used. Actin was used as the internal control. All the primer details were provided in Table 1.

Table 1. Primers used in the current study – for PCR and RT PCR analysis

Primer Abbreviation	Primer Sequence ((5'-3')	Primer Size (bp)	Amplicon size (bp)
GhZFP FP	CTTCAACGGTAAGTCCTTCTGG	22	- 400
GhZFP RP	GCCCGACCAGTTTTCTTTATTT	22	
Act FP	TGGCATCAC ACT TTCTACAA	20	
Act RP	CAACGGAATCTC TCA GCT CC	20	

Sequence analysis

The cDNA sequence data were analyzed using BLASTn and BLASTp at NCBI website (http://www.ncbi.nlm.nih.gov). Nucleotide translations were performed using translate tool at ExPASy (http://www.expasy.ch/). Multiple sequence alignment was done using ClustalW (www.ebi.ac.uk) and MEGA 4.0.2 software respectively.

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References

1. Berg JM, Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. Science. 1996; 271: 1081–1085.
2. Bernoux M, Timmers T, Jauneau A, Brière C, de Wit PJ, Marco Y, Deslandes L. RD19, an Arabidopsis cysteine protease required for RRS1-R-mediated resistance, is relocalized to the nucleus by the Ralstonia solanacearum PopP2 effector. The Plant Cell. 2008; 20: 2252–2264.
3. Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science. 1998; 281: 1001–1005.
4. Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R. The EAR-motif of the Cys2/His2-type zinc finger protein Zat7 plays a key role in the defense response of Arabidopsis to salinity stress. Journal of Biological Chemistry. 2007; 282: 9260–9268.
5. Cui J, Jander G, Racki LR, Kim PD, Pierce NE, Ausubel FM. Signals involved in Arabidopsis resistance to Trichoplusia ni caterpillars induced by virulent and avirulent strains of the phytopathogen Pseudomonas syringae. Plant Physiology. 2002; 129: 551–564.
6. Dangl JL, Jones JD. Plant pathogens and integrated defence responses to infection. Nature. 2001; 411:826–833.
7. Davletova S, Schlauch K, Coutu J, Mittler R. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. Plant Physiology. 2005; 139: 847–856.
8. Dong Wang, Yinghui Guo, Changai Wu, Guodong Yang, Yingying Li and Chengchao Zheng. Genome-wide analysis of CCCH zinc finger family in Arabidopsis and rice. BMC Genomics. 2008; 9:44.
9. Durrant WE, Dong X. Systemic acquired resistance. Annu. Rev. Phytopathol. 2004; 42:185–209.
10. Gao G, Guo X, Goff SP. Inhibition of retroviral RNA production by ZAP, a CCCH-type zinc finger protein. Science. 2002; 297: 1703–1706.
11. Gao LL, Anderson JP, Klingler JP, Nair RM, Edwards OR, Singh KB. Involvement of the octadecanoid pathway in bluegreen aphid resistance in Medicago truncatula. Molecular Plant-Microbe Interactions. 2007; 20:82-93.

12. Glazebrook J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review in Phytopathology. 2005; 43:205–227.
13. Hall TM. Multiple modes of RNA recognition by zinc finger proteins. Current Opinion in Structural Biology. 2005; 15: 367–373.
14. Ham BK, Park JM, Lee SB, Kim MJ, Lee IJ, Kim KJ, Kwon CS, Paek KH. Tobacco Tsip1, a DnaJ-type Zn finger protein, is recruited to and potentiates Tsi1-mediated transcriptional activation. The Plant Cell. 2006; 18: 2005–2020.
15. Ibeas JL, Lee H, Damsz B, Prasad DT, Pardo JM, Hasegawa PM, Bressan RA, Narasimhan ML. Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein. Plant J. 2000; 23:375–383.
16. Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 1996; 47: 377–403.
17. Jenkins TH, Li J, Scutt CP, Gilmartin PM. Analysis of members of the Silene latifolia Cys2/His2 zinc-finger transcription factor family during dioecious flower development and in a novel stamen-defective mutant ssf1. Planta. 2005; 220: 559–571.
18. Kamalay JC, Goldberg RB. Regulation of structural gene expression in tobacco. Cell. 1980; 19: 935–946.
19. Koizumi M, Yamaguchi-Shinozaki K, Tsuji H, Shinozaki K. Structure and expression of two genes that encode distinct drought-inducible cysteine proteinases in Arabidopsis thaliana. Gene 1993; 129: 175–182.
20. Kombrink E and Schmelzer E. The hypersensitive response and its role in local and systemic disease resistance. European Journal of Plant Pathology. 2001; 107:69–78.
21. Kong Z, Li M, Yang W, Xu W, Xue Y. A novel nuclear-localized CCCH-type zinc finger protein, OsDOS, is involved in delaying leaf senescence in rice. Plant Physiology. 2006; 141: 1376–1388.
22. Li J, Jia D, Chen X. HUA1, a regulator of stamen and carpel identities in Arabidopsis, codes for a nuclear RNA binding protein. The Plant Cell. 2001; 13: 2269–2281.
23. Li Z, Thomas TL. PEI1, an embryo-specific zinc finger protein gene required for heart-stage embryo formation in Arabidopsis. The Plant Cell. 1998; 10: 383–398.
24. Maathuis FJ, Filatov V, Herzyk P, Krijger GC, Axelsen KB, Chen S, Green BJ, Li Y, Madagan KL, Sanchez-Fernandez R et al. Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. The Plant Journal. 2003; 35: 675–692.
25. Moore M, Ullman C. Recent developments in the engineering of zinc finger proteins. Briefings in Functional Genomics and Proteomics. 2003; 1: 342–355.
26. Mukhopadhyay A, Vij S, Tyagi AK. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proceedings of the National Academy of Sciences, USA. 2004; 101: 6309–6314.
27. Murray M, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic acids research. 1980; 8:4321-4326.
28. Oh SK, Yi SY, Yu SH, Moon JS, Park JM, Choi D. CaWRKY2, a chili pepper transcription factor, is rapidly induced by incompatible plant pathogens. Molecular Cell. 2006; 22: 58–64.
29. Pande S, Narayana Rao J. Resistance of wild Arachis species to late leaf spot and rust in greenhouse trials. Plant Dis. 2001; 85:851–855.
30. Peleg Z, Blumwald E. Hormone balance and abiotic stress tolerance in crop plants. Current Opinion in Plant Biology. 2011; 14:290-295.
31. Phillips RS, Ramos SB, Blackshear PJ. Members of the tristetraprolin family of tandem CCCH zinc finger proteins exhibit CRM1-dependent nucleocytoplasmic shuttling. Journal of Biological Chemistry. 2002; 277: 11606–11613.
32. Ronald P, Leung H. The rice genome. The most precious things are not jade and pearls. Science 2002; 296: 58–59
33. Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K. Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. Plant Physiology. 2004; 136: 2734–2746.
34. Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K. Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. Proceedings of the National Academy of Sciences, USA. 2006; 103: 18822–18827.
35. Schumann U, Prestele J, O'Geen H, Brueggeman R, Wanner G, Gietl C. Requirement of the C3HC4 zinc RING finger of the Arabidopsis PEX10 for photorespiration and leaf peroxisome contact with chloroplasts. Proceedings of the National Academy of Sciences, USA. 2007; 104: 1069–1074.
36. Skriver K, Mundy J. Gene expression in response to abscisic acid and osmotic stress. The Plant Cell. 1990; 2: 503–512.
37. Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, Legrand M, Fritig B. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. Biochimie. 1993; 75: 687–706.
38. Sun J, Jiang H, Xu Y, Li H, Wu X, Xie Q, Li C. The CCCH-type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in Arabidopsis. Plant Cell Physiology. 2007; 48: 1148–1158.
39. Sutherland MW. The generation of oxygen radicals during host plant reponses to infection. Physiological Mol Plant Pathology. 1991; 39:79–93.
40. Takatsuji H. Zinc-finger transcription factors in plants. Cellular and Molecular Life Sciences. 1998; 54: 582–596.
41. Thomma BPHJ, Penninckx IAMA, Broekaert WF, Cammue BPA. The complexity of disease signaling in Arabidopsis. Curr. Opin. Immunol. 2001, 13:63–68.
42. Waditee R, Hibino T, Nakamura T, Incharoensakdi A, Takabe T. Overexpression of a Na+/H+ antiporter confers salt tolerance on a freshwater cyanobacterium, making it capable of growth in sea water. Proceedings of the National Academy of Sciences, USA. 2002; 99: 4109–4114.
43. Wang D, Guo YH, Wu CA, Yang GD, Li YY, Zheng CC. Genome wide analysis of CCCH zinc finger family in Arabidopsis and rice. BMC Genomics. 2008; 9: 44.
44. Wang X, Zafian P, Choudhary M, Lawton M. The PR5K receptor protein kinase from Arabidopsis thaliana is structurally related to a family of plant defense proteins. Proceedings of the National Academy of Sciences, USA. 1996; 93: 2598–2602.

45. Ying-Hui Guo, Yue-Ping Yu, Dong Wang, Chang-Ai Wu, Guo-Dong Yang, Jin-Guang Huang and Cheng-Chao Zheng. GhZFP1, a novel CCCH-type zinc finger protein from cotton, enhances salt stress tolerance and fungal disease resistance in transgenic tobacco by interacting with GZIRD21A and GZIPR5. New Phytologist. 2009; 183: 62–75.
46. Zhu B, Chen TH, Li PH. Activation of two osmotin-like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. Plant Physiology. 1995; 108: 929–937.
47. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiology 2004; 136: 2621–2632.

28

Progression of Transgenic Cotton (*Gossypium Hirsutum L.*) With Glyphosate Resistant Gene is a Combinational Approach to Combat with Weeds

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Abstract

***Background**: Transgenic cotton (Gossypium hirsutum L.) engineered with broad-spectrum herbicide glyphosate has significantly enhanced agricultural efficiency across the globe. This is because the biotic stresses are becoming more severe and have a direct impact on the growth and quality of the biological yield. Cotton is a cash crop that is successfully cultivated in approximately 50 countries worldwide. It contributes a valuable addition to agricultural GDP and has prime importance in the economy of Pakistan. In addition to its significance, cotton cultivation faces numerous problems including weeds. Manual weed management is too expensive and a hectic job. In the past, hoeing was the only solution to remove weeds which resulted in a higher cost of production. Weeds can cause yield losses of up to 50% if not managed properly and impart severe loss to the economy.*

***Results**: A local cotton cultivar CIM-343 was transformed through Agrobacterium mediated transformation method with herbicide resistant gene (cp4 EPSPS). The constitutive promoter CaMV 35S was used for gene expression in the transformed cotton plants. The designed construct was used to confer resistance against herbicide in the cotton cultivar CIM-343. Once integrated into the cotton genome, the gene product, CP4 EPSPS synthase develops resistance against glyphosate. CP4 has unexpected properties, such as kinetic and structural properties that depict its uniqueness among the known EPSP synthases.*

***Conclusion**: In this study, the transformed cotton variety depicted 75% better results in comparison to the non-transgenic variety. The findings of this study recommend that farmers should use the transgenic cotton cultivar resistant to glyphosate for general cultivation to improve the production of cotton and increase their return.*

***Keywords**: Cotton, Transgenic, Agrobacterium, Biotic a-Biotic stresses.*

Background

Being a fiber crop Cotton is cultivated across the globe and is well known as “white gold”. It provides raw material to textile industries and is also used as food and animal feed. Cotton seed is the main source of cooking oil in developing countries. The remaining seed cake as a result of oil extraction is used as animal feed rich in protein (Hari, 2007; Keshamma et al., 2008; John, 2011). Lint is the premier source of cotton fiber. Spinning industries are greatly influenced by fiber quality as it has a direct impact on the quality of the product and the cost of production. Fiber characteristics including fiber length, strength, micronaire value, and color are the key characteristics contributing to fiber quality (Akhtar et al., 2004). Cotton is mostly grown in tropical and subtropical climatic conditions. On a large scale, approximately more than 50 countries across the world are involved in cotton cultivation including India, America, China, Brazil, and Pakistan. Asia shares more than 70% of the global cotton production (Zhang et al., 2019).

Cotton production faces numerous problems, such as insect pests, weeds, and different micro pathogens. After insect pests, weeds directly affect cotton production worldwide (Keshamma et al., 2008). Production losses due to weeds in cotton are approximately 40-48%. Weed plants act as a host of various insect pests and pathogens and compete with the cotton plant for space, water, and nutrition. Most of the soil nutrition is used up by the weed plants leading to a shortage of nutrient supply resulting in production losses and poor quality. In the past, weeds were controlled manually by hoeing which is a hectic and time-consuming process.

Genetic engineering and other Biotechnological tools are widely used in crop improvement (John, 2011). Among the genetically modified crops cotton was the first crop released for commercial use (Jones et al., 1996; Wilkins et al., 2005). Genetically modified crops having herbicide resistance have a dramatic impact on the world's agriculture efficiency (Owen, 2001). By genetic transformation of a bacterial gene such as 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) into crop plants can impart glyphosate tolerance (Fitzgibbon and Braymer, 1990; Padgette et al., 1991). Foliar application of nonselective Glyphosate [N-(phosphonomethyl) glycine] herbicide kills mostly all types of weeds and is more cost-effective than manual weed control. The broad-spectrum effect of glyphosate over time kills broad and narrow leave weeds (annual, biennial, and perennial), grasses with herbaceous nature, and sedges (Malik et al., 1989; Franz et al., 1997). The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is nontoxic to mammals (animals and humans) because its production pathway is unique to microbes and plants. Therefore, the use of glyphosate around the world is more than any other agrochemical due to its distinctive properties, nontoxicity to animals, cheap and easy foliar application, and broad-spectrum weed control.

EPSPS enzyme is slightly modified in Agrobacterium strain (cp4) that inhibits the binding of glyphosate and also catalyzes the reaction of amino acid synthesis. Transgenic plants expressing the EPSPS gene have the backup ability to keep making amino acids that provide resistance when plants are sprayed with glyphosate (Dill 2005).

In this study, the glyphosate tolerance gene (cp4-EPSPS) was transformed, through Agrobacterium mediated transformation method, into a locally developed CIM-343 cotton variety. Gene expression in the cotton plant was regulated by the constitutive promoter (CaMV 35S). A designed construct having GTGene was used to confer resistance against broad-spectrum herbicides. Expression of GTGene is inhibited to the specific part of the plant, especially green tissues. Thus, plant roots do not express the EPSPS gene. According to biosafety roles, this transformation would not cause any contamination that is harmful to soil microbes or the environment.

Results

Designing of Expression Vector: pCAMBIA 1301 cp4EPSPS

The gene cassette of cp4EPSPS was amplified with the aid of a gene-specific primer. The amplified PCR product was ligated into pCAMBIA 1301 by using KpnI and BamHI restriction sites. The successful ligation of 1368 bp of gene cassette into 12456 bp of pCAMBIA was confirmed through restriction digestion. The 1.4 kb of insert and 12 kb of the vector were obtained through restriction digestion with KpnI and BamHI enzymes. The total genome size of the ligated product (gene + pCAMBIA) was 13824 bp (Fig.1).

Transformation of cp4EPSPS gene construct into cotton line through Agrobacterium

Agrobacterium-mediated transformation was used to incorporate cp4EPSPS gene into local cotton cultivar CIM-343. For this purpose, de-linted cotton seed was sterilized to avoid contamination by using 1 g/L HgCl2 and 1 g/\L of 10 % sodium dodecyl sulfate (SDS). Sterilized seeds were germinated in petri plates and mature embryos were isolated for transformation. A minor cut was made on epicotyl of embryos with sterilized blade. Injured embryos and transformed Agrobacterium strain (EPSPS) were co-cultivated for 1 hour at room temperature. After incubation the embryos were dried and cultured on MS medium at 28 oC for 3 days. Optimized glyphosate dose (120 µmol/L) was used in MS medium for plants selection. After 3 days, survived seedlings were sub-cultured on MS medium containing optimized glyphosate dose. In this study control plants died due to glyphosate and transgenic plants survived. This confirmed the gene transformation in these plants. After 7 weeks of selection,120 transgenic plants were obtained from 10,000 transformed embryos. Putative transgenic plants were shifted in pots that contain loamy soil (mixture of sand, silt and clay). Putative transgenic plants with stable growth were subjected to molecular analysis.

Molecular analyses of putative transgenic plants in successive generations

The survived plants in the soil after glyphosate selection were used for further molecular analysis to assess the presence of cp4EPSPS gene and their expression.

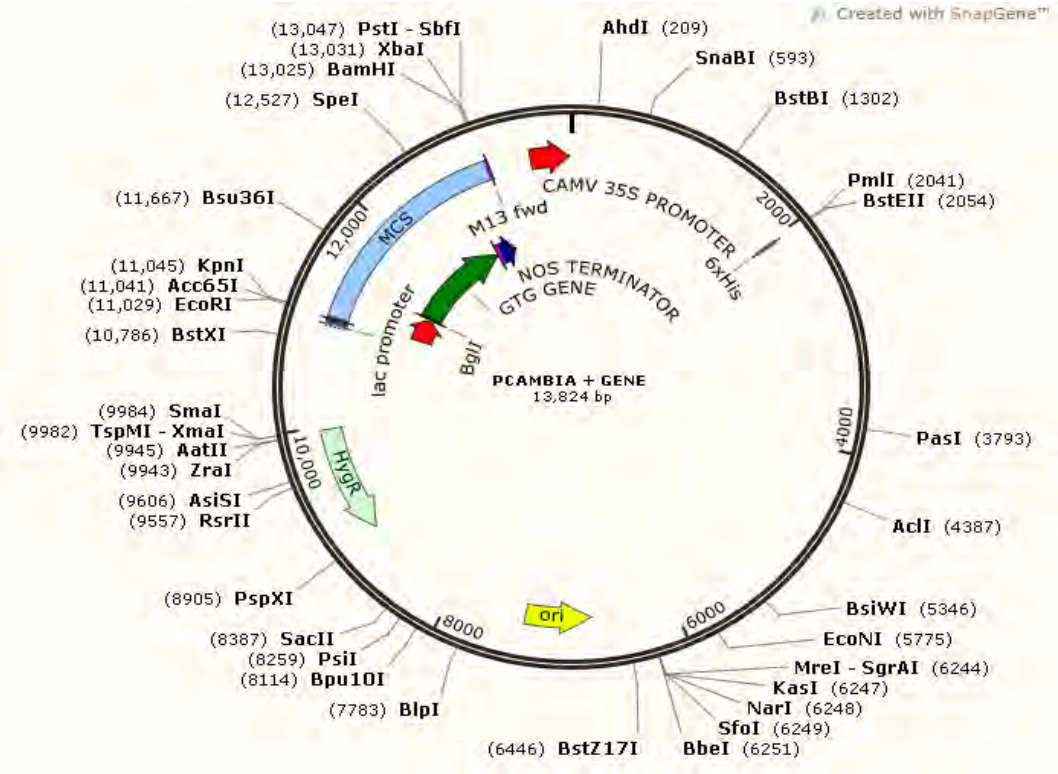


Fig. 1 *Construct map of codon optimized cp-4EPSPS cloned gene in pCAMBIA 1301 vector*

Dot Blot Analysis

DNA Dot Blot technique was used for the confirmation of putative transgenic plants. For this purpose, highly concentrated and good-quality genomic DNA was extracted from young leaves loaded on the Sensi Blot™ Plus hybond Nylon Membrane. Plasmid DNA was used as the positive control and DNA from non-transgenic plants was used as the negative control. Solution of BCIP/NBT tablets was used to develop color that confirmed the probe hybridization in positive transgenic plants. Colored spots on the membrane after the hybridization of the probe confirmed the integration of transgene in the cotton plant. Fig. 2.

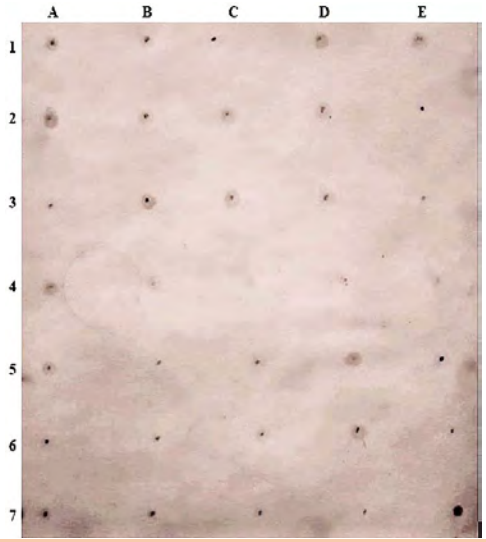


Fig. 2*: Dot Blot analysis of GTGene transformed cotton plants. Transgenic plant samples (A1, A2, A4-A7, B1-B3, C2, C3, D1-D3, D5, D6 and E1) are showing Color on membrane due to the integration of EPSPS gene in these plant genomes. D7 is the negative control (DNA from non-transgenic plant). E7 is the positive control (plasmid DNA).*

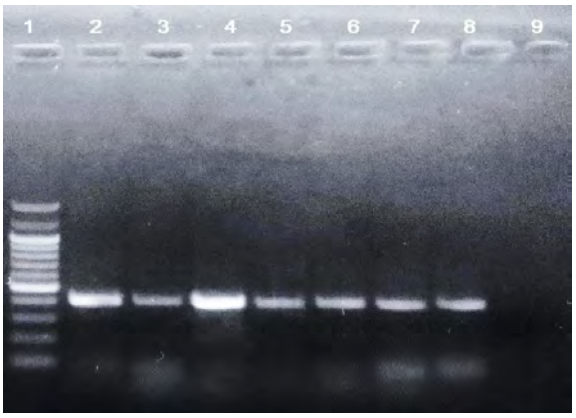


Fig. 3: *PCR amplification of GTGene from transformed cotton plants. Lane 1: 1 kb ladder (Thermo Scientific™); Lane 2; Positive control (plasmid DNA); Lane 9; Negative control (DNA from non-transgenic plants). Lane 3, 8: Amplification of 423bp from transgenic plants.*

Amplification of the cp4EPSPS gene in Cotton plants

Gene-specific short detection primers were used to detect transformed genes (cp4EPSPS) in putative transgenic plants. The amplified fragments were 423 bp in size. Only 6 plants out of 30 were found to be positive with amplification of the cp4EPSPS gene, and the control plant showed no amplification of the transgene in all three generations (Fig. 3). The amplified gene fragment was observed on 1.5 % agarose gel. Vir G primers were used to confirm the removal of Agrobacterium contamination by the complete absence of amplified band as done by Awan et al., 2015; Bajwa et al., 2013a; Bajwa et al., 2013b.

CP4EPSPS gene expression in Cotton Plants

The plants that showed amplification in PCR assay were used for further analysis. These plants expressing the cp4EPSPS gene were used for protein expression. Total protein from 6 transgenic cotton plants was extracted and analyzed by ELISA. ELISA results showed that the PCR confirmed transgenic plants expressed the cp4EPSPS gene protein in all generations while in negative control plants no protein was expressed.

Agronomic and fiber traits of Transgenic Plants

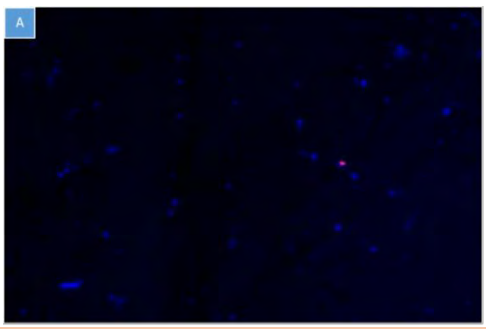
Confirmed transgenic plants were further subjected to observe their agronomical traits. Morphological traits showed significant results in all generations. The agronomical traits observed in this study are given in Table 1.

Table 1: Agronomic and Fiber traits of transgenic plants.

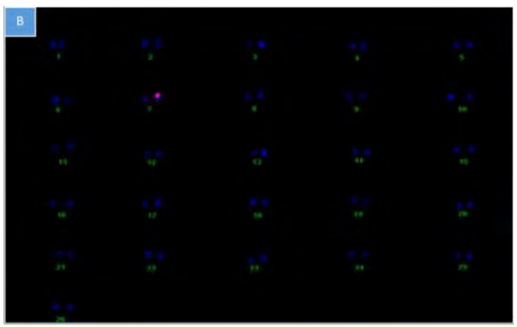
Sr No.	Agronomic traits	Observed data	Sr No.	Fiber traits	Observed data
1	Plant height	120-149 cm	**6**	Fiber Length	30.05 mm
2	Sympodial, Monopodial	15-20, 2-3	**7**	Fiber strength	30.8 g/tex
3	No of boll per plant	67-75	**8**	Micronaire	4.31
4	Average boll weight	3.5	**9**	Uniformity index	82.9%
5	seed cotton yield	210 g	**10**	Maturity	0.90

Determination of location and copy number of the transgene in cotton

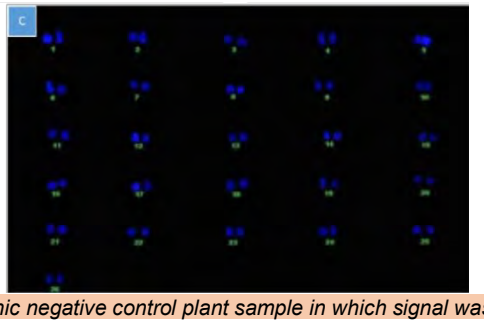
Transgenic plants transformed with GTGene were confirmed by PCR and then subjected to further analysis. Copy no of the transgene was determined by fluorescence in situ hybridization (FISH). The fluorescently labeled gene-specific probe was used for hybridization. After hybridization, a visible signal was obtained in the transgenic plant sample and the negative control plant showed no signal (Fig. 4).



A) Transgenic plant used as positive control.



B) One signal detected in CIM-343 transformed with EPSPS gene that showed one copy number.



C) Non-transgenic negative control plant sample in which signal was not detected.

Fig. 4: *FISH analysis of transformed cotton plants.*

Fig. 5*: Spray assay of glyphosate on GTG transformed plants (1900 ml/acre). A) Field before glyphosate spray assay. B) Field after herbicide spray assay all weeds died after 5 days of spray.*

Spray Assay of Glyphosate for Screening

Glyphosate assay was done on transgenic plant lines to observe the efficacy of transgene. Positive plants were subjected to leaf glyphosate spray assay. The results showed that transgenic plants with glyphosate tolerance gene survived at 1900 ml/acre (glyphosate 41 % w/w) dose of glyphosate spray while all weeds like Tribulus Terrestris, Trianthema portulacastrum, Portulaca oleracea, Corchorus tridens, Cyperus rotundus, Setaria viridis, Digera muricata, Euphorbia helioscopia, Amaranthus viridis, Echinochloa colon, Cynodon dactylon, Euphorbia prostrate, Cyperus dactylon and Sorghum halepense were killed at the mentioned dose of the glyphosate spray (Fig. 5).

Conclusion

Among biotic factors that contribute to major yield loss, weeds are more prominent and share approximately 0.1% of the world's flora. Weeds compete with crops for nutrients, water, light, and space that disturb the plant population which is the major contributing factor to yield loss. Weeds have a devastating effect on cotton yield, approximately more than 30% yield losses are observed in cotton (Khan and Khan 2003). Therefore, nowadays weed management is the major concern for cotton growers. In the past, different methods were used to control the weeds such as manual and mechanical hoeing, mulching, and crop rotation due to no availability of synthetic chemicals. Conventional methods are time taking, expensive, and hectic. With the discovery of synthetic herbicides in1930s, weed control methods were shifted towards target-oriented methods. To utilize the transgenic approach, GTGene was transformed in cotton plants. Codon optimization was done according to G. hirsutum to achieve maximum gene expression. By following the (Kiani et al., 2013; Rao et al., 2009) procedure, the cp4EPSPS gene with optimized codon was cloned in pCAMBIA 1301, by using restriction digestion with KpnI and Bam HI enzymes. After germination of sterilized cotton seed, mature embryos were isolated and subjected to Agrobacterium-inoculation after injury (Rao et al., 2009; Bakhsh et al., 2009). The seedlings were shifted to pots containing loamy soil. Afterward, acclimatization and screening were done with maker gene and PCR according to Bajwa et al (Rao et al., 2009). By using gene-specific short detection primers, 423 bp amplified fragment of cp4EPSPS was obtained, which confirmed the successful transformation of the transgene in cotton lines to develop broad-spectrum glyphosate-resistant cotton lines as done by Rao et al. (Cronn et al., 2002). The absence of VirG PCR amplified product ensured the successful elimination of Agrobacterium contamination. After PCR confirmation of cp4EPSPS gene expression, the transgenic plants were assessed by ELISA by using an Envirologic kit (cat #AP010 as done by Kiani et al. (2013). Transgenic line CIM-343 showed one copy number as indicated by FISH signals shown in Figure 4b, and in the non-transgenic plants, no signal was detected as shown in Figure 4c. The findings of this research are highly interrelated with the results of Rao et al.,

2012; Rao et al., 2013 in which similar signals were observed in transformed plants using the same methods of transformation and FISH. Moreover, a spray assay was performed to assess the gene expression of cp4EPSPS in transgenic plants. Fig.5 depicts the spray assay of transgenic plants in the field condition with 1900ml/acre of glyphosate. Plants survived after the spray assay confirmed the efficacy of cp4EPSPS gene in cotton plants.

Methods

GTGene Codon optimization according to G. hirsutum

The full-length nucleotide sequence of cp4EPSPS GTGene was retrieved from the Gene bank and analyzed for differences in codon bias. The codon usage was optimized according to the cotton plant to achieve maximum gene expression in cotton, through a freely available tool on the integrated DNA Technologies (IDT) website. Codons with fewer preferences have been replaced with their synonymous codons (Perlak et al., 1991).

Designing of cp4 EPSPS gene construct and cloning into pCAMBIA 1301

A transgenic cassette was designed from a combination of a promoter sequence (CAMV 35S), GTGene (cp4EPSPS), and transcriptional termination sequences (NOS) as shown in (Fig. 6). A selectable marker, kanamycin (neomycin phosphotransferase II, nptII) was used for maintenance in bacteria. The constitutive promoter used to express the GTGene in this construct was cauliflower mosaic virus promoter 35S (Kay and Mcpherson 1987). Restriction enzymes KpnI and BamHI were used for restriction digestion of pCAMBIA 1301 vector. The gene cassette of cp4 EPSPS was amplified with gene-specific primers. The amplified PCR product was ligated into pCAMBIA 1301 by using KpnI and BamHI restriction sites. The ligation was done through the optimized protocol of the Fermentas ligation kit (Cat #EL0014). The positive clones of the ligated product were confirmed through restriction digestion by using KpnI and BamHI restriction sites. The digested products were resolved on 1.5% agarose gel. Gene-specific primers were used for the confirmation of successful ligation into the vector.

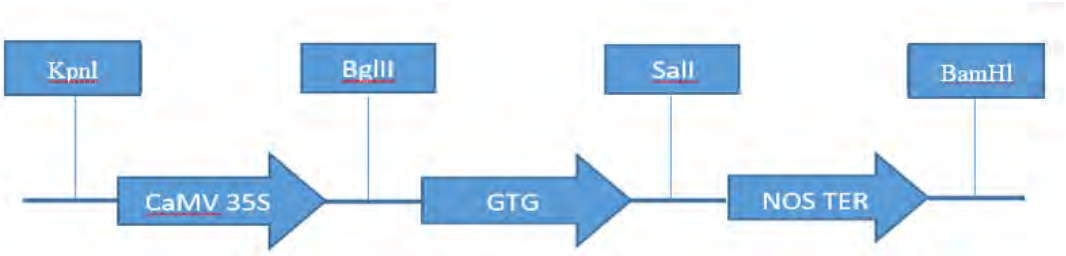


***Fig. 6**: A physical map of cp4EPSPS gene construct.*

Plant transformation with cp4EPSPS gene construct

CCRI locally developed variety (CIM-343) was used for the transformation of optimized synthetic cp4EPSPS (GTGene). By following Rao et al. (2009) procedure of transformation, the gene construct containing cp4EPSPS was transformed into Agrobacterium strain LBA4404 through electroporation and then into local cotton lines using transformed Agrobacterium with GTGene.

Dot Blot Analysis

Genomic DNA was extracted from fresh leaves by following Paterson et al. (1993) and Porebski et al. (1979) procedures and subjected to dot blot analysis for confirmation of GTGene integration in the cotton genome. Highly concentrated DNA 20 ng/µl for each sample was labeled with DIG High Prime DNA Labeling and Detection Starter Kit, and transformed on Sensi Blot™ Plus Nylon Membrane and cross-linked with UV light. After incubation of the membrane with Prehybe solution for 90 min, the probe was added and incubated for 15 hours at 65oC. After washing of membrane, BCIP/NBT tablets solution was used to develop color to confirm the probe hybridization.

PCR amplification and Confirmation of cp4EPSPS gene in cotton line

The transformation of cp4EPSPS gene into the cotton genome was done through Agrobacterium mediated transformation. Gene-specific primers were used for the confirmation of successful transformation. Short gene-specific detection primers were used for detection: Forward primer:

TCAATCTCCGTCTTGCTGGT and Reverse primer: TTGTGGCGATCATAGTTGCG. The annealing temperature of the PCR was 62°C. The bacterial DNA was used as positive control while DNA extracted from the Non-Transgenic cotton plant was used as a negative control. The amplified products were visualized on 1.5% agarose gel under ultraviolet light.

Enzyme-Linked Immunosorbent Assay (ELISA) of transformed plants

The plants confirmed for the presence of the transgene by PCR were analyzed for protein expression by an enzyme-linked immunosorbent assay (ELISA). Fresh leaves of transgenic plants were used to isolate the protein with a protein buffer using an Envirologix ELISA Kit (Cat # AP010). For quantification of total crude protein Bradford assay (Bradford 1976) was used. ELISA-kit quali plate was used to load a sample of crude protein, 20 µg were loaded in each well of the ELISA plate and ELISA was performed according to the manufacturer's instructions (Rao et al., 2011).

Fluorescence in situ hybridization (FISH)

To determine the copy number of PCR-confirmed transgenic plants, Fluorescence in situ hybridization (FISH) was performed following the procedure described by Rao et al. (2012). The probe was labeled by Mirus Label IT® FISH Cy3 Kit (cat# MIR6510, MJS BioLynx Inc. P.O Bag 1150, 300 Laurier Blvd. Brockville, ON K6 V 5W1, Canada). Radical from the germinated cotton seed was isolated to prepare the chromosomes to slide and then hybridized with a probe. The signals were detected under a Fluorescent microscope (Carl Zeiss AXIO 100) by using a suitable filter. The fluorescence signal was captured by a CCD camera and analyzed using software (Genus 3.7) provided by Applied Imaging Systems. By using the same package software, karyotyping of transgenic cotton plants was done.

Spray Assay of Herbicide of Transgenic Plants in Greenhouse and Field Condition

Transgenic cotton plants expressing the cp4EPSPS gene were firstly screened in the greenhouse and then in the field. After spray assay in the greenhouse, plant selection was done and seeds were collected from healthy plants that showed good resistance against glyphosate at 1000 ml/ha dose. Collected seeds from healthy transgenic T0, T1, and T2 plants were grown under field conditions and further assessed for their resistance to glyphosate. At this stage, the selection was done at 1900 ml/ha of Roundup-Ready glyphosate (field equivalent rate) dose on transgenic and non-transgenic control plants to screen the plants with the highest degree of tolerance. Data were recorded from both transgenic and control plants (Pline et al 2009).

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Reference

1. Akhtar KP, Hussain M, Khan AI, Haq AA, Iqbal MM, et al. Influence of plant age, whitefly population and cultivar resistance on infection of cotton plants by cotton leaf curl virus (CLCuV) in Pakistan. Field Crops Res. 2004;86, 15–21. doi: 10.1016/S0378-4290(03)00166-7.
2. Rao AQ, Bajwa KS, Puspito AN, Khan M, Abbas MA, Bakhsh A, et al. Variation in Expression of Phytochrome B Gene in Cotton (Gossypium hirsutum L). J Agric Sci Technol. 2013;15(5):1033–42.
3. Awan M, Abass M, Muzaffar A, Ali A, Tabassum B, Rao A, et al. Transformation of Insect and Herbicide Resistance Genes in Cotton (Gossypium hirsutum L.). J Agric Sci Technol. 2015.
4. Bajwa KS, Shahid A, Rao AQ, Dhab AH, Muzaffar A, Rehman HU, et al. Stable genetic transformation in cotton (Gossypium hirsutum L.) using marker genes. Adv Crop Sci. 2013;3:811–21.
5. Bajwa KS, Shahid AA, Rao AQ, Ashraf MA, Dahab AA, Bakhsh A. Expression of Calotropis procera expansin gene CpEXPA3 enhances cotton fibre strength. Australian Journal of Crop Science. 2013;7(2).
6. Bakhsh A, Rao AQ, Shahid AA, Husnain T, Riazuddin S, et al. Insect resistance and risk assessment studies in advance lines of Bt cotton harboring Cry1Ac and Cry2A genes. Am Euras J Agric Environ Sci. 2009;6(1):1–11.
7. Cronn R, Cedroni M, Haselkorn T, Grover C, Wendel JF, et al. PCR-mediated recombination in amplification products derived from polyploid cotton. Theor Appl Genet. 2002;104(2–3):482–9.
8. Dill, GM. Glyphosate-resistant crops: history, status and future. Pest Management Science: formerly Pesticide Science. 2005;61(3), 219-224.
9. Fitzgibbon JE, Braymer HD. Cloning of a gene from Pseudomonas sp. strain PG2982 conferring increased glyphosate resistance. Appl. Environ. Microbiol. 1990;56, 3382–3388.
10. Franz JE, Mao MK, Sikorski JA, et al. Glyphosate: a unique global herbicide. Washington DC: American Chemical Society. 1997;36–88.
11. Hari G. Multiple References to Non-Wood Fibers for Paper. Retrieved from Pulp & Paper Resources & Information Site. 2007.
12. John B. "Cotton subsidies, the WTO, and the 'Cotton Problem'," in The World Economy,..(Washington, DC: Wiley Blackwell), 2007;34,1534–1556. http://hdl.handle.net/10.1111/j.1467-9701.2011.01396.x
13. John, B. "Cotton subsidies, the WTO, and the 'Cotton Problem'," in The World Economy, (Washington, DC: Wiley Blackwell), 2011;34,1534–1556. http://hdl.handle.net/10.1111/j.1467-9701.2011.01396.x

14. Jones AL, Lai EM, Shirasu K, Kado CI et al. Vir B2 is a processed pilin-like protein encoded by the Agobacterium tumefaciens Ti plasmid. J. Bacteriol. 1996;178, 5706–5711.
15. Kay R, Mcpherson J. Hybrid pUC vectors for addition of new restriction enzyme sites to the ends of DNA fragments. Nucl Acids Res. 1987;15(6):2778.
16. Keshamma E, Rohini S, Rao KS, Madhusudhan B, Udayakumar M, et al. Tissue culture independent in plant a transformation strategy: an Agrobacterium tumenfaciens mediated gene transfer method to overcome recalcitrance in cotton (Gossypium hirsutum L.). J. Cotton Sci. 2008;12, 264–272.
17. Khan NU, Khan SU. Integrated weed management in upland cotton. Pakistan J Weed Sci Res. 2003;9(3–4):185–92.
18. Kiani S, Ali A, Bajwa KS, Muzaffar A, Ashraf MA, Samiullah TR, et al. Cloning and chloroplast-targeted expression studies of insect-resistant gene with ricin fusion-gene under chloroplast transit peptide in cotton. Electron J Biotechnol. 2013;16(6):13.
19. Malik J, Barry G, Kishore G. The herbicide glyphosate. BioFactors Oxf Engl. 1989;2(1):17–25.
20. Owen MDK et al. “Importance of weed population shifts and herbicide resistance in the Midwest USA corn belt,” in Proceedings Brighton Crop Protection Conference – Weeds (Farnham: British Crop Protection Council). 2001;407–412.
21. Padgette SR, Re DB, Gasser CS, Eichholtz DA, Frazier RB, Hironaka CM, et al. Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. J. Biol. Chem. 1991;266, 22364–22369.
22. Paterson AH, Brubaker CL Wendel JF et al. A rapid method for extraction of cotton (Gossypium spp) genomic DNA suitable for RFLP or PCR analysis. Plant Molecular Biology Reports. 1993; 11: 122–127.
23. Perlak FJ, Fuchs RL, Dean DA, McPherson SL, Fischhoff DA, et al. Modification of the coding sequence enhances plant expression of insect control protein genes. Proc Natl Acad Sci. 1991;88(8):3324–8.
24. Pline WA, Price AJ, Wilcut JW, Edmisten KL, Wells R, et al. Absorption and translocation of glyphosate in glyphosate-resistant cotton as influenced by application method and growth stage. Weed Sci. 2009;49(4):460–7.
25. Porebski, S., Bailey, L. G. and Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant molecular biology reporter, 15(1): 8-15.
26. Rao A, Batool F, Azam S, Shahid A, Husnain T, et al. Transgene copy number and phenotypic variations in transgenic Basmati rice. JAPS J Anim Plant Sci. 2012;22(4):1004–13.
27. Rao AQ, Bakhsh A, Kiani S, Shahzad K, Shahid AA, Husnain T, et al. The myth of plant transformation. Biotechnol Adv. 2009;27(6):753–63.
28. Rao AQ, Irfan M, Saleem Z, Nasir IA, Riazuddin S, Husnain T, et al. Overexpression of the phytochrome B gene from Arabidopsis thaliana increases plant growth and yield of cotton (Gossypium hirsutum). J Zhejiang Univ Sci B. 2011;12(4):326–34.
29. Wilkins TA, Arpat A, Sickler B, et al Cotton fiber genomics: Developmental mechanisms. Pflanzenschutz-Nachrichten Bayer. 2005;58, 119–139.
30. Zhang Y, Dong H, Academy S, et al. Yield and Fiber Quality of Cotton. 2019;1–9. doi:10.1016/B978-0-12-803581-8.11166-X.

Spatio-Temporal Cry1Ac Protein Expression and Bioassay Studies of Stabilized Transgenic Cotton Events

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Abstract

India has passed through a successful decade of Bt cotton cultivation and its impact is evidenced by an unprecedented adoption of this technology from Monsanto covering over 90% of the cotton area. Invitro meristamatic tip culture was used for transformation with two Cry1Ac constructs obtained from ICGEB, New Delhi and the University of Ottawa. From among the many positive events, three transgenic events (Event No. 32 with Cry1Ac of Ottawa University source and Event No. 76 and 78 with ICGEB source) have been identified and confirmed for gene integration and studied for detailed Spatio-temporal Cry1Ac protein production. Bioassay studies have been conducted in comparison to BGI and BGII events. The dynamics of protein expression and insect mortality in Event No. 78 (transformed with ICGEB Cry1Ac construct) are significantly superior to both the checks in all the tissues and intervals studied except in petals confirming the expression of the transgene. Event No. 32 (transformed with Altosaar Cry1Ac construct from the University of Ottawa) was on par with the checks. The stable expression of Cry1Ac protein in Event No. 78 can lead to the diversification of events and form a base for the public sector to use for gene pyramiding. This can help to develop multiple gene cotton varieties for cultivation. Event No. 78 is presently under confined field trial with the permissions of RCGM.

***Keywords**: Spatio-temporal, transgenic, cotton, Cry1Ac, protein, bioassay*

Background

Cotton has played an important role in human civilization and till today is an important commercial crop in the world. India is the country with the highest area (122.38 lakh hectares) and production (361 lakh bales of 170 Kg) in the world, All India Coordinated Cotton Improvement Project, Annual Report (2018-19). Of the whole large pest complex of cotton, larvae of American bollworm (Helicoverpa armigera) are the most devastating. Though development of cultivars resistant to this pest was of paramount importance in cotton throughout the world, in the absence of sound Host Plant Resistance (HPR), conventional plant breeding methods which are sought to improve resistance were ineffective (Dhaliwal et al., 1998; Lycett and Gierson, 1990). With the advent in Plant biotechnology field it allowed the scientists to transfer genes from soil bacterium (Bacillus thuringiensis) to plants, in the year 1987 pest resistance of plants containing Cry 1Ac gene was demonstrated, Barton et al. (1987) and in 1996 transgenic cotton harboring Cry 1Ac was de-regulated to the world market.

India has passed through well more than a decade of Bt cotton cultivation and the impact of Bt cotton is evidenced by an unprecedented adoption of this technology covering over 90% of cotton area as it delivers significant and multiple agronomic, economic, environmental and welfare benefits to Indian farmers and society. The defining feature of Bacillus thuringiensis (Bt) is its ability to produce proteinaceous crystals during sporulation. These proteins in the presence of alkaline conditions, specific proteases and receptors are lethal to lepidoteran insects and used as topical pesticides. The requirement of alkaline conditions, specific proteases and specific receptors makes Bt toxins harmless to mammals. Bt transgenic crops have been overwhelmingly successful and beneficial leading to higher yields and reducing the use of chemical pesticides and fossil fuels. Bt cotton released in 1996 is one of the most widely commercially accepted transgenic crop in the world. Cotton is a recalcitrant species and somatic regeneration for introducing transgene is very difficult with only Coker varieties which are not under cultivation have been reported to be responsive for regeneration (Davidonis and Hamilton, 1983; Kumaria et al., 2003; Trolinder and Goodin 1987, 1988 a, b). Though cotton is among the first crops in the application of transgenic plant techniques with first transgenic reported in 1987 not much progress has been made till date only due to the fact that plant regeneration via somatic regeneration

remained extremely difficult. A total of 53 cotton transgenic events have been reported worldwide out of which 22 events are reported to be not available. The only two events reported in the world from public sector in Chain also are not available (www.isaaa.org/gmapprovaldatabase). The most important pest is Heliothis armigera and Cry1Ac toxin being effective against this pest has become the most important transgenic event in the world.

Results

Spatio-temporal Cry 1Ac protein production in transgenic cotton events

Cry 1Ac protein production was quantified in three transgenic events (Event-32, Event-76, Event-78) and checks (BG-I, BG-II) in both vegetative and reproductive tissues over the chosen crop period of 60 to 150 days after sowing (DAS) at every 15 days interval.

Vegetative tissues (Leaves)

Cry 1Ac protein concentration in top leaves of was quantified from 60 DAS to 150 DAS at every 15 days interval (Table 1). The pattern of protein concentration in top leaves across all the events showed highest protein concentrations at 75 DAS. The second highest values were observed at 60 DAS followed by values at 90 DAS. From 90 days onwards the concentrations gradually decreased till 150 DAS. The protein concentrations in middle and bottom leaves were taken during their availability from 90 to 135 DAS at 15 days interval. The highest concentrations in all events was observed at 90 DAS and gradually reduced with least values at 135 DAS. The Cry 1Ac protein value of top leaves of Event-78 was 91% higher compared to the mean endotoxin concentration in checks, 128 % higher in middle leaves and 104% higher in bottom leaves. The Cry 1Ac values produced by Event No. 32 with Altosaar gene were comparable to the corresponding values of the checks. Though the protein production was higher than checks in Event No. 76 during the early stage of crop growth the values dropped at 105 DAS in the top leaves. Among the three new events studied Event No. 78 produced significantly high protein at all stages in all three leaves studied. The event mean value is 7.54 µg/g compared to 3.74 and 3.56 µg/g in checks BGI and BGII respectively. The event mean of Event 32 is 3.77 µg/g.

Reproductive tissues (Bract, Petal Staminal column Boll rind and Seeds)

Compared to leaves, reproductive tissues recorded considerably lower quantity of Cry 1Ac protein (Table 2). The protein concentrations in all the four reproductive tissues studied was highest at 90 DAS and gradually reduced till 150 DAS. Among the different reproductive tissues protein concentration was higher in bracts in all events except No. 76. The highest bract toxin was in Event-78 (5.57 µg/g) and lowest in Event-76 (0.29 µg/g). The protein concentrations in bract, stamina column and boll rind were higher in Event No. 78 than both the checks BG I and BG II while that in petal was higher than BG I and comparable to BG II. The protein concentrations of Event 32 were on par with checks. The concentrations of Event 76 similar to that observed in vegetative tissues were lesser than checks. The protein concentration in the seeds of Event 78 was significantly higher than both the checks with 6.21 µg/g compared to 2.32 µg/g in BG I and 5.82 µg/g in BG II (Table 3).

Bioassay studies for *Helicoverpa armigera* resistance

Bioassy studies were conducted on leaves and squares of the five events. The effect of Cry 1Ac expression on the survivability of Helicoverpa larvae were recorded three days after infestation to top and middle leaves at three intervals (60, 75 and 100 DAS). Larval mortality in top and middle leaves showed reduction at 100 DAS than 60 and 75 DAS. In line with the Cry 1Ac protein expression the Event-78 recorded 100 percent mortality at 60 and 75 DAS in both the leaves. This event had the highest mortality percentage and higher to both the checks at all intervals in both the leaves. Event-32 showed 100 percent mortality at 90 DAS and was on par with checks at other intervals. Bioassay in squares showed 100 percent mortality in Event-78 (Table 4 and 5).

Discussions

The insecticidal properties of Bt was recognized many years before the bacterium was identified. In the modern era the first commercial products that contain Bacillus thuringiensis were commercialized in 1938, later on in the year of 1987 pest resistance of plants that were transformed with Bt (B. thuringiensis) gene was demonstrated, Safiuddin (2009). Transgenic plants harboring Cry genes encode crystal proteins, d endo toxins which are potential insecticidal agents and cause disruption of mid gut cells when larvae ingests a Bt containing tissue, finally causing death and thereby providing an in-built mechanism of pest tolerance, Georgina et al., (2011). For the Bt-transgenic technology to be sustainable, it is important that the d endo toxin concentration is adequate in appropriate plant parts at the requisite time of the season to afford protection against target pests, primarily the bollworms, Kranthi

et al., (2011). Among the three new stabilized events the dynamics of protein expression and insect mortality in Event No. 78 are clearly superior to both the checks in all the tissues and intervals studied except in petals making it suitable for commercial use. Event 76 transformed with same gene cassette of Cry 1Ac as of event 78, showed lower expression and insect mortality. The characteristic of an event makes it unique for its transgene insertion point, copy number, expression dynamics (Guo et al., 2001; Hobbs et al., 1993; Rao, 2005). Checks BG-I and BG-II with same gene cassette of Cry 1Ac, showed higher toxin and percent mortality in BG-II compared to BG-I in line with the report of Hallad (2010). The Event No. 32 is comparable to the checks for the toxin production.

Table 1: Cry 1Ac (µg/g) protein expression in Bt cotton Events in various Vegetative tissues at different days of crop growth

Event	Treatments	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS	135 DAS	150 DAS	SEm±	CD @5%	CV (%)	Leaf Mean	Event Mean
Event No. 32	Top leaf	$^{C}4.72_{B}$	$^{D}5.90_{A}$	$^{GH}4.03_{C}$	$^{GH}3.51_{D}$	$^{D}3.36_{DE}$	$^{D}3.25_{EF}$	$^{B}3.12_{F}$	0.08	0.19	2.73	3.98	
Cry 1Ac	Middle leaf	NA	NA	$^{GH}4.08_{A}$	$^{EF}3.69_{B}$	$^{D}3.43_{C}$	$^{D}3.27_{D}$	NA	0.04	0.11	1.64	3.62	3.77
(Altosaar source)	Bottom leaf	NA	NA	$^{F}4.69_{A}$	$^{E}3.97_{B}$	$^{DE}3.25_{C}$	$^{EF}3.04_{D}$	NA	0.05	0.11	1.65	3.74	
Event No. 76	Top leaf	$^{C}4.36_{B}$	$^{B}6.99_{A}$	$^{FG}4.33_{B}$	$^{J}1.97_{C}$	$^{H}0.77_{D}$	$^{K}0.88_{D}$	$^{D}0.51_{E}$	0.06	0.14	3.52	2.83	
Cry 1Ac + Enhancer	Middle leaf	NA	NA	$^{F}4.52_{A}$	$^{E}3.83_{B}$	$^{D}3.42_{C}$	$^{D}3.30_{D}$	NA	0.05	0.11	1.31	3.76	3.17
(ICGEB source)	Bottom leaf	NA	NA	$^{F}4.50_{A}$	$^{H}3.34_{B}$	$^{G}2.61_{C}$	$^{I}1.27_{D}$	NA	0.05	0.11	2.1	2.93	
Event No.78	Top leaf	$^{A}10.49_{B}$	$^{A}12.02_{A}$	$^{C}8.22_{C}$	$^{C}6.31_{D}$	$^{B}5.94_{E}$	$^{B}5.72_{E}$	$^{A}5.39_{F}$	0.12	0.28	2.02	7.72	
Cry 1Ac + Enhancer	Middle leaf	NA	NA	$^{A}10.92_{A}$	$^{B}6.94_{B}$	$^{A}6.73_{B}$	$^{A}5.99_{C}$	NA	0.26	0.61	4.28	7.64	7.54
(ICGEB source)	Bottom leaf	NA	NA	$^{B}9.03_{A}$	$^{A}7.90_{B}$	$^{A}6.85_{C}$	$^{C}5.28_{D}$	NA	0.08	0.2	1.51	7.26	
BG-I	Top leaf	$^{B}5.59_{B}$	$^{D}5.88_{A}$	$^{E}5.06_{C}$	$^{D}4.65_{D}$	$^{C}3.83_{E}$	$^{DE}3.14_{F}$	$^{B}3.15_{F}$	0.11	0.22	3.31	4.47	
Cry 1Ac	Middle leaf	NA	NA	$^{FG}4.31_{A}$	$^{E}3.84_{B}$	$^{E}3.16_{C}$	$^{EF}2.98_{C}$	NA	0.12	0.29	4.98	3.57	3.74
(MON-531)	Bottom leaf	NA	NA	$^{I}3.60_{A}$	$^{I}3.08_{B}$	$^{E}3.11_{B}$	$^{F}2.95_{B}$	NA	0.08	0.18	2.66	3.18	
BG-II	Top leaf	$^{B}5.33_{B}$	$^{C}6.28_{A}$	$^{FGH}4.28_{C}$	$^{FG}3.56_{D}$	$^{G}2.55_{E}$	$^{H}2.15_{F}$	$^{C}1.21_{G}$	0.06	0.13	2.01	3.62	
Cry 1 Ac+ Cry 2Ab	Middle leaf	NA	NA	$^{I}3.65_{A}$	$^{GH}3.45_{A}$	$^{F}2.93_{B}$	$^{G}2.57_{C}$	NA	0.11	0.25	4.17	3.15	3.56
(MON 531+MON15985)	Bottom leaf	NA	NA	$^{D}5.47_{A}$	$^{D}4.69_{B}$	$^{DE}3.25_{C}$	$^{H}2.27_{D}$	NA	0.09	0.19	2.54	3.92	
SEm±		0.14	0.18	0.15	0.08	0.07	0.08	0.08					
CD @5%		0.31	0.38	0.31	0.16	0.14	0.16	0.17					
CV (%)		3.43	3.4	3.22	2.37	2.36	2.97	4.16					

Note: NA: Not assessed
Figures with similar alphabets (Super script) in a column do not differ significantly at by DMRT (P=0.05)
Figures with similar alphabets (Sub script) in a row do not differ significantly at by DMRT (P=0.05)
DAS : Days after sowing

Table 2: Cry 1Ac (µg/g) protein expression in Bt cotton Events in various reproductive tissues at different days of crop growth

Event	Plant tissue	90 DAS	105 DAS	120 DAS	135 DAS	150 DAS	Sem±	CD @5%	CV (%)	Mean
Event No.32 Cry 1Ac (Altosaar source)	Bract	$^{F}2.54_{A}$	$^{J}1.30_{D}$	$^{F}1.93_{B}$	$^{E}1.8_{B}$	$^{E}0.955_{D}$	0.03	0.08	2.2	1.71
	Petal	$^{E}2.86_{A}$	$^{F}2.02_{B}$	$^{J}0.99_{C}$	NA	NA	0.06	0.17	3.18	1.96
	Staminal coloumn	$^{G}1.9_{A}$	$^{H}1.55_{B}$	$^{H}1.42_{C}$	NA	NA	0.02	0.06	1.69	1.62
	Boll rind	NA	$^{B}3.30_{A}$	$^{A}3.01_{B}$	$^{A}2.84_{B}$	$^{D}1.856_{C}$	0.05	0.14	2.36	2.75
Event No.76 Cry 1Ac + Enhancer (ICGEB source)	Bract	$^{M}0.29_{A}$	$^{N}0.2_{B}$	$^{M}0.15_{B}$	$^{H}0.06_{C}$	$^{H}0.001_{C}$	0.003	0.009	2.98	0.14
	Petal	$^{F}2.43_{A}$	$^{K}1.21_{B}$	$^{L}0.77_{C}$	NA	NA	0.03	0.007	2.05	1.47
	Staminal coloumn	$^{L}0.62_{A}$	$^{O}0.005_{B}$	$^{N}0.001_{B}$	NA	NA	0.009	0.02	4.55	0.21
	Bollrind	NA	$^{O}0.004_{A}$	$^{N}0.003_{A}$	$^{I}0.002_{A}$	$^{H}0.001_{A}$	0.001	0.002	4.38	0.00
Event No.78 Cry 1Ac + Enhancer (ICGEB source)	Bract	$^{A}5.57_{A}$	$^{A}3.42_{B}$	$^{B}2.87_{C}$	$^{D}2.27_{D}$	$^{A}2.25_{D}$	0.069	0.15	1.9	3.28
	Petal	$^{H}1.6_{A}$	$^{I}1.38_{B}$	$^{I}1.13_{C}$	NA	NA	0.01	0.03	1.7	1.37
	Staminal coloumn	$^{D}2.93_{A}$	$^{E}2.61_{B}$	$^{I}1.16_{C}$	NA	NA	0.04	0.002	1.77	2.23
	Boll rind	NA	$^{E}2.62_{A}$	$^{D}2.48_{B}$	$^{D}2.27_{C}$	$^{B}2.1_{D}$	0.06	0.14	3.17	2.37
BG-I Cry 1Ac (MON-531)	Bract	$^{B}3.12_{A}$	$^{D}2.90_{B}$	$^{E}2.34_{C}$	$^{E}1.87_{D}$	$^{G}0.79_{E}$	0.04	0.09	2.05	2.20
	Petal	$^{K}1.25_{A}$	$^{M}1.07_{B}$	$^{J}0.97_{C}$	NA	NA	0.04	0.11	4.56	1.09
	Staminal coloumn	$^{J}1.34_{A}$	$^{L}1.14_{B}$	$^{J}0.96_{C}$	NA	NA	0.03	0.07	2.86	1.14
	Boll rind	NA	$^{I}1.42_{A}$	$^{H}1.39_{B}$	$^{F}1.29$	$^{EF}0.94_{D}$	0.02	0.05	1.93	1.26
BG-II Cry 1 Ac+ Cry 2Ab (MON 531+MON15985)	Bract	$^{C}3.04_{A}$	$^{C}2.95_{B}$	$^{C}2.83_{C}$	$^{G}2.18_{D}$	$^{C}1.94_{E}$	0.03	0.09	1.67	2.58
	Petal	$^{H}1.69_{A}$	$^{I}1.45_{B}$	$^{I}1.16_{C}$	NA	NA	0.02	0.06	2.04	1.43
	Staminal coloumn	$^{I}1.63_{A}$	$^{K}1.22_{B}$	$^{K}0.91_{C}$	NA	NA	0.05	0.11	4.32	1.25
	Boll rind	NA	$^{G}1.73_{A}$	$^{G}1.45_{B}$	$^{G}1.10_{C}$	$^{F}0.93_{D}$	0.06	0.1	4.81	1.30
	SEm±	0.04	0.03	0.03	0.02	0.03				
	CD @5%	0.09	0.07	0.06	0.05	0.07				
	CV (%)	2.47	2.85	2.74	2.12	3.99				

Note: NA: Not assessed
Figures with similar alphabets (lower case) in a column do not differ significantly at by DMRT (P=0.05)
Figures with similar alphabets (upper case) in a row do not differ significantly at by DMRT (P=0.05)
DAS : Days after sowing.

Table 3: Variation in Cry1Ac toxin expression in Seeds of Transgenic Events

Plant tissue	Toxin (µg/g) of seed
Event No.32 Cry 1Ac (Altosaar source)	2.25
Event No.76 Cry 1Ac + Enhancer (ICGEB source)	1.47
Event No.78 Cry 1Ac + Enhancer (ICGEB source)	6.21*
BG-I Cry 1Ac (MON-531)	2.32
BG-II Cry 1 Ac+ Cry 2Ab (MON 531+MON15985)	5.82
SEm±	0.03
CD @5%	0.07
CV (%)	1.14

Figures with similar alphabets (lower case) in a column do not differ significantly at by DMRT (P=0.05)

It is evident by earlier works on d endo toxin concentration that a minimum of 1.81 to 1.9 µg/g of Cry 1Ac toxin is necessary to bring about 90 per cent of mortality (LC90%) in Helicoverpa armigera as evident by bioassay tests conducted using Heicoverpa neonates from 19 different cotton growing districts of India, Anon, et al. (2004), where in toxin concentration of more than 1.9µ g/g in leaf tissues brought 100 per cent mortality of H armigera neonates. The pooled toxin expression in different tissues as detailed in Table 6 show more than 3 µg/g in seven out of the eight tissues studied in Event No. 78. This will safely ensure the insect mortality.

Table 4: Bio-efficacy of different transgenic events against American bollworm *Helicoverpa armigera* Hub in leaves

Event/Genotype	Plant part	Neonate mortality (%)			
		60 DAS	75 DAS	100 DAS	Mean
Event No.32 Cry 1Ac (Altosaar source)	Top Leaf	^{ab}96.30 (78.90)	^{a}100.0 (90)	^{c}85.69 (67.8)	94 (75.8)
	Middle Leaf	^{a}100 (90)	^{a}100.0 (90)	^{bc}89.29 (70.9)	96.4 (79.1)
Event No.76 Cry 1Ac + Enhancer (ICGEB source)	Top Leaf	^{b}92.13 (73.7)	^{b}86.32 (68.3)	^{c}86.71 (68.6)	88.38 (70.1)
	Middle Leaf	^{ab}96.3 (78.9)	^{a}96.62 (79.4)	^{d}79.39 (63.0)	90.67 (72.2)
Event No.78 Cry 1Ac + Enhancer (ICGEB source)	Top Leaf	^{a}100.0 (90)	^{a}100.0 (90)	^{ab}92.60 (74.2)	97.53 (81.0)
	Middle Leaf	^{a}100.0 (90)	^{a}100.0 (90)	^{a}96.43 (79.1)	98.8 (83.7)
BG-I Cry 1Ac (MON-531)	Top Leaf	^{c}85.19 (67.4)	^{b}86.67 (68.6)	^{e}71.45 (57.7)	81.1 (64.2)
	Middle Leaf	^{b}92.60 (74.2)	^{a}96.60 (79.4)	^{d}78.59 (62.4)	89.3 (70.9)
BG-II Cry 1 Ac+ Cry 2Ab (MON 531+MON15985)	Top Leaf	^{ab}96.30 (78.9)	^{a}96.60 (79.4)	^{bc}89.29 (70.9)	94.1 (75.9)
	Middle Leaf	^{a}100.0 (96)	^{a}100.0 (90)	^{ab}92.84 (74.5)	97.6 (81.1)
	SEm±	2.90	2.37	2.35	
	CD 5%	6.05	4.95	4.91	
	CV (%)	3.70	3.02	3.35	

Note: DAS: Days after sowing
Figures in the parentheses are arc sine transformations
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table 5: Bio-efficacy of different transgenic events against American bollworm *Helicoverpa armigera* Hub in squares

Event	Neonate Mortality (%)
Event No.32 Cry 1Ac (Altosaar source)	^{b}96.3 (78.84)
Event No.76 Cry 1Ac + Enhancer (ICGEB source)	^{b}29.6 (33.43)
Event No.78 Cry 1Ac + Enhancer (ICGEB source)	^{a}100.0 (90.05)
BG-I Cry 1Ac (MON-531)	^{a}96.3 (78.7)

BG-II Cry 1 Ac+ Cry 2Ab (MON 531+MON15985)	[a]100.0 (90.05)
SEm± CD @ 5% CV %	3.18 5.42 3.56

Note: DAS: Days after sowing
Figures in the parentheses are arc sine transformations
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table 6: Pooled concentrations of Cry1Ac toxin in different tissues of events

Events/ Genotypes	Top leaf	Middle leaf	Bottom leaf	Bract	Petals	Staminal column	Boll rind	Seeds
Event No.32 Cry 1Ac (Altosaar source)	4.72	5.9	3.73	1.51	2.44	1.73	3.05	2.25
Event No.76 Cry 1Ac (ICGEB source)	4.36	6.99	2.93	0.14	1.47	0.21	0.00	1.47
Event No.78 Cry 1Ac (ICGEB source)	10.49	12.02	7.26	3.28	1.22	2.23	2.37	6.21
BG-I Cry 1Ac (MON-531)	5.59	5.88	3.18	2.20	1.10	1.15	1.26	2.32
BG-II Cry 1 Ac+ Cry 2Ab (MON 531 + MON15985)	5.33	6.28	3.92	2.69	1.40	1.25	1.30	5.82

Note: DAS: Days after sowing
Figures in the parentheses are arc sine transformations
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Apart from the variability of protein dynamics between different events, in the single event also a pattern of variation in the expression over crop growth in the same tissue and the variation of toxin in different tissues as detailed in the results was observed in the present study. Such important pattern of Cry protein accumulation were also made viz. i) Attenuation in Cry protein concentration over the season (Adamczyk et al., 2009; Bakhsh et al., 2009; Chen et al., 2000; Finnegan et al., 1998; Fitt et al., 1998 ; Greenplate et al., 1998 ; Kranthi et al,. 2005 ; Mahon et al., 2002 ; Manjunatha et al., 2009; Olsen et al., 2005; Xia et al., 2005), ii) Variable Cry 1Ac protein concentration in different plant parts, being higher in leaves and notably lower in reproductive tissues (Bakhsh et al., 2009; Chen et al, 2000; Kranthi et al., 2005). The mechanism behind the 'spatio-temporal' variation in transgene expression is subtle and rather complicated, still it can be attributable to facts like site of transgene insertion, copy number, kind of promoter. Olsen et al.,(2005) is of opinion that initiation of squaring in cotton lowers the levels of Cry 1Ac transcripts and Xia et al., (2005) while working on temporal variation in Cry 1Ac gene expression hypothesized that over expression of Bt gene at earlier stages lead to gene regulation at post-transcriptional levels and consequently gene silencing.

Many research reports correlate concentration of Cry 1Ac protein in plant tissues with H. armigera mortality and hence a common phenomenon that the efficacy is relatively higher in early growing season and reduces significantly over the season. (Greenplate 1999; Greenplate et al, 2000; Xia et al, 2005). Similarly temporal differences in H. armigera mortality was also observed in this study in transgenic lines as well as in checks, although the extent of decline differed from event to event and from the results obtained it was clear that Event-78 can bring highest insect deaths in leaves (92.6 to 100 %) and in squares (100%) in comparison with checks BG-I (leaves – 71.45 to 96.6 % ; squares – 96.3 %), BG-II (leaves-89.29 to 100 % ; squares – 100%). Though it's a well-known fact that dual toxin Bt cottons do provide substantially better control of bollworms, Stewart et al, (2001), Event-78 with single gene showed higher levels of neonate mortality marked by BG-II.

The Event No. 78 with the permissions from RCGM has been evaluated under confined field trial during kharif 2019-20 and results for cry protein expression are found to be stable as observed in the present study in the field trial also.

Methods

Establishment of Plants

Three transgenic cotton events namely Event-32, Event-76 and Event-78 carrying Cry 1Ac in the genetic background of cultivar RAH-100 (released variety belonging to Gossypium hirsutum. L in T3 generation were used. Event-32 was transformed with Cry 1Ac gene from Dr. Altosaar, University of Ottawa, Canada (Altosaar source) and Event-76 and 78 were transformed with Cry 1Ac with promoter with enhancer obtained from Dr. Raj Bhatnagar, International Centre for Genetic Engineering and Biotechnology, New Delhi (ICGEB -source).

Delinted seeds of transgenic events (15 seeds/event) and two commercial checks Bunny (BG-I with Cry1Ac, Mon531 event) and Kanaka (BG-II, with Cry1Ac, Mon531 event + Cry2Ab, 15985 **event**) were surface sterilized by dipping in 0.2 per cent Mercuric chloride for 20±2 minutes with constant stirring followed by three washes with sterile water and germinated at 260C. The germinated seeds were sown in pots filled with soil, sand and vermicompost in the ratio of 1:1:1 and maintained in green house at 28±20 C and 60 to 80 per cent relative humidity..

Characterization of Events for Cry 1Ac Protein Production

Enzyme Linked Immunosorbent Assay technique was utilized to quantify Cry 1Ac protein production using commercially available ELISA kit (Envirologix Inc. Kit # 40923, Portland, USA). For characterizing events for Cry protein production, different vegetative tissues viz., top, middle, bottom leaves and reproductive tissues viz., petals, bracts, staminal column, boll rind, mature seeds were used for analysis as and when they are borne on transgenic plants.

Fresh tissue was weighed and ground using 1x extraction buffer provided with kit, further analysis was performed in accordance with the procedure furnished in the kit. Quantification of Cry 1Ac protein was carried out by plotting absorbance values of sample on the standard curve generated with standards on each ELISA plates and values are expressed as micrograms of Cry 1Ac protein/ gram of fresh tissue.

Bioassay studies for *Helicoverpa armigera* resistance

Laboratory bioassay with 1st instar larvae of Helicoverpa armigera were conducted using leaf feeding method to check efficacy of different events against target pest at 60, 75 and 100 DAS. Ten healthy neonate larvae were released on leaf and squares contained in autoclaved petri plates, wet cotton swab was wrapped around leaf pedicel to maintain leaf greenness throughout the study. Petri plates were sealed with parafilm and perforations were made to facilitate air movement. Mortality observations were recorded three days after release and per cent mortality was calculated by Abbott's formula (Abbott, W. S., 2004).

$$\text{Percent mortality} = \frac{\text{Percent Survivability in control} - \text{Percent Survivability in treatment}}{\text{Percent Survivability in control}} \text{X } 100$$

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References

1. Abbott, W. S. (1925) A method of computing the effectiveness of an insecticide. J. Econ Entomol., 18, 265-267.
2. Adamczyk, J. J., Willium, R. and Meredith, J. (2004) Genetic bases for viability of Cry 1Ac expression among commercial transgenic Bacillus thuringensis (Bt) cotton cultivars in the Unites States. The J Insect Sci.,8, 17-23.
3. Anonymous (2004) Monitoring for shifts in baseline susceptibility (development of tolerance/resistance) in the cotton bollworms (Helicoverpa armigera, and Earias vittella against Cry 1A(c) toxin in various cotton growing regions of the country. Annual Progress Report 2003 04., Central Institute for Cotton Research, Nagpur.
4. Bakhsh, A., Rao, A. Q., Shahid, A. A., Husnain, T. and Riazuddin, S. (2009) CaMV35S is a Developmental Promoter Being Temporal and Spatial in Expression Pattern of Insecticidal Genes (Cry1Ac & Cry2A) in Cotton. Res J Cell and Molecular Bio., 3(1), 56-62.
5. Barton, K.A., Whiteley, H.R. and Yang, N.S., (1987) Bacillus thuringiensis δ-endotoxin expressed in transgenic Nicotiana tabacum provides resistance to Lepidopteran insects. Plant Physiol., 85, 1103–1109.
6. Chen, S., Wu, J., Zhou, B., Huang, J. and Zhang. R., (2000) On the temporal and spatial expression of Bt toxin protein in Bt transgenic cotton. Acta Gossypii Sin.,12, 189-193.
7. Davidonis, G.H. and Hamilton, R.H., (1983) Plant regeneration from callus tissue of Gossypium hirsutum L. Plant Sci. Lett., 32, 89-93.
8. Dhaliwal, Harcharan S., Kawai Maki and Uchimiya Hirofumi., (1998) Genetic engineering for abiotic stress tolerance in plants. Plant Biotechnology, 15 (1), 1-10.

9. Finnegan, E. J., Lllewellyn, D. J. and Fitt, G. P., (1998) What's happing to the expression of the insect protection in field-grown Ingard cotton. In: ed. Proceedings 9th Australian Cotton Conference, 12– 14 August, Broadbeach, Queensland, Australia.
10. Fitt, G. P., Daly, J. C., Mares, C. L. and Olsen, K., (1998) Changing Efficacy of Transgenic Cotton Plant Patterns and Consequences. Sixth Australian Entomological Research Conference., Brisbane, Australia.
11. Greenplate, J. T., (1999) Quantification of Bacillus thuringiensis insect control protein Cry 1Ac over time in Bollgard cotton fruit and terminals: J Econom Entomol., 92, 1377–1383.
12. Greenplate, J. T., Head, G. P., Penn, S. R. and Kabuye, V. T., (1998) Factors potentially influencing the survival of Helicoverpa zea on Bollgard cotton. In: P. Dugger, and D. A. Richter, eds. Proceedings, Beltwide Cotton Conference, pp. 1030-1033. National Cotton Council of America, Memphis, TN.
13. Greenplate, J. T., Penn, S. R., Mullins, J. W. and Oppenhuizen, M., (2000) Seasonal Cry1Ac levels in DP50B: the Bollgard basis for Bollgard II. In: P. Dugger, and R. Richter, eds. Proceedings, Beltwide Cotton Conference, San Antonio, pp. 1039-1041. National Cotton Council of America, Memphis, TN.
14. Guo, W. Z., Sun, J., Guo, Y. F. and Zhang, T. Z., (2001) Investigation of different dosage of inserted Bt genes and their insect-resistance in transgenic Bt cotton. Acta Genet. Sin., 28, 668-676.
15. Hallad, Anand., (2010) Comparitive efficacy of first and second generation Bt transgenic cottons. M. Sc. Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India).
16. Hobbs, S. L. A., Warkentin, T. D. and Delong. C. M. O., (1993) Transgene copy number can be positively or negatively associated with transgene expression. Plant Mol. Biol., 21, 17-26.
17. Kranthi, K. R., Naidu, S., Dhawad, C. S., Tatwawadi, A., Mate, K., Patil, E., Bharose, A. A., Behere, G. T., Wadaskar, R. M. and Kranthi, S., (2005) Temporal and intraplant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera. Curr. Sci., 89, 291-298.
18. Kranthi, K.R., Venugopalan, M.V. and Yadav, M.S., (2011) CICR- Vision 2030, Director Central Institute for Cotton Research, Nagpur., http://www.cicr.org.in/pdf/cicr_vision_2030.pdf 1-5.
19. Kumaria, R., Sunnichan, V. G., Das, D.K., Gupta, S.K., Reddy, V. S., Bhatnagar, R. K., and Leelavati (2003) High frequency somatic embryo production and maturation into normal plants in cotton (Gossypium hirsutum) through metabolic stress. Plant Cell Rep., 21, 635-639
20. Lycett, G.W. and Grierson, D., (1990) Genetic engineering of crop plants. Butterworth Heinemann, London,UK,. 303 ISBN 0-40-804779-8.
21. Mahon, R., Finnergan, J., Olsen, K and Lawrence, L., (2002) Environmental stress and the efficacy of Bt cotton. Aust. Cotton Grower, 22, 18-21.
22. Manjunatha, R., Pradeep, S., Sridhar, S., Manjunatha, M., Naik, M. I., Shivanna, B. K. and Hosamani, V., (2009) Quantification of Cry1Ac protein over time in different tissues of Bt cotton hybrids. Kar J. Agric. Sci., 22, 609-610.
23. Olsen, K. M., Daly, J. C., Holt, H. E. and Finnegan, E. J., (2005) Season-long variation in expression of Cry1Ac gene and efficacy of Bacillus thuringiensis toxin in transgenic cotton against Helicoverpa armigera (Lepidoptera: Noctuidae). J. Econom. Entomol. 98: 1007-1017.
24. Rao, C. K., (2005) Transgenic Bt Technology: 3. Expression of Transgenes. http: //www. monsanto. co. uk/news/ukshowlib. phtml?uid¼9304.
25. Saifuddin, N., Wong, C.W. and Nuryasumira, A.A., (2009) Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. Eur. J. Chem., 6, 61-70.
26. Sanahuja, Raviraj Banakar, Richard M., Twyman, Teresa Capell and Paul Christou (2011) Bacillus thuringiensis: a century of research, development and commercial applications Georgina. Plant Biotechnology Journal,9,283–300.
27. Trolinder N. and Goodin J. (1987) Somatic embryogenesis and plant regeneration in cotton (Gossypium hirsutum L.) Plant Cell Rep., 6,231-234.
28. Trolinder N. and Goodin J. (1988a) Somatic embryogenesis in cotton (Gossypium.) I. Effects of source of explants and hormone regime. Plant Cell Rep., 12,31-42.
29. Trolinder N. and Goodin J. (1988b) Somatic embryogenesis in cotton (Gossypium.) II. Requirements for embryo development and plant regeneration. Plant Cell Tissues Organ Cult. 12:43-53.
30. Xia, L., Xu, Q and Guo, S., 2005, Bt insecticidal gene and its temporal expression in transgenic

Cloning and characterization of Green Tissue specific Promoter from upland cotton (*Gossypium hirsutum* L. cv. Suraj)

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Abstract:

Background: *Promoter sequence plays a key role in the regulation of gene expression. There are three types of promoters such as constitutive, inducible and tissue specific. Constitutive promoters are universally used in plant genetic engineering. For instance, the 35S CaMV promoter facilitates a higher expression of candidate transgene in transgenic crops. However, constitutive promoter has potential effects on GM plants such as metabolic burden. Green tissue-specific promoter is an excellent option to overcome the constitutive expression penalty. A number of transgenic crops were developed with insect resistance genes driven by green tissue specific promoters in rice, cotton, soybean, maize etc.*

Results: *In the present study, we carried out differential expression analysis of eight previously reported genes in green and non-green tissues of Gossypium hirsutum cv. Suraj and the gene coding for Ribulose 1, 5 bisphosphate carboxylase-oxygenase (RUBISCO) small subunit's (rbcs) once again proved to be high expressing gene in green tissues. Moreover, based on transcriptome analysis data of Gossypium hirsutum cv. Suraj, log2 fold values were on par with each other in leaf and bollrind. Hence, 1132bp region upstream of start codon of rbcs genewas isolated from the DNA and cloned in pBI121 vector replacing the 35S CaMV promoter. Deletion analysis of promoter sequence in tobacco showed that 643bp upstream region harbouring four light responsive elements (GATA/I-Box at 111bp and 305bp on +strand and G-box/Box II at 132bp, BOX 4 at 370bp and GATA motif at 437 bp on reverse strand upstream TSS) recorded the highest expression in green tissues when compared to 362bp and 1132bp upstream regions.*

Conclusions: *Histochemical analysis and quantification of gus activity in transgenic tobacco showed gus activity in the green tissues like leaf, shoot and apparently no gus expression in non-green tissues like root and ovule.*

Key words*: Gossypium hirsutum, Promoter, Green tissue-specific, Ribulose 1, 5 bisphosphate carboxylase-oxygenase, gus, Histochemical, Tobacco, boll rind*

Comment: Abstract can be accepted for inclusion in the conference. The full length paper may be considered for oral presentation.

Introduction

Indian farmers adopted Bt-cotton carrying *cry1Ac* and combinations with *cry2Ab* for effective control of bollworms for the past two decades and are still cultivated by them across the country. However, pink boll worm (*Pectinophora gossypiella Saunders)* has shown resistance against both genes since 2009-10 (Dhurua and Gujar 2011, Kranthi 2015). The key reason for the outbreak of pink bollworm resistance was due to poor or no refuge planting along with Bt-cotton in India (Mohan 2020). Further pink bollworm is generally considered as monophagous insect with a high affinity towards cotton flowers and bolls. The best approach to confer resistance against pink bollworm is

genetic engineering for the production of Bt toxin (Tabashnik and Carriere 2019). Nevertheless, the Bt toxin expression varies with different plant parts like highest in leaf followed by square, boll and very low in boll rind (Nagappa and Khadi 2018). Since the expression of Bt toxin is low in green boll rind, the pink boll worms survive well and enter the bolls. It is well known that the *cry1Ac* and *cry2Ab* are driven by constitutive promoter *35S CaMV*, and the cry protein is expressed throughout the plant in varying concentrations. The 35S constitutive promoter has many drawbacks like expression or over-expression at one place where it is not required and low expression where it is more crucial and this leads to severe consequences on the growth and development of the plant (Huang *et al.* 1999). For this reason, it is necessary to use the tissue specific promoter that would express more in the targeted tissues.

Unfortunately, in India there are no reports on green tissue specific expression of *cry* genes in cotton to fight against pink bollworm. Based on previous reports, eight promoters reported to be green tissue specific were selected for expression studies employing RT-PCR. Of these, *rbcs* (RUBISCO small subunit) and *chlorophyll a, b* genes were found to be highly expressed in green tissues (leaf and boll rind) compared to seed. However, the transcriptome sequencing data (unpublished) had shown log2fold change values on par with each other in leaf and boll rind. Hence, in the present study, we isolated and characterized 1132bp upstream region of *RUBISCO* small subunits (*rbcs*) gene (LOC107952825) harboring six light responsive elements which would express only in the green tissues including cotton green bolls thereby, the expression of Bt toxin in the boll rind will be increased many folds to overcome the pink boll worm occurrence. The upstream sequences were fused with reporter gene *gus* and validated the tissue specific expression.

Materials and Methods:

Relative gene expression studyBased on previous reports, eight promoters reported to be green tissue specific were selected for expression studies. The expression analysis was performed as mentioned above in three tissues of leaf, boll rind and seed @ 25 DPA using gene specific primers designed from their coding sequence (Table -1).

Table-1. List of green tissue specific genes and their primers

S. No	Gene Name	Primer sequence	Reference
1	CAB F	CATTGTACCCAGGTGGTAGTT	Liu *et al.*, 2018
	CAB R	ACCCGAACATGGAGAACATAG	
2	cFBPase F	CCATCTCTTGGCGAGTTCATAC	Li-Zhen *et* al., 2003
	cFBPase R	CCGTCCCAATTCTTTGCATTTC	
3	LLRRPK F	AGGAAGACTGCCACATACATTAG	Thilmony *et al* 2009
	LLRRPK R	GGAGGAATGAAGCCAGAGAAG	
4	MgIXMEC F	GTACTGCTGAGTTCTCTGGTTT	Wang *et al* 2016
	MgIXMEC R	GGCTTCATCCCTTGACATAAGA	
5	PD540 F	GGAAGGAAGGGTAAGGGAAAG	Cai *et al* 2007
	PD540 R	CCAGTCGTTGGTGTTGTAGATA	
6	PEPC1 F	GGCACTACAAAGGGAGATTCA	Liu *et al* 2018
	PEPC1 R	GTAGCTCATTCCTGCTCTCATC	
7	Psak F	TCGGTGCTCGATGTGATTT	Lin *et al* 2017
	Psak R	GGCCTTTCTGTTTGCTGATG	
8	Rbcs F	TCGAGCTTACCCACGTACTA	Song *et al.,* 2000
	Rbcs R	TTCTCAAGCTCCTCCAACAC	

Green Tissue specific promoter isolation:

i. Based on relative gene expression studies, two genes were selected for green tissue specific promoter isolation such as *Chlorophyll a-b binding protein of LHCII type 1-like* (LOC107953509) and *Ribulose bisphosphate carboxylase small chain, chloroplastic* (107952825). The $\log_2$ fold change values of the above genes were compared in green and

non green tissues based on the transcriptome data (unpublished) of Suraj variety. Genes showing relatively similar expression both in leaf and bollrind was selected.

ii. Isolation of *rbcs* promoter: DNA was isolated from five cotton varieties using CTAB extraction method. The *rbcs* promoter was isolated from *G. hirsutum* cv Suraj with promoter specific primers reported by Song *et al.,* (2000) and Sanger sequenced to find any variation in promoter sequence.

The primers used for promoter isolation are as follows:

Primer Code	Sequence	Bases
AEG11 RS FP	CGCTCATGTTAACAATTAATTCCTATAATC	30
AEG11 RS RP	CATCGTAGTACGTGGGTAAGCTCGAGTACT	30

The promoter sequences were cloned into TOPO isomerase vector and transformed into *E. coli* cells using TOPO XL-2 kit (Cat No. K8050-20, Invitrogen) and sequenced. Selection of upstream region for characterization of promoter region upstream 1132bp region of the gene was downloaded from NCBI covering six-light responsive *cis*-elements. Plant Care software was used to identify *cis*-regulatory elements reported earlier. Pair wise alignment of promoter regions. The upstream region of *rbcs* (LOC 107952825) and the *rbcs* promoter (Song *et al*., 2000) from Suraj were aligned using Global alignment tool NEEDLE.

Restriction digestion analysis of the plant vector pBI121

The binary vector pBI121 having *gus* reporter gene was driven by *35S CaMV* promoter was replaced with green tissue specific promoter region. The plasmid size is 14,758 bp and the promoter sequence is flanked by restriction sites to facilitate the replacement of the promoter sequence. The map of pBI121 is given below (Figure-1). Restriction enzymes were selected based on zero cutting on the upstream regions of the selected genes to release *35S CaMV* promoter sequence from the plasmid. The combination of *Hind*-III and Xba1 were used to double digest the plasmid and release the promoter fragment. The released fragment of promoter was replaced with deletion fragments of upstream gene sequence having different combination of *cis*-elements.

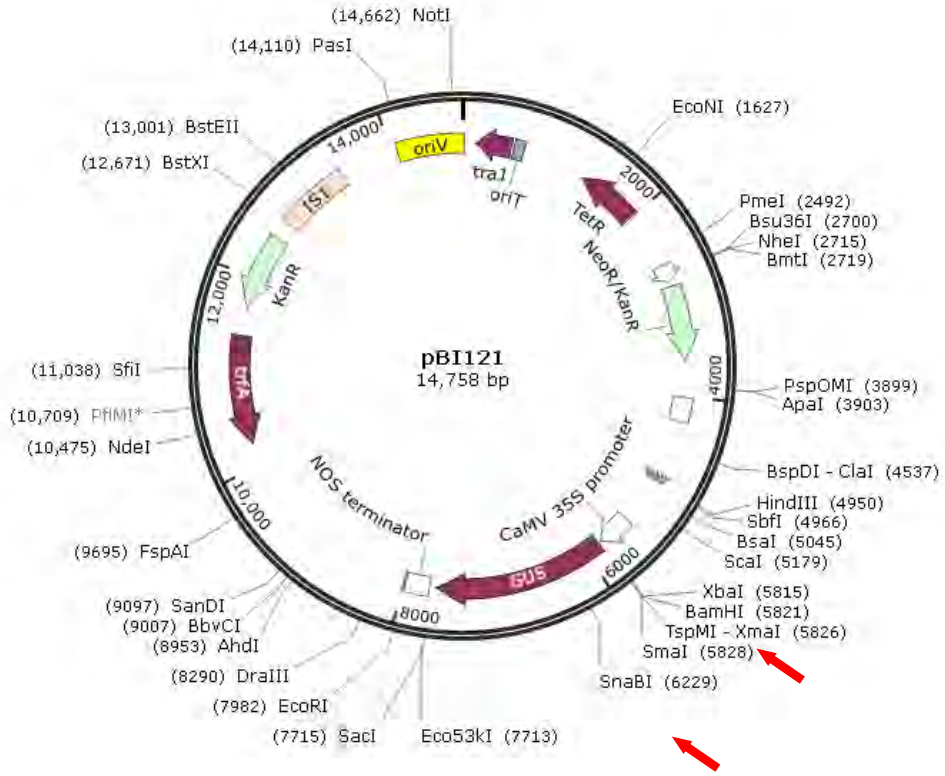

Figure-1: Restriction map of the plant vector pBI121

Vector construction:

Deletion primers were designed to identify and characterize the upstream region of *rbcs* gene. The reverse primer was designed immediately upstream of start codon and the forward primers were designed based on the light responsive elements present. The primers were designed with restriction enzyme sequences of *Hind* III in forward primer and *XbaI* in reverse primer at their 5' end to facilitate the sticky end production to be ligated in the opened plasmid pBI121. The primer sequences are given below in table-2.

Table-2: Deletion primers:

Ribulose bisphosphate carboxylase small chain	5'→ 3'	No. of nucleotides
CCCAAGCTTTCCAAATTTAGAAAATATTTCC	Forward	31
CCCAAGCTTATGCTTCCATCTTTTAGCTGCA	Forward	31
CCCAAGCTTAATCGGGAATGGCCAAATGGGA	Forward	31
CGCGGATCCTGCTATTACTGCTTACTAGT	Reverse	29

The deletion fragments were PCR amplified, eluted from gel and purified according to manufacturer's protocol (Catalogue Number K220001, Invitrogen). Both plasmid and PCR amplified fragments were digested with restriction enzymes at 37ºC for one hour and ligated at 16ºC. The ligated products were transformed into competitive DH5α cells and screened on kanamycin selection plates to grow transformed positive colonies. Twenty colonies from each construct plate were sub-cultured thrice and ten colonies were tested using colony PCR. Plasmid was isolated from colony PCR positive colonies and tested for release of respective promoter fragments from each construct. Plasmids from positive colonies were used to transform competitive *Agrobacterium tumefaciens* EHA105 cells and positive colonies were selected on Kanamycin and Rifampicin plates. The surviving positive colonies were tested with PCR and confirmed colonies were sub-cultured thrice and used for glycerol stocks and transformation of tobacco plants. The transformed bacterial colonies with uncut pBI121 plasmid were treated as positive control and were confirmed with *gus* 4 primer. The *gus* 4 primer sequence is as follows

***Gus* 4 primer sequence**

Primer code	Sequences 5'→3'	No. of nucleotides
Gus 4 F	CGCATTACCCTTACGCTGAA	20
Gus 4 R	AACGCGTAAACTCGACCC	18

Transformation of *Agrobacterium tumefaciens* with plasmid pBI121:

The custom designed constructs were ligated in pBI121 having *gus* gene and were transformed into EHA105 strain of *Agrobacterium* cells along with uncut pBI121 plasmid by Heat Shock method. The transformed *Agrobacterium* cells were selected on LB plates containing 50 mg/L Kanamycin and 25mg/L Rifampicin.

Transformation of tobacco explants:

Seed sterilization:

Tobacco seeds were disinfected by taking healthy seeds in 1.5 ml Eppendorf tubes and washed with sterile distilled water 2-3 times followed by incubation in systemic fungicide Bavistin (0.1%) for 20 min. Later the seeds were rinsed with 0.1% $HgCl_2$ followed by 0.1% SDS. Finally the seeds were rinsed with sterile distilled water 3-4 times; blot dried and inoculated on half strength Murashige and Skoog (1962) media.

Callus Induction and Regeneration:

Sterile tobacco leaves were cut into 0.5 cm X 0.5 cm pieces and incubated with 0.5 OD bacterial suspension of respective gene constructs on regeneration medium (Geethalakshmi *et al.* 2016). The explants were inoculated on sterile media containing Murashighe and Skoog (MS) media with

0.1mg/L 2, 4-D and 0.5 mg/L Kinetin for callus induction. Callus was observed in 2 weeks from which somatic embryos developed into shoots in one month.

Histochemical *Gus* assay:

Leaf sample was collected from rooted embryos and tested for *Gus* gene activity according to Jefferson *et al*. (1987). *Gus* positive plants were established in pots in green house which were also confirmed for transgene presence by PCR.

***GUS* quantification in transformed tobacco plants:**

Different tissues of *gus* positive plants like leaf, root, shoot, petal, pollen and seed were tested for *gus* activity and images of them were captured with an electric inverted microscope. Simultaneously ten lines from each construct was tested for MU (4-Methyumbelliferone) activity according to Gallagher *et al.*(1992) with three replications per line and total protein content was calculated using Bradford (1976) method. The MU fluorescence was studied with a multimode plate reader.

Results:

Candidate green tissue specific genes for promoter isolation:

Based on relative gene expression studies, two genes were selected for green tissue-specific promoter isolation such as *Chlorophyll a-b* binding protein of *LHCII type 1-like* and *Ribulose bisphosphate carboxylase* small chain, *chloroplastic*. Both are chloroplast genes and shown high expression in green tissues (data not shown). Of these two genes, *RUBISCO* small subunit was selected for promoter isolation for three reasons. Primarily, *RUBISCO* is showing very low or no expression in seeds and high-level expression in leaf and boll rind. The log_2 fold change values for both the genes are given in the table-3 (transcriptome data, unpublished). Secondly, the PCR amplicon of the same with the primers from Song *et al*. (2000) was reported 560bp long with two *cis*-acting elements, however, we could amplify 1081bp fragment with the same primers (Figure-2) in *G. hirsutum* cv Suraj. Thirdly, the 1132 bp upstream start codon region of *rbcs* gene was aligned with 1081bp promoter region isolated with song *et al.*(2000) primers and there was only 24.2 percent identity between the sequences of 419bp long (Figure 3).

Thus, it was considered a unique region in upstream with four *cis*-acting elements in our cultivar.

Table-3: Whole transcriptome analysis of candidate genes and log_2 fold changevalues.

S. No.	Gene Name	Locus	Log_2 Fold change (Seed vs Leaf)	Log_2 Fold change (Seed vs Boll rind)
1	LOC107901843	Chlorophyll a-b binding protein of LHCII type-1 like	7.3731	3.13773
2	LOC107952825	Ribulose bisphosphate carboxylase small chain, chloroplastic	6.77327	5.66147

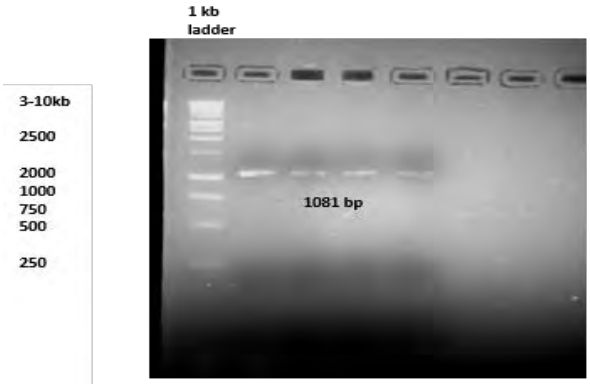

Figure-2: PCR amplicon of rbcs promoter sequence of 1081bps

Global alignment of promoter sequences using EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) and the highlighted region is the only identical portion between two sequences mentioned below.

Tools > Pairwise Sequence Alignment > EMBOSS Needle

```
EMBOSS_001      1 TCCAAATTTAGAAAATATTTCCAGAGTTGGAAATTGTATTTGTAATACCC    50
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001     51 TAATATTTGTACACTGCAGTGAACATGGACAATCATGTCATGATGCAGAA   100
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    101 CTATAAAATTGTGCCAACTCTCCTTCTTCTCCCCCTTTTTCTTCCAAAAC   150
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    151 TATATCCTCGAAAATGGTGACATCAAACTTCCTTTCGTTTATCAAAGGTT   200
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    201 CACCCCTCACATTACTGGGTCCTGTTCGCATCTCAAGGCAATCAAACATG   250
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    251 GAATCCATATATATGAATCTTCCCCAATCCTTGTTGTAGATTCTTTGGGG   300
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    301 ATACAAGCCTTTTTGTTTGTATTTAAATACAAAAAAGGGAAAAAGAAAAG   350
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    351 CGCCATCTATAACTGCATGCTAATGATTGCCAAAATCACTGTTTGTTTTG   400
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    401 TTGGAAGGCTTTCAATGTTGCGGGGTCTTGAAGTAAGAGCCTGTTTCAGT   450
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    451 AACTTGCCATTAATTCTCTCATACATTCCAATCCTAAAAAATGCTTCCAT   500
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    501 CTTTTAGCTGCAACAGTTTATAATCATCAGAAAGGTCTAGTCAGAATAAT   550
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    551 ATTACCTTCTCCTTTTTAACAGTTTTTTTTTTTTTAAATCATGCCACTAC   600
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    601 AACTGTAGCTGCGCTCATGTTAACAATTAATTCCTATAATCGACATCAAA   650
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    651 ATTATATGAAAGAATTAACACTTGGTTACCGAGTTACCATATTTGAAGAT   700
EMBOSS_001      1 --------------------------------------------------     0
EMBOSS_001    701 AAGGCGAAAGGTAAAAACACAAAAGGCAAGCATGACCAAGCAAACAAGGT   750
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1 ----------AAAACACAAAAGGCAAGCATGACCAAGCAAACAAGGT    37
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EMBOSS_001      751 ATGGACATAGATTTTTTTTGAATCGGGAATGGCCAAATGGGACCGTGAAG  800
                    ||||||||.|| ||||||||||||||||.||||||||||||||||
EMBOSS_001       38 ATGGACATCGA-TTTTTTTGAATCGGGAATGACCAAATGGGACCGTGAAG   86

EMBOSS_001      801 AGGGGACAAAGGAGAAATCAGGCATTCACGGTTTCCATTGGATGAAATGA  850
                    |||.||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       87 AGGTGACAAAGGAGAAATCAGGCATTCACGGTTTCCATTGGATGAAATGA  136

EMBOSS_001      851 GATAAGATCACTGTGCTTCTTCCACGTGGCAGGTTGCCAAAGATAAGGCT  900
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      137 GATAAGATCACTGTGCTTCTTCCACGTGGCAGGTTGCCAAAGATAAGGCT  186

EMBOSS_001      901 TTACCATTCAAGAAAAGTTTCCACCCTCTTTGTGGTCATAATGGTTGTAA  950
                    |||||||||||||||||||||||||||||||||||.|||||||||
EMBOSS_001      187 TTACCATTCAAGAAAAGTTTCCACCCTCTTTGTGGTCATTATGGTTGTAA  236

EMBOSS_001      951 TGTCATCTGATTTAGGATCCAACGGTCACCCTTTCTCCCAAACCAATCTC  1000
                    |||||||||||||||||||||||||||||.|||||||||||||||||
EMBOSS_001      237 TGTCATCTGATTTAGGATCCAACGGTCACCCTGTCTCCCAAACCAATCTC  286

EMBOSS_001      1001 TAAATGTTGTGAGCTTAGGCCAAATTTTATGACTATATATAGGGGATTGC  1050
                    |||||||.|||||||||.|||||||||||||||||||||||||||||||||
EMBOSS_001      287 TAAATGTGGTGAGCTTAGACCAAATTTTATGACTATATATAGGGGATTGC  336

EMBOSS_001      1051 ACCAAGGCAGTGACACTATTAAGGGATCAGTGAGACTCTTTTGTATAACT  1100
                     ||||||||||||||||||||||||||||||||||||||||||||||.||
EMBOSS_001      337 ACCAAGGCAGTGACACTATTAAGGGATCAGTGAGACTCTTTTGTATACCT  386

EMBOSS_001      1101 GTAGCATATAGTACTAGTAAGCAGTAATAGCA----------------- 1132
                     ||||||||||||.|||||||||||||||||
EMBOSS_001      387 GTAGCATATAGTAATAGTAAGCAGTAATAGCAATGGCCTCCTCCATGATC  436

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      437 TCATCGGCAACCATTGCCACCGTGAACCGCTCCTCCCCGGCTCCCAGTCA  486

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      487 CCAGGAAGGCCAACAACGACATCACTTCTCTTGCAAGCAACGGTGGGAGA  536

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      537 GTGCAATGCATGCAGGTACTTGGTGATGCATAAATACCACTTAAATTACC  586

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      587 CCAAGTGTTTGAACACAACAAATTACATAAATTGAATCAAATATATATCT  636

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      637 TGGCTTTTGAGTACAGGTGTGGCCGCCTCTTGGGAAGAAGAAGTTCGAGA  686

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      687 CTCTCTCATACCTGCCCGATCTTACTCCCGTACAGTTGGCTAAGGAAGTA  736

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      737 GATTACCTTCTTCGCTCTAAATGGATTCCTTGCTTGGAATTCGAATTAGA  786

EMBOSS_001      1133 ---------------------------------------------- 1132

EMBOSS_001      787 GGTATTTTGGAGCTCTAAATTATTCCATTCCTCCTCTTAATTTTTTTAGT  836

EMBOSS_001      1133 ---------------------------------------------- 1132
```

EMBOSS_001 837 GGGATATTTGAGTTGATTAAGTGTGTTTTATATGTATGTGCAGGAGGGAT 886

EMBOSS_001 1133 ---------- 1132

EMBOSS_001 887 TCGTGCACCGTAAGTACTCGAGCTTACCCACGTACTACGATGAAGGGCGA 936

EMBOSS_001 1133 ---------- 1132

EMBOSS_001 937 ATTCGTTTAAACCTGCAGGACTAGTCCCTTTAGTGAGGGT 976

#---------------------------------------
#---------------------------------------

Identification of cis- elements in the upstream region of *rbcs* small subunit gene

In the selected upstream region of *ribulose bisphosphate carboxylase* small chain, there are 32 cis-elements reported earlier of which only 15 *cis*-elements were reported to be light-responsive elements in different crops. Most of the green tissue-specific genes reported earlier were also having light-responsive elements that are driving tissue-specific expression. Hence, light-responsive elements were only considered for further characterization of the putative promoter region and design deletion primers. The light-responsive elements in the *rbcs* upstream region are shown in figure- 3

*Figure-3: cis-elements in the upstream region of the RUBISCO small subunit gene (*Underlined sequences are primer binding sites)*

Restriction digestion analysis of the plant vector, pBI121

pBI121 is a binary vector with *gus* reporter gene driven by a 35S cauliflower mosaic virus promoter (*35S::Gus*). The size of the plasmid is 14,758 bp and the promoter sequence is flanked by restriction enzymes which facilitate the replacement of the promoter sequence. *Hind*-III HF and *Xbal* released the promoter fragment of 865 base pairs and the linearized plasmid of 13,893 base pairs with sticky ends (Figure -4).

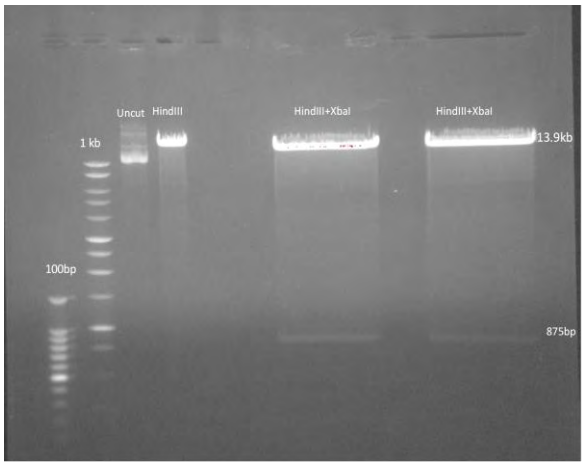


Figure -4. Restriction Digestion of pBI121

Similarly, deletion fragments of the upstream sequence of 1141bp sequence were also digested with *Hind*-III HF and *XbaI* to produce sticky ends. The selected region harbours 6 *cis*-elements reported to drive green tissue-specific expression in previous reports. Three gene constructs were generated after ligation of the linearized plasmid and deletion fragments having sticky ends. Each deletion fragment was isolated by amplification of Suraj DNA with 3 different forward primers and 1 common reverse primer. The amplified products were of the sizes, I-1141bp (6 *cis*-elements), J-651bp (4 *cis*-elements) and K-372bp (2 *cis*-elements). The core promoter element (TATA box) is at +44bp and CAAT box is at around 86bp upstream Transcription Start Site (TSS).

Green tissue-specific *cis*-elements underlying deletion fragments:

The shortest deletion fragment is 372bp and comprised of GATA/I-Box at 111 bp upstream TSS was reported to be a part of light-responsive elements in crops like cotton, *Arabidopsis*, tobacco and so on and G-box at 132 bp upstream TSS was reported to be involved in light responsiveness in various crops like wheat and peas. The 651bp deletion fragment is comprised of two more *cis*-elements besides the above two that is I box at 305 bp, BOX 4 at370 bp and GATA box at 437bp on reverse strand were also found to be involved in light responsiveness. However, the longest fragment of 1141bp comprised of the above four *cis*-elements besides two more regulatory elements, Box 4 at 537bp found to be part of a conserved DNA module involved in light responsiveness in *Petroselinum crispum* and TCT motif at 563 bp reported being part of a light responsive element in *Arabidopsis thaliana* (PLANT CARE).

Bacterial Transformation:

The transformed bacterial colonies were confirmed with *gus* 4 primer with amplicon size of 325bp (Figure-5).

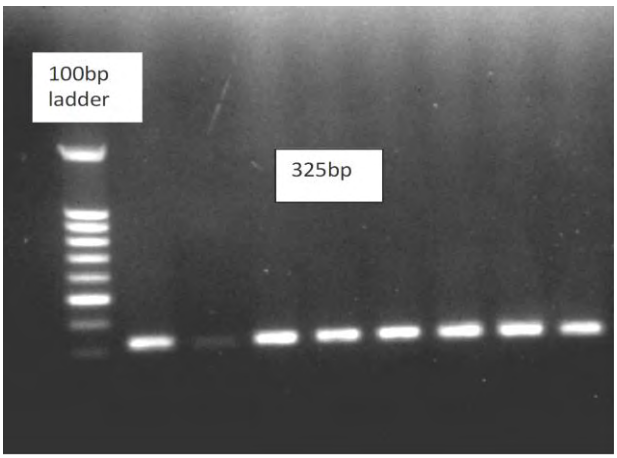


Figure-5. PCR amplification of gus gene in transformed colonies

Tobacco transformation, *gus* detection and quantification

Tobacco plants were transformed with *Agrobacterium* mediation and putative transformed plants were established in the soilrite condition. The T_0 tobacco plants were subjected to histochemical *gus* assay and the *gus* positive plants showed *gus* activity in the green tissues like leaf, shoot and no *gus* expression in non-green tissues like root and ovule. The microscopic images of these tissues are shown in figure-6. Furthermore, the positive plants were PCR tested for the presence of *rbcs* promoter fragment (Figure-7&8). The PCR amplified products were according to the size of inserted deletion fragment. PCR tested positive plants were further considered for *gus* gene quantification through fluorimetric analysis.

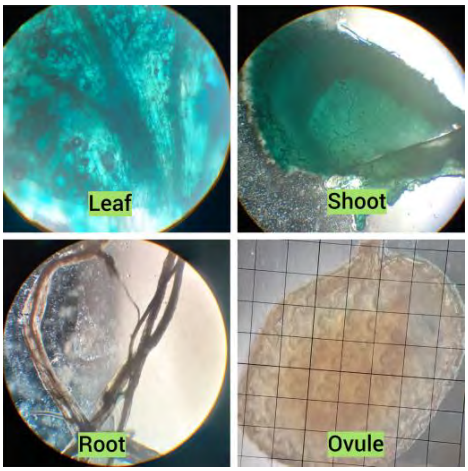
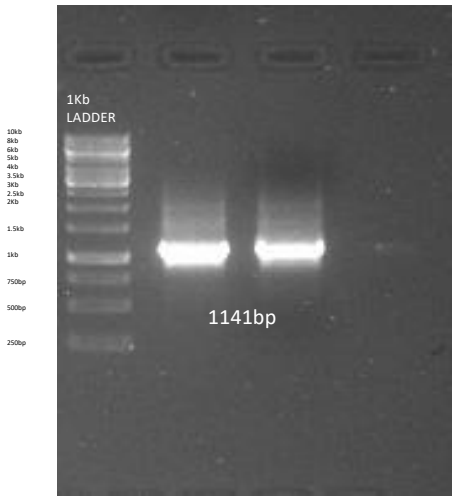


Figure-6. Gus gene expression in various tissues.



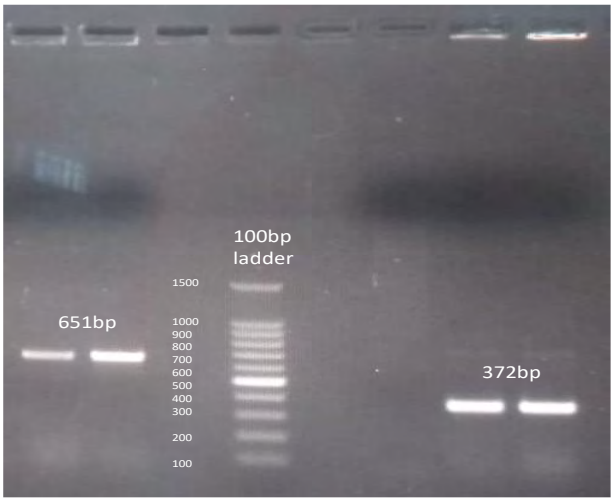


Figure 7 & 8: T_0 Confirmation of I-1141bp and T_0 J-651 & K-372 bp

Of all the three constructs transformed plants were subjected to histochemical analyses. The gene construct with 643bp upstream region of *rbcs* gene has shown highest *gus* expression in leaf tissues when compared to other constructs on par with positive control (table-4). In a similar manner there is no expression of *gus* gene in non green tissues like root and ovule and negative control.

Table-4: *Gus* quantification: Methyl Umbelliferone expression pattern:

Promoter sequence (I,J,K)	picoMoles/min/mg protein
I14R1	27.69147255
I14R2	13.06120668
J18R1	58.35791659
J18R2	68.87572595
K1R1	29.02027943
K1R2	32.74403673
NCR1	3.23657868
NCR2	4.33527186
PC1R1	71.11172672
PC1R2	70.61960585
PC2R1	70.49031076
PC2R2	72.38617145

*I- transgenic lines harboring 1141bp fragment; J- transgenic lines harboring 651bp fragment; K- transgenic lines harboring 372bp fragment; NC-Negative Control; PC- Positive Control

Discussion:

With the advent of high throughput RNA-Seq analysis, a number of the transcription factors, promoters or regulatory sequences were identified (Zhang *et al.* 2016). Rubisco is an important candidate gene for isolation of promoter region to express or over express foreign gene in the targeted cells or tissues and that would avoid the universal expression and negative effect on plant growth or yield (Gittins et al. 2000; Marraccini *et al*. 2003). The Rubisco is composed of eight small subunits encoded by the nuclear multigene family (*rbcS*), and eight large subunits encoded by a single gene (*rbcL*) in the multi-copy chloroplast genome (Chen *et al*. 2015).

Differential gene expression among green tissue and non-green tissues of eight previously reported green tissue-specific genes RT-PCR studies, resulted in two genes showing high expression in green tissues and no or low expressions in non-green tissues . As we envisaged both of them were found to be chloroplast genes and considered as best green tissue-specific promoters.

In the present study, we have characterized the 5'regions of the cotton *RUBISCO* gene and established that it contains an efficient promoter to express only in specialized cells which can be used for regulating the foreign gene in targeted cells. Serial deletion of the 5" upstream region was prepared and three gene constructs with different upstream regions of *rbcs* (1132, 643 and 362bps) were fused with *gus* plasmid. All the 3-constructs were introduced into tobacco plants. The expression of reporter gene was analysed to determine the pattern of expression provided by promoter fragments of various length. Among all three, 643bp sequences showed maximum *Gus* expression in the transient assay of transgenic tobacco. The 643bp deletion fragment comprised of total four *cis*-elements found to involve in light responsiveness. However, the longest fragment of 1132bp comprised of the above four *cis*-elements besides two more regulatory elements.

For tissue-specific expression two *cis*-acting elements such as I-box and the G-box are important (Donald and Cashmore, 1990 and Martinez-Hernandez *et al.* 2002). Song *et al.* (2000) reported that the 560-bp promoter fragment from the cotton *rbcs* gene was used to assemble the *gus* reporter gene construct which includes putative I-box (-287 to -274bp) and G-box (-260 to -252bp) sequences. In our promoter sequence, 643 bp upstream regions had four cis-elements including I-box in duplication, G-box and BOX 4 are positioned within -600nto -100bp transcription initiation site.

Putative transgenic tobacco plants were regenerated (T0) with independent gene construct fused with *rbcs-gus*. The integration and expression of *gus* gene were confirmed by PCR amplification and histochemical analysis. Interestingly there is no expression of *gus* gene in non-green tissues like root and ovule, whereas in leaves *gus* expression was recorded. Similar results were recorded by Song *et al.* (2000) by using *Gh-rbcs* promoter with *gus* gene. The transgenic cotton plants showed *gus* expression only on leaves. These results suggest that the sequence with *cis*-elements in the upstream region would be responsible for high-level expression of the transgene. Meier *et al.* (1995) expressed *gus* gene fused with *rbcs* promoter ranging from 0.6 to 3.0 kb of the *rbcs1, rbcs2* and *rbcs3* in tomato and these genes were sufficient to confer the temporal, organ-specific expression of the transgene. Bt *Cry9C* driven by PNZIP promoter, a green tissue-specific promoter from *Pharbitis nil* was used to develop insect-resistant transgenic cotton and the *Cry9C* protein accumulation was 100 times lower than the 35S CaMV driven transgenic lines (Ghasimi Hagh *et al.* 2009). The use of green tissue-specific promoters in cotton will minimize food safety concerns and significantly reduce the waste of plant resources that occurs by constitutive expression (Xue *et al.* 2018)

Gus expressions were quantified by Fluorometric assay with all the transformed six plants with three different gene constructs. The expression level of *gus* varies significantly among transgenic tobacco regenerated with different promoter fused with *gus*. The gene construct with 651bps upstream region of *rbcs* gene has shown the highest *gus* expression in leaf tissues when compared to other plants having 1141bps and 372bps promoter region. Song *et al.* (2000) suggested that plant-to-plant variation in the expression between different cell lines could have been caused by position effects that depend on the chromosomal location of the transgenic insertion, by co-suppression and or the presence of multiple copies of the transgene. Use of this green tissue specific promoter in transgenic cotton will increase cry genes many fold in leaves and green bolls and reduced or no accumulation in the seeds.

Acknowledgements

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References:

1. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*1976; 72, 248-254.
2. Cai M, Wei J, Li X, et al. A rice promoter containing both novel positive and Negative cis -elements for regulation of green tissue specific gene expression in transgenic plants. Plant Biotechnology Journal. 2007; 5: 664-674.
3. Chen Y, Wang B, Chen J, et al. Identification of Rubisco rbcL and rbcS in Camellia oleifera and their potential as molecular markers for selection of high tea oil cultivars. Front Plant Sci. 2015 Mar 31; 6:189. doi: 10. 3389/fpls. 2015.00189. PMID: 25873921; PMCID: PMC4379756.
4. Dhurua S, Gujar GT. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag Sci. 2011; 67:898–903.
5. Donald RGK and Cashmore AR. Mutation of either G box or I box sequences profoundly affects expression from the Arabidopsis rbcs-1A promoter. EMBO J. 1990; 9: 1717-1726.
6. Gallagher SR. Quantitation of GUS activity by fluorometry. In GUS Protocols: using the GUS gene as a reporter of gene expression (Gallagher, S.R., ed).Academic Press, Inc., New York.1992; pp. 47-59.
7. Geethalakshmi S, Hemalatha B, Saranya N. Optimization of Media Formulations for Callus Induction, Shoot Regeneration and Root Induction in Nicotiana benthamiana. J Plant Sci Res. 2016; 3(1): 150-154.
8. Ghasimi HZ, Rahnama H, Panahandeh J, et al. Green-tissue-specific, c(4)-pepc-promoter-driven expression of Cry1Abmakes transgenic potato plants resistant to tuber moth (Phthorimaea operculella, zeller). Plant Cell Rep. 2009; 28, 1869–1879.
9. Gittins JR, Pellny TK, Hiles ER, et al. Transgene expression driven by heterologous ribulose-1,5-bisphosphate carboxylase/oxygenase small-subunit gene promoters in the vegetative tissues of apple (Malus pumila Mill.). Planta, 2000, 210, 232–240.
10. https://www.ebi.ac.uk/Tools/psa/emboss_needle/
11. Huang F, Buschman LL, Higgins RA, et al. Inheritance to Bacillus thuringiensis toxin (Dispel ES) in European corn borer.Science.1999; 284:965–967.
12. Jefferson RA , Kavanagh TA and Bevan MW. GUS fusions: B-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J.1987; 6, 3901-3907.
13. Kranthi KR. Pink bollworm Strikes Bt-Cotton. Cotton Statistics and News. 2015, No.35:1-6.
14. Lin Z, Yan J, Yan H et al. Characterization of a strong green tissue-specific motif in rice photosystem I gene promoter Ppsak. Plant Biotechnol Rep. 2017; 11:87–95.
15. Liu W, Mazarei M, Ye R, et al. Switchgrass (Panicum virgatum L.) promoters for green tissue-specific expression of the MYB4 transcription factor for reduced-recalcitrance transgenic switchgrass. Biotechnology Biofuels. 2018; 11:122
16. Li-Zhen SI, Shou-Yun CAO, Cai CC. Isolation of a 1195 bp 5′-Flanking Region of Rice Cytosolic Fructose-1,6-bisphosphatase and Analysis of Its Expression in Transgenic Rice. Acta Botanica Sinica. 2003; 3: 359-364.
17. Marraccini P, Courjault C, Caillet V, et al. Rubisco small subunit of Coffea arabica: cDNA sequence, gene cloning and promoter analysis in transgenic tobacco plants. Plant Physiol. Biochem. 2003; 41, 17–25.
18. Martı´nez-Herna´ndez A, Lo´pez-Ochoa L, Argu¨ello-Astorga G, et al. Functional properties and regulatory complexity of a minimal RBCSlight-responsive unit activated by phytochrome, cryptochrome, and plastid signals. Plant Physiology. 2002; 128, 1223–1233.
19. Meier I, Kristie L, Fleming JA, et al. Organ-specific differential regulation of a promoter subfamily for the ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit genes in tomato. Plant Physiol. 1995; 107:1105-1118.
20. Mohan KS. SWOT analysis of refuge-in-bag for Bt-cotton in India, Current Science.2020; 119:1746-1751.
21. Murashige T, and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 1962;15: 473-497.
22. Nagappa BH and Khadi BM. Expression of Bt Gene (Cry1Ac) on Different Plant Parts at Different Stages in Bt Cotton Genotypes. Int. J. Curr. Microbiol. App. Sci.2018;7(01):3339-3353.doi:https://doi.org/10.20546/ijcmas. 018.701.396.
23. Song P, Heinen JL, Burns TH, et al. Expression of two tissue-specific promoters in transgenic cotton plants. J Cotton Sci. 2000; 4:217-23.
24. Tabashnik BE, Carrière YJ. Global patterns of resistance to Bt crops highlighting pink bollworm in the United States, China, and India. J. Econ. Entomol. 2019; 112(6): 2513–2523.
25. Thilmony R, Guttman M, Thomson JS et al. The LP2 leucine-rich repeat receptor kinase gene promoter directs organ-specific, light-responsive expression in transgenic rice. Plant Biotechnology Journal. 2009; 7: 867–882.
26. Wang Q, Zhu Y, Sun L, et al. Transgenic Bt cotton driven by the green tissue-specific promoter shows strong toxicity to lepidopteran pests and lower Bt toxin accumulation in seeds. Science China. 2016 ; 59:172-182.
27. Xue M, Long Y, Zhao Z, et al. Isolation and Characterization of a Green-Tissue Promoter from Common Wild Rice (Oryza rufipogonGriff.)Int. J. Mol. Sci. 2018; 19, 2009; doi: 10.3390/ijms19072009.
28. Zhang YQ, Lu X, Zhao FY, et al. Soybean GmDREBl increases lipid content in seeds of transgenic Arabidopsis. Sci. Rep. 2016; 6, 34307.

Molecular cloning and expression analysis of Chitin synthase A and B gene in *Helicoverpa armigera* of cotton

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Abstract

Background: *Chitin synthases(CHS) (EC 2.4.1.16) are enzymes of integral membrane belonging to β-glycosyltransferase 2 (GT2)family involved in the process of polymer synthesis, translocation and oligomerization of chitin nanofibirils. Insect development through staged molting phase is strongly modulated by the action of degradation and synthesis of chitin polymer. The presence of the structural polysaccharide chitin in the insect cuticle and the peritrophic membrane as well as the absence of chitin in higher plants and animals makes chitin a suitable selective target for insect control. The present study was perfomed to characterize the HaCHSA and HaCHSB protein coding sequence from the H armigera strains of cotton from India and to analyse the sequence for identification of alternative splice variants and expression analysis at different stages and tissues of insect development.*

Results: *Gene structure analysis indicated the occurrence of alternative splice variants for HaCHSA in Helicoverpa armigera of Cotton. The alternative splice variants were unique in Helicoverpa species with reference to position of occurrence and amino acid length which is otherwise conserved among CHSA variants in different insect species. HaCHSA1 and HaCHSA2 shared 98.9 % and 87.7 % aminoacid identify of full length and alternative spliced protein sequence respectively. Expression analysis of HaCHSA1 and HaCHSA2 was varied at different tissues and developmental stages. HaCHSA1 variant showed relatively higher expression in 3rd and 4th larval stages while HaCHSA2 noticed more transcript abundance in 1st and 2nd Instar larval stages. Both HaCHSA1 and HaCHA2 variants indicated lower transcript abundance in gut tissues of the 3rd and 4th Instar larvae. In contrast no alternative splicing transcripts were observed for chitin synthase B. Gene structure analysis revealed major difference in sequences and length of 15th and 16th exon among HaCHSB and HzCHSB protein coding sequence. The exon size of 306 and 230 nucleotides in HaCHSB and 350 and 171 nucleotides in case of HzCHSB were noticed. The resulted variation in nucleotide was apparent through changes in amino acid sequences and it has resulted in addition of coil coil domain downstream of the 5 transmembrane (5-TMS) domain in HaCHSB and which was absent in HzCHSB. Relatively higher expression of HaCHSB was observed in gut tissues and stages studied.*

Conclusion: *Study was able to identify the alternative splice variants for chitin synthase A in H armigera of Cotton. No alternative transcripts for HaCHSB were noticed similar to CHS2 of different insect species. Expression pattern of the CHS variants and structural features information of the study provided valuable insights on understanding of chitin synthase gene in H armigera*

Keywords: *Chitin synthase, Coil-coil domain, expression analysis, alternative splice variants, H armigera*

Background

Chitin, the linear homopolymer of β-1, 4-linked N-acetylglucosamine, is produced by the membrane bound enzyme glycosyltransferase or chitin synthase (CHS) and is the major structural polysaccharide present in the insect exoskeleton and gut lining (Merzendorfer and Zimoch, 2003, Merzendorfer, 2005). Chitin synthases(CHS) (CHS, EC 2.4.1.16) are enzymes of integral membrane belonging to f β-glycosyltransferase 2 (GT2)family involved in the process of polymer synthesis, translocation and oligomerization of chitin nano fibirils (Zhu et al 2016). Insect development through staged molting phase is strongly modulated by the action of degradation and synthesis of chitin polymer. The presence of the structural polysaccharide chitin in the insect cuticle and the peritrophic membrane as well as the absence of chitin in higher plants and animals makes chitin a suitable selective target for insect control. Based on the sequence similarity, differential tissue and developmental expression the CHS in insects are identified to be two isoforms viz., CHS1/CHSA and CHS2/CHSB. Insect cuticle and tracheal chitionous structures reported to be contributed by the action of CHS1 but chitinous structures of

peritrophic matrix(PM) of midgut epithelial lining was contributed by CHS2. Two major classes of chitin synthase (CHSA and CHSB) have been identified and characterized in several insect species. Among lepidopteran species, it is charactersied in Manduca sexta (Zhu et al., 2002; Hogenkamp et al 2005), Spodopetra frugiperda (Bolognesi et al 2005), Spodopetra exigua (Chen et al 2007; Kumar et al., 2008), , Plutella xylostella (Ashfaq et al 2007) Heliothine moths Helicoverpa zea, H.armigera (Shrik et al 2015), Spodoptera litura (Yu et al 2020). Unique tandem juxtaposed genomic arrangement of the two chitin synthase genes in both H. zea and H. armigera and alternative splicing of HzCHSA and HzCHSB in H zea was reported. But, the data on spatio-temporal expression pattern of both CHS genes of H. armigera and existence of alternative splicing of CHS genes was not discussed due to the non availability of the tissue for expression studies (Shrik et al 2015). H.armigera (HUBNER) is an important poylphagous pest infesting economically important field crops worldwide. In India, it is major devastating pest reported on cotton, legumes and vegetables causing significant damage (Kranthi et al 2001). The major chemical pesticide consumption of cotton during pre Bt era was deployed to control H. armigera. It has reported to be evolved resistance to every group of insecticides used to control this pest. In the present study CHS genes was isolated from strains of H.armigera on cotton at ICAR-CICR, Nagpur. The HaCHSA and HaCHSB coding sequence was PCR amplified and sequenced, expression pattern of both the genes and alternative spliced variants of HaCHSA at different stages and tissues of insect development were analysed.

Results

The HaCHSA and HaCHSB sequences from H.armigera of cotton was isolated by using over lapping primers (Table 1) designed based on the BAC clone (HQ840515) of Helicoverpa zea (Fig 1).

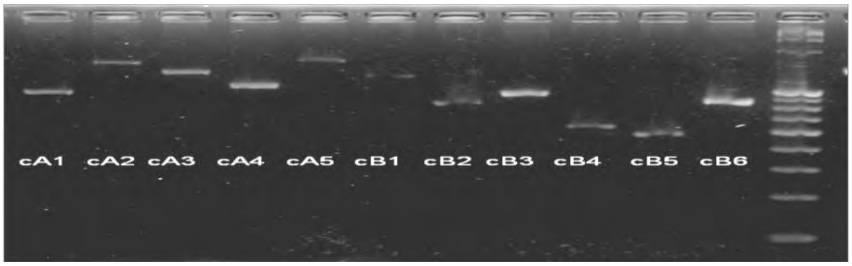

Figure 1: *PCR amplified cDNA fragments of H. armigera Chitin Synthase A and B*

Analysis of nucleotide sequences generated from sequencing of overlapping PCR products resulted in identification of open reading frame of HaCHSA (GenBank accession number : KP939100) containing 4695 nucleotides encoding 1565 amino acids(AKJ54482.1) and HaCHSB (KP939101.1) cds consisted of 4599 nucleotides encoding 1533 amino acids (AKJ54483.1). TMHMM v.2.0 prediction of HaCHSA and HaCHSB protein identified 16 transmembrane helices. Similar to other chitin synthase family of genes HaCHSA and B was predicted to have three domains: an N-terminal domain with nine transmembrane helices; a central catalytic domain which contains catalytically critical sequences, including aspartyl residues, and the 'signature motifs' EDR (positions 833–835 in the amino acid sequence), QRRRW (positions 870-874 in the amino acid sequence). It is also predicted that Five transmembrane spans (5-TMS) are predicted to appear consecutively after the putative central catalytic domain. C-terminal domain was found to be with additional transmembrane helices. also contains and WGTRE (positions 1053–1057 in the amino acid sequence) near the C-terminus, which are conserved in polymerizing β-glycosyltransferases from many species (Senthilkumar et al., 2008). A Coiled coil region was also predicted by SMART (Simple Modular Architecture Research Tool) through COILS program (Fig 2). No signal peptide was predicted, but two potential N-glycosylation sites was predicted using NetNGLyc 1.0 software, suggesting that the protein was glycosylated.

Figure 2: *A Coiled coil region (postion 1055-1089) in HaCHSB as detected by SMART*

A.

```
KP939100HaCHSA2     GTTTTAATTTCAAAAGAATATCTGCAACTTGAGCCAATCGGTTTAGTGTTCGT GTTCTTC      60
KT302158HaCHSA1     GTTTTAATTTCAAAAGAATATCTGCAACTTGAGCCAATCGGTTTAGTGTTCGT GTTCTTC      60
                    ************************************************************

KP939100HaCHSA2     TTCGCCTTGATTCTGGTGATCCAGTTCTCAGCTATGTTGTTCCATCGATTTGG AACCCTC     120
KT302158HaCHSA1     TTCGCYTTGATTCTGGTSATCCAGTTCTCAGCTATGTTGTTCCATCGATTTGG AACCCTC     120
                     ************************************************************

KP939100HaCHSA2     TCGCACATCTTAGCGTCTACAGAACTGAATTGGTTCTGTTCGAAGAAGTCCG ATGACTTA     180
KT302158HaCHSA1     TCRCACATCTTAGCGTCTACAGAACTGAATTGGTTCTGTTCGAAGAAGGTTA GCATACAC     180
                       ** *******************************************

KP939100HaCHSA2     TCTCAAGACGCTCTGCTAGATAAGAATGCAATAGCAATAGTGAAAGATCTGC AGAAACTG     240
KT302158HaCHSA1     TTCACGAAGACYATAGCCTATAAGAATGCAATAGCAATAGTGAAAGATCTGCA GAAACTG     240
                       *    * * *   ****************************************

KP939100HaCHSA2     AATGGTCTGGACGACGATTATGACAACGACTCGGGCTCGGGTCCACATAAC GTCGGCAGA     300
KT302158HaCHSA1     AATGGTCTGGACGACGATTATGACAACGACTCGGGCTCGGGTCCACATAAC GTCGGCAGA     300
                    ************************************************************

KP939100HaCHSA2     AGAAAGACCATTCATAATTTGGAGAAGGCCAGACAGAAGAAGAGGAACATAG GCACACTG     360
KT302158HaCHSA1     AGAAAGACCATTCATAAYYTGGAGAAGGCCAGACAGAAGAAGAGGAAYATAG GCACACTG     360
                    ***************** ************************** ************

KP939100HaCHSA2     GATGTCGCTTTCAAGAAGAGATTCTTCAACATGAATGCTAATGACGGACCAG              412

KT302158HaCHSA1     GATGTCGCTTTCAAGAAGAGATTCTTCAACATGAATGCTAATGACGGACCAG              412

                    ***************************************************

B.

KP939100HaCHSA2     VLISKEYLQLEPIGLVFVFFFALILVIQFSAMLFHRFGTLSHILASTELNWFCSKKSD DL     60
KT302158HaCHSA1     VLISKEYLQLEPIGLVFVFFFXLILXIQFSAMLFHRFGTLXHILASTELNWFCSKKVS IH     60
                    ******************** *** ************** ***************  .

KP939100HaCHSA2     SQDALLDKNAIAIVKDLQKLNGLDDDYDNDSGSGPHNVGRRKTIHNLEKARQKK RNIGTL     120
KT302158HaCHSA1     FTKXIAYKNAIAIVKDLQKLNGLDDDYDNDSGSGPHNVGRRKTIHXXEKARQKKR XIGTL     120
                      . : ************************************  ******** ****

KP939100HaCHSA2     DVAFKKRFFNMNANDGPX                                              138
KT302158HaCHSA1     DVAFKKRFFNMNANDGPX                                              138
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Figure 3: *Nucleotide (A) and Aminoacid (B) sequences of alternative transcript variants of CHSA1 and CHSA2 in H armigera*

Tissue specific and developmental stages expression patterns of HaCHSA1 and HaCHSA2

Expression analysis of HaCHSA1 and HaCHSA2 quantified using qPCR through primers designed for amplification of differential spliced exons. The expression levels of HaCHSA1 and HaCHSA2 at different tissues and developmental stages and were determined using qPCR. HaCHSA1 variant showed relatively higher expression in 3rd and 4th larval stages while HaCHSA2 noticed more transcript abundance in 1st and 2nd Instar larval stages (Fig. 4). Among the expression levels of HaCHSA1 maintained relatively higher expression levels in trachea, midgut in 4th instar larvae and rest of the pooled tissues in both 3rd and 4th Instar larvae but relative higher expression of HaCHSA2 was observed in tracheal tissues of 2nd instar larvae. Both HaCHSA1 and HaCHA2 variants indicated lower transcript abundance in gut tissues of the 3rd and 4th Instar larvae.

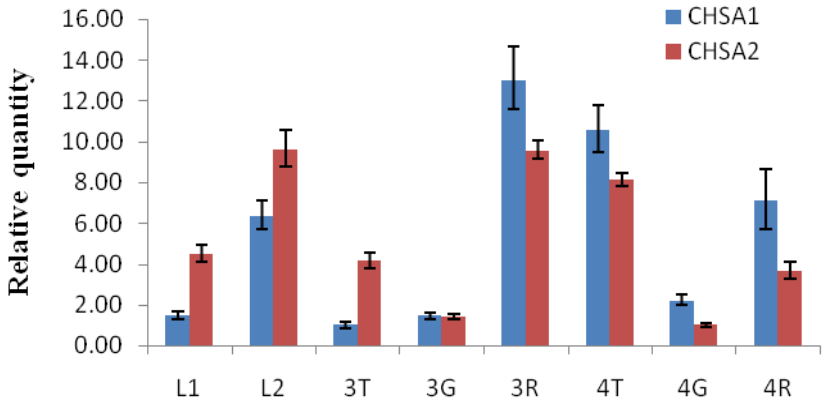

Figure 4: *Relative expression of HaCHSA1 and HaCHSA2 in different tissues and stages of H armigera*

Analysis of chitin synthase B of H.armigera (HaCHSB) and H.zea (HzCHSB)

HaCHSB (AKJ54483.1) from the present study showed 97.9 % similarity with the HzCHSB (ADX66427.1). Gene structure analysis was done to study the difference in the cDNA sequence and contributing variation in protein sequence of the HaCHSB and HzCHSB. Splign analysis (https://www.ncbi.nlm.nih.gov/ sutils/ splign) predicted 23 exons and it revealed major difference in sequences and length of 15th and 16th exon in both HaCHSB and HzCHSB coding sequence. The exon size of 306 and 230 nucleotides in HaCHSB and 350 and 171 nucleotides were noticed in HzCHSB. The resulted variation in nucleotide was apparent through changes in amino acid sequences as depicted through clustalW analysis (Fig. 5) and it has resulted in addition of coil coil domain in HaCHSB which is absent in HzCHSB.

```
HaCHSB    TLYIMYQSMLMFGTILGPGTIFLMMVGAMNAITQMSMSNALILNLVPILIFIVVCMTCKS   960
HzCHSB    TLYIMYQSMLMFGTILGPGTIFLMMVGAMNAITQMSMSNALILNLVPILIFIVVCMTCKS   960
                  ************************************************************
HaCHSB    ETQLFLASLITCAYAMVMMLVIVGIVLQIVEDGWLAPSSLFTAVIFGTFFVTAALHPQEI   1020
HzCHSB    ETQLFLASLITCAYAMVMMLVIVGIVLQIVEDGWLAPSSLFTAVIFGTFFVTAALHPQEI   1020
                  ************************************************************
HaCHSB    ICLLYLTVYYVTIPSMYMLLIIYSLCNLNNVSWGTREVVQKKTAKEMEQERKEAEEAKKK   1080
HzCHSB    ICLLYLTVYYVTIPSMYMLLIIYSLCNLNNVSWGTREVVQKKTAKVRIHSHTD-----FI   1075
                  ********************************************** :.:.:
HaCHSB    MDEKSIQKWFGKSDETSGSLEMSVAGLFKCMCCTNPKDHKEDLHLLQIATAIEKIDKRL   1140
          E
HzCHSB    AKIIFIQKWFGKSDETSGSLEMSVAGLFKCMCCTNPKDHKEDLHLLQIATAIEKIEKRLE  1135
                                                                      .
                  ********************************************************.****
```

Figure 5: Multiple sequence alignment of the HaCHSB and HzCHSB chitin synthases aminoacid sequences of corresponding 15th and 16th exons

Developmental stages specific expression of chitin synthase B (HaCHSB)

Expression analysis of HaCHSB was quantified at different tissues and developmental stages of H armigera using qPCR. HaCHSB showed expression in all the larval viz., 1st (L1), 2nd (L2), 3rd and 4th Instar larval stages. The increase in trend of expression with progress in developmental stages was noticed. Relatively higher expression in gut tissues of 2nd and 4th instars larval stages was observed. Among the studied stages and tissues, relatively lower expression of HaCHSB gene was noticed in tracheal tissues of 3rd instar (Fig 6).

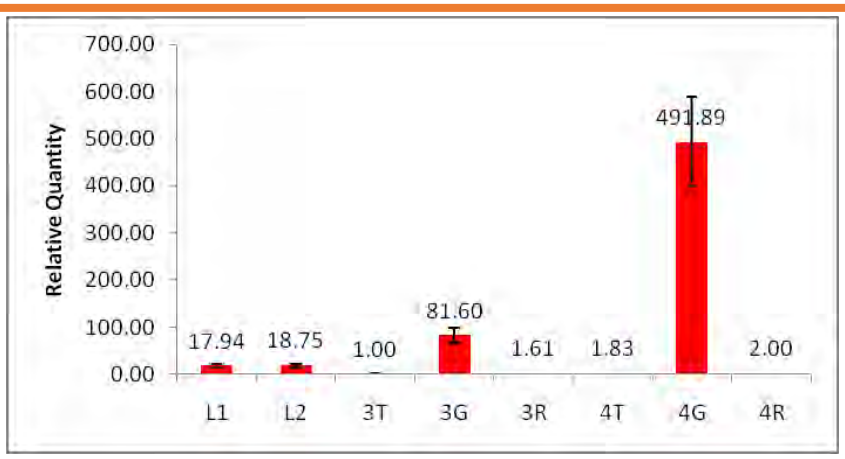

Figure 6: *Relative expression of HaCHSB in different tissues and stages of H armigera.*

Discussion

Chitin, the linear homopolymer of β-1, 4-linked N-acetylglucosamine, is produced by the membrane bound enzyme glycosyltransferase or chitin synthase (CHS) and is the major structural polysaccharide present in the insect exoskeleton and gut lining (Merzendorfer and Zimoch, 2003, Merzendorfer, 2005). Chitin synthases belongs to β-glycosyltransferase 2 (GT2) family which plays crucial role in developmental regulation of chitin biosynthesis in life cycle of insects. In the present study, we found, the presence of chitin synthase A and B, and alternative splice variants for HaCHSA in H armigera strains of cotton, in congruence with similar reports on CHS from different lepidopteron insects. The alternative splice variants were found to be unique to H.armigera with which is otherwise highly conserved among CHSA variants in different insect species. The alternative splice variants of CHSA differ in a 59 amino acid has been reported in many insect species (Zhu et al., 2002; Hogenkamp et al 2005; Bolognesi et al 2005; Chen et al 2007; Kumar et al., 2008; Ashfaq et al., 2007; Zhang et al 2010). Shrik et al (2015) also reported alternative splice variants in H. zea (HzCHSA1 and HzCHSA2) with predicted length alternate exons of 59 amino acids. In contrast, the alternative splice variants HaCHSA1 and HaCHSA2 is unique and differ from other insects produced a 138-aminoacids (Figure 2) as resultant of differential spliced exons. Expression analysis of alternative spliced exons of HaCHSA1 and HaCHSA2 showed relatively higher expression in tracheal and rest of tissues keeping minimal expression in the gut tissues. The expression pattern reaffirms the role of CHSAs in the chitin synthesis in the epidermal tissues of insect. The results from the present study are in congruence with the reports of SeCHSA and MsCHSA expression in epidermial tissues (Chen et al 2007; Hogenkamp et al 2005). It is also noted that, HaCHSA2 and HaCHSA1 played crucial role in chitin synthesis in early and late developmental stages respectively.

Enhanced gut specific expression of HaCHSB in different larval developmental stages of H armigera was noticed. The results supported the similar findings of class B CHS role in pertrophic membrane of lepidopteran insects (Qu et al 2010; Shrik et al 2015; Yu et al 2020). The predicted isoelectric point (pI) of HaCHSB is pH6.00, which is slightly more acidic than that of HaCHSA1 (7.03) and HaCHSA2 (6.56). The acid nature of predicted pI of HaCHSB are in congruence with the pI of chitin synthase B reported in lepidopteran insects viz., OfCHSB (5.79), MsCHSB (pI: 6.06), SeCHSB (pI: 5.64), SfCHSB (pI: 5.84) suggesting their probable role in the peritrophic matrix (Qu et al 2010). The chitin synthesis by HaCHSB might helps to maintain barrier functions of peritrophic membrane in H armigera (Kelkenberg et al., 2015).

Presence of coil coil domain immediately after 5 transmembrane (5-TMS) domain are regarded as one unique feature that is seen in CHSA proteins but not in CHSB. Coiled-coils are known to be potential sites for protein protein interactions and/or signals for vesicular trafficking (Melia et al., 2002). Similar to other members of Lepidoptera namely S. exigua , S.frugiferda , M. sexta , O. furnacalis and even in H. armigera coil-coil domain position in HaCHSA occurs seven amino acids downstream of 5 TMS (Table 1 and 2). But in contrast to the earlier studies, a coiled coil region (postion 1055-1089) in HaCHSB was predicted by SMART (Simple Modular Architecture Research Tool) through COILS program.

Table 1: Aminoacid sequence of the coil coil domain in CHSB sequences of lepidopteran insects

Organism	Aminoacid in coil coil domain and Position
HaCHSB	1055TREVVQKKTAKEMEQERKEAEEAKKKMDEKSIQKW1089
Se CHSB	1056 RETVQKKTAKEMEMEKKEAEEAKKKMENQSLRKL1089
Ms CHSB	1054VAQKKTAKEMEMDKKAAEEAKKKMDNQSI1082
HzCHSB	Nil
Of CHSB	Nil

Table 2: Coil Coil domain in CHSB and sequences of lepidopteran insects

Chitin synthase	End Position of 5th - Transmembrane helice(TMS)	Coil- coil domain (Position)	Chitin synthase	End Position of 5th - Transmembrane helice (TMS)	Coil- coil domain (Position)
HaCHSA	1033-1055	1062-1093 1128-1156	Ha CHSB	1026-1048	1055-1089
HzCHSA	1033-1055	1062-1093 1128-1156	HzCHSB	1026-1048	Nil
Se CHSA	1033-1055	1062-1094 1128-1156	Se CHSB	1026-1048	1056-1089
MsCHSA	1033-1055	1062-1094 1128-1156	Ms CHSB	1022-1044	1054-1082
OfCHSA	1033-1055	1062-1094 1128-1156	Of CHSB	1022-1044	Nil

Ha- Helicoverpa armigera; Hz; Helicoverpa zea; Se:Spodoptera exigua: Ms: Manduca sexta; Of; Ostrania furnicalis

Interestingly, among Helicoverpa spp, the detection of coil coil domain was observed only in HaCHSB but was absent in HzCHSB. SMART analysis of CHSBs predicted coil coil domain in three of the five lepidopteran insects (H armigera, S exigua, M sexta) but found none in O furnicalis, H zea. The presence of coil coil domain has been correlated to the presence of alternate exons. In contrast to earlier prediction and hypothesis, the HzCHSB has found no coil coil domain but has been reported with alternative splice variants (Shrik et al 2015). It is important to note that, the amino acid variation due to alternative spliced exons (15th and 16th exons) in HaCHSB has resulted in change in the aminoacid sequence that resulted to be coil coil domain. Hence, the role of coil coil domain in CHS in H armigera needs to further investigation.

Conclusion

Two chitin synthase (CHSs) HaCHSA and HaCHSB was identified in H armigera. The study was able to identify the alternative splice variants for chitin synthase A i.e., HaCHSA1 and HaCHSA2 in H armigera of Cotton. Coil coil region was predicted in HaCHSB which is absent in the HzCHSB. No alternative transcripts for HaCHSB were noticed similar to CHS2 of different insect species. Expression pattern of the CHS variants and structural features information of the study provided valuable insights on understanding of chitin synthase gene in H armigera.

Methods

H.armigera Rearing and Tissue Preparation

H. armigera larvae were collected from cotton fields at ICAR-Central Institute for Cotton Research (CICR), Nagpur and were reared on an artificial diet standardized at Insectary and biocontrol laboratory of ICAR-CICR, Nagpur. The 3rd and 4th Instar larvaes were dissected to obtain tracheal, gut and rest of the pooled tissues, washed in precooled DEPC water, processed immediately for isolation of total RNA.

Total RNA isolation and cDNA synthesis

For cloning, total RNA was isolated from the 3rd, 4th and 5th instar larvae using Purelink Total RNA isolation Kit (Life-Technologies, Rockville, MD), and equal concentration of total RNA was pooled. For tissue and stage specific expression analysis of CHSA and CHSB gene, total RNA was isolated from 1st, 2nd, 3rd and 4th Instar larval stages. In 3rd and 4th Instar larvae, RNA was isolated from the tracheal, gut and rest of the pooled tissues using Purelink Total RNA isolation Kit and stored in -800C till use. Total RNA was quantified using nano spectrophotometer. The first strand cDNA was prepared

using reverse transcriptase Superscript III (Invitrogen) from 1ug of pooled total RNA for cloning and from individual samples in triplicate for gene expression analysis as per manufacturer's instructions.

Primer designing, PCR amplification and sequencing of full length HaCHSA and HaCHSB

To obtain the sequence of the full-length cDNA of the Helicoverpa armigera CHSA and CHSB, the overlapping primers designed on the basis of chitin synthase BAC clone (HQ840515.1) available for Helicoverpa zea were used to obtain overlapping PCR products using the cDNA as a template (Table 1). The 50 µl of PCR reaction included the following: 1 µl cDNA, 5 µl of 10X HiFi buffer, 2 µl of dNTPs (2.5 mM), 2 µl of MgSO4 (50 mM), 0.8 µl of each primer (10 µM), 0.2 µl (1 U) of Platinum Taq DNA polymerase (Invitrogen) and 38.2 µl of dH2O. The PCR conditions were as follows: one cycle pre-denaturing at 94oC for 3 min. followed by 35 cycles of denaturing at 94oC for 20s, annealing at 57oC for 30s and extension at 68oC for 2 min. with a final 8 min. extension at 68oC. The amplified PCR fragments from each reaction were subjected to electrophoresis in 1.2 % agarose gels and purified using the Qiagen Gel Extraction kit (Qiagen). The purified PCR products were sequenced by the Sanger method (Eurofins). The resulting overlapping sequences were assembled to obtain the full-length HaCHSA and HaCHSB cDNA sequence in Bioedit software.

Bioinformatic analysis of HaCHSA and B nucleotide and protein sequence

The amino acid sequence of HaCHSA and B was deduced from the corresponding cDNA sequence using the translation tool at the ExPASy Proteomics website (http://expasy.org/tools/dna.html). Protein sequence analysis tools used in this study were obtained from the ExPASy Proteomics website (http://expasy.org/). ExPASy Proteomics Server was used to compute isoelectric point and molecular weight of the deduced protein sequences. The transmembrane helices of CHS proteins were analyzed using TMHMM v.2.0 (http://www.cbs.dtu.dk/services/ TMHMM-2.0/) and domain analysis was performed using SMART domain. NetNGlyc 1.0 Server was used to analyze the N-glycosylation sites. Coiled coil region was predicted by SMART (Simple Modular Architecture Research Tool) through COILS program. The signal peptide was predicted by SignalP 3.0 Multiple sequence alignments of deduced amino acid sequences were made using Multiple Alignment software (http://www.ebi.ac.uk/clustalw/index.html). Splign analysis (https://www.ncbi.nlm.nih.gov/sutils/splign) performed using genomic and cDNA sequences of CHSA and CHSB sequences of H. armigera and H zea.

Table 3 : Primers sequence used for isolation of full-length coding sequence of HaCHSA

Name	Sequence
CHSA 26143F	agt aga gag cca gcc tct c
CHSA38026R	cttccaccgtagatttcattggc
CHSA 45167F	caa agg aca gta caa gaa acg aa
CHSA 45901F	cgt ttg cca tcc cag aaa tag gaa
CHSA 45995R	cacaataaactgcgtactcgttggt
CHSA 46353F	atattcat agcggaatcg ctgc
CHSA 46680R	ctatggggctttgacgtgtgacg
CHSA 46951R	ctcttctacaacgatgctgtgg
CHSA 46752F	gta taa tca aaa gtt tgg gca ga
CHSA 47310R	catggaagtaacaattgtcgcca
CHSA 47101F	aagtggcagc tgcatacaaa tcg
CHSA 47998R	tttcgaattcgtagtaatcgg
CHSA 48709R	ctgatccgacaggatgaatac
CHSA 47821F	atc aaa tcg tcg gat cag atc ac
CHSA 48818F	gtc cca tgg tgt ggt atc aaa tg
CHSA 49326R	ctggtatgcgatgtagggactcg
CHSA 49261F	tcgccgattg taaacatact at
CHSA 50018R	cttcttcgtctttttaacttgc
CHSA49921F	taccctctat gtacttgctt tt
CHSA50587R	tctgggtacagtagaaagagtatc
CHSA50461F	acgtaaactc tctgttgggc cta
CHSA51356R	ctcttgagtaacctcatcgtatg
CHSA 51191F	caaggatctg aaagaattga ga
CHSA 52837 R	gaacacgaacactaaaccgattg
CHSA 52861F	ttcttcgcct tgattctggt ga
CHSA53481F	tactgaaccg caagatgaca ttg
CHSA 53597 R	cattccagtgactccatattc
CHSA 54107R	ttccgctgtcacaaaccttaaa

Relative quantification of HaCHSA1 and HaCHSA2, HaCHSB gene expression

Expression analysis of alternative splice variants HaCHSA1 and HaCHSA2, HaCHSB at different developmental stages were quantified using real time qRT-PCR. qPCR primers designed for differentially spliced exon variants of Ha CHSA1 (qCHSA1F-GGAACCCTCTCGCACATCT TA and qCHSA1R GCCATGGTCTTCGTGAAGTG) and Ha CHSA2(qCHSA2F-ATGACTT ATCTCAAGACGCTCTG and qCHSA2R-GTCGTTGTCATAA TCGTCGTCCA). HaCHSB qPCR primers (qCHSBF-AGTCCTGGATGCTTCTCTCT and qCHSBR-GGTCCTCACCTTGATCGT ATTG). The synthesized primers were subjected to qPCR using cDNA of samples to study gene expression. qRT-PCR conditions encompassed 950C for 2 minutes, 950C for 30 seconds, 600C for 30 seconds, 720C for 30 seconds with 35 cycles followed by 720C for 7 minutes final extension. Similar conditions were simulated for qPCR experiments with an additional step for melt curve segment. Raw Ct value from three biological replicates used to calculate the relative expression levels using the 2-ΔΔCt method (Livak and Schmittgen 2001). Relative expression of HaCHSA1 and HaCHSA2 was calculated in relation expression at 3rd instar gut tissues as a calibrator. Relative expression of HaCHSB was calculated in relation to expression at 2nd instar tracheal tissues as calibrator. The expression of calibrator was set as unit one. Normalisation of expression data was done using actin as normaliser gene.

Table 4: Primers sequence used for isolation of full-length coding sequence of HaCHSB

Name	Sequence
CHSB 8461	ttacattttt tatggtgtgc ta
CHSB8822	atggcgacg aaacccaaga ct
CHSB10056	cgcaagaaagtgaaggaatgta
CHSB9890	c tttagatcaacagtttataa cagt
CHSB11247	ctgaccattgttgatccgagct
CHSB10991	taaaggcaca atgttgatga at
CHSB 11745	ctcatgtacgatgtagaagcggt
CHSB 11581	ttaaaaagga ctcgttatta taca
CHSB 12731	ctccaacgctatatcagtgtc
CHSB 12511	tgtacttctt aaacaag
CHSB 13547	ctgggaccatcttttcctgt
CHSB 13342	aagtgaatc gtttcgtca
CHSB 14373	cttgatcgtattgtacataatgt
CHSB 14224	aaaaggc gacggaacac atgat
CHSB 15236	ctgatacataatataaagcgtgga
CHSB 15114	ttctttaaccagcgacg acgat
CHSB 16210	aatattatttttagcaataaaat
CHSB 16029	at catatgctta ctgtacctaa
CHSB 17141	cctaggtacatcgctggatacat
CHSB 17009	cg gtgcacctcc cgaagagaca
CHSB 18241	cacattcatctgatcatcgta
CHSB 18065	gaacgt atcaaaacgg acttgaa
CHSB 19740	cttcttagtgaagtaccagttaa
CHSB 19621	ttcattatat tcttcgggtc cat
CHSB 20953	cgcgccgggcgagttgaacatgaa
CHSB 20791	a actgatttct cgcctaccat
CHSB 21682	tcacgcgtaatggtcttggaagc
CHSB 22221	ttttttttcaattcttcctt

Acknowledgement

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References

1. Ashfaq, M.; Sonoda, S.; Tsumuki, H. (2007). Developmental and tissue-specific expression of CHS1 from Plutella xylostella and its response to chlorfluazuron. Pestic. Biochem. Phys., 89, 20–30.
2. Bolognesi, R., Arakane, Y., Muthukrishnan, S., Kramer, K.J., Terra, W.R. and Ferreira, C. (2005) Sequences of cDNAs and expression of genes encoding chitin synthase and chitinase in the midgut of Spodoptera frugiperda. Insect Biochem Mol Biol 35: 1249–1259.
3. Burkhard, P., Stetefeld, J. and Strelkov, S. V. (2001). Coiled coils: a highly versatile protein folding motif. Trends Cell Biol. 11,82 -88.

4. Chen, X., Tian, H., Zou, L., Tang, B., Hu, J. and Zhang, W. (2008) Disruption of Spodoptera exigua larval development by silencingchitin synthase gene A with RNA interference. BullEntomol Res 98: 613–619.
5. Chen, X., Yang, X., Senthil Kumar, N., Tang, B., Sun, X., Qiu, X.et al. (2007) The class A chitin synthase gene of Spodopteraexigua: molecular cloning and expression patterns. InsectBiochem Mol Biol 37: 409–417.
6. Hogenkamp, D.G., Arakane, Y., Zimoch, L., Merzendorfer, H., Kramer, K.J., Beeman, R.W. et al. (2005) Chitin synthase genes in Manduca sexta: characterization of a gut-specific transcript and differential tissue expression of alternately spliced mRNAs during development. Insect Biochem Mol Biol 35: 529–540.
7. Kelkenberg M, Odman-Naresh J, Muthukrishnan S, Merzendorfer H. (2015) Chitin is a necessary component to maintain the barrier function of the peritrophic matrix in the insect midgut. Insect Biochem Mol Biol. 56:21-8. doi: 10.1016/j.ibmb.2014.11.005. Epub 2014 Nov 20. PMID: 25449129.
8. KR Kranthi, D Jadhav, R Wanjari, S Kranthi, D Russell (2001) Pyrethroid resistance and mechanisms of resistance in field strains of Helicoverpa armigera (Lepidoptera: Noctuidae). Journal of Economic Entomology, 94(1); 253-263.
9. Kumar, N.S., Tang, B., Chen, X., Tian, H. and Zhang, W. (2008) Molecular cloning, expression pattern and comparative analysis of chitin synthase gene B in Spodoptera exigua. Comp Biochem Physiol B Biochem Mol Biol 149: 447–453.
10. Livak, K.J. and T.D. Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. Methods, 25 : 402-408
11. Melia TJ, Weber T, McNew JA, Fisher LE, Johnston RJ, Parlati F, Mahal LK, Sollner TH, Rothman JE.(2002). Regulation of membrane fusion by the membrane-proximal coil of the t-SNARE during zippering of SNAREpins. J Cell Biol. 2;158(5):929-40. doi: 10.1083/jcb.200112081. Epub 2002 Sep 3. PMID: 12213837; PMCID: PMC2173141.
12. Merzendorfer, H. (2006) Insect chitin synthases: a review. J Comp Physiol [B] 176: 1–15.
13. Merzendorfer, H. and Zimoch, L. (2003) Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. J Exp Biol 206: 4393–4412.
14. Qu, M. and Yang, Q. (2011) A novel alternative splicing site of class A chitin synthase from the insect Ostrinia furnacalis –gene organization, expression pattern and physiological significance.Insect Biochem Mol Biol 41: 923–931.
15. Qu, M., Liu, T., Yang, J. and Yang, Q. (2011) The gene, expression pattern and subcellular localization of chitin synthase B from the insect Ostrinia furnacalis. Biochem Biophys Res Commun 404: 302–307.
16. Shirk PD, Perera OP, Shelby KS, Furlong RB, LoVullo ED, Popham HJR. (2015) Unique synteny and alternate splicing of the chitin synthases in closely related heliothine moths. Gene. http://dx.doi.org/10.1016/j.gene.2015.08.001.
17. Tellam, R.L., Vuocolo, T., Johnson, S.E., Jarmey, J. and Pearson,R.D. (2000) Insect chitin synthase cDNA sequence, geneorganization and expression. Eur J Biochem267: 6025–6043.
18. Yu, H. Z., Li, N. Y., Xie, Y. X., Zhang, Q., Wang, Y., & Lu, Z. J. (2020). Identification and Functional Analysis of Two Chitin Synthase Genes in the Common Cutworm, Spodoptera litura. Insects, 11(4), 253. https://doi.org/10.3390/insects11040253
19. Zhang, J., Liu, X., Zhang, J., Li, D., Sun, Y., Guo, Y. et al. (2010a) Silencing of two alternative splicing-derived mRNA variants of chitin synthase 1 gene by RNAi is lethal to the oriental migratory locust, Locusta migratoria manilensis (Meyen). Insect Biochem Mol Biol 40: 824–833.
20. Zhu, Y., Specht, C.A., Dittmer, N.T., Muthukrishnan, S., Kanost, M.R.andKramer,K.J. (2002) Sequence of a cDNA and expression of the gene encoding a putative epidermal chitin synthase of Manduca sexta. Insect Biochem Mol Biol 32: 1497–1506.

Evaluation of Molecular markers linked to drought tolerance in Cotton

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Abstract

Background: Abiotic stresses like drought is the major limiting factors that influence plant growth and crop production. Drought stress due to water deficit reduces the cotton lint production. Thus, it is important to develop drought tolerant cotton cultivars.

Results: In this study RIL population (129 lines) derived from the interspecific cross of *G hirsutum* (28 I) X *G barbadense* (Suvin) were evaluated for SSR markers linked to osmotic potential (OP). The SSR markers BNL3259 on chromosome 14, BNL1153 on chromosome 25 and BNL2884 on chromosome 6 which were linked to osmotic potential (OP) was selected and they were validated among the parents and the same isogenic banding pattern was present in the RIL (129) population. Physiological parameters like canopy temperature (CT), chlorophyll content (SPAD value), Relative water content (RWC), Proline content were measured in the parents and RIL (129) population. Canopy temperature and chlorophyll content were measured in the RIL population in three replicates. Canopy temperature ranged between 27 to 31 °C and the Chlorophyll content ranged from 31 to 40 SPAD value. Relative Water Content (RWC) was calculated it ranged between 85 to 90% in the RIL population. The proline content in the RIL population was observed in leaf samples (1 gm fresh weight) ranged from 150 to 270 µmole/mg of tissue.

Conclusion: The molecular markers evaluated in this study will be used for marker assisted selection to transfer tolerant traits to high-yielding cultivars and to develop drought tolerant varieties.

Keywords: Drought tolerance; *G hirsutum; G barbadense;* interspecific cross; RILs; SSR markers.

Introduction:

The textile industry worldwide is largely based on cotton, the world leading natural fiber crop. Drought stress due to water deficit is a major threat to sustainable cotton production. Water deficit is the major limiting factor can adversely affect physiological and biochemical processes for plant growth and crop productivity (Khan *et al*., 2018; Hou *et al*.,2018). Genetic resources of cotton has diverse traits can be used via marker-assisted selection (MAS)-based breeding programs to develop abiotic stress tolerant cotton cultivars (Kushanov *et al*., 2021). QTLs were mapped for osmotic potential, carbon isotope ratio, chlorophyll a, b, total dry matter, seed cotton yield, harvest index, boll weight, boll number and economically important traits were linked with drought tolerance (Saranga *et al*., 2001). Integration of QTL information, physiological knowledge, and gene expression data is a significant step towards understanding genes controlling physiological responses that affect production and quality under stressful conditions.

QTLs were identified under water stress condition in cotton, using F3 families derived from the cross between *G. barbadense* cv. F-177 and *G. hirsutum* cv. Siv'on (Saranga *et al*., 2001, 2004, Levi *et al*.,2009a, 2009b). QTLs for osmotic potential BNL3259, BNL1153 and BNL2884 were mapped on Chromosome 14, 25 and 6 respectively (Babar *et al.,* 2009). Through marker assisted selection NILs were developed *G. barbadense* cv. F-177 and *G. hirsutum* cv. Siv'on population and they studied physiological parameters like osmotic potential, carbon isotope ratio, chlorophyll a, b (Levi *et al*., 2011,Saeed *et al*., 2011). Two QTLs for relative water content on chromosome 23 and 12,

one QTL for excised leaf water loss on chromosome 23 were identified for drought tolerant traits in upland cotton. (Saleem *et al*., 2015) Genetic map using 1295 simple sequence repeat markers amplified 1342 loci, distributed on 26 chromosomes, covering 3328.24 cM and identified thirteen QTL clusters on nine chromosomes 2, 3, 5, 6, 9, 14, 15, 16, and 21 (Zheng *et al*., 2016). 6 SNPs associated with leaf temperature, 2 QTLs on each chromosome A05, A11, and D03 and one QTL on chromosome A01 were identified in upland cotton at the seedling stage under drought stress (Li *et al*., 2019).

QTLs associated with productivity and quality under field drought conditions was genotyped in recombinant inbred line (RIL) population and ten traits of water stress deficit were used to assess on two recombinant inbred line (RIL) populations with the CottonSNP63K array (Ulloa *et al*., 2020). QTL (13) for plant height (PH),7 QTL for dry shoot weight (DSW) for abiotic stress tolerance were identified in the MAGIC-RILs using 11 parents, where tolerant and sensitive alleles recombined by transgressive segregation (Abdelraheem *et al*., 2021). QTL clusters for drought tolerance were reported, four QTL on chromosome A13 and three QTL on A01 for drought tolerance which, can be used for marker-assisted selection to develop cultivars with drought tolerance in cotton to tackle the climate resilience (Abdelraheem *et al*., 2021). Under water stress and irrigated conditions in 181 intra-specific recombinant inbred lines (RILs), 53 QTLs for plant height (PH), number of sympodial branches, boll number (BN), and boll weight (BW) were identified by QTL analysis (Boopathi *et al*., 2022). In this study, to genetically improve drought resilience of cultivated Upland drought tolerant cotton genotype 28 I (*Gossypium hirsutum* L.) with good fibre quality traits from Suvin (*Gossypium barbadense* L.*)* interspecific crosses was made and RILs were developed and F 11 population was subjected for validation of drought tolerance linked osmotic potential SSR markers through marker assisted selection.

Material and Methods

Plant materials

RILs of F11 generation of consisting of *G hirsutum* (28 I) X *G barbadense* (Suvin) 129 lines derived from an intraspecific cross between drought susceptible Suvin and drought tolerant 28 I parents developed by the single seed descent method was used.

Phenotyping

The RIL population was phenotyped for physiological parameters like canopy temperature (CT), chlorophyll content (SPAD value), Relative water content (RWC) and proline content. Canopy temperature is measured remotely by the infrared thermometer (IRT). Canopies emit long-wave infrared radiation as a function their temperature. The IRT senses this radiation and convert it to an electrical signal, which is displayed as temperature was measured in RIL population. Chlorophyll content (SPAD value) is measured in the SPAD-502 meter. SPAD-502 meter is a hand-held device that is widely used for the rapid, accurate and non-destructive measurement of leaf chlorophyll concentrations. Relative Water Content (RWC) was calculated using formula RWC (fresh weight – dry weight)/(turgid weight – dry weight) × 100 (Clark and Townley-Smith ;1986). The proline content in the RIL population was determined in leaf samples (1 gm fresh weight) by measuring the quantity of the coloured product of proline reaction with ninhydrin at 520 nm absorbance (Bates *et al*., 1973).

Genotyping of RILs

DNA extraction and PCR amplification

The genomic DNA was extracted from young leaves of RILs along with the parental lines by following CTAB method of DNA extraction (Paterson *et al.,* 1993). The isolated DNA quality was checked by gel electrophoresis and quantified by NanoDrop (Eppendorf BioSpectrometer). SSR markers linked to osmotic potential reported in previous studies in the same background was selected to validate among the parents. Initially, to identify polymorphic markers, the selected 10 markers were screened using genomic DNA samples of both the parents and 3 were found polymorphic which were having *G hirsutum* X *G barbadense* background. Later, the 3 polymorphic markers were used, for genotyping of entire RIL population consisting of 129 lines.

The polymerase chain reactions were performed in a total 20-µl reaction volume consisting of 2.0 µl of 10_PCR buffer, 2.0 µl of 2mM dNTP, 2.0 µl of 25 mM MgCl2, 2.0 µl each of forward and reverse primer (3 µM), 3.0 µl of 10 ng/µl high-quality template DNA, 1.0 µl of 1 U/µl Taq DNA polymerase and 6.0 µl of nuclease-free water. The PCR conditions were optimized and employed for amplification comprised of an initial denaturation of 94◦C for 5 min followed by 35 cycles consisting of denaturation at 94◦C for 40 s, annealing 52◦C for 45 s and extension at 72◦C for 1 min. After 35 cycles, all the samples

were kept for a final extension at 72◦C for 10 min. The thermal cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany) was used for PCR amplification. The amplified products along with 5 µl of loading dye were seperated in 3.5% agarose gel electrophoresis. The resolved PCR products were detected by ethidium bromide staining in agarose gels and documented using gel documentation system (Bio-Rad, USA).

Results

SSR markers closely linked to drought tolerance in cotton were validated in recombinant inbred lines (RILs) derived from *G hirsutum* (28 I) X *G barbadense* (Suvin). SSR markers linked to osmotic potential (OP) a drought tolerance trait in interspecific crosses between *G hirsutum* (28 I) X *G barbadense* cotton were selected from earlier reports for validation (Table 1) for validation.The SSR markers BNL3259 on chromosome 14, BNL1153 on chromosome 25 and BNL2884 on chromosome 6 which were linked to osmotic potential (OP) which produced polymorphism among the parents and they were validated (Fig 1). SSR primers BNL3259, BNL1153 and BNL2884 were screened with RIL (129) population produced same isogenic banding pattern (Fig 2,3 &4).

The RILs were phenotyped for physiological parameters. Canopy temperature and chlorophyll content were measured in the *G hirsutum* X *G barbadense* (28 I X Suvin) RIL population in three replicates. Canopy temperature ranged between 27 to 31 ◦C (Fig 5) and the Chlorophyll content ranged from 31 to 40 to the higher value (Fig 6). Relative Water Content (RWC) was calculated it ranged between 85 to 98% and stable in the *G hirsutum* X *G barbadense* (28 I X Suvin) RIL population in three replicates using Clark and Townley-Smith (1986) formula: RWC (fresh weight – dry weight)/(turgid weight – dry weight) × 100(Fig 7). The proline content were determined in leaf samples (1 gm fresh weight) has increased in the RIL population and ranged from 150 to 270 µmole/mg of tissue by measuring the quantity of the coloured product of proline reaction with ninhydrin at 520 nm absorbance (Fig 8).

Discussion

Drought tolerance in various crop improvement using genomic tools is essential to mitigate the climate change (Cattivelli et al.,2008). Marker validation is referred to determining the target phenotype in independent populations and different genetic backgrounds. Marker-assisted selection for drought tolerance is a two-step process. The process starts with the detection of molecular markers linked to drought tolerance and markers flanking QTL influencing resistance. Subsequently, those markers ought to be validated in different mapping populations and genotypes so that the effectiveness of the markers in identifying the desired genotypes can be evaluated in advance (Kushanov *et al.,* 2021). The current study evaluates the previously reported drought tolerant linked markers in a different RIL mapping population for their application.

Table 1: SSR markers linked to Osmotic potential in the Cotton Genome

S. No	SSR marker	Chromosome no.	Forward primer	Reverse primer
1.	CIR 199	C1	CAGAATTTGACCGTTTC	GCCATGATATTTCGGT
2.	NAU 2474	C1	CTATTACCTCCGCCGTAGTG	CTGAGCTAATGCAAGAAGCA
3.	BNL 3594	C6	AGGGATTTTGATTGTTGTGC	TGAATTCAAAACAAATGTTAGCC
4.	JESPR 066	C2	CTGGACTAACTATTTGGTATCCCTC	GATCTGGACTACCGCTAATCAC
5.	MUSS 181	C1	AGCTACAAAGCAGCAATGGG	CATTCATTTCTCAAAGGGGG
6.	CIR 054	C1	TTTCCCTGGTATGCTG	CAATTTCTTCCTCTCGTT
7.	JESPR 273	C25	GGTGTGAGTTATCGCCAAAGG	CTCGCATATAAACGCAACTCG
8.	BNL 1153	C25	CTTTATCCGGAGACGGAACA	CTAACTTTTGCTCACCCCCA
9	BNL3259	C14	TTTTGAAATTCCAGCGAAGG	GTCAATACCTGCTTCTCCACG
10	BNL2884	C6	TCAACTCATACCAAATCAATTC	CCCTGTTTTGTTCAATGGGT

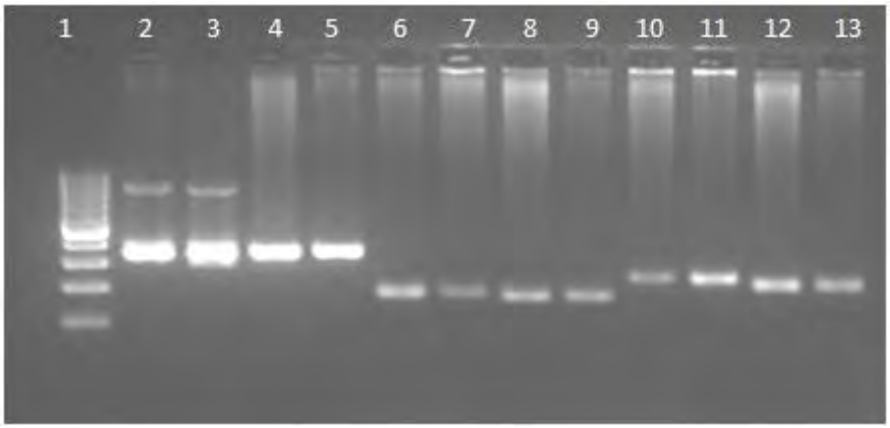
The QTLs for drought tolerant traits like osmotic potential, carbon isotope ratio, chlorophyll a, b were transferred through marker-assisted selection to near-isogenic lines (NILs) derived from interspecific crosses Gossypium barbadense (GB) cv. F-177 and Gossypium hirsutum (GH) cv. Siv'on), introgressed an additional QTL region, expressing higher carbon isotope ratio and chlorophyll a, b, lower expression in osmotic adjustment (OA) (Levi *et al*., 2011,Saeed *et al*.,2011).Similarly in our study

the QTLs were identified and they were transferred in the RIL population derived from *G hirsutum* X *G barbadense* (28 I X Suvin). The SSR markers BNL3259, BNL1153, BNL2884 and were found consistently linked to drought tolerance in this and previous studies .The increased values of the osmotic solutes and chlorophyll content in the progenies (RILs) could contribute to withstand drought. Ten traits of water stress deficit were used to assess on two recombinant inbred line (RIL) populations with the CottonSNP63K array (Ulloa *et al*., 2020).In cotton validation of a major QTL conferring resistance to a defoliating isolate of Verticillium wilt was identified and mapped on chromosome 21 (Zhang *et al.,* 2014). Screening of molecular markers for validation was reported for sucking pest resistance in two recombinant cotton inbred lines (RIL'S) derived from wide hybridization of wild relatives in cotton (Sridhar *et al*., 2017)

Conclusion

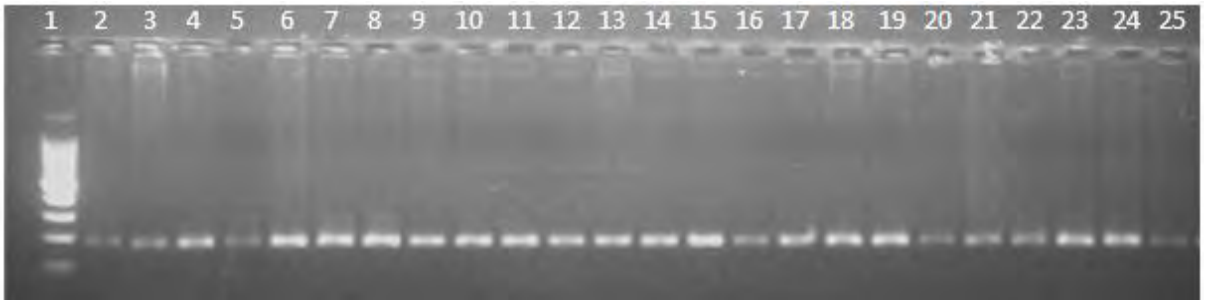
The molecular markers evaluated in this study will be used for marker assisted selection to transfer tolerant traits to high-yielding cultivars and to develop drought tolerant varieties.

Fig 1: Screening of parents *G hirsutum* (28 I) X *G barbadense* (Suvin) with SSR markers

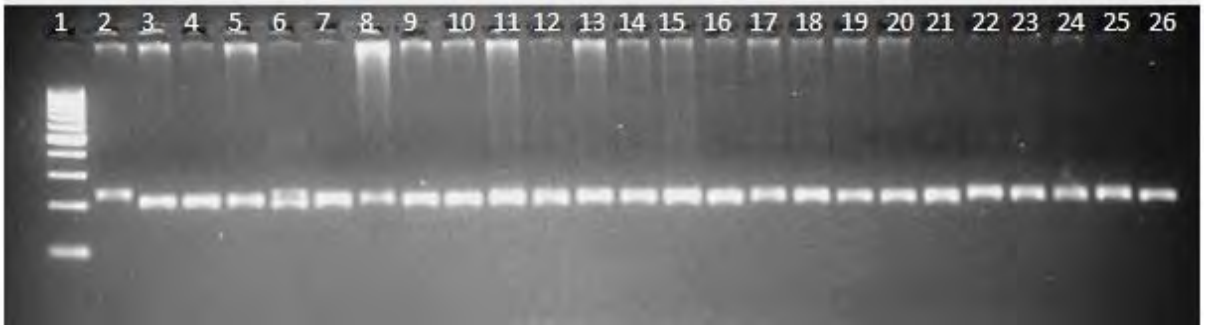


Lane 1:100bp marker 2&3: BNL 1153 (Suvin); 4&5: BNL 1153 (28I);6&7: BNL 2284 (Suvin) ; 8&9: BNL 2284 (28I);10&11: BNL 3259 (Suvin),12&13 : BNL 3259 (28I)

Fig 2: Screening of RIL population with SSR primer BNL 3259

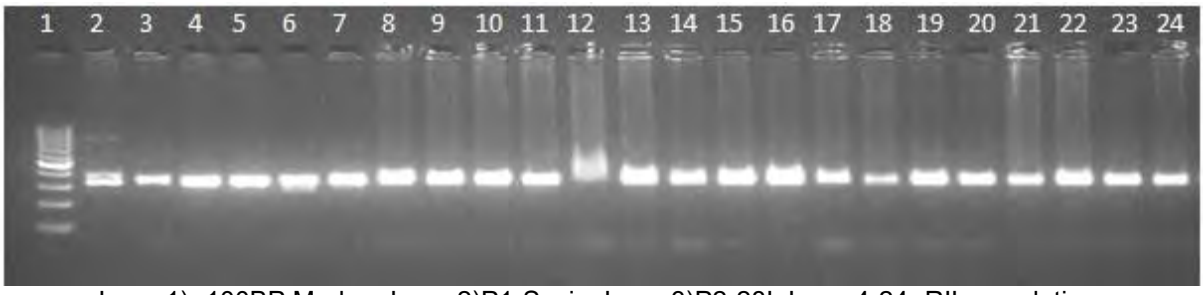


Lane 1) -100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, Lane 4-25- RIL population

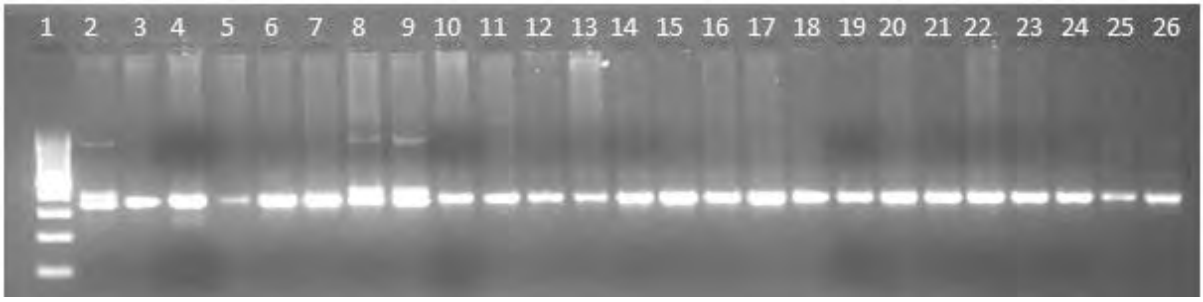


Lane 1)-100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, 4-26

Fig 3: Screening of RIL population with SSR primer BNL 1153

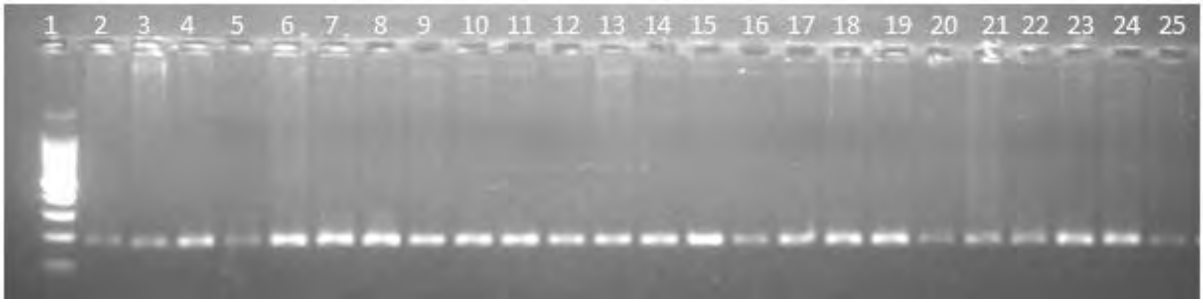


Lane 1) -100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, Lane 4-24- RIL population

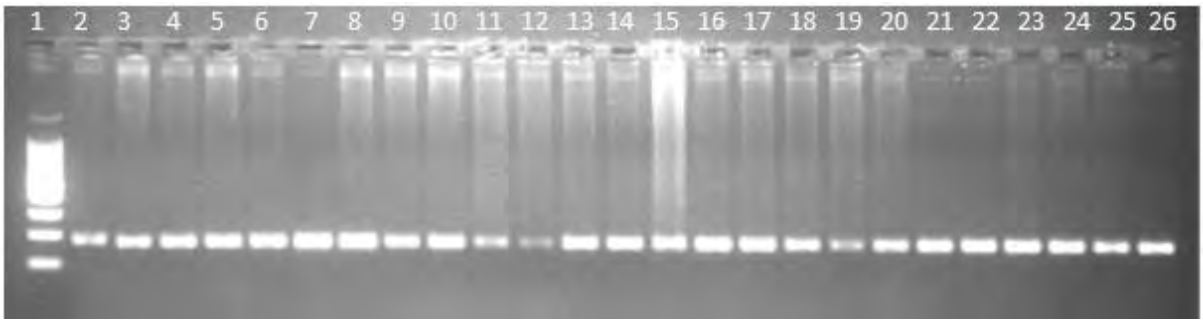


Lane 1) -100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, Lane 4-26- RIL population

Fig 4: Screening of RIL population with SSR primer BNL 2284



Lane 1) -100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, Lane 4-25- RIL population



Lane 1) -100BP Marker, Lane 2)P1-Suvin, Lane 3)P2-28I, Lane 4-26- RIL population

Fig 5: Frequency distribution of canopy temperature in RIL population of cross G hirsutum (28 I) X G barbadense (Suvin)

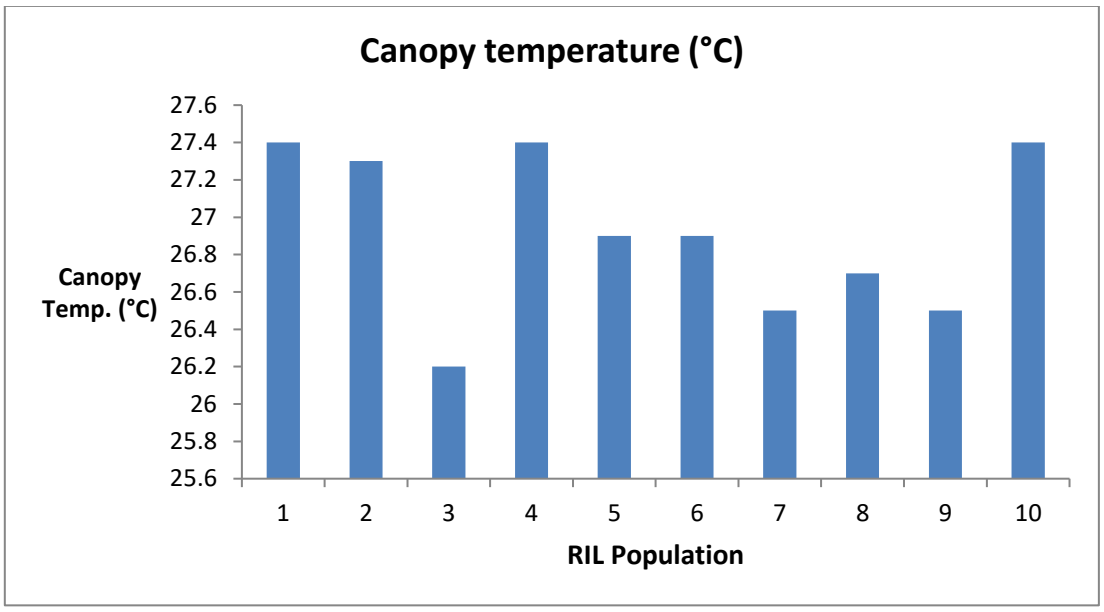


Fig 6: Frequency distribution of Chlorophyll content in RIL population of cross G hirsutum (28 I) X G barbadense (Suvin)

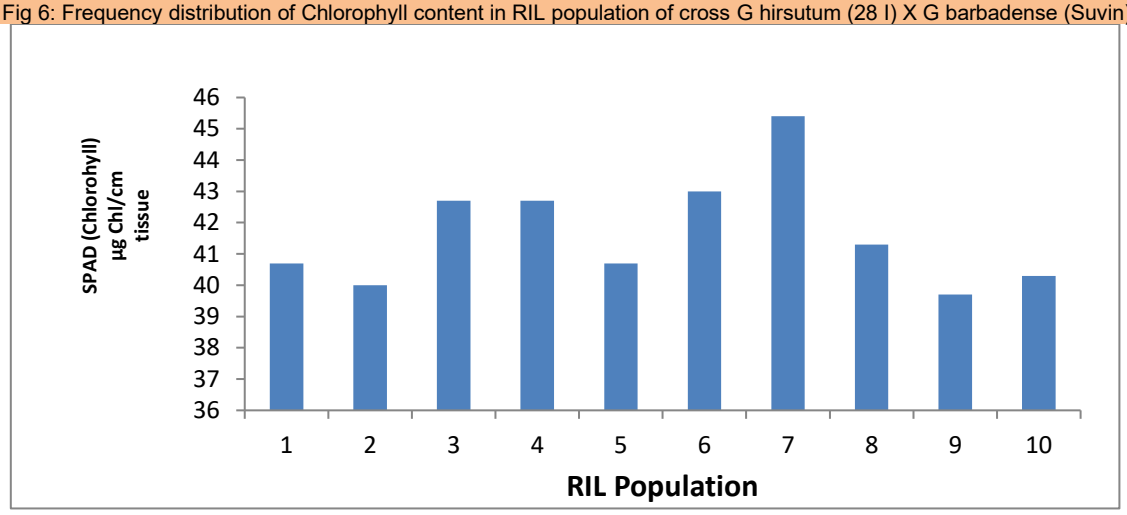


Fig 7: Frequency distribution of relative water content in RIL population of cross G hirsutum (28 I) X G barbadense (Suvin)

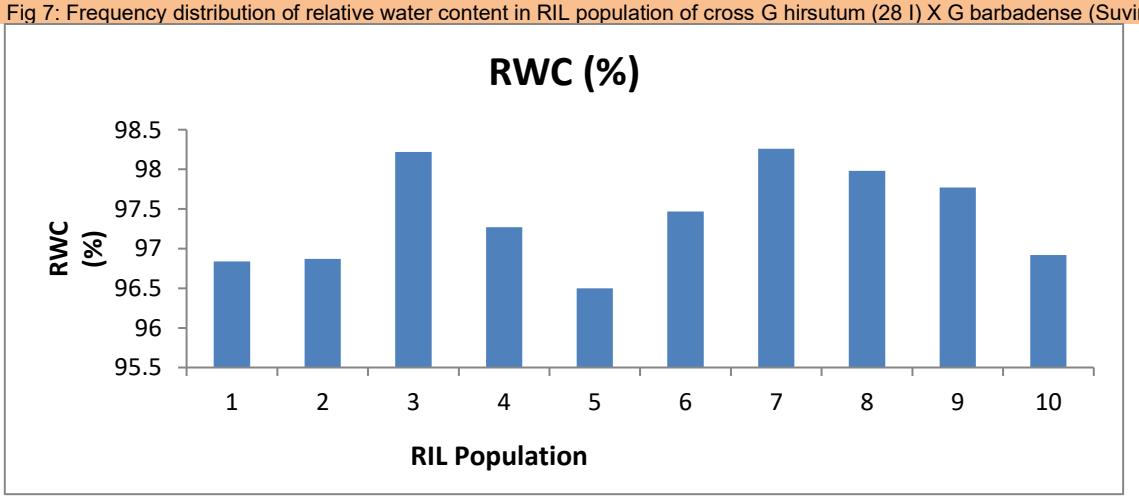
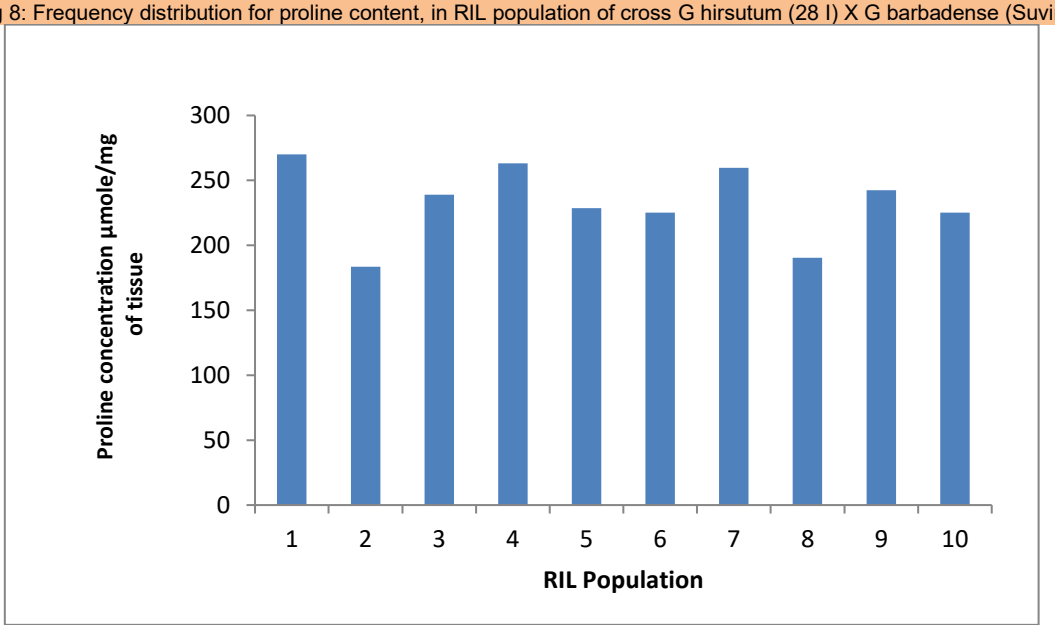


Fig 8: Frequency distribution for proline content, in RIL population of cross G hirsutum (28 I) X G barbadense (Suvin)



References:

1. Abdelraheem A, Thyssen GN, Fang DD, et al.GWAS reveals consistent QTL for drought and salt tolerance in a MAGIC population of 550 lines derived from intermating of 11 Upland cotton (Gossypium hirsutum) parents. Mol Genet Genomics. 2021; 296(1):119-129.doi: 10.1007/s00438-020-01733-2.
2. Babar M, Saranga Y, Iqbal Z, et al. Identification of QTLs and impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from f_6 population using a phylogenetic approach.African Journal of Biotechnology.2009; Vol. 8 (19), 4802-4810.
3. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies.Plant Soi. 1973; 39: 205-207.
4. Boopathi NM, Tiwari GJ, Jena SN, et al. Identification of Stable and Multiple Environment Interaction QTLs and Candidate Genes for Fiber Productive Traits Under Irrigated and Water Stress Conditions Using Intraspecific RILs of Gossypium hirsutum var. MCU5 X TCH1218. Front. Plant Sci. 2022; 13:851504. doi: 10.3389 / fpls. 2022.851504.
5. Cattivelli L, Rizza F, Badeck FW, et al. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res. 2008; 105 .1–14.
6. Clarke JM and Townley-Smith TF. Heritability and relationship to yield of excised leaf water retention in durum wheat. Crop Sci. 1986; 26: 289- 292.
7. Hou S , Zhu G , Li Y, et al. Genome-Wide Association Studies Reveal Genetic Variation and Candidate Genes of Drought Stress Related Traits in Cotton (*Gossypium hirsutum* L.) .Front Plant Sci. 2018; Sep 3; 9: 1276.doi: 10.3389/ fpls.2018.01276.
8. Khan A , Xudong P ,Ullah N ,et al. Coping with drought stress and adaptive mechanisms and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. Biol Res. 2018; 14; 51(1):47.doi:10.1186/s40659-018-0198-z.
9. Kushanov FN, Ozod ST, Ernazarova DK , et al.Genetic Diversity, QTL Mapping, and Marker-Assisted Selection Technology in Cotton (Gossypium spp.). Front Plant Science. 2021; Dec 16; 12: 779386. doi: 10.3389/fpls.2021.779386.
10. Levi A, Paterson AH, Cakmak I, et al. Metabolite and mineral analyses of cotton near-isogenic lines introgressed with QTLs for productivity and drought-related traits. Physiol Plant. 2011; 141(3):265-75.doi: 10.1111/j.1399-3054.2010.01438.
11. Levi A, Lianne OA, Paterson AH, et al. Photosynthesis of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. Plant Science. 2009; 177. 88–96.
12. Levi A, Ovnat L, Paterson AH, et al.Photosynthesis of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. Plant Sci 2009 a; 177: 88–96.
13. Levi A, Paterson A, Barak V, et al.Field evaluation of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. Mol Breed 2009b; 23: 179–195.
14. Li HM, Liu SD , Chang WG , et al. Analysis of Drought Tolerance and Associated Traits in Upland Cotton at the Seedling Stage. Int J Mol. Science. 2019; 9; 20(16):3888.doi: 10.3390/ijms20163888.
15. Paterson AH, Brubakar CL, Wendel JF. A rapid method for extraction of cotton (Gossypium spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol Biol Rep 1993; 11: 122–127.
16. Saleem MA, Malik TA, Shakeel A, et al.QTL mapping for some important drought tolerant traits in upland cotton. The Journal of Animal & Plant Sciences. 2015; 25(2): 502-509.
17. Saranga Y, Jiang C, Wright R, et al.Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. Plant Cell Environ.2004; 27: 263–277.
18. Saranga Y, Menz M, Jiang CX, et al.Genomic dissection of genotype x environment interactions conferring adaptation of cotton to arid conditions. Genome Res. 2001; 11(12):1988-95.doi: 10.1101/gr.157201.

19. Sridhar V, Reddy PS, Reddy SS, et al.Development and validation of molecular markers for sucking pest resistance in Cotton [Abstract].In Abstracts of the NGBT conference, Bhubaneswar, Odisha, India: Can J biotech. Oct 02-04, 2017, Volume 1, Special Issue (Supplement), Page 288. https://doi.org/10.24870/cjb.2017-a272
20. Ulloa M , Luis M, Santiago D , et al.Enhancing Upland cotton for drought resilience, productivity and fiber quality comparative evaluation and genetic dissection. Mol Genet Genomics.2020; Jan, 295 (1) :155-176.doi: 10.1007/s00438-019-01611-6
21. Zhang X, Yuan Y, Wei Z, et al. Molecular mapping and validation of a major QTL conferring resistance to a defoliating isolate of verticillium wilt in cotton (Gossypium hirsutum L.). PLoS ONE.2014; 9(4):e96226.doi:10.1371/journal.pone.0096226.
22. Zheng JY, Oluoch G, Riaz Khan MK, et al. Mapping QTLs for drought tolerance in an F2:3 population from an inter-specific cross between *Gossypium tomentosum* and *Gossypium hirsutum*. Genet Mol Res. 2016;15(3). doi: 10.4238/gmr.15038477.

Cotton Yield Under Different Nitrogen Rates and Sources Following Soybean Harvest in the Brazilian Cerrado

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Abstract

Background: *Brazil stands as the world's second largest cotton exporter with 1.6 million hectares planted and 2.8 million tons of fiber produced, the state of Mato Grosso being the main producer. Practically, all of the cotton in Mato Grosso is a second season crop – sown after soybean harvest. Cotton yield is reduced under low nutrient availability, especially nitrogen (N) due to its role in plant physiological processes and fiber yield. Therefore, we evaluated the effect of N rates and sources on cotton yield in Mato Grosso state, Brazil.*
Results: *The experiment was established in 2016 cropping season under a Typic Haplustox and as a complete randomized block design with four replicates. The treatments included the application of two N sources (urea versus calcium ammonium nitrate – YaraBela®) and four N rates (48, 96, 144 and 192 kg ha-1), plus the control, without N application. For the 2019 cropping season (fourth season), seed cotton yield and total N in plant shoots enhanced with increasing N rates (P<0.01). Considering all the N rates, YaraBela® increased seed cotton yield and N accumulated in plant shoots by 273 kg ha-1 and 48 kg N ha-1 compared to urea (5,028 vs 4,755 kg ha-1; 453 vs 405 kg N ha-1), respectively. Maximum yield for YaraBela® (5,650 kg ha-1) was reached at rate 144 kg N ha-1, whereas for urea (5,666 kg ha-1) at rate 192 kg N ha-1.*
Conclusion: *Therefore, cotton grown as a second crop is responsive to higher doses of N. Applying YaraBela® (compared to urea) increases N use efficiency by the crop, enhancing seed cotton yields..*

Keywords: *Gossypium hirsutum L.; mineral nitrogen; Oxisol; second-crop*

Background

Brazil is one of the world's leading cotton exporter and producer. In 2019, Brazil planted 1.6 million hectares of cotton and produced 2.8 million tons of fiber, of which, approximately, 70% came from Center-West state of Mato Grosso, within the Cerrado biome (CONAB, 2020). Most of the production in Mato Grosso is rain-fed second season crop (called safrinha), sown from January to the beginning of February and following soybean harvest.

Cotton is a high nutrient demanding crop and yields may be limited if nutrients are not adequately provided and accumulated in the plant during its growth (Rochester et al., 2012), notably in soils from the Cerrado, which are naturally poor in fertility. Mato Grosso is the biggest fertilizer consumption market in Brazil - 22% of total fertilizer deliveries in 2019 (ANDA, 2020) - and fertilizer for cotton accounts for 20-25% of production costs (ANDA, 2020; IMEA, 2020). Thus, in order to increase fertilizer use efficiency, it is of importance to understand the maximum nutrient uptake rate, extraction efficiency and exportation by the crop, and, also, the dynamics and potential losses of nutrients in soil (e.g. N volatilization) (Borin et al., 2018). A recent study under field conditions in the Cerrado shows that for each megagram of produced seed cotton, the plant uptakes 68 kg N and exports (through harvest), approximately, 50% of that amount (Borin et al., 2018). However, few information is available on nutrient requirements for cotton production, following soybean in the Cerrado, especially for nitrogen (N), which may be supplied partially to cotton from soybean, requiring new studies about N rate in this new cropping system.

N fertilizer use efficiency in tropical agro-ecosystems is commonly low (Dourado-Neto et al., 2010). Cunha et al. (2018), considering eighteen main agricultural crops in Brazil from 2013 to 2016 verified that only 58% of N applied as fertilizers were efficiently used by the crops. N losses by N-NH3 volatilization may reach up to 40% of total N applied to crops depending on the type of source used

(Cantarella et al 2007; Duarte et al., 2017; Mazzetto et al., 2020). These losses are also dependent on climate conditions (e.g. precipitation events) and soil conditions (e.g. humidity, cation exchange capacity and temperature) (Rodrigues & Kiehl, 1986; Sangoi et al., 2003). N sources, such as ammonium sulfate and ammonium nitrate, have shown lower N-NH3 losses compared to urea in Center-South region of Brazil and under maize field production (Fontoura and Bayer, 2010). However, studies in the Center-West Brazil are few, and, therefore, necessary to understand the best management for N fertilization for cotton production.

For this purpose, this study aimed to evaluate the effect of N rates and sources on cotton yield following soybean harvest in Mato Grosso state, Brazil.

Results

There was no interaction between the two N sources and the rates of N for seed cotton yield, dry matter yield and N accumulated in dry matter. However, differences were observed for sources and rates of N. Cotton yield was higher for YaraBela® (5,028 kg ha-1) compared to urea (4,755 kg ha-1). The cotton yield averaged among the N fertilized treatments (factorial – 4,891 kg ha-1) was higher than control (3,508 kg ha-1) (Figure 1). For dry matter yield (DMY), only the sources differed from each other – YaraBela® (21,955 kg ha-1) increased DMY compared to urea (19,368 kg ha-1). N accumulated in cotton dry matter followed the same pattern as seed cotton yield and DMY – YaraBela® increased the N in cotton shoots (Figure 2).

Considering both N sources, cotton yield and N accumulated in shoots were enhanced with increasing N rates (Figure 3A) and reached the highest yield and accumulation (5,337 kg ha-1; 466 kg N ha-1) at the rate of 192 kg N ha-1. Lower yields and N accumulation were seen at rate 48 kg N ha-1 (4364 kg ha-1; 368 kg N ha-1). According to all data and following the regression model (Figure 3A), the maximum yield is reached at a rate higher than 192 kg N ha-1 (300 kg N ha-1; y = -0.0174x2 + 10.426x + 3940.3). When YaraBela® and urea are evaluated separately, (Figure 3B) and following the regression models, the rate of N for maximum yield would be at 165 kg N ha-1 (y = -0.0608x2 + 20.111x + 3634) and higher than 192 kg N ha-1 (265 kg N ha-1; y = -0.0276x2 + 14.641x + 3480.6), respectively. Within the study data and not considering the regression model, maximum yield for YaraBela® (5,650 kg ha-1) was reached at rate 144 kg N ha-1, whereas for urea (5,666 kg ha-1) at rate 192 kg N ha-1. In this case, for each kg of N ha-1 used, YaraBela® and urea produced 39 and 30 kg ha-1 of seed cotton, respectively.

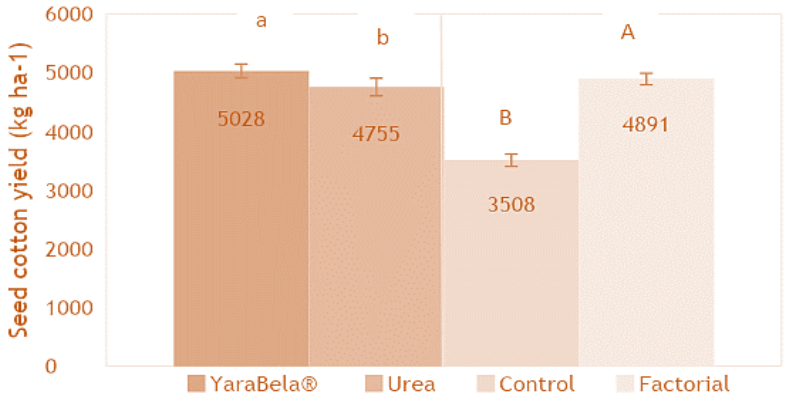

Figure 1. Seed cotton yield in response to the application of YaraBela®, urea, control (without N), and average of N fertilized treatments (factorial)

Notes: Results in Paiaguás Farm (SLC Agrícola Group), Diamantino, Mato Grosso, Brazil. Means followed by different lowercase letters (YaraBela® vs Urea) differ from each other; means followed by different uppercase letters (Control vs factorial) differ from each other (LSD, P<0.10).

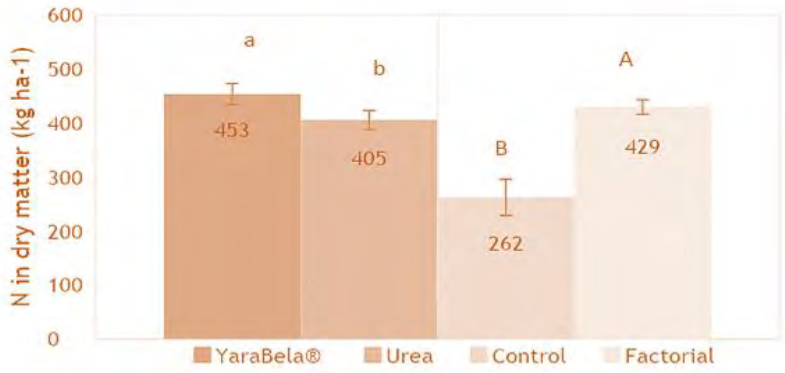

Figure 2. Nitrogen accumulated in cotton dry matter (stems, leaves and reproductive structures) in response to the application of YaraBela®, urea, control (without N), and average of N fertilized treatments (factorial)

Notes: Results in Paiaguás Farm (SLC Agrícola Group), Diamantino, Mato Grosso, Brazil. Means followed by different lowercase letters differ from each other; means followed by different uppercase letters differ from each other (LSD, P<0.10).

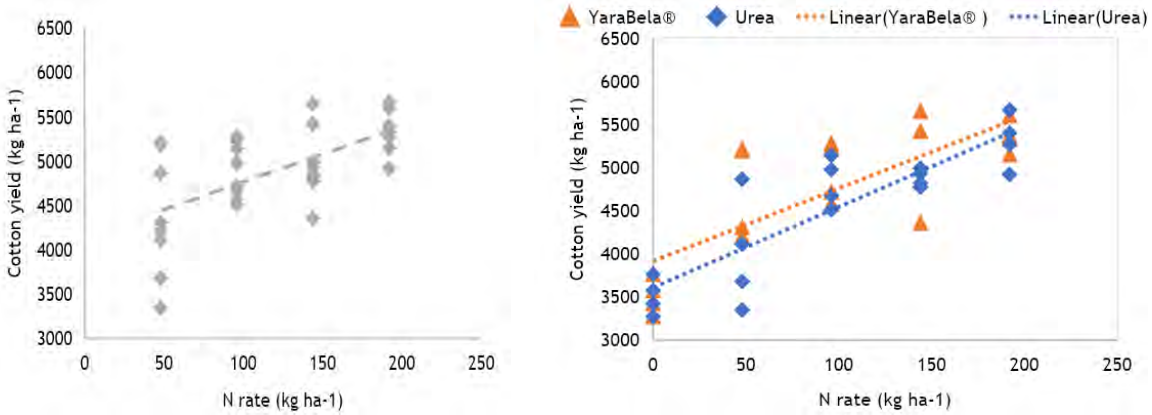

*Figure 3. Seed cotton yield considering all N rates and sources (A), and considering, separately, the sources YaraBela and urea (B). ***: significant at 1% by F test*

Discussions

Higher yields for YaraBela®, compared to urea (Figure 1), may be highly associated with lower N-NH3 volatilization along the cropping years, and, consequently, with higher N uptake by the plant, as observed by the N accumulated in cotton shoots (Figure 2) . N-NH3 from cropping systems can be both an economic burden to farmers (lower yields), and an environmental issue – ammonia has a direct effect on eutrophication, acidification and loss of biodiversity in global ecosystems (Erisman et al., 2008). N-NH3 emissions from urea-based fertilizers are higher than from other fertilizers due to the rapid hydrolysis of urea which causes rise in pH in the direct vicinity of the application zone, especially in soils with high urease enzyme activity due to crop residues (Cantarella et al., 2018; Fontoura et al., 2010). On the other hand, ammonium nitrate-based fertilizers reduce N-NH3 emissions due to its acid-based reaction. In Mato Grosso, urea-based fertilizers are commonly applied as broadcast and over no-till cropping systems, and N-NH3 losses may be higher than 40% (Cantarella et al 2007; Duarte et al., 2017; Mazzetto et al., 2020).

Cumulative N-NH3 volatilisation is negatively correlated with soil pH and rainfall for urea-based fertilizers (Mazzetto et al., 2020). In this study, although the accumulated rainfall across the cropping year (812 mm) was considered enough for cotton, it's distribution was irregular. Possibly, it resulted in microsite hotspots for N-NH3 volatilisation. Looking at Figure 4 and at the red arrows indicating N application events (which were previously planned to fit plant demand), it is possible to understand how challenging it is to match N applications with precipitation events that will lower N-NH3 volatilization. As seen in Figure 4, there were no precipitation events in the last 2-3 days before applying N to crop, which also is the best timing (and most commonly practiced) for farmers to put their tractors in the field, since soil humidity is lower and operations are more efficient. Precipitation events are also important after the application of urea-based fertilizers to avoid volatilization (Cantarella et al., 2007). In this study, and one

day after the application of N (in all three events), there was only 6 mm of rain (1.96 mm, 0.00 mm, 4.10 mm for 1st, 2nd and 3rd N application events, respectively). It is known that higher peaks of N-NH3 volatilization happen in the first two to three days after N application (Cantarella et al., 2007; Fontoura et al., 2010). Thus, combining N application with high probability of rainfall events in Mato Grosso is a very difficult task. Added to that, the planning of field operations and available structure is limited in Mato Grosso since cotton farms have on average 5.2 thousand hectares, and some outliers of approximately 50 to 100 thousand hectares that represent 35% of cotton areas in Mato Grosso (IMA, 2020).

Considering the soybean-cotton production system, the second crop is prefered by farmers due to its higher economic and technical relevance. Because of this, short cycle soybean cultivars are prefered to ensure that cotton is sown in the right timing. As a result, the residual N left from the soybean residues to cotton may be limited. Apart from N that may be obtained from soybean residues to cotton, it is estimated that, approximately, 75 kg N ha-1 year-1 is mineralized in Cerrado soils for every 1% soil organic matter in the 0-30 cm layer (Borin et al., 2018). It is suggested that for every megagram of seed cotton yield produced, 35 kg N ha-1 should be applied (Borin et al., 2018). This is alligned to what was measured in this trial – for each megagram of seed cotton, 34 kg N ha-1 was needed as urea (5,666 kg ha-1 at rate 192 kg N ha-1). However, in the case of YaraBela®, this lowers down to 25 kg N ha-1 for every megagram of seed cotton produced (5,650 kg ha-1 at rate 144 kg N ha-1) from the fourth season of the trial.

Conclusion

Nitrogen fertilization is a key practice and necessary for higher cotton yields grown under tropical Cerrado conditions and following soybean harvest. Cotton was highly responsive to nitrogen application and increasing nitrogen rates enhanced seed cotton yield and nitrogen accumulated in shoots. However, seed cotton yields and N uptake by the plant differ depending on the source of nitrogen used. Ammonium nitrate-based fertilizer (YaraBela®) increased nitrogen uptake and seed cotton yield compared to urea-based fertilizer. In order to achieve similar target yield, the rate of nitrogen when using YaraBela® is smaller (144 kg N ha-1) than when urea is used (192 kg N ha-1) and, therefore, for each kg of N ha-1 used, YaraBela® and urea produced 39 and 30 kg ha-1 of seed cotton, respectively. These results show that YaraBela® potentially improves nitrogen use efficiency compared to urea, leading to lower agronomic losses (e.g., N-NH3 volatilization), and, consequently, to higher nitrogen uptake by the crop. It should be emphasized that long-term experiments are important to better understand the dynamics of nitrogen in the soil and the effect of applying different sources of nitrogen-based fertilizers to different cropping systems. More research that targets on nitrogen use efficiency and losses is necessary to provide information on the fate of nitrogen inputs, especially in tropical field conditions and under different rotational systems.

Methods

The study was established during 2016 cropping season in Paiaguás farm, SLC Agrícola Group, located at Diamantino, Mato Grosso, Brazil (14º04'57.7''S, 57º27'42.5''W and altitude of 270 m), and continued for four cropping seasons. The climate is classified as Aw (Köepen Classification), with dry winter and rainy summer and average temperature of 25.9 ºC. Figure 4 shows daily precipitation events and average temperatures from the 2019 cropping season. Total precipitation along cotton 2019 season was 812 mm. The soil was classified as Latossolo Vermelho distrófico (according to Brazilian Soil Classification System - EMBRAPA, 2013) or a Typic Hapludox (according to Soil Survey Staff, 2014). The data presented here are from the 2019 cropping season (fourth cropping season), and soil chemical properties and particle-size distribution before cotton was sown for the season can be found in Table 1.

The experiment was set up as randomized block design with four replicates, arranged in a factorial [(2 x 4) + 1], with two N sources (urea and YaraBela® calcium ammonium nitrate) and four N rates (48, 96, 144 and 192 kg ha-1), plus the control, without N application. N was broadcasted and applied at 30 (25% of total N), 60 (45% of total N) and 80 (30% of total N) days after plant emergence. Treatments were applied to the same plots in the consecutive cropping seasons of 2017, 2018, and 2019. Nitrogen levels were determined according to 40, 80, 120 and 160% of the amount of N applied by farmers in the region, which is, approximately for 120 kg ha-1 of N. Each experimental plot was composed by six rows of 15 m long spaced by 0.90 m from each other.

Cotton was sown on January 23, 2019 using the variety TMG 47 B2RF and with a population of 100,000 plants ha-1. 100 kg P2O5 ha-1, 111 kg K2O ha-1 and 44 kg S ha-1 were applied at sowing and for all

treatments. Disease, insect pest, weed management and growth were controlled as needed and carried out with insecticides, fungicides, herbicides and plant growth regulators.

To measure dry matter yield and total N accumulated in cotton shoots (stems, leaves and reproductive structures), ten plants from each experimental unit were randomly selected at 110 days after plant emergence. The plants were weighed, dried in an oven at 60º C for 120 hours to obtain the dry mass. Total N was determined following Malavolta et al. (1997). N accumulated in cotton shoots was calculated by multiplying dry matter yield and N content. Cotton yield was determined by manually harvesting (fiber and seed) ten meters of the two central rows and weighed in a field scale on July 25, 2019. Maximum yield by the N sources was determined based on the N curve response.

Precipitation	Temperature
39.25	21.90
39.67	22.26
51.72	22.98
0.83	23.88
6.89	23.52
2.29	24.97
15.07	24.16
0.00	23.28
0.00	26.20
0.00	24.73
0.00	24.89
0.00	24.37
2.64	23.55
0.00	23.72
7.53	22.98
11.18	24.23
0.00	24.79

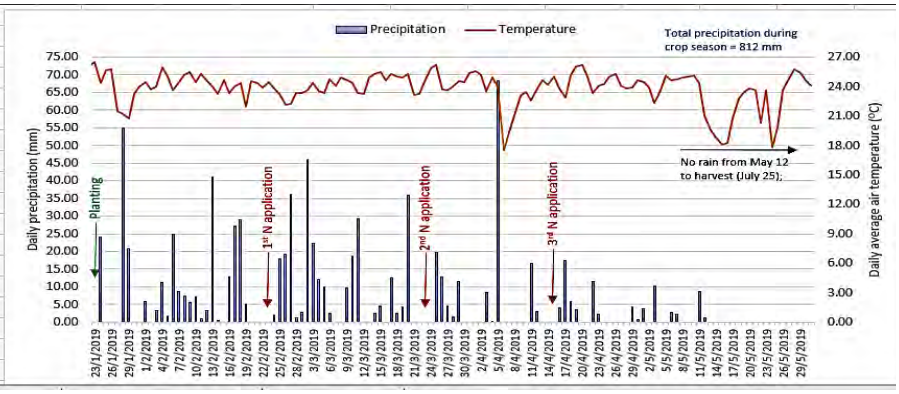

Figure 4 Precipitation and average air temperature at the experimental site and during cotton cropping season 2019

Statistical analyses were performed using R v.3.2.2 (R core team, 2015), with a statistical significance threshold of P≤0.05. Data were tested for normality using the Shapiro-Wilk test. Regression analysis was used to determine the maximum cotton yield by each N source using the software Sigmaplot_ Version 10 software (Systat Software Inc., Chicago, USA, 2007).

Table 1 Soil chemical properties and particle-size distribution at the 0.00-0.20 and 0.20–0.40 m layers before cotton was planted for the 2019 cropping season

Soil layer	pH	P	S	K	Ca	Mg	CEC	SOM	Sand	Silt	Clay
----m----		---mg dm^{-3}---		----------mmolc dm^{-3}----------				----------g kg^{-1}----------			
0.0–0.2	5.2	117	5	2.5	32	12	69	25	230	34	736
0.2-0.4	5.2	42	6	2.0	29	10	53	23	262	33	705

**pH measured in $CaCl_2$ (0.01 mol L^{-1}); SOM = soil organic matter determined by wet dichromate oxidation and using the 1.72 factor from van Bremelen ; CEC – cation exchange capacity ; P, K, Ca and Mg determined by the resin method; S determined in calcium phosphate (0.01 mol L^{-1}).*

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References

1. ANDA. Associação Nacional para Difusão de Adubos. Anuário estatístico do setor de fertilizantes 2019-2020. São Paulo, 2020.
2. BORIN, A.L.D.C.; Ferreira, G.B.; Carvalho, M.C.S.; Ferreira, A.C.B. Nutrição e adubação do algodão na região do Cerrado. In: Flores, R.A.; Cunha, P.P.; Marchão, R.L.; Moraes, M.F. Nutrição e Adubação de Grandes Culturas na Região do Cerrado. Goiânia: Gráfica UFG, 2018. 620 p.
3. Cantarella, H. Nitrogênio. In: Novaes, R.F; Alvares, V.V.H.; Barros, N.F.; Fontes, R.L.; Neves, J.C.L. (editores). Fertilidade do Solo. Viçosa, MG. Sociedade Brasileira de Ciência do Solo. 2007. P.375-470.
4. Cantarella, H.; Otto, R.; Soares, J.R.; Silva, A.G.B. Agronomic efficiency of NBPT as a urease inhibitor: A review. Journal of Advanced Research. 2018. v. 13, p.19–27.

5. CONAB. Acompanhamento da safra brasileira de grãos, v. 7, safra 2019/20 – Quinto levantamento, Brasília, 2020. p. 1-112, fevereiro

6. Cunha, J.F.; Francisco, E.A.B.; Prochnow, L.I. Balanço de nutrientes na agricultura brasileira: 2013 a 2016, Piracicaba, SP: IPNI. 2018. P. 3-14 (Informações Agronômicas, 162).

7. Dourado-Neto, D.; Powlson, D.; Abu Bakar, R.; Bacchi, O.O.S.; Basanta, M.V.; Cong, P.T.; Keerthisinghe, G.; Ismaili. M.; Rahman, S.M.; Reichardt, K.; Safwat, M.S.A.; Sangakkara, R.; Timm, L.C.; Wang, J.Y.; Zagal, E.; van Kessel, C. Multiseason recoveries of organic and inorganic nitrogen-15 in tropical cropping systems. Soil Science Society of America Journal 2010. 74, 139–152,

8. Duarte, A.P.; Cantarella, H.; Kappes, C. Adubação de Sistemas Produtivos: Milho Safrinha e Soja. In: Paes, M.C.D. Construindo sistemas de produção sustentáveis e rentáveis: palestras do XIV Seminário Nacional de Milho Safrinha, Cuiabá, MT, 21 a 23 de novembro de 2017. Sete Lagoas: Associação Brasileira de Milho e Sorgo. 2017. p. 173-195.

9. Erisman, J.W.; Sutton, M.A.; Galloway, J.; Klimont, Z.; Winiwarter, W. How a century of ammonia synthesis changed the world. Nature Geoscience, v.1, p. 636–639. 2008.

10. Fontoura, S.M.V.; Bayer, C. Ammonia volatilization in no-till system in the South-Central region of the state of Paraná, Brazil. Revista Brasileira de Ciência do Solo. 2010. v. 34, p. 1677-1684.

11. IMA. Instituto Mato Grossense do Algodão. Levantamento de Safra 2020. Maio, 2020.

12. IMEA. Instituto Mato Grossense de Economia Agrícola. Custo de Produção do Algodão – Safra 2020/21. Março, 2020.

13. Malavolta, E.; Vitti, G.C.; Oliveira, S.A. Avaliação do estado nutricional das plantas: princípios e aplicações. 2ª. ed. Piracicaba: Potafos; 1997.

14. Mazzetto, A.M.; Styles, D.; Gibbons, J.; Arndt, C.; Misselbrook, T.; Chadwick, D. Region-specific emission factors for Brazil increase the estimate of nitrous oxide emissions from nitrogen fertiliser application by 21%, Atmospheric Environment. 2020. v. 230, 117506.

15. Rochester, I.J.; Constable, G.A.; Oosterhuis, D.M.; Errington, M. Nutritional requirements of cotton during flowering and fruiting. In: Oosterhuis, D.M.; Cothren, J.T. (editors). Flowering and fruiting in cotton. Cordova: The cotton foundation, Number Eight. 2012. p. 35-50,

16. Rodrigues, M.B.; Kiehl, J.C. Volatilização de amônia após emprego de uréia em diferentes doses e modos de aplicação. Revista Brasileira de Ciência do Solo. 1986. v. 10, p.37-43.

17. Sangoi, L.; Ernani, P.R.; Lech, V.A.; RAMPAZZO, C. Volatililização de N-NH3 em decorrência da forma de aplicação de ureia, manejo de resíduos e tipo de solo, em laboratório. Ciência Rural, 2003. 33:687-692

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Effect of Nitrogen Rate on Cotton Crop Growth, Yield and Fibre Quality

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Abstract

Background: *Nitrogen application rate is an important practice for optimizing cotton production. CB-15 is a newly released cotton variety for which N rate is not determined.*

Results: *Experiments were conducted at three Cotton Research Centers located at Sreepur, Gazipur; Jagadishpur, Jashore and Sadarpur, Dinajpur in 2018-2019 growing season to determine the effects of variable doses of nitrogen on yield and fiber quality of cotton variety CB-15. The influence of seven nitrogen rates (0, 40, 80, 120, 160, 200, 240 kg/ha) were compared in RCB Design with three replications. Cotton growth, yield and fiber quality data were collected. Nitrogen rate had a positive effect on seedcotton yield. Nitrogen rates at all levels significantly ($p < 0.05$) increased the cotton yield when compared with control. At Sreepur, Gazipur and Sadarpur, Dinajpur the highest seedcotton yield (3187 and 2736 kg/ha respectively) were obtained from 160 kg N/ha while at Jagadishpur, Jashore the highest seedcotton yield (2963 kg/ha) was obtained from 200 kg N/ha. The estimated regression equations of cotton yield in response to N rates for Sreepur was $y = -0.075x^2 + 27.03x + 725.1$ ($R^2=0.898$), for Sadarpur was $y = -0.044x^2 + 17.61x + 1013$ ($R^2=0.9489$) and for Jagadishpur was $y = -0.041x^2 + 18.24x + 913.1$ ($R^2=0.9473$). The higher value of R-square revealed that the yield was predictable. N had significant positive association with fiber strength.*

Key Words:
Nitrogen fertilizer, cotton growth, cotton yield, cotton fiber quality, regression equations of cotton yield

Background

Cotton in an important cash crop and main raw material for the textile industries in Bangladesh. Sustainability of textile value chain in Bangladesh largely depends on the steady supply of lint. In 2019, Bangladesh has imported 17.52 lac tons of lint from India (25%), USA (10%), Australia (9%), Mali (9%), Burkina Faso (8%), Benin (8%), Brazil (7%), Uzbekistan (6%), Turkmenistan (4%), Cameroon (3%), Ivory Coast (2%), Chad (2%) and others (7%) while the local production was 31207 tons that met 1.78 per cent of national requirement. To ensure uninterrupted cotton supply and avoiding market vulnerability, increasing cotton production in Bangladesh is one of one best alternative solution to achieve sustainable textile sector. For increasing cotton production in Bangladesh, Cotton Development Board released CB-15, an open pollinated high yielding variety for growing all over the Bangladesh. However, for optimizing the yield of CB-15 its N fertilizer rate was not determined.

All over the cotton growing countries, nitrogen (N) is a major nutrient element limiting cotton production (Bondada and Oosterhuis, 2001; Rochester, 2011; Devkota et al., 2013; Iren and Aminu, 2017a & b; Sattaret al., 2017,). Nitrogen deficiency can reduce leaf size, number of fruiting nodes, boll retention, yield and fibre quality (Hallikeri et al., 2010; Tang et al., 2012; Bhati and Manpreet, 2015; Iren and Aminu, 2017a & b; Sattar et al., 2017). While excess N can cause excessive vegetative growth, delay maturity, create difficulty in defoliation, increase pest problems, and ultimately reduce the crop yield and fiber quality (Tewolde and Fernandez, 1997; Cisneros et al., 2001; Howard et al., 2001; Hons et al, 2003). Cotton fiber quality has direct effect on processing performance, yarn quality, and end products in the textile industry. Producing high-yielding and high-quality cotton requires careful fertilizer management. Nitrogen (N) nutrient can affect lint yield and fiber properties (Fritschi et al., 2003; Bauer and Roof 2004; Girma et al., 2007, Main et al., 2011).

The objective of this study was to investigate the effect of nitrogen (N) application rates on seed cotton yield and fiber quality of cotton cultivar CB-15.

Results

Location Effect of nitrogen on CB 15 growth and yield

The results revealed that location had significant effect ($p<0.05$) on plant height at harvest, number of monopodial branch/plant, number of sympodial branch/plant, number of boll/plant, individual boll weight and seed cotton yield (Table 1). The maximum plant height (128.0cm) was produced at Jagadishpur and the minimum plant height (101.70 cm) was recorded at Sreepur. The lowest monopodial branch/plant (1.2) was recorded at Sreepur and the highest monopodial branch/plant (2.9) was recorded at Sadarpur. The highest number of sympodial branch/plant (16.7) was found at Jagadishpur and the lowest number of sympodial branch/plant (15.10) was found at Sadarpur. The highest number of boll/plant (24.10) was produced at Sreepur and the lowest number of boll/plant (21.20) was recorded at Sadarpur. The lowest single boll weight (4.99 g) was recorded at Jagadishpur and the highest single boll weight (5.30 g) was recorded at Sreepur. The maximum seed cotton yield (2403kg/ha) was recorded at Sreepur and minimum seed cotton yield (2195 kg/ha) was recorded at Sadarpur.

Table 1. Effect of Location of N on CB-15 yield and yield contributing characters

Location	Plant Height (cm)	Monopodial branch/plant	Sympodial branch/plant	Boll/ plant	Boll weight (g)	Yield (kg/ha)
Sreepur	101.7	1.2	16.3	24.10	5.30	2403
Jagadishpur	128.0	1.9	16.7	24.0	4.99	2249
Sadarpur	118.6	2.9	15.1	21.2	5.18	2195
5% LSD	6.01	0.37	0.93	1.56	0.15	185
CV%	8.1	34.2	9.4	10.4	4.7	12.8

Treatment effect

The effect of various levels of N fertilizers on yield and yield contributing characters of CB-15 are found significant ($p<0.05$) on plant height at harvest, number of monopodial branch/plant, number of sympodial branch/plant, number of boll/plant, individual boll weight and seed cotton yield (Table 2).

Table 2. Effect of various levels of N fertilizer on yield and yield components of CB-15

N rates (kg/ha)	Plant Height (cm)	Monopodial branch/plant	Sympodial branch/plant	Boll/ plant	Boll weight (g)	Seed cotton Yield (kg/ha)
0	83.0	1.5	10.0	10.1	4.02	942
40	102.1	1.6	13.6	18.0	4.42	1763
80	113.9	1.8	15.8	21.4	4.88	2258
120	124.0	1.6	17.1	24.3	5.27	2594
160	140.7	1.7	18.5	33.3	5.78	3062
200	138.2	1.8	18.3	32.8	5.83	2945
240	140.4	2.3	18.3	30.9	5.63	2926
5% LSD	7.11	0.44	1.10	1.85	0.17	219
CV%	8.1	34.2	9.4	10.4	4.7	12.8

The minimum plant height (83cm) was recorded from control treatment (0 kg N/ha) and the maximum plant height (140.7 cm) was recorded from the treatment of 160 kg N/ha. The relationship between plant height vs. seed cotton yield is given in Figure1.

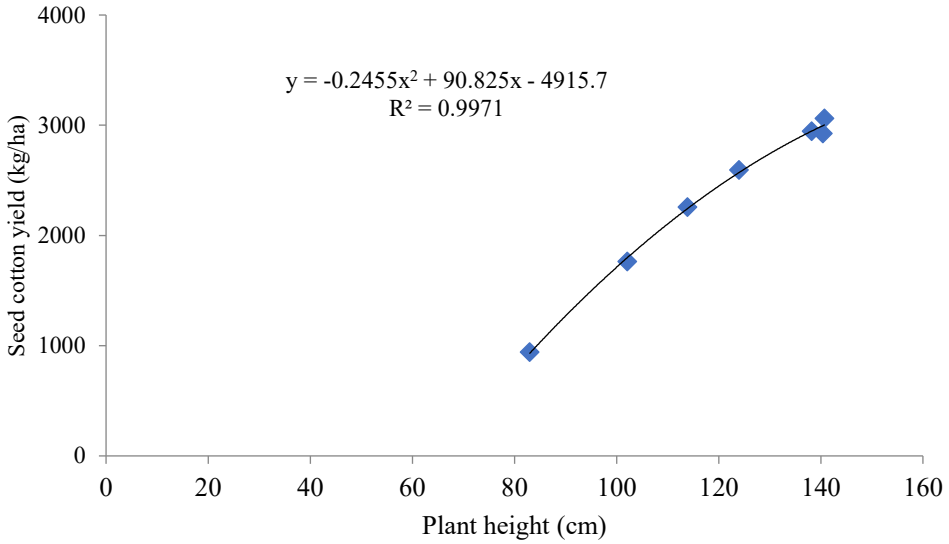

Figure 1. *The relationship between plant height vs. seed cotton yield*

The highest monopodial branch/plant (2.3) was recorded from 240 kg N/ha and the lowest number of monopodial branch/plant (1.5) was recorded from no nitrogen. The relationship between monopodial branches per plant vs. seed cotton yield is given in Figure 2.

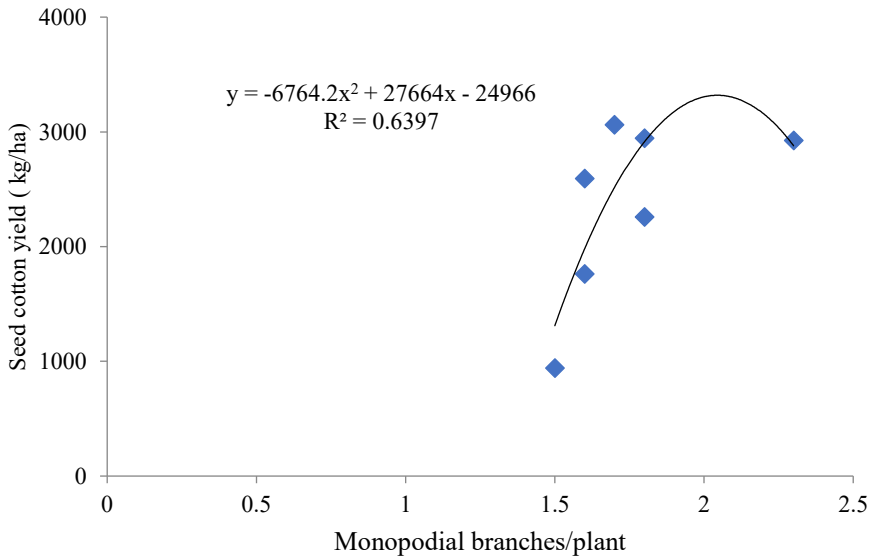

Figure 2. *The relationship between monopodial branches/plant vs. seed cotton yield*

The lowest sympodial branch/plant (10.0) was found in control treatment (0 kg N/ha) and the highest sympodial branch/plant (18.5) was found in of 160 kg N/ha. The relationship between sympodial branches per plant vs. seed cotton yield is given in Figure 3.

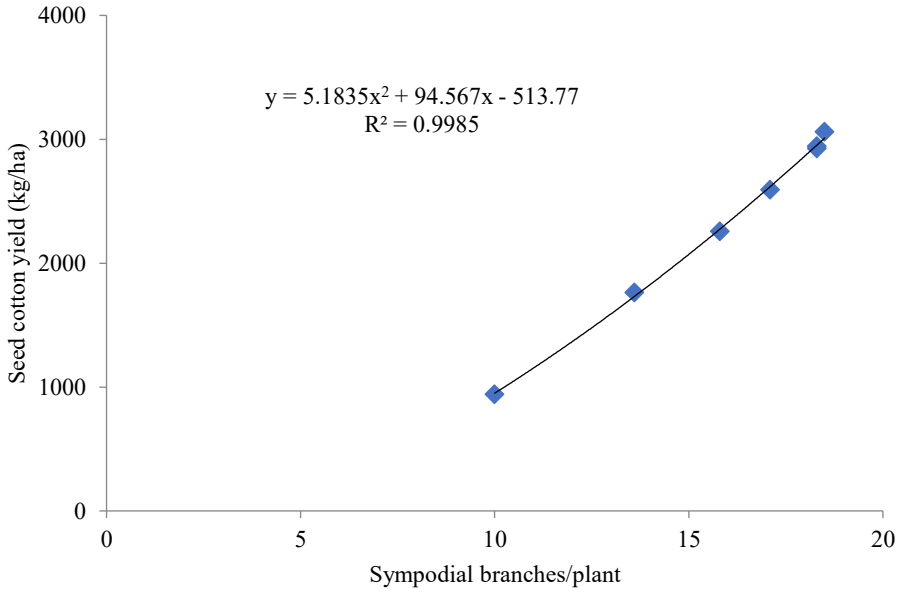

Figure 3. *The relationship between sympodial branches/plant vs. seed cotton yield*

The lowest boll/plant (10.1) was recorded from control treatment (0 kg N/ha) and the highest boll/plant (33.30) was recorded from the treatment of 160 kg N/ha. Gangaiah et. al., 2013 reported that application of 180 kg N/ha produced mean boll number of 54/plant which was 40% greater over no nitrogen fertilizer application. The relationship between boll/plant vs. seed cotton yield is given in Figure 4.

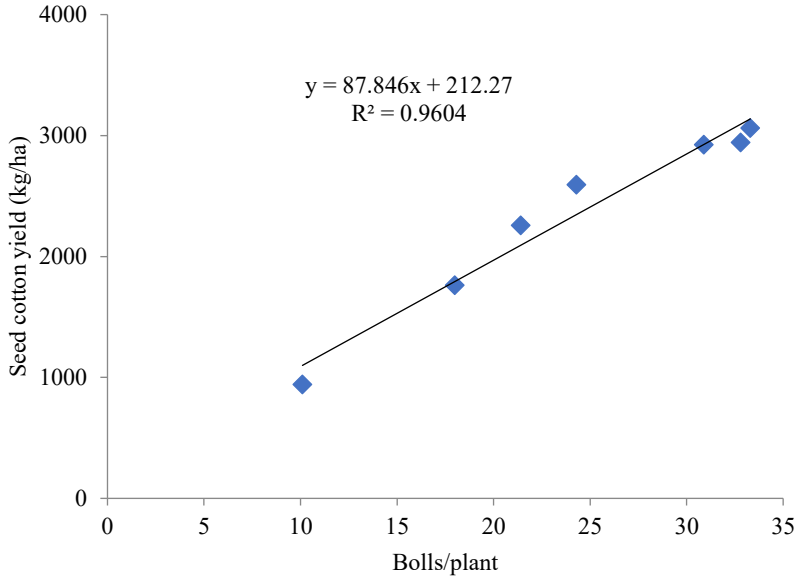

Figure 4. *The relationship between bolls/plant vs. seed cotton yield*

The lowest single boll weight (4.02 g) was recorded from no nitrogen and the highest single boll weight (5.83 g) was recorded from the treatment of 200 kg N/ha. The relationship between single boll weight vs. seed cotton yield is given in Figure 5.

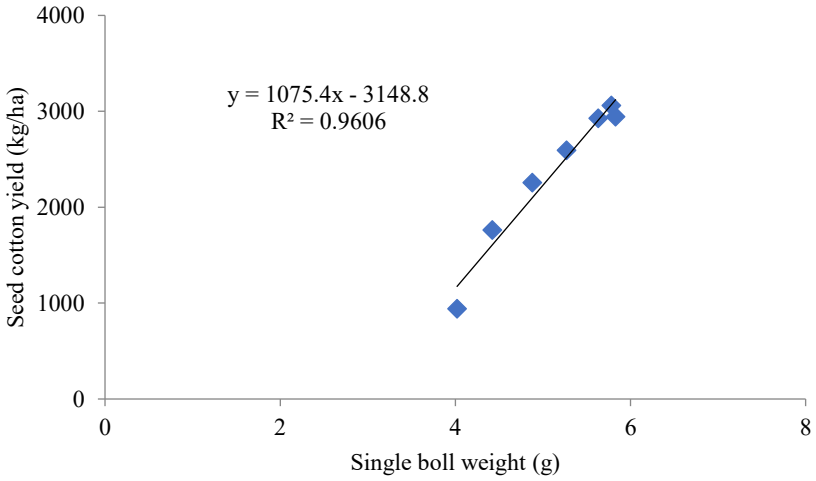

Figure 5. *The relationship between single boll weight vs. seed cotton yield*

The minimum seed cotton yield (942 kg/ha) was recorded from control treatment (0 kg N/ha) and the maximum seed cotton yield (3062 kg/ha) was recorded from the treatment of 160 kg N/ha.

Interaction Effect

The interaction effects of location × N were found significant ($p<0.05$) on plant height at harvest, number of monopodial branch/plant, number of sympodial branch/plant, number of boll/plant, individual boll weight and seed cotton yield (Table 3). The highest plant height at Sreepur Cotton Research Center (125.6 cm), Jagadishpur Cotton Research Center (150.8 cm) and Sadarpur Cotton Research Center (143.0 cm) were obtained from 240, 200 and 160 kg N/ha respectively. The highest number of monopodial branch/plant at Sreepur Cotton Research Center (1.8) and Sadarpur Cotton Research Center (4.1) were obtained from 240 kg N/ha while at Jagadishpur Cotton Research Center N rates from 40 kg/ha to 240 kg/ha had no significant effect on monopodial branch/plant. The highest number of sympodial branch/plant at Sreepur Cotton Research Center (19.4), Jagadishpur Cotton Research Center (20.3) and Sadarpur Cotton Research Center (16.8) were obtained from 240, 160 and 120 kg N/ha respectively. The highest number of boll/plant at Sreepur Cotton Research Center (31.8), Jagadishpur Cotton Research Center (32.9) and Sadarpur Cotton Research Center (32.4) were obtained from 200, 160 and 160 kg N/ha respectively. The highest single boll weight at Sreepur Cotton Research Center (5.99 g), Jagadishpur Cotton Research Center (5.93 g) and Sadarpur Cotton Research Center (5.07 g) were recorded from 200, 160 and 200 kg N/ha respectively.

Table 3. Location × nitrogen (N) interaction effect on CB-15

Location	N rates (kg/ha)	Plant Height (cm)	Monopodial branch/plant	Sympodial branch/plant	Boll/ plant	Boll weight (g)	Yield (kg/ha)
Sreepur, Gazipur	0	58.6	0.3	9.9	11.3	4.24	730
	40	70.5	0.7	13.9	16.8	4.72	1717
	80	103.8	1.2	16.5	22.3	5.13	2338
	120	110.8	1.3	17.6	24.1	5.42	2870
	160	121.4	1.2	18.6	31.6	5.92	3187
	200	120.9	1.7	18.4	31.8	5.99	3123
	240	125.6	1.8	19.4	30.3	5.69	2858
Jagadishpur, Jashore	0	84.5	1.1	9.8	10.7	3.90	975
	40	109.1	2.1	13.6	17.6	3.95	1450
	80	119.6	2.0	15.8	20.5	4.53	2212
	120	134.1	2.1	18.0	23.7	4.96	2396
	160	147.5	2.0	20.3	32.9	5.93	2862
	200	150.8	2.0	19.6	32.2	5.90	2963
	240	150.4	2.1	19.5	30.3	5.75	2883
Sadarpur, Dinajpur	0	77.7	2.9	9.9	9.1	3.93	1004
	40	104.9	2.3	13.3	14.0	4.43	1662

	80	113.7	3.1	15.0	15.2	4.97	2158
	120	115.4	2.7	16.8	18.7	5.53	2420
	160	143.0	2.7	17.7	32.4	5.77	2736
	200	136.9	2.4	16.7	29.9	6.07	2722
	240	138.8	4.1	16.0	28.9	5.57	2664
5% LSD		15.90	0.98	2.45	4.14	0.39	491
CV%		8.1	34.2	9.4	10.4	4.7	12.8

N availability and crop N uptake may vary considerably with soil properties, weather conditions, and interactions between these factors, optimal N rates vary from year to year and field to field (Tremblay, 2004; Olfs et al., 2005; van Es et al., 2005; Melkonian et al., 2007; Zhu et al., 2009). Optimum N rates for cotton production varied by soil type; production, climate, and various other soil and crop management factors (Boquet and Breitenbeck, 2000; Boquet, 2005). Cotton yield is affected by the different weather parameters such as temperature (Ghosh et al., 2014), rainfall (Gupta and Pandey, 1991), humidity (Singh et al., 2009) etc. The soil properties and weather parameters were different at Sreepur, Sadarpur and Jagadishpur (Table 1 and Table 2, respectively). The highest seed cotton yield at Sreepur Cotton Research Center (3187 kg/ha), Jagadishpur Cotton Research Center (2963 kg/ha) and Sadarpur Cotton Research Center (2736 kg/ha) were obtained from 160, 200 and 160 kg N/ha respectively. Saleem et al. (2010) obtained maximum seed cotton yield (3002 kg/ha) by applying N at the rate of 120 kg/ha. Rashidi et al. (2011) reported that application of N at the rate of 200 kg/ha produced the highest seed cotton yield (4363 kg/ha). Alitabar et al. (2013) found that application of 225 kg/ha N produced the maximum seed cotton yield (1731.06 kg/ha).

Optimum level of N was determined by equating the inverse price ratio with marginal product (Table 4) which indicated that it was profitable to apply N in the range of 160-200 kg/ha at Sreepur and Sadarpur cotton research centers and in the range of 200-240 kg/ha at Jagadishpur cotton research center.

Table 4. Marginal product and inverse price ratio at different levels of N application

Location	N rates (kg/ha)	Yield (kg/ha)	Total product due to N	Marginal product	Inverse price ratio
Sreepur	0	730			
	40	1717	987	24.68	0.68
	80	2338	1608	15.53	0.68
	120	2870	2140	13.30	0.68
	160	3187	2457	7.93	0.68
	200	3123	2393	-1.60	0.68
	240	2858	2128	-6.63	0.68
Jagadishpur	0	975	0		
	40	1450	475	11.88	0.68
	80	2212	1237	19.05	0.68
	120	2396	1421	4.60	0.68
	160	2862	1887	11.65	0.68
	200	2963	1988	2.53	0.68
	240	2883	1908	-2.00	0.68
Sadarpur	0	1004			
	40	1662	658	16.45	0.68
	80	2158	1154	12.40	0.68
	120	2420	1416	6.55	0.68
	160	2736	1732	7.90	0.68
	200	2722	1718	-0.35	0.68
	240	2664	1660	-1.45	0.68

Notes: Price of N= 39.13 Taka/kg, price of seed cotton= 57.50 Taka/kg

Regression analysis

CB-15 yield response to N fertilizer at Sreepur Farm is presented in Figure 6. The estimated equation for CB-15 yield in relation to N is y = -0.075x2 + 27.03x + 725.1(R2=0.898). The higher value of R-square revealed that the yield is predictable.

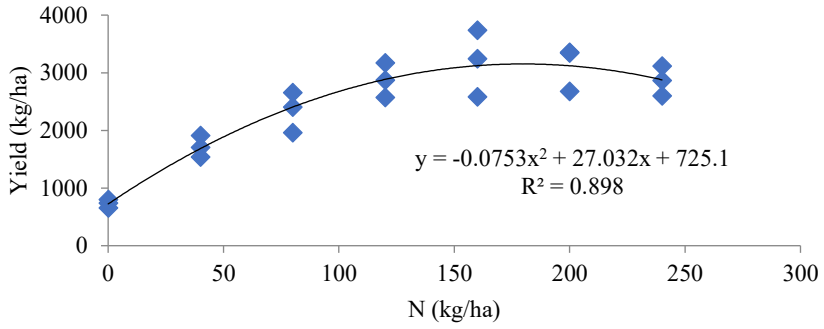

Figure 6. *CB-15 yield in response to N fertilizer at Cotton Research Center, Sreepur, Gazipur*

CB-15 yield response to N fertilizer at Jagadishpur Farm is presented in Figure 7. The estimated equation for CB-15 yield in relation to N is y = -0.041x2 + 18.24x + 913.1(R2=0.9473). The higher value of R-square revealed that the yield is predictable.

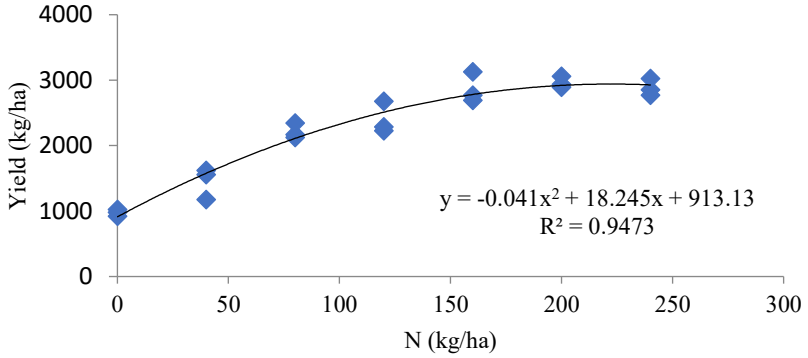

Figure 7. *CB-15 yield in response to N fertilizer at Cotton Research Center, Jagadishpur, Jashore*

CB-15 yield response to N fertilizer at Sadarpur Farm is presented in Figure 8. The estimated equation for CB-15 yield in relation to N is y = -0.044x2 + 17.61x + 1013(R2=0.9489). The higher value of R-square revealed that the yield is predictable.

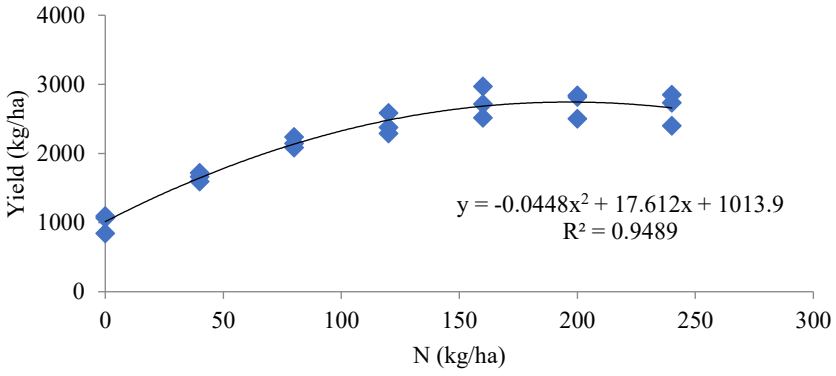

Figure 8. *CB-15 yield in response to N fertilizer at Cotton Research Center, Sadarpur, Dinajpur*

Predicted yield and marginal product at different locations obtained from fitting the regression equation were given in Table 5. It was estimated that maximum profitable yield at Sreepur, Jagadishpur and Sadarpur cotton research centers could be obtained by applying N at the rate of 176, 214 and 192 kg/ha respectively.

Table 5. Predicted yield and marginal product at different locations

Location	Regression equation	N rates (kg/ha)	Predicted yield (kg/ha)	Total product due to N	Marginal product	Inverse price ratio
Sreepur	y = -0.075 x 2 + 27.03 x + 725.1	176	3159	2429	0.71	0.68
Jagadishpur	y = -0.041 x 2 + 18.24 x + 913.1	214	2939	1964	0.73	0.68
Sadarpur	y = -0.044 x 2 + 17.61 x + 1013	192	2772	1768	0.76	0.68

Price of N= 39.13 Taka/kg, price of seed cotton= 57.50 Taka/kg

Fiber Quality

The correlation matrix among N and fiber quality of CB-15 was given in Table 6. Studies on correlation revealed that N had significant association with fiber strength ((r = 0.326) while the association among N and fiber length, uniformity index, short fiber index, elongation, micronaire, reflectance and yellowness were not significant. The highly significant negative association was found between fiber length and short fiber index (r=-0.566) and between micronaire and reflectance (r=-0.534). The N nutrition effect on certain fiber properties had been studied (Murray et al., 1965; Hearn, 1976; Koli and Morrill, 1976;Constable and Hearn, 1981; Bowman and Westerman, 1994; Rochester et al., 2001; Bogiani et al., 2011; Gottardo, 2012; and Sofiatti et al., 2013 and Kappes et al., 2016). Some studies found that N nutrition increased both lint yield and fiber length (Hearn, 1976; Constable and Hearn, 1981). Some other studies concluded that N nutrition does not affect any of the fiber quality parameters (Murray et al., 1965; Bowman and Westerman, 1994; Bogiani et al., 2011; Gottardo, 2012; and Sofiatti et al., 2013 and Kappeset al., 2016). Reports of cotton nutrition effect on fiber properties are sometimes contradictory that may vary depending on genotype, weather and soil (Jenkins et al., 1990; Minton and Ebelhar, 1991; Jones and Wells, 1998; Pettigrew, 2003; Reddy et al., 2004).

Table 6. Correlation matrix among the N and fiber quality

	N	Fiber length	Uniformity Index	Short Fiber Index	Fiber strength	Elongation	Micronaire	Reflectance
Fiber length	0.326ns							
Uniformity Index	0.413ns	0.988ns						
Short Fiber Index	0.237ns	-0.566**	-0.446ns					
Fiber strength	0.776*	0.544ns	0.628ns	-0.06214ns				
Elongation	0.185ns	0.525ns	0.561ns	-0.20139ns	0.745ns			
Micronaire	0.385ns	0.564ns	0.667ns	-0.36786ns	0.663ns	0.631ns		
Reflectance	-0.429ns	-0.524ns	-0.542ns	0.003202ns	-0.615ns	-0.499ns	-0.534**	
Yellowness	-0.512ns	-0.122ns	-0.135ns	-0.65616ns	-0.274ns	0.002ns	0.185ns	-0.379ns

*=significant ($p < 0.05$), **= highly significant ($p < 0.01$), ns=non-significant

In Bangladesh during 2018-2019 growing season American cotton was grown over 44185 ha of land of which 60% area was plain and 40% area was hill slope. Among the plain land, 36% area was located at Jashore region, 17% in Rangpur region and 7% in Dhaka region. Earlier recommended N application rates for the plain areas of Bangladesh were uniform (Islam et al., 2013; Ahmmed et al., 2018). The optimum N doses for Sreepur, Jagadishpur and Sadarpur will be applicable for Dhaka, Jashore and Rangpur region respectively and will be useful for the management of location specific N.

Conclusion

The results from this study indicate that nitrogen fertilizer rate as well as location have significant effect on plant height at harvest, number of monopodial branch/plant, number of sympodial branch/plant, number of boll/plant, individual boll weight and seed cotton yield of cotton variety CB-15. The optimum nitrogen rate of cotton variety CB-15ranges from 160-200 kg/ha for different locations. The finding of this study will be helpful for planning of resource allocation for increasing cotton production in Bangladesh.

Methods

Field experiments were conducted at 3 Cotton Research Center located at Sreepur, Gazipur; Jagadishpur, Jessore and Sadarpur, Dinajpur in 2018-2019. To know the effect of 7 different rates of N (0, 40, 80, 120, 160, 200, 240 kg/ha) on CB-15, experiments were set up in RCBD with 3 replications. Unit plot size was 5.4 × 4.5 m and plant spacing was 90 × 45 cm. The seed was sown on second week of July, 2018. Urea was applied as the source of N. Cotton Development Board recommended rates of TSP (280 kg/ha), Gypsum (150 kg/ha), Zinc sulphate (25 kg/ha), Magnesium sulphate (25 kg/ha), borax (25 kg/ha) and one-third urea (as per treatment) and one-third MoP (105 kg/ha) were applied as basal. The rest of Urea (as per treatment) and MoP were applied in three equal splits as top dressing at 25, 50 and 70 days after sowing.

Two irrigations were applied in the month of October and November. Intercultural operations such as weeding, thinning, gap-filling, earthling-up, insects and pest management were done in all plots uniformly. Cotton growth data were collected from 10 randomly selected plants at each plot. Average boll weight was calculated by dividing the weight of cotton obtained from 10 randomly picked bolls. Seed cotton was harvested from three middle rows to determine the plot yield. Data collected on different parameters were analyzed statistically by using CropStat 7.2 developed by International Rice Research Institute. The law of diminishing return was used to determine the optimum level of nitrogen by equating the inverse price ratio with marginal product (Sharma and Sharma, 1981).

The status of the initial soil was presented in Table 6.The characteristics of Sreepur soil are clay loam, moderately acidic (pH=5.6) with very low nitrogen (0.084%), low organic matter (1.68%) and low content of other nutrients. The characteristics of Jagadishpur soil are sandy loam, neutral in soil reaction (pH=7.20) with very low nitrogen (0.010%), very low organic matter (0.20%) and low content of other nutrients. The characteristics of Sadarpur soil are sandy loam, neutral in soil reaction (pH=6.73) with very low nitrogen (0.005%), low organic matter (1.03 %) and low content of other nutrients.

At Sreepur, the maximum average air temperature (34.1 °C) was recorded in the month of September, 2018 and the minimum average air temperature (14.7 °C) was recorded in the month of January, 2019. The maximum average relative humidity (80.1%) and the maximum average rainfall (11.5 mm) were recorded in the month of July, 2018. The minimum average relative humidity (57.5%) and no rainfall were recorded in the month of January, 2019. At Sadarpur, the maximum average air temperature (33.8 °C) was recorded in the month of August, 2018 and the minimum average air temperature (11.3 °C) was recorded in the month of December, 2018. The maximum average relative humidity (78.2%) and the maximum average rainfall (6.4 mm) were recorded in the month of September, 2018. The minimum average relative humidity (71.2%) and no rainfall were recorded in the month of January, 2019. At Jagadishpur, the maximum average air temperature (34.6 °C) was recorded in the month of September, 2018 and the minimum average air temperature (10.3 °C) was recorded in the month of January, 2019. The maximum average relative humidity (81.7 %) and the maximum average rainfall (13.9 mm) were recorded in the month of July, 2018. The minimum average relative humidity (72.6%) and no rainfall were recorded in the month of November, 2018.

Table 6. Initial soil status of Experimental plot

Location	pH	OM (%)	N (%)	K meq/100 g soil	P µg/g soil	S µg/g soil	Mg meq/ 100g soil	Zn µg/g soil	B µg/g soil	Soil Texture
Sreepur	5.60	1.68	0.084	0.12	6.87	0.004	0.82	1.33	0.43	Clay loam
Jagadishpur	7.20	0.20	0.010	0.12	2.41	0.002	0.79	0.92	0.13	Sandy loam
Sadarpur	6.73	1.03	0.05	0.38	5.50	4.50	1.20	1.12	0.18	Sandy loam

Mean monthly weather data for the cotton growing season 2018-2019 is presented in Table 7.

Table 7. Average monthly weather data for cropping season 2018-2019

Location	Month	Temperature (°C)		Relative humidity (%)	Rainfall (mm)
		Maximum	Minimum		
Sreepur	July-2018	32.6	26.7	80.1	11.5
	August-2018	33.8	27.1	75.0	4.5
	September-2018	34.1	27.0	74.7	2.5
	October-2018	32.0	23.6	67.9	1.5
	November-2018	30.3	19.8	65.4	0.4
	December-2018	26.1	16.2	64.0	0.4
	January-2019	27.2	14.7	57.5	0.0

Sadarpur	July-2018	33.3	27.0	77.6	5.5
	August-2018	33.8	26.9	78.0	4.9
	September-2018	33.3	26.1	78.2	6.4
	October-2018	31.3	21.5	76.3	0.3
	November-2018	29.6	15.7	75.3	0.0
	December-2018	25.5	11.3	77.4	0.3
	January-2019	25.8	13.7	71.2	0.0
Jagadishpur	July-2018	33.3	26.6	81.7	13.9
	August-2018	33.8	26.7	79.3	3.7
	September-2018	34.6	26.0	80.2	2.5
	October-2018	33.0	21.8	80.4	2.5
	November-2018	31.0	16.8	72.6	0.0
	December-2018	26.0	12.0	79.2	0.4
	January-2019	26.9	10.3	74.6	0.0

References

1. Bauer, P.J. and Roof, M.E. Nitrogen, aldicarb, and cover crop effects on cotton yield and fiber properties. Agron. J., 2004; 96:369–376.
2. Ahmmed et al. Fertilizer Recommendation Guide-2018. Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka 1215. 223p. 2018.
3. Alitabar, R.A., Salimbeck, R., Alishah, O. and Andarkhor, S.A.A. The Effects of Nitrogen and Row Spacing on Growth and Yield of Cotton Varieties. International Journal of Agriculture: Research and Review. 2013; 3:120-125.
4. Bhati, A.S. and Manpreet, S. Effect of split application of nitrogen and potassium on yield, nutrient uptake and nutrient use efficiency in Bt-cotton. Annals of Plant and Soil Research, 2015; 17(1):71-73.
5. Bogiani JC, Ferreira GB, Smiderle OJ, MourãoJúnior M, Borin ALDC. Adubação com potássio e nitrogênio de cultivares de algodoeiro sob sistema de cultivoadensado. In: Anais do 8º Congresso Brasileiro de Algodão; 1ª Cotton EXPO [CD-ROM]; 2011; São Paulo. Campina Grande: Embrapa Algodão; 2011; p.1451-58.
6. Bondada, B. R. and Oosterhuis, D. M. Canopy Photosynthesis, specific leaf weight, and yield components of cotton under varying nitrogen supply. Journal of plant Nutrition, 2001; 24: 469-477.
7. Boquet, D.J. Cotton in Ultra-Narrow Row Spacing: Plant Density and Nitrogen Fertilizer Rates. Agronomy Journal, 2005; 97: 279-287
8. Boquet, D.J. and Breitenbeck, G.A. Nitrogen Rate Effect on Partitioning of Nitrogen and Dry Matter by Cotton. Crop Science, 2000; 40:1685-1693.
9. Boquet, D.J., Breitenbeck, G.A. Nitrogen rate effect on partitioning of nitrogen and dry matter by cotton. Crop Sci. 2000; 40:1685–1693.
10. Bowman, R.K., and R.L. Westerman. Nitrogen and mepiquat chloride effects on the production of nonrank, irrigated, short season cotton. J. Prod. Agric. 1994; 7:70–75.
11. Cisneros, J.J.andGodfrey,L.D. Mid-season pest status of the cotton aphid (Homoptera: Aphididae) in California cotton: Is nitrogen a key factor? Environ. Entomol. 2001. 30:501–510.
12. Constable, G.A., and A.B. Hearn. Irrigation for crops in a subhumid environment: VI. Effect of irrigation and nitrogen fertilizer on growth, yield and quality of cotton. Irrig. Sci. 1981; 3:17–28.
13. Devkota, M., Martius, C., Lamers, J.P.A., Sayre, K.D., Devkota, K.P. and Vlek, P.L.G. Tillage and nitrogen fertilization effects on yield and nitrogen use efficiency of irrigated cotton. Soil and Tillage Research, 2013; 134: 72-82.
14. Fritschi, F.B., B.A. Roberts, R.L. Travis, D.W. Rains, and R.B. Hutmacher. Response of irrigated Acala and Pima cotton to nitrogen fertilization: Growth, dry matter partitioning, and yield. Agron. J., 2003; 95:133–146.
15. Gangaiah,B., Ahlawat,I.P.S. and Babu, M.B.B.P. Response of nitrogen fertilization on Btand non Btcotton.SAARCJ.Agri., 2013; 11(1):121-132.
16. Ghosh, K., Rajavel, M., Samui, R.P., Singh, G.P. and Karmakar, C. Forewarning incidence of American boll worm (Heliothis armigera H.) of cotton at Akola in Vidarbha region of Maharashtra. Mausam, 2014; 65 (1): 73-82
17. Girma, K., Teal, R.K., Freeman, K.W., Boman, R.K. and Raun,W.R. Cotton lint yield and quality as affected by applications of N, P, and K fertilizers. J. Cotton Sci., 2007; 11:12–19.
18. Gottardo LCB. Resposta do algodoeiroadensadoaonitrogênio no cultivoemsucessão [dissertação]. Piracicaba: Escola Superior de Agricultura Luiz de Queiroz; 2012.
19. Gupta, B.R.D. and Pandey, V. Influences of weathers parameters and agroclimatic elements on cotton yield at Surat. Mausam, 1991; 42: 107-110.
20. Hallikeri, S. S., Halemani, H., Patil, V. C., Palled, Y. B., Patil, B. C. andKatageri, I. S. Effect of nitrogen levels, split application of nitrogen and detopping on seed cotton yield and fibre quality in Bt-cotton. Karnataka J. Agric. Sci., 2010; 23(3): 418-422.
21. Hearn, A.B. Response of cotton to nitrogen and water in a tropical environment: III Fiber quality. J. Agric. Sci. 1976; 86:257–269.
22. Hons, F.M., Lemon R.G. and Mc Farland,M.L. Managing nitrogen fertilization in cotton. Department of Soil and Crop Science, Taxes A & M university. 2003.
23. Howard, D.D., Gwathmey, C.O., Essington, M.E., Roberts, R.K. and Mullen. M.D. Nitrogen fertilization of notill cotton on loess-derived soils. Agron. J., 2001; 93:157–163.
24. Iren, O. B. and Aminu, Y. G. Soil nutrient dynamics and yield of cotton (Gossypium hirsutum L.) following an amendment with cattle manure. International Journal of Plant & Soil Science, 2017 a; 16(2): 1-10.
25. Iren, O. B. and Aminu, Y. G. Nutrient concentrations, cotton (Gossypium hirsutum L.) growth and biomass yield as influenced by different nitrogen rates in cattle manure. Journal of Organic Agriculture and Environment, 2017 b; 5(1): 5- 14.

26. Islam, M. K., Khalequzzaman, Ibrahim, K. M. and Akhteruzzaman, M. Effect of time and split application of NPK on upland cotton production. Bangladesh Journal of Progressive Science and Technology, 2013; 11(1): 093-098.
27. Jenkins, J.N., McCarty Jr., J.C., Parrott, W.L., Fruiting efficiency in cotton: boll size and boll set percentage. Crop Sci. 1990; 30, 857–860.
28. Jones, M.A., Wells, R. Fiber yield and quality of cotton grown at two divergent population densities. Crop Sci. 1998;38, 1190–1195.
29. Kappes, Claudinei, Zancanaro, Leandro, & Francisco, Eros ArturBohac. Nitrogen and Potassium in Narrow-Row Cotton. RevistaBrasileira de Ciência do Solo, 2016; 40, e0150103. https://doi.org/10.1590/18069657rbcs20150103
30. Koli, S.E., and L.G. Morrill. Effect of narrow, row, plant population and nitrogen application on cotton fiber characteristics. Agron. J. 1976; 68:794–797.
31. Main, C.L., Barber, L.T., Dodds, D.M., Duncan, S.R., Edmisten, K.L., Jones, M.A., Whitaker, J.R., Morgan, G., Osborne, S., Boman, R.K., Norton, R. and Nichols, R.L. Cotton cultivar response to nitrogen fertilization. Proc. Beltwide Cotton Conf., Atlanta, GA.4-7 January. Natl. Cotton Council, Memphis, TN. 2011.
32. Melkonian, J., H.M. van Es, A.T. DeGaetano, J.M. Sogbedji, and L. Joseph. Application of dynamic simulation modeling for nitrogen management in maize. In: T.W. Bruulsema, editor, Managing Crop Nitrogen for Weather: Proceedings of the Symposium “Integrating Weather Variability into Nitrogen Recommendations,” Indianapolis, IN. 15 Nov. 2006. Int. Plant Nutr. Inst., Norcross, GA. 2007; 14–22 p.
33. Minton, E.B., Ebelhar, M.W., Potassium and aldicarb-disulfoton effects on verticillium wilt, yield, and quality of cotton. Crop Sci. 1991. 31, 209– 212
34. Murray, J.C., R.M. Reed, and E.S. Oswalt. Effect of fertilizer treatments on the fiber properties of cotton. Agron. J. 1965; 57:227.
35. Olfs, H.W., K. Blankenau, F. Brentrup, J. Jasper, A. Link, and J. Lammel. Soil- and plant-based nitrogen-fertilizer recommendations in arable farming. J. Plant Nutr. Soil Sci. 2005; 168:414–431
36. Pettigrew, W.T., Relationships between insufficient potassium and crop maturity in cotton. Agron. J. 2003; 95, 1323–1329
37. Rashidi, M. and Gholami, M. Response of Yield and Yield Components of Cotton to Different Rates of Nitrogen Fertilizer. Academic Journal of Plant Sciences, 2011; 4, 22-25.
38. Reddy, K.R., Koti, S., Davidonis, G.H., Reddy, V.R. Interactive effects of carbon dioxide and nitrogen nutrition on cotton growth, development, yield and fiber quality. Agron. J. 2004; 96:1148–1157.
39. Rochester, I. J. Assessing internal crop nitrogen use efficiency in high-yielding irrigated cotton. Nutrient Cycling in Agroecosystems, 2011; 90(1): 147-156.
40. Rochester, I.J., M.B. Peoples, and G.A. Constable. Estimation of the N fertilizer requirement of cotton grown after legume crops Field Crops Res. 2001; 70:43–53.
41. Saleem, M.F., Bilal, M.F., Awais, M., Shahid, M.Q. and Anjum, S.A. Effect of Nitrogen on Seed Cotton Yield and Fiber Qualities of Cotton (Gossypium hirsutum L.) Cultivars. The Journal of Animal & Plant Sciences, 2010; 20:23-27.
42. Sattar, M., Safdar, M. E., Iqbal, N., Hussain, S., Waqar, M. Q., Ali, M. A., Ali, A., Ali, M. A. and Javed, M. A. Timing of nitrogen fertilizer application influences on seed cotton yield. International Journal of Advanced Science and Research, 2017; 2 (1): 6-9.
43. Sharma, A.N. and V.K. Sharma. Element of Farm Management. Prentice Hall of India (Pvt.) Ltd., New Delhi, India, 1981; pp: 30-39.
44. Singh, S.P., Sekhon, B.S., Brar, J.S., Dhaliwal, L.K. and Chahal, S.K. Effect of weather parameters and plant geometry on sucking pest dynamics in Bt and non Bt cotton. Journal of Agrometeorology, 2009; 11: 129-234.
45. Sofiatti V, Bogiani J, Pedrosa MB, Azevedo D, Alencar A, Silva L. Adubaçõesnitrogenada e potássica de cobertura no algodoeiroemsistemaadensado no oeste da Bahia. In: Anais do 9º. CongressoBrasileiro de Algodão; 2013; Brasília. Brasília, DF: AMPA/Abrapa; 2013.
46. Tang, H.Y., Yang, G.Z., Zhang, X.L. and Siddique, K. Improvement of Fertilizer N Recovery by Allocating More N for Later Application in Cotton Gossypium hirsutum L.). International Journal of Basic & Applied Sciences, 2012; 12: 32-37.
47. Tewolde, H., and Fernandez, C. J. Vegetative and reproductive dry weight inhibition in nitrogen- and phosphorus-deficient Pima cotton. J. Plant Nutrition, 1997; 20(2–3): 219–232.
48. Tremblay, N. Determining nitrogen requirements from crops characteristics: Benefits and challenges. Recent Res. Dev. Agron. Hortic. 2004; 1:157–182.
49. van Es, H.M., C.L. Yang, and L.D. Geohring. Maize nitrogen response as affected by soil type and drainage variability. Precis. Agric. 2005; 6:281–295.
50. Zhu, Q., J.P. Schmidt, H.S. Lin, and R.P. Sripada. Hydropedological processes and their implications for nitrogen availability to corn. Geoderma 2009; 154:111–122.

Detopping is an Option to Reduce Field Duration of Cotton without Affecting the Yield and Quality of Cotton Fiber

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Abstract

Background: *Cotton (Gossypium hirsutum L.) is an important textile industrial crop. Detopping is a management technique for reducing field duration while increasing fiber yield of cotton. The study was conducted in the experimental field of Cotton Research, Training and Seed Multiplication Farm, Sreepur, Gazipur, Bangladesh during Kharif season of 2018–2019. The experiment included five genotypes (BC-479, BC-495, BC-514, JA-13/R and CB-12) and four detopping times (No detopping, detopping at 80, 90 and 100 days after sowing).*

Results: *Detopping practices significantly reduced plant height, monopodial, sympodial, secondary branches plant-1and field duration but increased flowering, bolls numberplant-1 and seed cotton yield. Genotype BC-479 produced the highest seed cotton yield (3.90 t ha-1) and the genotype gave 18 percent more seed cotton while detopping at 90 days after sowing (DAS). The genotype with detopping at 90 DAS also showed minimum field duration (155 days), which 30 days earlier than the duration required for the genotype without detopping. Fiber quality (staple length, strength, uniformity index and micronaire) was also significantly improved by significantly affected by detopping, genotype and their detopping irrespective of genotype.*

Conclusion: *Based on the results detopping is suggested as a good practice to reduce field duration and also to increase yield and quality of cotton fiber.*

Key Words: *Cotton (Gossypium hirsutum L.), detopping, field duration, lint quality, yield*

Background

Cotton (Gossypium hirsutum L.) is a cash crop that provides fiber, oil and fuel wood, and contributes a major part of income for farmers in the world. More than sixty countries of the world are growing cotton in tropical areas (Nawaz et al., 2019). Cotton is the second important cash crop as well as the main raw materials for the textile industry in Bangladesh. Cotton is a long duration crop which is cultivated in Bangladesh during July to February as a sole crop. Due to long duration of cotton, the crop cannot be fitted in the existing three crops based cropping patterns in the country. Therefore, cotton cultivation is being pushed to the marginal lands only. If the duration of cotton can be reduced to some extent, the crop can be fitted in the three crops based cropping pattern.

Detopping is one of the most important management practices in cotton plants. Cultural practices including detopping plays a very essential role in improving cotton yield. The main aim of detopping is to get good architecture, so that the plant can get required sunlight with a minimum of mutual shading and thus the picking efficiency can be increased with crop maturity. Rathinaval et al. (2003) found the detopping technique as a good practice for reducing field duration of cotton. Detopping at 75 cm plant height increased number of fruiting branches, percentage of boll on sympodial branches, boll weight, seed cotton yield and highest number of boll retention (Obasi and Msaakpa, 2005). Increased sympodial branches plant-1, number of bolls plant-1 and also seed cotton yield 15.1 to 21.1 percent at 75 DAS detopping (Kataria and Valu, 2018). The field experiment was conducted with a view to exploring the feasibility of using detopping technique in cotton to reduce the field duration of cotton and improving the yield or at least without affecting the yield.

Results

Plant height (cm) at maturity

Plant height of cotton varied significantly due to genotype, detopping and their interaction (Table 1). The tallest plant height (108.43 cm) was observed in the genotype CB-12 whereas the shortest plant height (95.98 cm) was found in genotype BC-479. The tallest plant height (117.79 cm) was obtained in no detopping (control) and the shortest plant height (88.45 cm) was found in detopping at 80 DAS. When detopping interacted with genotype, genotype CB-12 showed the tallest plant height of 135 cm with no detopping whereas the genotype JA-13/R produced the shortest plant height of 86.67 cm with detopping at 80 DAS.

Main stem node of a first fruiting branch (NFB)

The NFB was significantly different among the genotypes but it was not influenced by detopping and interaction of genotype and detopping (Table 1). Genotype BC-514 showed the lowest NFB (4.13) followed by BC-479 whereas the highest NFB (4.68) was obtained in genotype JA-13/R.

Monopodial branches plant-1

The study observed that all treatments were significantly influenced by monopodial branch plant-1 (Table 1). The maximum number of monopodial branches plant-1(1.06) was recorded in genotype CB-12 and the lowest one (0.67) was found in BC-479. Maximum monopodial branches plant-1(0.97) was recorded in detopping at 100 DAS followed by detopping at 90 DAS and the minimum one (0.82) was found in no detopping. Among the interactions, the maximum monopodial (1.20) was showed genotype BC-514 with detopping at 80 DAS and minimum (0.50) was found genotype BC-479 with detopping at 80 DAS.

Sympodial branches plant-1

The most important yield contributing character sympodial branch was significantly influenced by genotype and detopping. The maximum sympodial branches plant-1 (14.91) was recorded in BC-479 and the minimum (12.63) in CB-12 (Table 1). Treatment of no detopping showed the maximum sympodial branches plant-1 (14.77) and the minimum (13.22) was recorded in detopping at 100 DAS and there was no significant difference in interaction.

Secondary fruiting branches plant-1

The results showed that secondary fruiting branches were significantly influenced in all treatments. As shown in Table 1, the maximum secondary fruiting branches plant-1 (3.06) was recorded in genotype BC-495 and minimum (2.10) in BC-479, whereas maximum secondary fruiting breaches plant-1(2.76) was recorded in no detopping and minimum one (2.43) was found in detopping at 90 DAS. The maximum and minimum secondary fruiting breaches plant-1 (3.63) and (2.00) were recorded in genotype BC-495 with detopping at 100 DAS and genotype BC-479 with detopping at 90 days after sowing, respectively.

Days to the first flowering

The cotton genotypes were found indeterminate in flowering habits. The time required for the first flowering of the tested cotton genotypes was significantly different (Table 1). The shortest duration (55.92 days) was recorded in genotype BC-479 and the longest duration (61 days) was recorded in CB-12. No significant difference was observed due to detopping and its interaction with genotype.

Number of bolls plant-1

The number of bolls plant-1varied significantly due to all treatments. The results revealed that the maximum number of bolls plant-1 (38.08) was produced in genotype BC-479 and the minimum one (30.58) in CB-12. Detopping at 90 DAS and no detopping recorded the maximum (37.20) and minimum (32.87) bolls plant-1, respectively. The maximum bolls (40.33) was obtained in genotype BC-479 when detopping was done at 90 DAS and minimum one (24.33) in genotype CB-12 when no detopping (Figure 1).

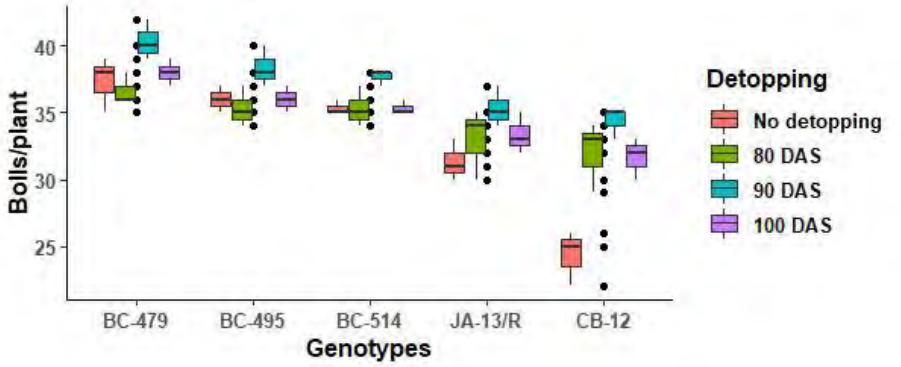

Figure 1. *Effect of Bolls plant-1on genotypes with different detopping times*

Average boll weight

The results revealed significant differences in boll weight in genotypes (Table 1). The genotype CB-12 recorded maximum (5.06 g) average boll weight, whereas minimum boll weight (4.81g) was recorded in BC-479 followed by the genotype BC-514 which was recorded 4.96 g boll weight, but there was no significant difference in detopping and their interaction.

Seed cotton yield

The seed cotton yield was significantly influenced by genotypes, detopping and their interaction. The genotype BC-479 represented the highest seed cotton yield (3.90 t ha-1) followed by BC-495 and BC-514. The genotype (CB-12) was produced the lowest the seed cotton yield 2.93t ha-1 (Table 1).

The highest seed cotton yield (3.79 t ha-1) was recorded in detopping at 90 DAS and the lowest (3.07t ha-1) was observed in no detopping.

The results also indicated that genotype BC-479 gave the highest seed cotton yield (4.15 t ha-1) when detopping at 90 DAS and with the earlier or later detopping seed cotton yield significantly decreased. A similar trend in yield (4.11 t ha-1) of seed cotton was observed in genotype BC-495 for detopping at 90 DAS but the lowest seed cotton yield (2.67 t ha-1) was obtained in genotype CB-12 with no detopping.

Table 1. Effect of genotype and detopping on plant height, days to first flowering, NFB, Monopodial, Sympodial, Secondary fruiting branches plant-1 , Average boll weight (g) and Seed cotton yield (t/ha).

Genotypes	Plant height at maturity (cm)	Days to first flowering	Node of a first fruiting branch (NFB)	Monopodial branches plant-1	Sympodial branches plant-1	Secondary branches plant-1	Bolls weight (g)	Cotton yield (t ha-1)
G1	95.98 c	55.92 d	4.37 bc	0.67 d	14.91 a	2.10 c	4.81 b	3.90 a
G2	100.17	58.67 c	4.47 ab	0.98 b	13.93 bc	3.06 a	5.03 a	3.79 a
G3	102.18 b	59.50 bc	4.13 c	0.97 b	14.25 b	2.54 b	4.96 ab	3.58 b
G4	104.55	59.92 b	4.68 a	0.88 c	13.47 c	2.24 c	5.05 a	3.38 c
G5	108.43 a	61.00 a	4.32 bc	1.06 a	12.63 d	2.93 ab	5.06 a	2.93 d
Detopping								
D1	117.79 a	59.20	4.33	0.82 b	14.77 a	2.76 a	4.94	3.07 d
D2	88.45 d	58.80	4.56	0.89 b	13.89 b	2.57 b	4.94	3.54 c
D3	97.87 c	58.67	4.32	0.96 a	13.47 c	2.43 b	4.99	3.79 a
D4	104.95 b	59.33	4.36	0.97 a	13.22 c	2.53 b	5.05	3.67 b
Interaction								
G1×D1	55.67	4.47	0.77 def	16.20	2.23 d-h	103.53 d-g	4.59	3.50 fg
G1×D2	55.67	4.47	0.50 g	15.07	2.10 fgh	87.07 jk	4.62	3.92 a-
G1×D3	56.00	4.20	0.80 de	14.57	2.00 h	93.60 g-k	4.95	4.15 a
G1×D4	56.33	4.33	0.60 fg	13.80	2.07 gh	99.73 e-h	5.07	4.05
G2×D1	59.00	4.33	0.90 cd	14.60	3.07 b	111.00 cd	5.09	3.08 hij
G2×D2	58.67	4.93	0.80 de	13.73	2.90 bc	87.80 ijk	5.03	3.96 a-
G2×D3	58.00	4.20	1.13 ab	13.53	2.63 cd	98.27 f-i	4.90	4.11 ab
G2×D4	59.00	4.40	1.07 abc	13.87	3.63 a	103.60 d-g	5.09	4.00

G3×D1	58.67	3.73	0.73 def	15.00	2.60 cd	116.53 bc	4.87	3.04 hij
G3×D2	59.00	4.13	1.20 a	14.00	2.50 c-f	89.20 ijk	5.02	3.66 ef
G3×D3	61.00	4.53	0.80 de	13.67	2.47 d-g	98.00 f-i	4.90	3.81
G3×D4	59.33	4.13	1.13 ab	14.33	2.60 cd	105.00 def	5.07	3.82 b-
G4×D1	61.67	4.80	0.70 ef	14.60	2.27 d-h	122.87 b	5.05	3.08 hij
G4×D2	59.67	4.87	0.90 cd	13.87	2.13 e-h	86.67 k	5.01	3.27 gh
G4×D3	58.33	4.67	0.90 cd	13.07	2.53 cde	102.27 d-g	5.12	3.68 def
G4×D4	60.00	4.40	1.00 bc	12.33	2.03 h	106.40 def	5.04	3.50 fg
G5×D1	61.00	4.33	1.00 bc	13.43	3.63 a	135.00 a	5.08	2.67 k
G5×D2	61.00	4.40	1.03 abc	12.80	3.23 b	91.53 h-k	5.05	2.88 jk
G5×D3	60.00	4.00	1.17 ab	12.50	2.53 cde	97.20 f-j	5.11	3.18 hi
G5×D4	62.00	4.53	1.03 abc	11.77	2.30 d-h	110.00	4.98	2.97 ij
LSD(0.05)	1.0024ns	0.2928ns	0.0813**	0.4766ns	0.1809**	4.6084*	0.1816ns	0.1303*

Notes: G1=BC-479, G2=BC-495, G3=BC-514, G4=JA 13/R, G5=CB-12; D1= No detopping, D2= Detopping at 80 DAS, D3= Detopping at 90 DAS, D4= Detopping at 100 DAS

Field duration

Field duration was significantly influenced by genotypes, detopping, and their interaction. The results showed the shortest field duration (164.92 days) was recorded in genotype BC-479 and the longest (187.00 days) in CB-12 which was the most popular variety in the country. In case of detopping, the shortest and longest field duration 172.07 and 187.80 days were observed with detopping at 90 DAS and no detopping, respectively. The interaction effect showed minimum field duration (155 days) in genotype BC-479 when detopping was done at 90 DAS and maximum duration (195 days) was found in the genotype CB-12 when no detopping was done (Figure 2).

Quality parameters

Lint qualities are very important for the textile industry. The results of lint quality were significantly influenced by genotype, detopping and their interaction (Table 2). The results showed that the longest fiber length (30.73 mm) observed in genotype CB-12 and shortest on BC-495. The longest fiber length (30.02 mm) observed detopping at 100 DAS and shortest (29.52) in detopping at 90 DAS. Among the longest length (32.11 mm) was observed in genotype CB-12 with detopping at 100 DAS and genotype BC-495 showed shortest length (28.42 mm) with detopping at 90 DAS.

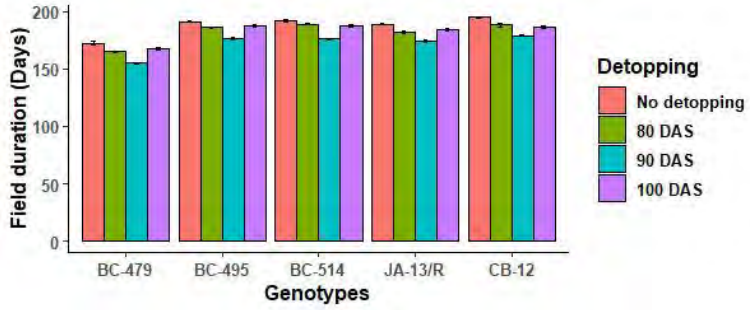

Figure 2. *Field duration of cotton as influenced by genotype and detopping*

The maximum value of fiber strength (31.78 g/tex) observed in genotype BC-479 and minimum (29.85 g/tex) was genotype JA-13/R. The maximum strength (35.13 g/tex) was showed in detopping at 100 DAS and minimum (30.54 g/tex) at no detopping. The maximum strength (35.13 g/tex) showed in genotype CB-12 with detopping at 100 DAS and minimum one (28.37 g/tex) obtained JA-13/R genotype with detopping at 90 DAS.

The highest value of Uniformity index (84.33 %) was showed in genotype CB-12 and lowest one (83.13 %) genotype BC-495. The highest uniformity (83.80 %) observed detopping at 80 DAS and lowest one (83.25 %) at 90 DAS. Among the interaction, the highest uniformity (85.27 %) showed genotype CB-12 with detopping at 100 DAS and lowest (82.34 %) was genotype BC-495 with detopping at 100 DAS.

The highest value of micronaire (5.58) observed in genotype JA-13/R and lowest one (4.80) genotype BC-479. The highest micronaire (5.38) showed no detopping and lowest on (5.15) detopping at 100 DAS. In interaction of genotype and detopping, the highest value of micronaire (6.16) showed in

genotype BC-495 with detopping at 80 DAS and lowest one (4.37) genotype BC-479 with detopping at 100 DAS.

Discussions

Detopping is a management practice for reduced field duration of the cotton crop. Significantly differences among genotypes were found where field duration ranges from 164 to 187 days and after detopping it was 155 to 189 days. During detopping practices at 80, 90 and 100 DAS, the genotypeBC-479 showed field duration 4, 10 and 2 percent earlier, respectively, BC-495 genotype 2, 7 and 1 percent earlier, respectively, BC-514 genotype 1, 8 and 2 percent earlier, respectively, JA-13/R genotype 3, 7 and 2 percent earlier, respectively and CB-12 genotype 3, 8 and 4 percent earlier, respectively over no detopping. In a similar study, Rathinavel et al. (2003) found the detopping technique as a good practice for reducing field duration of cotton. Although the main aim of detopping is to get good architecture so that the plant can get required sunlight with the minimum of mutual shading and in this way, the picking efficiency is increased with the progression of crop maturity. Xu et al. (2001) and Dai et al. (2003) explained vegetative growth and strengthening of reproductive growth as the driving factors for early maturity in cotton in case of detopping.

As the amount of sunlight received by the plant canopy is higher in case of detopping, the practice is also very effective to increase the yield of cotton. In this study, significant cotton yield differences were observed among the genotypes used ranging from 2.93 to 3.90 t ha-1. The highest seed cotton yield (3.90 t ha-1) was produced by the genotype BC-479. While detopping was done at 80, 90 and 100 DAS, cotton yield in the genotype increased by 12, 18 and 15 percent, respectively. A similar yield increase was also found in all other genotypes due to detopping practice. According to Renou et al. (2011), detopping practices improved the yield due to more biomass allocation to reproductive organs, such as green and opened bolls (Yang et al., 2008). Similar results were reported by Singh and Sandhu (1996) where detopping also recorded significantly higher seed cotton yield over no detopping reflecting an increase of 17.8 percent. Brar et al. (2002) reported that plant height significantly decreased by detopping and yet it significantly increased sympodial branches, total and open bolls plant-1 as compared to no detopping and eventually seed cotton yield.

Table 2. Effect of detopping and genotype on cotton fiber length, fiber strength, uniformity index and micronaire

Genotypes	Fiber	Fiber	Uniformity	Micronaire
G1	30.55 ab	31.78 a	83.38 c	4.80 e
G2	29.28 d	30.79 d	83.13 d	5.50 b
G3	30.08 b	31.56 b	83.87 b	5.43 c
G4	29.51 c	29.85 e	83.45 c	5.58 a
G5	30.73 a	31.32 c	84.33 a	5.08 d
Detopping				
D1	29.77 b	30.54 d	83.67 b	5.38 a
D2	30.01 a	31.17 b	83.80 a	5.36 b
D3	29.52 c	30.68 c	83.35 c	5.22 c
D4	30.02 a	31.85 a	83.71 ab	5.15 d
Interaction				
G1×D1	29.75 g	30.39 g	83.56 e	4.93 no
G1×D2	29.18 i	30.52 g	83.04 f	4.91 o
G1×D3	29.54 h	33.77 b	83.38 e	4.37 p
G1×D4	29.71 g	32.46 c	83.54 e	4.98 l
G2×D1	30.04 f	32.25 cd	83.82 d	5.54 d
G2×D2	30.07 f	32.06 d	83.87 d	6.16 a
G2×D3	28.42 k	29.25 i	82.34 g	5.34 g
G2×D4	28.60 j	29.59 h	82.48 g	4.95 mn
G3×D1	30.21 e	30.96 f	83.98 cd	5.81 c
G3×D2	30.57 c	33.63 b	84.30 b	5.51 e
G3×D3	30.01 f	30.49 g	83.81 d	5.39 f
G3×D4	29.55 h	31.15 f	83.38 e	5.02 k
G4×D1	29.19 i	29.25 i	83.49 e	5.36 fg
G4×D2	29.53 h	30.87 f	83.37 e	5.11 i
G4×D3	29.19 i	28.37 k	83.04 f	6.01 b
G4×D4	30.11 ef	30.93 f	83.89 d	5.81 c
G5×D1	29.68 g	29.86 h	83.49 e	5.26 h
G5×D2	30.69 b	28.77 j	84.39 b	5.08 j
G5×D3	30.43 d	31.52 e	84.16 bc	5.00 kl

G5×D4	32.11 a	35.13 a	85.27 a	4.98 lm
LSD(0.05)	0.0537**	0.1456**	0.1175**	0.0137**

Notes: G1=BC-479, G2=BC-495, G3=BC-514, G4=JA 13/R, G5=CB-12; D1= No detopping, D2= Detopping at 80 DAS, D3= Detopping at 90 DAS, D4= Detopping at 100 DAS

In the study plant height (cm) was significantly different at maturity stage. The tallest plant height (135 cm) was observed in genotype CB-12 without detopping whereas the shortest plant height (86.67 cm) was observed in JA-13/R with detopping at 80 DAS. Alam et al. 1996) reported that shorter plant height is desirable for cotton plants. According to Farooq et al. (2013), positive correlation and positive indirect effects of seed cotton yield in plant highest. Several authors (Khan et al. 2009; Batool et al. 2010; Suinaga et al. 2006; Taohua and Haipeng, 2006; Meena et al., 2007) studied the stability and adaptability and observed varied values for plant height and other yield components of Gossypium hirsutum cultivars.

Monopodial, sympodial and secondary fruiting branches plant-1 showed significantly highest values in genotype CB-12 without detopping and they decreased with detopping practices but highest bolls plant-1 (40.33) was observed in genotype BC-479 with detopping at 90 DAS because this practice inhibited vertical plant growth and subsequently promoted lateral growth including branching. The similar results are in conformity with the findings of Anonymous (2010) and Kumari and George (2012). Shwetha et al. (2009) observed maximum bolls plant-1asproduced by detopping practices compared with no detopping. Further, Venkatakrishnan and Pothiraj (1994) reported that detopping decreased the number of monopodial branches but increased sympodial branches due to the reason that it breaks apical dominance and leads to increased lateral fruiting branches number.

Lint quality character of cotton is an important character for textile industry. In the study all genotypes showed medium of good lint quality. The highest Fiber length, strength and uniformity index 32.11mm, 35.13g/tex and 85.27% showed genotype CB-12 with detopping at 100 DAS, respectively and highest value of micronaire 6.16 showed genotype BC-495 detopping at 80 DAS. According to study Rathinavel et al. (2001) reported that lint quality of cotton fiber in American upland cotton medium staple length (25-29 mm), strong >29 g/tex, fiber fineness (3 -3.9), uniformity >45 was Good.

It was clear from the studies of detopping on cotton and other crops that crop yield was unaffected due to detopping, in turn, yield levels were increased in some occasions as the plant stature changes on account of termination of apical dominance in cotton. Further, the results can be better used as canopy modifier under excess growth conditions.

Conclusion

Genotype BC-479 with detopping at 90 days after sowing took minimum field day while produced maximum yield and yield contributing character and fiber quality. Therefore, the present study strongly suggests the use of the genotype BC-497 with detopping practice at 90 DAS to improve the yield and quality of cotton and also to reduce the field duration. So that it can be fitted in the existing cropping patterns of Bangladesh with a provision to have a rabi crop after cotton harvest.

Methods

An experiment was carried out during Kharif season (Monsoon) 2018-2019 at the Central Cotton Research, Training and Seed Multiplication Farm, Sreepur, Gazipur, Bangladesh which is geographically situated 24.090N latitude and 90.260E longitudes. The design followed in the experiment was Randomized Complete Block Design (RCBD) with factorial arrangement having three replications. The experiment composed of two factors- Factor A: Five genotypes viz. G1: BC-479, G2: BC-495, G3: BC-514, G4: JA-13/R and G5: CB-12and Factor B: Four detopping practices viz. D1: no detopping (control), D2: detopping at 80 DAS, D3: detopping at 90 DAS and D4: detopping at 100 DAS. The plot was 16.2 m2 where spacing 90 cm and 45 cm between row and plant, respectively were used. The distance between plots and replications were 1 m and 2 m, respectively. Data were recorded on plant height (cm), main stem node of first fruiting branch (NFB), number of monopodial branches plant-1, number of sympodial branches plant-1, days to first flowering (days), number of bolls plant-1, average boll weight (g), seed cotton yield (t ha-1), field durations (days). Lint quality was measured as staple length (mm), strength (g tex-1), uniformity (%) and micronaire. The collected data were statistically analyzed using analysis of variance (ANOVA) technique and Least Significant Difference was considered for comparing the treatment means by computer package R studio.

Abbreviations

DAS: Days after sowing, T. aman : Transplant aman

NFB: Main stem node of a first fruiting branch, CBD: Cotton Development Board

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References

1. Alam AKMR, Mandol MS, Hossain N, Sarker R. Genotype-location interaction for yield and some agronomic characters in upland cotton (G. hisutum L.). Bangladesh J Plant Breed Genet. 1996;9:7-10.
2. Anonymous. Manipulation of morphoframe through nipping at grand growth stage and mimic the effect using action specific chemicals-maleic hydrazide in cotton. Acharya N. G.Ranga Agricultural Uni., Guntur. Annu Rep. 2010:pp: 51.
3. Batool S, Khan NU, Makhdoom K, et al. Heritability and genetic potential of upland cotton genotypes for morpho-yield traits. Pakistan J Bot. 2010;42(2):1057-1064.
4. Brar AS, Novtej S, Deol JS. Influence of plant spacing and growth modification practices in yield and its attributing characters of two cotton cultivars (G. hirsutum). J Res Punjab Agric Univ. 2002;39(2):181-183.
5. Dai J, Zheng W, Yang. JS. Review on growth and application of cotton monopodial branches. China Cott 30. 2003;6:2-5.
6. Farooq J, Anwar M, Riaz M, et al. Association and path analysis of earliness, yield and fiber related traits under cotton leaf curl virus (CLCuV) intensive conditions in Gossypium hirsutum L. Plant Knowl Journal, South Cross Publ Group, Aust. 2013;2(1):43-50.
7. Kataria GK, Valu MG. Effect of detopping and growth retardants on physiological parameters and yield of Bt cotton (Gossypium hirsutum L.). J Cott Res Dev. 2018;32(2):226-230.
8. Khan NU, Marwat KB, Hassan G, et al. Study of fiber quality traits in upland cotton using additive-dominance model. Pakistan J Bot. 2009;41(3):1271-1283.
9. Kumari SR, George M. Physiological manipulation of plant morphoframes by MH and through nipping for enhancing cotton yield. Int J Appl Agric Hortic Sci. 2012;3(6):677-79.
10. Meena RA, Monga D, Kumar R. Undescriptive cotton cultivars of north zone: an evaluation. J Cott Res Dev. 2007;21:21-23.
11. Nawaz B, Sattar S, Malik T. Genetic analysis of yield components and fiber quality parameters in upland cotton. Int Multidiscip Res J. 2019;9:13-19. doi:10.25081/imrj.2019.v9.5284
12. Obasi MO, Msaakpa TS. Influence of topping, side branch pruning and hill spacing on growth and development of cotton (Gossypium barbadense L.) in the Southern Guinea Savanna location of Nigeria. J Agric Rural Dev Trop Subtrop. 2005;106(2):155-165.
13. Rathinavel K, Gopalakrishnan N, Dhamayanthy KPM. Detopping with defoliation treatment – maximizes seed yield in cotton. Online. 2003:pp.1.
14. Rathinavel K, Manickam S, Sabesh SM. Training Manual on DUS Test in Cotton with reference to PPV and FR Legislation, 2001. Cent Inst Cott Res Reg Reg Station Coimbatore. 2005:1-141.
15. Renou A, Téréta I, Togola M. Manual topping decreases bollworm infestations in cotton cultivation in Mali. Crop Prot. 2011;30(10):1370-1375. doi:10.1016/j.cropro.2011.05.020
16. Shwetha NS, Halepyati AS, Pujari BT. Effect of detopping , removal of monopodia and plant spacings on nutrient uptake , quality parameters and economics of Bt cotton (Gossypium hirsutum L .). Karnataka J Agric Sci. 2009;22(4):892-893.
17. Singh J, Sandhu BS. Effect of detopping in cotton on the efficacy of chemical control of boll worms. J Indian Soc Cott Improv. 1996;21:56-57.
18. Suinaga FA, Bastos CS, Rangel LEP. Phenotypic adaptability and stability of cotton cultivars in Mato Grosso State, Brazil. Pesqui Agropecu Trop. 2006;36(3):145-150.
19. Taohua Z, Haipeng Z. Comparative study on yield and main agri-characters of five hybrids colored cotton varieties. J Anhui Agric Univ. 2006;33:533-536.
20. Venkatakrishnan AS, Pothiraj P. Effect of nutrient management, foliar spray and detopping on yield attributes and seed cotton yield. Madras Agric J. 1994;81(1):519-520.
21. Xu, H. L, Li GF, He XH, Yang D. Regularity of accumulation anddistribution of dry matter in monopodial branches retained cotton plants. J Agcultre Sci. 2001;17(3):153-157.
22. Yang Y, Ouyang Z, Yang Y, Liu X. Simulation of the effect of pruning and topping on cotton growth using COTTON2K model. F Crop Res. 2008;106(2):126-137. doi:10.1016/j.fcr.2007.10.019

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Effect of Foliar Potassium Fertilization on Cotton Seed Yield and Quality

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Abstract

Background: *The experiment was conducted at Central Cotton Research Farm, Sreepur, Gazipur during cotton growing season of 2009-2010. Cotton variety cv. CB-10 was used under experiment. Eight levels of potassium (0, 30, 40, 50, 60, 70, 80 and 90 g K L-1 water) were sprayed at reproductive stage of cotton as treatment. . Foliar K sprayings were done at the time of flower emergence to boll formation stage for three times at ten days intervals. Potassium was derived from Mop The design of the experiment was randomized completely block design (RCBD) with three replications.*

Results: *Result revealed that, foliar application of potassium has significant influence on different traits of cotton. Foliar application of potassium may improve the performance of cotton plant by developing boll weight and better quality cotton seed.*

Conclusion: *Foliar application of potassium may improve the performance of cotton plant by developing boll weight and better quality cotton seed.*

Keywords:
Seed cotton, cotton boll, k fertilizer, upland and hill cotton

Background

Upland cotton (Gossypium hirsutum) and hill cotton (Gossypium arboreum) are mostly cultivating in Bangladesh from very old era for its quality industrialized fibre. Potassium plays an important role in photosynthesis, water balance, balance between mono and divalent cations, translocation of carbohydrates and resistance against insects and diseases (Brar and Tiwani, 2004). Potassium also affects on respiration, chlorophyll II development, carbon decide assimilation, and carbon movement (Sangakkara et al., 2000). Effect of potassium on fibre quality characteristics tended to more critical than its effect on lint yield, especially when deficiency is expected in the field. Growth rate and maturity of cotton cultivars were reported to be important factors associated with potassium and its effect on fibre quality (Pettigrew et al., 1996; Pettigrew, 1999).
Potassium deficiencies can limit the accumulation of crop biomass. This has been attributed to a reduction in the partitioning of assimilate to the formation of leaf area and a decrease in the efficient use of intercepted radiation for the production of above ground biomass (Colomb et al., 1995). This in turn reduces lint yield, lint index and fibre and seed quality.

Potassium influences cotton yield by affecting late season growth. Cotton requires from 3 to 5 kg K ha-1 day-1 during bill fill, and an average mature cotton crop is estimated to require a total of 110 to 250 kg K ha-1 (Halevy, 1976). Thus cotton appears to be more sensitive to low soil K availability than most major field crops (Cope, 1981), and often shows signs of K deficiency on soils not considered K deficient for other crops (Cassman et al., 1989). Furthermore, the cotton root system has a low density relative to other major crops (Gerik et al., 1987), and K, which is relatively immobile in soil, moves slowly by diffusion (Barber, 1984). Therefore, the sensibility of cotton to the soil K supply, coupled with the large requirement for K and its relative immobility in soil, could lead to a deficiency even on soils that test high in extractable K (Oosterhuis, 1995).

Foliar K has shown potential to remediate early symptoms of plant K deficiency in cotton and may be used to supplement soil application as a means to maximize lint yield (Howard et al., 1998). However, the level of response to foliar K applications depends on choice of K source, buffering the spray solution,

applying K with other agrochemicals and concentration of K in the solution. The present study is planned to evaluate the appropriate concentration of foliar K to obtain good quality lint and seed of cotton.

Results and discussion

Growth attributes

Growth of cotton in relation to foliar K application was not significant and it had little impact on plant height (Table 1). However, taller plants were observed at higher K concentrations and shorter plants at lower K levels.

Table 1. Effect of different concentration of foliar K on growth of cotton plant

Foliar potassium concentration (g L-1water)	Plant height (cm)	Branch plant-1	
		Monopodial	Sympodial
0	86.42	1.78	12.37
30	86.67	1.87	12.42
40	86.75	1.87	12.78
50	88.42	1.80	13.12
60	87.40	1.80	12.75
70	90.92	1.75	13.08
80	85.75	1.80	12.67
90	90.25	1.80	12.58
CV (%)	8.67	13.62	6.22

Notes : Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Similar phenomena also observed in case of monopodial and sympodial branch of cotton. It could be possible to explain that foliar application of K at reproductive stage increased the nutrient content of the plants and increased the plant growth (Poole et al., 1983). Further, activation of different enzymes by foliar K and its involvement in ATP production is important in increasing photosynthesis and growth of plants (Troch and Thomson, 1993). Conversely, growth slowed at lower foliar K concentration because of lower ATP production at K deficient condition (Blevins, 1985).

Boll number and yield

Foliar application of 50 g K L-1 water significantly increased the number of bolls per plant compared with the untreated control (Table 2). However, application of foliar K from 40 to 90 g K L-1 water produced similar boll number. Increase in boll number in foliar K sprayed plants is the resultant of reduction in boll shedding as K regulates flower abscission and yield of cotton. Guinn (1985) indicated that K deficiency increases boll shedding through an increase in ethylene production. Results for boll numbers to foliar K application in this study were similar other authors (Coker et at., 2001; Pervers et al., 2004).

Table 2 Effect of foliar K application on yield contributing characters and yield of cotton

Foliar potassium concentration (g L-1 water)	Boll number plant-1	Boll weight (g)	Seed cotton yield (t ha-1)
0	15.93 c	3.09 e	0.93 c
30	16.53 bc	3.58 c	1.04 bc
40	18.43 abc	3.38 d	1.22 a
50	20.93 a	4.26 a	1.26 a
60	20.45 a	3.87 b	1.21 a
70	19.58 ab	4.04 ab	1.18 ab
80	18.43 abc	4.00 ab	1.16 ab
90	18.33 abc	3.90 b	1.15 ab
CV (%)	9.60	8.25	7.69

Notes : Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Boll weight and yield

Foliar application of K also showed significant increase in boll weight of cotton. The highest boll weight (4.26 g) was recorded at 50 g K L-1 water and the lowest (3.09 g) in potassium deficient treatment

(Table 2). The reason of decrease in boll weight in K deficient treatment is that K deficiency resulted in early abscission of leaves and carbohydrates accumualted in main stem leaves, so the top bolls of cotton plant developed incompletely, and the boll weight was lower in K deficient plants. The results of the present study are in line with other authors (Gormus, 2002; silva et al., 1996; Waraich et al., 2011) that higher accumulation of K at the later stages of plant growth are related to higher boll weight of cotton.

Seed cotton yield

Foliar applied K significantly increased seed cotton yield. The highest seed cotton yield (1.26 t ha-1) was found at 50 g K L-1 water which was similar to yields obtained from other treatments except control and 30 g K L-1 water (Table 2.2). Yield increases could be attributed to the effect of K on new growth and nutrient uptake (Fan et al., 1999), which caused favorable effects on number of bolls per plant and boll weight, leading to higher seed cotton yield. In present study, there also exists positive relationship between boll number and boll weight with seed cotton yield of cotton (Fig. 1 and 2). Lower cotton yield in control treatment was attributed in part to a reduction in boll mass that was mostly ascribed to K deficiency (Pettigrew et al., 1996). Li et al. (1999) reported that cellulose synthesis and dry matter accumulation were increased by foliar K applications which indicate that K deficiency during the reproductive period changes the structure of fruit bearing organs and decreases cotton yield. Results obtained in this study are similar to those of Ghourab et al. (2000) and Gormus (2002) but were in contrast with those of Minton and Ebelhar (1991).

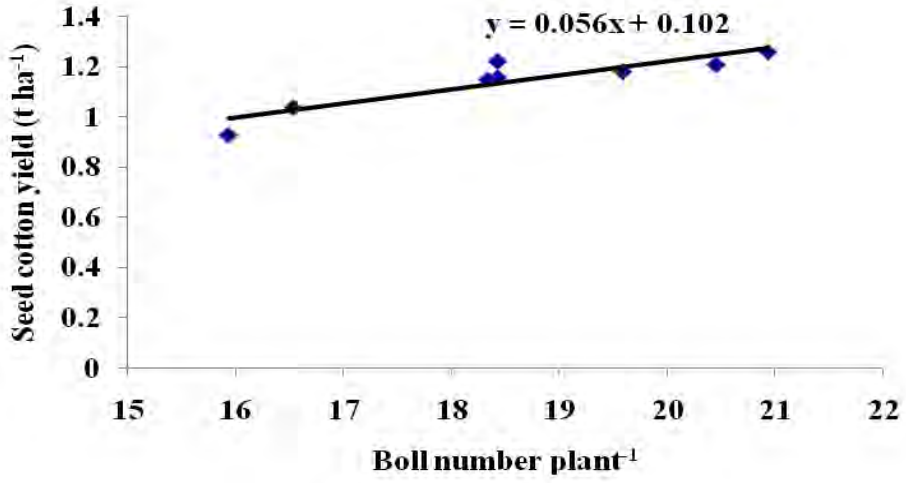

Fig. 1. *Relationship between boll numbers and yield of cotton.*

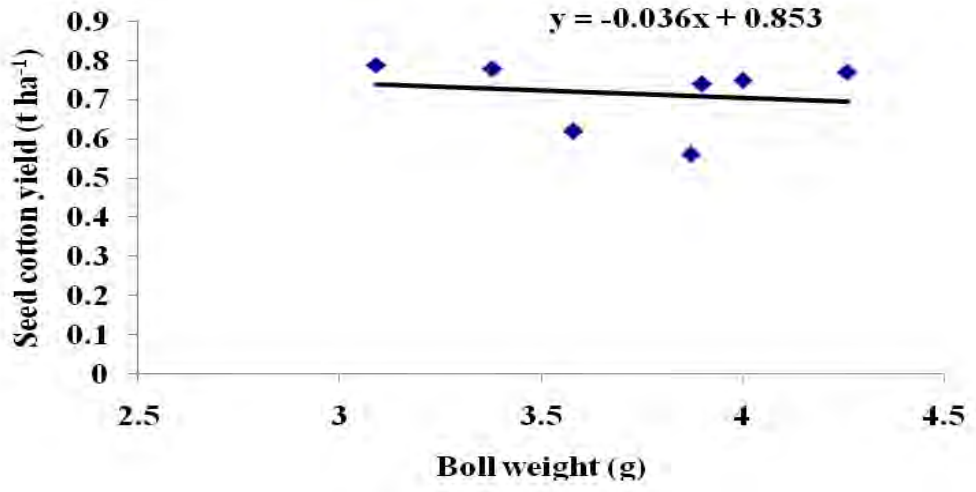

Fig. 2 *Relationship between boll weight and yield of cotton.*

Ginning outturn

Application of foliar K increased ginning out turn and it was the highest (40.38%) at 50 g K L-1 water (Table 3). Increase in the ginning out turn with the increasing K application is in agreement with Read et al. (2006) and they reported that it is better to apply K at early boll development stage. The possible reason of increased ginning out turn may be due to increased cellulose synthesis and dry matter accumulation under foliar K application (Li et al., 1999).

Table 3 Effect of foliar K application on yield contributing characters and yield of cotton

Foliar potassium concentration (g L-1 water)	Ginning outturn (%)	Lint yield (t ha-1)	Seed yield (t ha-1)
0	35.59 ab	0.37 b	0.56 b
30	36.07 ab	0.42 ab	0.62 b
40	37.30 ab	0.44 a	0.78 a
50	40.38 a	0.47 a	0.79 a
60	39.78 a	0.44 a	0.77 a
70	36.36 ab	0.42 ab	0.75 a
80	35.34 ab	0.41 ab	0.75 a
90	35.65 ab	0.41 ab	0.74 a
CV (%)	8.33	6.52	8.08

Notes: Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Lint yield

Foliar K also significantly lint yield suggesting that it increases yield when soil test K level is not adequate or when recommended rate of K is not applied in soil. However, a large level of K concentration ranging from 30 to 90 g L-1 water produced similar lint yields of cotton. Results showed that the lowest lint yield (0.37 t ha-1) was found from control treatment and the highest yield (0.47 t ha-1) from foliar spray of 50 g K L-1 water. These observations were consistent with earlier report of Coker et al. (2009).

Seed yield

All most similar effect of foliar K was observed that the foliar K significantly increased the seed yield of cotton. The highest seed yield (0.79 t ha-1) was recorded at 50 g K L-1 water and the lowest (0.56 t ha-1) at control plants. Keino et al. (1999) reported that foliar K stimulated seed yield through increased carbohydrate flow to the developing seed. Seed yield of cotton under foliar K application also increased due to increase in number of seeds per boll (Oosterhuis, 1995).

Span length and uniformity of fibre

Foliar K had significant effect on fibre quality of cotton (Table 4). Maximum values for fibre length (12.19 mm) and uniformity ratio (45.67%) were found under 50 g K L-1 water sprayed. On the other hand, minimum values for those parameters were observed when no K was sprayed. The reason for most fibre length in K deficient plants may be the less water pressure inside of the developing fibre in conjunction with the deposition of carbohydrate polymers which drives elongation (Waraich et al., 2011). Cassman et al. (1990) started that increasing K fertilization to cotton increases fibre length and Dhindas et al. (1975) argued that reduction in fibre length produced by K deficiency is consistent with K serving as an osmoticum, producing turgor pressure for fibre elongation.

Table 4. Effect of foliar application of K on fiber characteristics

Foliar K concentration (g K L^{-1} water)	50% span length (mm)	Uniformity ratio (%)	Micronair value	Pressly strength (PSI)
0	10.92 bc	41.17 d	4.08 c	82.98
30	11.68 abc	42.33 cd	4.20 bc	83.15
40	11.94 ab	44.50 ab	4.33 ab	82.43
50	12.19 a	45.67 a	4.52 a	84.56
60	11.94 ab	44.50 ab	4.37 ab	83.26
70	11.68 abc	44.33 abc	4.35 ab	83.61
80	11.68 abc	43.50 bc	4.25 bc	83.14
90	11.68 abc	43.00 bcd	4.20 bc	83.75
CV (%)	4.23	4.52	3.75	2.26

Notes: Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Uniformity is related to fibre length in that the uniformity index is the ratio between the mean length and the upper one-half mean length of the fibre sample (USDA, 2001). Fibre uniformity significantly increased with K application. Present results of fibre uniformity are in contrast with the results of Gormus (2002). He found that fibre uniformity was non-significant to K application. For variation in this parameter, Jones and wells (1998) argued that the indeterminate growth habit of cotton, and cultivar variation in development rate, may cause fibre properties to vary in different study.

Micronaire value of fibre

Micronaire values were increased significantly by foliar K application. The highest micronaire (4.52) was found from cotton lint under the condition of 50 g K L-1 water sprayed. However, most of the treatments showed the similar positive response in improvement of micronaire values. This result is in agreement with the findings of Nascimento and Athayde (1999) who found that varying concentration of foliar K increased micronaire values of cotton fibre.

Pressly value of fibre strength

In our studies, the fibre strength was not significantly increased with increase in foliar K concentration. However, numerically higher strength (84.56 PSI) was observed at foliar K spraying at the rate of 50 g k L-1 water during reproductive stage of cotton. This lack of response of fibre strength to K application is in contrast to the findings of Ebelhar (1991) who reported significant increase in fibre strength by the rate of K application.

Grain quality

Grain quality of cotton seed was improved by foliar K application. The applied K caused significant increase in seed oil content compared with the untreated control (Table 5). The foliar K increased seed oil content from 17.88% in control to 20.30% in 50 g K L-1 water. The favorable effects of K on seed oil content were also reported by other authors (Eid et al., 1997; Abou et al., 2000). This could be attributed to role of K in different biochemical pathways that favored higher oil content in cotton seed.

The foliar K also increased seed protein content as compared with the untreated control (Table 5). The positive effect of K in protein increment is associated with N metabolism (Bisson et al., 1994; Bednarz and Oosterhuis, 1999; Pettigrew, 1999). These are manifested in metabolites formed in plant tissues and directly influence the growth, development and oil content in seed. Similar results were obtained by Abou et al. (2000) and Ghourab et al. (2000).

Table 5 Effect of foliar K application on protein and oil content of cotton seed

Foliar potassium concentration (g K L-1 water)	Oil content (%)	Protein content (%)
0	20.94 c	17.88 c
30	22.24 b	18.98 bc
40	23.44 b	19.03 bc
50	25.25 a	20.30 a
60	23.27 b	18.99 bc
70	23.27 b	19.06 b
80	22.92 b	18.73 bc
90	23.41 b	18.12 bc
CV (%)	5.10	4.94

Notes: Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Seed index

The hundred seed weight of cotton increased significantly with the increase of foliar K and it was the highest (10.00 g) at 50 g K L-1 water foliar application of K which was followed by 60, 70 and 80 g foliar K fertilizations (Table 6). Results suggested that K requirement increases by many folds at seed developing stage of cotton. This K requirement can also be met by pre-plant soil K application but there is every possibility to fix K to soil which impairs the K availability at the later crop growth (Abaye, 1996). Therefore, when foliar K application was done, seed index was significantly higher than the control and other levels of K application.

Table. 6 Effect of Foliar K application on seed quality attributes of cotton

Foliar K concentration (g L-1water)	Seed index (g)	Germination (%)	Root length (cm)	Shoot length (cm)	Electrical conductivity (µS cm-1 g-1)
0	8.93 d	82.00 d	9.73 c	10.60	141.00 a
30	9.15 d	86.00 c	9.93 bc	10.60	117.33 b
40	9.26 cd	86.00 c	10.67 a	10.50	111.33 b
50	10.00 a	86.40 bc	10.93 a	10.83	107.00 b
60	9.10 ab	91.50 a	10.90 a	10.90	113.17 b
70	9.92 ab	90.30 a	10.77 a	10.83	111.50 b
80	9.74 ab	88.22 ab	10.47 ab	10.73	112.33 b
90	9.63 bc	87.35 ab	10.60 a	10.67	116.00 b
CV (%)	4.48	7.35	3.46	9.00	8.24

Notes: Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Seed germination

Germination capacity of cotton seed improved due to application of foliar K application (Table 6). Germination percentage (91.50%) reached peak at 50 g K L-1 water and it was similar to other germination percentages obtained from seeds of other higher K levels. Improvement in germination capacity of cotton seed under higher foliar K might be attributed to increase in seed index. Higher seed index increased mitochondrial protein in bolder seed indicates higher respiratory rate and greater energy (ATP) production which give higher growth potential of growing seedling (Ferguson et al., 1990).

Seedling characteristics

Shoot length of cotton seedling did not influence markedly due to foliar application of K to parent cotton plants. However, effect of foliar K on root length was observed under higher K levels. The beneficial effect of foliar K in seedling growth might be due to enrichment of seed K which had favorable effects on the metabolism of nucleic acids, protein and growth substances (Vlasynk et al., 1978).

Seed quality according to electrical conductivity

Electrical conductivity of seed leachates decreased under foliar K application and it was lowest (107.00 µS cm-1 g-1) at 50 g K L-1) water (Table 5). Lower electrical conductivity indicates better seed quality (Lowe and Pies, 1972). The favorable effects of foliar K in seed quality enhancement are manifested through involvement of K in metabolism processes, which might reflected in improvement in seed quality of cotton.

Seed protein content is associated with seed quality as there exists negative relationship between protein content and electrical conductivity of seed leachates (Fig. 3). This indicates that protein content of seed increases cell membrane integrity and improves quality of cotton seed.

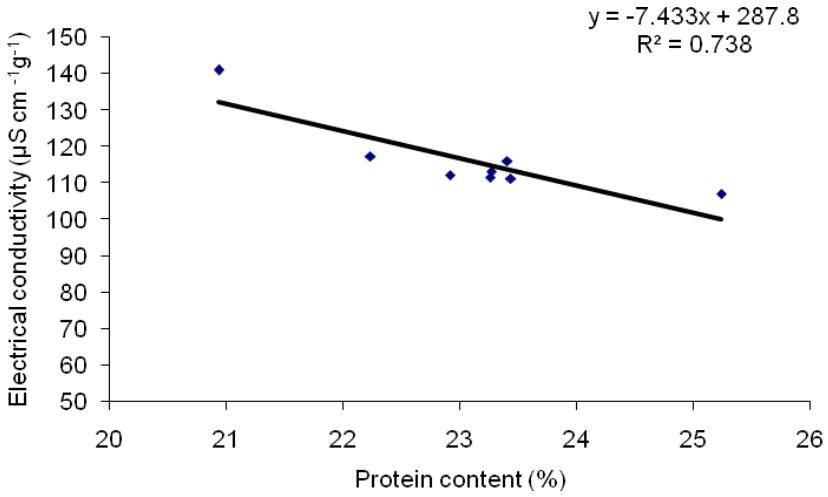

Fig. 3. *Relationship between seed protein content electrical conductivity of cotton seed leachate.*

Conclusion

Cotton showed the best performance towards foliar application of at 40 g k L-1 and50 g k L-1 that of control under present experiment. So, it may be said that, foliar application of potassium may improve yield, fiber properties and seed quality of cotton.

Methods

Second field experiment was conducted at Central Cotton Research Farm, Sreepur, Gazipur during 2009-2010 growing season. The characteristics of soil of experimental site, climatic condition and crop management practices followed as described in earlier experiment. Same cotton variety (CB-10) was used in the experiment and eight levels of potassium (0, 30, 40, 50, 60, 70, 80 and 90 g K L-1 water) were applied as foliar spray. Foliar K sprayings were done at the time of flower emergence to boll formation stage for three times at ten days intervals. Potassium was derived from Mop. The design of the experiment was randomized completely block design (RCBD) with three replications. Unit plot size was 7m x 3m and the distance between the plots was 1.00 meter. The treatments were assigned randomly in each replication. Data on different growth parameter, yield, and fibre and seed quality were recorded as procedures described in first experiment.

All data were subjected to statistical analysis by analysis of variance (ANOVA). Microsoft EXCEL and MSTAT software programs were used wherever appropriate and the means were compared according to Duncan's Multiple Range Test (DMRT). Functional relationships among the parameters were established through correlation and regression analysis by using SPSS software program.

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References

1. Abady, E. M. I., S. E. Seadh, A. N. Attia and A. E. A. E. Saidy. 2008. Impact of foliar fertilization and its time of application on yield and seed quality of soybean Proc.. 2nd Field crops conf. FCAI. ARC. Egypt.
2. Abaye, A. O. 1996. Potassium fertilization of cotton. NO. 418-0. Virginia Cooperative Extension Service, Blackberg. VA. 25.
3. Abou El-Nour, M. S., M. A. Saeed and M. A. Morsy. 2000. Effect of potassium fertilization under two planting dates on yield, yield components and some technological and chemical properties of Giza 80 cotton cultivar. Egypt. J. Agric. Res. 78: 1219-1231.
4. Aladakatti Y. R., S. S. Hallikeri, R. A. Nandagavi, N. E. Naveen, A.Y. Hugar and D. Blaise, 2010, Yield and fiber qualities of hybrid cotton (Gossypium hirsutum) as influenced by soil and foliar application of potassium, Karnataka J. Agric. Sci.2: 133-136.
5. Alam S. M. 1999. Nutrient uptake by plants under stress condition. In: Handbook of Plant and Crop Stress . (Ed.): M. Pessarakli. Marcel Dakker Publishers, New York. U.S.A. pp: 285-313
6. Anonymous. 2007. Potassium nutrient for cotton. Cotton catchments communities cooperative research centrte. pp.1-3.
7. Anonymous. 2012. Statistical year book. Bangladesh Bureau of Statistics. Dhaka. pp.63-68.
8. Barber, S. A. 1984. Soil Nutrient Bioavailability. A Mechanistic Approach. John Wiley. NY.
9. Bednarz, C. W. and D. M. Oosterhuis. 1999. Physiological changes associated with potassium deficiency in cotton. J. Plant Nutr. 22: 303-313.
10. Blevins, D. G. 1985. Role of protein in protein metabolism in plants. In Potassium in Agriculture. Am. Soc. Agron. 413-424.
11. Brar, M. S and A. S. Brar. 2004. Foliar nutrition as a supplement to soil fertilizer application to increase yield of upland cotton. Indian J. Agric. Sci. 74: 472-475.
12. Cassman, K. G., B. A. Roberts, B. A. Kerby, T. A. Bryant, and D. C. Higashi. 1989. Soil potassium balance and cumulative cotton response to annual potassium additions on a vermiculitic soil. Soil Sci. Soc. Am. J. 53: 805-812.
13. Cassman, K. G., T. A. Kerby, B. A. Roberts, D. C. Bryant and S. L. Higashi. 1990. Potassium nutrition effects on lint yield and fiber quality of Acala cotton. Crop Sci. 30: 672-677.
14. Chakravotry, S. C. and S. Singh. 1979. Effect of fertilizers (NPK)on seed weight, protein content and oil composition of cotton. Indian agric.23: 165-171
15. Coker, D. and D. M. Oosterhuis. 2001. Effect of soil and foliar potassium fertilizer on yield of water deficit stressed cotton. Proc 2001 Cotton Meeting and Summaries of Research in Progress. University of Arkansas Agricultural Experiment Staation, Special Report (in press).
16. Cope, J. T. J. 1981. Effects of 50 years of fertilization with phosphorus and potassium on soil tests levels and yields at locations. Soil Sci. Soc. Am. J. 45: 342-347.
17. Crozier, C. R., B. Walls, D. H. Hardy and J. S. Barnes. 2004. Response of cotton to P and K Soil Fertility Gradients in North Crolina (Online). J. Cotton Sci. 8: 130-141.
18. Ebelhar, M. W. 1991. Producing quality cotton in 1991 - Plant nutrition management. Proc. 1991 Beltwide Cotton Prod. Conf., Natl.Cotton Council of Amer. Memphis TN. p. 55-58.
19. Eid, E. T., M. H. Abdel-Aal, M. S. Ismail and M. M. Wassel. 1997. Proc.FAO-IRCRNC. Pp.189-190.Joint meeting of working groups 3 and 4 (Cotton nutrition and growth regulators) 20-23 March.1995,Cairo,Egypt.
20. Elgala, A. M., A. El-Leboudi, and A. A. Sakar, 1976. Growth and nutritional status of cotton plant subjected to foliar spray with certain nutrient solution. Agric. Res. Rev. 54: 141-152.

21. Elham, H. L., A. Ahmad and F. Fatemeh. 2012. Effect of boron foliar application on some morphological traits and seed yield of indigo (Indigofera tinctoria L.). American-Eurasian J. Agric. & Environ. Sci. 12: 1102-1104.
22. Essington, M. D., H. S. Howard G. Lessman. 2002. Potassium fertilization of cotton produced on loess-derived soil. Better Crops. 86: 13-16.
23. Fan, S., X. Yuzhang and Z. Chaojun. 1999. Effects of nitrogen, phosphorus and potassium on the development of cotton bolls in summer. Acta Gossypii Sinica.11: 24-30.
24. Ferguson, J. M., D. M. TeKrony and D. B. Egli. 1990. Changes during early soybean seed and axes deterioration I. Seed quality and mitochondrial respiration. Crop Sci. 30: 175-179.
25. Girma, K., K. T. Roger, W. F. Kyle, R. K. Boman, and W. R. Raun. 2007. Cotton lint yield and quality as affected by applications of N, P, and K. Fert. J. of Cotton Sci. 11: 12–19.
26. Gormus, O. 2002. Effect of rate and time of potassium application on cotton yield and quality in Turkey. Agro. and Crop Sci. 188:382-388..
27. ISTA. 2006. International Rules for Seed Testing. The international Seed Testing Association. Zurich, Switzerland.
28. Keino, J., C. A Beyrouty. And D. M. Oosterhuis. 1999 Differential uptake of potassium by plant roots. In D.M. Oosterhuis and G. Berkowitz (eds.) Frontiers in potassium nutrition: New Perspectives on the Effects of Potassium on Crop Plant physiology. Published Potash and Phosphate Institute and Am. Soc. Agron.
29. Mandol, M. S. 2011. Influence of monopodia removal, plant spacing and N P K application on yield and quality of cotton lint. (phd thesis). Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706
30. Minton, E. B and M. W. Ebelhar. 1991. Potassium and aldicarb-disulfoton effects on verticillium wilt, yield, and quality of cotton. Crop Sci. 31: 209-212.
31. Mullins. G. L. and C. H. Burmester, 1990. Dry matter, nitrogen, phosphorus and potassium accumulation by four cotton cultivars. Agron. J. 82: 729-736.
32. Oosterhuis, D. M. 1995. Potassium nutrition of cotton in the USA with particular fererence to foliar fertilization. In proc. First World Cotton Research Conference Cotable. CA, Forrester NW(eds.) CSIRO, Brisbane. Australia. 133-146.
33. Palomo, G. A., S. G. Avila and J. F. Chavez. 1999. Use with new cotton cultivars: yield, yield components and fiber quality. Agrociencia 33 Gonzalez.
34. Parvez, H., M. Ashraf and M. I. Makhdum. 2004. Influence of potassium rates and sources on seed cotton yield and yield components of some elite cotton cultivars. J. Plant. Nutri. 27: 1295-1317.
35. Perry, D. A. 1972. Seed and seedling vigour. Seed Biol. 1: 313-387.
36. Pervez, H., M. Ashraf and M.I. Makhdum. 2005. Response of cotton to potassium fertilizer on effectiveness of fruiting sites in aridisols. J. Plant Nutrition 28: 1023-1039.
37. Pettigrew, W. T. 1999. Potassium deficiency increases specific leaf weights and leaf glucose levels in field grown cotton. Agron. J. 91: 962-968.
38. Sangakkara, U. R., M. Frehner and J. Nosberger. 2000. Effect of soil moisture and potassium fertilizer on shoot water potential, photosynthesis and partitioning of carbon in mungbean and cowpea. Crop Sci. 185: 201-207.
39. Sardar, A., M. Ashraf, M. E. Akhter. 2003. Effect of potash on boll characteristics and seed cotton yield in newly developed highly resistant cotton varieties. Pakistan J. Biol. Sci. 6: 813-815.
40. Snyder, C. S., W. N. Miley,. D. M. Oosterhuis, and R. L. Maples. 1991. Foliar application of potassium to cotton. University of Arkansas, Cooperative Extension Service. Cotton Comments. 4-91.
41. Temiz, M., Y. K. Koca, F. Aydin and E. Karahan. 2009. Effect of foliar potassium and micronutrient additions on yield and fiber quality of cotton (Gossypium hirsutum L.). J. Food Agric. & Environ.7: 118 - 122 .

Effect of Phosphorus on Yield and Fiber Quality of Cotton Variety CB-15

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Abstract

Field experiments were conducted at three Cotton Research Centers located in Sreepur, Gazipur; Jagadishpur, Jashore and Sadarpur, Dinajpur during kharif (monsoon) season in 2018-19 to determine the effect of phosphorus on yield and fiber quality of cotton variety CB-15. The treatments wereT0 = 0 (Control), T1 = 20 kg P/ha, T2 = 40 kg P/ha, T3 = 60 kg P/ha, T4= 80 kg P/ha, T5 = 100 kg P/ha. The experiments were set up in RCB design with three replications. The highest seed cotton yield at Sreepur, Jagadishpur and Sadarpur were obtained by applying phosphorus 60, 100 and 80 kg/ha respectively. Phosphorusrate hadsignificant positive correlation with fiber strength and elongation. Phosphorus application to cotton crop is of vital importance in improving both seed cotton yield and quality.

Keywords: *Cotton, Phosphorus fertilizer, yield and fiber quality*

Background

Phosphorus (P) is the second most limiting nutrient in cotton (Gossypium hirsutum L.) production after nitrogen. It is a constituent of cell nuclei, essential for cell division and development of meristematic tissue (Russell, 2001) and has a well-known impact on photosynthesis as well as synthesis of nucleic acids, proteins, lipids and other essential compounds (Guinn, 1984; Taiz and Zeiger, 1991). Cotton growth and maturity are altered by cultivars, seasonal management and environmental conditions (Gwathmey and Craig, 2003). Pis an integral component of several important compounds in the plant cells, including the sugar phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up plant membranes (Taiz and Zeiger, 2003).P is one of the major nutrients necessary for crop growth and development. However, there are cases where cotton response to P has been positive and economical (Gill et.al. 2000). The P requirements of cotton are considered very low because of its deep root system and indeterminate growth habit (Malik et.al. 1996). Cotton fiber quality is mainly influenced by genotype of the cultivars but agronomic practices and environmental conditions are the secondary factors influencing fiber quality (Subhan et al., 2001).Sharma et al. (1991) stated that fiber quality improved by P application. Vieira et al. (1998) found that fiber length of cotton was increased by P application. The P content of Bangladesh soils is being depleted gradually due to crop removal particularly, in intensive cultivation. Application of P fertilizers is recommended for all soils and crops in Bangladesh to obtain better yield (Ahmed et al., 2018)).The present investigation, therefore, was carried out for optimization of P rates on yield and yield contributing characters of upland cotton and to determine response of cotton to P fertilization.

Results and Discussions

Location Effect on CB-15

Location effect of P fertilizers on yield and yield contributing characters of CB-15 are given in Table 1. The maximum seed cotton yield (2452kg/ha) was recorded from Dinajpur Farm and minimum seed cotton yield (2325 kg/ha) was found in Sreepur Farm.

Table 1. Effect of Location of P on CB-15 yield and yield contributing characters

Location	Plant height (cm)	Monopodia/ plant	Sympodia/ plant	Boll/plant	Single boll weight (g)	Seed cotton yield (kg/ha)
Sreepur	90.9	0.8	15.1	23.4	5.05	2325
Jagadishpur	127.9	1.8	16.3	23.8	4.98	2449
Sadarpur	107.0	2.7	13.6	23.5	5.22	2452
5% LSD	5.73	0.31	0.62	1.73	0.16	141
CV%	7.7	31.3	6.1	10.9	4.7	8.9

Treatment effect

The effect of various levels of P fertilizers on yield and yield contributing characters of CB-15 are given in Table 2. The lowest plant height (83.40 cm) was recorded from control treatment and the highest plant height (120.10 cm) was recorded from the treatment of 80 kg P/ha. The relationship between plant height vs. seed cotton yield is given in Figure1.

Table 2. Effect of various levels of P fertilizers on yield and yield contributing characters ofCB-15

Treatment	Plant height (cm)	Monopodia/ plant	sympodia/ plant	No.boll/ plant	Boll Wt(g)	Yield (kg/ha)
0	83.4	1.3	10.2	9.9	3.97	971
20	110.4	1.4	14.4	19.3	4.62	1900
40	115.0	1.5	15.4	24.6	5.09	2392
60	119.0	1.4	17.0	29.2	5.52	2978
80	120.1	1.6	17.0	30.9	5.75	3093
100	121.3	1.7	16.6	29.2	5.60	2969
5% LSD	6.28	0.34	0.68	1.90	0.17	155
CV%	7.7	31.3	6.1	10.9	4.7	8.9

The lowest monopodial branch/plant (1.30) was recorded from control treatment and the highest monopodial branch/plant (1.70) was recorded from the treatment of 100 kg P/ha. The relationship between monopodial branches per plant vs. seed cotton yield is given in Figure 2.

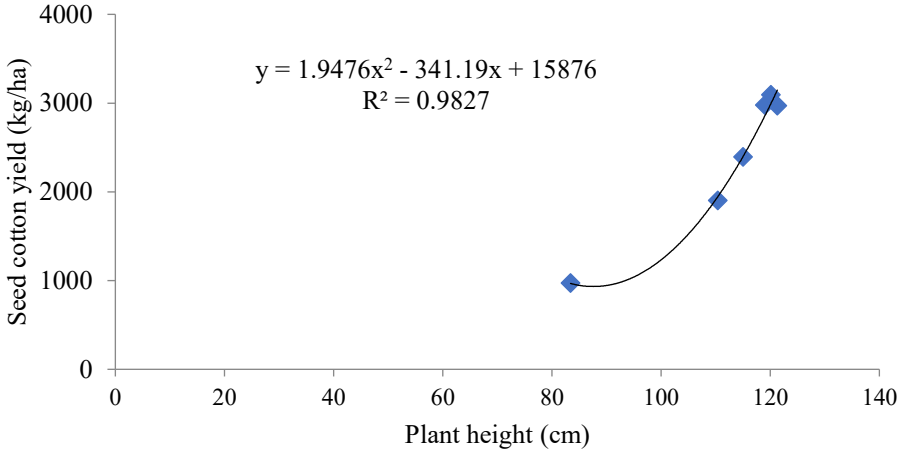

Figure 1. The relationship between plant height vs. seed cotton yield

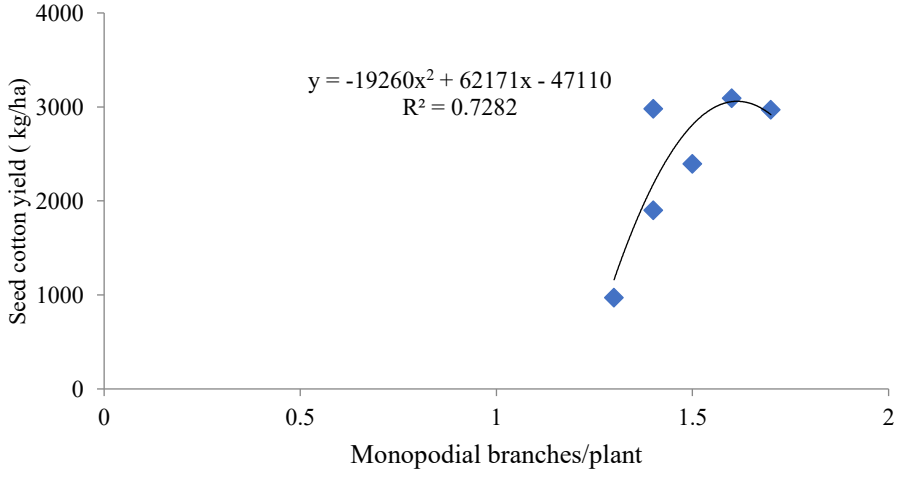

Figure 2. *The relationship between monopodial branches/plant vs. seed cotton yield*

The maximum sympodial branch/plant (17.0) was found in treatment T4 and T5 and minimum sympodial branch/plant (10.20) was found in control treatment which was followed by the treatment T3 (60kg P/ha). The relationship between sympodial branches per plant vs. seed cotton yield is given in Figure 3.

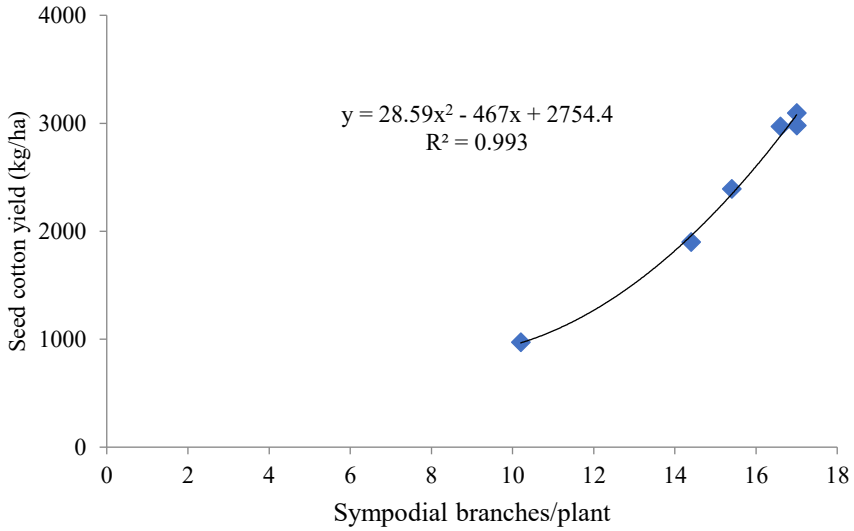

Figure 3. *The relationship between sympodial branches/plant vs. seed cotton yield*

The lowest boll/plant (9.90) was recorded from control treatment and the greater number of boll/plant (30.90) was collected from the treatment of 80 kg P/ha. The lowest single boll weight (3.97 g) was recorded from control treatment and the highest single boll weight (5.75 g) was recorded from the treatment of 80 kg P/ha. Maqshoof et al. (2009) reported that boll weight increased to the highest value of 3.66 g with P application of 34 kg/ha.

The maximum seed cotton yield (3093kg/ha) was found inT4 treatment and minimum seed cotton yield (971 kg/ha) was recorded from the control treatment. A significant increase in seed cotton yield in soils having phosphorus < 12 mg/kg of soil in the Punjab province has been reported (Gill et al., 2000, Makhdum et al., 2001).

Interaction Effect

Interaction effect of Location and Treatment of P fertilizers on yield and yield contributing characters of CB-15 are given in Table 3.The highest seed cotton yield at Sreepur, Jagadishpur and Sadarpur were obtained by applying phosphorus 60, 100 and 80 kg/ha respectively.
The estimated regression equations of CB-15 yield in relation to P fertilizer for Sreepur, Jagadishpur and Sadarpur were y = -0.264x2 + 48.96x + 846.30 (R2=0.8538), y = -0.335 x2 + 52.93.x + 1033 (R2=0.9645) and y = -0.326x2 + 50.44x + 1125 (R2=0.8884) respectively. The higher values of R-square (Figure 4, 5 and 6) revealed that seed cotton yields were predictable.

Table 3. Location × treatment interaction effect on CB-15

Location	P rates (kg/ha)	Plant Height (cm)	Monopodial branch/plant	Sympodial branch/plant	Boll/ plant	Boll weight (g)	Yield (kg/ha)
Sreepur Gazipur	0	57.7	0.0	10.7	10.2	4.06	973
	20	93.1	0.8	14.4	21.9	5.01	1605
	40	97.6	0.9	15.7	23.5	5.20	2042
	60	98.1	1.0	17.0	29.4	6.10	3275
	80	97.3	1.0	16.4	29.0	5.37	3034
	100	101.6	1.0	16.7	27.1	5.32	3024
Jagadishpur Jessore	0	94.0	1.5	10.1	9.4	3.83	937
	20	122.0	2.0	14.9	19.5	4.40	2132
	40	131.7	1.5	16.0	22.1	4.57	2601
	60	139.2	2.0	19.0	30.7	5.27	2964
	80	140.1	2.1	18.6	29.7	5.93	3019
	100	140.0	1.9	18.8	31.1	5.90	3041

Sadarpur Dinajpur	0	73.2	2.8	10.0	10.5	4.03	986
	20	111.3	2.2	13.8	19.9	4.53	2313
	40	107.3	3.2	13.5	24.2	5.27	2548
	60	116.5	1.7	15.4	26.3	5.80	2745
	80	114.2	2.6	14.7	30.5	5.93	3227
	100	119.7	3.7	14.4	29.2	5.73	2896
5% LSD		14.0	0.8	1.5	4.2	0.39	346
CV%		7.7	31.3	6.1	10.9	4.7	8.9

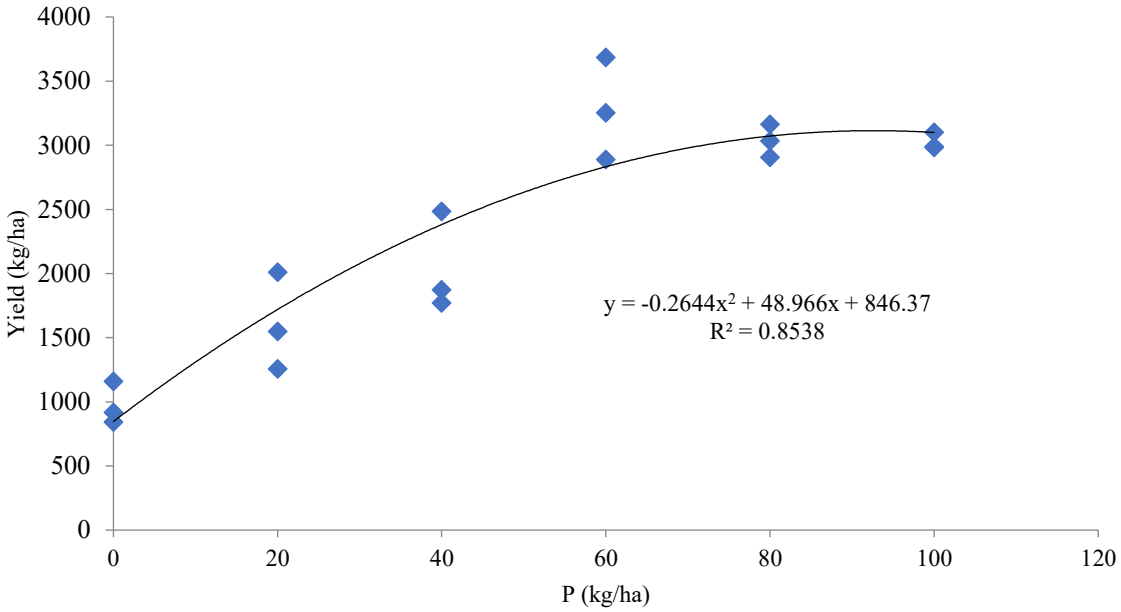

Figure 4. CB-15 yield in response to P fertilizer at Cotton Research Center, Sreepur, Gazipur

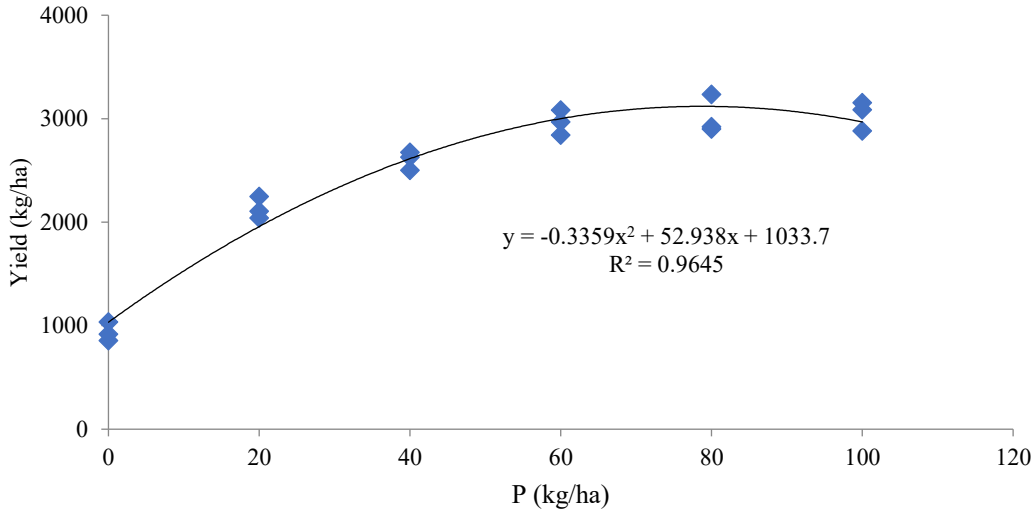

***Figure 5.** CB-15 yield in response to P fertilizer at Cotton Research Center, Jagadishpur, Jashore*

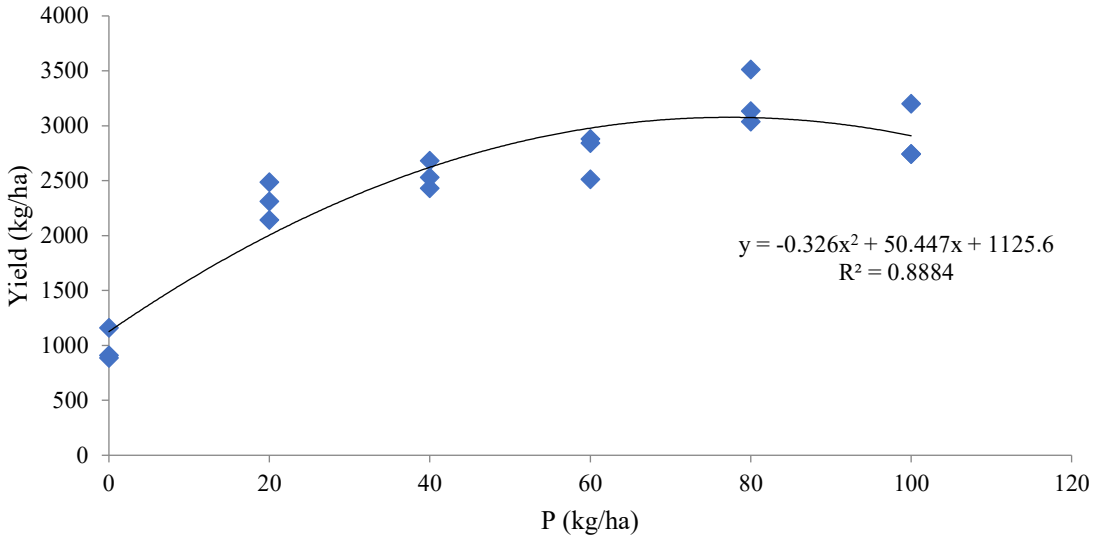

Figure 6. *CB-15 yield in response to P fertilizer at Cotton Research Center, Sadarpur, Dinajpur*

Predicted yield and marginal product for different locations obtained from fitting the regression equation and Optimum level of P was determined by equating the inverse price ratio with marginal product (Table 4). It is estimated that maximum profitable yield at Sreepur, Jagadishpur and Sadarpur cotton research centers could be obtained by applying P at the rate of 89, 76 and 74 kg/ha respectively. Further study is needed to confirm the prediction.

Table 4. Predicted yield and marginal product at different locations

Location	Regression equation	P rates (kg/ha)	Predicted yield (kg/ha)	Total product due to N	Marginal product	Inverse price ratio
Sreepur	y = -0.264x2 + 48.96x + 846.30	89	3110	2137	2.17	2.09
Jagadishpur	y = -0.335 x2 + 52.93.x + 1033	76	3117	2180	2.22	2.09
Sadarpur	y = -0.326x2 + 50.44x + 1125	74	3074	2088	2.53	2.09

Price of P= 120.00 Taka/kg, price of seed cotton= 57.50 Taka/kg

Fiber Quality Related Parameters

Fiber strength was highest with the highest P fertilizer while fiber fineness, uniformity ratio, length and maturity coefficient did not change (Mehetre et al., 1990). Malik et al. (1992) observed that phosphorus had no consistent effect on fiber properties. El-Debaby et al. (1995) reported that higher p rate resulted in a slight reduction in lint percentage. Sharma et al. (1991) stated that fiber quality improved by phosphorus application. Vieira et al. (1998) found that fiber length of cotton was increased by phosphorus application. Keeping in view the above contradictory reports, a study was planned to see the effect of phosphorus application rates on quality determining traits of diverse cotton cultivars. The correlations among P and fiber quality of CB-15 are given in Table 5. The results showed that P has positive correlation with fiber strength and elongation.

Table 5. Correlation matrix among the N and fiber quality

	P	Fiber length	Uniformity Index	Fiber strength	Elongation	Micronaire value	Reflectance
Fiber length	0.789ns						
Uniformity Index	0.766ns	0.965**					
Fiber strength	0.923**	0.807ns	0.720ns				
Elongation	0.912**	0.480ns	0.440ns	0.808ns			
Micronaire value	0.548ns	0.841*	0.902*	0.516ns	0.186ns		
Reflectance	-0.676ns	-0.229ns	-0.239ns	-0.670ns	-0.775ns	-0.188ns	
Yellowness	0.624ns	0.721ns	0.849*	0.418ns	0.357ns	0.886*	-0.242ns

*=significant ($p < 0.05$), **= highly significant ($p < 0.01$), ns=non-significant

Phosphorus is the second most important nutrient (next to nitrogen) that has been found limiting crop production in Bangladesh. Deficiency of phosphorus is widespread in Bangladesh soils. Its deficiency is also quite high in crop growing areas (Sultana et al., 2015). The findings of this study will be useful for location specific P management for cotton production in Bangladesh.

Conclusion

The present study concludes that in case of Cotton Research Center, Sreepur Gazipur, Jagadishpur Jashore and Sadarpur Dinajpur the optimum doses of P for cotton variety CB-15 are 80 kg/ha respectively. For CB-15, P has positive correlation with length, uniformity index, strength, elongation, micronaire and yellowness while it has negative correlation with reflectance. Phosphorus application to cotton crop is of vital importance in improving both seed cotton yield and quality.

Methods

Field experiments were conducted at 3 Cotton Research Center located at Sreepur, Gazipur; Jagadishpur, Jessore and Sadarpur, Dinajpur in 2018-2019. At each location one field experiments were set up.

The experiments were set up in RCBD with 3 replications at each location. The status of the initial soil are presented in Table 6. The treatments wereT0 = 0 (Control), T1 = 20 kg P/ha, T2 = 40 kg P/ha, T3 = 60 kg P/ha, T4= 80 kg P/ha, T5 = 100 kg P/ha.

The experiment was set up second week in the month of July, 2018 in a plot size 5.4 × 4.5m. The row spacing was 90 × 45 cm. Cotton variety CB-15 was used as a test material. Total amount of TSP (as per treatment) gypsum, zinc sulphate, magnesium sulphate, borax and one-third urea and one-third MoP were applied as basal. The rest of Urea and MoP were applied in three equal splits as top dressing at 25 DAS (Days After Sowing), 50 DAS and 70 DAS. The weather data for the cotton growing season is presented in Table 7.

Two irrigations were applied in the month of October and November. Intercultural operations such as weeding, thinning, gap-filling, earthling-up, insects and pest management were done in all plots uniformly.

Table 6. Initial soil status of Experimental sites

Location	pH	OM (%)	N (%)	K meq/100 g soil	P µg/g soil	S µg/g soil	Mg meq/100g soil	Zn µg/g soil	B µg/g soil	Soil Texture
Sreepur Gazipur	6.10	1.58	0.18	0.42	5.68	0.01	1.31	1.34	0.60	Clay loam
Jagadishpur Jashore	7.70	0.27	0.01	0.14	3.28	1.05	0.48	0.88	0.20	Sandy loam
Sadarpur Dinajpur	6.70	0.90	0.04	0.17	9.90	6.40	1.76	3.11	0.12	Sandy loam

Cotton growth data were collected from 10 randomly selected plants at each plot. Average boll weight was calculated by dividing the ten bolls. Seed cotton was harvested from three middle rows to determine the plot yield. Cotton growth data were collected from 10 randomly selected plants at each plot. Average boll weight was calculated by dividing the weight of cotton picked from 10 randomly picked bolls. Seed cotton was harvested from three middle rows to determine the plot yield. Fiber characters like strength, fineness, uniformity and elongation of each plant were measured using by high volume instrument (HVI-900). Mean genotypic values of these characters were calculated. Data collected on different parameters were analyzed statistically by using CropStat 7.2 developed by International Rice Research Institute. The law of diminishing return was used to determine the optimum level of nitrogen by equating the inverse price ratio with marginal product (Sharma and Sharma, 1981).

Table 7. Average monthly weather data for cropping season 2018-2019

Location	Month	Temperature (°C)		Relative humidity (%)	Rainfall (mm)
		Maximum	Minimum		
Sreepur	July-2018	32.6	26.7	80.1	11.5
	August-2018	33.8	27.1	75.0	4.5
	September-2018	34.1	27.0	74.7	2.5
	October-2018	32.0	23.6	67.9	1.5
	November-2018	30.3	19.8	65.4	0.4

	December-2018	26.1	16.2	64.0	0.4
	January-2019	27.2	14.7	57.5	0.0
Sadarpur	July-2018	33.3	27.0	77.6	5.5
	August-2018	33.8	26.9	78.0	4.9
	September-2018	33.3	26.1	78.2	6.4
	October-2018	31.3	21.5	76.3	0.3
	November-2018	29.6	15.7	75.3	0.0
	December-2018	25.5	11.3	77.4	0.3
	January-2019	25.8	13.7	71.2	0.0
Jagadishpur	July-2018	33.3	26.6	81.7	13.9
	August-2018	33.8	26.7	79.3	3.7
	September-2018	34.6	26.0	80.2	2.5
	October-2018	33.0	21.8	80.4	2.5
	November-2018	31.0	16.8	72.6	0.0
	December-2018	26.0	12.0	79.2	0.4
	January-2019	26.9	10.3	74.6	0.0

References

1. Ahmed et al. Fertilizer Recommendation Guide. Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka 1215. 2018; 223p
2. El-Debaby A. S., G. Y. Hammam and M. A. Nagib. Effect of planting date, N and Papplication levels on seed index, lint percentage and technological characters of Giza 80 cotton cultivar. Ann. Agric. Sci. 1995; 33:455–64.
3. Gill, K.H., Sherazi, S.J.A., Iqbal, J., Ramzan, M., Shaheen, M.H. and Ali,Z.S. “Soil Fertility Investigations on Farmers Fields in Punjab”, Soil Fertility Research Institute, Department of Agriculture, Govt. of Punjab, Lahore, Pakistan. 2000. Pp . 133-135.
4. Guinn, G. Potential for improving production efficiency with growth regulants. In Proc. of the Beltwide Cotton Production Res. Conference Atlanta, GA, USA, January 8-12 J.M. Brown. 1984; 67- 71. Memphis, USA: National Cotton Council.
5. Malik, M.N.A., Chaudhry, F.I. and Makhdum, M.I. “Investigation on phosphorus availability and seed cotton yield in silt loam soils”, J. An. Plant Sci., 1996; 6(12), 21-23.
6. Mehetre S. S., A. V. Tendulkar and R. S. Darade. Effect of foliar application of diammonium phosphate and naphthalene acetic acid on seed cotton yield and fiber properties of Gossypium hirsutum L. cotton. J. Ind. Soc. Cotton Improv., 1990; 15:145–7.
7. Malik M. N. A., M. I. Makhdum and F. I. Chaudhry. Influence of phosphorus fertilization on crop growth, seed cotton yield and fibre quality. PakistanJ. Sci. Ind Res., 1992; 35:288–90.
8. Subhan, M., H. U. Khan and R. O. Ahmed. Population analysis of some agronomic and technological characteristics of upland cotton (Gossypium hirsutum L.). Pakistan J. Biol. Sci., 2001; 1:120-123.
9. Sharma C. P., P. N. Sharma and C. Chatterjee. Manganese deficiency in maize affects pollen viability. Plant Soil. 1991; 138:139–42.
10. Vieira D. J., N. E. M. Beltrao and V. G. Ribeiro. Effect of nitrogen and phosphorus fertilizers on yield of herbaceous cotton in southwestern Bahia: Iyapora, BA, 1995/96. Pesquisaem Andamento-Centro Nacional de Pesquisa do Algodao No. 1998; 77, 3.
11. Singh, P. Cotton breeding. Kalyani Publishers Ludhiana New Delhi Noida (U.P.) Hyderabad Chennai Kolkata Cuttack India. 2004; pp:295.
12. Sultana, J., Siddique, M. N. A. & Abdullah, M. R. Fertilizer recommendation for Agriculture: practice, practicalities and adaptation in Bangladesh and Netherlands. International Journal of Business, Management and Social Research, 2015. 01(01), 21- 40.
13. Taiz, L. and E. Zeiger. Plant Physiology: Mineral Nutrition. Redwood City, CA: The Benjamin Cumm. Pub. Co. 1991.
14. Taiz, L. and E. Zeiger. Plant Physiology. Panima Publishing Corporation, New Delhi, India.73p. 2003.
15. Russell, E. W. Soil Condition and Plant Growth. London: The English Language Book Society and Longman. 2001.

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Effect of Timing of Last Irrigation on Growth, Yield and Water Productivity in Cotton Under Gezira Conditions

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Abstract

Background: *A field experiment was conducted for two seasons (2015/2016 and 2016/2017) at Gezira Research Station Farm (GRSF) to determine the optimum timing of the last irrigation for the newly released Bt. cotton (Seeni1) along with non Bt. cotton (Hamid) on the basis of scheduling irrigation approach. The experiment was executed in a split plot design with the two cotton cultivars comprising the main-plots and eight timings of last irrigation as sub-plots. All treatments were replicated three times.*

Results: *Delay of final irrigation significantly increased number of sympodia per plant and plant height. Irrespective of cotton cultivar, 27 WAS recorded the highest number of bolls/plant in both seasons. Cotton yield, water productivity and fiber quality were highly affected by irrigation treatments. Delay of final irrigation up to 21 WAS resulted in higher crop and water productivities. Moreover, delaying the last irrigation after 21 weeks showed no improvement in cotton fiber quality.*

Conclusion: *These results indicate that excessive irrigation might not produce more yield or improve cotton quality.*

Keywords: *Irrigation, Sudan, productivity, cotton quality*

Background

Water availability is generally the most important natural factor limiting expansion and development of agriculture in arid and semi-arid regions. There is great need for judicious use of river water through a better understanding of the crop yield-water application interaction. This include efforts to improve crop water use efficiency by changing irrigation methods, applied amounts (deficit irrigation), crops, tillage practices, and other management methods (Aujla et al. 2005; Buttar et al. 2006; Ibragimov et al. 2007).

Cotton (Gossypium hirsutum L.) is the major fiber crop in the Sudan. Due to crop intensification in Gezira Scheme after the adoption of the new Act of 2005, water becomes the major limiting factor for crop production. With increasing concern about water shortage with regard to summer season's crops in the Gezira there is a renewed interest in increasing the water use efficiency in cotton. Sowing of cotton, in the Gezira, is recommended from the beginning of July, which coincides with the onset of rainy season. During this period irrigation is supplementary to meet the crop water requirement. According to sowing date, harvesting of cotton starts early December and continues until middle of March; irrigation water is available to farmers at two weeks intervals.

Many researchers proved that cotton yields can actually be reduced by application of excessive water (Letey and Dinar, 1986; Grimes, 1994; Grimes et al. 1969; Jackson and Tilt, 1968; Karam et al. 2006; Wanjura et al. 2002). In the Sudan, previous work on Barac (67) B showed that terminating irrigation after 8 – 14 applications did not result in a significant yield reduction, however, for quality reasons irrigation water could be terminated after 10 irrigations without affecting adversely the yield or quality (Farah et al. 1986). On the other hand, water stress during the late stages of growth reduces cotton yield which can be attributed to reduction in net CO2 assimilation and abscission of almost all young bolls (Faver et al., 1996; Grimes et al. 1970). Therefore, this study was conducted to determine the timing of last irrigation and its effects on growth, yield, fiber quality characteristics and water use efficiency of cotton grown on heavy clay soils of Gezira Scheme, Sudan.

Results and discussion

Plant height and number of sympodia/plant

Effect of timing of last irrigation on plant height (cm) and number of sympodia/plant of Bt. and non-Bt. cotton cultivars are presented in Table (1). Plant height (cm) was significantly affected by cultivar and time of last irrigation as well. Cultivar Hamid significantly recorded taller plants when the last irrigation was delayed to 27 WAS compared to other treatments, but this increase was significant only during the first season (2015/2016). Time of last irrigation had significant effect on number of sympodia, while cultivar and the interaction had no significant effect. Irrespective of cultivar, the first and second of last irrigation treatments (13 and 15 WAS) showed lower number of sympodia/plant compared to other treatments. Similar to plant height, there was an increase in number of sympodia/plant with the delay in last irrigation.

Yield components

Yield components (number of bolls/plant, boll weight and GOT%) as affected by cultivar and timing of last irrigation are presented in Table (2). No interaction effects were observed in means of irrigation treatments and cultivars. Bt. cotton had a significantly (P≤ 0.01) higher values of ginning out turn (GOT%) compared to non Bt. cotton in both seasons (Table 2). Similarly timing of last irrigation had significantly affected number of bolls/plant in both seasons. No significant responses were observed among irrigation treatments and cultivars for both GOT% and boll weight.

Crop and water productivities

The crop and water productivities associated with the different timings of last irrigation are presented in Table 3. Timing of last irrigation had significantly (p≤ 0.001) affected seed cotton yield and lint cotton yield (p≤ 0.01). Irrespective of cultivar, the first and second irrigation treatments (13 and 15 DAS) showed lower seed cotton yield (kg/ha) and lint yield (kg/ha) than other treatments, while delaying the last irrigation to 27 WAS significantly increased seed cotton yield, but this increase was significant only during the second season (2016/2017). The results were contradicted with that obtained by Farah et al. (1981). They reported that no differences were observed when the irrigation was stopped after 8-14 irrigations on Barac 67 (B).

Table 1. Effect of timing of last irrigation on growth parameters of Bt and non Bt-cotton during 2015/2016 and 2016/2017 seasons

Factor	Plant height (cm)	No of sympodia /plant	Plant height (cm)	No of sympodia /plant	Plant height (cm)	No of sympodia /plant
	2015/2016		2016/2017		Combined	
Timing of last irrigation (WAS)						
13	78	9.7	79	10.5	79	10.1
15	83	10.0	84	11.7	84	10.9
17	85	11.0	87	12.3	86	11.7
19	85	12.2	94	13.4	90	12.8
21	92	12.0	90	12.8	91	12.4
23	89	11.7	90	13.6	90	12.7
25	89	12.3	91	13.8	90	13.1
27	102	11.8	93	14.0	98	13.0
SE±	1.54	0.54	1.82	0.73	2.2	0.8
L.S.	***	**	***	***	***	**
Cultivar						
Hamid	94	11.2	91	13.2	93	12.2
Seeni1	81	11.5	86	12.4	84	12.0
SE±	1.01	0.43	2.91	0.63	2.8	3.0
L.S.	**	NS	NS	NS	NS	NS
CV%	4.3	11.7	5.0	12.2	6.5	12.5

Notes: L.S. = level of significance: **, *** = significant at p≤ 0.01 and 0.001 levels, respectively. NS= non significant

Table 2. Effect of timing of last irrigation on yield components of Bt. and non Bt-cotton during 2015/2016 and 2016/2017 seasons

Factor	No. of bolls/plantnt	Boll weight (g)	GOT (%)	No. of bolls/plant	Boll weight (g)	GOT (%)	No. of bolls/plant	Boll weight (g)	GOT (%)
	2015/2016			2016/17			Combined		
Timing of last irrigation (WAS)									
13	8.1	4.8	38	9	4.6	36	8.6	4.6	37
15	8.4	4.2	38	10	4.6	37	7.2	4.4	38
17	11.3	4.3	37	10	4.8	38	10.7	4.6	38
19	10.1	4.6	36	11	4.9	38	10.6	4.7	37
21	11.0	4.3	38	10	4.6	38	10.5	4.5	38
23	11.2	4.6	38	11	4.7	37	11.1	4.7	38
25	10.5	4.5	38	10	4.7	37	10.3	4.6	38
27	14.4	4.7	37	11	4.6	38	12.7	4.7	38
SE±	0.39	0.21	0.85	0.53	0.42	0.64	0.65	0.5	0.9
L.S.	***	NS	NS	*	NS	NS	**	NS	NS
Cultivar									
Hamid	10.0		4.6	34	10	4.6	10.0	4.6	34
Seeni1	11.3		4.3	41	10	4.7	10.7	4.5	41
SE±	0.29		0.07	0.38	0.89	0.19	0.90	0.2	0.4
L.S.	NS		NS	**	NS	NS	NS	NS	**
CV%	8.9		11.5	5.6	12.9	10.9	11.5	11.8	6.5

Notes: L.S. = level of significance: *,**, *** = significant at p≤ 0.05, 0.01 and 0.001 levels, respectively. NS= non significant

It is clear from Table 3 that WP was significantly affected by irrigation treatment (p≤ 0.001) and no interactions effects were observed. Irrespective of timing of last irrigation, the two cultivars obtained similar values of WP (0.59 kg/m3 and 0.58 kg/m3 for Hamid and Seeni1, respectively) during both seasons. This could be due to the similar amount of water that was consumed by each cultivar (4305 and 4342 m3/ha, respectively). It is clear from Table (3), that timing of last irrigation offers beneficial effects to water productivity. During the first season, the three irrigation treatments (15, 17 and 19 WAS) significantly scored higher WP (0.63, 0.65 and 0.66 kg m-3, respectively) compared to the last three irrigation treatments (23, 25 and 27 WAS) which gave 0.56, 0.52 and 0.52 kg m-3, respectively. The increased WP of 15 WAS treatment resulted from decreasing water input rather than increasing yield. However, for 17 and 19 WAS irrigation treatments, higher values of WP could be attributed to both decreasing irrigation water input and increasing seed cotton yield as shown in Table 4. On the other hand, no differences were observed among the treatments 13, 15, 19 and 21 WAS which gave 0.59, 0.63, 0.65 and 0.58 kg m-3, respectively. The lowest WP values were obtained when the last irrigation was delayed beyond 23 WAS.

Table 3. Effect of timing of last irrigation on yield and irrigation water productivity (WPi+r) of Bt. and non Bt-cotton during 2015/16 and 2016/17 seasons

Factor	Seed yield (kg/ha)	Lint yield (kg/ha)	WPi+r (kg/m3)	Seed yield (kg/ha)	Lint yield (kg/ha)	WPi+r (kg/m3)
	2015/2016			2016/2017		
Timing of last irrigation (WAS)						
13	1669	634	0.59	1748	629	0.57
15	1776	675	0.63	1793	663	0.56
17	2021	748	0.66	1857	706	0.55
19	2031	731	0.65	2453	932	0.71
21	2155	819	0.58	2267	861	0.64
23	2252	855	0.56	2308	854	0.59
25	2212	840	0.52	2320	858	0.57
27	2405	890	0.52	2071	787	0.49
SE±	77.2	30.0	0.021	127.2	54.0	0.035
L.S.	***	***	***	**	**	**
Cultivar						
Hamid	2105	716	0.60	2087	710	0.59
Seeni1	2026	831	0.58	2117	868	0.58
SE±	59.6	25.0	0.011	51.7	21.0	0.019
L.S.	NS	**	NS	NS	**	NS

CV%	9.2	10.4	9.4	14.8	12.5	9.4

Notes: L.S. = level of significance: **, *** = significant at p≤ 0.01 and 0.001 levels, respectively. NS= non significant

During the second season, treatment 19 WAS obtained significantly higher WP (0.71 kg m-3) compared to other irrigation treatments. However, no differences were observed between 19 and 21 WAS which gave 0.64 kg m-3. The higher WP achieved by 19 and 21 WAS was due to higher yield (2031 and 2155 kg ha-1, respectively). Although the last three irrigation treatments (23, 25 and 27 WAS) produced similar yield compared to 19 WAS, the former treatments significantly obtained lower WP which could be due to higher irrigation water consumed by these treatments.

Cotton quality parameters

The Main quality parameters such as length, strength and micronaire (Mic.) for Bt (Seeni1) and non Bt. Cotton (Hamid) are presented in Table (5). The quality parameters (length and strength) showed almost, the same result for both cotton varieties, because it is genetically controlled. Micronaire values were not clearly affected for the non Bt. variety (Hamid). On the other hand, low micronaire values compared to standard, were indicated for the Bt. variety (seeni1). Therefore, irrigation of Bt cotton after 21 weeks had no effect on quality improvement as indicated by the low micronaire value (second pick) as compared to the variety standard.

Table 4. The combined analysis of timing of last irrigation on yield and irrigation water productivity (WPi+r) of BT and non Bt-cotton

Factor	Seed yield (kg/ha)	Lint yield (kg/ha)	WPi+r (kg/m3)
Timing of last irrigation (WAS)			
13	1709	632	0.60
15	1785	669	0.62
17	1939	727	0.62
19	2242	832	0.58
21	2211	840	0.53
23	2280	855	0.50
25	2266	849	0.45
27	2238	839	0.45
SE±	135.0	57.0	0.02
L.S.	***	***	***
Cultivar			
Hamid	2096	713	0.56
Seeni1	2072	850	0.53
SE±	62.6	31.5	0.01
L.S.	NS	**	**
CV%	10.2	12.7	9.2

Notes: L.S. = level of significance: **, = significant at p≤ 0.01 levels,. NS=non significant

Table 5. Fiber characteristics of Bt. and non-Bt. cotton varieties (First and Second pick) as affected by timing of last irrigation at Gezira Research Station Farm during 2015/2016

Irrigation	Pick 1			Pick 2		
	Length(mm)	Mic.	Strength g/tex	Length(mm)	Mic.	Strength g/tex
			Hamid			
13-15	29	5.0	31	27	5.0	28
17-19	30	5.1	32	28	5.0	28
21-23	31	5.0	29	29	5.1	29
25-27	31	5.1	31	29	5.0	29
			Seeni-1			
13-15	30	4.9	30	29	4.5	31
17-19	30	4.9	29	29	4.6	30
21-23	30	5.3	29	28	4.0	27
25-27	30	5.2	30	28	4.4	27

Conclusion

The results revealed that the cotton yield was significantly affected by the time of the final irrigation (Fig. 1). The yield from the treatments was significantly different at P≤ 0.01 level. There were significant yield reductions in the first (13WAS) and second (15WAS) irrigation treatments; the third irrigation treatment (17WAS) was slightly higher than 13 and 15WAS by 12 and 8%, respectively. The other five irrigation

treatments (19, 21, 23, 25 and 27WAS) gave similar seed cotton yield that ranged between 2211 and 2280 kg/ha.

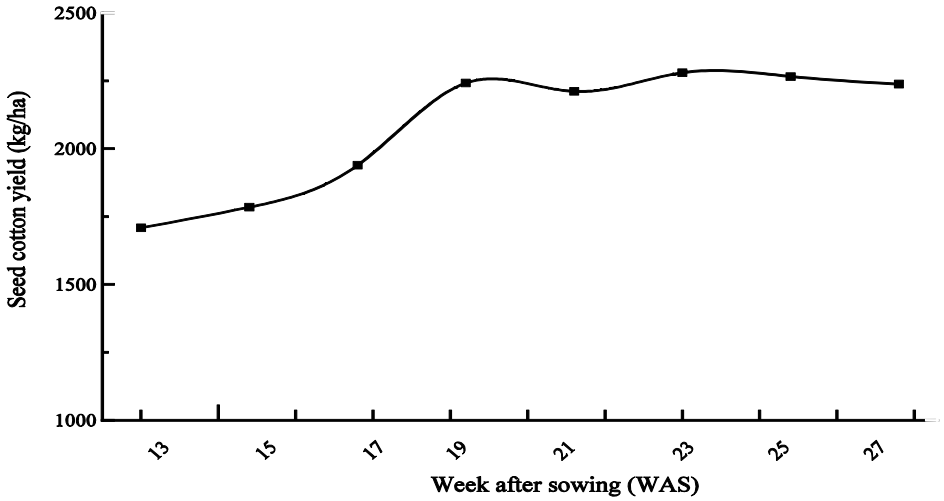

Fig. 1. *Evolution of seed cotton yield during the two growing seasons (combined)*

The plotting of seed cotton yield against plant height, number of bolls per plant and number of symbodia per plant (Fig. 2) showed strong positive and linear correlation between cotton yield and plant height (R2= 0.72) and between cotton yield and number of symbodia per plant (R2= 0.95) which in turn depends on optimum plant height.

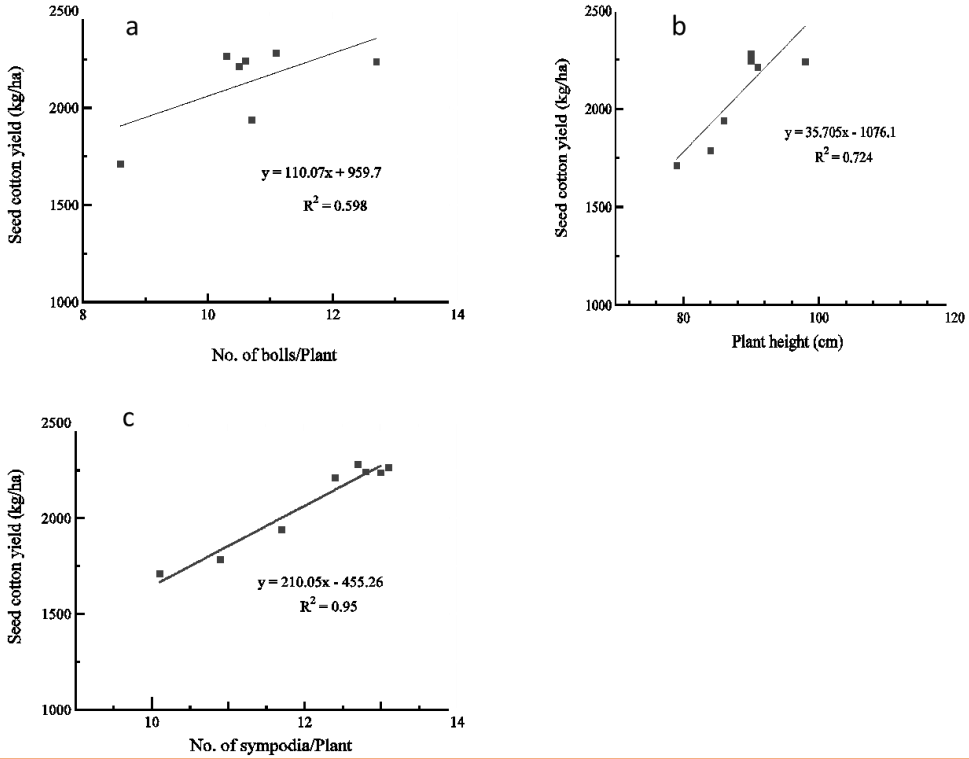

Fig. (2) *Relationship of seed cotton yield with (a) number of bolls per plant, (b) plant height and (c) number of symbodia per plant*

Based on the results achieved during the two consecutive seasons (2015/2016 and 2016/2017) under GRSF, the maximum seed cotton yield associated with higher water productivity can be achieved when the irrigation is terminated at 21 weeks, as no improvements were observed in quality beyond this period (21WAS).

Methods

This study was conducted for two seasons (2015/2016 – 2016/2017) in the Gezira Research Station Farm (GRSF) at Wad Medani. The main objective of this research was to evaluate the effect of different timings of last irrigation on growth attributes, yield, yield components, fiber quality and water productivity of Bt. and non-Bt. cotton cultivars under Gezira conditions. Treatments were combination of two cotton cultivars (Seeni1 and Hamid) and eight timings of last irrigation. The first irrigation treatment was started 13 weeks after sowing (WAS), then every two weeks. Split plot design was used with three replications. Cultivars were assigned to the main plot while irrigation treatments as sub plot. The sub plot size was 57.6m² (4.8 x 12m).

Sowing was carried-out on the third week of July. Sowing was done by hand in ridges spaced 80cm apart and intra-row spacing of 30cm between holes within the ridges. Seedlings were thinned to 2plants/hole four weeks after sowing. Nitrogen fertilizer in the form of urea at the rate of 186 kg/ha was applied 6 weeks after planting. Herbicides were applied before sowing then the experimental plots were hand weeded four times during the growing season.

Plant height (cm) was measured on five plants randomly selected in each plot at harvesting. Number of bolls and number of sympodial branches were recorded from five plants sample. Boll attributes such as boll weight (g) and ginning out turn (%) were determined from ten bolls randomly taken from each plot prior to harvest. Seed cotton yields were taken from the net area of four central ridges at distance of 12.0m (38.4 m²) in each plot. The harvested cotton was weighed to obtain seed cotton and lint yield (kg/ha) in each plot. Main fiber quality parameters, such as length, strength and micronaire (Mic.) were carried out by the cotton fiber testing laboratory according to standards.

Total applied water (TAW) is the sum of irrigation water applied plus rainfall received during the growing season. Irrigation water applied was measured using water flow meter. For computation of water productivity (WP), seed cotton yields per hectare in different treatments were divided by the respective total applied water and expressed as kg m-3.

Data collected were subjected to statistical analysis using split plot analysis of variance (Mstatc). Levels of significance for 0.05, 0.01 and 0.001 probabilities for the main factors (cultivar and irrigation treatments) and their interactions responses were calculated. Significantly different means were separated by the Duncan's Multiple Range Test (DMRT) for significance at the 0.05 level of probability.

References

1. Farah, S.M., Gandoul, G.I., Hassan A.T. 1986. Effects of the date of final irrigation on the yield of cotton. Annual report, Gezira Research Station, pp. 7-8.
2. Farah, S.M., Asim, A.A. 1981. Effect of water stoppage on yield and quality of cotton variety Barac 67 (B). Annual report, Gezira Research Station, pp. 7-9.
3. Faver, K.L., Gerick, T.J., Thaxton, P.M., El-Zik, K.M. 1996. Later season water stress in cotton. II. Leaf gas exchange and assimilation capacity. Crop Sci. 36, 922-928.
4. Grimes, D.W. 1994. Efficient irrigation of pima cotton. In: Proceedings of 1994 Beltwide Cotton Conferences, vol. 1, National Cotton Council of America, Memphis, TN, pp. 90-93.
5. Grimes, D.W., Miller, R.J., Dickens, L. 1970. Water stress during flowering of cotton. California Agric. 24, 4-6.
6. Grimes, D.W., Yamada, H., Dickens, W.L. 1969. Function for cotton (Gossypium hirsutum L.) production from irrigation and nitrogen fertilization variables. I. Yield and evapotranspiration. Agron. J. 61, 769-773.
7. Jacksoon, E.B., Tilt, P.A. 1968. Effects of irrigation intensity and nitrogen level on the performance of eight varieties of upland cotton, Gossypium hirsutum L. Agron. J. 60, 113-17.
8. Karam, F., Rafic, L., Randa, M., Daccache, A., Mounzer, O., Rouphael, Y. 2006. Water use and lint yield response of drip irrigated cotton to length of season. Agric. Water Manage. 85 (3), 287-295.
9. Letey, J., Dinar, A. 1986. Simulated crop-water production functions for several crops when irrigated with saline waters. Hilgardia 54(1).
10. Wanjura, D.F., Upchurch, D.R., Mahan, J.R., Burke, J.J. 2002. Cotton yield and applied water relationships under drip irrigation. Agric. Water Manage. 55(3), 217-237.

Effects of Direct Sowing Under Mulch-Based Cropping System (DMC) on Ferruginous Soil (Lixisoil) Chemical Characteristics in the South Sudan Area of Burkina Faso

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Abstract

Background: *To better understanding of the effects of direct sowing under mulch-based cropping system (DMC) in Burkina Faso's cotton production systems, a randomized blocks of Fisher experimental design were implemented at Farako-Bâ research station from 2010 to 2019. The study was conducted on lixisoil to evaluate the DMC effects on biomass production, crops yields and soil chemical properties in a maize and cotton rotation system associated with cover plant. Conventional tillageand direct seeding without cover plantwere compared to DMC under B. ruziziensis (GERM. & EVRARD), DMC under B. ruziziensis + M. cochinchinensismulch and DMC under C. juncea (L.) mulch used in association with maize.*

Results: *The biomass production, crops yields and soil chemical characteristics were evaluated. The results showed that over 10 years production, compare to Conventional Tillage and Direct Seeding, Direct sowing under mulch-based cropping system permit an equivalent yield of maize and cotton. The use of cover plant allows to increase the biomass production compared to Conventional Tillage and Direct Seeding. Maize yield has not varied significantly with the cover plants use. After 10 years of maize and cotton rotation, the improvement raised from +27 to +38% for organic matter and from +15 to +29% for nitrogen with DMC including legumes such as M.cochinchinensis and C.juncea compared to Conventional Tillage on0-5 cm depth. No significant differences were found on soil acidity like P2O5 and K2Ocontent. Although DMC with C. juncea used as cover crop did not provide the best biomass production, but it contributes to increase nitrogen and organic matter of the soil and has better soil mineral balances in 10 years of rotation. The 5-10 cm and 10-20 cm were little influenced by DMC systems.*

Key Words:
Cover plants, tillage, direct sowing under mulch-based cropping system, crop rotations, mineral content, legumes

Background

Conservation agriculture (CA) has long been practiced around the world (AFD, 2006). In Burkina Faso, as well as in some sub-Saharan African countries, it is difficult to be adopted in terms of inadequate crop systems (Coulibaly et al., 2018). Many authors presented conservation agriculture as an alternative to sustainable production systems in terms of its potential to improve the physical, chemical and biological characteristics of soil (Yadav et al., 2017, Corsiet al., 2019).It's characterized by three principles: minimum tillage, permanent soil cover and crop diversification by cultural associations and rotations (Corsiet al., 2019).In the cotton growing systems, conservation agriculture takes on another dimension because the quality of fiber can't be associated with any biomass production that could influence his quality.

In West African cotton systems, the state of soil fertility is a major constraint on ensuring production sustainability (Ouattara et al., 2018). Extension of crop areas can't be adopted because of overcrowding in cities and countryside. The acidity, the organic matter and mineral content in soils are below the average recommended levels according to soil chemical interpretation standards (Bado, 2002,

Coulibaly et al., 2012). Crop systems must focus on maintaining or improving the soil organic matter and mineral content and their availability for crops. Factors that contribute to the degradation of soil fertility include the lack of organic restitution, cropping practices such as annual ploughing of fields which affect the soil structure while exposing it to the phenomenon of degradation (Bado, 2002; Doamba et al., 2011). However, its application in cotton-based production systems requires an adaptation of the principles in order to ensure cereals and cotton production that meets market standards. Crop and weed crop residues are insufficient for good soil cover in sub-Saharan Africa. It is important to use cover plants in combination with cereals in a context of cropland scarcity to improve the amount of biomass on plots. In the direct sowing under mulch-based cropping system (DMC), cover plants are used based on their highly variable characteristics from one species to another (Diakhaté et al., 2018). Therefore, it is necessary to identify the contributions of cover plant species, including their potential for producing aerial and underground biomass that may contribute to increased soil content of organic matter and other minerals. In DMCthe chemical characteristics of the soil, namely organic matter levels, soil levels of nitrogen, phosphorus, and potassium, are thought to be partly the result of cover plants and the roots biomass decomposition (Gamour et al.,2014). The residues degradation in the soilis a function of climate, soil characteristics and residues quality. The degradation rate of mulch depends on the organic matter quality. The nature of organic matter varied between graminaceae (B. ruziziensis) and dicotyledon (M. cochinchinensis and C. juncea) (Gama-Rodrigues, 2007). In the DMC, this degradation constitutes an important source to improve nitrogen, phosphorus and potassium rate of soil. In addition to their biomass production potential, some cover plants are atmospheric nitrogen-fixing. Many cover plants as Brachiaria sp, Panicum sp, dicotyledon, namely Crotalaria sp, Cajanus sp and Mucuna spare using by the farmers (Husso et al., 2008, Botton et al., 1958). The objective of this study is to determine the effects of DMC using three cover plants, on the crops yields and the ferruginous soil chemical characteristics variations.

Results

Variations of the yieldsof maize associated to cover crops

Cover plants associated with maize did not decrease the maize yields compared to Conventional Tillage (Table 1).The DMC allows an equivalent yield of maize compared to Conventional Tillage and Direct Seeding. Over five years of maize production in rotation with cotton, the average yields ranged from 2791 kg ha-1(DMC/ B. ruziziensis + M. cochinchinensis) to 3221 kg ha-1(Conventional Tillage). Maize yields increased regardless of treatments (Table 1).

Variations of cotton yields in systems of production

Statistical analyses indicate that cotton yields from 2011 to 2019 were not influenced by the treatments applied (Table 2). Compared to Conventional Tillage, DMC did not give significant improvements in cotton yields. Over the five years of cotton production in rotation, average yields ranged from 1191 kg ha-1(DMC/B. ruziziensis) to 1390 kg ha-1(Conventional Tillage). Overall, trends indicate stability in cotton yields with Conventional Tillage compared to the Direct Seeding and DMC that decline slightly.

Table 1. Evolution **of the maize yields** from 2010 to 2018 under different treatments

Treatments	2010	2012	2014	2016	2018	Average
			kgha-1			
ConventionalTillage(CT)	2368±448	2408±444	3921±180	3339±133	4065±105	3221±185
Direct Seeding	1938±305	2026±449	3875±165	3004±790	3649±285	2897±300
DMC/B. ruziziensis	2120±205	2213±382	3775±286	3289±433	3336±326	2945±265
DMC /B. ruziziensis + M. coch	2071±346	2302±396	3800±202	2604±381	3172±255	2791±168
DMC /C. juncea	2197±417	2325±509	4107±56	3298±530	3269±371	3039±286
F	0,263	0,110	0,467	0,384	1,643	0,431
Probability (0.05)	0,897 (ns)	0,977 (ns)	0,759 (ns)	0,817 (ns)	0,215 (ns)	0,784 (ns)

Notes: ns= not significant; DMC=Direct sowing under mulch-based cropping system

Table 2. Evolution of cotton yields from 2011 to 2019 under different treatments

Treatments	2011	2013	2015	2017	2019	Average
			kgha-1			
Conventional Tillage	1361±152	1415±187	1437±211	1165±113	1516±75	1390±76
Direct Seeding	1673±101	1246±203	986±87	975±26	1207±284	1218±115
DMC /B. ruziziensis	1302±86	1204±125	1281±184	985±22	1184±113	1191±86
DMC /B. ruziziensis + M. coch.	1322±189	1439±29	1131±152	1132±58	1205±124	1250±89
DMC /C. juncea	1514±135	1293±42	1007±82	1159±55	1135±104	1230±65
F	1,300	0,566	1,558	2,107	0,910	0,715
Probability (0.05)	0,314 (ns)	0,691 (ns)	0,236 (ns)	0,131 (ns)	0,483 (ns)	0,594 (ns)

Notes: ns= not significant; DMC=Direct sowing under mulch-based cropping system

Effects of direct sowing under mulch-based cropping system biomass production

The average biomass production of maize and total (maize + covers plants) was affected by the compared treatments (Table 3). The maize biomass production varied between 4210 kg ha-1(DMC/ B. ruziziensis +M. cochinchinensis) and 5497 kg ha-1(Conventional Tillage) in average (Table 3). The best maize biomass production was obtained with Conventional Tillage. The DMC allows to increase the total biomass compared to Conventional Tillage. The average of total biomass varied between 4746 kg ha-1(Direct seeding) and 7413 kg ha-1(DMC/ B. ruziziensis +M. cochinchinensis). The use of cover plants increased total biomass production. In average, the association of B. ruziziensis, B. ruziziensis + M. cochinchinensis and C. juncea contribute to significantly increase the total biomass. A better total biomass production was observed with the association of B. ruziziensis + M. cochinchinensis cropping with maize (Table 3).

Effects of direct sowing under mulch-based cropping system on soil pH

After 10 years of maize associated to cover plants in rotation with cotton, a little variation in the pH water was observed. The pH was between 5.54 (DMC/C. juncea mulch) and 5.69 (Direct seeding). With the Conventional Tillage the pH was 5.60 and 5.62 with DMC/B. ruziziensis mulch and 5.63 with DMC /B. ruziziensis + M. cochinchinensis mulch. Compared to the Conventional Tillage, Direct Seeding and direct sowing under mulch-based cropping system did not cause a significant change of soil pH.

Table 3. Biomass production by maize straws and cover crops (kg ha-1) from 2010 to 2018 under different treatments

Treatments	2010		2012		2014		2016		2018		Average	
	Maize straw	Cover crops	Maize straw	Cover crops	Maize straw	Cover crops	Maize straw	Cover crops	Maize straw	Cover crops	Maize straw	Cover crops
Conventional Tillage	5444 a	-	5230 a	-	6510 a	-	5136 a	-	4897 a	-	5497 a	-
Direct seeding	4786 a	-	4948 a	-	5729 a	-	4157 ab	-	4311 a	-	4746 ab	-
DMC/ B. ruziziensis	4684 a	2450 a	5148 a	1788 a	5313 a	3917 a	3755 b	2481 a	4522 a	2695 ab	4568 b	2666 a
DMC/ B. ruz.+M. coch.	4257 a	2751 a	4443 a	1196 a	5104 a	2733 b	3022 b	3521 a	4458 a	3808 a	4210 b	2802 a
DMC/C. juncea	4467 a	1616 b	4473 a	1497 a	5260 a	1433 c	3788 b	2009 a	4347 a	1712 b	4466 b	1654 b
F	2.023	8.577	0.329	1.369	2.688	33.889	3.801	2.322	0.433	9.112	3.425	14.435
Probability (0.05)	0.150	0.010	0.854	0.308	0.078	0.000	0.029	0.160	0.782	0.009	0.040	0.002

Notes: S=significant, ns=not significant; DMC=Direct sowing under mulch-based cropping system. Values followed by the same letter in each column do not differ statistically according to Fisher test at 5% level of probability

Effects of direct sowing under mulch-based cropping system on soil organic matter content

Statistical analysis shows no significant difference ($p > 0.05\%$) between treatments regardless of the different soil layers (Figure 1). Soil organic matter increase with Direct Seeding and Direct sowing under mulch-based cropping system (DMC) compared to Conventional Tillage. In 10 years on 0-5 cm depth, DMC/B. ruziziensis, DMC/ B. ruziziensis + M. cochinchinensis and DMC/ C. juncea gave respectively 27%, 34% and 38% improvement of organic matter compared to Conventional Tillage. Direct Seeding without cover plants gave 33% increase of organic matter compared to Conventional Tillage.On 5-10 cm depth, compared to Conventional Tillage, the soil organic matter content improvement was 5%, 14% and 17% respectively with Direct Seeding, DMC / B. ruziziensis + M. cochinchinensis and DMC/C. juncea. On 10-20 cm depth, only DMC showed increases of soil organic matter content. These increases were in the order of 23% (DMC/B. ruziziensis) and 9% (DMC/B. ruziziensis+M. cochinchinensis and DMC/C. juncea) compared to Conventional Tillage. High soil organic matter levels were observed with the use of C.juncea as a cover plant on the 0-5 cm and 5-10 cm depth and with

DMC/ B.ruziziensis on the 10-20 cm depth. The organic matter content of the soil follows a gradient from the upper (0-5 cm) to the deep depth (5-10 cm and 10-20 cm).

Effects of direct sowing under mulch-based cropping system on nitrogen (N total) content of soil

The analyses show N total levels is equivalent on the different depth and follow a gradient from the upper to the deep depth (Figure 4). Compared to Conventional Tillage, Direct Seeding and DMC have improved total nitrogen levels in soil. These increases are21% and 3% respectively on the 0-5 cm and 5-10 cm depth with Direct Seeding compared to Conventional Tillage. With DMC systems increases over Conventional Tillage were in the range of 15 to 29% on the 0-5 cm depth, 0 to 9% on the 5-10 cm depth and 4 to 19% on the 10-20 cm depth. In the DMC systems, the best N levels were observed with treatments containing legumes as M. cochinchinensis and C. juncea.

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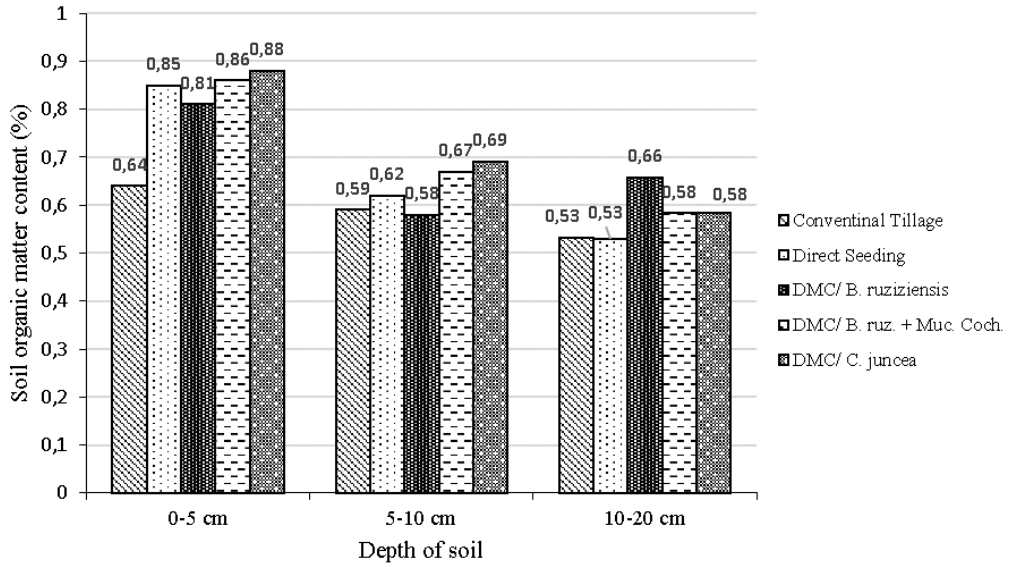


Figure 1. *Effects of treatment son soil organic matter content (%) in 2019*

Effects of direct sowing under mulch-based cropping system on P total, P assimilable, K total and K available

The analyses of variance revealed no significant differences between DMC, Direct Seeding and Conventional Tillage for P total and P assimilable levels regardless of soil depth (Table 4). On the 0-5 cm, 5-10 cm and 10-20 cm depth, Conventional Tillage gave higher P total than those of Direct Seeding and DMC. On the other hand, we notice on 0-5 cm depth, high levels of P assimilable with DMC/B. ruziziensis+M. Cochinchinensis and DMC/C. juncea. On the 0-5 cm, 5-10 cm and 10-20 cm depth considered, K total and K available levels were statistically equivalent. On 0-5 cm, K availability was improved with Direct Seeding and DMC system compared to Conventional Tillage (Table 5).

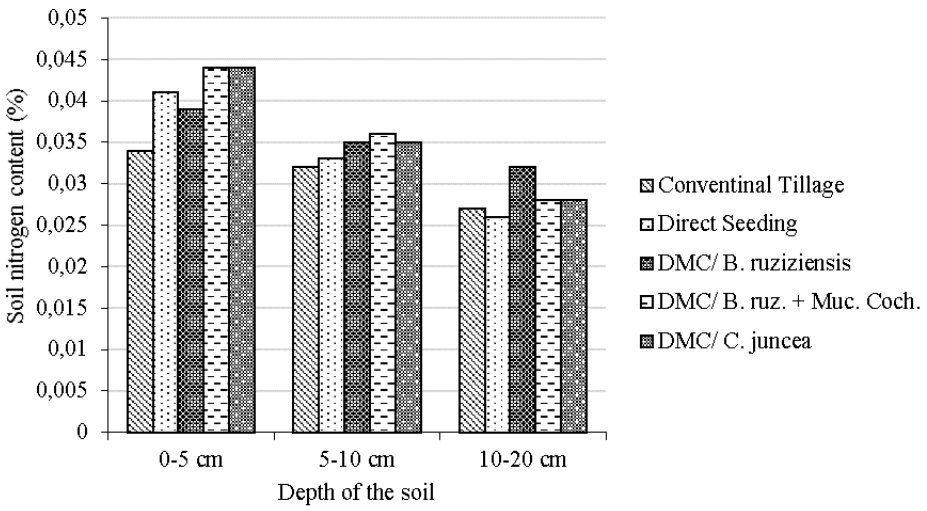


Figure 2. *Effects of treatments on soil nitrogen content (%) in 2019*

Table 4. Effects of treatments on P total and P assimilable in soil (2019)

Treatments	0-5 cm		5-10 cm		10-20 cm	
	Ptotal	Pass.	Ptotal	Pass.	Ptotal	Pass.
	mgkg-1of soil					
Conventional Tillage	150.94±34	12.17±4	154.61±36	11.91±2	144.61± 34	7.27± 1
Direct Seeding	101.15±11	12.01±2	98.45±4	10.49±3	120.86 ± 30	7.10 ± 1
DMC /B. ruziziensis	124.17±29	10.88±1	120.86±27	5.13±1	119.11± 26	5.43 ± 1
DMC /B. ruziziensis + M. coch.	104.68±10	14.43±4	92.88±5	9.81±2	100.18± 9	6.21 ± 1
DMC /C. juncea	135.44±28	14.66±2	127.46±34	9.14±2	117.46± 30	5.57± 1
F	0.736	0.315	0.959	1.678	0.34	0.51
Probability (0.05)	0.582 (ns)	0.863 (ns)	0.458 (ns)	0.207 (ns)	0.847 (ns)	0.730 (ns)

Notes: ns= not significant; DMC=Direct sowing under mulch-based cropping system

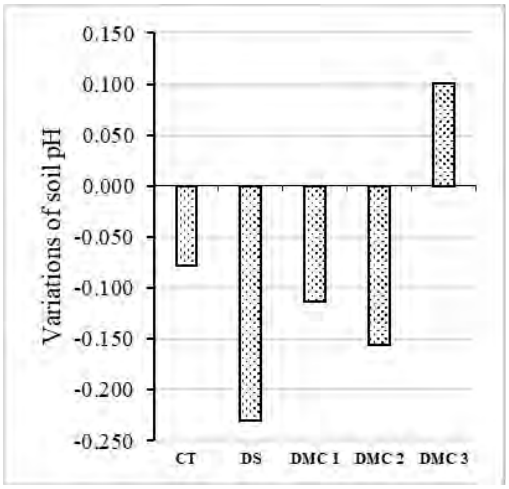
Tableau 5. Effects of treatments on K total and K available level in soil (2019)

Treatments	0-5 cm		5-10 cm		10-20 cm	
	Ktotal	Kavailable	Ktotal	Kavailable	Ktotal	Kavailable
	mgkg-1of soil					
Conventional Tillage	1988±273	89±12	1733±160	99±4	2002 ± 194	75± 2
Direct Seeding	1867±139	131±21	1878±200	103±3	2050 ± 191	77 ± 9
DMC /B. ruziziensis	1685±99	146±9	2090±328	82±1	1990 ± 113	70 ± 2
DMC /B. ruziziensis + M. coch.	1966±332	128±14	1976±284	1001±2	1733 ± 134	68 ± 6
DMC /C. juncea	1709±64	141±20	1736±91	82±3	2076 ± 162	69 ± 3
F	0.458	1.982	0.455	0.31	0.718	0.637
Probability (0.05)	0.766 (ns)	0.149 (ns)	0.767 (ns)	0.867 (ns)	0.593 (ns)	0.644 (ns)

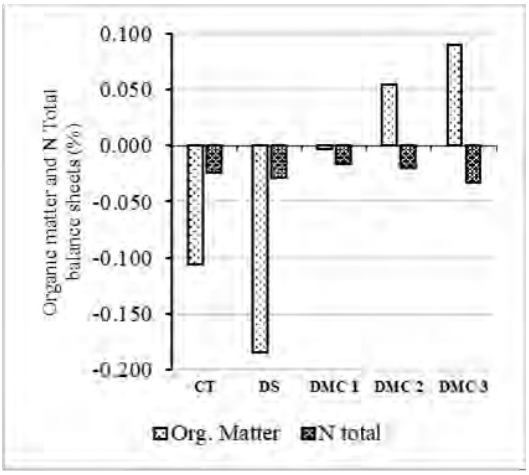
Notes: ns= not significant; DMC=Direct sowing under mulch-based cropping system

Soil chemicals characteristics reviews over 10 years of operation (0-5 cm)

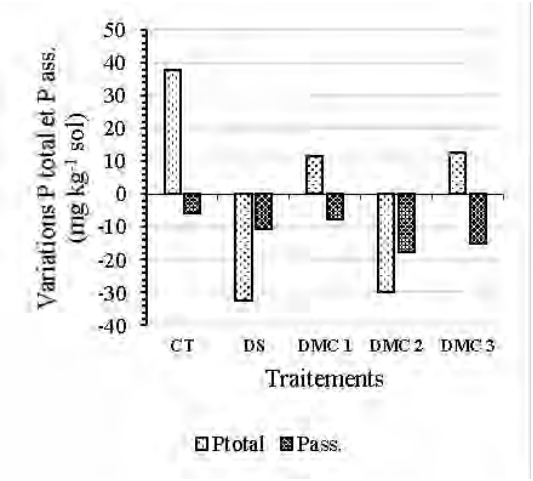
Over 10 years of crop rotations (maize-cotton) the pH of soil has changed slightly, depending on the treatments (Figure 3a). Soil pH balances variation between 2010 and 2019 indicate a decrease of soil pH on the upper depth (0-5 cm) with Conventional Tillage as well as Direct Seeding alone and DMC/B. ruziziensis and DMC/B.ruziziensis+M.cochinchinensis. The balance of pH was from -0.055 to - 02 units. On the other hand, with the use of C. juncea as a cover plant in the DMC system, the soil acidity balance shows an increase of one unit towards neutrality.



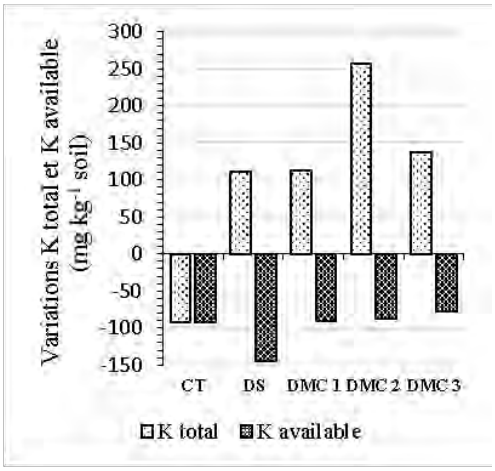
3a. Soil pH balance (0-5 cm)



3b. Organic matter and N Total balance (0-5 cm)



3c. P Total and P ass balance sheets. (0-5 cm)



3d. K Total and K available balance (0-5 cm)

Notes: CT=Conventional Tillage; DS=Direct Seeding; DMC 1=DMC/B. ruziziensis; DMC 2=DMC/B. ruziziensis + M. cochinchinensis; DMC 3=DMC/C. juncea

Figure 3. *Soil chemicals characteristics from 2010 to 2019*

As for soil organic matter contents, only DMC/B. ruziziensis associated with M.cochinchinensis and DMC/C.juncea provided stability in levels or a little increase of +0.054% (DMC/B. ruziziensis + M. cochinchinensis) and +0.90% (DMC/C. juncea) (Figure 5b).

The total nitrogen (N total) in the soil decreased with Conventional Tillage, Direct Seeding and DMC/B.ruziziensis, DMC/B.ruziziensis+M.cochinchinensis and DMC/ C.juncea. The use of legume cover plants (M.cochinchinensis and C.juncea) did not gave positive balance of nitrogen content. P total balance sheets show improvements of 11.53; 12.54 and 37.91 mgkg-1 of soil respectively with DMC/B.ruziziensis, DMC/C.juncea and Conventional Tillage. However, these improvements of P total were not inducing any improvement in the availability of this element in the soil (Figure 5c).

K total balances in soil indicate improvements with Direct Seeding, DMC/B.ruziziensis, DMC/B.ruziziensis+M.cochinchinensis and DMC/C.juncea compared to Conventional Tillage. These positive K total balances in soil were not consistent with an improvement in soil availability (Figure 5d).

Discussions

During the 10 years of crop rotation, the efficiency on cotton and maize yields of direct seeding with or without a cover plant was statistically equivalent to conventional tillage. The use of B.ruziziensis, B.ruziziensis+M.cochinchinensis and C.juncea as cover plants associated with maize did not affect the maize yields. After 10 years cultivation, including 5 years of cotton production, the cotton yields indicate that Direct Seeding, direct sowing under mulch-based cropping system were equivalent to Conventional

Tillage. These results confirm Souza et al. (2019), Diakhaté et al. (2018), Koulibaly et al.(2017) and Chervet et al. (2016) studies which found an equivalence between Direct Seeding and Conventional Tillage after 5 years or 20 years of direct sowing under mulch-based cropping system. Biomass production has been significantly improved by cover plants. This has ensured fairly good soil cover in direct sowing under mulch-based cropping system. Nunes et al. (2006) had estimated that in DMC, biomass production of at least 6000 kg/ha is required to ensure good soil cover and according to Ouattara et al. (2018), 2 t/ha of biomass was required to improve soil water balances compared to Conventional Tillage. B.ruziziensis has ensured a better production of biomass and better when associated with M.Cochinchinensis. The contribution of biomass production of C.juncea was lower than B. ruziziensis only and B. ruziziensis + M. cochinchinensis.

With the DMC, soil acidity varied very little compared to Direct Seedling and Conventional Tillage. The balance sheets indicate general decline of pH with the Conventional Tillage, Direct Seeding and DMC/B.ruziziensis, DMC/B.ruziziensis+M.cochinchinensis. With C.juncea as a cover plant in DMC, the increase of pH to neutral pH has been observed.

In 2019, the levels soil organic matter was relatively higher withDMC tested compared to Direct Seeding and Conventional Tillage on 0-5 cm and 10-20 cm depth. The increasing of organic matter with DMC is attributed to the degradation of cover plant and his root residues. These results confirm studies of Razafimbelo et al.(2006)and Yadav et al.(2017)that obtained same trends in tropical climate conditions. The accumulation of organic matter in the 0-5 cm depth compared to the 5-10 cm and 10-20 cm depth is due to the more pronounced accumulation and degradation in the tropical area of residues and a higher root density in this depth. Conventional Tillage contributes to create conditions for a rapid decomposition of soil organic matter. The best organic matter content of the soil was recorded with the use of C.juncea as a cover plant. This related to C. juncea characteristics whose decay of residues by microorganisms would be slower compared to B. ruziziensis and M. cochinchinensis. The contribution of legume cover plants was only found in the first 10 centimeters of soil depth. The legumes used as cover plants, namely M. cochinchinensis and C. juncea, has led to increases soil nitrogen content, particularly on the 0-5 cm and 5-10 cm depth. This is related to the atmospheric nitrogen-fixing characteristics of the legumes cover plants used. These results are similar to those of Rakotoarisoa et al. (2010) which featured legume cover plants with a strong root system that could improve nitrogen levels on different depth of soil. B.ruziziensis, which has a powerful root system fasciculate in association with M.cochinchinensis, has contributed to a better colonization of depth through its important and fasciculate root system that can reach in deep depth. The high levels of P total and P assimilable with Conventional Tillage are partly related to the faster degradation of organic matter. In the tropical climate area. Conventional Tillage creates the conditions for substrate mineralization by soil microorganisms. The results of Bottinelli (2010) indicates a significant reduction in organic matter levels in soil under continuous ploughing related to the mineralization process.

Conclusion

Undercover plant-covered seeding systems are also efficient because they provide the production stability. Frequent ploughing is not the only alternative, especially in the conditions of climate change that hinder the proper establishment of crops. The acidity of the soil in the conventional tillage as with DMC has experienced little variation. DMC systems have improved K total in soil levels while reducing K available losses. B.ruziziensis alone or associated with M. cochinchinensis and C. juncea contribute effectively to the production of biomass. These cover plants have stabilized or improved the status of organic matter in the soil. The contribution of legumes (M. cochinchinensis and C. juncea) was low perceived on the nitrogen content of soil in the upper layers. DMC presents itself as applicable practices in cotton-based production systems in the south-Sudan area of Burkina Faso because requires a slight adaptation of production method. B. ruziziensis alone or associated with M. cochinchinensis ensure a good biomass production.

Methods

The experimental site was located at Farako Bâ research station in a southwest of Bobo Dioulasso (Figure 4). The average annual rainfall is 1200 mm of water. The soil studied is lixisoil characterized by low organic matter(less than 1%), total N and P content. Texture were sandy in 0-40 cm depth and clayey in 40 to 60 cm depth. The pH varied from 5.3 to 5.6.

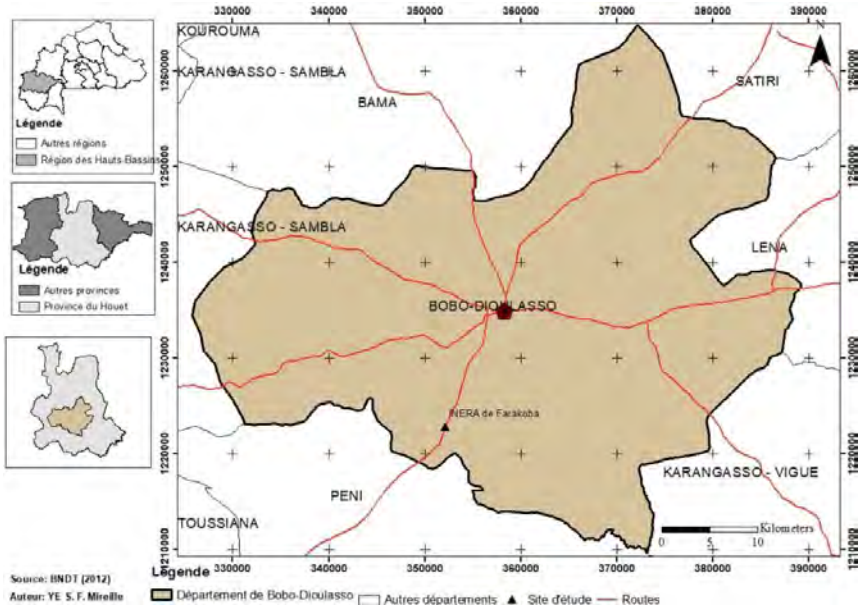


Figure 4: *Location map of Farako-Bâ research station*

From 2009 to 2019, the amount of rainfall varied (Figure 5). Compared to the 10-years average of 1070 mm of water, the 2010, 2013, 2014, 2018 and 2019 rainy seasons showed an increase in the amount of rain that fell. These increases range from 58 to 239 mm of water. The lowest rainfall was recorded in 2011 and 2017 with 831 and 744.6 mm of water respectively.

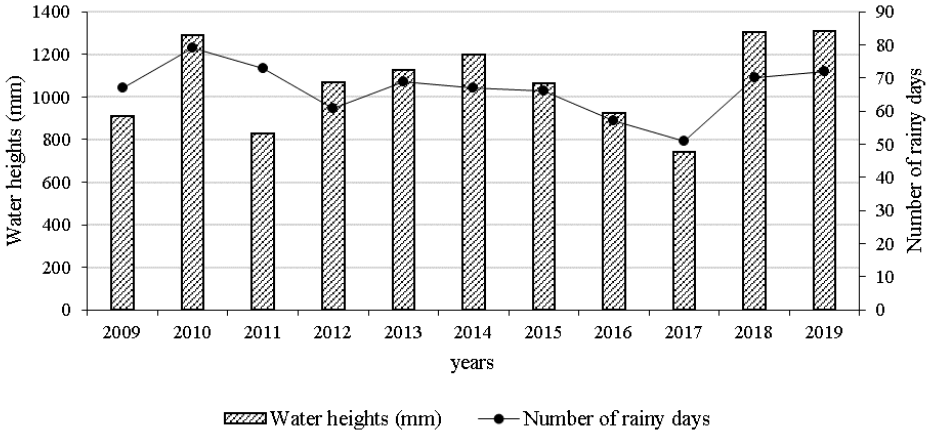


Figure 5: *Annual rainfalls of Farako-Bâ research station from 2009 to 2019*

Plant material used included the maize (Zea mays L.) variety SR21with 95-110days of cycle (planting to maturity) and 5100kgha-1 as potential yield and the cotton (Gossypium hirsutum L.) FK37 with 120days of cycle (seeding to capsule opening) and 2600 kgha-1 as potential yield. These varieties are adapted to the local conditions and currently used by many farmers in Burkina Faso.

Three species of cover plants that differ in their characteristics were used in pure or in combination with other crops. The biomass production of B. ruziziensis (GERM. & EVRARD) can reach 6000 to 8000kg/ha under tropical climate conditions. Its fasciculated root system is made up of many roots, dense and constitutes an important source of organic matter both on the surface and in the depth from 1.8 m to 2 m and can help to unpack soils quickly (Hussonet al., 2008). The root system is capable of restructuring the soil, injecting carbon in depth and efficiently recycling leached nutrients.

The M. cochinchinensis is a legume used as a green manure and plant cover species, is also grown in combination with cereals to improve biomass produced. It is a species that has a good level to fix atmospheric nitrogen.

The C. juncea (L.) variety, an annual legume up to 4 m long, has also been used as a cover plant. Its potential biomass can reach 3 to 6 tha-1 with nitrogen levels of 3.6% and a return capacity of 100 to 220 units of nitrogen per hectare.

The maize, cotton and M. cochinchinensis seeds were provided by the seed production service at the Farako-Bâ experimental research center. The seeds of B. ruziziensis and C. junceawas provide by the Brazilian Agricultural Research Organization at Secretariat of International Relations (Embrapa Cotton).

Mineral fertilizer used are NPK-S-B (14-18-18+6S+1B) and Urea (46% of N) and have brought44N, 27P, 27K, 9S and 1.5B per hectare on cotton and 74N, 36P, 36K, 12S and 2B per hectare on maize associated with cover plants. The fertilizers used were brought with from cotton companies and are national research recommendations for cotton-based farming systems.

Five treatments including three direct sowing under mulch-based cropping system (DMC) with cover plants associated with maize was compared and defined below:

- Conventional Tillage (CT) (annual ploughing): sowing of cotton after annual plough.

-Direct Seeding (DS) without cover plant: direct sowing of cotton without mulch

-DMC/ B. ruziziensis mulch (DMC 1): direct sowing of cotton under maize and B. ruziziensis mulch,

-DMC/ B. ruziziensis + M. cochinchinensis mulch (DMC 2): direct sowing of cotton under maize and B. ruziziensis+ M. cochinchinensis mulch

-DMC/ C. juncea mulch (DMC 3): direct sowing of cotton under maize and C. juncea mulch

The approach adopted in this study is to define the possibilities of biomass production with the use of cover plants associated with maize in cotton and maize biennial rotation systems. The growing of maize and cover plants provide a biomass for the direct sowing of the cotton the following campaign.

A randomized blocks of Fisher experimental design with five treatments and four replications was used for this study. The basic plot consists of 11 lines of 20m; an area was 176 m². The rehearsal area was 880 m². The maize, cover plants and cotton residues are kept on the plots. At the head of rotation, maize is associated with cover plants (grasses and/or legumes) to improve biomass production on the plot that will be valued as a plant cover for cotton seedlings. After 15 to 20 days of sowing maize, B. ruziziensis and C. juncea were sown continuously on line and M. cochinchinensis into 40 cm distant pockets between maize lines. Cotton and maize were sown to the same sizes of 0.80 m between the lines and 0.40 m between the pockets, with theoretical density of 62,500 plants/ha. NPKSB fertilizer was applied 15 days after the crop was lifted and urea at 40 days after lifting. Weeding was carried out on demand and phytosanitary treatments using products based on Indoxacarb 150 gl-1, Zeeta-cypermethryne 12 gl-1 - profenfos 200 gl-1 and Acetamipride 32 g/l - cypermethryne 144 gl-1 for the three insecticide treatment windows. The maize associated with cover plants was planted during the 2010, 2012, 2014, 2016 and 2018, for cotton seedlings, it was introduced during the 2011, 2013, 2015, 2017 and 2019 rain season.

The variables studied consisted of the biomass of maize and cover plant, changes in crop yields, soil acidity (pH), soil organic matter levels, P total and P assimilable, K total and K available. The balances of the chemical parameters were assessed from the initial soil reserves to the implementation of the test and the reserves after 10 years of application of the different systems. The balance sheets reflect the amount between the soil reserves in 2019 and the reserves for the implementation of the study in 2010.

The chemicals analyses of the soils were carried out at the Soil-Water-Plant laboratory at the Farako Bâ research center. The pH water was determined with pH-meter with glass electrode following a 1/2.5solution ratio. The Walkley-Black (1934) method for organic carbon determination was used. The organic matter rate is obtained from the formula Carbon rate *1.724. Total nitrogen was measured by the Kjeldahl method (Hillebr and et al., 1953). Total Phosphorus was measured on the condensed mineralization (Anderson and Ingram 1989). Assimilable phosphorus was determined by the BRAY 1 method (Dickman et al., 1940). Total potassium was measured at the flame spectro-photometer from the remnant of the filter from the mineralization of soil test shots.

Data were collected and entered with the Excel2010 version. The analysis of variance and standard deviations were performed using XLSTAT 2016 and R software. Fisher's 5% test was used to compare the average.

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References

1. Akal S. P., Ter-Rumum A. and Enoch M. I. Effects of Time of Intercropping of Mucuna (Mucuna cochinchinensis) in Maize (Zea mays) for Weed and Soil Fertility Management. Int. J. Agric. Biol., 2012; Vol. 14, No. 3, Pages 469–472.
2. Agence Française de Développement Le semis sous couverture végétale permanente (SCV). Paris, France, 2006. 68 pages.
3. Bado B., V. Rôle des légumineuses sur la fertilité des sols ferrugineux tropicaux des zones guinéenne et soudanienne du Burkina Faso. Thèse de doctorat : Université Laval-Québec, 2002; p.197.
4. Bayer C., Mielniczuk J., Amado T. J. C., Martin-Neto L. et Fernandes S. V. Organic matter storage in a sandy clay loam Acrisol affected by tillage and cropping systems in southern Brazil. Soil and Tillage Research, 2000; 54: pp. 101-109.
5. Botton H., Hallé N. Les Plantes de couverture. Guide pratique de reconnaissance et d'utilisation des Légumineuses enCôte d'Ivoire (suite et fin). In : Journal d'agriculture tropicale et de botanique appliquée, 1958; vol. 5, n°1-3. pp. 45-172. doi: https://doi.org/10.3406/jatba.1958.2454
6. Bottinelli N. Evolution de la structure et de la perméabilité d'un sol en contexte de non labour associé à l'apport d'effluent d'élevage : rôle de l'activité lombricienne. Sciences de la Terre. Agrocampus - Ecole nationale supérieure d'agronomie de rennes, 2010; 165 pages.
7. Chervet A., Ramseier L., Sturny W. G., Zuber M., Stettler M., Weisskopf P., Zihlmann U., Martínez G. I. et Keller T. Rendements et paramètres du sol après 20 ans desemis direct et de labour. Recherche Agronomique Suisse 2016; 7 (5) : 216–223.
8. Corsi S. and Muminjanov H. Conservation Agriculture: Training guide for extension agents and farmers in Eastern Europe and Central Asia. Rome, FAO. 2019; 140 pages.
9. Grandière I., Razafimbelo T., Barthès B., Blanchart E., Louri J., Chenu C. Wolf N., Albrecht A., et Ch. Feller. Distribution granulo-densimétrique de la matière organique dans un sol argileux sous semis direct avec couverture végétale des Hautes Terres malgaches. Étude et Gestion des Sols, Volume 14, 2, 2007; pages 117 à 133.
10. Coulibaly K, Vall E, Autfray P., Nacro H., B, Sedogo P., M. Effets de la culture permanente coton-maïs sur l'évolution d'indicateurs de fertilité des sols de l'Ouest du Burkina Faso. Int. J. Biol. Chem. Sci., 2012; 6(3): 1069-1080. doi : http://dx.doi.org/10.4314/ijbcs.v6i3.13
11. Coulibaly K., Vall E., Naudin K., Nacro H. B. et Havard M. Effets du travail minimum du sol en sec sur la flexibilité du calendrier agricole et le rendement du maïs en zone soudanienne du Burkina Faso. Tropicultura, 2018; 36, 4, 608-620.
12. Gamour G., Dorel M., Toc Hoa Quoc, Meynard C., Risede J.M. A trait-based characterization of coverplantsto assess their potential to provide a set of ecological services in banana cropping systems. European Journal of Agronomy 2014; 52:218-228.
13. Diakahté D., Chopart J. L. et Ralisch R. Traits fonctionnels de Brachiaria (TRIN.) brizantha (HOCHST.) Stapf, B. decumbens STAPF ET B. ruziziensis (GERM. & EVRARD) NDAB cultives en association avec Pennisetum glaucum (L.) Leeke R. BR. en climat tropical sec. Agronomie Africaine 2018; 30(3) : 225 - 235.
14. DickmanS. R., BrayH. R. Colorimetric determination of phosphate. Ind.Eng. Chem,Anal.Ed, 1940; 12 :665-668.
15. Doamba S., M., F., Nacro H., B., Sanon A., Sedogo P. M. Effet des cordons pierreux sur l'activité biologique d'un sol ferrugineux tropical lessivé (Province du Kouritenga au Burkina Faso). Int. J. Biol. Chem. Sci., 2011; 5(1): 304-313 DOI: ttp://dx.doi.org/10.4314/ijbcs.v5i1.68065.
16. Gama-Rodrigues, A.C.; Gama-Rodrigues, E.F.; Brito, E.C. Decomposition and nutrient release from cover crop residues in passion-fruit plantation. Revista Brasileira de Ciência do Solo, 2007; 31:1421-1428.
17. Hillebrand W. F, LundellG. E. F., Bright H.A., Hoffman J. I. Applied Inorganic Analysis.John Wiley & Sons, Inc:New York, USA;1034. 1953.
18. Husson O., Charpentier H., Razanamparany C., Moussa N., Michellon R., Naudin K., Razafintsalama H., Rakotoarinivo C., Rakotondramanana, Seguy L. Fiches techniques plantes de couverture : Graminées pérennes (Brachiaria sp.). Manuel pratique du semis direct à Madagascar. Volume III. Chapitre 3. § 4.1. 20 pages. 2008.
19. Husson O., Charpentier H., Michellon R., Naudin K. Enjalric F., Razanamparany C., Moussa N., Andrianasolo H., Razafintsalama H., Rakotoarinivo C., Rakotondramanana, Seguy L. Fiches techniques plantes de couverture, Les principales plantes de couverture utilisées à Madagascar. Manuel pratique du semis direct à Madagascar. 2008; Volume III. 87 pages.
20. Koulibaly B., Ouattara A., Dakuo D., Ouattara K., Traoré O., Geraldo J. D. and Lompo F. Efficacy of the association of cover crops with maize and direct sowing short-term effect on crops yield in maize-cotton cropping system in wester Burkina Faso. African Journal of Agricultural Research, 2017; Vol. 12 (52), pp. 3577-3584.
21. Nunes U., R., Andrade Júnior V. C., Silva E., de B., Santos N., F., Costa H., A., O., Ferreira C., A. Production de paille de plantes de couverture et de rendement des haricots en sans labour. Brazilian Agricultural Research, Brasilia, DF, c. 2006; 41: p. 943-978.
22. Ouattara B., Coulibaly K., Kohio E., Doumbia S., Ouédraogo S., et Nacro H., B. Effets du Système de Culture sous couverture Végétale (SCV) sur les flux hydriques d'un sol ferrugineux à l'Ouest du Burkina Faso. Int. J. Biol. Chem. Sci. 2018; 12(4) : 1770-1783.
23. Rafael Torres de Souza R. T., Assis F. C., Valadão D. D. V. J., Guimarães P. R., Paula V. R. R. Maize-crotalaria intercropping systems. Semina: Ciências Agrárias, Londrina, 2019; v. 40, n. 4, p. 1455-1468.
24. Rakotoarisoa J., Oliver R., Dusserre J., Muller B., Douzet J. M., Michellon R., Moussa N., Razafinjara L. A., Rajeriarison C., et Scopel E. Bilan de l'azote minéral au cours du cycle du riz pluvial sous-systèmes de culture en semis direct sous couverture végétale en sol ferrallitique argileux à Madagascar. Etude et Gestion des Sols, 2010; Volume 17, 2, 2010-pages 169 à 186.
25. Razafimbelo T.M., Albrecht A., Basile I., Borschneck D., Bourgeon G., Feller C., Ferrer H., Michellon R., Moussa N., Muller B., Razanamparany C., Seguy L., et Swarc M., Oliver R. Effet de différents systèmes de culture à couverture végétale sur le stockage du carbone dans un sol argileux des Hautes Terres de Madagascar. Étude et Gestion des Sols, 2006; Volume 13, 2, pages 113 à 127.
26. Sà J. C. D. M., Cerri C. C., Dick W. A., Lal R., Filho S. P. V., Piccolo M. C. et Feigl B. E., - Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a brazilianoxisol. Soil Science Society of America Journal, 2001; 65, pp. 1486-1499.

27. WalkleyA, BlackR, N. Anexamination of the method Dedtjar efffor determining soil organic matter and to proposed modification of the chromicacidtitration method. Soil Science. 1934. 37:29-38.
28. Yadav M. R., Parihar C. M., Rakesh Kumar, Yadav R.K., Jat S. L., Singh A.K., Ram H., Meena R.K., Singh M., Meena V.K., Yadav N., Yadav B., Kumawat C., and Jat M. L. Conservation Agriculture and Soil Quality– An Overview. International Journal of Current Microbiology and Applied Sciences. ISSN: 2319-7706. 2017; Volume 6 Number 2: pp. 707-734. http://dx.doi.org/10.20546/ijcmas.2017.602.080

Enhancing Nitrogen Use Efficiency in Bt-cotton

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Abstract

Background: *Cotton is one of the most widely cultivated fiber crops across a broad range of climates with varied soils and cultivation practices. Nitrogen is the major essential nutrient element limiting cotton growth and development. In most intensive agricultural production systems, 50- 75% of the N applied to the field is not used by the crop and is lost by leaching into the soil. Some microorganisms are able to improve soil fertility by metabolizing the N that is not absorbed by plants. It is however a lengthy process which involves a major risk because mineral N, especially nitrate and ammonical form are very soluble and can run off into the surface water or flow into the groundwater. Knowing its practical implications leading to higher N applications for higher yields irrespective of time of applications results in reduced N use efficiency and wastage of nutrients causing environmental pollution. It may also leads to excessive growth, unbalanced source sink relationships and dropping of fruiting bodies. In this way an experiment was designed with seven treatments with three replications in randomized complete block design. The treatments consists of T1: N0 (Control): 100% P &K and No fertilizer N application, T2: Band application of 100 % RDN in 2 splits at basal & flowering, T3: Band application of 75 % of RDN in 2 splits at basal & flowering,T4: Spot application of 75 % of RDN in 2 splits at basal & flowering, T5: Spot application of 75 % of RDN in 4 splits at basal, squaring, flowering & boll development stages, T6: T5 +Foliar application of 1% urea, 3 times: squaring, flowering & boll development stages, T7: T6 + raising of sun hemp between rows of cotton & incorporated before flowering.*

Results: *Experimental results indicated that Seed cotton yield was significantly increased in spot application of 75 %RDN in 4 splits at basal, squaring, flowering & boll development stages, + Foliar application of 1% urea, 3 times at squaring, flowering & boll development stages(T6)(2205 kg/ha) as compared to any other treatments. This may be because of improved nitrogen use efficiency in this treatment due to spot application methods & split applications in 4 doses as compared to recommended method with 100% RDN(T2). Though 25% RDN is more in T2 it was failed to produce higher yield than any other treatments including T6. Same trend was followed in all the three years and pooled analysis. Improved NUE by spot method enhanced the applied N to the root zone of the crop, split applications specifically supplied the nitrogen requirements in the critical period of the crop stage, and further foliar applications additionally supplied the nitrogen for better biosynthesis to produce the sink. It also justified that the 25% reduced levels of nitrogen in T6 was compensated by increased NUE as compared to T2. Further, in-situ incorporation of sun hemp though improved the SCY during two years of study but failed to produce same trend during third year (T7). Nitrogen use efficiency computed based on the seed cotton yield obtained per kg of nitrogen applications was zero in no fertilizer N application (T1),whereas 29.4 kg/kg N in T6 which was significantly highest as compared to any other treatments. Soil fertility analysis indicated that in T7 where raising of sun hemp and in-situ incorporation was made in the soil improved the soil fertility by increasing the available N, P and K status as compared to other treatments. This will indicate the recoupment of soil nutrients due to in- situ incorporation though crop removal was obvious. Soil uptake studies results indicated that the nitrogen uptake was considerably reduced in no nitrogen were applied (T1) as compared to other treatments. This may be because of reduced availability and non application of nutrient N to soil. It was also evident that the NPK uptake was drastically increased in T7 where sun hemp was grown and incorporated in to the soil as compared to any other treatments. Economic analysis of the study with gross returns (Rs 1, 21,310/ha), net returns (Rs 54,226/ha) and B: C ratio (1.79) was significantly highest with spot application of 75 % of RDN in 4 splits at basal, squaring, flowering, boll development and foliar application of 1 % urea 3 times at squaring, flowering and boll development stages) (T6) as compared to any other treatments. Increased returns in this treatment were due to significantly increased SCY, boll numbers and boll weight.*

Conclusion: *Based on the three years pooled data on seed cotton yield, economic analysis and soil fertility data it can be concluded that Bt- cotton cultivation can be sustained with productivity, profitability and by maintenance of soil fertility by way of with 75% RDN application (100% P & K on basal), N applied in four splits (basal, squaring, flowering and boll development) along with foliar application of 1% urea at squaring, flowering & boll development stage, and raising of two rows of sun hemp as an intercrop in between cotton and in-situ incorporation at around 35-40 DAS(T7) can*

produce the seed cotton on par with as per package(T2). Here soil fertility can better be improved with sun hemp in-situ incorporation.

Key Words: *Spot applications, split applications, nitrogen use efficiency, Insitu green manuring*

Background

Cotton is one of the most widely cultivated fiber crops across a broad range of climates with varied soils and cultivation practices (Shah et al., 2017). Nitrogen is the major essential nutrient element limiting cotton growth and development. In most intensive agricultural production systems, 50% to 75% of the N applied to the field is not used by the plant and is lost by leaching into the soil (Raun and Johnson, 1999). Some microorganisms are able to improve soil fertility by metabolizing the N that is not absorbed by plants. It is however a lengthy process which involves a major risk because mineral N, especially nitrate and ammonical form are very soluble and can run off into the surface water or flow into the groundwater. Knowing its practical implications leading to higher N applications for higher yields irrespective of time of applications results in reduced N use efficiency and wastage of nutrients. It may also leads to excessive growth, unbalanced source sink relationships and dropping of fruiting bodies. In addition to that it results in substantial N losses and the creation of environmental pollution.

Due to indeterminate growth habit of cotton crop, better understanding of cotton growth and development under N supply at the rational nutrient regime is important in the continuing efforts of increasing higher yields. Nitrogen is required in larger amounts than other nutrients by cotton (Chen et al., 2010). Synchronization of N uptake for demands along with supply is crucial for generating the optimum N use efficiency in cotton (Khan et al., 2017). It was proved beyond doubt that nitrogen is an essential nutrient for most of the biosynthesis in the plant. The N demand by cotton is strongly related to yield potential, which was intern associated with N supply (Macdonald et al., 2017). Several times N deficiency cause stress for cotton resulting in reduced growth and production (Lokhande and Reddy, 2015). Several low cost methods can also help to improve the N availability to the crop by way of method of application, time of application, foliar applications and in-situ incorporation of green manures.

Results and discussion

Cotton requires relatively larger amount of nitrogen compared with the demand for other elements to obtain maximum yield (Beltrao, 1999). Nitrogen is the nutrient most absorbed by cotton. Therefore, it pays an important role in plant growth & development, especially during vegetative stage, stimulates growth and flowering. It regulates the plant cycle, increases productivity and improves quality when applied in appropriate doses (Beltrao, 1999). Hence from the studies of 2019-20 indicated that growth parameters such as plant height, number of monopodials per plant and sympodials per plant was significantly increased with 100% RDN with band application in two splits(T2) as compared to other treatment where the dosage of application was less (75% RDN) (Table 2). Growth was significantly reduced in T1 where crop was applied without nitrogen (control). However, from the treatments of T4, T5, T6 and T7 were also showing on par growth parameters with T2 due to improved nitrogen utilization pattern & N use efficiency by spot application methods (as in T4), increased no of split applications (as in T5), further addition of foliar nutrition of nitrogen (as in T6 and T7). From the table of NUE it was clear that these treatments were showing higher values as compared to T2 (recommended method). This was again similar to the results of Castro, 2004 that the nitrogen use efficiency depends on the method of N application, time of application and doses of nitrogen.

Yield and yield components :(Pooled mean of three years)

Studies made for three years 2017-18, 2018-19 and 2019-20 and pooled mean of three years on seed cotton yield (SCY) and their components is presented in Table 2. Seed cotton yield was significantly increased in T6 (2205 kg/ha) as compared to any other treatments. This may be because of improved nitrogen use efficiency in this treatment due to spot application methods & split applications in 4 doses as compared to T2 (recommended method), where it was followed with recommended practice with 100% RDN. Though 25% RDN is more in T2 it was failed to produce higher yield than any other treatments including T6. Same trend was followed in all the three years and pooled analysis. Improved NUE by spot method enhanced the applied N to the root zone of the crop, split applications specifically supplied the nitrogen requirements in the critical period of the crop stage (Lokhande and Reddy, 2015) further foliar applications additionally supplied the nitrogen for better biosynthesis to produce the sink. It also justifies that the 25% reduced levels of nitrogen in T6 was compensated by increased NUE as compared to T2. Further, in-situ incorporation of sun hemp though improved the SCY during two years of study but failed to produce during third year (T7). During 2019-20 there was excess rainfall especially during incorporation time resulted in negative impact on the crop. This method especially fails either due to deficit or excess rainfall during incorporation time. Statistical analysis of data indicated that

T7(2143 kg/ha), T2 (2170 kg/ha), T4 (2094 kg/ha) and T5 (2079 kg/ha) recorded on par seed cotton yield with T6 (2205 kg/ha).

Number of bolls per sq meter and single boll weight which are the main yield components also shows the difference between the treatments. Number of bolls were significantly increased in T7 (124.9/sq m) as compared to any other treatments (Table 2). However, it was on par with T6(122.2/sq m), T2(121.6/sq m) and T5 (118.7 sq/m). It shows that the methods used to increase the nitrogen use efficiency in T5 and T6 (spot applications and split applications) were improved to produce the boll numbers on par with T7. Similar trend was also observed with individual boll weight. Hence the yield improved by enlarging the boll weight and the number of bolls per unit area due to increased N use efficiency.

Nitrogen use efficiency which was computed based on the seed cotton yield obtained per kg of nitrogen applications. Hence the NUE which was zero in T1 as per computation can able to record 29.4 kg/kg N in T6 which was significantly highest as compared to any other treatments (Table 2). However, it was on par with T7(28.6 kg/kg N), T5 (27.7 kg/kg N), T4 (27.9 kg/kg N) and T3 (26.4 kg/kg N). But it was significantly higher than present recommended practices (T2: 21.7kg/kg N). Similar trend was followed in all the years. Further, the data shows that different methods used to improve the NUE there was an addition of nutrient efficiency from T3 to T4 to T5 to T6 and further to T7 treatments. Hence the extra N efficiency that was contributed by each method is given in Table 1.

Table 1: NUE contributions from each methodology (From pooled data)

Particulars	Seed cotton yield (Kg/ha)	Boll Numbers /sq m	Boll weight (g)	NUE (kg/kg N)
Nitrogen (100% RDN) (T2-T1)	756	38.1	0.8	21.7
Net loss due to 25% N (T3-T2)	-10.6	-11.1	-0.14	4.7
Spot application (T4-T3)	117	3.0	0.09	1.5
Split applications (T5-T4)	-15.0	5.2	-0.06	-0.2
Foliar applications (T6-T5)	126.0	3.5	0.23	1.7
In-situ incorporation of sun hemp (T7-T6)	-62.0	2.7	-0.03	-0.8

Soil fertility analysis :(Initial, 2017-18, 2018-19 and 2019-20)

Soil fertility status as evaluated with availability of nutrients after harvest of each cropping season is presented in Table 3. Soil available NPK nutrient status after harvest of 2019-20 trial was not significantly affected by different treatments. Available soil N status was the lowest in treatment T1 where N was not applied as compared to other treatments in all the years. However, there was no much variation in the available P & K status in all the treatments and in all three years as the recommended quantity of P and K was applied to all treatments. In T7 where raising of sun hemp and in-situ incorporation was made in the soil improved the soil fertility by increasing the available N, P and K status as compared to other treatments. This will indicate the recoupment of soil nutrients due to in- situ incorporation though crop removal was obvious.

Nutrient up take studies: (Pooled analysis)

Nitrogen status of agricultural crops is assessed by analyzing the actual N concentration of above ground dry matter and the critical N concentration. The critical N concentration is defined as the minimum demand of a crop for necessary N concentration to achieve the highest growth rates and potential yield (Chakwizira et al., 2016). The results of all the three years revealed that nitrogen uptake was considerably reduced in T1 (No N was applied) as compared to other treatments (Table 4). This may be because of reduced availability and non application of nutrient N to soil. It was also evident that the NPK uptake was drastically increased in T7 where sun hemp was grown and incorporated in to the soil as compared to any other treatments (Zhao et al., 2005). This was also due to increased availability of nutrients in that treatment. Process of sustainable cotton production can be enlightened by way of improving soil fertility and productivity on long way and improving precision of nutrient applications, supply of nutrients to the crop when it was required or split methods can help to update the requirement of cotton production practices.

Economic analysis of NUE methodology

Economic analysis of three years pooled data on gross returns, net returns and B: C ratio are presented in Table 5 & 6. Gross returns (Rs 1, 21,310/ha), net returns (Rs 54,226/ha) and B:C ratio (1.79) was significantly increased with T6 (Spot application of 75 % of RDN in 4 Split: Basal, Squaring, Flowering,

Boll development + Foliar application of 1 % urea 3 times: Squaring, Flowering and Boll development stages) as compared to any other treatments. Increased returns in this treatment were due to significantly increased SCY, boll numbers and boll weight. Cost of cultivation of treatments increased serially from T3 (63,805/ha) to T7 (Rs 67,651/ha). This was due to addition of factors serially from T3 to T7 for increasing NUE. Though gross returns were significantly increased in T6, due to increased cost of cultivations net returns were reduced as compared to T2. However, net returns of T6 (Rs 54,226/ha) were on par with T2 (Rs 54,411/ha), T4 (Rs 50,383/ha), T5 (Rs 49,305/ha) and T7 (Rs 50,240/ha) treatments.

Table 2. Seed cotton yield and its parameters as influenced by time and method of application of N in Bt-cotton (2017, 2018 and Pooled)

Treatments	Seed Cotton Yield (kg/ha)				No. of Bolls/sq.m				Boll weight (g)				NUE (kg kapas/kg of N)			
	Y1	Y2	Y3	All	Y1	Y2	Y3	All	Y1	Y2	Y3	All	Y1	Y2	Y3	All
T_1: No nitrogen (Control)	1202	1884	1156	1414	88.3	94.0	68.3	83.5	4.70	4.75	4.70	4.72	0.00	0.00	0.0	0.0
T_2 :100 % of RDN(Band application in 2 splits at Basal & Flowering)	1692	2621	2197	2170	120.3	137.1	107.3	121.6	5.52	5.63	5.40	5.52	16.92	26.21	22.0	21.7
T_3: 75 % of RDN(Band application in 2 splits at Basal & Flowering)	1640	2310	1982	1977	109.7	120.7	101.0	110.5	5.32	5.53	5.30	5.38	21.87	30.80	26.4	26.4
T_4 : 75 % of RDN + Spot application in 2 splits at Basal & Flowering)	1645	2486	2152	2094	107.7	127.4	105.3	113.5	5.33	5.58	5.50	5.47	21.94	33.14	28.7	27.9
T_5 : 75 % of RDN + (Spot application in 4 Split: Basal, Squaring, Flowering, Boll development)	1665	2564	2009	2079	118.3	129.1	108.7	118.7	5.37	5.67	5.20	5.41	22.20	34.18	26.8	27.7
T_6 :T_5 + Foliar application of 1 % urea (3 times: Squaring, Flowering, Boll development)	1680	2617	2317	2205	120.3	131.3	115.0	122.2	5.58	5.73	5.60	5.64	22.40	34.89	30.9	29.4
T_7 :T_6 + raising of Sunnhemp between rows incorporated before flowering	1749	2625	2056	2143	128.7	137.4	108.7	124.9	5.62	5.92	5.30	5.61	23.32	35.01	27.4	28.6
SE.m	68	90	101	41	5.8	4.3	1.59	3.0	0.24	0.11	0.19	0.11	0.87	0.88	1.28	0.99
C.D. at 5%	209	277	310	126	18.0	13.3	4.89	9.30	NS	0.34	NS	0.34	2.69	2.70	3.94	3.10

Notes: Y1=2017, Y2=2018, Y3=2019, All=3 years pooled; FYM @ 5t/ha and 100% P & K are common to all treatments; RDF: 100:50:50 N:P2O5:K2O kg/ha

Table 3. Soil Nutrient status as influenced by time and method of application of N in Bt-cotton (2017, 2018 and 2019)

Treatments	Available Nitrogen (Kg/ha)				Available P_2O_5 (kg/ha)				Available K2O (kg/ha)			
	Ini.	Y1	Y2	Y3	Ini.	Y1	Y2	Y3	Ini.	Y1	Y2	Y3
T_1: No nitrogen (Control)	226.5	210.6	195.5	225.5	35.8	33.9	30.6	33.6	515.1	510.3	506.4	527.0
T_2 :100 % of RDN(Band application in 2 splits at Basal & Flowering)	222.4	225.5	229.9	253.0	35.2	34.0	32.1	31.6	520.4	525.6	526.8	511.5
T_3: 75 % of RDN(Band application in 2 splits at Basal & Flowering)	220.4	215.4	207.2	231.0	31.8	30.3	33.3	29.9	510.5	507.1	512.2	531.5
T_4 : 75 % of RDN + Spot application in 2 splits at Basal & Flowering)	224.5	213.8	203.9	231.0	35.6	32.6	31.1	34.2	525.6	512.2	514.2	539.0
T_5 : 75 % of RDN + (Spot application in 4 Split: Basal, Squaring, Flowering, Boll development)	227.8	217.1	205.7	242.0	33.4	31.7	32.8	33.4	515.4	517.0	515.5	526.0
T_6 :T_5 + Foliar application of 1 % urea (3 times:Squaring, Flowering, Boll development)	230.5	218.5	202.8	241.0	32.9	34.0	30.9	32.5	510.9	520.5	513.8	514.0
T_7 :T_6 + raising of Sunhemp between rows incorporated before flowering	224.8	220.2	210.6	264.0	33.8	34.4	34.2	34.3	515.2	515.5	523.8	558.0
SE.m	5.8	6.5	5.3	21.3	1.32	1.62	1.74	3.1	3.6	4.0	4.5	21.7
C.D. at 5%	NS	NS	16.42	NS	NS	NS	NS	NS	NS	NS	NS	NS

Notes: Ini=Initial, Y1=2017, Y2=2018, Y3=2019, All=3 years pooled; FYM @ 5t/ha and 100% P & K are common to all treatments; RDF: 100:50:50 N:P2O5:K2O kg/ha

Table 4. Nutrient uptake studies (kg/ha) as influenced by time and method of application of N in Bt-cotton (2017, 2018,2019 and Pooled)

Treatments	Nitrogen uptake (Kg/ha)				P uptake (kg/ha)				K uptake (kg/ha)			
	Y1	Y2	Y3	All	Y1	Y2	Y3	All	Y1	Y2	Y3	All
T_1: No nitrogen (Control)	57.5	85.5	57.4	66.8	10.88	13.78	19.1	14.6	43.5	69.7	60.7	58.0
T_2 :100 % of RDN(Band application in 2 splits at Basal & Flowering)	100.9	149.5	87.9	112.8	16.70	23.13	21.3	20.4	83.5	110.0	74.2	89.2
T_3: 75 % of RDN(Band application in 2 splits at Basal & Flowering)	97.0	122.0	73.1	97.4	14.63	19.92	18.9	17.8	73.9	95.1	72.8	80.6
T_4 : 75 % of RDN + Spot application in 2 splits at Basal & Flowering)	99.3	140.8	74.2	104.8	17.6	23.20	19.6	20.1	77.4	106.8	78.9	87.7
T_5 : 75 % of RDN +(Spot application in 4 Split: Basal, Squaring, Flowering, Boll development)	105.1	146.4	81.2	110.9	19.25	23.28	16.6	19.7	78.2	146.9	70.8	98.6
T_6 :T_5 + Foliar application of 1 % urea (3 times: Squaring, Flowering, Boll development)	112.3	150.8	93.3	118.8	18.04	26.45	24.5	23.0	88.2	152.5	91.0	110.6
T_7 :T_6 + raising of Sun hemp between rows incorporated before flowering	119.9	158.9	101.3	126.7	20.83	26.01	27.3	24.7	93.9	143.1	82.6	106.5

Notes: Y1=2017, Y2=2018, Y3=2019, All=3 years pooled

Table 5. Cost of cultivation and gross returns as influenced by time and method of application of N in Bt cotton (2017, 2018, 2019 and Pooled)

Treatments	COC (RS/ha)				Gross returns (RS/ha)			
	Y1	Y2	Y3	All	Y1	Y2	Y3	All
T_1: No nitrogen (Control)	59028	61525	59079	59877	62487	107370	63562	77806
T_2 :100 % of RDN(Band application in 2 splits at Basal & Flowering)	62533	67070	65337	64980	87984	149372	120817	119391
T_3: 75 % of RDN(Band application in 2 splits at Basal & Flowering)	61951	65523	63941	63805	85297	131659	109028	108662
T_4 : 75 % of RDN + Spot application in 2 splits at Basal & Flowering)	61976	66940	65539	64818	85557	141686	118360	115201
T_5 : 75 % of RDN + (Spot application in 4 Split: Basal, Squaring, Flowering, Boll development)	62074	67635	65573	65094	86580	146139	110477	114399
T_6 :T_5 + Foliar application of 1 % urea (3 times: Squaring, Flowering, Boll development)	62647	69990	68614	67084	87343	149152	127435	121310
T_7 :T_6 + raising of Sunnhemp between rows incorporated before flowering	62992	71401	68561	67651	90931	149647	113098	117892
SE.m	340	319			3533	5130	5531	2253
C.D. at 5%	1047	983			10885	15806	17042	6942

Notes: Y1=2017, Y2=2018, Y3=2019, All=3 years pooled; FYM @ 5t/ha and 100% P & K are common to all treatments; RDF: 100:50:50 N:P2O5:K2O kg/ha

Table 6. Net returns and B:C ratio as influenced by time and method of application of N in Bt cotton (2017, 2018,2019 and Pooled)

Treatments	Net returns (RS/ha)				B:C ratio			
	Y1	Y2	Y3	All	Y1	Y2	Y3	All
T_1: No nitrogen (Control)	3458	45845	4483	17929	1.06	1.75	1.08	1.29
T_2 :100 % of RDN(Band application in 2 splits at Basal & Flowering)	25451	82302	55480	54411	1.41	2.23	1.85	1.83
T_3: 75 % of RDN(Band application in 2 splits at Basal & Flowering)	23347	66135	45087	44856	1.38	2.01	1.7	1.69
T_4 : 75 % of RDN + Spot application in 2 splits at Basal & Flowering)	23582	74747	52821	50383	1.38	2.12	1.81	1.77
T_5 : 75 % of RDN + (Spot application in 4 Split: Basal, Squaring, Flowering, Boll development)	24506	78505	44904	49305	1.39	2.16	1.69	1.75
T_6 :T_5 + Foliar application of 1 % urea (3 times: Squaring, Flowering, Boll development)	24695	79162	58821	54226	1.39	2.13	1.86	1.79
T_7 :T_6 + raising of Sun hemp between rows incorporated before flowering	27938	78246	44537	50240	1.44	2.1	1.65	1.73
SE.m	3193	5003	5531	2220	0.05	0.08	0.08	0.04
C.D. at 5%	9839	15417	17042	6841	0.15	0.24	0.26	0.11

Notes: Y1=2017, Y2=2018, Y3=2019, All=3 years pooled;

Methods

A field experiment was conducted during 2017-18 to 2019-20 for three years at the Agricultural Research Station, Dharwad, which lies in the northern transitional zone-8 of Karnataka. The experimental site is located at 15o28' 11.57''N latitude and 75o 1' 17.92'' East longitudes with an altitude of
678 m above the mean sea level. The soil of the experimental site was medium deep black soil, low in organic carbon (0.58 %) available N (225 kg/ha), available P2O5 (31kg/ha) and available K2O (485 kg/ha). The experiment was laid out in a randomized complete block design with three replications. The treatments consists of T1: N0 (Control): 100% P &K and No fertilizer N application, T2: Band application of 100 % RDN in 2 splits at basal & flowering, T3: Band application of 75 % of RDN in 2 splits at basal & flowering,T4: Spot application of 75 % of RDN in 2 splits at basal & flowering, T5: Spot application of 75 % of RDN in 4 splits at basal, squaring, flowering & boll development stages, T6: T5 +Foliar application of 1% urea, 3 times: squaring, flowering & boll development stages, T7: T6 + raising of sun hemp between rows of cotton & incorporated before flowering. Recommended doses of farm yard manure, P and K fertilizers were applied uniformly to all the treatments. Only nitrogen was applied as per the doses, methods and split applications as and when the dates appear. Cotton seeds were sown with a spacing of 90x60 cm. Plant stand was maintained with gap filling and rouging, keeping only one hill per spot. In the treatment T7 two rows of sun hemp was sown in between cotton and maintained for 35-40 days. Sun hemp was uprooted and a furrow was made in between cotton rows and incorporated in to the soil. Foliar application of 1% urea was given to T6 & T7 at mentioned stage of the crop. Plant protection was made to the crop as and when required with the recommended practices. Representative composite soil sample was drawn from the experimental site at a 0-15 cm soil depth before the initiation of experiment. Than after soil sampling was done after harvest of every cropping season and used for analysis of soil available nutrients such as N, P and K by following standard laboratory procedures and methods. Observations on growth parameters such as plant height, number of monopodial branches and sympodial branches per plant were recorded just before harvest of crop. Yield components such as number of bolls per sq meter, seed cotton yield per plot from the net plot area and single boll weight from 20 random boll sampling was recorded at the time of harvesting. Total dry matter accumulation per plant was recorded from one plant collected randomly from each treatment oven dried and recorded its weight. The same plant sample was used for preparation of plant sample for nutrient content of N, P and K from each treatment. Economic analysis of the data was made with accounting of expenditures made to calculate cost of cultivations by each of the operations; labour wages paid & input costs. Gross returns, net returns and B:C ratios were computed based on the yield obtained and prevailing market price.

References

1. Beltrao, N.E. de M. (1999) - Brazilian Cotton from: Situation and prospects. In: Beltrao, N.E. de M.- Agribusiness cotton in Brazil. Brasília, Embrapa Communication for technology transfer,1:15-27.
2. Chakwizira E, de Ruiter JM, Maley S, Teixeira E (2016). Evaluating the critical nitrogen dilution curve for storage root crops. Field Crop Research 199:21-30.
3. Chen WP, Hou ZN, Wu LS, Liang YC, Wei CZ (2010). Effect of salinity and nitrogen on cotton growth in arid environment. Plant and Soil 326(1-2):61-73
4. Lokhande S, Reddy KR (2015). Cotton reproductive and fiber quality responses to nitrogen nutrition. International Journal of Plant Production 9(2):191-210.
5. Khan A, Najeeb U, Wang LS, Tan DKY, Yang GZ, Munsif F, … Hafeez A (2017b). Planting density and sowing date strongly influence growth and lint yield of cotton crops. Field Crop Research 209:129-135.
6. Macdonald BCT, Chang YF, Nadelko A, Tuomi S, Glover M (2017). Tracking fertilizer and soil nitrogen irrigated cotton: uptake, losses and the soil N stock. Soil Research 55(3):264-272.
7. Raun, W.R.; Johnson, G.V. Improving nitrogen use efficiency for cereal production. Agron. J. 1999, 91, 357-363.
8. Shah AN, Iqbal J, Tanveer M, Yang GZ, Hassan W, Fahad S, … Wu YY (2017). Nitrogen fertilization and conservation tillage: a review on growth, yield, and greenhouse gas emissions in cotton. Environmental Science and Pollution Research 24(3):2261-2272.
9. Zhao, D.; Reddy, K.R.; Kakani, V.G.; Read, J.J. and Kotil, S. (2005) - Selection of optimum reflectance ratios for estimating leaf nitrogen and chlorophyll concentrations of field-grown cotton. Agronomy Journal, 97: 89-98.

Environmental Conditions on Cotton Yield and Fibre Quality Under Narrow Row Systems

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Abstract

Background: *Cotton is a forced annual crop with an indeterminate growth habit and a complicated fruiting pattern.There are effectively three major components influencing yield: variety, cultural inputs including management, and environment. The objective of this study was to assess the effect of different environmental conditions during the critical period (CP) on yield and cotton fiber quality of different genotypes in Argentina in a narrow row system.*

Results: *Six experiments were carried out at the INTA ReconquistaArgentina, during three-seasons in different sowing dates and with different genotypes. The environmental conditions recorded during CP were: rainfall (Rf), average temperature (Tmed), average maximum temperature (Tmax), average minimum temperature (Tmin) and global solar radiation (Rad). Fiber cotton yield (FCY) was determined with several of its components as seed cotton yield (SCY), ginning turn-out percentage (% GO), number of bolls.m-2 (NB), weight per boll (WB). Also, some cotton quality fiber parameters were used such as upper-half mean length (UHML), strength (Str) and micronaire (Mic). Several correlation and regression analyses, between yield components and environmental conditions, were performed. The best adjustment between yield, fibre quality and environmental conditions were obtained with CP of 15 days previous until 15 post first flower opened. It was observed a wide variability in the results that could be explained by the difference in environmental data recorded in the seasons evaluated. FCY, its components and some fiber quality parameters were significantly affected by Tmed, Tmin, Tmax, Rad and Rf during CP. Global solar radiation, presented positive correlation on both components and yield, mean while temperature presented negative correlation with yield and their components.*

Key Words: *Temperature, Radiation, Rainfall, Narrow row, Environment*

Background

Cotton production for the 2019/20 season in Argentina showed an increase in terms of national sown area compared with the last four seasons. Officially, about 460.000 ha (MAGYP, 2020) was sown with an average yield of 2.5 t. ha-1 of seed fiber cotton reaching a national production of about 300.000 tn. The competitiveness of cotton is mainly due to increased taxes on other summer crops like soybean and corn.

Narrow-row cotton has become popular in Argentina in the last few years reaching about 90 % of the national sown area. By reducing distance between rows and increasing plant population, plants became smaller and harvested with stripped machines reducing harvesting cost compared with the previous traditional cotton system (Paytas and Tarrago, 2011).

Cotton is a perennial with an indeterminate growth habit and a complicated fruiting pattern. Of particular importance is that the cotton plant has a very dynamic response to management and changes in the environment. There are effectively three major components influencing yield: variety, cultural inputs including management, and environment (Oosterhuis, 1999).

In cotton, temperature is a primary controller of the rate of plant growth, developmental events, and fruit maturation (Baker, 1965). Temperature is an environmental factor that regulates the rate of phenological development and biomass accumulation (Burke and Wanjura, 2010). Temperature effects on yield are complex; crop responses to changes in temperature depend on the temperature optima for

photosynthesis, growth, and yield processes and these optimal values are all different (Conroy et al., 1994; Polley, 2002).

Above average temperatures during the day can increase photorespiration and decrease photosynthesis and carbohydrate production. Oosterhuis (1999) indicates that there is no sharp threshold but rather a gradual decline to more than 50% decrease at about 32 to 35 °C. On the other hand, hot night temperatures, i.e above 20 ° C, can significantly increase respiration. Furthermore, abiotic factors such as rainfall, temperature, and irradiance can alter seed and fiber development (Bradow and Davidonis, 2000).

Growth, development, yield and fiber quality of cotton with different sowing date have been subject of many studies (Ali et al., 2009; Bozbek et al., 2006; Hebbar et al., 2002; Winkler et al., 2018). These studies revealed that a delay in sowing date reduced the potential productivity of cotton crop.

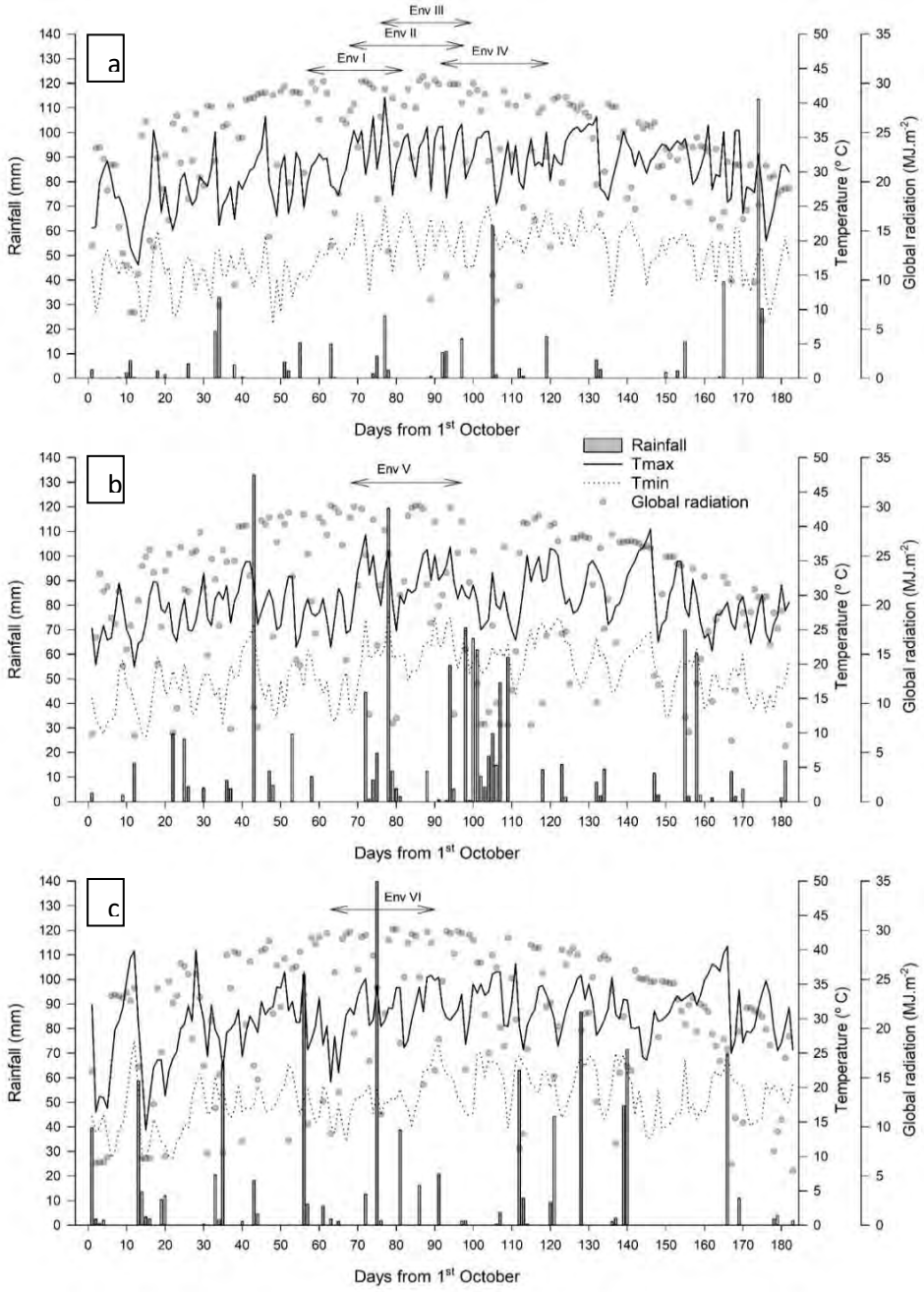
The critical period (CP) for yield determination is located in a time window between 10 days before the opening of the first flower and 10 days after cut out. The approximate duration of this period is 45 days (Paytas and Ploschuk, 2013).

The objective of this study was to evaluate the effect of the environmental conditions (temperature, rainfall, and solar radiation) during the CP on yield and cotton fiber quality of different genotypes in Argentina in a narrow row system.

Results

Environmental conditions

In Table 1, conditions of precipitation, radiation, maximum and minimum temperatures and the monthly averages recorded during the duration of the tests as well as the historical averages of the INTA Reconquista weather station (1970 - 2020) are showed. The differences between the first two experimental seasons can be highlighted, while during the 2017/18 season between October and March 419.8 mm were recorded, during the 2018/19 season in the same months 1204.2 mm were recorded. The 2019/20 season presented values nearly to the historical average. Concerning solar global radiation, since it is a variable related to rainfall, a similar behavior to that analyzed was observed. Furthermore, temperatures, in general, were higher than the historical average, which may indicate an effect of global warming, especially on minimum temperatures. Finally, Figure 1 also shows the times when the different CP were recorded in each of the environments and the temperature, radiation and rainfall conditions that were registered at those times, generated due to the different planting dates.



Notes: Daily maximum (solid) and minimum (dotted) temperature (lines), rainfall (bars) and global radiation (circles) during season 2017/18 (a), 2018/19 (b) and 2019/20 (c). Each arrow shows the critical period for each environment (Env I, II, III, IV, V, VI).

Figure 1: *Temperature, rainfalls and radiation during three seasons*

Table 1. Average rainfall, global radiation, maximum and minimum temperature reached during seasons 2017/18, 2018/19, 2019/20 and average historical (Hist.) at INTA (1970-2020).

	Rainfall (mm)				Global radiation (MJ.m-2)				Tmax (° C)				Tmin (° C)			
	17/18	18/19	19/20	Hist.	17/18	18/19	19/20	Hist.	17/18	18/19	19/20	Hist.	17/18	18/19	19/20	Hist.
Oct	24,0	86,5	145,8	129,5	602,1	601,3	542,9	583,6	25,8	26,4	26,5	26,3	14,7	15,2	15,0	14,5
Nov	82,3	203,9	230,7	146,3	762,6	687,3	653,4	671,9	29,2	29,0	30,0	28,4	15,4	18,0	18,3	16,7
Dec	65,2	226,6	234,1	154,5	783,1	739,7	750,0	735,3	32,8	31,2	31,1	30,9	19,9	19,9	18,5	19,1
Jan	112,1	473,1	137,1	148,7	728,0	586,8	734,2	720,4	31,8	30,9	31,8	32,0	20,5	22,2	20,7	20,6
Feb	16,5	39,5	211,1	151,5	680,1	635,1	661,1	597,8	33,1	31,1	30,9	30,8	19,7	19,8	19,1	19,9
Mar	199,7	174,6	89,0	157,7	598,3	536,5	551,5	545,9	29,8	27,7	32,1	28,9	17,1	17,3	18,9	18,3

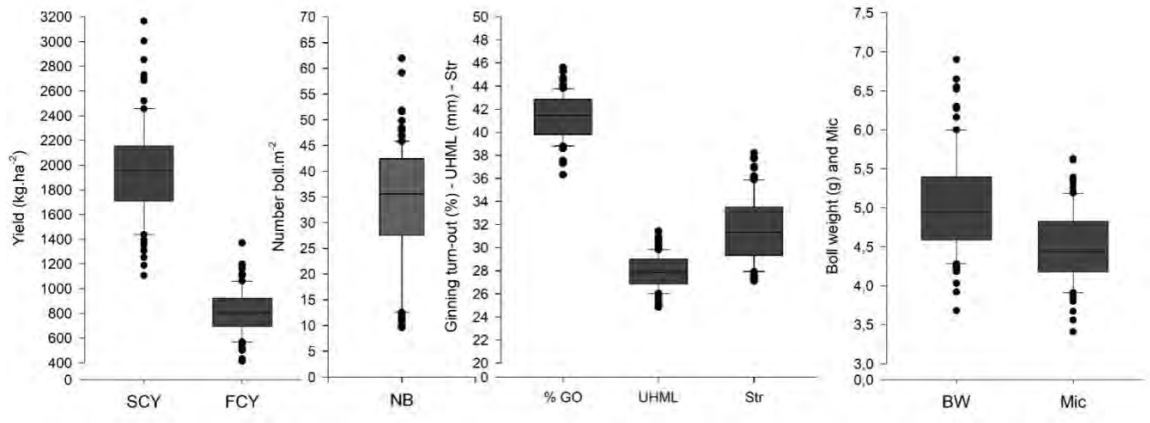
Degree days (DD) required to reach both 1st open flower and 1st open boll for the different cultivars were very stable in the different environments and they presented low variations (Table 2). It can be observed that the cultivar that presented the shortest cycle was DP 402, while the cultivar that presented the longest cycle in all environments was DP 1238. It is also important to note that among all the cultivars available in Argentina, there are no large differences in terms of length of the crop cycle.Lastly, it is important to remark that although late sowing dates presented similar DD to reach the development stages, calendars days in that environments were lower.

Table 2. Average of total day degrees (DD) required to reach 1st flower and 1st boll open for each

Cultivars	DD to 1st Flower	CV (%)	DD to 1st Boll open	CV (%)
DP 1238	745,1	4,2	1472,3	3,0
DP 402	653,4	3,6	1337,3	5,0
G2000	662,9	4,5	1392,5	4,4
Guaraní	663,4	1,9	1429,4	3,3
Guazuncho 4	669,9	3,2	1425,3	0,3
NuOpal	720,9	3,0	1439,0	2,6
Porá 3	709,0	5,7	1437,8	1,5

cultivar. CV: variation coefficient (%)

In Figure 2, through box plots, it can be seen the variation for all genotypes in the 6 environments evaluated. SCY presented a median of 1958 kg.ha-1, with minima of 1105 and maxima of 3165, FCY varied between 412 and 1371 kg.ha-1, showing a median of 801 kg.ha-1. Concerning the % GO, it presented a median of 41.5 %, varying between 36.3 and 45.6 %. It is important to note here that this is the parameter that had the greatest variation among cultivars and it was evaluated after a manual harvest, this is because, if it is compared with the national average, it seems to be above it.
For the yield components, it can be seen that NB was the one that showed the greatest variation, with a median of 35 and a minimum and a maximum of 10 and 62, respectively. On the other hand, BW had a median of 5 g, with a minimum of 3,7 and a maximum of 6,9. When observing some of the fiber quality parameters, median values of 27.8, 4.4 and 31.3 were presented for UHML, Mic and Str, respectively. Besides, it can be noted that Mic is presented greater variation, with a minimum of 3.4 and a maximum of 5.6. To Str and UHML, they registered minimum values of 27 or 24.8 and maximum values of 38 and 31.4, correspondingly.



Notes: Box plots (n=90) for seed cotton yield (SCY), fiber cotton yield (FCY), number of bolls (NB), ginning turn out (% GO), Upper half mean length (UHML), fiber strength (Str), boll weight (BW) and micronaire.

Figure 2: *Genotypes characteristics in six environments*

FCY presented significant and positive correlations with % GO, SCY, NB, WB, Mic and Rad, while with Rf, Tmax, Tmed and Tmin presented negative correlations. % GO as well as FCY registered positive correlations with SCY, NB, WB, Mic and negative correlations with Tmed, Tmax, Tmin and Rf. SCY, like FCY and % GO, showed significant positive correlations with NB, WB and Rad, whereas negative correlations with Tmed, Tmax, Tmin and Rf (Table 3).

Table 3. Pearson correlation table among the variables analyzed in the experiments.

	Tmed (º C)	Tmin (º C)	Tmax (º C)	Rf (mm)	Rad (MJ.m-2)	Mic	Str	UHML (mm)	WB (g)	NB (n.m-2)	SCY (kg.ha-1)	% GO	FCY (kg.ha-1)
Tmed	1	+0,90***	+0,76***	+0,14ns	-0,25**	-0,49**	-0,04ns	+0,01ns	-0,65***	-0,37***	-0,62***	-0,61***	-0,68***
Tmin		1	+0,40**	+0,48***	-0,63***	-0,29**	-0,06ns	+0,05ns	-0,49***	-0,67***	-0,71***	-0,57***	-0,74***
Tmax			1	-0,42***	+0,42***	-0,60***	+0,01ns	-0,05ns	-0,64***	+0,22*	-0,25*	-0,43***	-0,32**
Rf				1	-0,83***	+0,25*	+0,16ns	+0,28**	+0,2ns	-0,81***	-0,34**	-0,07ns	-0,30**
Rad					1	-0,18ns	-0,01ns	- 0,13ns	-0,02ns	+0,82***	+0,50***	+0,18ns	+0,48***
Mic						1	-0,06ns	- 0,09ns	+0,47***	-0,15ns	+0,19ns	+0,39**	+0,25*
Str							1	+0,62***	+0,05ns	+0,01ns	+0,11ns	+0,04ns	+0,11ns
UHML								1	-0,05ns	-0,22*	-0,09ns	-0,07ns	-0,09ns
WB									1	-0,12ns	+0,33**	+0,43***	+0,39***
NB										1	+0,67***	+0,33**	+0,65***
SCY											1	+0,44***	+0,98***
% GO												1	+0,60***
FCY													1

Notes: Medium temperature during PC (Tmed),maximum temperature during PC (Tmax), minimum temperature during PC (Tmin), rainfall during PC (Rf), global radiation during PC (Rad), seed cotton yield (SCY), fiber cotton yield (FCY), number of bolls (NB), ginning turn out (% GO), Upper half mean length (UHML), fiber strength (Str), boll weight (BW) and micronaire (Mic).

*= p<0.05, **= p<0.01, **= p<0.001.

At this point, it can be seen that both NB and WB were significant for SCY, but NB correlated more strongly, therefore, it was the yield component that best explained SCY. NB correlated significantly and positively with Rad and Tmax, while negatively with UHML, Rf, Tmin and Tmed. On the other hand, WB correlated positively only with Mic and negatively and like most of the parameters with Tmed, Tmax and Tmin.

Mic was the parameter with the highest number of significant correlations, both with yield components and environmental measurement evaluated. This could be explained by the fact that Mic is more dependent on the environment, whereas UHML and Str are more dependent on the genotype. On the other hand, a high positive correlation between UHML and Str can be highlighted. Concerning environmental variables, Rad correlated significantly and positively with Tmax and negatively with Tmed, Tmin and Rf. Rf presented a positive correlation with Tmax, while negative with Tmed and Tmin. Finally, temperatures correlated positively and significantly.

Discussions

This is the first rigorous experiment carried out in Argentina that quantifies the effects of rainfalls, solar radiation and temperatures during the CP of cotton crop in several genotypes and seasons. With the data collected and analyzed the effects of environmental conditions present during the CP on yield components and fiber quality were partially demonstrated. It is necessary to explain, that there were also significant correlations with other periods, but the coefficients of determination were lower, therefore, they were not included in the paper.

Through the different seasons in which the experiments were carried out, it had the particularity of recording, on the one hand, in the 2017/18 season, the driest season in history (1970-2020) with only 300.1 mm registered from October to February. On the other hand, in the subsequent season (2018/19), January was registered as the highest rainfall record in history with 473.1 mm in 31 days. The differences in rainfall also generated important modifications in terms of solar global radiation and temperature, which made it possible to see the wide variation in terms of results in the different environments evaluated.

Degree days (DD) to reach the stages of both 1st open flower and 1st open mouth agree with Constable and Shaw (1988) in their review in Australia. Also, in this work, DD required to reach the different stages were constant. This does not coincide with Hebbar et al. (2002) who found that DD required during flowering to boll opening was significantly reduced with delayed dates of sowing. The effect of higher temperatures during the crop cycle on late sowing dates caused development stages to be reduced (data not show). This decreased the period of boll filling and could have an effect on the decrease in yield. This is in according with Hodges et al. (1993) who observed that most of the shortening of development time occurs during the boll growth period, resulting in smaller bolls, lower yields, and poor quality lint.

The wide variability observed in the results can be explained by the difference in environmental data recorded in the seasons evaluated. This made that, environmental or year component, was the one that contributed most to the sum of squares in most of the parameters analyzed in the ANOVA (data not shown). These results agree whitKrieg (1997), who reported that about 70% of the variation in yield

from year to year is dependent upon the environment and only 30% of the variation is subject to management.

FCY correlated positively and highly significantly with both SCY and % GO. NB contributed to a higher proportion of SCY than WB. Positive and highest impact of NB than WB on seed cotton yield has also been identified in other studies (Ali et al., 2009; Azhar et al., 1999; Rauf et al., 2004). Yield components variables did not present significant correlations with the fiber quality components as UHML and Str.

UHML was strongly positively correlated with Str, but they do not show correlation with Mic. Many are the works that show positive correlations between UHML and Str, and negative correlations with Mic (Asif et al., 2008; Basal et al., 2009; Karademir et al., 2010). In the case of this paper, probably the combined variability effect of both environment and genotypes on Mic hid the negative associations of UHML and Str with Mic. The effect of the environment on Mic can be demonstrated because Mic was the fiber quality parameter significantly affected by temperature, solar radiation and precipitation.

Concerning the relationship between the analyzed environmental variables, yield and its components, it was observed that Rad increased FCY and its components. This can be explained with the study realized by Reddy et al. (1996) who demonstrated that photosynthesis of fruiting cotton plants increased as CO2 and solar radiation increased. Furthermore, late sowing dates experienced a shorter reproductive period due to increased air temperatures and reduced canopy photosynthesis due to less radiation interception (Khan et al., 2017).

The negative effect of temperature on yield and its components coincides with that explained by Oosterhuis (1999) who found a negative correlation between yield and high temperature during flowering and early boll development. Furthermore, excessively high temperatures decreased seed size, fibers per seed, and fiber length (Oosterhuis, 1999). Additionally, Singh et al. (2007) demonstrated that high temperature had a strong negative correlation with lint yields, with yields decreasing about 110 kg. ha-1 for each 1 ºC increase in maximum daily temperature.

Besides, the temperature variable that best explained the decrease in yield was Tmin, often associated with night temperature. In this subject and under controlled experiment, Zeiher et al. (1995) demonstrated that the poor boll set was associated with elevated night temperature. Additionally, if one considers the results of Powell (1969), it may be that pollen sterility is related to minimum temperatures rather than maximum temperature. That is if during the critical period the night temperature does not drop low enough after a hot day, sterility results. This idea is supported also, by the results of Fisher (1973) who compared temperature and boll set over 7 years.

Conclusion

Cotton yield, its components and some fiber quality parameters were significantly affected by temperature, solar radiation and rainfall during the CP.

On one part, the effect of global solar radiation, in general, was positive and direct on both components and yield. This means that when higher radiation values were registered, the yield was higher. On the other part, the effect of temperature on yield and their components were negative, which means that when higher temperatures were recorded, the yield was lower.

Further studies should be carried out to analyze the specific effects of each of the environmental factors on the yield and fiber quality of cotton in narrow furrow systems.

Methods

The experiments were carried out at the research station of the National Institute of Agricultural Technology (INTA), located in Reconquista (29°15'56.19"S, 59°44'32.14"O), Santa Fe Province, Argentina, during three-seasons, from 2017 to 2020 involving different sowing dates and different genotypes (Table 4).

Each experiment was arranged in a randomized complete block design with 3 replications. Each plot consisted of eight rows (10 m long) and 52 cm distance between rows. The cotton seeds were mechanically sown by using cotton seeder. Seed rate was approximately 30 kg.ha-1, after emergence, plants were thinned to eleven plants per meter when the seedling had three true leaves, thus seed density per hectare was 200.000 pl.ha-1.

The soil was an Aquertic Argiudoll (Science and Administration, 1975) with silt loam texture and belonging to the Soil Order Mollisols. Their mean properties were: pH = 5.7, organic matter = 1.54 %,

N = 0.1 %, available P = 44.2 kg. ha-1, and available K = 35.5 kg.ha-1. Nitrogen (18 %) and phosphorus (46 %) at the rate of 100 kg of N and P2O5 ha-1 were applied. Additional nitrogen (50 kg N.ha-1) was applied30 days after planting. Fertilizer application, control of insects and weeds were similar in all environment and they were practiced during the growing season according to the local recommendations. All the environments were cultivated under rainfall conditions.

Table 4. Description of different environments and genotypes utilized during the experiment.

Season	Environment	Sowing dates	Genotypes
2017/18	I	17/10	DP 402 – DP 1238 – NuOpal – Guazuncho 2000
	II	31/10	
	III	13/11	
	IV	28/11	
2018/19	V	17/10	DP 402 – DP 1238 – NuOpal – Guazuncho 2000 – Guarani – Guazuncho 4 – Porá 3
2019/20	VI	10/10	DP 402 – DP 1238 – NuOpal – Guazuncho 2000 – Guarani – Guazuncho 4 – Porá 3

The treatments included seven genotypes: Guazuncho 2000 RR (INTA), NuOpal BG/RR (Monsanto), DP 402 BG/RR (Delta Pine), DP 1238 BG/RR (Delta Pine), Guarani BG/RR (INTA), Guazuncho 4 BG/RR(INTA), Porá 3 BG/RR (INTA). The cultivars employed for the experiments were representative of the most widely used by Argentine cotton farmers.

Meteorological measurements as temperature, solar radiation and precipitation were daily taken from a meteorological station located 100 meters from the experiment site. The date of the phenological events of 1st open white flower and 1st open bollwas taken. Then the day degrees (DD) were calculated using the base temperature of 12ºC (Constable and Shaw, 1988).

Seed-cotton yield (SCY) was collected after a hand-harvest in an area of 8 m2 on the center two rows of each plot. To calculate the yield components, 25 bolls were randomly selected within the harvested area and weighed to calculate the average weight per boll (BW). Then, using the data of BW and total weight harvested in each plot, the number of bolls per unit area (NB) was estimated. Lint percentages (% GO) and fiber-cotton yield (FCY) were calculated after using a 10-saw laboratory gin with 250 grams' samples of the harvested seed cotton. After ginning, 50 grams' lint samples were used for determination of various quality parameters. Fiber samples were tested for the fiber quality traits: upper-half mean length (UHML), strength (Str) and micronaire (Mic) using a high volume instrument (HVI) system (APPA lab).

Several correlation and regression analyses, between yield components and environmental conditions, were performed using InfoStat computer software (Di Rienzo et al., 2011), to determine the critical period (CP) where the genotypes' yield was most affected for environmental conditions. Meteorological measures were evaluated on one side with the average of maximum temperature (Tmax), minimum (Tmin) and medium or average (Tmed) for the period and on the other side with the sums of precipitation (Rf, in mm) and global solar radiation (Rad, in MJ.m-2) for the same period. The CP most critical was selected with correlation models based on subject considerations and statistical criteria such as adjusted r2(Neter et al., 1996). Best adjustment between yield, fibre quality and environmental conditions were obtained with CP of 15 days previous until 15 post first flower opened.

References

1. Ali, H., Afzal, M.N., Muhammad, D. Effect of sowing dates and plant spacing on growth and dry matter partitioning in cotton (Gossypium hirsutum L.). Pak. J. Bot 2009; 41, 2145-2155.
2. Asif, M., Mirza, J.I., Zafar, Y.J.P.J.o.B. Genetic analysis for fiber quality traits of some cotton genotypes. 2008; 40, 1209-1215.
3. Azhar, F., Khan, A., Mahmood, I.J.I.J.o.A. Biology, Path coefficient analysis of some advanced generation progenies of Gossypium hirsutum L. 1999; 1, 1-3.
4. Baker, D.N.J.C.S. Effects of Certain Environmental Factors on Net Assimilation in Cotton 1. 1965; 5, 53-56.
5. Basal, H., Unay, A., Canavar, O., Yavas, I.J.S.J.o.A.R. Combining ability for fiber quality parameters and within-boll yield components in intraspecific and interspecific cotton populations. 2009; 7, 364-374.
6. Bozbek, T., Sezener, V., Unay, A.J.J.A., 2006. The effect of sowing date and plant density on cotton yield. 5, 122-125.
7. Burke, J.J., Wanjura, D.F. Plant responses to temperature extremes, Physiology of cotton. Springer, 2010; pp. 123-128.
8. Conroy, J., Seneweera, S., Basra, A., Rogers, G., Nissen-Wooller, B.J.F.P.B. Influence of rising atmospheric CO2 concentrations and temperature on growth, yield and grain quality of cereal crops. 1994; 21, 741-758.
9. Constable, G., Shaw, A.J.A.P. Temperature requirements for cotton. 3. 1988.
10. Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., González, L., Tablada, M., Robledo, y.C.J.G.I., FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar, 2011. InfoStat versión 2011; 8, 195-199.

11. Fisher, W. Association of temperature and boll set, Proc. Beltwide Cotton Prod. Res. Conf., Phoenix, AZ, 1973; pp. 9-10.
12. Hebbar, K., Venugopalan, M., Rao, M., Gadade, G., Chatterji, S., Mayee, C.J.I.J.o.P.P. Effect of sowing dates and fertilizer levels on phenology, growth and yield of cotton. 2002; 7, 380-383.
13. Hodges, H.F., Reddy, K., McKinion, J., Reddy, V.J.B. Temperature effects on cotton. 1993.
14. Karademir, E., Karademir, Ç., Ekinci, R., Gençer, O. Relationship between yield, fiber length and other fiber-related traits in advanced cotton strains. 2010.
15. Khan, A., Najeeb, U., Wang, L., Tan, D.K.Y., Yang, G., Munsif, F., Ali, S., Hafeez, A.J.F.C.R., Planting density and sowing date strongly influence growth and lint yield of cotton crops. 2017; 209, 129-135.
16. Krieg, D. Genetic and environmental factors affecting productivity of cotton, Proc. Beltwide Cotton Conf., New Orleans, LA, 1997; pp. 7-10.
17. MAGYP, 2020. Ministerio de agricultura ganaderia y pesca.
18. Neter, J., Kutner, M.H., Nachtsheim, C.J., Wasserman, W. Applied linear statistical models. 1996.
19. Oosterhuis, D.M.J.S.R.-U.o.A.A.E.S. Yield response to environmental extremes in cotton. 1999; 193, 30-38.
20. Paytas, M., Tarrago, J. Cotton genotypes performance under rainfed and irrigated conditions in two regions of northern Argentina, World Cotton Research Conference-5, Mumbai, India, 7-11 November 2011. Excel India Publishers, 2011; pp. 309-311.
21. Paytas, M.J., Ploschuk, E.L. Cultivos Industriales. Algodón. 2013.
22. Polley, H.W.J.C.s. Implications of atmospheric and climatic change for crop yield and water use efficiency. 2002; 42, 131-140.
23. Powell, R.J.C.G.R. Effect of temperature on boll set and development of Gossypium hirsutum. 1969; 46, 29-36.
24. Rauf, S., Khan, T.M., Sadaqat, H.A., Khan, A.I.J.I.J.A.B., Correlation and path coefficient analysis of yield components in cotton (Gossypium hirsutum L.). 2004; 6, 686-688.
25. Reddy, K.R., Hodges, H.F., McCarty, W.H., McKinion, J.M. Weather and cotton growth: Present and future. MSU-MAFES. 1996.
26. Science, U.S., Administration, E. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. Soil Conservation Service, US Department of Agriculture. 1975.
27. Singh, R.P., Prasad, P.V., Sunita, K., Giri, S., Reddy, K.R.J.A.i.A. Influence of high temperature and breeding for heat tolerance in cotton: a review. 2007; 93, 313-385.
28. Winkler, H.M., Scarpin, G.J., Dileo, P.N., Paytas, M.J., Fecha de siembra óptima en variedades comerciales de algodón. EEA Reconquista. 2018.
29. Zeiher, C., Matumba, N., Brown, P., Silvertooth, J. Response of Upland cotton to elevated night temperature II, Proceedings of 1995 Beltwide Cotton Conferences, 1995; pp. 4-7.

Evaluation of Cotton Varieties under Organic and Conventional Cultivation Practices

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Abstract

An experiment was conducted during 2017-18 growing season at the Cotton Research Center, Gazipur, Bangladesh to evaluate growth and yield of cotton varieties (Gossypium hirsutum L.) CB-12, CB-14, CB-15, Ispahani-01 and Rupali-1 under organic and conventional cultivation practices. The experiment was conducted in split plot design and replicate thrice, where cultivation practices were assessed in the main plot and variety in the sub-plot. The result showed that seed cotton yield and cotton plant height at all stages (seedling, flowering, boll open and at harvest) lower in organic cultivation practice compared to conventional practice. The reduction of seed cotton yield was estimated from 63.93 to 84.59% and plant height at harvest from 41.53 to 49.95%. This reduction due to the lowest number of sympodial branch plant-1, bolls plant-1 and single boll weight (g) in organic cultivation practice compared to conventional practice. Seed cotton yield of hybrid variety {Ispahani-01 (972.22 kg ha-1) and Rupali-1 (818.36 kg ha-1)} was lower compared to open pollinated variety {CB-12 (2122.53 kg ha-1), CB-14 (2276.23 kg ha-1), CB-15 (2164.66 kg ha-1)} in both production practices due to lowest number of plant population and poor plant establishment. Lower number of harmful insects/plant- i.e. Jassids (Amrasca biguttula), Spodoptera (Spodoptera litura) and Spotted Bollworm (Earias 1itelli) and higher number of beneficial insects/plant- i.e. Ladybird beetle, Spider and Damsel/Dragon fly were counted in organic cultivation practice compared to conventional practice. Organic cultivation practice shortened days to first open boll (153.87 DAS) compared to conventional cultivation practice (164.87 DAS). Fiber quality i.e. Short Fiber Index (SFI) increases but Fiber length (UHML) and Uniformity Index (UI) decreases in organic cultivation practice compared to conventional practice. Cultivation practices had no significant effect on Ginning out turn (GOT).

Keywords: *Cultivation practice, Growth, Seed Cotton Yield, Cotton variety.*

Background

Cotton crop is the major source of income to the small-scale farmers, especially in the ecologically constraint areas-i.e. Charland areas, Hilly areas and Barind Tract areas of Bangladesh. Major portion of cotton are produced in these areas, where cropping intensity (151%) is lower compared to other areas (average 190%) (Anon., 2011; Parvin, et al. 2017). These marginal lands are low in organic matter, soil fertility and water content (Anon., 2012). Conventional cotton cultivation practice consumes huge amount of different chemicals (like- chemical fertilizers, herbicides, growth regulators, defoliators etc.) that reduces soil fertility. Cotton alone consumes 15% of the total global pesticides and 25% of insecticides in the world directly harm to the environment, ecological balance and farmers health and also increases production cost that affects farmer's economy. On the other hand, organically grown cotton without use of GMO seed, synthetic chemical fertilizers, pesticides, growth regulators and defoliant improved soil and environment, human health, eco-balance between pest and beneficial insect and reduce insecticide resistance. It is estimated that organic cotton cultivation reduced production cost by 30-40%. Government of Bangladesh has incorporated sustainable agriculture-i.e. Organic, Good Agricultural Practice (GAP), Integrated Pest Management (IPM), Integrated Crop Management (ICM) etc. in its Agricultural Policy-2018.

Organic cotton is now grown in 19 countries with 1.8 million MT lint in 2017/18 in the world of which China, Tanzania, Uganda, Turkey and the USA were the top five producers (Anon., 2019a). Cotton is not only used as fiber but also used as edible oil for human consumption, oil cake as feed for dairy and fisheries and as fertilizer. All the cotton products and by-products directly or indirectly entered into the human food chain. Bangladesh is the largest cotton consumer and apparel producer in the world, the

value of cotton import was US$ 5.85 billion in 2018 (Karim, 2020). Cotton and its value addition play's vital role in the economy of Bangladesh covers 13% of the GDP and contribute about 86% foreign earnings. Bangladesh imports nearly 8 million bales of cotton every year, mainly from India, the US and from African countries. Of the imports, nearly 6 percent is organic cotton that comes from India and the US. GOTS report shows 73% increase in production and consumption of organic certification in Bangladesh in 2019 (Anon., 2020). On the contrary, domestic production is only 0.17 million bales that meets only 2% of the total demand (Anon., 2019b). With this small production capacity and market demand for organic cotton, Bangladesh can be 100% organic cotton producing country. In view of this, the present study was undertaken with the objectives to evaluate existing cotton varieties for growing under organic cultivation practice compared to conventional practice.

Results and Discussions

Plant height of cotton was significantly affected by cultivation practices at different growth stages of cotton. In all growth stages plant height of cotton was reduced significantly in organic cultivation practice compared to conventional cultivation practice. Among the variety CB-14 performed better growth both in organic cultivation practice and conventional cultivation practice in all growth stages of cotton (Table 1).

The reduction in plant height between cultivation practices was estimated from 41.53 to 49.95%. This reduction in plant height may occur due to lower accumulation of plant nutrient and other growth factors by cotton plants in organic cultivation practice (Table 2).

Both Sympodial and monopodial branches of cotton plant significantly differed within the cultivation practices and among the varieties. Organic cultivation practice produced lower number of sympodial (9.79 Plant-1) and monopodial (0.40 Plant-1) branches in cotton compared to conventional cultivation practices. Among the variety CB-14 showed highest number of sympodial branches (14.22 Plant-1) and CB-15 produced highest number of monopodial branches (1.03 Plant-1). On the other hand, Rupali-1 produced lowest number of sympodial (11.82 Plant-1) and monopodial branches (0.27 Plant-1). Number of cotton plants ha-1 had no significant difference among the cultivation practices but significant difference was observed in plant number among the varieties. Highest number of cotton plant (29861.10 plants ha-1) was counted in open pollinated CB-14 variety and lowest (12861.10 plants ha-1) from hybrid variety Ispahani-01. It was observed that heavy rainfall during cotton sowing and seedling stage affects germination and plant establishment of cotton hybrids (Rupali-1 and Ispahani-01) both in organic and conventional cultivation practices compared to open pollinated cotton varieties (CB-12, CB-14 & CB-15). Days to first open boll have significant effect on cultivation practices. Organic cultivation practice shortened days to first open boll (153.87 DAS) compared to conventional cultivation practice (164.87 DAS) (Table 3).

Table 1: Effect of cultivation practice and variety and their interaction on plant height in cotton.

Treatments	Plant height (cm) at different stages			
	@seedling	@flowering	@boll open	@harvest
Cultivation practice				
Organic	10.24	63.47	72.75	81.45
Conventional	10.86	122.06	138.07	150.75
LSD (0.05)	0.327	6.50	4.39	3.48
Variety				
CB-12	10.92	91.45	101.46	114.63
CB-14	11.27	103.52	116.53	128.15
CB-15	10.80	88.03	100.70	108.55
Ispahani-01	10.15	87.40	103.67	108.35
Rupali-1	9.62	93.43	104.70	120.82
LSD (0.05)	0.52	10.28	6.94	5.50
Interaction				
CP*VAR	0.73	NS	NS	7.78

Table 2: Interaction effect of cultivation practice and variety on plant height in cotton at harvest.

Variety	Cultivation Practice		Reduction in plant height as cultivation practice (%)
	Organic	Conventional	
CB-12	77.07	152.20	49.36
CB-14	92.37	163.93	43.65
CB-15	80.10	137.00	41.53
Ispahani-01	77.13	139.57	44.74
Rupali-1	80.60	161.03	49.95

Table 3: Effect of cultivation practice and variety and their interaction on different morphological parameters in cotton.

Treatments		Number of plants ha-1	Sympodial branch plant-1	Monopodial branch plant-1	1st open boll (days after sowing)
Cultivation practice					
Organic		24444.40	9.79	0.40	153.87
Conventional		23549.40	15.63	1.42	164.87
	LSD (0.05)	NS	1.01	0.43	1.947
Variety					
CB-12		28858.00	13.27	0.95	157.67
CB-14		29861.10	14.22	0.82	158.67
CB-15		29759.30	12.25	1.03	159.00
Ispahani-01		12861.10	12.01	0.58	160.67
Rupali-1		18645.10	11.82	0.27	160.83
	LSD (0.05)	1707.45	1.59	NS	NS
Interaction					
CP*VAR		NS	NS	NS	4.353

Number of bolls plant-1, single boll weight (g) and boll number per sympodial branch also significantly affected by cultivation practices and lowest Number of bolls plant-1 (7.45) single boll weight (4.90 g) and bolls per sympodial branch (0.77) was recorded from organic cultivation practice compared to conventional cultivation practice. Among the variety number of bolls plant-1 was also differed significantly and highest (15.33) bolls plant-1 was recorded from CB-12 and the lowest (12.02) from Rupali-1. Cotton hybrid varieties (Rupali-1 and Ispahani-01) showed lowest number of boll plant-1 both in organic and conventional cultivation practices compared to open pollinated varieties (CB-12, CB-14 & CB-15) (Table 4).

Table 4: Effect of cultivation practice and variety and their interaction on different yield variables in cotton.

Treatments		Bolls plant-1	Bolls sympod-1	Single boll weight (g)	Seed cotton yield (kg ha-1)
Cultivation practice					
Organic		7.45	0.77	4.90	761.67
Conventional		20.59	1.33	5.17	2579.94
	LSD (0.05)	0.94	0.102	0.25	184.63
Variety					
CB-12		15.33	1.08	4.87	2122.53
CB-14		14.93	1.02	5.17	2276.23
CB-15		13.98	1.09	5.08	2164.66
Ispahani-01		13.85	1.07	5.02	972.22
Rupali-1		12.02	0.97	5.05	818.36
	LSD (0.05)	1.49	NS	NS	291.93
Interaction					
CP*VAR		NS	4.08	NS	412.85

Seed cotton yield was significantly influenced by cultivation practices, variety and their interaction. Highest seed cotton yield was recorded from conventional cultivation practice (2579.94 kg ha-1) and lowest from organic cultivation practice (761.67 kg ha-1). Seed cotton yield was reduced from 63.93 to 84.59% in organic cultivation practice compared to conventional cultivation practice. Among the varieties highest seed cotton yield was recorded from conventional cultivation practice compared to organic cultivation practice. This highest seed cotton yield was contributed by number of bolls plant-1, single boll weight (g), bolls per sympodial branch and number of sympodial branch plant-1. Among the

varieties lowest seed cotton yield (818.36 kg ha-1) was recorded from hybrid variety Rupali-1 and highest (2276.23 kg ha-1) from open pollinated variety CB-14, which is statistically similar with CB-12 and CB-15. (Table 4 and Table 5).

Table 5: Interaction effect of cultivation practice and variety on seed cotton yield.

Variety	Cultivation Practice		Yield loss as cultivation practice (%)
	Organic	Conventional	
CB-12	981.17	3263.89	69.94
CB-14	1167.28	3412.04	65.79
CB-15	1140.43	3162.04	63.93
Ispahani-01	300.926	1643.52	81.69
Rupali-1	218.519	1418.21	84.59

Number of harmful insects plant-1 were significantly influenced by the cultivation practices. The Lowest number of harmful insects plant-1- i.e. Jassids (Amrasca biguttula), Spodoptera (Spodoptera litura) and Spotted Bollworm (SBW) (Earias vitella) were recorded from organic cultivation practice compared to conventional cultivation practice. Abundance of beneficial insects were also influenced by the cultivation practice. The highest number of beneficial insect plant-1- i.e. Ladybird beetle (LBB), Spider and Damsel/Dragon fly were counted in organic cultivation practice compared to conventional practice. This lower number of harmful insects in organic cultivation practice due to higher presence of beneficial insects and avoiding use of synthetic chemicals for insect control (Table 6).

Ginning Out Turn (GOT), Seed index and lint index didn't influence significantly by the cultivation practices and interaction of cultivation practice and variety but these parameters were significantly influenced among the varieties. The highest GOT (43.13%) was recorded from cotton variety Ispahani-01 and lowest (38.56%) from CB-14. Lint index also significantly differed among the varieties, the highest (7.37) was recorded in Ispahani-01 and the lowest (5.98) from CB-14 (Table 7).

Table 6: Effect of cultivation practice and variety and their interaction on Insect population and abundance of beneficial insects.

Treatments	Harmful Insects			Beneficial Insects		
	Jassids	Spodoptera	SBW	LBB	Spider	Dragon/ damsel fly
Cultivation practice						
Organic	0.60	0.27	0.27	1.20	1.20	0.87
Conventional	1.47	1.33	1.33	0.20	0.13	0.13
LSD (0.05)	0.368	0.482	0.368	0.341	0.311	0.241
Variety						
CB-12	0.83	1.00	0.67	0.50	0.50	0.33
CB-14	1.00	0.83	0.67	0.50	0.67	0.50
CB-15	1.17	0.50	0.83	0.83	0.67	0.83
Ispahani-01	0.83	1.00	0.83	0.83	0.83	0.33
Rupali-1	1.33	1.17	1.00	0.83	0.67	0.50
LSD (0.05)	NS	NS	NS	NS	NS	0.381
Interaction						
CP*VAR	NS	NS	NS	NS	NS	NS

Table 7: Effect of cultivation practice and variety and their interaction on different ginning characters in cotton.

Treatments	Seed index	Lint Index	Ginning out turn (GOT%)
Cultivation practice			
Organic	9.23	6.68	41.26
Conventional	9.57	6.86	41.16
LSD (0.05)	NS	NS	NS
Variety			
CB-12	9.43	6.42	40.27
CB-14	9.40	5.98	38.56
CB-15	9.60	6.95	41.42
Ispahani-01	9.47	7.37	43.13
Rupali-1	9.10	7.14	42.69
LSD (0.05)	NS	0.57	0.77
Interaction			
CP*VAR	NS	NS	NS

Fiber quality of cotton i.e. Upper Half Mean Length (UHML), Uniformity Index (UI%) and Short Fiber Index (SFI) were significantly influenced by cultivation practices and organic cultivation practice produce lower UHML, UI and SFI. But other fiber characters like- fiber strength (Str.), fiber elongation (%) and fiber fineness (µg/inch) didn't influence by cultivation practices. All the fiber quality parameters of cotton like- UHML, UI, SFI, Strength, Elongation. and Micronaire were significantly differed among the varieties. CB-14 showed highest fiber length (31.11 mm), uniformity (84.71%) and elongation (6.61%), also cotton hybrid Rupali-1 showed highest short fiber index (8.68) and lowest elongation (5.96%) and better fiber fineness (4.14 µg/inch) compared to other varieties. Fiber strength (33.54 g/tex) was highest in CB-15 and lowest (29.84 g/tex) in cotton hybrid Ispahani-01. But the interaction of cultivation practice and variety didn't have any significant effect on the cotton fiber quality parameters (Table-8). Swezey and Goldman (1996) also studied the effect of organic growing conditions on fiber quality and didn't found any differences in fiber length, strength and micronaire. However, organic cotton had a higher percentage of spotted cotton. But literature shows that, if nitrogen is not applied to cotton when needed, micronaire is enhanced and staple length reduced which is similar with finding of the present study.

Table 8: Effect of cultivation practice and variety and their interaction on different fiber characters in cotton.

Treatments	UHML (mm)	UI (%)	SFI	Str. (g/tex)	Elong. (%)	Mic. (µg/inch)
Cultivation practice						
Organic	29.32	83.23	8.17	31.71	6.24	4.49
Conventional	30.33	84.09	7.68	31.83	6.35	4.38
LSD (0.05)	0.51	0.45	0.26	NS	NS	NS
Variety						
CB-12	29.99	84.05	7.58	31.76	6.30	4.72
CB-14	31.11	84.71	7.25	33.46	6.61	4.29
CB-15	30.36	84.13	7.63	33.54	6.50	4.55
Ispahani-01	28.78	82.65	8.49	29.84	6.11	4.47
Rupali-1	28.88	82.75	8.68	30.24	5.96	4.14
LSD (0.05)	0.80	0.70	0.406	1.42	0.28	0.267
Interaction						
CP*VAR	NS	NS	NS	NS	NS	NS

UHML-fibre length, UI-uniformity, SFI-Short fiber index, Str.-fibre Strength, Elong.-fibre elongation, Mic.-micronaire (fibre fineness)

Conclusions and Recommendations

Open pollinated cotton variety CB-14 can be recommended for organic cultivation practice. Further assessment should be done on plant spacing, source and dose of organic fertilizer. This production practice can be applied in the Chittagong Hill Tracts of Bangladesh. In these areas cotton have been growing from times immemorial and farmers are accustomed in traditional cultivation practice following organic practice in crop protection and management. Also, cropping intensity (139%) is in these areas are lower and lands are comparatively less polluted compared to other areas of the country.

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Methods

The experiment was conducted at the Cotton Research Centre, Gazipur, Bangladesh during 2017-2018 growing season to evaluate cotton varieties feasible for growing under organic cultivation practice compared to conventional cultivation practice. The location of the experimental site was high land belongs to the Salna series and is classified as Shallow Red-Brown Terrace type which falls under the order Inceptisols of soil taxonomy and located between 24.09o N latitude and 90.26o E longitude with an elevation of 8.4 meter above the sea level under the Agro Ecological Zone of Madhupur Tract (Anon., 2012; Brammer, 1996). Soil of the experimental field was loamy, soil pH 6.6, organic matter content 1.01, Total N% 0.051, K 0.29 meq/100g soil, Mg 2.68 meq/100g soil, P 12.68 µg/g soil, S 4.70 µg/g soil, B 0.16 µg/g and Zn 6.35 µg/g soil. The experiment was laid out in split plot design and replicated thrice.

Five cotton varieties (open pollinated variety CB-12, CB-14, CB-15 and hybrid variety Ispahani-01 and Rupali-1 were used) and two cultivation practices viz. i) organic cultivation practice without synthetic chemicals and conventional cultivation practice with synthetic chemicals. The cultivation practice arranged in the main plot and varieties in the sub-plot. The treatments of the experiment are as follows.

Factor-A: Cultivation Practice (main plot)

CP-1= Organic cultivation practice and

CP-2= Conventional cultivation practice

Factor-B: Variety (Sub/split plot)

V1 = CB-12

V2 = CB-14

V3 = CB-15

V4 = Ispahani-1

V5 = Rupali-1

The unit plot size of the experiment was 3.6m×6.0m (21.6 m-2) maintaining 2.5m distance between main plot and 1m between subplot. Fuzzy cotton seed of variety CB-12, CB-14 and CB-15 and delinted (without fuzz) seed of Ispahani-01 and Rupali-1 was sown on 02 August 2017 in the experimental plots. The experimental land was prepared finely by tractor using one disc and two harrows. The experimental field was fertilized as per cultivation practices. Conventional cultivation practice plot was fertilized at basal with 3 tons of cowdung ha-1 during plowing and recommended N, P, K, S, Zn and B at the rate of 26.0-37.5-37.5-9.0-6.0-1.0 kg ha-1, respectively were incorporated into the soil before sowing as urea, triple super phosphate, muriate of potash, gypsum, zinc sulphate and borax. Remaining recommended dose of N, K and B were applied at the rate of 26.0-56.0-1.0 kg ha-1 at 25 DAS, 26.0-56.0-0.5 kg ha-1 at 40 DAS and 26.0-37.5-0.5 kg ha-1 at 60 DAS. Also remaining recommended dose of P and S were applied at 40 and 60 days after sowing at the rate of 22.5-10.0 and 7.5-7.0 kg ha-1 respectively (Anon, 2016). On the other hand, Organic cultivation practice plots were fertilized with 4 tones cowdung, 1.5 tones wood ash, 0.5 tones poultry litre, 0.5 tones cotton seed oil cake, 150 kg bone meal and 2.5 kg Azotobacter per hectare during land preparation. Green manuring was done by using sun hemp (Crotalaria juncea) in the experimental field a month before cotton sowing. Thinning and other intercultural operations were done as and when necessary. Cotton crop in the conventional practice was sprayed with Hychem and Cuplan (Thiamethoxam & Chlorantraniliprole) to control bollworm and Hymedor (Imidacloprid 200 SL) and Suntap (Thiocarbamate) to control jassid and other sucking insects using Knapsack sprayer at seedling stage and power sprayer at flowering to boll open stage. Also fungicide Proud 25 EC (Propiconazole) was sprayed to control boll rot disease. But the insect pest in the organic field was controlled by using pheromone trap, yellow trap, molasses trap and spraying botanical insecticides and mahogany (Swietenia mahagoni) seed extract. Sunflower (Helianthus annuus), marigold (Tagetes sp.) and maize (Zea mays) seed was sown on the border of organic plots and one row mungbean was intercropped between two rows of organic cotton and incorporated in the soil after mungbean harvest. Plant height of cotton was recorded at seedling, flowering, boll open and at harvest. Yield and yield contributing characters of cotton including boll number plant-1, single boll weight (g), 100 seed weight (g), number of monopodial and sympodial branch plant-1, seed cotton yield (kg) was recorded. Seed index, lint index and Ginning out turn was calculated using the following formula-

$$\text{Ginning out turn (\%)} = \frac{\text{Weight of lint}}{\text{Weight of seed cotton}} \times 100$$

Seed index = Weight of 100-seed

$$\text{Lint Index} = \frac{\text{Weight of lint}}{\text{Weight of seed}} \times \text{Seed index}$$

All the data were analyzed following analysis of variance (ANOVA) technique using CROP-STAT software. Means were separated by Least Significance Difference (LSD) test at 5% level of significance (Gomez and Gamez, 1984).

References

1. Anonymous. 2020. GOTS Annual Report-2019, Global Organic Textile Standard (GOTS), https://www.global-standard.org/information-centre/news/355-annual-report-2019-released.html.
2. Anonymous. 2019a. Organic Cotton Market Report-2019, Textile Exchange, https://textileexchange.org/wp-content/uploads/2019/11/Textile-Exchange-Organic-Cotton-Market-Report-2019.pdf.
3. Anonymous. 2019b. Tula Gobeshona o Samprasaron Pratibedon-2018-19 (Cotton Research and Extension Report-2018-19). Cotton Development Board, 5th & 6th Floor, 3rd Building, Khamarbari, Farmgate, Dhaka, Bangladesh.
4. Anonymous. 2016. Tula Gobeshona o Samprasaron Pratibedon-2016-17 (Cotton Research and Extension Report-2016-17). Cotton Development Board, 5th & 6th Floor, 3rd Building, Khamarbari, Farmgate, Dhaka, Bangladesh. P. 1-136.
5. Anonymous. 2012. Fertilizer Recommendation Guide-2012. Bangladesh Agricultural Research Council, Dhaka, Bangladesh. P-274.
6. Anonymous. 2011. Cropping Intensity, 1980-81 to 2010-11, Bangladesh, Bangladesh Bureau of Statistics (BBS) Parishankhyan Bhaban, Agargaon, Dhaka-1207, Bangladesh
7. Brammer H., 1996. The Geography of Soils of Bangladesh, University Pub. Ltd., Dhaka Gomez , K.A. and A.A. Gomez. 1984. Statistical Procedure for Agricultural Research. 2nd edn. John Willey and Sons. Inc. New York. P. 304-307.
8. Karim, R., 2020. MOFA for Relaxing Fumigation Test of Cotton Imported from USA, The
9. Financial Express, Tropicana Tower (4th floor), 45, Topkhana Road, GPO Box : 2526 Dhaka-1000, Bangladesh.
10. Khokon, M. A., 2020. Presidents Report, Cotton, Yarn & Fabrics, BTMA Newsletter, March, 2020, https://www.btmadhaka.com/newsletter-march-2020/.
11. Parvin, N., A Khatun, M. K. Quais and M. Nasim. 2017. Cropping Pattern, Intensity and Diversity in Dhaka Region, Bangladesh Rice J. 21 (2) : 123-141, 2017
12. Swezey, S. L. and P. Goldman. 1996. Conversion of cotton production to certified organic management in the Northern San Joaquin Valley: Plant development, yield, quality and production costs. Proceedings of the Beltwide Cotton Conferences, National Cotton Council of America, P. O. Box 12285, Memphis, TN 38182, USA.

Impact of Mineral Amendments and Cotton Compound Fertilizer Enriched with CaO on Cotton Yield

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Abstract

Background: *Tropical ferruginous soils are known to be fragile with low levels of organic matter, high levels of sand, and low cation exchange capacity. Improving these different parameters is the key to improve yield. The objective of this study is to improvecrop productivity. To achieve this objective, a trial was installed in thirty producers field during two campaigns (2018-2019 and 2019-2020) in different agro-ecological zones with rainfall range between 550 to 1250 mm with different types of amendments (Tilemsi Rock Phosphate (TRP) and agricultural lime) and two types of cotton compound fertilizer: 14N - 18P2O5 - 18K2O + 6S +1B2O3and 14N - 18P2O5 - 18K2O + 5S + 1B2O3 + 2.5CaO.*

Results: *Use ofamendments improved soil pH in all producers field. Spatial and temporal distribution of rainfall was not good during campaigns. In 2018-2019, control treatment with recommended mineral fertilization (RMF): 200 kg of cotton compound fertilizer ha-1 plus 50 kg of urea ha-1yield was 1338 kg ha-1. Using TRP at 300 kg ha-1 plus RMF resulted in increased yield in all areas. The difference was 141 kg ha-1, or 10.54% increase over control yield. In contrast, yield increasedwith 1000 kg of Stones agricultural lime ha-1plus FMR was 69 kg ha-1, or 4.90% over the control. In 2019-2020, control treatment yield was 1407 kg ha-1. The use of new cotton compound fertilizer enriched with CaO result in yield increase of 186 kg ha-1, or 13% increase over the control. TRP at 300 kg ha-1 plus RMF resulted in yield increase of 140 kg ha-1 or 10%. Stones agricultural lime plus RMF was 128 kg ha-1, or 9% increase over the control.*

Conclusion: *The new cotton compound fertilizer enriched with CaO and mineral amendments helps to correct soil acidity and improve cotton yield.*

Keywords: *Amendment, ferruginous soils, soil acidity, productivity, producers, Mali*

Background

The cotton sector plays an essential role in Mali's economic and social activities in terms of income generation, organization of rural world and modernization of production systems. Nearly 4 million producers live off cotton revenues and benefit from the backward effects of cotton system (FAO, 2013). According to the same source, cotton accounts for 8.2% of country's Gross Domestic Product (GDP).

Although seedc otton yields have gone from 225 kg ha-1 in 1961/1962 to 1344 kg ha-1 in 1990/1991, since then, a drop in yields has been noted with an average of 941 kg ha-1 over the last ten years (CMDT, 2018). At present, in cotton zone, production increase is linked to area increase, while yield keeps on decreasing. This decline concern various stakeholders in the cotton sector (Sissoko, 2009).

In Mali, cotton is grown on tropical ferruginous soils which generally have a natural tendency to acidification, i.e. the replacement of mineral cations by H+ ions. This soil acidification has two adverse effects on plants: the solubilization of aluminium and the loss of cationic nutrients in the soil. Work carried out by Cotton Program (2012) has shown that 17% of soils in cotton zone have a pH below 5.5, threshold below which acidity significantly reduces crop growth and yields (Kamprath, 1970). The rest of soils (83%) have a pH less than or equal to 6.5 and are therefore considered acidic.

Among the factors that can contribute to lower yields, soil pH plays a very important role. Common solutions for acidity correction consist of adding limestone (CaCO3), CaO or Ca(OH)2, which are more effective when the economic situation of farmer allows it. Calcium (Ca++) alone accounts for 70% of

sum of exchangeable cations (Dabin, 1985). The most commonly used method is liming (McLean, 1971), which increases the calcium content and pH of the soil and stimulates microbial growth, which has a positive impact on the availability of nitrogen as well as other nutrients. The evaluation of the effect of agricultural lime or dolomite with a content of 15-23% MgO and 30% CaO brought to the cotton-growing area of Mali during the 2014-2015 agricultural season has allowed an improvement in soil acidity of 0.8 points on highly acid soils (pH 4.5) and the increase in yield reached 13.20% with 300 kg ha-1 and 25.76% with 500 kg ha-1 in the farming area (Cotton Program, 2014). Amendments can be used as an alternative for soil acidity correction and yield improvement.

The present study was carried out to evaluate the interest of amendments and the enrichment of cotton compound fertilizer formula in CaO with a view to improving cotton productivity.

Results

Effect of different treatments on the number of plants at harvest

In 2018-2019, the average number of plants per hectare was 52784, or 63% of the theoretical density (83333 plants ha^{-1}). The lowest density was observed in Fana with 44917 plants ha^{-1}, i.e. 53.90%. The best densities were observed in Sikasso. Depending on the treatments, the density varied between 60000 and 66067 plants ha_{-1} (Table 1). For whole CMDT zone, plot densities for the three treatments ranged from 51098 to 54205 plants ha^{-1}. The analysis of variance did not show a significant difference between treatments for the number of plants per hectare at harvest.

In 2019-2020, analysis of variance showed no significant difference between treatments for the number of plants per hectare at harvest. The average density was 43,335 plants per hectare, 52% compared to the theoretical density (83,333 plants ha-1). The lowest density was observed at Fana with 34 900 plants ha-1 and the highest density was observed at Bougouni with 56 917 plants ha-1 , i.e. 68.30% (Table 2). For the whole CMDT area, densities in the plots of the four treatments varied between 41,561 and 44,562 plants ha-1. Densities were lower in the 2019-2020 season

Table 1: Average number of plants at harvest in the plots of the different treatments during the 2018-2019 season

Treatment	Bougouni	Sikasso	Koutiala	Fana	Kita	Moyenne CMDT
T1	48,542	63,900	49,000	46,792	61,333	54,205
T2	47,667	66,067	53,417	45,708	45,583	53,049
T3	48,167	60,000	48,833	44,917	49,333	51,098
Probability	0.927	0.267	-	0.878	-	0.237
CV (%)	6.60	8.68	-	11.30	-	10.019
Meaning	NS	NS	-	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: FMR + 1000 kg Stones agricultural lime ha^{-1}; T3: T3: FMR + 300 kg granulated PNT ha^{-1}

Table 2: Average number of seedlings at harvest in the different plots of the different treatments during the 2019-2020 season

Treatment	Bougouni	Sikasso	Koutiala	Fana	Kita	Average CMDT
T1	55,028	39,133	39,389	35,867	38,389	41,561
T2	53,139	37,100	38,500	39,033	48,444	43,243
T3	56,917	38,100	41,778	34,900	46,111	43,561
T4	49,583	45,333	41,972	35,867	50,056	44,562
Probability	0.278	0.277	0.606	0.544	-	0.676
CV (%)	12.01	17.22	13.17	12.76	-	13.9
Meaning	NS	NS	NS	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: 200 kg (14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5 CaO) ha^{-1} + 50 kg urea ha^{-1}; T3: FMR + 300 kg granulated PNT ha^{-1}; T4: FMR + 1000 kg Stones agricultural lime ha^{-1}.

Effect of the different treatments on the average number of bolls per plant

The analysis of variance did not show a significant difference between treatments for the mean number of bolls per plant (Table 3). The mean number of bolls per plant ranged from 12 to 14.

Effect of different treatments on mean boll weight (PMC)

The analysis of variance did not show a significant difference between treatments for PMC. The PMC ranged from 3.2 to 3.8 (Table 4).

Table 3: Average number of bolls per plant in the plots of the different treatments during the 2019-2020 season

Treatment	Bougouni	Sikasso	Koutiala	Fana	Kita	Average CMDT
T1	16.2	13.3	10.1	10.5	11.1	12.2
T2	16.9	16.1	12.2	11.5	12.2	13.8
T3	17.5	14.7	11.1	12.0	14.2	13.9
T4	16.2	13.7	13.8	13.1	13.3	14.0
Probability	0.575	0.255	0.501	0.114	-	0.301
CV (%)	11.29	15.5	13.4	12.92	-	12.9
Meaning	NS	NS	NS	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: 200 kg (14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5 CaO) ha^{-1} + 50 kg urea ha^{-1}; T3: FMR + 300 kg granulated PNT ha^{-1}; T4: FMR + 1000 kg Stones agricultural lime ha^{-1}.

Table 4: Average weight of capsules in the different plots of the different treatments during the 2018-2019 seasons

Treatments	Mean boll weight en (g)					
	Bougouni	Sikasso	Koutiala	Fana	Kita	Average CMDT
T1	3.4	3.6	3.3	3.6	3.2	3.4
T2	3.6	3.8	3.4	3.8	3.3	3.6
T3	3.5	3.6	3.5	3.3	3.4	3.5
Probability	0.182	0.264	-	0.107	-	0.057
CV (%)	4.5	5.68	-	7.74	-	6.27
Meaning	NS	NS	-	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: FMR + 1000 kg Stones agricultural lime ha^{-1}; T3: T3: FMR + 300 kg granulated PNT ha^{-1}

Effect of different treatments on yield

In 2018-2019, the analysis of variance did not show a significant difference between treatments for yield (Table 5). The average yield of fertilizer treatment was 1338 kg ha^{-1}. The use of PNT at the 300 kg ha^{-1} rate resulted in an arithmetic increase in yield in all zones. It was 141 kg ha^{-1} or 10.54% compared to the control. In contrast, the average yield increase with the use of Stones agricultural lime was 69 kg ha^{-1}, or 4.90% compared to the control.

In 2019-2020, the analysis of variance did not show a significant difference between treatments for yield (Table 6). The average yield of the fertilizer treatment was 1407 kg ha^{-1}. The use of the new CaO-enriched fertilizer formula resulted in an arithmetic yield increase of 186 kg ha^{-1}, or 13% compared to the control (extension compound fertilizer). The use of PNT at 300 kg ha^{-1} resulted in a yield increase of 140 kg ha^{-1} or 10% compared to the control. In contrast, the average yield increase with the use of Stones agricultural lime was 128 kg. ha^{-1}, or 9% compared to the control.

Table 5: Yields obtained in the different plots for the different treatments in the 2018-2019 crop year

Treatments	Yield in kg ha^{-1} byfiliale and in Bougouni coordination office					
	Bougouni	Sikasso	Koutiala	Fana	Kita	Moyenne CMDT
T1	1,115	1,422	1,214	1,580	1,358	1,338
T2	1,365	1,498	1,179	1,528	1,467	1,407
T3	1,195	1,473	1,338	1,770	1,617	1,479
Probability	0.267	0.751	0.432	0.356	-	0.138
CV (%)	16.24	11.80	15.49	14.08	-	13.71
Meaning	NS	NS	NS	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: FMR + 1000 kg Stones agricultural lime ha^{-1}; T3: T3: FMR + 300 kg granulated PNT ha^{-1}

Table 6: Yields obtained in the different plots of the different treatments during the 2019-2020 season

Treatments	Yield in kg ha^{-1} by filiale and in Bougouni coordination					
	Bougouni	Sikasso	Koutiala	Fana	Kita	Average CMDT
T1	1,517	1,137	1,041	2,015	1,324	1,407
T2	1,601	1,467	1,155	2,166	1,577	1,593
T3	1,619	1,360	1,121	1,940	1,693	1,547
T4	1,387	1,343	1,213	2,190	1,544	1,535
Probability	0.497	0.315	0.578	0.710	-	0.156
CV (%)	18.52	20.26	18.82	18.78	-	18.12

Meaning	NS	NS	NS	NS	-	NS

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: 200 kg (14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5 CaO) ha^{-1} + 50 kg urea ha^{-1}; T3: FMR + 300 kg granulated PNT ha^{-1}; T4: FMR + 1000 kg Stones agricultural lime ha^{-1}.

Discussions

The spatial and temporal distribution of rainfall was poor during the two agricultural seasons 2018-2019 and 2019-2020. The analysis of rainfall in June, the most favorable month for cotton sowing, showed a very high variability between the decades of the same year, with often decades without rain. The start was difficult and the cumulative rainfall recorded during July, August and September was in excess. Based on data collected from 80 weather stations in Mali, Sivakumar (1988) showed that from July onwards, the frequency and quantity of rainfall increased steadily and exceeded crop evapotranspiration. These water surpluses recorded during this period of wintering affect cotton plant flowering. The soil is often waterlogged and the root system of the cotton plant remains for a long time in excess of field moisture, which can lead to the loss of flower buds and bolls (FAO, 2014). When rainfall is well distributed, the cotton plant needs about 600 mm of water during its crop cycle (Cetin and Bilgel, 2002).

Correction of pH by using both types of amendments was observed in all zones during both seasons. This pH correction by the use of amendments was also observed by Koulibaly et al. (2009) in Burkina Faso. In Rwanda, Nabahungu et al. (2005) also showed that the use of agricultural lime can correct soil acidity.

Density is a very important factor in yield development. However, the number of plants per hectare was low in the different areas during the two campaigns, below 55000 plants ha-1, i.e. 66% of the theoretical density. In a study conducted in Mali, Rapidel et al. (2006) obtained densities varying between 34000 and 48300 plants ha-1. In Burkina Faso, Kabore, (2014) also observed densities varying between 47150 and 52708 plants ha-1. This can be explained by several factors. A late onset of rainfall is observed in many areas, with farmers sowing cotton on soils that are often dry or with insufficient moisture to ensure good emergence of cotton plants. Cultivation operations are poorly assured and working equipment is often of poor quality and most probably the soil bio-physico-chemical conditions. Germination can be affected if soil is very acidic.

An improvement in yield has also been noted with both types of amendments. The use of PNT at 300 kg ha-1 resulted in a 10% yield improvement over the control. On the other hand, improvement with Stones agricultural lime at 1000 kg ha-1 varied between 5 and 10% compared to the control. Soenen et al, (2015) also reported that gypsum as an amendment improves deep rooting and crop yield. Koulibaly et al. (2009) also showed that soil amendments associated with mineral fertilisers improve cotton yield.

Tropical ferruginous soils are fragile and naturally predisposed to acidification. Mineral and/or organic amendments are essential for sustainable farming.

Conclusion

The onset of the cropping campaign has been seldom optimal in Mali. Some plantings were late. Excess water was recorded during certain periods of the season. However, the use of the amendments associated with the recommended mineral fertilization and the new formula of the cotton compound fertilizer enriched with CaO improved pH and yield in all zones.

These amendments and the new formula of the enriched compound fertilizer can be offered to cotton producers.

Methods

Sites

During the two campaigns, the tests were installed in the four subsidiaries and in the Bougouni coordination at 30 producers in CMDT zone. The soils are classified as leached tropical ferruginous soils with low clay (< to 10g/100g), organic matter (< 1 g/100g) and exchangeable bases contents. The cation exchange capacity is low (< 10 $Cmol^+$ /kg). The content of assimilable phosphorus is low (< 10 g/100g). Soils are acidic (pH < 6). Soil pH was improved with Stones agricultural lime, TNP and the CaO-enriched compound fertilizer (Tables 7 and 8). The pH was very often above 6.2 after amendments and enriched fertilizer.

The average rainfall recorded in the different zones is shown in Table 9. There was a high variability both in a cumulative annual rainfall and in a number of rainy days per year. This results in a poor spatial and temporal distribution of rainfall during the different campaigns. Rainfall starting is late and significant amounts are recorded in two to three months during the wintering period.

Table 7: pH measured in the different plots in CMDT zone during the 2018-2019 season

Sites	Earlycampaign	T1 at harvest	T2 at harvest	T3 at harvest
Sikasso	5.5	5.9	6.7	6.5
Fana	6.0	6.0	6.9	6.9
Bougouni	5.6	5.7	7.5	6.7
Kita	5.5	5.7	7.0	6.6

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: FMR + 1000 kg Stones agricultural lime ha^{-1}; T3: T3: FMR + 300 kg granulated PNT ha^{-1}

Table 8: pH measured in the different plots in CMDT zone during the 2019-2020 campaign

Sites	Earlycampaign	T1 at harvest	T2 at harvest	T3 at harvest	T4 at harvest
Sikasso	6.1	6.1	6.2	6.2	6.2
Fana	6.2	6.1	6.2	6.3	6.3
Bougouni	6.2	6.2	6.3	6.3	6.3
Koutiala	6.3	6.2	6.3	6.3	6.3
Kita	5.9	6.0	6.1	6.2	6.2

Notes: T1: 200 kg (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg urea ha^{-1} (FMR); T2: 200 kg (14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5 CaO) ha^{-1} + 50 kg urea ha^{-1}; T3: FMR + 300 kg granulated PNT ha^{-1}; T4: FMR + 1000 kg Stones agricultural lime ha^{-1}.

Genetic materials

The plant material proposed by CMDT was used to conduct the tests. A total of six varieties were used: NTA 93-15, NTA 90-5, NTA MS334, STAM 59A, STAM 279A, BRS 293. Some morpho-physiological, agronomic and technological characteristics of the varieties used are shown in Table 10.

Two types of cotton compound fertilizers have been used: 14N-18P_2O_5-18K_2O+6S+1B_2O_3 the formula currently use and the new formula enriched in CaO, 14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5CaO. Urea $CO(NH_2)2$, (46% N) was also used. Stones agricultural lime (79.77g/100gCaO, < 0.01g/100gMgO and < 0.01g/100g P_2O_5) and granulated Tilemsi Natural Phosphate (30g/100g P_2O_5 + 40g/100gCaO) were used as an amendment.

Table 9: Recorded rainfall and number of rainy days in different zones during the 2018-2019 and 2019-2020 campaigns

Rainfall	Bougouni	Sikasso	Koutiala	Fana	Kita
2018-2019					
Cumul in mm	1,050 – 1,217	973 – 1,467	986	524 - 986	935 - 962
Nomber of days	52 -54	37 - 66	52	29 - 72	38 - 40
2019-2020					
Cumul 1n mm	877-1,376	863-1,181	630-980	622-873	981-983
Nomber of days	50-64	36-66	37-61	31-59	40-43

Table 10: Some morpho-physiological, agronomic and technological characteristics of the varieties grown in the different zones

Feature	NTA 90-5	NTA 93-15	NTA MS334	STAM 59A	BRS 293
Morpho-physiologique					
Nomber of BV	-	2,1	1,7	-	1,9
Average position 1e BF	-	5,9	6,1	-	5,8
Height	112	-	129,6	110	124,9

Agronomics					
Yield cotton seed kg/ha	1569	1379	1505	1599	1731
Ginning yield (%)	43,5	44,9	43,2	43,8	43
PMC (g)	-	4,3	3,9	-	4
Seed index (g)	-	8,6	8,2	9,4	7,6
Fibertechnology					
Length (mm)	30,7	29,5	28,7	29,1	28,9
Micronaire index	4,12	3,8	4,2	4,1	3,8
Reflectance (%)	73,9	76,7	76,4	75,8	77,4
Yellow index (+b)	10,2	10,1	9,8	9,2	9,6

Source: Cotton Programme coton, 2018

Experimental design

The experimental design used was the dispersed block. The test area per grower was ¾ hectare (7500 m²) in 2018-2019 and one hectare (10000 m²) in 2019-2020. Agricultural lime and PNT were applied prior to tillage. Cotton was sown at 0.80 m x 0.30 m spacing and two seedlings were planted per pocket. The recommended mineral fertilization of cotton plant was used (200 kg cotton compound fertilizer ha^{-1} + 50 kg urea ha^{-1}). The cotton compound fertilizer was applied 15 days after emergence (JAL) and the urea was brought to the ridge at the 45th JAL.

The treatments used in 2018-2019 for the cotton tests were: T1: 200 kg of (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg of urea ha^{-1} (FMR); T2: FMR + 1000 kg of Stones agricultural lime ha_{-1}; T3: FMR + 300 kg granulated PNT ha_{-1}. These plots were materialized and cultivated in maize during the 2019-2020 season.

In 2019-2020, the same treatments from 2018-2019 were used plus another treatment with the new formula of cotton compound fertilizer. The four treatments used were: T1: 200 kg of (14N-18P_2O_5-18K_2O+6S+1B_2O_3) ha^{-1} + 50 kg of urea ha^{-1} (FMR); T2: 200 kg of (14N-18P_2O_5-18K_2O+5S+1B_2O_3+2.5 CaO) ha^{-1} + 50 kg of urea ha_{-1}; T3: FMR + 300 kg of granulated PNT ha^{-1}; T4: FMR + 1000 kg of Stones agricultural lime ha^{-1}.

Measured parameters

Estimates were based on : rainfall (different amounts of rain were measured with a rain gauge), pH (soil samples taken were sent to the laboratory for determination), number of plants at harvest (three squares of 5 lines of 10 m each were placed in each treatment for counting), number of bolls (the count was made on 5 plants in each square), average boll weight (determined from the 5 plants in each square) and cotton yield (determined from the 3 squares placed in each treatment)

Data processing

The analysis of variance (ANOVA) was performed using Stat Box 6.5 software to determine the difference between treatments. Newman and Keuls test was used to separate the means when the ANOVA revealed significant differences between treatments at 5% probability level.

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References

1. Cetin O, Bilgel L. Effets of different irrigation methods on shedding and yield of cotton. Agricultural Water Management, 2002; 54 (1): 1-15. DOI: https://doi.org/10.1016/S0378-3774(01)00138-X.
2. CMDT. Rapport bilan de la campagne 2014/2015 de la Compagnie Malienne pour le Développement des Textiles. 2015; Pages 45.
3. CMDT. Rapport sur le processus de production agricole : réunion préparatoire de la commercialisation campagne 2019/2020. Pages 62. 2018.
4. Dembélé, D., Traore, K., Quansh, C., OseiJnr, M.E., BA, D.B., Ballo, M. Optimizing Soil Fertility Management Decision in Mali by Remote Sensing and GIS. Donnish Journal of Agricultural Research 2017; Vol 3 (4): 022-034.
5. FAO. Revue des Politiques Agricoles et Alimentaires au Mali. Rapport pays, Février 2013; Pages 217.
6. FAO. Gestion intégrée de la production et des déprédateurs du coton. Guide du facilitateur pour les champs écoles des producteurs. 2014; Pages 79, ISBN 978-92-5-208280-4

7. Kaboré I. Itinéraires techniques recommandés et pratiques paysannes courantes dans la zone cotonnière Ouest du Burkina Faso : cas du coton et du maïs. Mémoire de Master, Université Polytechnique de Bobo-Dioulasso, Burkina Faso, 127 pages. 2014.
8. Kamprath E. J. Exchangeable aluminum as a criterion for liming leached mineral soils. Soil Sci. Soc. Am. Proc 1970; 24: 252-254.
9. Kanté S. La gestion de la fertilité des sols par classe d'exploitation au Mali-Sud. Thèse de Doctorat. Wageningen University and Research center Department of Plant Sciences. Pays Bas, 2001; 213 pages.
10. Koulibaly B., Traoré O., Dakuo D., Zombré P. N. Effets des amendements locaux sur les rendements, les indices de nutrition et les bilans culturaux dans un système de rotation coton-maïs dans l'ouest du Burkina Faso. Biotechnol. Agron. Soc. Environ. 2009; 13(1), 103-111
11. Lawson, A. J. Effet de différentes pratiques de taille sur l'amélioration des performances agronomiques du cotonnier Gossypium hirsutum L. Mémoire Ingénieur, Université de Parakou, Bénin, 2008; 57 pages.
12. Nabahungu LN, Ruganzui V, Mukuralinda A, Zaongo C, Ntizo S. Différentes sources du phosphore inorganique, fumier et chaux sur l'amélioration des sols acides du Rwanda. African Crop Science Conférence Proceedings, 2005; Vol. 7. pp. 1103-1108. ISSN 1023-070X/2005
13. Programme coton. Rapport de la commission scientifique du CNRA. 2014; pp ; 52
14. Rapidel B, Defeche C, Traore B, Lançon J, Wery J. In-field development of a conceptual crop functioning and management model: A case study on cotton in southern Mali. European Journal of Agronomy, 2006; 24: 304-315. DOI: https://doi.org/10.1016/j.eja.2005.10.012
15. Sissoko F. Analyse des flux d'eau dans les systèmes de culture sous couverture végétale en zone soudano sahélienne : cas du coton semé après une culture de sorgho/brachiaria au Sud du Mali. Thèse de Doctorat unique. Université de Montpellier, Montpellier, France, 2009; 169 pages.
16. Soenen, B., Lagrange, H., Bouthier, A., Helias, R., Rauzy, Y., Berton, G., Castillon, P. Mise en evidence de l'intérêt du gypse pour lutter contre la toxicité aluminique sous prairie de longue durée ; Arvalis Institut du végétal. Rencontres Comifer-Gemas. Lyon 18 et 20 novembre 2015. France.
17. Van Der Pol F. et Traoré B. Soil Nutrient depletion by agricultural production in Southern Mali. Fertilizer Research 1993; 36: 79-90.

Seedling Age Effect on Yield and Lint Quality of Cotton

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Abstract

Background: *Sowing of cotton seed in due time is very important to achieve expected yield of cotton but in Bangladesh condition cotton seed sowing takes place during rainy season i.e. in July to August .Due to climate change heavy and continuous rainfall in cotton sowing season, seeds cannot be sown in proper time as well as it hamper seed germination, seedling damage and it led to repeated gap filling, thereafter causes age and canopy coverage difference among direct sowing and gap-filling plants, which ultimately affect the yield. To minimize the age gap among direct sowing plants and transplanted seedling and achieve the expected yield of cotton, a trial on seedling transplantation has been undertaken in the experimental field of the Cotton Research, Training and Seed Multiplication Farm, Sreepur, Gazipur during June 2018 to February 2019 to find out the effective age of cotton seedling for transplanting. Five categories of seedlings viz…10, 15, 20, 25 and 30 days old were tested along with direct seed sowing as control treatment.*

Results: *Survival rate of the seedlings among the age categories ranges from 79 to 96%. The highest survival rates of the seedlings were observed in direct seed sowing as well as seedling of 25 days age category and the lowest at 30 days age category seedlings. Survive rate of cotton plant decreased with increasing the age of seedling transplantation. The seedling age did not affect yield and yield contributing agronomic traits like plant height and boll weight. The seedling age categories had insignificant effect on lint quality characteristics like length, strength and micronaire value of the lint.*

Conclusion: *The findings can help farmer to raise cotton seedling on seedbed and maintain seedling age not exceeding 25 days under the adverse weather condition during sowing time.*

Keywords: Gossypium hirsutum, plant traits, yield, lint

Background

Due to huge demand, cotton is a important crop in Bangladesh, despite having only total 8.5 million hectare cultivable land most of which engage in food crop production to feed more than 160 million peoples of the country. Cotton a a high land crop has to compete with other high value crops like vegetables and fruit crops in Bangladesh, more over cotton crop occupied farmland more than six month were as other quick growing vegetables need only three month, so farmers of Bangladesh are only interested to grow cotton when they can get more profit than other competitive crops.

A large number of labor forces of the country are employed in cotton cultivation and cotton processing mills (Mahmood 1999). In spite of such importance of cotton in Bangladesh, its yield is low (1.5 t ha-1) which is far below compared to that in cotton growing countries (FAO1997). This situation of cotton in Bangladesh demands high cotton yield per unit area. Cotton yield can be increased by the development of new varieties, proper agronomic practices and pest management.

Cotton varieties/hybrids that cultivated in Bangladesh vary in their plant agronomic traits, yield and lint quality (Amin et al. 2008a). Abundance and infestation of insect pests affect plant agronomic traits, yield and quality of fiber (Amin et al. 2008b). Proper sowing or transplanting time is a very important component of integrated pest management. Sowing of cotton seed in due time could play significant role to achieve expected yield. In recent years due to climate change heavy and continuous rainfall in sowing time,cotton farmers are facing great challenge in cotton seed sowing in optimum time as well as keeping optimum seedling the per unit area, for this reason usually they have to spend more money

for purchasing additional costly hybrid seed and ultimately they get poor yield. Heavy rainfall during July and August months in Bangladesh hinders sowing of seeds in proper time and maintaining optimum plant population per unit area. As a result, the expected yield cannot be achieved. Excessive rainfall affects germination of seeds and repeated gap filling there after causes age difference among direct sowing and gap-filled plants, which ultimately affect the yield. Therefore, this study was conducted to evaluate the effect of seedling age on plant population which ultimately ensure the yield and lint quality of cotton.

Results

Among the cotton seedling age ranges from 10 to 30 days and direct seed sowing survival rate ranges from 79 to 96% Highest survival rate was observed in direct seed sowing treatment and lowest was 79% in case of seedling age 30 days. There was no significant effect in case of yield, no. of sympodial branch plant, no. of monopodial branches/plant, Plant population/ha, Plant height, No. of bolls/plant, Single boll weight/plant. T6 Direct seed sowing showed highest seed cotton yield that is 2.36 ton/ha that is statistically similar to other treatments (Table 1). Maximum single boll wt. of 5.9 was recorded from treatment T1 which was followed by the treatment T1 (5.66) and T5and T2 respectively. Highest no. of ball/plant was found T6 (26.33) that is similar to all treatments. There is no significance different in case of plant population ha -1 and plant height at harvest.

Survival rate of cotton plant decreases with increasing the age of seedling transplantation. (Fig. 1). According to Kulvir Singh, 2013 age of seedling for transplanting has significant effects on yield and yield contributing characters. There was a decline in lint yield contributing characters. There was a decline in lint yield as the age of the seedlings increased. Seedlings of 10 days age performed better as compared to old seedlings (30 days) because of their better ability to withstand transplanting shock. This might be due to certain unfavorable effects on root growth experienced in 30 days old seedlings resulting into their less efficiency as compared to 10 days old seedlings. Such seedlings, when transplanted exhibited improved seed cotton yield primarily due to better leaf area indices, which helped in more sink production resulted in more bolls/plant and eventually yield was increased. There was no significant difference among the treatments in terms of lint quality.

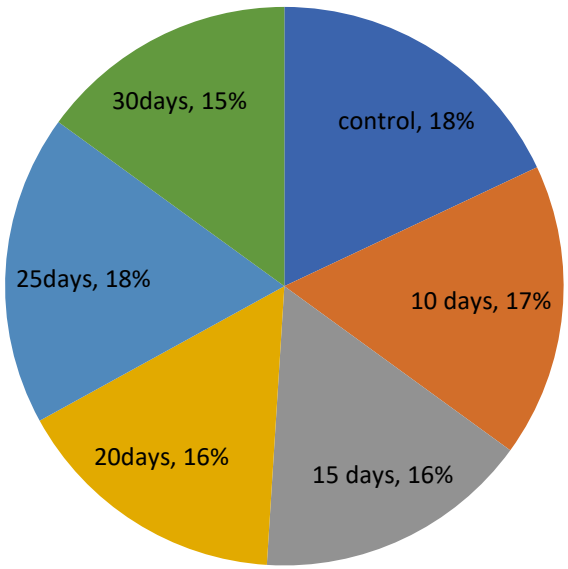

Figure 1: Survival rate of different age categories of cotton seedling.

Table 1: Effect of cotton seedling age on cotton plant agronomic traits.

Plant agronomic traits						
Seedling age (Day)	Population (Number ha ^{1})	Plant height (cm)	Monopodial branch plant $^{-1}$	Sympodial branches plant $^{-1}$	Bolls plant $^{-1}$	Boll weight (g)
10	30.45 ab	119.0a	1.1 b	14.5 ab	21.9 ab	5.7 a
15	29.8 ab	97.7 a	0.9 bc	15.5 a	22 ab	5.2 a
20	29.2 ab	123.1 a	0.9 bc	14.9 a	21.2 ab	5.1 a
25	33.1 a	94.1 a	0.3 c	14.2 b	17.5 b	5.5 a
30	27.2 ab	93.9 a	0.4 c	14.8 ab	22.6 ab	5.3 a
Control	24.7 b	102.8 a	2.0 a	14.9 ab	26.6 a	5.9 a

Notes: Means within a column followed by same letter (s) are not significantly different by LSD at< 0.05.

Table 2. Effect of cotton seedling age on cotton yield and lint quality

Lint quality traits				
Seedling age (days)	Yield (t ha^{-1})	Length (mm)	Strength (g/tex)	Micronaire value
10	2.3 a	31.8 a	29.3 a	4.8 a
15	2.2 a	31.3 a	31.5 a	4.8 a
20	2.0 a	31.2 a	29.9 a	4.9 a
25	2.1 a	30.8 a	29.8 a	4.8 a
30	1.9 a	30.9 a	30.2 a	4.8 a
Control	2.4 a	31.6 a	31.7 a	4.7 a

Notes: Means within a column followed by same letter (s) are not significantly different by LSD at< 0.05.

Discussions

In Bangladesh farmers have several land use option for high land particularly those land are use for cotton cultivation as the land are suitable for other field and horticultural crops also, usually a good number of farmers are taking decision on the cotton cultivation specially on the prevailing weather condition during the sowing time of cotton. Due to climate change specially distribution of rainfall is being varied year on year, in Bangladesh cotton seed sowing usually take place in the rainy season i.e. on July and August, in recent years excessive rainfall affect cotton seed germination, so farmers have to go for repeated seed sowing in the same field, that increase production cost as well as delaying of cotton sowing, which also affect the proper sowing time of following crop of cotton. So many farmers are changing their decision on cotton cultivation in the particular year when excessive rainfall occurs during cotton sowing period. To overcome this challenge they need the technology how cotton seeding could survive with the excessive rainfall condition. Hence seedling raising in seedbed then transplanting in the main field can provide multiple benefit to the farmers in terms of hybrid seed cost saving due to better germination of seed on seed bed, maintaining optimum plant population as well as synchronized growth of the plant which ultimately contribute good yield and leaving the main field for previous crop for better yield and quality as well as . But the major challenge is the accurate weather forecast and the extra effort for the seedbed preparation seedling raising and transplanting of the seedlings. The experiment was conducted on clay loam red soil of Cotton Research Centre, Sreepur, Gazipur, Bangladesh i.e. at the central part of the country, now cotton farmers need hands on training on seedling raising and transplanting.

Conclusion

Direct seed sowing produced the highest seed cotton yield followed by 10 days age seedling yield 2.3 ton/ha. Highest single boll weight was found in treatment 6 that is 5.9. Highest boll/plant was found in treatment 6 that is 26.63. Survive rate of cotton plant decreased with increasing the age of seedling for transplantation. As it is a new technology for the cotton farmers of Bangladesh so extensive hands on training need for the cotton farmers on seedling raising and transplanting to get the benefit of the technology.

Methods

Study site and condition

The study was conducted during june 2018 to February 2019 in the Field of Entomology, Cotton Research, Training and Seed Multiplication Farm, Sreepur, Gazipur, Bangladesh. The study site is

located in the middle of Bangladesh which occupied 24022' North latitude and 90041' East longitude. The study area has a tropical climate followed by mean maximum and minimum temperatures, relative humidity and rainfall of 36.0 and 12.7 0C, 65.8% and 237.6 cm, respectively (Amin et al.2016).

Raising of seedling

Cotton Gossypium hirsutum L, var. DM-3 seeds were collected from Lal Teer Company Ltd. Dhaka. Seeds were sown in seedbed with five days differences viz., 10days, 15days, 20days, 25days, 30 days and then transplanted to the main plot along direct seed sowing in the month of August 2018. The distance from row to row was 90 cm and plant to plant 45 cm. Intercultural operations such as irrigation and weeding etc. were done whenever necessary but the crops were kept free from pest management practices.

Experimental design and cultivation of cotton

The experiment was laid out in randomized complete block design with three replications. Five categories of seedlings such as T1-10days,T2-15days,T3-20days,T4- 25days,T5- 30 days age were transplanted and direct seed sowing was done as control treatment. The experiment was set up in the month of August, 2018 and the plot size 4.5m x 3.6m. Intercultural operations and observation of cotton agronomic traits: Thinning and earthing up were completed by 20 days after emergence. In case of first thinning, two seedlings per hill were kept after 10 days of emergence. Second thinning was done 20 days after emergence keeping one seedling per hill. N, P, K, S was fertilized @ 120:45:131:27 kg ha-1 . One forth of Urea and half of MOP were applied during final land preparation as basal dose. The rest amount of urea was applied in three equal splits at 20, 40 and 60 days after sowing as top dressing. Similarly, rest amount of murate of potash was applied at the time of second and third application of nitrogen application. The experimental field was kept weed free up to 60 days after emergence of seedling by hand weeding. Mulching between two rows was done by power tiller. The third week of November and first week of December irrigation were given due to drought situation. Scouting based spray was followed for pest management. Ten plants were selected randomly from each plot and tagged for taking data. Harvesting of seed cotton from the net plot and border are done in three number of picking. Data on plant height at harvest, number of monopodial brunch plant -1, number of sympodial brunch plant -1, number of boll plant -1, individual boll weight, seed cotton yield were taken during experiment.

Data analysis

Analysis of variance (ANOVA) followed by LSD was used for analyzing data.

Acknowledgement

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References

1. Amin Mr, Ahad MA, Rasel NA, Hossain SMA and Tithi DA. 2008b. Effect of some systemic insecticides on the abundance of major insect pests and predators associated with cotton plants. Journal of Agroforestry and Environment. 2: 143-146.
2. Amin MR, Tith DA, Azad HMS and Hossain SMA. 2008a. Management of cotton pest using seed treating insecticide and pyrethroid at different locations of Bangladesh. Journal of Science and Technology. 7: 123.128.
3. Amin, M.R. S. Namni, M.R.U. Miah, M.G. Miah, M.Zakaria, S.J. Suh and Y.J. Kwon. 2015. Insect inventories in a mango-based agroforestry area in Bangladesh: Foraging behavior and performance of pollinators on fruit set. Ent. Res. 45:217-224.
4. FAO.1997. FAO Production Yearbook, 1997. Food and Agriculture organization, Rome, Italy.
5. Mahmood, N.T. 1999. Cotton leaf curl virus disease and its status in Pakistan. Pro. ICAC-CCRL- Reg Consult on insecticide resist. Manag. In Cotton. June 28-July 1, pp:234.
6. Singh, K. 2013. Effect of transplanting and seedling age on growth, yield attributes and seed cotton yield of Bt cotton (Gossypium Hirsutum). Indian Journal of Agricultural Sciences 83 (5): 508-13,

Soil Fertility in Cotton-Based Cropping Systems in Côte d'Ivoire

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Abstract

Background: *Soil fertility is a major parameter in crop productivity. In tropical areas, the decline in soil fertility is one of the constraints that negatively impacts yields in the absence of fertilization. This is the case of cotton in Côte d'Ivoire, whose yields are considered low (often below 1000 kg). This study was carried out to assess the fertility potential of the soils cultivated by the 4 major cotton companies.*

Results: *For this purpose, a total of 150 cotton farms (35 farms per company) over the whole cotton basin cotton companies were selected for soil sampling (0-20). The physico-chemical analyses of the composite soil samples formed in the laboratory included particle size, pH-water, organic matter, total N, assimilable P, exchangeable bases and CEC. The results showed that the soils studied had a sandy texture. The pH values are slightly acidic to neutral. These soils have very low levels of organic matter, total N, P, Ca, K, and low values of cation exchange capacity. On the basis of these fertility parameters, three groups were identified at the level of the cotton companies. The soils of SECO and CIDT have the same physico-chemical characteristics and differ from the other two cotton companies, Ivoire Coton and COIC.*

Conclusion: *Soils in the study area have become poorer whose organic matter status urgently needs to be upgraded through better use of crop residues and manure.*

Keywords: Fertility, Soil, Cotton, Productivity, Côte d'Ivoire

Background

Cotton cultivation, like other speculation, is subject to several constraints, including increasingly erratic rainfall and declining soil fertility. The causes of the decline in soil fertility in the cotton-growing zone of Côte d'Ivoire are, among others, overexploitation of land, land pressure and unsuitable cultivation practices (Zagbaï et al., 2006).

The disappearance of long-term fallow land also leads to a degradation of the production environment. In the long term, there is therefore a downward trend in yields (N'Goran et al., 2009; Intercoton, 2010).

In addition, the fertilizer doses (200 kg/ha) and fertilizer formulas (15N 15P 15K+ 6S+1B) currently popularized in the cotton zone have been developed for more than three decades (Bouchy, 1970; Latham, 1971; Deat, 1975; Sement, 1983). In this time interval, it is very likely that there have been changes in the chemical equilibrium of the mineral elements in the cultivated soils with the appearance of new deficiencies in the cotton-growing regions.

The evaluation of these constraints (decrease in mineral element contents and appearance of new deficiencies) is an indispensable step that will make it possible to identify appropriate techniques for efficient soil fertility management in cotton-based cropping systems for sustainable production in the cotton basin.

Moreover, since the pioneering work of Fritz and Vallerie (1971) and Braud (1973), cotton research in Côte d'Ivoire in the field of fertilization has changed very little. Little information exists on the edaphic characteristics (physical, chemical and biological) of the different cotton production zones.

However, the above-mentioned parameters play a major role in specific soil fertilization techniques to increase crop yields. In the current cropping system, yields are still low, often below 1000 kg (Intercoton, 2018). It is in this context that this soil characterization study of the four (4) major cotton companies in Côte d'Ivoire was initiated in order to make recommendations for efficient management of soil fertility. The objective of this work is to evaluate the physical-chemical parameters of the soils for sustainable farm management in cotton-based production systems in Côte d'Ivoire.

Results

The interpretation standards of Landon (1991) and INERA (Kambiré, 2000), the fertility scales of Dabin (1970), the threshold values proposed by Boyer (1972; 1982) for tropical soils were used.

Soil granulometry of the cotton companies' soils

The results on the physical characteristics of the soils of the different cotton companies are recorded in Table 1. In general, the granulometric analysis showed that the soils studied have an essentially sandy texture in the superficial horizons. Clay and silt contents are minimal in these superficial horizons. There is a high level of coarse sand in these horizons.

Table 1: Physical characteristics of soils of cotton companies

Parameters	Cotton companies			
	IC	COIC	SECO	CIDT
Texture (%)	Sandy	Sandy	Sandy	Sandy
Clay (A)	13,86	9,41	12,69	9,52
Coarse Silt	17,18	7,47	11,70	11,26
Fine Silt	13,64	9,00	10,71	12,03
Coarse sand	30,94	43,82	33,36	35,41
Fine sand	24,35	30,30	31,54	31,79

Notes: IC: Ivoire Coton; COIC: Compagnie Ivoirienne de Coton; SECO: Société d'Exploitation Cotonnière; CIDT: Compagnie Ivoirienne pour le Développement du Textile

Mean of Total Nitrogen and Organic Matter

For the company Ivoire Coton, the total nitrogen rate varied from 0.02 to 0.13% with an average of 0.08 against the threshold value of 0.2% (Table 2a). Referring to the threshold, all the soils prospected are totally deficient in nitrogen (100%). Organic matter levels varied from 0.33% to 2.44% with 83.23% below the threshold value of 2%. An average organic matter rate of 1.26% was obtained against a threshold value of 0.2%.

Table 2b shows the total nitrogen content at SECO level. This rate varied from 0.03 to 0.21% with an average of 0.11 against the threshold value of 0.2%. Compared to this standard in the literature, 93.75% of soils are deficient in nitrogen. Organic matter levels ranged from 0.62 to 3.87% with an average of 2.09% compared to the literature threshold value of 2%. Approximately 50% of the soils studied had organic matter levels below this threshold value.

Table 2c shows the total nitrogen rate at the level of the COIC cotton company which varied from 0.03 to 0.13% with an average of 0.08 against the threshold value in the literature which is 0.2%. All the soils studied are nitrogen deficient (100%). Organic matter levels ranged from 0.5 to 2.0% with an average of 1.15% compared to a threshold value of 2% in the literature. Based on this threshold value, 97.29% of the soils in this company are largely poor in organic matter.

For CIDT, the total nitrogen rate varied from 0.03 to 0.29% with an average of 0.11 against the threshold value of 0.2% (Table 2d). Compared to this threshold value, 89.36% of the surveyed soils are totally deficient in nitrogen. Average organic matter levels varied from 0.39% to 5.47% with an average of 1.82% against a threshold value of 2%. Approximately 61.70% of the company's soils are low in organic matter.

Exchangeable Cations

Calcium

In the Ivoire Coton zone, the calcium concentration varied from 0.46 to 3.36 Cmol+/kg (Table 2a) with an average of 1.19 Cmol+/kg against the threshold value in the literature, which is 4 Cmol+/kg. With reference to this threshold value, all the cotton farms surveyed have soils that are very deficient in calcium (100%).

Table 2b shows the calcium concentrations in the soils of the SECO cotton company. These concentrations ranged from 0.31 to 5.40 Cmol+/kg with an average of 2.0 Cmol+/kg. This average value is below the threshold recommended in the literature (4 Cmol+/kg). Compared to the above threshold, 93.75 % of the surveyed sites have calcium deficient soils.

At the COIC company level, calcium values ranged from 0.40 to 3.37 Cmol+/kg with an average of 1.76 Cmol+/kg against the threshold value of 4 (Table 2c). Compared to this threshold value, all the soils of this company are well deficient in calcium (100%).

Table 2d shows the calcium concentrations in the CIDT area. These values ranged from 0.66 to 6.58 Cmol+/kg with a mean of 2.18 well below the threshold recommended in the literature. As in the other cotton companies, in relation to the threshold, 87.23% of the soil is deficient in calcium.

Magnesium

The average concentration of exchangeable magnesium in all the cotton companies ranged from 0.58 to 0.86 Cmol+/kg against the threshold value of 0.5 Cmol+/kg. The proportion of farms with a calcium level below the threshold ranged from 10.63 to 40.54% for the four major cotton companies. This proportion was higher at the level of the COIC cotton company. In general, magnesium is not yet deficient in the various soils of the cotton companies (Tables 2a, b, c, and d).

Potassium

In the Ivoire Cotton zone, the potassium concentration ranged from 0.04 to 0.25 Cmol+/kg (Table 2a) with an average of 0.09 Cmol+/kg against the threshold value of 0.2 Cmol+/kg. With reference to this threshold value, 94.11% of the cotton farms surveyed have soils with a high potassium deficiency.

Table 2b shows the potassium concentrations at the level of the cotton company SECO. These concentrations ranged from 0.06 to 2.72 Cmol+/kg with an average of 0.20 Cmol+/kg. This average value is practically equal to the threshold value recommended by the literature, which is 0.2 Cmol+/kg. 78,12 % of soils have levels lower than or equal to this threshold value.

At COIC, potassium values ranged from 0,07 to 0,24 Cmol+/kg with an average of 0,14 Cmol+/kg against the threshold value of 0,2. Compared to this threshold value, 91% of the soils of this company are deficient in potassium (Table 2c).

Potassium concentrations in the CIDT area are shown in Table 2d. These potassium values ranged from 0.04 to 0.61 Cmol+/kg with a mean of 0.16 well below the threshold recommended in the literature (0.2). As in the other cotton companies, most of the soils prospected are deficient in potassium (82.97%).

pH ET CEC

For all 4 cotton companies, the average pH varied from 6.32 to 6.80. In general, the pH ranges from slightly acidic to neutral.

For all 4 cotton companies, the average CEC value varied from 5.87 to 8.82 Cmol+/kg against a threshold value of 15 Cmol+/kg. All the soils of these 4 large cotton companies (IC, SECO, COIC and CIDT) have low cation exchange capacities. (Tables 3a, b, c and d).

Cation saturation rates

In the Ivoire Coton zone, the saturation rate varied from 13.25 to 55.28% (Table 3a) with an average of 25.44% against the threshold value of 60%. Compared to this threshold rate, all the cotton farms surveyed have a low saturation rate.

Table 3b shows the cation saturation rates of the soils of the SECO cotton company. These rates varied from 30 to 102% with an average of 54.03, below the threshold value recommended by the literature. Compared to the threshold rate, the level of soil desaturation is not as alarming in this company.

At the COIC company, saturation rates varied from 20.22 to 90.62 % with an average of 43.55 % against the threshold value of 60 %. With reference to the threshold rate, 91.89% of the soils in this company are desaturated (Table 2c).

Soil saturation rates in CIDT area are shown in Table 3d. These rates ranged from 23.31 to 95.23% with an average of 47.96% below the threshold recommended by the literature (V= 60%). As in other cotton companies, 74.46% of the soil is desaturated.

Assimilable phosphorus

In the Ivoire Coton zone, concentrations of assimilable phosphorus varied from 10 to 70 ppm (Table 2a) with an average of 28 ppm compared to the threshold value in the literature, which is 15 ppm. Compared with this threshold value, 5.88 of the cotton farms surveyed had phosphorus-deficient soils.

Table 2b shows the concentrations of assimilable phosphorus in the soils of the SECO cotton company. These concentrations ranged from 2 to 15 ppm, with an average of 8.44 ppm, which is below the threshold recommended in the literature, which is 15 ppm. Based on this threshold value, 96.87% of the sites surveyed are deficient in this nutrient.

At COIC, the concentrations of assimilable phosphorus varied from 6 to 42 ppm with an average of 19.92 ppm. 21.62% of the company's operations are deficient in assimilable phosphorus compared with the threshold of 15 ppm (Table 2c).

Table 2d gives the concentrations of available phosphorus in the CIDT area. These concentrations ranged from 2 to 42 ppm with an average of 13.30 ppm below the threshold recommended in the literature. Compared to this threshold value, 53.19 % of the prospected soils are deficient in this element.

Assessment of soil fertility levels

Most of the physico-chemical soil parameters are very low in these four cotton companies (Tables 2a, b, c and d). Organic matter (OM), total nitrogen (N), cation exchange capacity (CEC), potassium (K+), assimilable phosphorus (Pass.) and base saturation rate (V) are limiting for all the soils of all the cotton companies.

It is clear that all the soils have more than two severe limitations. In the soils of the Ivoire Coton cotton company, the cation exchange capacity (CEC), potassium (K+) and saturation rate (V) are severe limitations. Two severe limitations have been recorded in SECO's soils, namely CEC and assimilable phosphorus. In COIC cotton soils, CEC and potassium are the severe limitations. CEC and potassium (K+) constituted the severe limitations in CIDT soils.

The soils of each cotton company have at least two severe limitations, which implies that all these soils belong to class IV with a very low level of fertility.

Table 2a: Physico-chemical characteristics of soils and level of soil fertility of the Ivoire Coton Company

Physio-chemical properties	Unit	Threshold value	Range	Average	% of samples below threshold	Level of limitation
Soil pH (pH)	-	5,5	5,80-6,90	6,36	0	WL
Organic Matter (OM)	%	2	0,33-2,44	1,26	83,23	LL
Total Nitrogen (N)	%	0,2	0,02-0,13	0,08	100	LL
Cation exchange capacity (CEC)	Cmol/kg	15	3,2-16,2	8,82	91,17	SL
Potassium (K^+)	Cmol/kg	0,2	0,04-0,25	0,09	94,11	SL
Calcium (Ca^{2+})	Cmol/kg	4	0,46-3,66	1,19	100	-
Magnesium (Mg^{2+})	Cmol/kg	0,5	0,34-1,41	0,70	26,47	-
Base saturation rate (V)	%	60	13,25-55,28	25,44	100	SL
Assimilable phosphorus (P.ass)	ppm	15	10-70	28,09	5,88	WL
Limiting factors			OM, N, CEC, K^+,Ca^{2+},V.			
Soil class			IV			
Fertility level			Very low			

Notes: WL= Without limitation; LL: Low Limitation; ML: Moderate Limitation; SL: Strict limitation; STL: Strong limitation

Table 2b: Soil physico-chemical characteristics and level of soil fertility of the SECO cotton company

Physio-chemical properties	Unit	Threshold value	Range	Average	% of samples below threshold	Level of limitation
Soil pH (pH)	-	5,5	5,59-7,54	6,62	0	WL
Organic Matter (OM)	%	2	0,62-3,87	2,09	50	LL
Total Nitrogen (N)	%	0,2	0,03-0,21	0,11	93,75	WL
Cation exchange capacity (CEC)	Cmol/kg	15	2,4-13,52	5,87	100	SL
Potassium (K^+)	Cmol/kg	0,2	0,06-2,72	0,20	78,12	SL
Calcium (Ca^{2+})	Cmol/kg	4	0,31-5,40	2,04	93,75	-
Magnesium (Mg^{2+})	Cmol/kg	0,5	0,28-1,90	0,73	31,25	-
Base saturation rate (V)	%	60	30-102	54,03	59,37	LL
Assimilable phosphorus (P.ass)	ppm	15	2-15	8,44	96,87	SL
Limiting factors				OM, N, CEC, Ca^{2+}, V, P.ass,		
Soil class				IV		
Fertility level				Very low		

Notes: WL=Without limitation; LL: Low Limitation; ML: Moderate Limitation; SL: Strict limitation; STL: Strong limitation

Table 3c: Physico-chemical characteristics of soils and level of soil fertility of the cotton company COIC

Physio-chemical properties	Unit	Threshold value	Range	Average	% of samples below threshold	Level of limitation
Soil pH	-	5,5	5,40-7,08	6,39	2,70	WL
Organic Matter (OM)	%	2	0,5-2,08	1,15	97,29	ML
Total Nitrogen (N)	%	0,2	0,03-0,13	0,08	100	LL
Cation exchange capacity (CEC)	Cmol/kg	15	2,88-9,84	6,31	100	STL
Potassium (K^+)	Cmol/kg	0,2	0,07-0,24	0,14	91	SL
Calcium (Ca^{2+})	Cmol/kg	4	0,40-3,37	1,76	100	-
Magnesium (Mg^{2+})	Cmol/kg	0,5	0,22-1,33	0,58	40,54	-
Base saturation rate (V)	%	60	20,22-90,62	43,55	91,89	SL
Assimilable phosphorus (P.ass)	ppm	15	6-42	19,92	21,62	LL
Limiting factors				OM, N, CEC, K^+, Ca^{++}, V.		
Soil class				IV		
Fertility level				Very low		

Notes: WL=Without limitation; LL: Low Limitation; ML : Moderate Limitation; SL: Strict limitation; STL: Strong limitation

Table 3d: Physico-chemical characteristics of soils and level of soil fertility of the cotton company CIDT

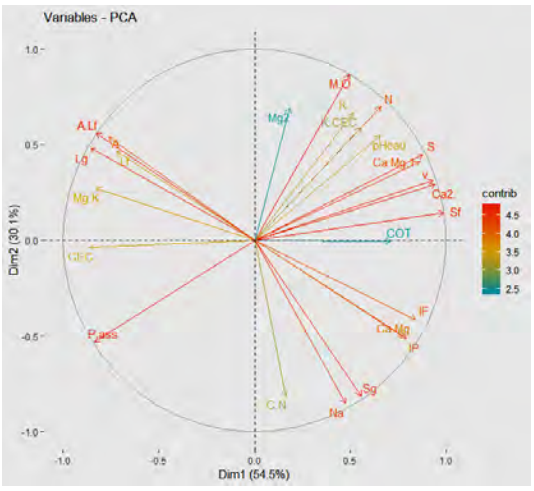
Physio-chemical properties	Unit	Threshold value	Range	Average	% of samples below threshold	Level of limitation
Soil pH (pH)	-	5,5	5,97-7,64	6,80	-	WL
Organic Matter (OM)	%	2	0,39-5,47	1,82	61,70	LL
Total Nitrogen (N)	%	0,2	0,03-0,29	0,11	89,36	WL
Cation exchange capacity (CEC)	Cmol/kg	15	2,08-22,6	7,20	97,87	SL
Potassium (K^+)	Cmol/kg	0,2	0,04-0,61	0,16	82,97	SL
Calcium (Ca^{2+})	Cmol/kg	4	0,66-6,58	2,18	87,23	-
Magnesium (Mg^{2+})	Cmol/kg	0,5	0,35-2,37	0,86	10,63	-
Base saturation rate (V)	%	60	23,31-95,23	47,96	74,46	ML
Assimilable phosphorus (P.ass)	ppm	15	2-42	13,30	53,19	ML
Limiting factors				OM, N, CEC, K^+, Ca^{++}, V, P.ass		
Soil class				IV		
Fertility level				Very low		

Notes: WL=Without limitation; LL: Low Limitation; ML : Moderate Limitation; SL: Strict limitation; STL: Strong limitation

Soil grouping based on current physico-chemical characteristics.

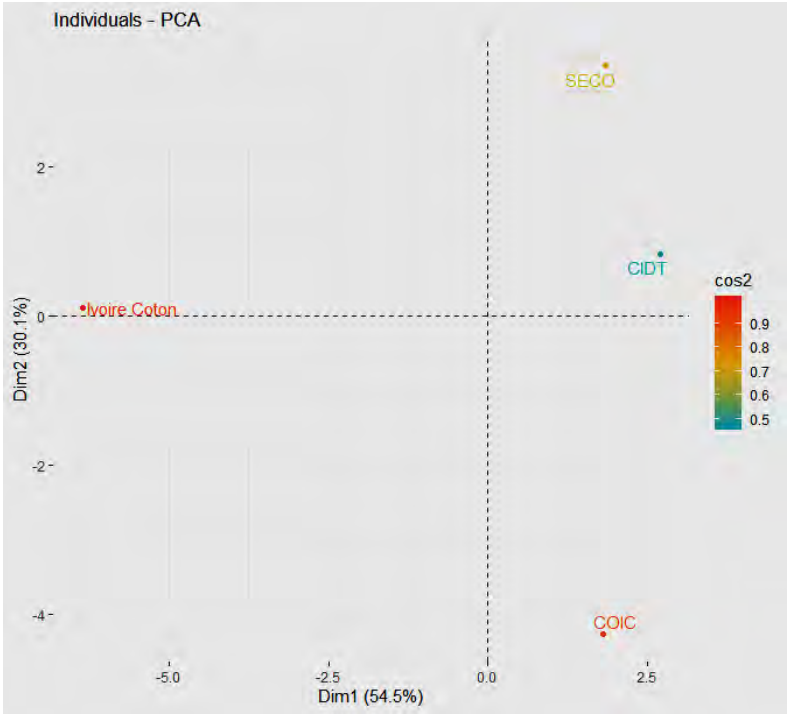
The principal component analysis made it possible to determine the relationships between the physical and chemical parameters of the soils of the different cotton companies. Two components or factor axes representing 84.59% of the information contained in the dataset were obtained. On the first axis (Dim 1) with more than 50% of the total variance explained (Figure 1), strong positive correlations were obtained between variables such as Clay (A), Coarse Silt (Lg), Fine Silt (Lf), Mg/K ratio, Cation Exchange Capacity (CEC) and Assimilable Phosphorus (P. ass). These variables are negatively correlated with variables such as Total Organic Carbon (TOC), Fine Sand (Sf), Calcium content (Ca2+), sum of Exchangeable Bases (S), saturation rate (V) and pH water. On the second axis (Dim 2), Organic Matter (OM), Magnesium (Mg2+), Potassium (K+), K/CEC ratio and Nitrogen are negatively correlated with C/N ratio, Sodium (Na+) and Coarse Sand (Sg).

In relation to the individual points representing the different cotton companies, the Ivory cotton company is diametrically opposed to the CIDT on the first factorial axis (Figure 2). This clearly shows that their soils have totally different physico-chemical characteristics. Similarly, the soils of the COIC cotton company differ from those of SECO on axis 2. The analysis of Figure 3, shows that the soils of the cotton company COIC are characterised by Lg, A, Lf, Mg/K, CEC and also by P. ass. On the other hand, those of CIDT are characterised by TOC, Sf, Ca2+, V, S, pH-water. Those of SECO are characterised by M.O, Mg2+, K/CEC, K. The soils of the cotton company COIC are characterized by coarse sands (Sg), Sodium (Na) and C/N ratio.



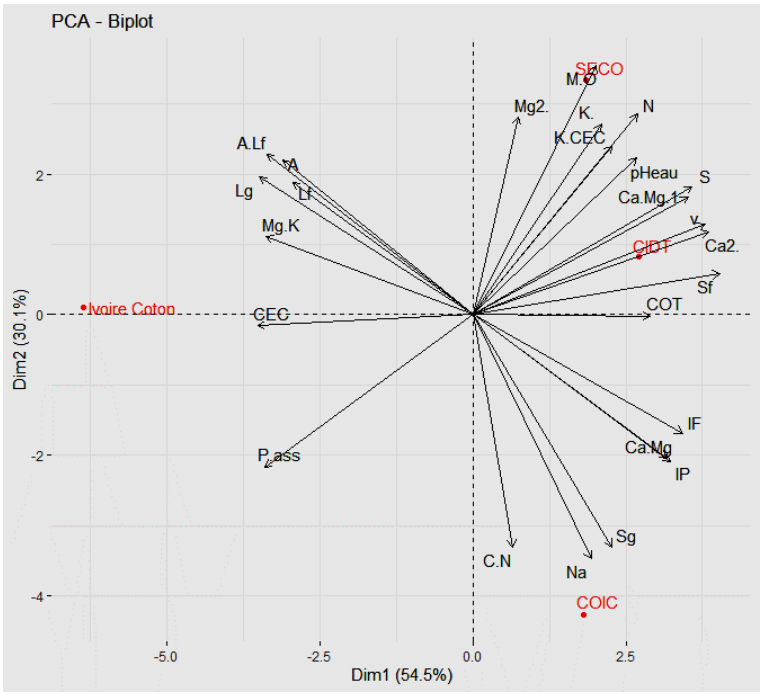
Notes: The colours indicate the level of contributions (contrib) of the physico-chemical variables

Figure 1: Circle of correlations of the physico-chemical variables of the soils of the cotton societies



Notes: The colours indicate the level of quality of the representation (Cos²) of the cotton companies.

Figure 2: Factorial map of the dispersion of cotton companies after PCA of the physico-chemical soil variables



Notes: The red and black colours indicate respectively the cotton companies and the physico-chemical variables of their soils.

Figure 3: Superimposition of cotton companies on the physico-chemical parameters of the soils of the cotton companies in the factorial plan

The hierarchical ascending classification (Figure 4) clearly distinguishes three major groups of soils at the soil level in the cotton basin: the SECO and CIDT soils have the same physico-chemical characteristics and are distinct from the other two cotton companies, Ivoire Coton and COIC.

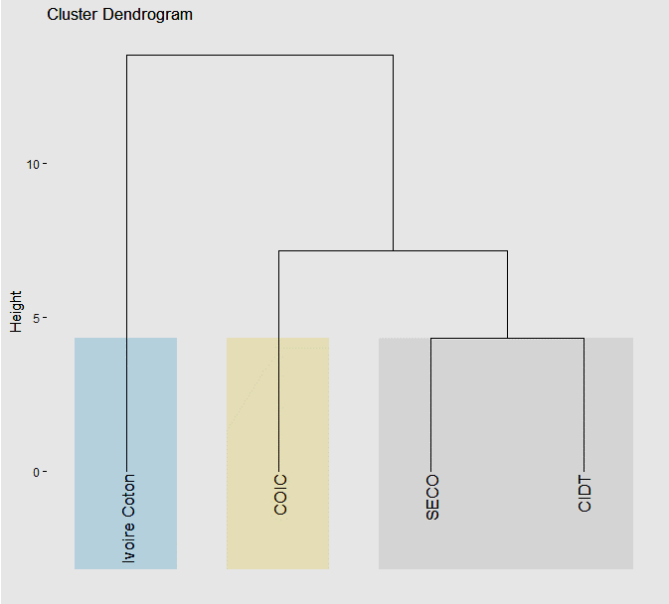


Figure 5: Dendogram of cotton companies

Discussions

The upper layers of the soils of the different cotton companies have a sandy texture. This sandy soil texture gives these soils good drainage, good air circulation and easy root penetration. But this condition has disadvantages. These soils have a low water retention capacity (Mulaji et al., 2016). Clay contents are minimal in these superficial horizons (between 9.41 and 13.86%). These low clay contents are not without drawbacks on the soils studied (Baize, 2000). According to this author, clay is indeed the most active granulometric fraction because it has multiple functions (association with organic matter, cohesion of aggregates, fixation of cations and anions on exchange sites, water retention, etc.). Indeed, this low clay content makes these soils less fertile. For low clay contents (<20%), more organic matter is needed to compensate for the lack of colloids. Moreover, the high proportion of sand would be related to the effects of ploughing which cause the leaching of fine particles (Koualibaly et al., 2014).

Soil pH determines the type of activity (acidic or basic) that exists or predominates. The pH values obtained in all cotton companies are above the threshold (pH>5.5) and are slightly acidic to neutral. This indicates that the chemical (nutrient bioavailability) and microbiological reactions in these soils are going well. Generally, most crop plants grow well in neutral or slightly acidic soil, i.e. 5.5 <pH<7 (Landon, 1991 and Baize, 2000). Low pH values in soils limit plant growth through reduced nitrification, phosphorus deficiency, aluminum and manganese toxicity, and high availability of some minor elements.

Organic matter contributes to the improvement of soil fertility. It is a good indicator for good plant health. In this study, it constitutes a limitation even if it has not yet become severe in all the cotton companies. The values found are low, which could be an obstacle to the good productivity of the cotton growers. This could be explained by the agricultural practices used in the different cotton companies, which are overexploitation of the soil and insufficient organic and chemical amendments (Baize, 2000; Sawadogo, 2006; N'goran et al., 2015). This situation of low organic matter content exposes these soils to degradation by water erosion during heavy rainfall in humid tropical environments (Mulaji et al., 2016).

CEC is another indicator of potential soil fertility. The cation exchange capacity of the soil represents the size of the reservoir allowing the reversible storage of certain cationic fertilizing elements (potassium, magnesium, calcium, etc.). It is linked to the clay-humic complex. The CEC value of a soil depends on the quantities of clay and organic matter it contains, but also on the nature of these elements and the soil pH. The average CEC values obtained for all four cotton companies are generally low. They are well below the threshold as described by Sawadogo (2006). This could be explained by the low clay and organic matter contents (Baize, 2000). Indeed, according to this author, soil organic matter plays a major role both agronomically and environmentally, i.e. adsorption and retention of water, exchangeable bases, phosphorus, nitrogen and trace metal elements. The low CEC values of soils do

not give them a high buffering capacity (Baize, 2000). This would be unfavourable to efficient mineral nutrition of cotton in these production areas.

Concerning the exchangeable bases (Ca, Mg and K) for all the soils studied, apart from the values found at the Mg level, the values obtained are lower than the threshold values determined for tropical soils (Landon, 1991; Boyer, 1982).

The saturation rate of the adsorbent complex given by the three alkaline and alkaline-earth cations is a valuable pedological and agronomic indicator of the chemical richness of the soil, which determines the biological activity, the quality of the structure, and the reserves of fertilizing elements. The average saturation rates obtained indicate that the soils are less fertile. These states of the soils under cotton could be explained by the agricultural practices used in the different cotton companies in the cotton basin, which are overexploitation of the soil (cotton monoculture on the same plot without rotation or fallow) and insufficient organic and chemical amendments (N'goran et al., 2015; Fagaye et al., 2015). These low values obtained at the level of exchangeable bases are not without disadvantages on the mineral nutrition of the cotton plant due to the imbalances that would exist at the level of the three essential cations (Mulaji et al., 2016). Therefore, these soils under cotton trees could experience problems of potassium deficiency.

Conclusion

At the end of this study, whose objective was to assess the physico-chemical parameters of soils for sustainable farm management in cotton-based cropping systems in Côte d'Ivoire, a state of degradation of most soils was noted.

The measurement of physical parameters revealed that the soils studied have an essentially sandy texture in the surface horizon. This gives them a very low retention capacity in mineral elements brought by fertilisation.

The chemical fertility parameters are largely low with particular emphasis on organic matter, CEC, calcium and potassium. CEC and exchangeable cations are largely low and cannot add value to fertilizer inputs.

The ascending hierarchical classification has made it possible to group the 4 major cotton companies in terms of fertility: the soils of SECO and CIDT have the same physico-chemical characteristics and are different from the two other cotton companies, Ivoire Coton and COIC.

As a major recommendation, the low levels of organic matter can be corrected by making good use of crop residues and local production of organic matter, which is the basis for improving and maintaining soil fertility levels, and consequently improving crop yields, which is the guarantee of food security.

Methods

Study site

The study was conducted in the four major cotton companies in Côte d'Ivoire which are : Ivoire Coton, COIC, CIDT and SECO (Figure 1). The Ivorian cotton zone is characterised by a tropical climate. Two rainfall regimes (monomodal and bimodal) characterise this zone, with average rainfall varying between 895 and 1197 mm per year (Kouamé, 1992).

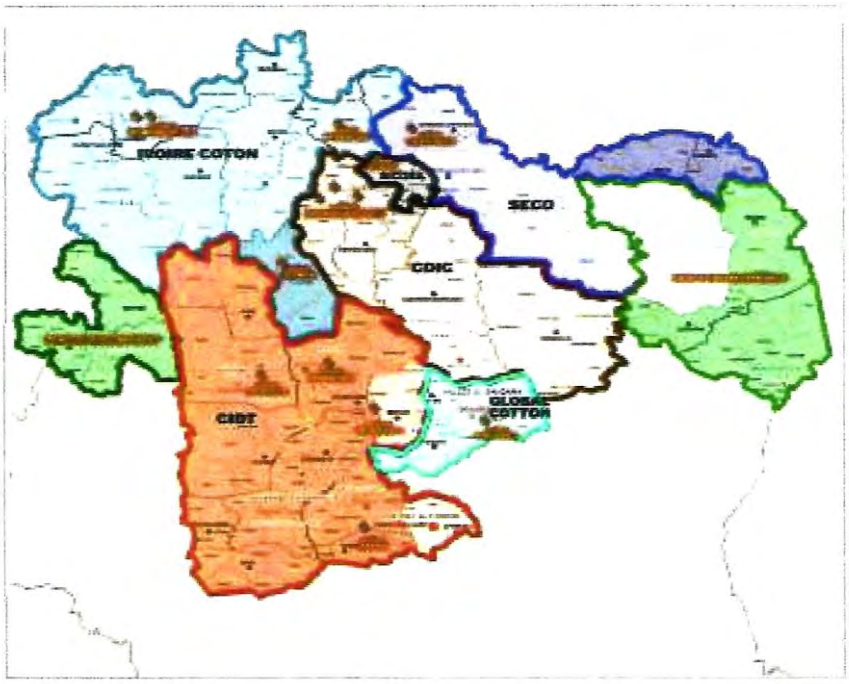


Figure 5: Map of the cotton zone of Côte d'Ivoire

The vegetation of the cotton zone is subdivided into two main types of landscape, namely the forest landscape corresponding to the Centre-West and East belonging to the Guinean domain and the savannah landscape corresponding to the Northern part of the cotton zone which belongs to the Sudanese domain.

Between these two domains, there is a transition zone called the forest-savanna contact zone corresponding to the centre of the cotton zone, characterised by the presence of shreds of mesophilic forest, and large meshes of savanna separated by islands of gallery forests (Brou, 2005). The cotton zone is essentially made up of moderately and highly desaturated ferrasols (N'goran et al., 2018; Dekoula, et al., 2014; FAO, 2014).

Selection of study plots

At the level of each cotton company, thirty (35) farms were prospected. With a view to covering as much as possible of the growing area of each cotton company, three major cotton producing localities were chosen. In each locality, ten (10) farms were selected. The system also took into account the 4 cardinal orientations (North, South, East and West) for the choice of the 10 farms.

Experimental arrangement and soil sampling

Soil sampling was carried out according to a total randomisation scheme in the 0-20 cm horizons, influenced by organic matter and recognised as the source of about 80% of the nutritional intake of annual crops such as cotton (N'guessan et al., 2016).

Soil samples were taken in 2014 and 2015 using an auger. On each cotton farm, 20 incremental soil samples were taken per hectare. In order to obtain results that are more representative of the reality of the soil in each farm, a composite sample was taken from the mixture of the elementary samples taken in equal parts.

Conditioning of the soil samples after field collection was carried out at the CNRA station in Bouaké. It consisted first of all of air drying, crumbling, sieving with a 2mm sieve to collect fine soil and bagging of the soil samples. These samples were transported to the Eaux-Sol-Végétaux laboratory of the Ecole Supérieure d'Agronomie (ESA) at the Institut National Polytechnique Félix Houphouët Boigny de Yamoussoukro (INPHB), whose methods are recognized.

Laboratory analysis

The physico-chemical analyses carried out according to standard methods (FAO, 1984), concerned 11 quantitative variables: granulometry, pH, Carbon (C), Nitrogen (N), Assimilable phosphorus (P ass.),

(K+, Ca2+, Mg2+, Cation exchange capacity (CEC), base saturation rate (V). The carbon determined was multiplied by 1.724 to estimate the organic matter under cultivated vegetation (Soltner, 2014).

Method for assessing soil fertility levels

The fertility of the studied soils was defined on the basis of the chemical fertility classes listed in Table I (Amonmide et al., 2019).

Ranking of soil chemical fertility levels

- Class I, high fertility level : soils are in this class when the characteristics have no or only slight limitations.
- Class II, medium fertility level: soils are in this class when the characteristics have no more than 3 moderate limitations possibly associated with low limitations.
- Class III, low fertility level: soils are in this class when the characteristics present more than 3 moderate limitations associated with a single severe limitation.
- Class IV, very low fertility level: soils are in this class when their characteristics present more than one severe limitation.

Table I: Evaluation Criteria for Soil Fertility Classes

Characteristics	Fertility level				
	Very high (without limitations)	High (low limitations)	Medium (Medium limitations)	low (severe limitations)	very low (very severe limitations)
	Level 0	Level 1	Level 2	Level 3	Level 4
O.M (%)	> 2	2-1,5	1,5-1	1-0,5	< 0,5
N (%)	> 0,08	0,08-0,06	0,06-0,045	0,045-0,03	< 0,03
P ass ($Cmol^+/kg$)	> 20	20-15	15-10	10-5	< 5
K^+ ($Cmol^+/kg$)	> 0,4	0,4-0,3	0,3-0,2	0,2-0,1	< 0,1
Cation Sum ($Cmol^+/kg$)	> 10	10-7,5	7,5-5	5-2	< 2
V(%)	> 60	60-50	50-30	30-15	< 15
CEC ($Cmol^+/kg$)	> 25	25-15	15-10	10-5	< 5
pH water	5,5-6,5	5,5-6	5,5-5,3	5,3-5,2	< 5,2

Sources : Dabin (1956), in Amonmide et al. 2019.

Statistical analysis

The various averages and graphs were produced using Excel 2016. A principal component analysis (PCA) was performed on the physico-chemical soil data to determine possible correlations. An ascending hierarchical classification made it possible to clearly distinguish the major soil fertility groups according to the practices of the cotton companies. These analyses were carried out with the R software. Studio under Software R3.5.2.

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References

1. Amonmide I., Dagbenonbakin G., Agbangba C. E. et Akponikpe P. Contribution à l'évaluation du niveau de fertilité des sols dans les systèmes de culture à base du coton au Bénin. Int. J. Biol. Chem. Scl. 2019; 13(3) : 1846-1860.
2. Baize, D. Guide des analyses en pédologie, 2nd Ed. France, INRA Editions. 2000. 257 P.
3. Bouchy, C. Contribution à l'étude des déficiences minérales en culture cotonnière de Côte d'Ivoire, Coton et Fibres Tropicales. 1970; Vol.25, No. 2, pp. 235-251.
4. Bouyer, S. & Dabin, B. Etudes pédologiques du delta central du Niger, Agronomie Tropicale, 1963; No 12, pp. 1300-1304.
5. Boyer, J. Soil Potassium. In soils of the humid tropics; 102-135, National Academy of Science publ. (Washington). 1972.

6. Boyer, J. Les sols ferrallitiques, Tome X : facteurs de fertilité et utilisation des sols, Initiations – Documentations Techniques, 1982; No 52, ORSTOM, Paris, France.
7. Braud, M. Le diagnostic foliaire et la nutrition potassique du cotonnier, In Institut International de la Potasse (Eds), le potassium dans les cultures et las sols tropicaux. Compte rendu du 10ème colloque organisé en décembre à Abidjan république de Côte d'Ivoire, 1973; pp. 265-276.
8. Brou, T. Y. Climat, mutations socio-économiques et paysages en Côte d'Ivoire. HDR, Université de Lille, 212 p.XI, 2005; pp 221- 237. ORSTOM – France.
9. Chiapo A. C. Analyse de la filière coton dans le contexte de la libéralisation : cas de la zone de Korhogo (Côte d'Ivoire). Ecole Supérieure d'Agronomie (ESA)-INPHB – Diplôme d'Agronomie Approfondie 2001; 77 p.
10. Dabin B. Les facteurs chimiques de la fertilité des sols (bases échangeables ; sels ; Utilisation des échelles de fertilité) – chapitre XI, 1970; pp 221- 237. ORSTOM – France.
11. Deat, M. Le redressement de la fertilité sur des terres à vocation cotonnière présentant des carences en éléments majeurs", Coton et Fibres Tropicales, 1975; Vol. 30, No. 2, pp. 245-262.
12. Dekoula C. S., Kouame B., N'goran E. K., Yao, F. G., Ehounou J. N., et Soro, N. Impact de la variabilité pluviométrique sur la saison culturale dans la zone de production cotonnière en Côte d'Ivoire. European Scientific Journal, ESJ, 2018; Vol 14 No12 : pp.143-156.
13. Fagaye, S., Coulibaly, D., Cissé, O., Patrick, D. Evaluation de l'arrière effet de la culture du coton sur la production céréalière en zone cotonnière du Mali, In M., Fok, N. Ousmane et K. Siaka (Eds.), AGRAR-2013 : 1re conférence de la recherche africaine sur l'agriculture, l'alimentation et la nutrition, Yamoussoukro : L'agriculture face aux défis de l'alimentation et de la nutrition en Afrique : quels apports de la recherche dans les pays cotonniers, 2015; pp. 149-160.
14. FAO. Méthodes d'analyse physique et chimique des sols et des eaux, Bullettin pédologique de FAO N° 10 Rome, 280 p, 1984.
15. FAO. Word Reference Base for Soil Resources. Food and Agriculture Organization of the United Nations, Rome. 2014.
16. Fritz, A. & Vallerie, M. Contribution à l'étude des déficiences minérales des sols sous culture cotonnière au Nord-Cameroun." Coton et fibres tropicales, 1971; Vol 26, No.3, pp. 273-301.
17. Intercoton. Bulletin d'information de la filière cotonnière ivoirienne. N° 47. www.intercoton.org. Consulté le 23 Mars 2019. 2018.
18. Intercoton. La filière coton en Côte d'Ivoire – Importance et difficultés www.intercoton.org, 2010; 31p.
19. Kambire F. Effets des techniques d'utilisation de la Dent IR.12 sur la production du coton et du maïs en zone cotonnière ouest du Burkina-Faso, Mémoire de fin d'études, Diplômes d'ingénieur du développement rural, option agronomie, 2000; 117p.
20. Kouamé, B. Adéquation de différentes modèles globaux pluie-débit pour déterminer les apports en eau dans les zones de transition et de forêt de la Côte d'Ivoire : Essai de régionalisation des paramètres. Thèse de doctorat, 1992; P.200.
21. Koulibaly B., Traoré O., Dakuo D., Lalsaga R., Lompo F. et Zombre P. Acidification des sols ferrugineux et ferrallitiques dans les systèmes de production cotonnière au Burkina Faso. Int. J. Biol. Chem. Scl. 2014; 8(-) : 2879-2890.
22. Landon, J. R. Booker Tropical Soil Manual, A handbook for soil survey and agricultural land evaluation in the tropics and subtropics, paperback, longman, Booker Tate limited, Oxon, Royaume Uni, 1991; p 474.
23. Latham, M. Rôle du facteur sol dans le développement du cotonnier en Côte d'Ivoire, Cahier ORSTOM, séries Pédologie, 1971; Vol.9, No 1, pp. 29-42.
24. Mulaji, C. Disa-Disa, P., Kibal, I. & Culot, M. Diagnostic de l'état agropédologique des sols acides de la province de Kinshasa en république démocratique du Congo (RDC), Comptes rendus Chimie, 2016; http://dx.doi.org/10.1016/j.cri.2015.08.010.
25. N'guessan, K. J-C., Akotto, O. F., Snoeck, D., Camara, M., & Yao-Kouamé, A. Potentiel de fertilité des vergers de cacaoyer Thebroma cacao L. (Malvacea) en Côte d'Ivoire. International Journal of Innovation and Applied Studies, IJIAS, 2016; Vol. 13 No 3, pp. 868-879.
26. N'goran, K. E., N'guessan, E., Tehia, K. E., Zohouri, G. P. & Ochou, O. G. Valorisation de la fumure organique dans les systèmes de culture à base de cotonniers et sécurité alimentaire en Côte d'Ivoire, In M., Fok, N. Ousmane et K. Siaka (Eds.), AGRAR-2013 : 1re conférence de la recherche africaine sur l'agriculture, l'alimentation et la nutrition, Yamoussoukro : L'agriculture face aux défis de l'alimenation et de la nutrition en Afrique : quels apports de la recherche dans les pays cotonniers, 2015; pp. 187-195.
27. Sawadogo, H. Fertilisation organique et phosphaté en système de culture Zaï en milieu soudano-sahelien du Burkina Faso. Thèse de doctorat, faculté universitaire des sciences agronomiques de Gembloux, Belgique, 2006; P.219.
28. Sement, G. La fertilité des systèmes culturaux à base de cotonnier en Côte d'Ivoire. Neuf années d'expérimentation et d'observations multilocales (1973-1982), 1983; 40 p.
29. Sery, Z. H. Evaluation de l'impact du coton sur le developpement rural : cas de la région de Korhogo au Nord de la Côte d'Ivoire. Thèse de doctorat, faculté universitaire des sciences agronomiques de Gembloux, Belgique, 2005; P. 267.
30. SODEXAM. Découpage agro climatique, Côte d'Ivoire. 2008.
31. Soltner D. Les bases de la production végétale : Tome 1. Le sol et son amélioration. Collections Sciences et Techniques Agricoles Tome I, 26e édition, Paris, 2014; 472 p.
32. Yoro G. R. Les principaux sols de Côte d'Ivoire et leur corrélation avec les groupes de référence de la base mondiale de données des sols (WRB). Quatorzième réunion du sous-comité Ouest et Centre Africain de Corrélation des Sols, 9-13 Octobre 2000, Cotonou. 2000; 19p.
33. Zagbaï, H.S., Berti, F. & Lebailly, P. Impact de la dynamique cotonnière sur le développement rural. Etude de cas de la région de Korhogo, au nord et au centre de la Côte d'Ivoire", Biotecnol. Agron. Soc. Environ. 2006; Vol.10, No. 4, pp. 325-334.

Status of Current Cotton Fibre Quality Parameters in South Africa

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Abstract

Background: *Genetically modified cotton, or so-called Bt-cotton (currently Bollgard®2 with Roundup Ready Flex) has been planted since 1996 in South Africa and currently all cotton planted in South Africa is Bt-cotton, except for refuge areas, giving a 100% adoption rate for GMO. Fibre quality is a key factor determining lint price and demand for local consumption and export to achieve quality of cotton textile products. Fibre quality is characterized by fibre length (mm), strength (grams/tex) and micronaire (unitless), and the textile industry has a preference for long and strong fibres of moderate micronaire for producing high-quality yarns. Fibre quality of Bollgard cotton produced in the different cotton producing areas in South Africa should be of acceptable standard to spinners to produce quality fabric. Quality influences financial return and cotton producers need to avoid discounts by achieving seedcotton of not a preferred quality. Cultivars cultivated in South Africa during the 2018/2019 season included DP1531 B2RF, DP1541 B2RF, DP1240 B2RF, PM3225 B2RF and Candia BGRF, all containing Bollgard 2 in stacked gene combination (CrylAc and Cry2Ab proteins), with Roundup Ready Flex.*

Results: *During the 2018/2019 season six locations were planted with one ha, each of the four commercial cultivars to determine yield and fibre qualities achieved in each specific area. The highest fibre strength of 32.6 gms/tex was obtained from the cultivar DP1240 B2RF, planted in a new cotton producing area in the North-West Province (Locality 1, i.eSchweizer-Reneke), where cotton is cultivated under dryland conditions. This cultivar DP1240 B2RF, showed the highest fibre strength in five out of the six test locations. Micronaire values were acceptable for all cultivars, except for the cultivar Candia which produced fibre with low micronaire of 3.3 at the 2nd locality in the North-West Province (Groot Marico), while it was 3.4 at the 3rd locality (North-West Province - Koppies). The cultivar DP1541 B2RF at this 3rd locality gave a micronaire of 3.3. Quality parameters of cotton produced in South Africa falls within the acceptable and desired ranges by spinners for lengths, strengths and micronaire.*

Conclusion: *These results proved that environment has a high inflence on cotton growth and development and subsequently fibre quality. Cultivar and locality selection are critical factors when making decisions on commercial production of cotton.*

Keywords: Cotton (Gossypiumhirsutum), fibre length, fibre strength, micronaire, South Africa

Background

Cotton is cultivated mainly for its fibre, which is using in the spinning, knitting and weaving industries (Tausifet al., 2018). The first step in the processing of the cotton pickings is taken at the gin, when the fibre of around 36 % of the mass (the so-called Gin out Turn), is separated from the seed. Fibre quality is the key factor that determines fibre price and the quality of cotton textile products (Wang et al., 2014). Fibre quality is characterized by fibre length (inches), strength (gms/tex) and micronaire (unitless) and the textile industry prefers long and strong fibres of moderate micronaire for producing high-quality yarns (Long et al. 2010). For the past several years, the size of the South African cotton crop increased, with the 2018/19 production year the biggest cotton crop since the 1998/99 production year.In South Africa, 24 921 ha were produced under irrigation and 16 792 ha under dryland, producing a total of approximately 220 000 lint bales of 200kg lint each, during the 2018/19 production year. Fibre quality of a cultivar may differ substantially when planted in different environments (Bradowet al. 1997; Davidoniset al. 2004; Campbell and Jones 2005). The cultivars compared in this study include all the current available cultivars in South Africa. The Candia BRF (Bollgard 2 (sic!) with Roundup Ready Flex) originates from Australia, and have a shorter growing season with an early boll-set period, producing a smaller compact plant. It does well under irrigation as well as dryland conditions and the acceptance of Candia is around 60-64% of the current crop. The DP 1541 B2RF, grows more vigorously, with a

medium to longer growth pattern, while DP1531 B2R also tends to have a shorter growth pattern. DP1240 B2RF is adapted for most conditions, and appears to do well under dryland conditions. Delta 18 RF (has only Roundup Ready Flex technology) is planted for refuge areas. Apart from the smooth varieties, small-scale growers plant a hairy variety, PM3225 B2RF, which does well under dryland conditions and gives good quality fibre when handpicked.

Results and discussion

Fibre quality

Fibre lengths of the five commercial cultivars for the 2018/2019 season were in the acceptable range.

Strengths of Bt-cotton at the cotton strip trials in South Africa during the 2018/2019 season at different cotton producing locations were in acceptable strengths falling within the range of (28.3- 32.6 gms/tex). The highest value in fibre strength of 32.6 gms/tex was achieved forfibre samples originating from the cultivar DP1240 B2RF from a new cotton producing area in the North-West Province (Locality 1 - SchweizerReneke) under dryland conditions, followed by 32.0 gms/texfor the same cultivar from a2nd locality in the North-West Province (Groot Marico) cultivated under irrigation. This cultivar (DP1240 B2RF) resulted in the overall highest fibre strength achieved when compared to all localities except for those from a fourthth locality in the Free State Province (Koppies), the latter, which is a new marginal cotton producing area. Mean strengths over seven localities were 30.7 gms/tex for DP1240 B2RF which superseded DP1531 B2RF (29.2 gms/tex), DP1541 B2RF (29.5gms/tex), PM3225 B2RF and Candia BGRF (29.8 gms/tex).

Micronaires were acceptable except for the cultivar Candia from the 2ndlocation (North-West Province), which gave a micronaire of 3.3, while the cultivar DP1541 B2RF at a 4thlocation (Free State Province - Koppies) had a micronaire of 3.3 and the Candia cultivar planted at this locationproduced a micronaire of 3.4 (Table 1).

Table 1. Fibre qualities (lengths (inches), strengths (gms/tex) and micronaires) of the cotton cultivars at different locations in the 2018/2019 season in South Africa

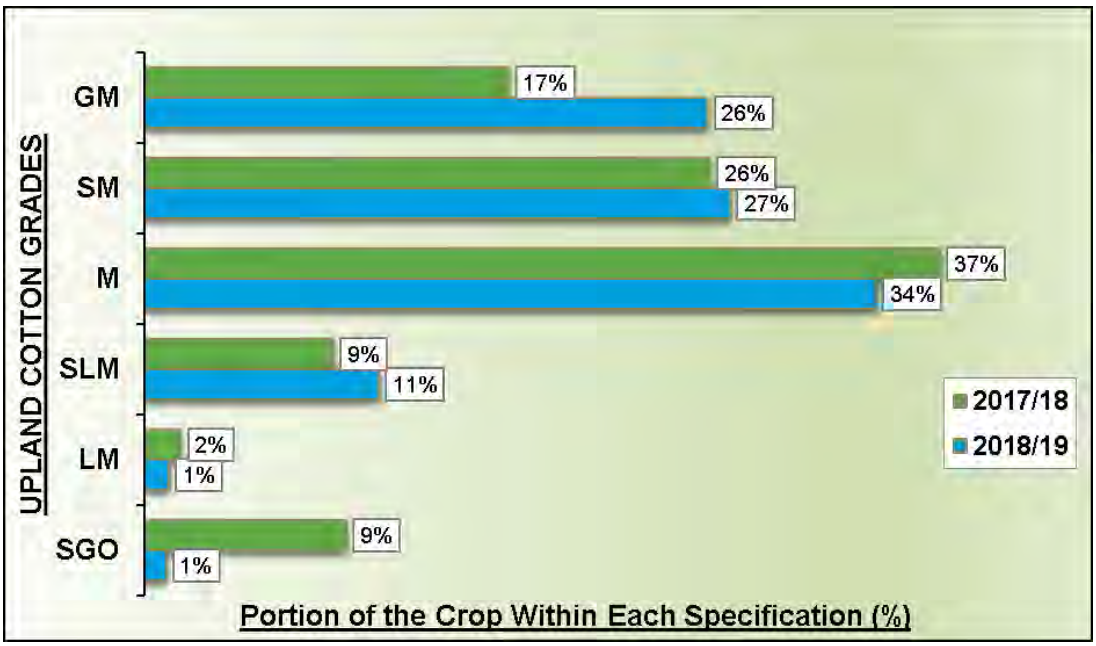
Cultivars	Location (Provinces)						
	Christiana (North-West)	Koppies (Free State)	Groot-Marico (North-West)	Groblersdal (Mpuma langa)	Vaalharts (Northern- Cape)	Schweiser- Reneke (North-West)	Pongola (KwaZulu-Natal)
Length (inches)							
DP1240B2RF	1 " 3/32	1 " 5/32	1 " 3/16	1 " 1/18	1 " 3/16	1 " 3/16	1 " 1/8
DP1531B2RF	1 " 3/32	1 " 7/32	1 " 3/16	1 " 5/32	1 " 7/32	1 " 3/16	1 " 7/32
DP1541B2RF	1 " 3 /2	1 " 3/16	1 " 5/32	1 " 5/32	1 " 3/16	1 " 5/32	1 " 5/32
Candia BGRF	1 "1/8	1 "7/32	1 "3/16	1 "5/32	1 "7/32	1 "7/32	1 "1/4
PM3325 B2RF	*	*	*	*	*	*	1 " 1/8
Strength (gms/tex)							
DP1240B2RF	30.1	29.3	32.0	29.9	30.3	32.6	32.0
DP1531B2RF	28.5	30.4	29.3	29.3	28.3	29.4	30.9
DP1541B2RF	29.1	28.7	30.7	28.7	29.6	30.2	28.7
Candia BGRF	29.8	30.0	31.0	28.4	29.2	30.5	29.5
PM3225 B2RF	*	*	*	*	*	*	31.7
Micronaire							
DP1240B2RF	4.5	3.9	4.0	3.5	4.2	4.8	4.7
DP1531B2RF	4.5	3.5	3.6	3.7	3.9	4.3	4.1
DP1541B2RF	4.9	3.3	4.0	3.9	4.4	4.6	4.8
Candia BGRF	3.9	3.4	3.3	3.7	3.8	4.1	3.5
PM3225 B2RF	*	*	*	*	*	*	4.2

Notes: *Cultivar not planted in these regions

Overall performance of the cotton crop

Favourable weather conditions experienced during the first half of the 2018-19 season have helped to improve the grade and quality performance of the local cotton crop. Figure 1 shows a 10% increase of

the top two grades (Good Middling & Strict Middling) and an 8 % decrease in the lowest grade – SGO (Strict Good Ordinary).



Notes: GM=Good Middling, SM=Strict Middling, M: Middling, SLM=Strict Low Middling, LM=Low Middling and SGO=Strict Good Ordinary)

Figure 1. Visual grades for the 2017/2018 season vs the 2018/2019 season

Values of fibre length is shown in Figure 2. The ever-improving fibre length in the long staple group (1 3/16" and better) makes out 46% of the total crop.

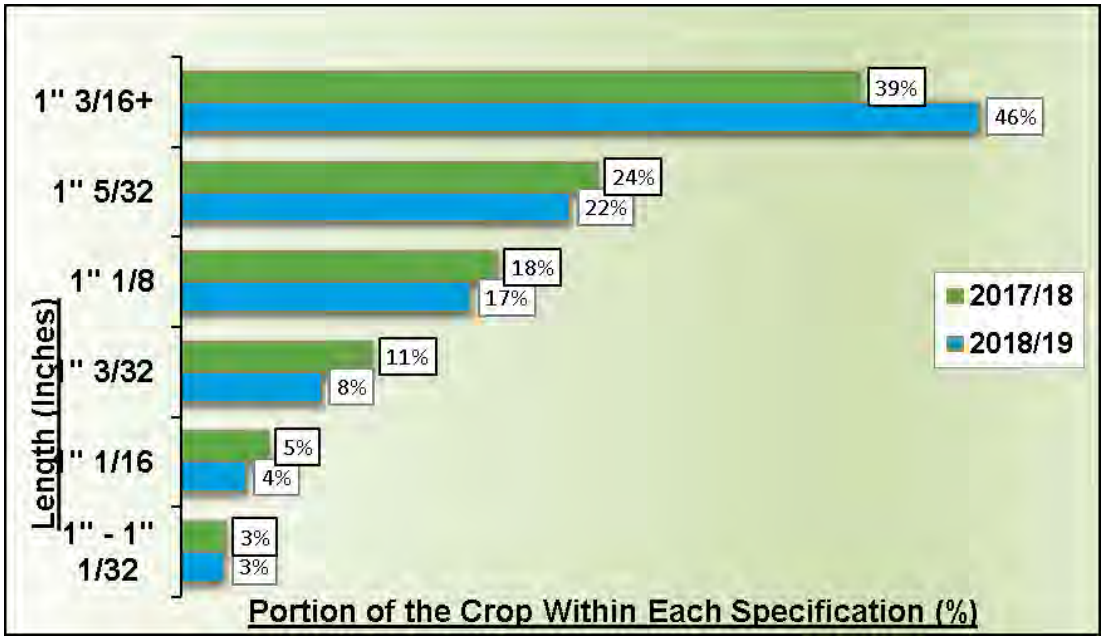


Figure 1. Length for the 2017/2018 season vs the 2018/2019 season (given as staple length)

Micronaire performance is presented in Figure 3. The "premium range" (3.80-4.29) cottons improved by much as 14%, probably because of better climatic conditions.

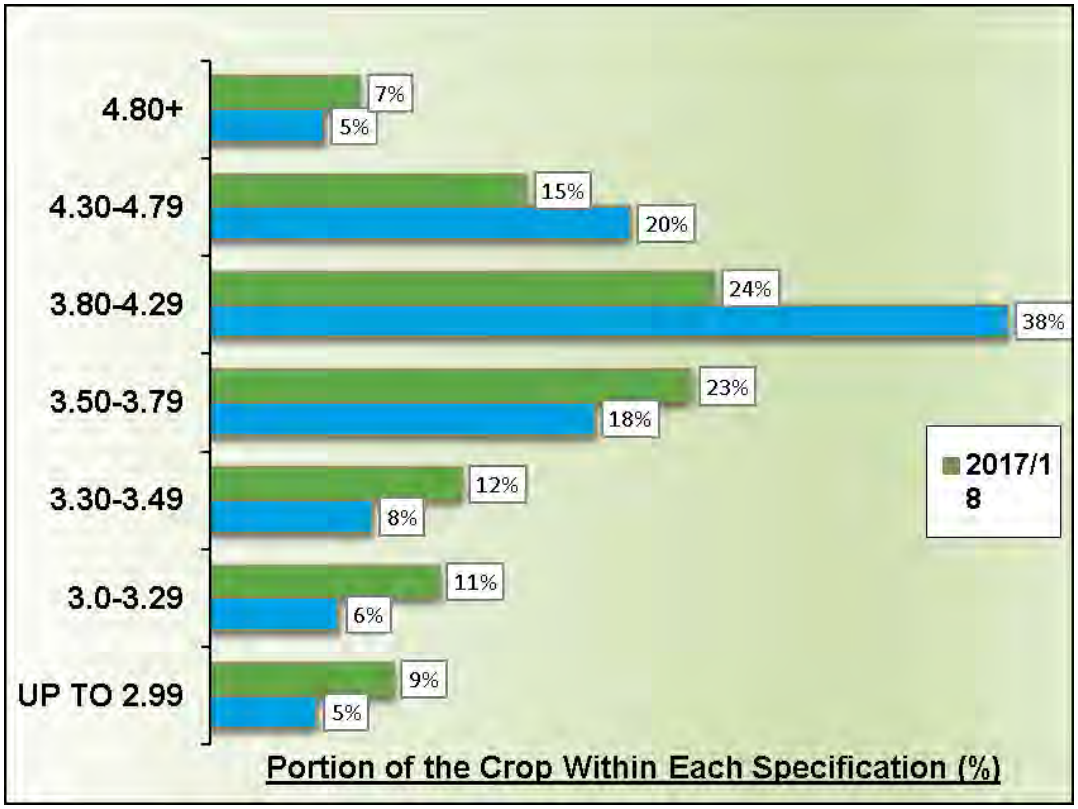


Figure 3. Micronaire for the 2017/2018 season vs the 2018/2019 season

Results on fiber strength performance is given in Figure 4. The results highlight the overall improvement of the fibre strength of cotton crop during 2018/19, with an increase of up to 61% in the groups of 28 gms/tex and better, when compared to 41% achieved the previous season.

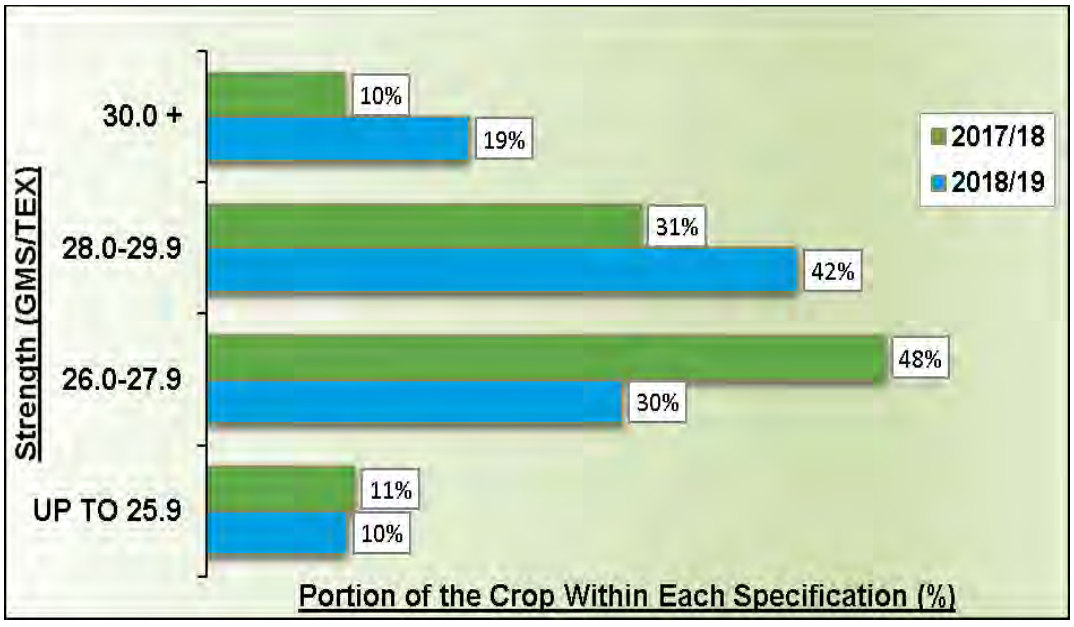


Figure 4. Strength for the 2017/2018 season vs the 2018/2019 season

Values of short fibre index is presented in Figure 5. Short fibre index achieved by the various gins of the two past seasons that indicates the care taken to not over-gin seed cotton and lower cotton lint quality.

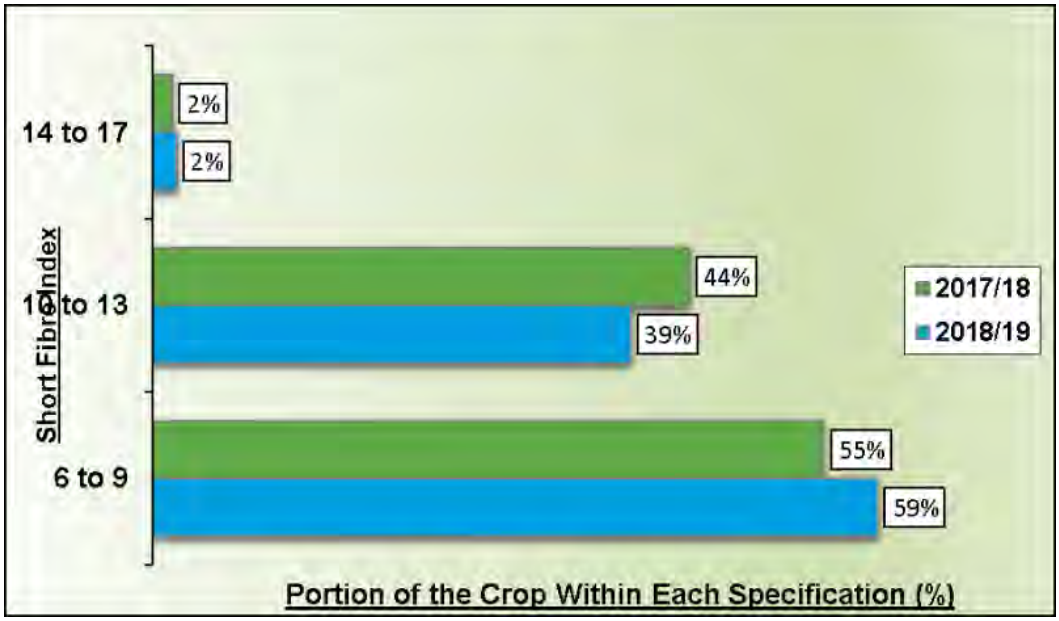


Figure 5. Short Fibre Index (SFI)for the 2017/2018 season vs the 2018/2019 season

Spinning Consistency Index (SCI) is given in Figure 6. The 2018/19 season shows a better overall distribution of the SCI when compared to that of the previous season.

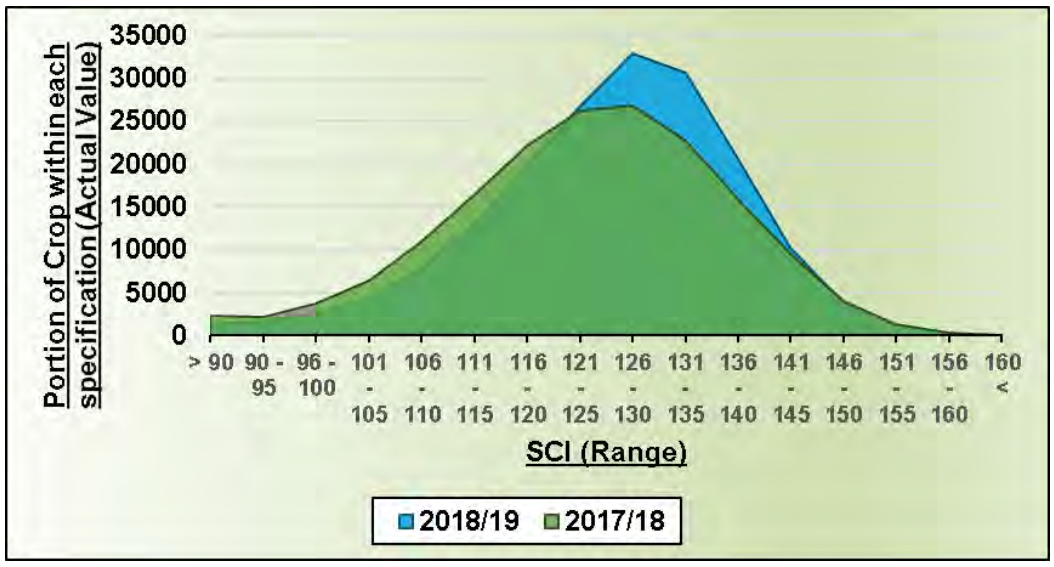


Figure 6. Distribution of the Entire South African Crop Based on SCI for the 2017/2018 season vs the 2018/2019 season

Current cultivar adoption rates in South Africa.

Candia BGRF makes out around 67% of all plantings in South Africa (See **Figure 7**). Currently the lifetime of Candia germplasm is limited, and Candia has been purchased by a private local buyer for further seed multiplication for around 4-5 years. Following on this period, producers would like to access similar varieties with a short growing season and hope to have access to 3rd generation technology in the near future.

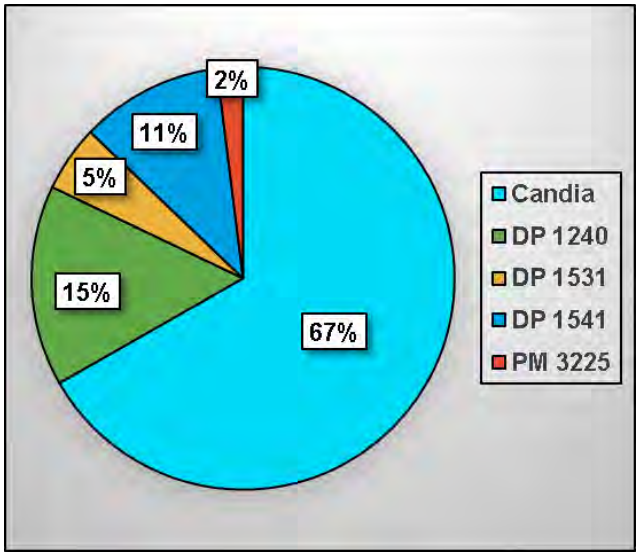


Figure 7: The Cultivar Adoption Rates per cultivar for the 2018/2019 season

Conclusion

There was a downward trend in the fibre strength achieved of the cotton crop, leading into the 2018/2019 season, which in turn has begun an upward movement. Special care is being given to cotton produced in marginal growing areas to ensure that the strength continues to improve. The length of the cotton fibre produced continues to fall within the medium to long staple group and the micronaire falls predominantly in the 3.5 - 4.8 range. Environmental factors heavily influence the quality of cotton fibre produced in South Africa and cultivar selection is critical in marginal growing such asKoppies. The concern remains that the preference for the Candia cultivar among South African farmers will lead to seed being in short supply.

Methods

During the 2018/2019 season, in South Africa, several new cotton-producing areas were identified and strip trials were conducted. Trials consisted of five cultivars namely Candia BGRF (containing Bollgard 2 sic), DP1240 B2RF, DP1531 B2RF, DP1541 B2RF and PM3225 B2RF at six different localities whose climatic conditions are shown in Table 2. Four rows per cultivar were planted, with approximate surface areas of 1ha each. Cotton was machine harvested in bales for yield determination, while fibre samples for quality determination were obtained at four random blocks in each strip trial by handpicking.

Table 2. Agroclimatic information on different localities for the 2018/2019 cotton season

Locality	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)
Vaalharts	34.5	15.7	86.0
Koppies	32.1	15.4	39.2
Loskop	32.6	17.8	26.7
Pongola	27.9	15.4	199.0
Groot Marico	34.0	15.8	229.0
Schweizer-Reneke	33.1	13.7	150.0

Acknowledgement

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References

1. Bradow JM. Bauer PJ. Hinojosa O. Sassenrath-Cole G. Quantitation of cotton fibre -quality variations arising from boll and plant growth environments. European Journal of Agronomy. 1997; Vol 6. 191-204.
2. Davidonis GH, Johnson AS, Landivar JA, Fernandez CJ. Cotton Fiber Quality is Related to Boll Location and Planting Date. Agron. J. 2004; 96:42–47
3. Campbell BT& Jones MA. Assessment of genotype × environment interactions for yield and fiber quality in cotton performance trials. Eyphytica, 2005; 144: 69–78
4. Long RL, Bange MP, Gordon SG, Van der Sluijs MHJ, Naylor GRS, and Constable GA. Fiber Quality and Textile Performance of Some Australian Cotton Genotypes. CropSci. 2010; 50:1509–1518
5. Tausif M, Jabbar A, Naeem MS, Basit A, Ahmad F & Cassidy T. Cotton in the new millennium: advances, economics, perceptions and problems. Textile Progress, 2018; 50:1, 1-66.
6. Wang X, Zhang L, Evers JB, Mao L, Wei S, Pan X, Zhao X, Van der Werf W and Li Z. Predicting the effects of environment and management on cotton fibre growth and quality: a functional – structural plant modelling approach. Published by Oxford University Press on behalf of the Annals of Botany Company (www.aobplants.oxfordjournals.org). 2014.

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Sustainable Cotton Production in Argentina: Addressing the Innovations and Challenges

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Abstract

Background: *Historically, cotton production in Argentina was considered a driving force of the primary sector, the industrial sector and linked services. Cotton faces challenges related to competitiveness between natural and synthetic fibres as well as aspects of sustainability of production. The objective of the work is to share and analyse the various components of innovation involved in the expansion and sustainable production of rainfed cotton over the past 15 years (2005/06 to 2019/20) in the province of Santa Fe, as a case study, in the Republic of Argentina.*

Results: *Through a retrospective analysis, it is possible to visualize the innovations that occurred in that period that allowed the crop to be competitive.*

Santa Fe has demonstrated its ability to innovate in the field of research and development with key impacts on mechanization and agronomic management of cotton cultivation. In the same way, innovation in the organizational aspect of the production chain through extension, training, communication, administrative and political management actions in support of the producer and the industrial chain sector. Numerous challenges ahead include germplasm improvements, implementation of sustainability indicators and new institutional organizational actions.

Keywords: *Cotton; Innovations; Development; Organization; Sustainability*

Background

In the last decades, the behaviour of the cotton industry has varied widely between different countries. While in some, it has experienced a setback or stagnation, in others it has expanded significantly. Cotton faces challenges related to competitiveness between natural and synthetic fibres as well as aspects of sustainability of production. This last term clearly refers to sustainability from its three pillars: social, environmental and economic. Other components that add to the dynamics of cotton are the expansion into new planting areas conditioned by the availability of land and water; the introduction of new technologies, including mechanization, seeds and new inputs; the need to strongly consolidate the relationship between producers, ginners, the textile industry and the rest of the actors, both private and public. The case of Argentina is not different from this initial proposal.

For more than 200 years, cotton has been the most important productive system in the Northern provinces of Argentina. Historically, cotton production in Argentina was considered a driving force of the primary sector, the industrial sector and linked services (Delssin, 2003). However, its production trend has varied markedly over the different decades. In the 1997/98 season, a historical production record was achieved, reaching a maximum area of 1.133.500 hectares nationwide, from which a period of accelerated decline in the planted area began, reaching 160.000 hectares in the 2002/03 season. There are several reasons that discouraged cotton production, including unfavourable prices, competition with soybean and adverse environmental conditions (Paytas and Ploschuk, 2013). However, in recent years the planting area has increased significantly. Through retrospective analysis, technological innovations can be mentioned: i) the development of “stripper” type harvesting machines with low acquisition and maintenance costs, and better prices than manual harvesting; ii) the development, research and extension associated with the expansion of a new planting system called “narrow rows and high densities” that managed to significantly stabilize production; iii) the successful organization of the public and private sector or “cluster” from the producer to the garment manufacturing (Paytas, 2010).

The objective of the work is to share and analyze different components of innovation involved in the expansion and sustainable production of rainfed cotton over the past 15 years in the province of Santa Fe, as a case study, in the Republic of Argentina.

Results

The cotton production area has showed a progressive increase from 2005/06 (less than 15.000 hectares) to the current 2019/20 season (approximately 80.000 hectares), with a significant peak during the 2013/14 season (greater than 150.000 hectares). The average yield obtained in the province of Santa Fe was 1.795 kg.ha-1 of raw cotton and average fibre yield of 30% in the 2018/19 season (Zorzon, 2019).

This situation was initially favoured by the implementation of a technological innovation developed locally by INTA Reconquista (Santa Fe), linked to harvesting. The mechanization through the “Stripper” Type harvester called Javiyu (which means “cocoon” in the Guaraní language) managed to solve the first problem of manual harvesting of the fibre due to its high costs and social problems (Pilatti, 2007). It has a height-adjustable platform, made up of spikes that comb and remove the capsules with a pre-cleaning system to reduce the percentage of trash prior to ginning, thus reducing fibre contamination (Scarpin, 2016; 2017). The public-private association managed to promote the mechanization of the crop, going from 75% of manual harvesting in 2004/05 to 100% of mechanical harvesting today. This harvesting system was implemented in Argentine systems and in other cotton countries. More than 450 units were manufactured locally and 71 of them were exported to other countries. Currently, a new prototype of self-propelled stripper harvesting and roll forming was developed (Bianchi, 2018).

At the same time, the development of a technological cotton package (know how) was promoted under “narrow rows and high densities” system conferring to the mechanical harvesting. Efforts were concentrated on the optimal adjustment of the spatial configuration (Paytas, 2005). Distances between rows of 52 cm and densities of 200.000 plants per hectare (Paytas, 2011) became the most implemented system by producers. The new production model led to increases about 45 kg.ha-1of fibre yield per year from the 2005/06 season to the present.

Optimal planting dates (Winkler, 2018) that begin in October, end in November, and allow the critical stage of flowering to coincide with the best environmental offer. In this way, ensuring the increase observed in recent years as well as incorporating the concept of integrated fibre management (Scarpin, 2017).

Soil health studies that began with the implementation of soil analysis routinely by producers and research programs that adjust the doses and critical moments of the main nutrients. Service crops, rotations and zero tillage are components of the package (Mieres, 2019). In the 2018/19 season, the survey indicated that 87% of the total area has been fertilized, being the highest recorded in the last 18 years (along with the 2007/08 season) (Zorzon, 2019).

The diagnosis by productive zones indicates that the most important limitations to produce cotton in all the zones are the low content of organic matter that results in less water retention and greater susceptibility to water erosion. Other limitations include the low availability of phosphorous and nitrogen, potassium and magnesium exchangeable and with high levels of exchangeable sodium in some areas (Mieres, 2018).

The main environmental limitations for cotton production have implications on various physiological processes. Water stress due to low water availability or commonly called drought can affect differentially depending on the phenological stage in which it occurs, its duration and intensity (Paytas, 2013). The same concept applies for periods of light stress (Paytas, 2016) and high temperatures (Colombo, 2018). In each case, mitigation and compensation strategies are proposed including: i) continue research for each region and its agro-environmental conditions to find out the yield potentials and the increasingly important limiting factors; ii) continue approaches that include soil health management, drainage or irrigation systems, integrated pest management, integrated fiber management, simulation models of cotton cultivation developed for each region. The new production areas in Santa Fe are occasionally affected by periods of water and salinity stress (Mieres, 2016); they are called “Los Bajos Submeridionales”.

Integrated pest management addressed by research programs that identify and propose management practices in a mostly GM cotton adopted system (99%) (Sosa, 2012). It is also remarkable the progress of the control and eradication plan for the cotton boll weevil (Sosa, 2009, Almada, 2017) during cultivation and its post-harvest management (Almada, 2015), as one of the main pests. Weeds and

their control (Menapace and Szwarc, 2019) is another factor that annually generates significant economic losses and the development of management strategies is key. Regarding diseases, it is important to diagnose them and design sustainable strategies for control that requires the precise identification of the causative agent for later studies on dynamics of pathogen sporulation changes (Roeschlin, 2017;Lorenzini, 2019).

Genetic improvement aims to identify materials adapted to various production environments through traditional breeding or biotechnological tools (Dileo, 2019) as well as methods that induce mutagenesis. The percentage of ginning, quality attributes and characteristics of plasticity and adaptability are the aspects that are prioritized in the genetic improvement program.

Discussions

How do we achieve the implementation of innovations by the productive sector? The implementation of cotton development and competitiveness programs must consider a Training Programme so that the farmer and the technician get to know the innovations of the sector and implement them according to their particularities. Continuous communication and education through information and communication tools are essential. During the 2018 season, more than 100 farmerswere trained through practical and conceptual summer courses. In the last couple of years around 250 professionals attended our postgraduate training course organize between INTA and the National University of the Northeast.

The previous paragraph is not feasible without the organization of the sector. What are the steps that we begin to go through in Santa Fe? From the organizational point of view, it is essential to consolidate the association that brings together all the members of the cotton production chain. In the case of Santa Fe, it is called Association for Promotion of Cotton Production and it is almost two decades old. Within the framework of the association, sectoral policies are proposed that boost competitiveness through credits for production inputs, agricultural machinery, financing, and sanitary control. A network of technical advisers was created to promote the implementation of practices for cotton boll weevil control and agronomic management.

From the concept of sustainability, we still have a way to go. Is it possible to incorporate sustainability indicators into our usual production practices? The first local studies of how technological innovations implemented in the production of raw cotton impact, from 1980 to 2018, on socio-economic environmental sustainability, through an “energy” analysis (Vitti Scarel, 2019) is being carried out. This type of approach will need to be considered when evaluating any type of environmental intervention, as well as the ICAC SEEP proposals.

The implementation of the traceability program is another example that allows us to visualize a well-organized chain. Having sustainable production protocols from the field to the garment is essential. The pilot project of 100% Santa Fe traceability carried out during three agricultural seasons in Santa Fe (Lopez, 2014), aimed to design a sustainable production strategy (social, environmental, economic). In these cases, the cotton production from Santa Fe fields ended up in clothes or hospital sheets. Public-private effort ensured that the finished product was sustainable without having to go through high-cost certification company processes. Finally, the implementation of HVI (high Volume Instrument) analysis for all the cotton from Santa Fe is essential to continue the improvement in marketing and fair prices to farmers, thus the current official Quality Fibre Laboratory is strongly central as well as training and management related.

Similar approaches in terms of innovation in research, development, extension and organization seen in Australia, India, the United States, China, and Brazil are clearly synonymous of successes in the cotton sector.

Conclusion

In the last 15 years, Santa Fe has demonstrated its ability to innovate in the field of research and development with key impacts on mechanization and agronomic management of cotton cultivation. In the same way, innovation in the organizational aspect of the production chain by strengthening the Association for the Promotion of Cotton Production with training, communication, administrative and political actions in support of the farmerand the industrial chain sector. Numerous challenges ahead include germplasm improvement, implementation of sustainability indicators and new institutional organizational actions.

Methods

This work addresses the qualitative and quantitative aspects of the implementation of development, research and extension strategies in the cotton sector in the province of Santa Fe (Argentina) during the last 15 years (2005/06 to 2019/20).

Thus, an analysis of the technological innovations achieved through INTA (National Institute of Agricultural Technology) at Reconquista (Santa Fe), in association with the cotton sector is initially presented. It includes mechanization and the technological package (Know how) of agronomic management adapted to environmental conditions. In addition, the comprehensive eco-physiological approach of abiotic and biotic components regardto a climate change scenario and new production areas. This compilation of information shows the main technologicaladvances for strengthening cotton production.

Subsequently, the progress made in terms of strengthening the organization of the sector or “cluster” is included, which embraces extension and training.

Finally, a description of the traceability proposal is made from the field to the garment to validate the sustainable production of cotton and the added valueto local communities.

Acknowledgement

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References

1. Almada, M; Cremona, D; Sager, M; Fernández, A; Vitti, D; Szwarc, D; Paytas, M. Post-harvest cotton regrowth control strategies for weevil management (Anthonomus grandis Boheman). In: XV Jornadas fitosanitarias Argentinas. October 9, 10 and 11, 2015.
2. Almada M; Vitti D; Szwarc D; Paytas, M. Mico insecticides in the north of Santa Fe. New evaluationsagainstthecottonbollweevil2016/17season. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2017; ISSN 2591-3379. 2016/17.
3. Bianchi, E. From the Javiyú project in 2006 to the La Lola project in 2014. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2018; ISSN 2591-3379.
4. Colombo F; Zuil S; Paytas M. Effects of stress from high temperatures and nitrogen fertilization on cotton yield. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2018; ISSN 2591-3379.
5. Delssin, E. Cotton in Santa Fe (A story linked to development). Reconquista Santa Fe Agricultural Experiment Station. Argentina. INTA Editions. 2003; ISSN 1667-5673
6. Dileo P, Scarpin G, Winkler H, Fernandes I, Senna R, Lorenzini F, Rodriguez G, Paytas M. Identification of Contrasting Genotypes for Characteristics of Agronomic Importance in Cotton (Gossypium hirsutum). XVII Latin American Congress of Genetics. In: Journal of Basic and Applied Genetics I Vol XXX Suppl. 2019. (1): 299-326; October 2019.
7. López M, Paiz D, Paytas M. Collaborative Project: 100% Cotton Traceability Test from Santa Fe. In: Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2016; No. 16. 42 pp.
8. Lorenzini F, Winkler H, Dileo P, Scarpin G, Cracogna M, Paytas M, Roeschlin R. Bacteriosis of cotton: Identification and isolation of the causal agent of Angular Cotton Spot in the North of Santa Fe. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2019; ISSN 2591-3379. 2018/19.
9. Menapace P and Szwac D. Herbicides and weed management. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2019; ISSN 2591-3379. 2018/19.
10. Mieres, L .; Szwarc, D .; Almada, M .; Vitti, D .; Sosa, M .; Paytas, M .; Sager, M .; Bianchi E. and Sager G. Cotton in "The Basses". Comprehensive analysis of cotton production in the area of influence of the BajosSubmeridionales. In Revista Voces y Ecos N ° 34. EEA INTA Reconquista. 2015. INTA Editions. 2016; ISSN 0328-1582.
11. Mieres L, Scarpin G, Paytas M. Analysis of soils in cotton lots, limitations and potentials by region. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2018; ISSN 2591-3379.
12. Mieres L, Scarpin G, Paytas M. Service cultivation and fertilization at planting in cotton. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2019; ISSN 2591-3379. 2018/19.
13. Paytas, M. Ultra-narrow and ultra-dense cottons in direct sowing in the north of Santa Fe. In: Revista Asociación para la Promoción de la Producción Algodonera (APPA). Year 4 No. 5. Argentina. 2005; p. 21-22.
14. Paytas, M; Mieres, L; Regonat A; Gregoret O. Narrow row cotton: Can we increase yield by enhancing assimilate supply? In: CongressoBrasileiro De Algodão, 8; Evolution of the chain for the construction of a setor forte: Anais. Campina Grande, PB: EmbrapaAlgodão, 2011; p.1782-1787. Brazil.
15. Paytas, M; Ploschuk, E. Chapter Cotton. In Book: De La Fuente, E; Gil A; Kantolil, A 2013. Industrial crops. Editorial Faculty of Agronomy University of Buenos Aires. 2013; 835p.
16. Paytas, M. Changes in the structure of the cotton plant: Effect of the configuration between furrows and water stress on yield and its components. In: CongressoBrasileiro De Algodão, 9; Brasilia, Brazil. 2013.
17. Paytas M, Yeates S, Bange M. Developing management options for cotton grown in variable solar radiation regions: yield recovery in response to reduced radiation during flowering and cultivar. In Book of: World Cotton Research Conference 6. Goianas, Brazil. May 2016.
18. Pilatti O. New technologies for the cultivation and harvesting of cotton. INTA Informa Newsletter 2007.

19. Rodriguez L, Mieres L, Paytas M. Effect of potassium and dolomite on the growth of capsules and fiber in cottons with high yield potential. In XV Latin American Congress of Plant Physiology. 2014.
20. Roeschlin R, Paytas M. Diseases that affect cotton cultivation in Argentina. Identification and study of the diversity of the causative agent of bacteriosis in cotton. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2017; ISSN 2591-3379. 2016/17.
21. Scarpin, G., Lorenzini, M., Ahumada C., Paytas, M. Pollution and quality of cotton fiber depending on the environment and harvesting heads. In: Industrial Crops - Cotton. 2016; INTA ISSN 1853-7677.
22. Scarpin G, Colombo F, Bianchi E, Ahumada C, Paytas M. Stripper Harvesting Methods And Varieties For Narrow Row Cotton Systems: Effects On Fiber Quality. At: 11th Brazilian Cotton Congress. August 2017.
23. Scarpin G, Dileo P, Paytas M. Cotton partitioned quality. Is there variation in the quality of fiber within the same plant? In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2017; ISSN 2591-3379. 2016/17.
24. Sosa M, Vitti, D, Almada M. Bollweviladvances (AnthonomusgrandisBoheman) (Coleoptera: Curculionidae) in thedepartament of General Obligado (Santa Fe). XIII Jornadas Fitosanitarias Argentinas. Termas de Rio Hondo. 2009.
25. Sosa, M., Almada, M., Vitti, D. Estimation of the richness of arthropod species in genetically modified cotton crops in northern Santa Fe. Congreso. VIII Congreso Argentino de Entomología. San Carlos de Bariloche, Río Negro. INTA-Bariloche, CONICET-INIBIOMA, SociedadEntomológica Argentina. 2012.
26. Vitti Scarel D. Environmentalassessment of cottonproduction. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2019; ISSN 2591-3379. 2018/19.
27. Winkler, H.; Scarpin, G; Dileo, P.; Roeschlin, R.; Paytas, M. Effect of the interaction of different sowing dates and genotypes on cotton fiber yield and quality. In: XXXII Argentine Meeting (RAFV) and XVI Latin American Congress of plant physiology November 2018. Córdoba.
28. Zorzon, C. Cotton in the province of Santa Fe Period 2018/2019. In Revista Asociación para la Promoción de la Producción Algodonera (APPA). 2019; ISSN 2591-3379. 2018/19.

Using Technology and Training to help farmers grow clean cotton while reducing cost of inputs

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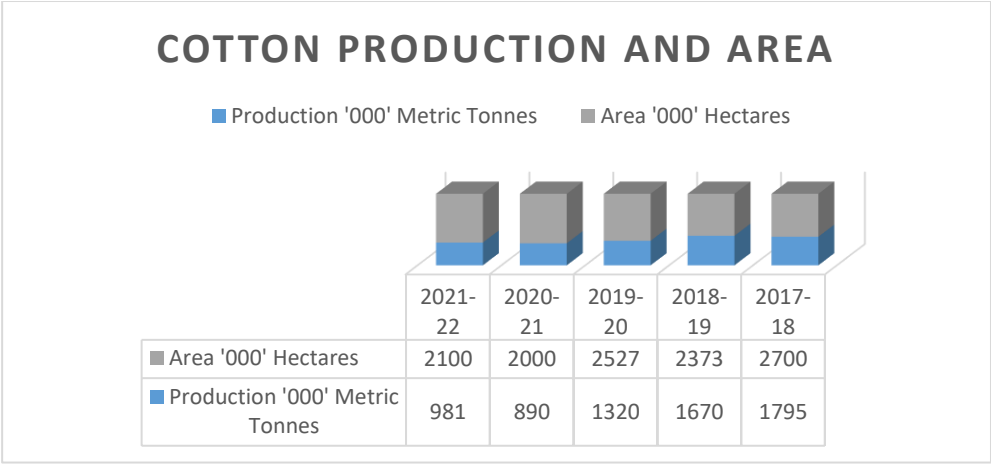
Abstract

***Background**: Agriculture in Pakistan is regarded as 2nd largest sector following service sector and this sector contributes 22.7% to GDP (GOP, 2022). Agriculture is a way to alleviate food shortage and to mitigate the fatal consequences of poverty. There are a host of factors responsible for reducing efficiency of this sector, including conventional management practices, limited or no adoption of modern farming techniques, and lack of technological innovations, rapid urbanization, water scarcity, and climate change (Waqas et al. 2017). The situation of rapid depletion of ground water is alarming (Punthakey et al. 2016), One of major reasons of water scarcity is poor irrigation management (Qureshi et al. 2010).*
Artistic Milliner's Milliner Cotton Initiative (MCI) aims to assist the farmers and all relevant stakeholders in the supply chain to produce better quality cotton. MCI has helped farmers and all other stakeholders in the cotton supply chain by enabling them to trace the cotton from farm to retail, while using smart farming to help reduce costs and increase productivity. WWF Pakistan is the implementation partner for Artistic Milliners in this project.
***Conclusion**: By ensuring clean cotton production practices in the field and arranging continuous cotton picking trainings for cotton pickers, it has been possible to retain best agricultural practices. Precision farming resulted in expected desired outcomes with climate friendly approach.*
***Keywords**: Smart Farming, Traceability, Alternate livelihood, Clean cotton*

Introduction

Cotton is considered as white gold for its fiber and its impact of global economy could be extended over $600 billion worldwide in context of textile industries. (Ashraf et al., 2018). In Pakistan, cotton is second largest cash crop after wheat; while the cotton production increased to 8.329 million bales from past year (GOP, 2022). It is a major source of cotton lint as raw material to textile sector and significant source of foreign exchange (1). Pakistan is ranked 6th in terms of cotton lint production worldwide (ICAC, 2021-22). Cotton crop is suffering worst impacts due to excessive usage of fertilizer and pesticide application along with inefficient production practices (Zulfiqar et al., 2017). Long-term sustainability of cotton production system is at risk due to decline in yields, even using excessive resources and having bad impact on environment (Makhdum et al., 2011). Because of dilemma in Agricultural sector caused by various factors, Artistic milliners has promoted an initiative "Milliners Cotton Initiative" that aim at best cotton picking practices, provide resources and skills to cotton pickers for alternative sources of income, traceability and precision farming.



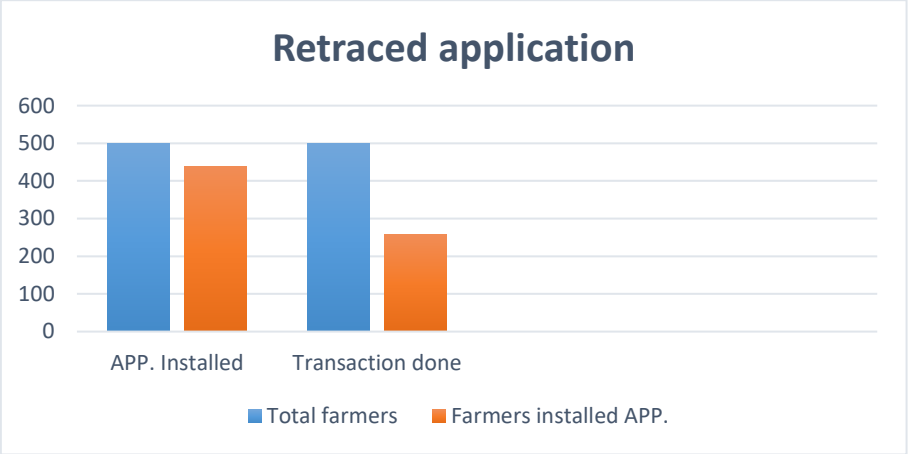
	2021-22	2020-21	2019-20	2018-19	2017-18
Area '000' Hectares	2100	2000	2527	2373	2700
Production '000' Metric Tonnes	981	890	1320	1670	1795

Graph 1. International Cotton Advisory Committee (https://www.icac.org/home/index/)

Artistic milliners is a textile business aiming at transforming the supply chain by linking farms to textile industry and international partners; thus, promoting the economically feasible, environmental friendly cotton growing and picking strategies. In 2020, Artistic Milliners started an initiative in collaboration with WWF in southern Punjab District Rahim yar khan that aimed at improving block chain traceability by introducing a mobile application for transparent traceability of cotton seed from farm to ginning factory. There are total of 500 farmers and 200 females (cotton pickers) registered under the umbrella of Milliners cotton initiative. The initiative establishes livelihood program and clean cotton picking trainings. This program helps in boosting sustainable program by increasing capacity building of cotton pickers, farmers and cotton ginners. There have been various trainings arranged by MCI staff for livelihood activities such as kitchen gardening, apiculture, poultry farming, embroidery. Clean cotton picking practices are introduced in the field in order to produce high quality cotton lint and cotton picking bags were distributed among female cotton pickers.

Traceability

Traceability is maintained by introducing retraced application that covers accurate record and processing of transaction data from farmers to ginning mills and mills to textile industry. Farmers have installed application and put the data of total raw cotton transaction to ginning mills, while ginning mills put tags on cotton bales and ship the bales to Artistic Milliners with proper transaction ID's. There is no need of middleman while selling the raw cotton to ginning mills. Farmers are linked with ginning mills and transport facility is available for those farmers who are unable to ship by themselves. It is not in compliance for farmers to use smartphone and some of farmers don't have any access to the internet connectivity, so some of farmers are unable to install the application and some farmers could not put data into the retraced application.



Best cotton picking practices

Cotton picking trainings are provided by MCI staff to cotton pickers that address how to pick clean cotton. White cotton picking bags are given to MCI registered females so that chances of contamination could be minimized. Personal protective equipment is used by cotton pickers including white gloves and picking pattern is comprehensible by all registered females. Technical approach is also a part of training in such a way that picking should be started from lower part of cotton plant and picking should be started when dew get dry from cotton plants. The recommended picking time starts when there

Precision Farming

Smart farming has been practiced and promoted as significant approach towards productive and efficient farming enterprises. Information and communication technologies help farmer to access the real time data and decisions could be made accordingly. In MCI project, crop2X is helping farmers to adopt the modern technology so that farmer could utilize weather forecasting data, pest dynamics, growth stages, irrigation patterns and application of chemical fertilizers. In conventional farming, farmer use excessive water based on month or days, over-usage of fertilizers and pesticide without considering the need accurately. Satellite crop monitoring is being possible by installing the weather station in MCI registered farmer cotton field and Normalized Difference Vegetation Index (NDVI) helps farmers to identify the healthy plants and to observe the reasons for weak plants. GPS tracking of cotton field alert the farmers about onset attack of pest attack and preventive measures could be taken by the farmers

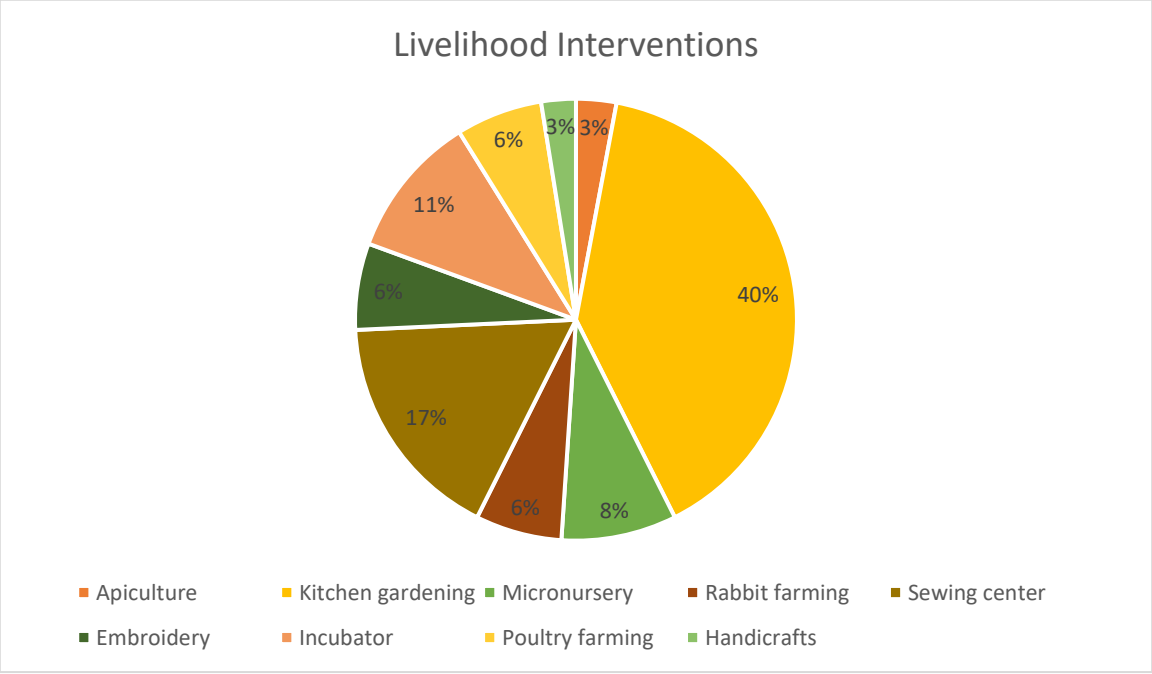
to escape the loss. It helps to attain the objective of minimum disturbance of ecosystem by minimizing the chemicals and efficient use of water due to scarcity of water in region.

Table 1: Data collected by crop2X report and verified by control union.

Sr. No	Factors	Conventional farming	Smart farming
1	Pesticide cost (Rs.)	5500	3600
2	Fertilizer cost (Rs.)	21700	10100
3	Irrigation hours	20	12
4	Yield (Mound)	21	28

Livelihood Interventions

In MCI project, various villages are selected and 300 female cotton pickers are registered in this project as female beneficiaries. Livelihood support is provided to the beneficiaries so that they could participate in earning capital for their family and educate their children and meet some of basic needs. Livelihood experts introduce numerous interventions on local and domestic levels. MCI project is mainly focused on providing trainings to cotton pickers about clean cotton picking practices. There have been many cases in which women have to bound within their homes due to social taboos, so for such beneficiaries, home-based interventions are established like embroidery. Interventions including kitchen gardening, apiculture, sewing center, cuniculture, micro nursery, embroidery and poultry farming. There have been many success stories; For instance, incubator was provided to a group of 10 females for poultry eggs hatching, after first hatching, nearby females were astonished and they bought their own incubator.



References

1. Zulfiqar, F., Datta, A. and Thapa, G.B., 2017. Determinants and resource use efficiency of "better cotton": An innovative cleaner production alternative. Journal of cleaner production, 166, pp.1372-1380.
2. Makhdum, A.H., Khan, H.N. and Ahmad, S., 2011, February. Reducing cotton footprints through implementation of better management practices in cotton production; a step towards Better Cotton Initiative. In Proceedings of the Fifth Meeting of the Asian Cotton Research and Development Network, Lahore, Pakistan (Vol. 1).
3. GOP. Agricultural Statistics of Pakistan 2021-22; Ministriy of Finance: Islamabad, Pakistan. Available online: https://www.finance.gov.pk/survey_2022.html.
4. Waqas, A., Hong, C., Maroof, A.M., Rana, R.M., Saima, A. and Ali, A., 2017. Comparison of organic farming and conventional farming in the Punjab, Pakistan. Int. J. Sci. Eng. Res, 8, pp.29-38.
5. Punthakey, J., Khan, M., Ahmad, R.N., Riaz, M., Javed, M., Zakir, G., Usman, M., Amin, M., Culas, R., Baig, I.A. and Blackwell, J., 2016. Optimising canal and groundwater management to assist water user associations in maximizing crop production and managing salinisation in Australia and Pakistan.
6. Qureshi, A.S., McCornick, P.G., Sarwar, A. and Sharma, B.R., 2010. Challenges and prospects of sustainable groundwater management in the Indus Basin, Pakistan. Water resources management, 24(8), pp.1551-1569.

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Coping for sustainable cotton production through developing of climate resilient cotton varieties in mutant's background in Pakistan

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Abstract

Cotton has special significance in Asia Pacific region as a leading fiber crop with its cultivation on 20.5 million hectares in three major cotton producing countries i.e. China, India and Pakistan. Pakistan is one of the most affected countries by climate change, with its disastrous effects of extreme period of heat stress i.e. 40-50 % fruit abortion during 2013-14 and 33 % shortfall in Cotton Production during 2016-17. The devastating effects of climate change in cotton production in Pakistan can be further visualized that during the year in 2019, 25 % short fall in area, 40.2 % in yield and 25 % in yield per acre against the year 2004. Poor resilience of main grown GMO cotton varieties against extreme periods of heat stress are considered as main cause this drastic fall. Using the approach of induced mutation breeding, NIAB Faisalabad, Pakistan has demonstrated its capabilities in developing cotton mutants that can withstand under the changing climatic conditions. The results of thermos-tolerant cotton varieties in the background of mutants derived (i.e. NIAB-878, NIAB-545, NIAB-1048, NIAB-444, NIAB-1089, NIAB-1064, NIAB-1042 in comparison i.e. FH-142 and FH-Lalazar for their phenological & physiological traits conferring heat tolerance will be presented in the said event. NIAB-878 excelled in heat tolerance by maintaining the highest anther dehiscence (82 %) and minimum cell injury percentage (39 %) along with illustrating of maximum stomatal conductance (27.7 mmol CO2 m-2s-1), transpiration rate (6.89 µmol H2O m-2s-1), net photosynthetic rate (44.6 mmol CO2 m-2s-1) and physiological water use efficiency (6.81 mmol CO2/ µmol H2O). The author would like to share the gradual adaptation potential (above 41 % share in seed production) of these climate resilient cotton varieties among the farming community with their ability to sustain their yield under the changed climatic scenario with recovery against biotic & abiotic stresses.

Key Words: *Sustainable, Climate change, cotton productivity, Phenological & Physiological traits*

Introduction

Cotton has a special significance and plays an important role in the economies of Australia, China, India, Iran (Islamic Republic of), Myanmar, Pakistan, Viet Nam and Bangladesh. This leading fibre crop is grown on 20.5 million hectares in the three main cotton producing countries of the Asia and Pacific region i.e. China, India and Pakistan, with their annual contribution of about 60-65 % in total world cotton production. Bangladesh, Myanmar, Viet Nam and Iran (Islamic Republic of), have a very nominal role in total world cotton production. However, emerging demands from Viet Nam and Bangladesh for their cotton mill use, signifies the increased role of cotton production in the economy of regional countries. The huge yield gap differences exist amongst the top three cotton producing countries of the region (i.e. China;1484 Kg/ha, India; 529 Kg/ha and Pakistan; 700 Kg/ha), but also amongst other countries of the region (i.e. Viet Nam;453 Kg/ha, Bangladesh; 608.0 Kg/ha, Iran (Islamic Republic of); 594 Kg/ha and Myanmar; 653 Kg/ha). These yield differences amongst regional

countries are being further aggravated due to changing climate associated with higher temperature stresses. Pakistan is likely to be the country that is most affected by climate change as far as agriculture and cotton production are concerned. Year to year variation in yields of the cotton crop due to climate change is not only impacting the farming industry negatively, it is also straining the positive development of cotton based industries in the region. The cotton belts in countries like Bangladesh, China, Iran (Islamic Republic of) and Pakistan, are located in high temperature zone, where the maximum temperature often exceeds 40°C during the cotton growing season. This increased trend of temperature inhibits the growth of the plant, causes increased photorespiration, leads to poor control of insect/pests, and enhances the requirements for inputs like irrigation and fertilizer with higher cost of production. The disastrous effects of extreme period of heat stress are very prominent in Pakistan during the cotton growing season 2013-14, there was early termination of crop with 40-50 % abortion of fruiting parts. The quality of lint and cotton seed in affected in general, while seed cotton yield per hectare is particularly affected due to changing climatic conditions (high temperatures/heavy rainfalls). The sensitivity of commercial cotton varieties against extreme periods of heat stress coupled with enhanced requirements for inputs like water and fertilizer, poor seed germination is considered as major factors/causes of erratic trend of cotton production in region. The impact of global warming on cotton production (Anonymous, 2013a, 2013b & 2013c), effects of unexpected periodic episodes of extreme heat stress on cotton in China (Liu, et al., 2006; Zhou et al., 1996) India (Anonymous. 2013c) and Pakistan are gaining significance as emerging challenges for researchers and cotton producers in coming years. Reduced pollen viability (Burke et al., 2004), the physiological response of cotton to high temperatures (Bibi etal,2003), less fertilization efficiency (Snider et al., 2009), reduced boll size and seed number per locules (Pettigrew, 2008), increased fruit shedding (Hodges et al., 1993; Reddy et al., 1991a &1999) are the main effects associated with reduction cotton yield associated due to extreme periods of heat stress. Screening of cotton cultivars for high temperature (Liu, et al.,2006), use of cellular membrane thermo stability for heat tolerance in upland cotton (Rahman et al., 2004), genetic diversity for stomatal conductance in Pima cotton (Radin et al.,1994), multi-level determination of heat tolerance in upland cotton under the field conditions (Cottee, et al., 2007 & 2010), screening of upland cotton under the field conditions (Karademir etal, 2012), High temperature stress on floral development and yield of cotton (Oosterhuis etal,2008 & 2011) influence and emphasis of high temperature, breeding for heat tolerance in cotton to evolve heat tolerant cotton varieties (Singh etal 2007) and multi-model projections of future climate and climate change impacts uncertainty assessment for cotton production in Pakistan (Rahman etal, 2018) are well documented in early findings. Using the approach of induced mutation breeding, NIAB, Faisalabad, Pakistan has demonstrated its capabilities in developing cotton mutants that can withstand the changing climatic scenario of heat stress. So far, NIAB has developed 14 cotton cultivars through the use of induced mutation include the famous cultivar of NIAB-78, NIAB-Krishma, NIAB-111 and most recently, two high yielding and fine fiber cotton varieties i.e. NIAB-KIRAN and NIAB-878, NIAB-545 & NIAB-1048. The cotton mutants, developed through the use of induced mutation, showed enhanced resilience against high temperatures under the field conditions and also showed significant variations in their rooting length after days of sowing (i.e. 30,60,90,120 and 150). Thus, the results of cotton mutants for their various phenological and physiological traits studied under the current investigation suggest, that these cotton mutants developed in the background of induced mutation has the ability to sustain their yield against the changing climatic scenario of high temperature are being presented under the present studies.

Materials and Methods

Seven advanced cotton mutant genotypes i.e. NIAB-878, NIAB-545, NIAB-1011/48, NIAB-444, NIAB-1011/89, NIAB-1011/64, NIAB-1011/42 along with 02 controls (i.e. FH-142 and FH-Lalazar) were evaluated for their responses to high temperature under field conditions at Nuclear Institute for Agriculture & Biology in RCBD. Data regarding morphological/ phenological parameters conferring for heat tolerance was recorded. The same set of genotypes were also evaluated for their heat tolerance, under field conditions at Central Cotton Research Institute, Multan using physiological attributes. Sowing of genotypes at Nuclear Institute for Agriculture & Biology was done in the 1st week of May during 2016-17, whilst sowing of material at Central Cotton Research Institute Multan, was

done in the month of mid-April (to coincide their fruiting phase with the hottest period of high temperature of the season). Data regarding various other phonological traits i.e. plant height, number of squares/flowers, total bolls formed/opened/un-opened were recorded on five guarded plants of each genotype in four repeats after 75 days of planting with 15 days' interval up to 150 days was recorded.

Data regarding physiological parameters that potentially contribute towards heat tolerance in cotton like: relative cell injury, electrical conductivity, anther dehiscence, pollen viability, gas exchange characteristics (stomatal conductance, transpiration rate, net photosynthesis rate and physiological water use efficiency) and some morphological characteristics like; 1st sympodia node number/its height, sympodia node number bearing 1st effective boll, sympodia node height bearing first effective boll, percent boll set on first position along sympodia, percent boll set on second position along sympodia, total fruiting positions/ intact points, number of locules and seeds/boll up to 10th sympodia of the genotypes were also recorded. Porometer was also used to measure leaf transpiration rate, diffusive resistance, relative humidity, leaf temperature etc. Gas exchange characteristics (stomatal conductance, transpiration rate, net photosynthesis rate and physiological water use efficiency) were also computed from the data generated by Porometer. At maturity, data were also recorded on plant population, plant height, number of bolls per m^2, yield potential and average yield per plant etc. Analysis of seed parameters that may contribute towards heat tolerance was also done. Seed protein contents were measured by using Micro Kajeldhal method as reported by AOAC (1990). Seed coat percentage, seed embryo percentage and their indexes were calculated as follows. Agronomic practices were kept uniform throughout the experiment period. Seed coat & seed embryos percentages were calculated by using the formula:

i. Seed Coat (%) = (Coat dry weight/seed dry weight) × 100
ii. Seed Embryo (%) = (Embryo dry weight/seed dry weight) × 100
iii. Seed Coat Index (SCI): SCI = Coat dry weight/Seed dry weight
iv. Embryo Index (EI): EI = Embryo dry weight/Seed dry weight

Results

The results of various phenological attributes conferring for heat tolerance i.e. fruit retention capacity/ number of bolls, locules and number of seeds up to 10th sympodia of the plant, plant height, squares/flowers and number of bolls formed /opened days after sowing (15 days' interval), yield /plant (g) up to 10th sympodia, seed coat (%), embryo and %, Protein % and final yield data (Kg ha^{-1}) for the studied cotton genotypes are given in the Tables-1, 2 &3. Data regarding various physiological parameters conferring to heat tolerance; such as relative cell injury, electrical conductivity, anther dehiscence, pollen viability, gas exchange characteristics (i.e. stomatal conductance, transpiration rate, net photosynthesis rate and physiological water use efficiency) are given in Figures 2-12.

From the given data in the Table-1, it is evident that maximum fruit retention capacity up to 10th sympodia was shown by NIAB-1089 (56 %) followed by NIAB-545 (47%), NIAB-878B (46%) and NIAB-1011/48 (42%) against standard varieties (i.e. FH-142 & FH-Lalazar) with very less fruit retention capacity (25%). The highest number of bolls, locules and number of seeds up to 10th sympodia of the plant (21 bolls; 79 locules and 357 seeds) was shown by NIAB-1089, followed by NIAB-444 (17 bolls; 69 locules and 336 seeds) and NIAB-545 (16 bolls; 65 locules and 316 seeds) as compared to standard varieties i.e. FH-142 (9 bolls; 36 locules and 189 seeds) & FH-Lalazar (7 bolls; 29 locules and 141 seeds). The highest value of yield per plant (g) up to 10th sympodia were revealed by NIAB-1089 and NIAB-444 (40 g) followed by NIAB-545 (35 g) and NIAB-878B (32 g) about 45 to 57 % higher than standard varieties (i.e. FH-142; 22 g & FH-Lalazar; 17 g).

Data recorded for other plant phenological traits days after planting (DAP) with 15 days' interval is given in the Table-2. NIAB-1042 showed the highest value for plant height (171 cm) against the minimum plant height value of 147 cm for cotton genotype NIAB-444. For all other genotypes height values ranged of from 149 to 168 cm. NIAB-1011/64 produced maximum number of bolls (92) at 150 DAP followed by NIAB-1089 (89) and NIAB-545 (88) against FH-Lalazar with minimum number of

bolls (65) and FH-142 (84). Data on flowers per plant were consistent for all genotypes except FH-Lalazar which showed 3 flowers at interval of 75 DAP. Flowering was completely ceased in FH-Lalazar at 135 DAP. Data on number of squares per plant yielded interesting results because all genotypes showed variation even at 150 DAP. There was increase in number of squares for all genotypes between 75 DAP and 90 DAP. After this interval number of squares were started to decline till 135 DAP. After this interval all genotypes showed increase up to 150 DAP and ranged between 6-10 squares per plant. NIAB-1011/64 surpassed all genotypes with 82 opened bolls per plant against FH-Lalazar (60) and FH-142 (75) after 150 DAP also as given in the Table-3. From the given data in Table-3 it is also evident that NIAB-1011/89 showed maximum number of unopened bolls (11) followed by NIAB-1011/64 (10) against FH-Lazar (5) and FH-142 (7) after 150 DAP. From the yield results data as given in theTable-1, it is also evident that NIAB-1011/48 surpassed all the genotypes by illustrating the highest yield of 5477 Kg ha-1 followed by the yield of NIAB 1011/64 (5475 Kg ha-1) and NIAB-878B (5384 Kg ha-1) against the yield of FH-142 (4465 Kg ha-1) and FH-Lalazar (3328 Kg ha-1) respectively.

From the data values given as Figure-1, it is evident that the maximum seed coat percentage (42%) was shown by NIAB-878B, followed by NIAB-1011/48 (40%) against minimum seed coat percentage value of FH-Lalazar (37%). For embryo percentage, NIAB-1089 showed maximum portion of embryo (4.76%) followed by NIAB-545 (4.39%) and NIAB-1011/64 (4.27%) as compared to standard FH-Lalazar which showed 3.18% embryo. Regarding seed protein percentage, NIAB-1011/48 showed maximum content (28 %) followed by NIAB-1011/64 (27.13 %) and NIAB-1089 (27.04 %) against FH-Lalazar was 24.59% also given as Figure-1.

Data regarding various physiological parameters conferring for heat tolerance are given as Figures 2-8. Data given in Figure-2 illustrates that, NIAB-878B excelled in heat tolerance by maintaining the highest anther dehiscence (82%), pollen viability (97%) and lowest cell injury percentage (39 %) as compared to other genotypes and standard varieties. Lowest anther dehiscence (53 %), pollen viability (55 %) and maximum cell injury percentage (87 %) was shown by NIAB-Bt-2 and found to be the most susceptible genotype to heat stress. Data regarding gas exchange characteristics like stomatal conductance (gs), transpiration rate (E) and net photosynthetic rate (PN) varied among the genotypes as given in Figures 9-12. The stomatal conductance (gs) varied from 21.3 to 27.7 m mol CO2 m-2 s-1, transpiration rate (E) from 5.04 to 6.89 µ mole H2O m-2s-1 and net photosynthetic rate (PN) from 32.0 to 44.6 m mol CO2 m-2s-1. The physiological water use efficiency (PN/E) varied from 5.25 to 6.81 m mol CO2/µ mol H2O in different genotypes. Amongst the evaluated genotypes, NIAB-878B maintained the highest values of net photosynthetic rate and physiological water use efficiency under the prevailing high temperature conditions. NIAB-878B also surpassed all genotypes by manifesting the maximum value of stomatal conductance (27.7 mmol CO2 m-2s-1), transpiration rate (6.89 µmol H2O m-2s-1), net photosynthetic rate (44.6 mmol CO2 m-2s-1) and physiological water use efficiency (6.81 mmol CO2/ µmol H2O) under prevailing high temperature conditions. NIAB-Bt-2 considering the most susceptible genotype to heat tolerance showed minimum stomatal conductance (15.6 mmol CO2 m-2s-1), transpiration rate (3.99 µmol H2O m-2s-1), net photosynthetic rate (20.3 mmol CO2 m-2s-1) and physiological water use efficiency (5.20 mmol CO2/ µmol H2O) among all studied genotypes.

Discussion

The studies were conducted in identifying/screening of thermos- tolerant and sensitive cotton genotypes, under the prevailing high temperature under the field conditions using various morphological and physiological attributes conferring for heat tolerance in cotton. Amongst 07 advanced cotton genotypes i.e. i.e. NIAB-878, NIAB-545, NIAB-1011/48, NIAB-444, NIAB-1011/89, NIAB-1011/64, NIAB-1011/42 (F_8 generation) along with 02 controls (i.e. FH-142 and FH-Lalazar) evaluated for their responses to high temperature under controlled conditions revealed that maximum fruit retention capacity, highest number of bolls/locules and number of seeds, maximum seed cotton yield per plant up to 10^{th} sympodia of the plant was shown by NIAB-1011/89 against standard sensitive cotton varieties (i.e. FH-142 & FH-Lalazar). The standard cotton varieties illustrated very less fruit retention capacity, lowest number of bolls/ locules/ number of seeds and yield per plant up

to 10th sympodia of the plant showing their inability to survive under the prevailing high temperature with ultimate loss/shedding of bolls. Fruit retention ability up to 10th sympodia of the genotypes studied was used as marker in developing of heat tolerant cotton mutants, due the reason that the reproductive organs of the plant are mostly affected under peak of high temperature (July-August) up to this part of plant. In the months of July – August day (40 ^{0C} +) and night temperature (27 ^{0C} +) often exceed the optimal limit. Maximum seed coat percentage value was shown by NIAB-878, followed, by NIAB-1011/48 against minimum seed coat percentage value of FH-Lalazar standard. The data when compared with other studied parameters showed that seed coat in cotton might act as environment protectant, against high temperature in cotton particularly, in protecting of development of cotyledons in developing bolls of the cotton plant. Stronger the development of cotyledon, the stronger the development of seed vigour with ultimate in gain weight of seed cotton per boll, resulting vigorous germination and crop growth stand. Embryo percentage studies results of genotypes showed that, that maximum portion of embryo was shown by NIAB-1011/89, followed by NIAB-545 in comparison with standard FH-Lalazar reflecting, the least effect of high temperature in the development of embryo during the peak hours of high temperature (July –August).

Higher the values of anther dehiscence/pollen viability and lower values for cell injury percentage makes the cotton plant most resilient against the high temperature tolerance. NIAB-878B excelled in heat tolerance by maintaining the highest values for anther dehiscence, pollen viability and minimum cell injury percentage as compared to other genotypes and standards i.e FH-142 & FH-Lalazar. NIAB-878 also surpassed all the genotypes & standards by manifesting of maximum stomatal conductance, transpiration rate, net photosynthetic rate, physiological water use efficiency under prevailing high temperature conditions with its ability to survive under the water deficit conditions under the high temperature stress. Based on the overall performance of cotton genotypes regarding heat tolerance under controlled conditions, NIAB-878B, NIAB-1011/89 and NIAB-1011/48 were found as tolerant and can better withstand the heat stress as compared to susceptible genotypes i.e. FH-142 and FH-Lalazar. Secondly based on the results of cotton genotypes for their screening for physiological, gas exchange characteristics, phenological traits, seed related parameters under the field conditions advanced line NIAB-878B was found to be resilient against high temperature as compared to standards. The said material developed against high temperature stress is proving of extreme worth in making of successful cultivation of cotton in heat prone cotton growing areas of the country like Pakistan, with maximum return to end-users.

Conclusion

Current studies were conducted during the year 2016-17 in identifying/screening of thermo- tolerant and sensitive cotton genotypes, under the prevailing high temperature of the field conditions using various morphological and physiological traits conferring for heat tolerance in cotton. Amongst evaluated cotton mutants, NIAB-1011/89 revealed that maximum fruit retention capacity, highest number of bolls/locules and number of seeds, maximum seed cotton yield per plant up to 10th sympodia of the plant against the standard sensitive cotton varieties (i.e. FH-142 & FH-Lalazar). The standard cotton varieties illustrated very less fruit retention capacity, lowest number of bolls, locules/ number of seeds and yield per plant showing their inability to survive under the prevailing conditions of high temperature with ultimate loss/shedding of bolls and yield (Kg/hac) at maturity. Maximum seed coat percentage was shown by NIAB-878B, followed, by NIAB-1011/48 against minimum seed coat percentage value of FH-Lalazar standard. The studied parameters showed that seed coat in cotton, might act as environment protectant against high temperature in cotton particularly, during the development of cotyledons at cotton boll development stage. The developed vigorous cotyledons, ultimately lead in gain weight of seed cotton per boll with vigorous germination and crop growth stand. Further maximum portion of developed embryo in NIAB-545 in comparison with standard FH-Lalazar reflecting, the least effect of high temperature in the development of embryo during the peak hours of high temperature (July –August) in Pakistan.

The results of physiological studies showed that NIAB-878B excelled in heat tolerance by maintaining the highest values for anther dehiscence, pollen viability and minimum cell injury percentage as compared to other genotypes and standards i.e FH-142 & FH-Lalazar. Higher the values of anther

dehiscence/pollen viability and lower values for relative cell injury percentage makes the cotton plant most resilient against the high temperature tolerance. NIAB-878 surpassed all the genotypes & standards by manifesting of maximum stomatal conductance, transpiration rate, net photosynthetic rate, physiological water use efficiency under prevailing high temperature of field conditions, with its ability to survive under the water deficit conditions under the high temperature stress. Based on the overall performance of cotton genotypes regarding heat tolerance under field conditions, NIAB-878B, NIAB-1011/89 and NIAB-1011/48 were found as tolerant and can better withstand the heat stress as compared to susceptible genotypes i.e. FH-142 and FH-Lalazar. The said material developed against high temperature stress, also proving of extreme worth in making of successful cultivation of cotton in heat prone cotton growing areas of the country like Pakistan with maximum return to end-users.

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Table-1: Results of various morphological, yield and fruit retention traits for determining heat tolerance in cotton genotypes studied at NIAB, Faisalabad 2016-17

Traits name	NIAB-545	NIAB-878	NIAB-1089	NIAB-1011/64	NIAB-444	NIAB-1042	NIAB-1011/48	FH-142*	FH-Lalazar*
1st Syp-node number	13	12	12	12	11	14	13	10	11
1st Syp-node height (cm)	23	23	24	25	25	27	26	20	20
Syp-height bearing 1st intact boll (cm)	30	36	29	37	31	41	37	37	32
TFP-up to 10th sympodia of the plant	35	29	35	32	47	29	26	28	27
TIP-up to 10th sympodia of the plant	17	13	19	10	18	11	11	7	7
SPs-up to 10th sympodia of the plant	18	16	15	22	29	18	14	21	20
Fruit Retention Capacity (%)	47	46	56	30	38	38	42	25	25
TNB-up to 10th sympodia	16	16	21	12	17	12	11	9	7
TNL-up to 10th sympodia	65	60	79	47	69	46	46	36	29
TNS-up to 10th sympodia	316	282	357	225	336	230	223	189	141
Yield (g) up to 10th sympodia of the plant	35	32	40	26	40	26	24	22	17
Plant Height (cm)	160	168	149	160	147	171	162	164	168
Number of Bolls m^{-2}	916	853	891	892	871	926	948	782	723
Yield (Kg ha^{-1})	5004	5384	4997	5475	4745	5121	5477	4465	3328

- *Syp-Sympodia, TFP- Total Fruiting Points, TIP- Total Intact Points, SPs-Shedding Points, FRC-Fruit Retention Capacity, TNB-Total number of bolls, TNL-Total number of locules, TNS-Total number of seeds*
- ** for control/standards*

Table-2: Results of different phenological parameters studied in cotton genotypes at NIAB, Faisalabad -2016-17

Genotypes	Days after planting (75)				Days after planting (90)				Days after planting (105)				Days after planting (120)				Days after planting (135)				Days after planting (150)			
	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P	PH	NB/P	NF/P	NS/P
NIAB-545	93	14	6	38	106	32	6	47	128	56	6	35	149	63	3	16	152	83	1	3	160	88	1	10
NIAB-878	93	7	4	42	108	23	6	39	146	48	7	36	161	56	2	13	162	70	1	3	168	74	1	7
NIAB-1089	89	14	5	39	99	32	6	45	127	57	6	31	139	70	1	14	145	86	2	5	149	89	0	7
NIAB-1011/64	92	13	5	43	102	29	7	49	129	56	6	36	149	64	3	18	155	86	2	5	160	92	1	6
NIAB-444	92	12	5	42	101	26	6	40	120	50	5	32	141	57	3	20	145	72	2	4	147	78	1	7
NIAB-1042	99	9	4	38	116	28	6	46	150	48	8	39	162	65	2	15	162	79	1	2	171	85	1	10
NIAB-1011/48	95	10	4	38	108	26	6	41	130	52	7	29	150	50	2	15	157	73	1	4	162	74	1	7
FH-142*	95	9	5	43	112	26	7	43	140	47	9	30	153	57	3	16	156	75	1	3	164	84	1	10
FH-Lalazar*	86	9	3	40	101	21	6	45	128	40	5	34	151	50	4	17	152	64	0	3	168	65	1	10

- PH = Plant Height, NB/P = Number of bolls/plant, NF/P = Number of flowers/plant, NS/P = Number of squares/plant
- * for control/standards

Table-3: Phenological data on number of unopened and opened bolls per plant with 15 days' interval at NIAB, Faisalabad-2016-17

Genotypes	Days after planting (105)		Days after planting (120)		Days after planting (135)		Days after planting (150)	
	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant	No. of unopened bolls/plant	No. of opened bolls/plant
NIAB-545	43	12	35	29	20	63	7	81
NIAB-878	41	6	37	20	18	53	6	69
NIAB-1089	46	12	37	33	24	63	11	78
NIAB-1011/64	46	10	39	25	27	58	10	82
NIAB-444	38	12	31	27	23	49	7	71
NIAB-1042	42	6	43	22	17	63	5	80
NIAB-1011/48	42	10	29	22	25	49	9	65
FH-142 *	36	11	37	21	21	56	7	75
FH-Lalazar *	30	10	33	17	21	43	5	60

- * for control/standards

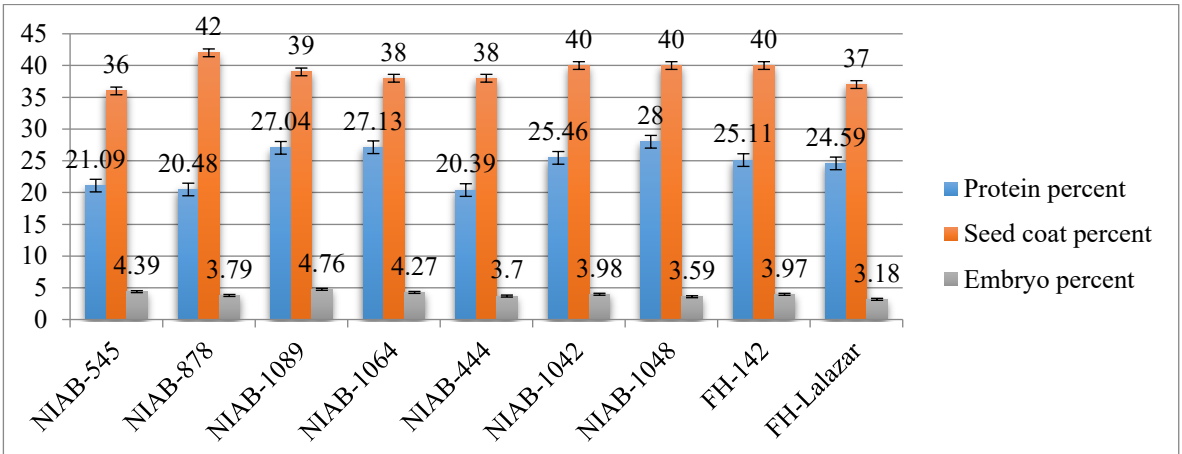

Figure-1: Showing the results for the analysis of seed traits for determining heat tolerance in cotton genotypes at NIAB, Faisalabad-2016-17

Figure- 2

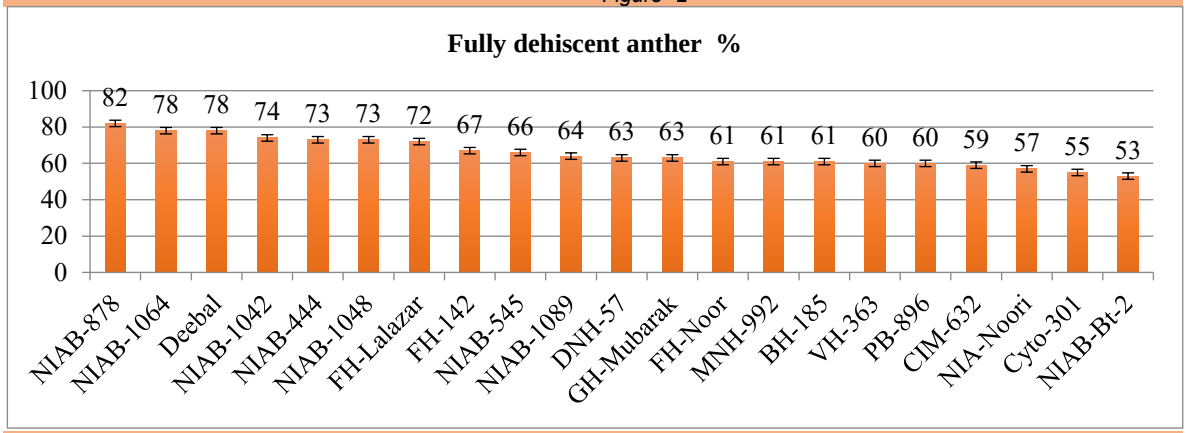

Figure- 3

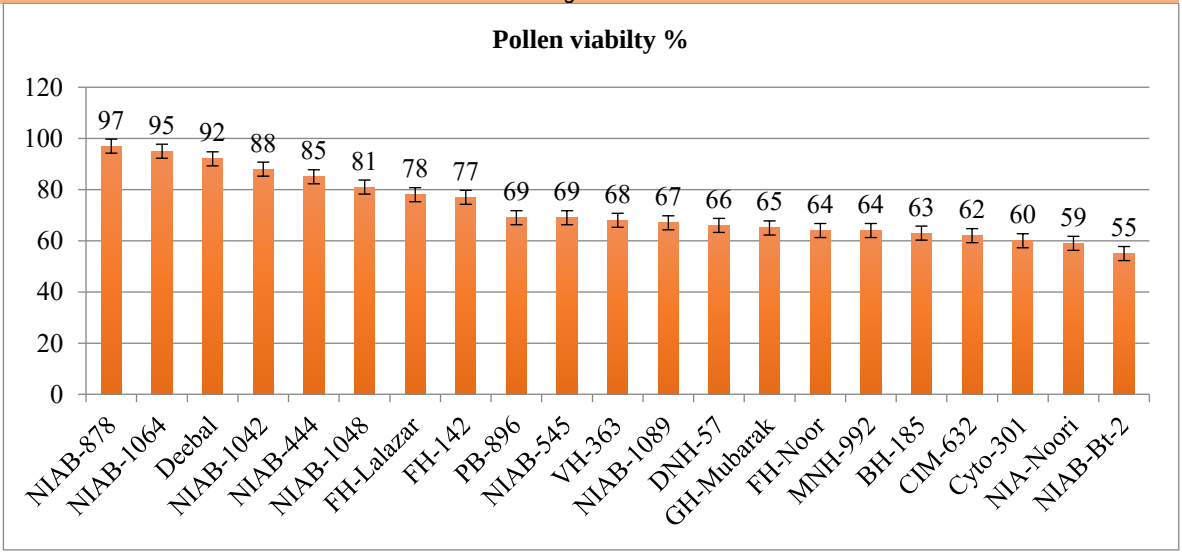

Figure-4

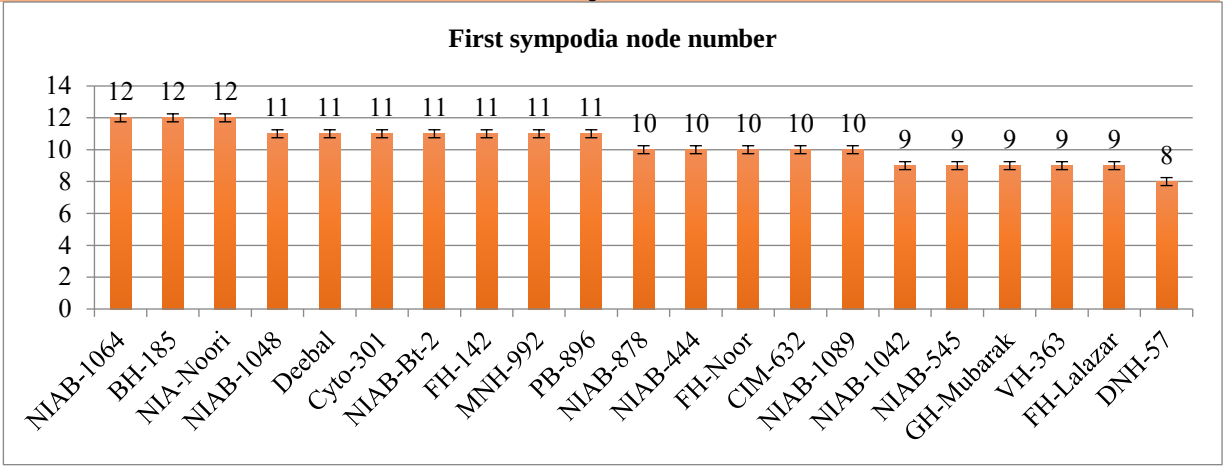

Figure-5

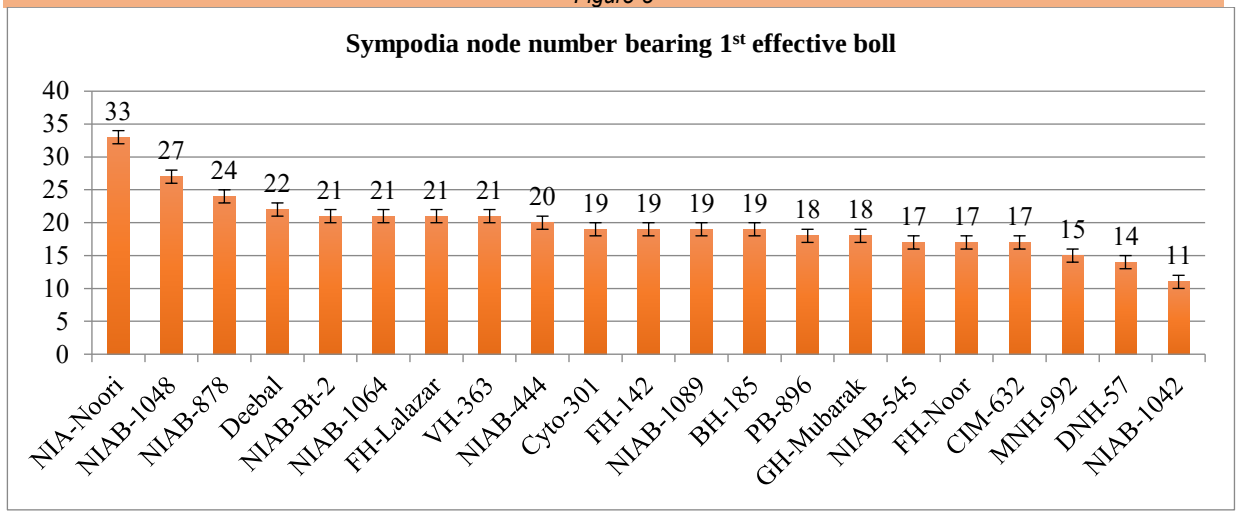

Figure-6

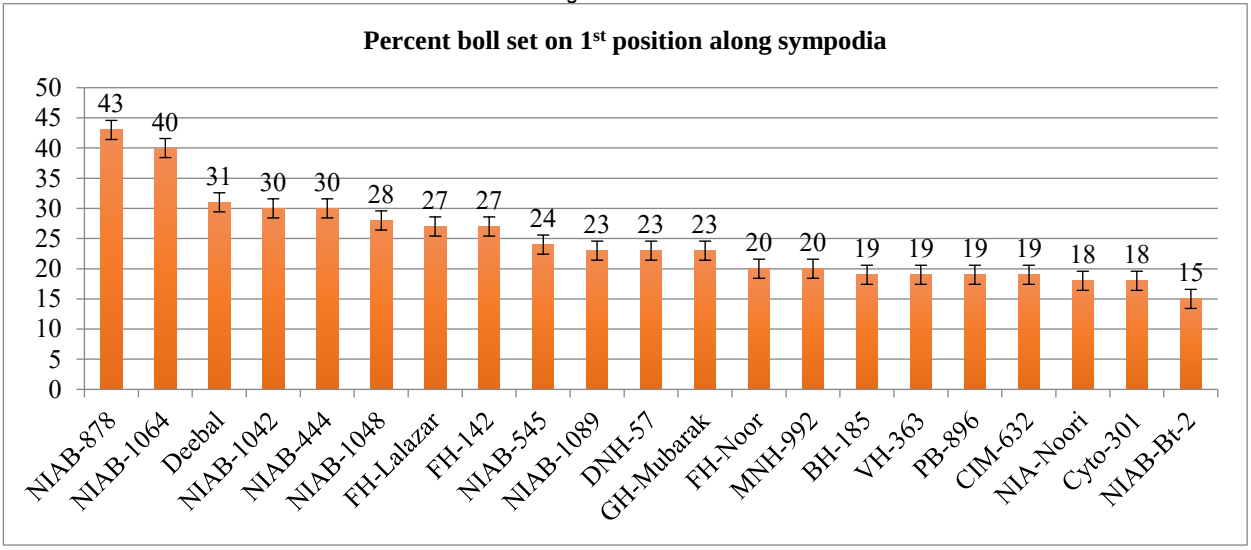

Figure-7

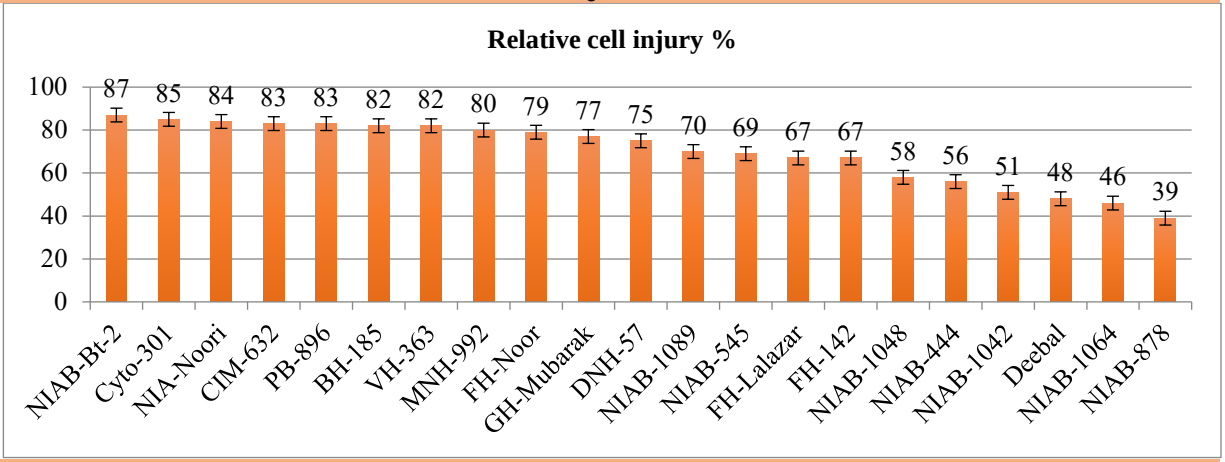

Figure-8

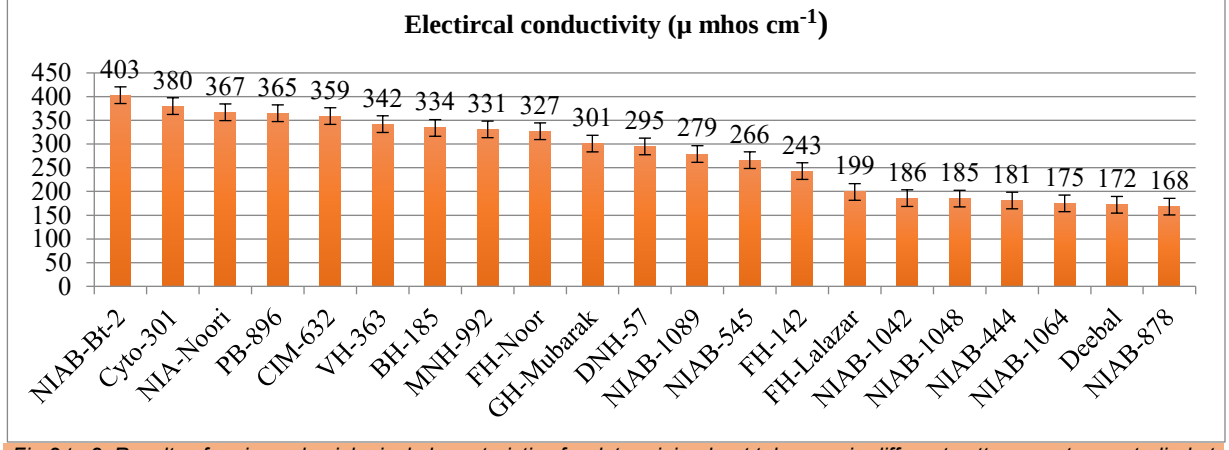

Fig 2 to 8: Results of various physiological characteristics for determining heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

Figure-9

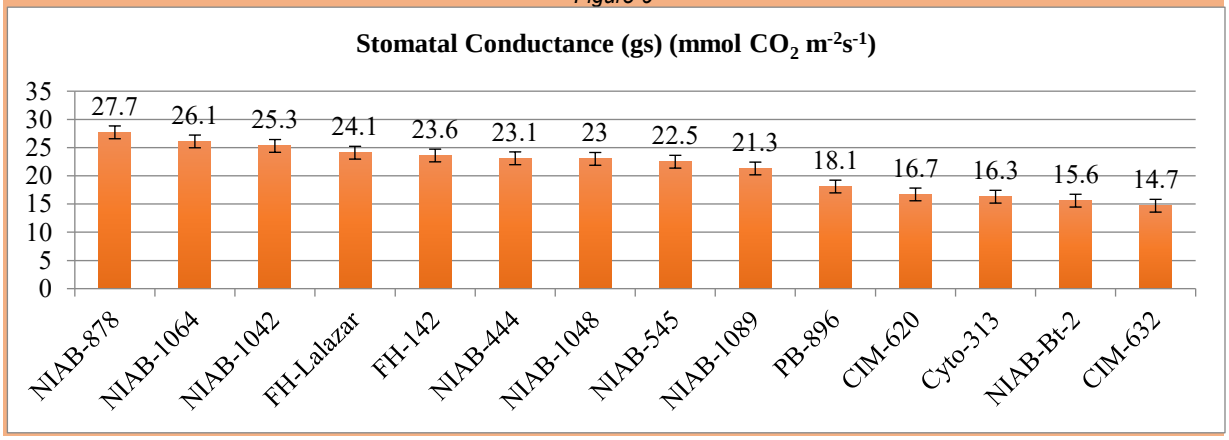

Figure-10

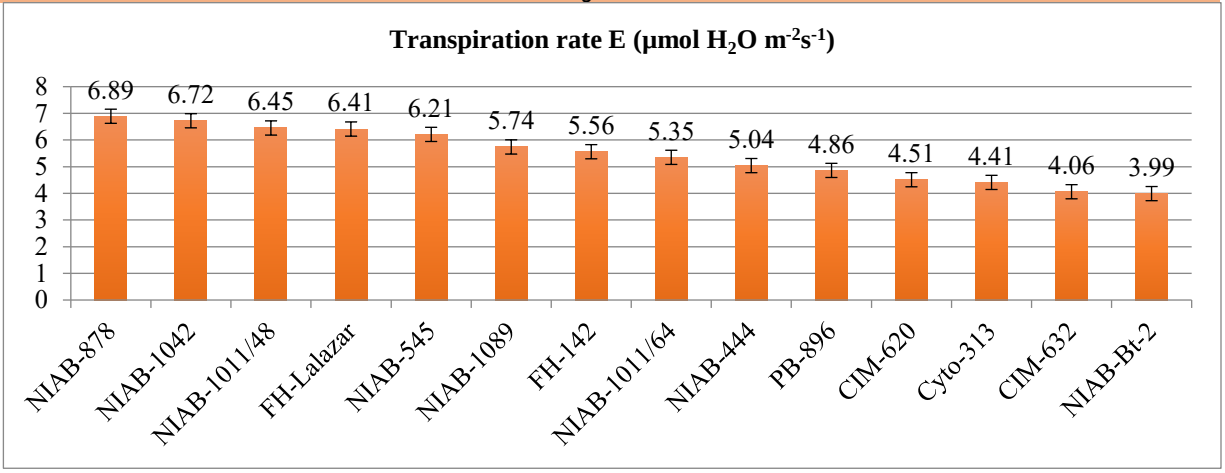

Figure-11

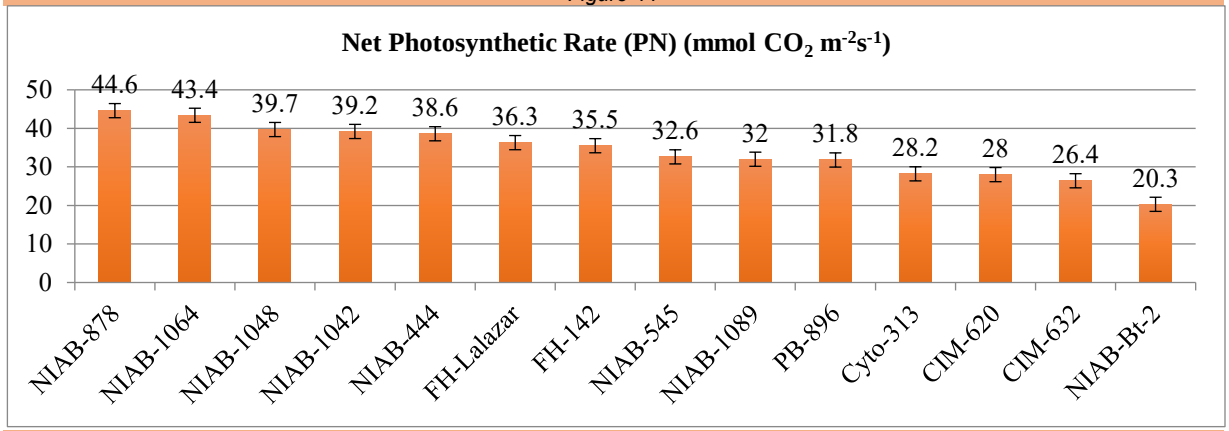

Figure-12

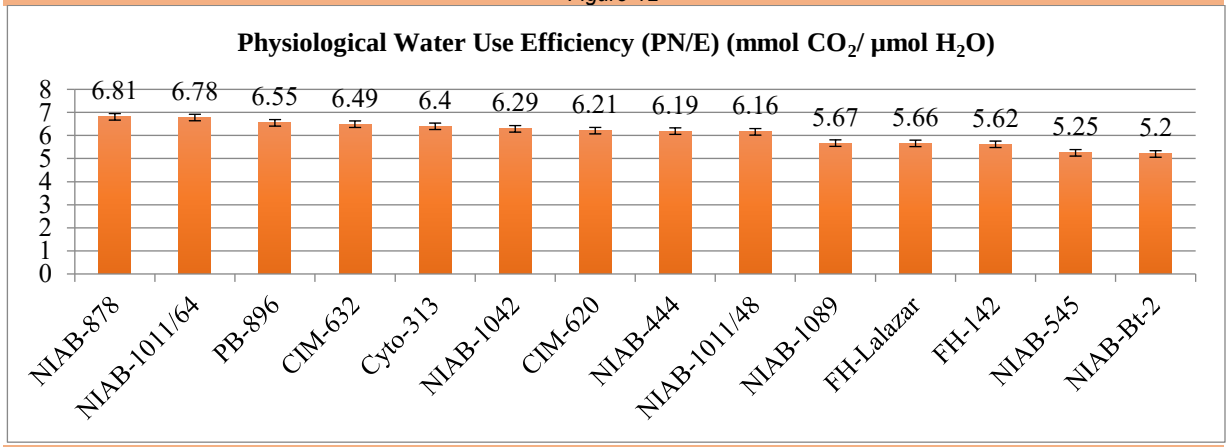

Fig 9 - 12: Results of gas exchange characteristics for determining heat tolerance in different cotton genotypes studied at CCRI, Multan-2016-17

Acknowledgements

The lead author is extremely thankful to the management of PARC, ALP, Planning Division, Pakistan in awarding me the national project on cotton ALP (CS-197) under which current investigations were carried in developing of heat tolerant cotton mutants for the need of country under the changing climatic scenario. Last but not east, a lot of thanks to the management of PAEC, HQ Islamabad and my team members at NIAB for their encouraging support in conducting of such studies in developing of heat tolerant cotton mutants with their ability to unlock their yield potential under the changing climatic scenario

References

1. Anonymous. 2013a. Global warming and cotton production: ICAC, press release, 03 October, 2013. (https://www.icac.org).
2. Anonymous. 2013b. Cotton Crop Assessment Reports, 2013-14.
3. Anonymous. 2013c. Impact of heat and other stresses on boll development in cotton plant (www.cicr/org.in).
4. Bibi A. C., D. M. Oosterhuis, R. S. Brown, E. D. Gonias and F. M. Bourland. 2003. The physiological response of cotton to high temperatures for germplasm screening. In Oosterhuis, D. M. (ed.) Summaries of Arkansas cotton research. Arkansas Agric. Exp. Stn., Fayetteville, AR. 521:87-93.
5. Burke, J. J., J. Velten, and M. J. Oliver. 2004. In vitro analysis of cotton pollen germination. Agron. J. 96:359–368.
6. Cottee, N. S., D. K. Y. Tan, M. P. Bange, J. T. Cothren, and L. C. Campbell. 2010. Multi-level determination of heat tolerance in cotton (*Gossypium hirsutum* L.) under Field Conditions. Crop Sci. 50:2553-2564.
7. Cottee, N. S., D. K. Y Tan, J. T. Cothren, M. P. Bange, and L. C. Campbell. 2007. Screening cotton cultivars for thermo tolerance under field conditions. In: Proc. 4th World Cotton Research Conference. Lubbock, TX, USA, 10-14th September.
8. Rahman H. M, A. Ahmad, X. Wang, A. Wajid, W. Nasim, M. Hussain, B. Ahmad, I. Ahmad, Z. Ali, W. Ishaque, M. Awais, V. Shelia, S. Ahmad, S. Fahd, M. Alam, H. Ullah, G. Hoogenboom. 2018. Multi-model projections of future climate and climate change impacts uncertainty assessment for cotton production in Pakistan. Agricultural and Forest Meteorology; Vols. 253–254, 94-113
9. Hodges, H. F., K. R. Reddy, J. M. McKinion, and V. R. Reddy. 1993. Temperature effects on cotton. p. 1-15. Mississippi Agricultural & Forestry Experiment Station, Mississippi State, MS.
10. Karademir E., C. Karademir, R. Ekinci, S. Basbag and H. Basal. 2012. Screening cotton varieties (*Gossypium hirsutum* L.) for heat tolerance under field conditions. African Journal of Agricultural Research 7(47): 6335-6342.
11. Liu, Z., Y. Yuan, S. Liu, X. Yu, and L. Rao. 2006. Screening for high-temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. J. Integr. Plant Biol. 48:706-714.
12. Oosterhuis D. M. and Snider J. L. 2011. High temperature stress on floral development and yield of cotton: stress physiology in cotton. In: Oosterhuis, D. M. (ed.), Stress physiology in cotton, 1-25. The cotton foundation, Cordova, Tennessee.
13. Oosterhuis D. M., F. M. Bourland, A. C. Bibi, E. D. Gonias, D. Loka and D. K. Storch. 2008. Screening for temperature tolerance in cotton. In Oosterhuis, D. M. (ed.) Summaries of Arkansas cotton research. Arkansas Agric. Exp. Stn., Fayetteville, AR. 573:37-41.
14. Pettigrew, W. T. 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. Crop Sci. 48:278–285.
15. Radin J. W, Z. M. Lu, R. G. Percy and E. Zeiger. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc Natl. Acad. Sci. USA 91, 7217–7221.
16. Reddy K. R., G. H. Davidonis, A. S. Johnson and B. T. Vinyard. 1999.Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. Agron. J 91, 851-858.
17. Reddy K. R., H. F. Hodges and V. R. Reddy. 1992a. Temperature effects on cotton fruit retention. Agron. J. 84: 26-30.
18. Reddy V. R, K. R. Reddy and H. F. Hodges. 1991a. Temperature effects on growth and development of cotton during the fruiting period. Agron. J. 83, 211-217.
19. Rehman H, S. A. Malik and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermo stability. Field Crops Res. 85, 149-158.
20. Singh R. P., P. V. Prasad, K. Sunita, S. N. Giri and K. R. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. Adv. Agron. 93: 313-385.
21. Snider, J. L., D. M. Oosterhuis, B. W. Skulman and E. M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. Physiol. Plant. 137:125-138.
22. Zhou G.Y, F. X. Yang, X. Q. Fu, X. L. Wang. 1996. The influence of climatic differences in the Yangtze River valley on cotton yield and fiber quality. China.

Soil Fertility Management in Cotton Growing Areas of Pr-Pica Countries: Assessment and Prospects

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Abstract

In cotton and cereal-based cropping systems, the degradation of soil fertility and its acidification is increasing with the insufficiency or absence of organic restitutions. Thus, the soils in cotton basins are increasingly acidic (pH < 6.5) with organic matter levels below 1%. Faced with these problems common to the cotton-producing countries of West and Central Africa, the agronomy commission has undertaken experiments in the PR-PICA member countries in order to help improve soil productivity and producers' incomes. From 2016 to 2020, the work carried out in the research stations and at farmers' sites focused on (i) the evaluation of two new formulas of less acidifying fertilisers enriched with calcium and/or magnesium in the cotton-growing areas of West and Central Africa (ii) the evaluation of mineral and organic amendments in the management of soil acidification in cotton growing in the PR-PICA countries, (iii) the evaluation of the effectiveness of 1ertiliza application of organic manure near the cotton plants in the PR-PICA countries. The results obtained show improvements in seed cotton yields with the application of new fertilizer formulas. This work highlighted the effectiveness of a new, less acidifying fertiliser formula enriched with Calcium (14-18-18+5S-1B+2.5CaO) and currently disseminated in the different PR-PICA countries. In the fight against soil acidification, the use of mineral fertiliser combined with organic amendment, granulated rock phosphate and dolomite have improved cotton yields in the different PR-PICA countries. The application of organic fertiliser at 2 t/ha 1ertiliza next to the cotton plants had the best efficiency on seed cotton yield in the 6 PR-PICA countries compared to the broadcast application of 5t/ha. The results of this work at the regional level made it possible to formulate recommendations in the different countries for the sustainable improvement of cotton production.

Key words: 1ertilization, acidity, amendment, rock phosphate, productivity profitability, cotton

Introduction

The cotton sector plays an essential role in economic and social activities in West and Central African producer countries in terms of income generation, organisation of the rural world and modernisation of production systems. Cotton is grown by many small family farms and nearly 4 million producers live directly from cotton income and benefit from the after-effects of the cotton system (FAO, 2013). The contribution of the cotton sector to gross domestic product (GDP) varies between 2% and 15% in the cotton producing countries of West and Central Africa (OCDE, 2015. Cotton cultivation accounts for up to 50% of export earnings in countries such as Burkina Faso and Mali, and more than 80% in Benin (CMA/AOC, 2002). Despite its economic importance, it is faced with several limiting factors, including the decline in soil fertility. The fertility of a soil is measured by its capacity to produce various products useful to humans in a sustainable manner. The sustainable maintenance of soil fertility is vital, while its decline results in a progressive loss of yields (Kasongo et al., 2013). Furthermore, the cultivation of soils leads to a rapid loss of organic matter and a progressive decrease in the physical, chemical and biological fertility of the

soil (Mulaji, 2011).
In the production areas of West and Central Africa, cotton is grown on soils that are mostly of the tropical ferruginous type, which generally have a natural tendency to acidify. The results of studies in Mali, for example, have shown that 17% of the soils in the cotton zone have pH water values below 5.5, the threshold below which acidity considerably reduces crop growth and yields (Pieri, 1989; Dembele et al., 2017). Soil acidification has two adverse effects on plants: solubilisation of aluminium and loss of cationic nutrients in the soil. Thus, the simplest and most effective way to combat soil acidity by improving the adsorbent complex is to use organic (manure, compost) and mineral amendments such as calcium, magnesium and rock phosphate. The usefulness of limestone amendments in improving soil fertility is well recognised by scientists (FAO, 1987; Gillet et al., 1992; Schlecht et al., 2006).

In response to the above constraints affecting soil fertility and yields in cotton agrosystems, as of 2015, various research works were undertaken in the member countries of the Regional Programme for Integrated Cotton Production in Africa (PR-PICA) which are Benin, Burkina Faso, Cote d'Ivoire, Mali, Senegal and Togo. The main objective was to evaluate the agronomic effects of different types of fertilisers in cotton production in order to improve the productivity and income of producers in these countries. Specifically, the aim was to (i) evaluate two new formulas of less acidifying fertilisers enriched in calcium and/or magnesium in cotton growing areas (ii) evaluate different types of mineral and organic amendments in the management of soil acidification in cotton growing and (iii) evaluate the effectiveness of organic manure application near cotton plants in PR-PICA countries. This paper presents the main achievements of the work carried out within the framework of the PR-PICA in order to better orient cotton growing in the different countries through a regional approach.

Materials and Methods

Study areas

The research work was conducted in 6 PR-PICA countries (Mali, Burkina Faso, Benin, Cote d'Ivoire, Senegal, Togo) in research stations and farmers' test plots (Fig. 1). During the period 2015 to 2020, the main activities focused on three studies implemented in these countries in close collaboration between research institutes, agro-industrial fertiliser firms and cotton companies.

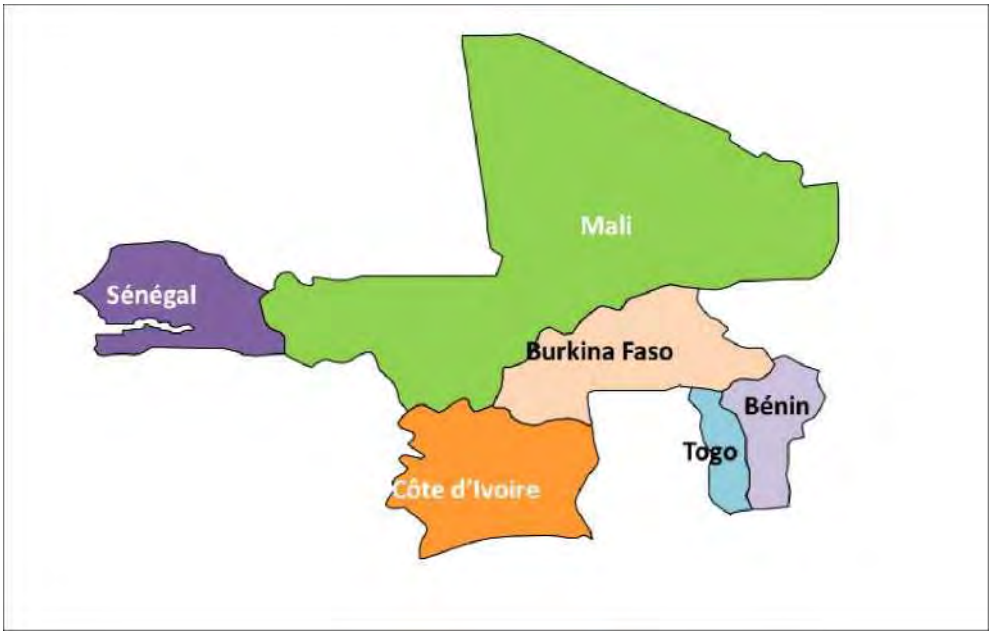

Figure: Map of the 6 PR-PICA countries that conducted the experiments and tests

Experiment: Evaluation of two new formulas of less acidifying fertilizers enriched in calcium and/or magnesium in West and Central African cotton zones

In the context of updating the 30-year-old cotton fertiliser formulas in the different countries, from 2015 to 2017, this study was conducted for 3 years in research stations and in the farmers' environment. A total of 246 tests were carried out in 2017 in Benin (30), Burkina Faso (45), Cote d'Ivoire (42), Guinea (9), Mali (35), Senegal (49) and Togo (36). These tests were conducted in a scattered block design with the following three treatments studied: T1- Mineral fertiliser control in each country, T2- 14N-18 P2O5-18K2O+5S+1B +2,5 CaO, et T3- 14N-15 P2O5-15 K2O +5S+1B+2,5 CaO + 2,5MgO. The two new formulas of enriched and less acidifying fertilizers (14-18-18+6S+1B+2,5 CaO, 15-15-15+5S+1B+2,5 CaO+2,5 MgO) The two new formulas of enriched and less acidifying fertilizers were manufactured by the firm TOGUNA (Mali) for the experiments. The cotton fertiliser formulas (NPK+S+B) disseminated in the countries are 14-18-18+6S+1B (Benin, Burkina Faso, Mali), 15-15-15 +6S+1B (Cote d'Ivoire), 14-23-14 +5S+1B (Senegal), et 12-20-18 +5S+1B (Togo).

Experiment 2: Evaluation of different types of mineral and organic amendments in the management of soil acidification in cotton growing in the PR-PICA countries

During three years of experimentation (2018 to 2020), this study was also conducted at research stations and in farmers' fields in the PR-PICA countries. A Fischer block design with four replicates was set up on station with the 6 treatments defined below:

T1 - 200 kg/ha NPK+S+B + 50 kg/ha Urea (FMR)
T2 - 200 kg/ha 14-18-18+5S+1B+2,5 CaO (PR-PICA) + 50 kg/ha Urea
T3 - 300 kg/ha PNT granulate (Toguna) + Recommended Mineral Fertilization (FMR)
T4 -300 kg/ha Phosphate Naturel (pays) + FMR
T5 - 500 kg/ha dolomie/chaux + FMR
T6 - 5 t/ha compost + FMR
In total, 15 and 12 experiments were conducted in 2019 and 2020 respectively in the 6 countries (Mali, Burkina Faso, Benin, Cote d'Ivoire, Senegal, Togo). The number of on-farm tests in these countries was 184 and 60 in 2019 and 2020 respectively. On an area of 0.75 ha, each test was conducted in a scattered block design with the three treatments studied defined as follows: T1: 200 kg/ha NPK+S+B + 50 kg/ha Urea (FMR)
T2: 200 kg/ha 14-18-18+5S+1B+2,5 CaO (PR-PICA) + 50 kg/ha Urea
T3 : 300 kg/ha PNT granulate (Toguna) + FMR.
In these experiments, the phosphate-lime amendment based on granulated Natural Phosphate (NP) was composed of 27.04% P2O5; 49.63% CaO; 1.6% MgO and 1.4% SO3. The samples needed for the experiments were made available to the countries by the agro-industrial company TOGUNA (Mali), which granulated this amendment.

Experiment 3. Evaluation of the efficiency of organic manure application near cotton plants in PR-PICA countries

This work was carried out at research stations in 2019 and 2020 in the 6 countries using a Fisher block design with 4 replications and the following treatments:
T1: control (without fertiliser)
T2: 200 kg NPKSB pays /ha + 50 kg Urea /ha (FMR)
T3: 1 t/ha of compost (as a localised input) + FMR
T4: 2 t/ha of compost (as a localised input) + FMR
T5: 5 t/ha of compost broadcast harrowing (uniform application) + FMR

Plant material
For these experiments, the cotton varieties used in the different countries have yield potentials of 2.5 to 4 t/ha of seed cotton (Table 1).

Table 1: Cotton varieties with yield potential used in experiments and tests by country

Variety	Country						Yield
	Benin	Burkina	Mali	Togo	Cote	Senegal	Potential (t/ha)
ANG 956							4
OKP 768							4
KET782							4
FK37							2,5
STAM 59A							2,5
NTA 90-5							3
N'TA-MS334							3
FK 64							3
NTA 93-15							3
STAM 279A							3
STAM 129A							3
Y 331							4
X442							4

Data collected

The number of plants was counted on two central lines of each treatment for each trial after disbudding and at harvest. At 30 days after emergence (DAE), ten (10) plants, five (5) of which were on each of the two central lines, were marked with string in each trial at the research stations. The height of the cotton plants was measured per marked plant using a graduated ruler (cm) from the first cotyledonary node at 30, 60 and 90 DAE. The height measurements were repeated on the same marked plants. Height has a great influence on the development of the reproductive organs. Before harvesting and on the two central lines of each trial treatment, the open and ripe bolls were picked and the seed cotton was separated from the carpels. After this operation the number of harvested carpels was counted and the weight of the seed cotton was weighed with a precision balance to determine the average capsular weight (PMC) per object. At harvest, the two central lines of each elementary plot were picked. The seed cotton was weighed, and the yield was assessed. After harvesting, plant mapping was carried out on ten (10) plants, five (5) of which were on each of the two central lines. The plant mapping concerns the insertion level of the first fruiting branch (NiPBF), the number of vegetative branches (NBV) and the number of fruiting branches (NBF). These yield components are crucial for yield improvement.

Data analysis

The collected agronomic and morphological data were subjected to analysis of variance using R (version 3.6.1), XL STAT version 2017. The uniformity of the data distribution was checked before the analyses. The pairwise.t.test was used for the separation of significantly different means at a probability of 5%.

Rainfall situation during the experiments

Tables 2 show the cumulative (lower and upper bound) rainfall recorded and the number of rainy days during the experiments in the different PR-PICA countries. It can be seen that the lowest level of rainfall was observed in Mali and Senegal during the conduct of the trials. In contrast, the highest rainfall was always observed in Benin (Table 2).

Table 2: Minimum and maximum cumulative rainfall in the different countries during the 2018 - 2019 and 2020 seasons

Pays	2018		2019		2020	
	Cumul pluviometrie (mm)	Nombre de Jour pluvieux	Cumul pluviometrie (mm)	Nombre de jours pluvieux	Cumul pluviometrie (mm)	Nombre jours de pluvieux
Benin	800-1400	40-80	1295-1472	53 - 70	1159-1460	63 - 79
Burkina Faso	826-965	42-56	1051 - 1323	64 - 73	854 - 1229	53 - 72
Cote d'ivoire	896-1320	69-72	1041-1148	64-80	775-1337	54-76
Mali	755-1032	40-54	841 - 1114	68 - 76	844 - 1062	74 - 76
Senegal	588-967	35-54	486 - 1132	47 - 61	679 - 1173	44 - 64
Togo	993-1249	50-90	803 - 1520	61 - 94	943 - 1437	53 - 80

Sowing period of the test plots in the different PR-PICA countries

The traditional sowing period for cotton in West and Central African countries was the third dekad of May and all of June. In these countries, at least 10% to 15% of sowing was done in

May. May sowing is increasingly rare in the West and Central African production areas. During the experiments covered by this paper, the majority of sowings were carried out in June. However, July planting has been observed in recent years in the PR-PICA countries and this is linked to the climatic changes currently being experienced in the West and Central African cotton zones (Table 3).

Table 3: Sowing period during the experiment on the evaluation of two new formulas of less acidifying fertilizers enriched with calcium and/or magnesium in the cotton zones of West and Central Africa

	Période de semis					
	Juin			Juillet		
Pays	1	2	3	1	2*	3
Bénin						
Burkina Faso						
Côte d' Ivoire						
Mali						
Sénégal						
Togo						

Some results of soil analysis at research stations in PR-PICA countries conducted in 2019 - 2020.

Table 4 shows the results of the analysis of soil samples taken at the stations in the 6 PR-PICA countries (Table 4). The analysis of soil texture indicates that the soil in the different countries is of the sandy-loam type with a clear sandy tendency in some countries such as Cote d'Ivoire, Mali and Togo where the sand content is above 70%. The percentage of clay ranges from 4% to 13% at the 0-30 cm horizon. These soil types are characterised by an easy drainage condition but with a tendency to dry out quickly. The indicators used to assess soil fertility are organic matter and constituent mineral elements such as nitrogen, phosphorus and potassium (N, P, K). The results of the analysis show that the soil is characterised by a low organic matter rate in all countries except Cote d'Ivoire where the organic matter rate is above 1%. Furthermore, the C/N ratio was below 15 in all countries, indicating the state of decomposition of the organic matter. The average water pH was below 6 in all countries indicating a soil acidity status slightly above the threshold of 5.5 below which acidity significantly reduces crop growth and yield (Kamprath,

1970).

Table 4 : Soil analysis at research stations in PR-PICA countries conducted in 2019 - 2020.

Characteristics	Benin	Burkina Faso	Cote d'Ivoire	Mali	Senegal	Togo
Clay (%)	12.63	13.73	13.5	8.36	23.98	4.41
Silt (%)	17.51	32.68	12.5	17.67	22.89	7.74
Sand (%)	69.85	53.59	74	73.97	52.91	87.85
OM (%)	0.93	0.53	2.27	0.69	-	0.45
Total nitrogen (%)	0.06	0.03	0.12	0.11	-	0.08
C/N	9	9.92	11	13.74	-	-
P ass (mg/kg)	9	78.54	7	14.94	-	10.5
pH water	5.23	5.56	5.02	5.85	7.8	5.76

Results

In the following, we present some results from the experiments of the three studies conducted at the research stations or at farmers' sites.

Study on the evaluation of two new formulas of less acidifying fertilizers enriched in calcium and/or magnesium in the cotton zones

Table 5 shows the average yields per fertiliser formula compared across countries. Compared to the control fertiliser formulas popularised in the different countries, the Ca-enriched fertiliser formula (14-18-18+5S+1B+2.5CaO) improved seed cotton yields in Benin (+8%), Burkina Faso (+11%), Cote d'Ivoire (+9%), Mali (+29%), Senegal (+19%) and Togo (+2%). The overall increase in seed cotton yield was on average +12% for all PRPICA countries with the Ca- enriched fertiliser (14-18-18+5S+1B+2.5CaO). For the CaO and MgO-enriched fertiliser (14N- 15 P2O5-15 K2O +5S+1B+2.5 CaO + 2.5MgO), yield increases were around +11% in Benin, +9% in Burkina Faso, +7% in Cote d'Ivoire and +12% in Mali. The 14N-15 P2O5-15 K2O +5S+1B+2.5 CaO + 2.5MgO fertiliser improved yields by +6% compared to the control fertilisers disseminated in the different countries. The yield of seed cotton was better with the application of the new CaO-enriched fertilizer formula in all countries except Benin, where the application of the Calcium and Magnesium-enriched fertilizer formula resulted in a yield increase compared to the other treatments.

Table 5: Seed cotton yields according to the fertiliser formulas tested (2019 and 2020).

	Benin	B. Faso	C. Ivoire	Mali	Senegal	Togo
			kg ha^{-1}			
T1- Country control fertilizer	1534	1358	1830	1052	949	1119
T2-14-18-18+6S+1B+2,5 CaO	1658	1504	2000	1357	1127	1138
T3-15-15-15+5S+1B+2 5 CaO+2,5 MgO	1700	1485	1959	1175	921	

Study on the evaluation of mineral and organic amendments in the management of soil acidification in cotton growing

Estimation of the number of cotton plants at harvest

Figure 2 shows the average number of cotton plants counted at harvest during the two years of

experimentation (2019 and 2020). In all countries, the average number of plants recorded at harvest was lower than the theoretical number of plants/ha (83333) per country. The average number of plants ranged from 30697 plants/ha to 66819 plants/ha for an average of 49693 plants/ha, i.e. 60% of the theoretical plant density (83333 plants/ha) (Fig. 2).

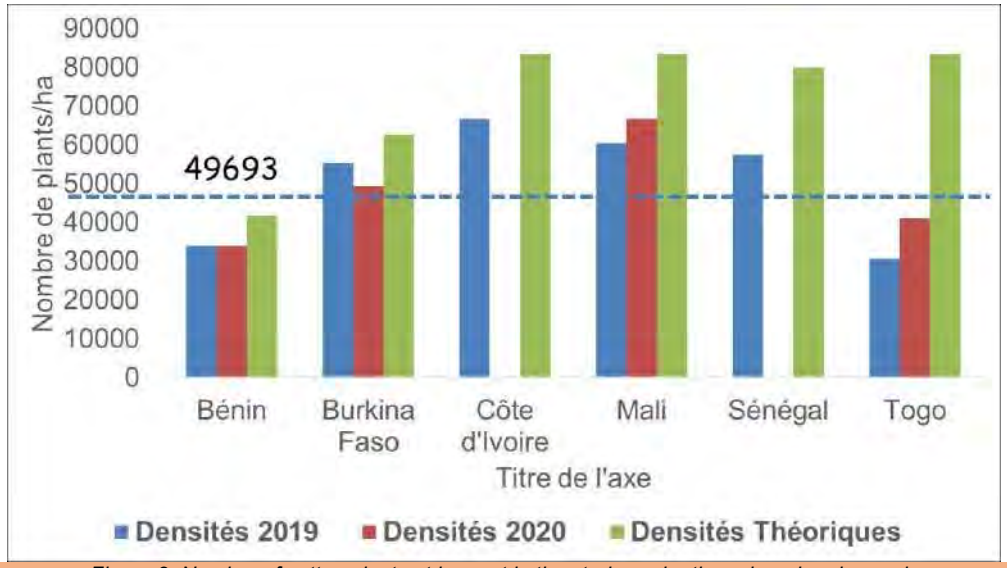

Figure 2: Number of cotton plants at harvest in the study evaluating mineral and organic amendments for soil acidification management in cotton.

Variations in the number of bolls at harvest with different fertilizers

The highest boll numbers were obtained in Senegal and Cote d'Ivoire. The average number of bolls/ha ranged from 437599 to 524778 with an average of 483961 bolls/ha in Senegal and from 424707 to 439453 with an average of 431982 bolls/ha in Cote d'Ivoire. In the other countries, the average number of bolls/ha was 215509 in Benin, 345176 in Burkina Faso, 287876 in Mali and 224392 in Togo (Table 6).

Table 6: Number of bolls at harvest by manures applied at the research stations

Objets	Benin	Burkina Faso	Cote d'Ivoire	Mali	Senegal	Togo
			Capsules ha^{-1}			
T1	202 083	314 363	429 199	282 903	524 778	196 614
T2	231 388	345 600	424 707	292 688	449 786	243 489
T3	172 222	360 226	439 453	289 947	455 663	240 234
T4	205 625	354 674	-	287 910	535 119	199 869
T5	232 118	342 321	-	290 707	437 599	209 635
T6	249 618	353 870	434 570	283 099	500 821	256 510
Average	215 509	345 176	431 982	287 876	483 961	224 392

Legend: T1: 200 kg/ha NPK+S+B + 50 kg/ha urea (Recommended mineral fertilisation, FMR), T2: 200 kg/ha 14-18-18+5S+1B+2.5 CaO (PR-PICA) + 50 kg/ha urea, T3: 300 kg/ha granulated NWP (Toguna) + FMR, T4: 300 kg/ha Natural Phosphate (country) + FMR, T5: 500 kg/ha dolomite/lime + FMR, T6: 5 t/ha FO + FMR.

Average capsular weight (PMC) per object

Table 7 shows the average PMC obtained per object during the two years of experimentation. The average capsular weight was different per object in comparison and per country. The highest average capsular weight (5.07 g) was observed in Togo in 2019 with the application of 500 kg/ha of dolomite plus the recommended mineral fertilisation. On the other hand, the lowest PMC was also observed in Togo (2.93) followed by Mali (2.95) with the application of 300 kg/ha of country rock phosphate (Table 7).

Table 7: Average capsular weight in the evaluation study of mineral and organic amendments in the management of soil acidification in cotton production.

Objets	Benin		Burkina Faso		Cote d'Ivoire	Mali		Senegal	Togo	
	2019	2020	2019	2020	2019	2019	2020	2019	2019	2020
T1	4.24	4.6	4.04	4.05	4.20	3.10	3.50	3.71	4.65	3.63
T2	4.19	4.44	4.12	4.1	3.50	3.10	3.78	3.76	4.26	3.15
T3	4.18	4.53	3.89	3.91	3.30	3.15	3.51	3.66	3.84	3.44
T4	4.44	4.63	3.90	3.77		2.95	3.81	3.48	4.89	2.93
T5	4.59	4.54	4.17	3.95		3.3	3.80	3.76	5.07	3.17
T6	4.36	4.50	4.28	4.09	3.60	3.55	4.21	3.90	4.72	3.96
Moyenne	**4.333**	**4.54**	**4.067**	**3.9783**	**3.65**	**3.192**	**3.76**	**3.712**	**4.57**	**3.38**

Legend: T1: 200 kg/ha NPK+S+B + 50 kg/ha urea (Recommended mineral fertilisation, FMR), T2: 200 kg/ha 14-18-18+5S+1B+2.5 CaO (PR-PICA) + 50 kg/ha urea, T3: 300 kg/ha granulated NWP (Toguna) + FMR, T4: 300 kg/ha Natural Phosphate (country) + FMR, T5: 500 kg/ha dolomite/lime + FMR, T6: 5 t/ha FO + FMR

Variations in seed cotton yields according to manures

Tables 8 and 9 shows the average yield obtained per object and per country. During the 2019-2020 season, the best seed cotton yield was obtained in plots amended with 5 t/ha of compost combined with the recommended mineral manure in four countries (Benin, Burkina, Mali, Togo). In Cote d'Ivoire and Senegal, however, it was the application of the popularised mineral manure that led to a significant increase in yields (Table 8). The average yield obtained in 2019 was above 1000 kg/ha in all countries except Mali where the average yield was 918 kg/ha.

Table 8: Cottonseed yields according to fertiliser and soil amendment application at research stations in 2019

Objets	Benin	Burkina Faso	Cote d'Ivoire	Mali	Senegal	Togo
	kg/ha (coton graine)					
T1	1 317	1 282	1 350	877	1 346	915
T2	1 218	1 439	1 160	907	1 149	1 037
T3	1 300	1 381	1 102	913	1 286	924
T4	1 376	1 396		849	1 204	977
T5	1 331	1 427		959	1 089	1 062
T6	1 475	1 521	1 174	1 005	1 240	1 212
Average	**1 336**	**1 408**	**1 197**	**918**	**1 219**	**1 021**

Legend: T1 : 200 kg/ha NPK+S+B + 50 kg/ha urea (Recommended mineral fertilisation, FMR), T2 : 200 kg/ha 14-18-18+5S+1B+2.5 CaO (PR-PICA) + 50 kg/ha urea, T3 : 300 kg/ha granulated NWP (Toguna) + FMR, T4 : 300 kg/ha Natural Phosphate (country) + FMR, T5 : 500 kg/ha dolomite/lime + FMR, T6 : 5 t/ha FO + FMR

In 2020, the use of organic fertiliser combined with mineral fertiliser had the best yields in three of the four countries that conducted the study (Burkina, Mali, Togo). In Benin, the use of dolomite in combination with mineral fertiliser had the highest yield. The average yield in 2020 was above 1000 kg/ha in all sites except Togo where the average was 763 kg/ha (Table 9).

Table 9: Seed cotton yields according to manures in the research stations2020

Objets	Benin	Burkina Fas	Mali	Togo
kg/ha (coton graine)				
T1	1 540	1 009	1 209	704
T2	1 760	973	1 492	719
T3	1 651	1 049	1 172	728
T4	1 786	1 072	1 578	711
T5	1 820	1 131	1420	717
T6	1 768	1 231	1 822	763
Average	1 721	1 078	1 449	724

Legend: T1: 200 kg/ha NPK+S+B + 50 kg/ha urea (Recommended mineral fertilisation, FMR), T2: 200 kg/ha 14-18-18+5S+1B+2.5 CaO (PR-PICA) + 50 kg/ha urea, T3: 300 kg/ha granulated NWP (Toguna) + FMR, T4: 300 kg/ha Natural Phosphate (country) + FMR, T5: 500 kg/ha dolomite/lime + FMR, T6: 5 t/ha FO + FMR

Variation in seed cotton yields on the farm

Of the three manures compared among farmers, treatment T2 (200 kg/ha 14-18-18+5S+1B+2.5 CaO) had the highest yield in all countries. The average yield was above 1000 kg/ha in all countries except Senegal and Togo (Table 10).

Table 10: Yields of seed cotton according to manures in farmers' fields (2020)

Traitement	Benin	Burkina Faso	Cote d'Ivoire	Mali	Senegal	Togo
			kg/ha (coton	graine)		
T1	1 652	942	1 510	1 407	770	674
T2	1 804	1 087	1 680	1 593	891	722
T3	1 764	1 141	1 604	1 547	833	649
Average	1 740	1 057	1 598	1 516	831	682

Legend: T1: 200 kg/ha NPK+S+B + 50 kg/ha Urea (FMR), T2: 200 kg/ha 14-18-18+5S+1B+2,5 CaO (PR-PICA) + 50 kg/ha Urea, T3: 300 kg/ha Granulated rock phosphate (PNT) (Toguna) + Recommended mineral fertilisation (FMR).

Effectiveness of localised organic manure application in cotton production in PR-PICA countries

Number of plants at harvest

The estimated average number of plants at harvest in the different countries is shown in Table 11. The highest number of plants was observed in the plots where 5t/ha of local organic manure was broadcast in combination with the recommended mineral fertilisation in Benin, Cote d'Ivoire, Senegal and Togo. On the other hand, in Burkina and Mali, the highest number of seedlings at harvest was observed respectively in the plots that received the localised application of 1t/ha and 2t/ha of local organic manure. On average, more plants were observed at harvest in Senegal and fewer at harvest in Benin (Table 11).

Table 11: Number of plants at harvest in 2020 at research stations in the study of the

effectiveness of localised application of organic fertiliser in cotton production in the PR-PICA countries.

Objets	Benin	Burkina Faso	Cote d'Ivoire	Mali	Senegal	Togo
			Plants ha^{-1}			
T1	30729		39974	63055	73108	33924
T2	31597	56220	42676	61204	73701	32257
T3	30324		44010	66018	71322	32778
T4	31019	58487	42676	65895	72063	33264
T5	32639	57020	46224	65278	76291	34097
Average	31 262	57 242	43 112	64 290	73 297	33 264

Legend: T1: Control (without fertiliser), T2: 200 kg NPKSB pays/ha + 50 kg Urea /ha (FMR), T3: FO (1t/ha in localised application) + FMR, T4: FO (2t/ha in localised application) + FMR, T5: 5t/ha of broadcast organic manure + FMR

Average capsular weight (PMC)

The average capsular weight was different between objects and countries. The highest average capsular weight was observed in Cote d'Ivoire during the two years of experimentation with an average of 5.36g and 5.18g in 2019 and 2020 respectively (Table 12). On the other hand, the lowest MAP was observed in Mali with 3.62g in 2019 and in Burkina with 3.66g in 2020 (Table 12).

Table 12: Average capsular weight in the study to evaluate the effectiveness of organic manure application near cotton plants in PR-PICA countries in 2019 and 2020 at the research stations

Objets	Benin		Burkina	Cote d'Ivoire		Mali		Senegal		Togo	
	2019	2020	2020	2019	2020	2019	2020	2019	2020	2019	2020
T1	4.15	4.12		5.10	5.20	3.32	3.41	3.39	3.62	5.17	3.11
T2	4.20	4.77	3.79	5.30	4.90	3.87	3.23	3.99	5.92	4.13	5.31
T3	4.85	4.78		5.40	5.30	3.62	3.69	3.71	5.05	5.08	5.97
T4	4.70	4.90	3.65	5.30	5.20	3.76	3.64	3.63	4.57	6.19	5.08
T5	4.30	4.88	3.56	5.70	5.30	3.57	3.96	3.99	5.22	7.41	5.81
Average	**4.44**	**4.69**	**3.66**	**5.36**	**5.18**	**3.62**	**3.58**	**3.74**	**4.87**	**5.59**	**5.05**

Legend: T1: Control (without fertiliser), T2: 200 kg NPKSB pays/ha + 50 kg d'uree /ha (FMR), T3: FO (1t/ha in localised application) + FMR, T4: FO (2t/ha in localised application) + FMR, T5:

Poids Moyen Capsulaire (PMC) en gramme

5t/ha of broadcast organic manure + FMR

Cotton yield

Seed cotton yields differed between years, objects and countries (Table 13). Overall, the control object without fertiliser application was less productive in all countries compared to the other treatments. Thus, in Burkina Faso and Togo, the best yield was obtained with the broadcast application of 5t/ha of compost during the two years of experimentation. On the other hand, in Benin and Cote d'Ivoire, the best yield was obtained with the localised application of the equivalent of 1t/ha of compost near the cotton plants in 2020. In all countries, the localized application of 1t/ha and 2t/ha of compost near the cotton plants improved the yield (above 1000 kg/ha) in all countries except Togo where the yield was below 1000 kg/ha for the T3 treatment in

2019 and the two treatments T3 and T4 in 2020 (Table 13).

Table 13: Seed cotton yields in the study to evaluate the effectiveness of organic manure application near cotton plants in PR-PICA countries in 2019 and 2020 at the research stations

	Benin		Burkina Faso	Cote d'Ivoire		Mali		Senegal		Togo	
Treatment	2019	2020	2020	2019	2020	2019	2020	2019	2020	2019	2020
	kg/ha (coton graine)										
T1	1089	1366		928	820	884	1058	1324	863	677	484
T2	1933	2229	1162	930	888	1212	1212	2030	2209	678	861
T3	2218	2041		1012	1120	1218	1618	1958	2208	826	899
T4	2192	2476	1274	1114	1295	1182	1517	1854	2170	1003	867
T5	2149	2152	1292	1289	1220	1213	1934	2036	2123	1177	977
Average	1916.2	2052.8	1242.667	1054.6	1068.6	1141.8	1467.8	1840.4	1914.6	872.2	817.6

Legend: T1: Control (without fertiliser), T2: 200 kg NPKSB pays/ha + 50 kg d'uree /ha (FMR), T3: FO (1t/ha in localised application) + FMR, T4: FO (2t/ha in localised application) + FMR, T5: 5t/ha of broadcast organic manure + FMR

Conclusion and Outlook

At the end of the three years of experimentation, it was found that both new fertiliser formulations improved seed cotton yields compared to controls in PR-PICA countries. On the other hand, the new calcium-enriched fertilizer formulation (14-18-18-5S-1B-2.5CaO) was more effective and gave the best seed cotton yield in most PR-PICA countries. The study on mineral and organic amendments also showed that the application of 5t/ha of organic manure increased seed cotton yields in all countries. In addition, the application of granulated rock phosphate and dolomite improved yields at the stations and among farmers. CaO-enriched fertiliser (14-18-18+5S+1B+2.5CaO) was found to be effective in improving cotton yields at farmers' sites. Recommendations were made for the extension of this less acidifying CaO- enriched fertilizer in PR-PICA member countries. Today, this fertilizer formula is being popularized by cotton companies. The localised application of 2t/ha of organic manure improves the efficiency of mineral manure. Overall, the localised application of compost to the cotton plants makes it possible to valorise the small quantities of organic manure often used on the farms. In the future, research activities will continue on the evaluation of new zinc-enriched fertiliser formulas. The evaluation of the effects of sowing dates and sowing densities on cotton yields and the effect of different nitrogen doses on cotton yields in the PR-PICA countries are underway.

References

1. FAO, 2013. Revue des Politiques Agricoles et Alimentaires au Mali. Rapport pays, Fevrier 2013. Pages 217.
2. Doumbia, M. D, 2009. Bilans mineraux des ecosystemes cotonniers. Rapport 15e Session du Comite de Programme : 1-19.
3. FAO, 1987, Guide sur les engrais et la nutrition des plantes. Rome.
4. Gillet Y., Scaglia J., 1992. Les sols acides au Rwanda. Atelier International.
    a. IBSRAM/ISABU/FACAGRO, Bujumbura, Burundi.
5. Kasongo L.M.E., Mwamba M.T., Tshipoya M.P., Mukalay M.J., Useni S.Y., Mazinga K.M., Nyembo K.L., 2013. Reponse de la culture de soja (Glycine max L. (Merril) a l'apport des biomasses vertes de Tithonia diversifolia (Hemsley) A. Gray comme fumure organique sur un Ferralsol a Lubumbashi, R.D. Congo. Journal of Applied Biosciences 63 : 4727-4735.
6. Kamprath E. J., 1970. Exchangeable aluminum as a criterion for liming leached mineral soils. Soil Sci. Soc. Am. Proc 24: 252-254.
7. Mulaji K.C., 2011. Utilisation des composts de biodechets menagers pour l'amelioration de la fertilite des sols acides de la province de Kinshasa (Republique Democratique du Congo). These de doctorat, universite de Liege- Gembloux Agro-Biotech, 220 p.

8. Rapport, 2012 de la conference des ministres de l'agriculture de l'Afrique de l'Ouest et du Centre a Dakar, Avenue Bourguiba - BP. 15799 Dakar-Fann pp33
9. Rapport OCDE, 2015 sur l'Importance economique et sociale du coton en Afrique de l'ouest : role du coton dans le developpement, le commerce et les moyens d'existence, pp72
10. Pieri C. 1989. Fertilite des terres de savanes. Bilan de trente ans de recherche et de developpement agricoles au sud du Sahara. Paris, Ministere de la Cooperation et CIRAD-IRA, 444 pages.
11. Schlecht, E., A. Buerkert, E. Tielkes et A. Bationo., 2006, A critical analysis of challenges and opportunities for soil fertility restoration in Soudano-sahelian West Africa. pp1-28, Advances in Integrated soil fertility management in Sub-saharan Africa: challenges and opportunities. Springer, 10911 p.
12. Van Der Pol F. et Traore B., 1993. Soil Nutrient depletion by agricultural production in Southern Mali. Fertilizer Research 36: 79-90.

Spectral Characterization and Mitigation of Leaf Reddening in Bt Cotton Genotypes though Proximal Sensor Based Nitrogen Management

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Abstract

Background: *Study was undertaken for spectral characterization of red leaf and to mitigate leaf reddening in Bt cotton genotypes with different nitrogen management practices through proximal sensors. Further correlation of spectral values, leaf nitrogen content and seed cotton yield with red leaf index was studied. Field experimentation was carried during Kharif (monsoon season) of 2019 and 2020 with two intra hirsutum hybrids in main plot and eight proximal sensor based N management practices in sub plot.*

Results: *Spectral characterization of red leaves was recorded through GreenSeeker (NDVI) and SPAD meter (SPAD value) and quantitative estimation of degree of leaf reddening was visualized by intensity of red leaf colour at 130 DAS. Genotypes show non-significant (P>.005) difference for NDVI and SPAD value and significant (P<.005) difference for red leaf index (1.03 and 1.59 with First Clasas and Ajeet 155, respectively). Proximal sensor based N management practices significantly (P<.0001) influenced spectral values and red leaf index.N supplementation at 1.1 – 1.5 RI, 81 – 90 % SI and RDF recorded comparable and significantly higher NDVI (0.78, 0.77 and 0.74, respectively) and SPAD value(31.70, 31.23 and 28.72, respectively) as compared to rest of treatments. Proximal sensor based N management practices (1.1 – 1.5 RI and 81 – 90 % SI) significantly reduced red leaf index (0.18 & 0.23) as compared to RDF (1.12) and N omission (2.23). Interactions were found non-significant. NDVI and SPAD value of red leaf, N content in leaf (%) and seed cotton yields were strongly and negatively correlated with red leaf index.*

Conclusion: *The results showed that the nitrogen supplementation at 1.1 – 1.5 RI and 81 – 90 % SI reduced the incidence of leaf reddening and also resulted in recording higher spectral values like NDVI and SPAD meter readings.*

Key words: *Bt cotton, Proximal sensors, Red leaf index, NDVI of red leaf, SPAD value of red leaf*

Introduction

Red leaf disease in cotton is commonly known as *lal patti* in India, copper top, phloem wilt and sudden wilt in US and red leaf and red wilt in other countries. Atkinson (1892) first time observed red leaf syndrome and recognized yellow and red leaf blight as a physiological disorder and attributed it to mineral deficiency, although exact element is not identified. Leaf reddening is more of a physiological disorder and outcome of the interaction of variety/ hybrid, environmental factors and nitrogen deficiency including micronutrients. Symptoms of leaf reddening differ from region to region in India. In West Punjab and Maharashtra reddening is caused mainly due to nitrogen deficiency while in South-West Punjab and Eastern Rajasthan; it appears to be due to sudden drop in temperature. And in Southern states like Karnataka, Andhra Pradesh and Tamil Nadu magnesium deficiency leads to leaf reddening (Subba Rao, 1975). In Dharwad region, of Karnataka, leaf reddening initiates at bud formation stage and

continues at an increasing rate. Initiation of leaf reddening is usually accompanied by sudden drop in temperature and high wind velocity, coupled with nutritional deficiency (Dastur, 1967 and Bhatt et al., 1982). Furthermore, it is also assumed that apart from external factors there might be other internal factors such as accumulation of photosynthates and formation of anthocyanins, due to variation in the external factors. Mechanical injury and insect damage particularly jassids, cause the development of red pigments. It is also reported that the extent of reddening would also depend on the genotype of cotton grown (Dastur et al., 1952).

After introduction of Bt cotton leaf reddening has become a serious physiological problem. Rapid translocation of saccharides and nutrients from leaves alter the source-sink relationship due to synchronized boll development (Wright, 1999; Hebbar et al., 2007). Leaf reddening in cotton causes due to non-synchronization of nutrients between source and sink. The red leaf syndrome is wide spread but its occurrence is not consistent. In American upland cotton occurrence of reddening at post flowering stage will not affect the yielding ability but when it occurs at flowering or vegetative stage yield reduces drastically. The yield is reduced as the plant growth is arrested because of poor rate of photosynthesis in developing red leaves (Zade and Dhopte, 1987).

Seed cotton yields can be reduced to the extent of 30 – 60 per cent due to leaf reddening depending on variety and reddening intensity (Pagare, 2011). In early planted crop, supply of nitrogen along with phosphorous and potassium to the leaf reduce the formation of anthocyanin (Basavaneppa et al., 2015, Honnali and Chittapur, 2017 and Sathyanarayanrao et al., 2014). A survey on constraints of Bt cotton cultivation showed that 93 per cent farmers are facing leaf reddening problem (Hosamani et al., 2017).

Number of remedial measures has been proposed from time to time by several workers to combat this problem. These include early sowing to escape the cool night at boll development stage and foliar application of urea @ 2 % DAP and $MgSO_4$ @ 1.0 %. Research has been carried to mitigate leaf reddening through supply of nitrogen, phosphorous and potassium in split applications but no work has been carried on time of application on real time basis.

Sensors utilize the optical characteristics of plants and their associated vigor and health properties (Walsh, 2015).Crop reflectance sensors provide an accurate and spatially-intensive method for diagnosing and applying the correct N rate (Scharf and Oliveira, 2008). Hosmath et al. (2012) also reported that 89 per cent farmers experiencing leaf reddening problem from peak flowering to boll development stage. Hence the present investigation was undertaken for spectral characterization of red leaf and to mitigate leaf reddening in Bt cotton genotypes through real time nitrogen application using proximal sensors. The incidence of leaf reddening is higher in *G*. h*irsutum* and *G.* b*arbadense* than the *G. arboreum and G. berbaceum* types (Alston, 1959). In the present investigation two intra-hirsutum hybrids, *G. hirsutum* genotypes Ajeet 155 and First Class were selected.

Materials and Methods

Experimental site, cultivar and index calculation

A field experiment was carried out at Main Agricultural Research Station, Dharwad, Karnataka during *Kharif* (monsoon) season of 2019-20 and 2020-21 to study the spectral characterization and mitigation of leaf reddening in Bt cotton genotypes through proximal sensor based nitrogen management. The experimental site lies between 15º 30'6" N latitude, 74º 59'13.2" E longitude and at an altitude of 678 m above mean sea level. Highest amount of rainfall was received during early squaring to early blooming stage during both the years (492.0 and 357.4 mm received in 19 and 11 rainy days, respectively) followed by mid flowering to peak flowering/boll initiation stage (285.0 and 223.4 mm received in 9 rainy days, respectively). The highest mean monthly maximum temperature (29.2 ºC and 29.2 ºC, respectively) was observed during mid-flowering to peak flowering/boll initiation stage and boll development to 1st picking stage, respectively. The lowest mean monthly temperature (17.7 ºC and 16.1 ºC, respectively) was observed during the boll development to 1st picking stage, respectively. Mean monthly

maximum relative humidity was 90.5 per cent during early blooming to mid flowering stage at 2019 and 94.1 per cent during early squaring to early blooming in 2020. Mean monthly minimum relative humidity values were 63.0 and 46.1 per cent at boll development to harvest stage during 2019 and 2020, respectively.

Soil was neutral to slightly alkaline in reaction (pH 7.65) with normal electrical conductivity (0.31 dSm^{-1}), low in organic carbon content (0.45 %), medium in available nitrogen (278.30 kg ha^{-1}) and phosphorous (34.35 kg P_2O_5 ha^{-1}) and high in available potassium (357.65 kg K_2O ha^{-1}). The experiment was established in split plot design with three replications and comprising of sixteen treatment combinations. Two genotypes Ajeet 155 (G1) and First Class (G2) in main plot and eight proximal sensor based N management practices [N supplementation at 60 - 70, 71 – 80 and 81 – 90 per cent Sufficiency Index (SI) (N_1 – N_3), N supplementation at 1.1–1.5, 1.6 – 2.0 and 2.1 – 2.5 Response Index (RI) (N_4 – N_6), RDF (150:75:75 N: P_2O_5: K_2O kg ha^{-1}) (N_7) and N omission (0:75:75 N: P_2O_5: K_2O kg ha^{-1}) (N_8)] in sub plot. Two nitrogen rich plots with application of 200 per cent RDF and two absolute control plots were maintained for respective genotypes.

Sufficiency index was calculated from SPAD value recorded through SPAD 502 meter and response index from NDVI recorded through GreenSeeker proximal sensor. SPAD and GreenSeeker values were recorded at 7 days interval from early squaring upto mid bloom stage.

$$\text{Sufficiency Index (SI)} = \frac{\text{SPAD value of test plot}}{\text{SPAD value of N rich plot}} \times 100$$

$$\text{Response Index (RI)} = \frac{\text{NDVI value of N rich plot}}{\text{NDVI value of test plot}}$$

Well decomposed FYM @ 10 t ha^{-1} was incorporated in to the soil three weeks prior to sowing. Bt cotton genotypes of Ajeet 155 and First Class were sown at a spacing of 90 cm × 60 cm on 21st June and 26th June of 2019 and 2020, respectively and raised through recommended package of practices. For all the treatments except N omission fifty per cent of nitrogen fertilizer and full dose of phosphorous and potassium was applied at the time of sowing as basal dose. In N omission plot full dose of phosphorous and potassium was supplied. In conventional nitrogen nutrition (RDF) plot the remaining 50 per cent of nitrogen in the form of urea was applied in two splits at fixed growth stages of 30 and 60 DAS. In the rest of the treatments (N1 to N6) nitrogen top dressing was done at the rate of 30 kg ha^{-1} whenever SI and RI values fall in the set range (N_1, N_5& N_6: No top dressing, N_2: one top dressing at 70 and 61 DAS during 1st and 2nd year, N_3: two top dressings (55 and 70 DAS during 1st year, 41 and 61 DAS during 2nd year); N_4: two top dressings (55 and 70 DAS during 1st year, 41 and 53 DAS during 2nd year).

Spectral Characterization of red cotton leaves: Spectral properties of prominent red cotton leaves were measured on-field using GreenSeeker and SPAD 502 meter active proximal sensors. NDVI readings were recorded from a height of 0.75 – 1 m above cotton red leaf canopy using GreenSeeker on cloud free days between 9:00 am to 12:30 pm. SPAD meter reading were recorded from the red cotton leaves of ten randomly selected plants. Both NDVI and SPAD readings of cotton reddening were recorded at 130 DAS.

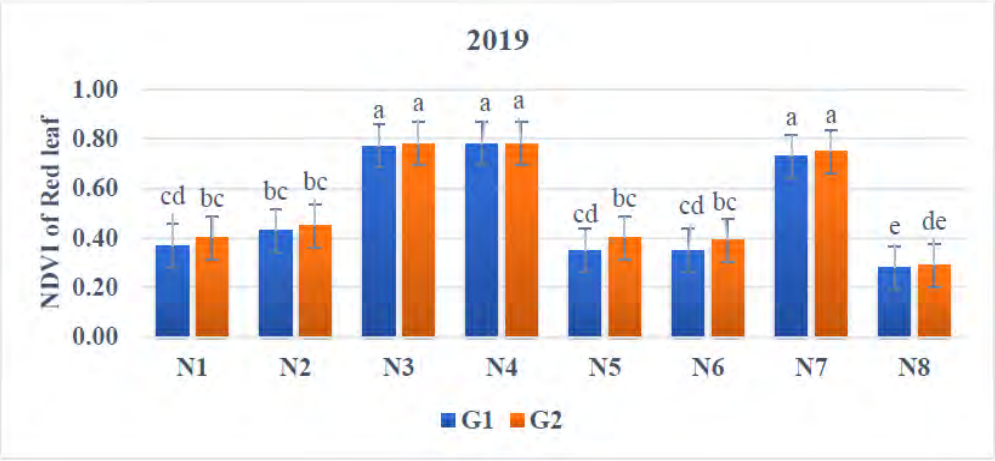
Red leaf index: Leaf reddening was recorded by visual observation. For quantitative estimation of degree of leaf reddening, observations were recorded at 130 DAS as outlined by Dastur *et al*. (1952). The number of leaves showing signs of reddening, partly or wholly were divided into five categories on the visual observations. Grade 'zero' - When all the leaves were green or less than three leaves showed signs of reddening, Grade 'one'- When three leaves showed reddening, Grade ‘two’-When more than three leaves were showing signs of reddening but young leaves were green, Grade 'three’-When all the leaves were showing reddening in patches and Grade ‘four’-When the whole plant turned red.

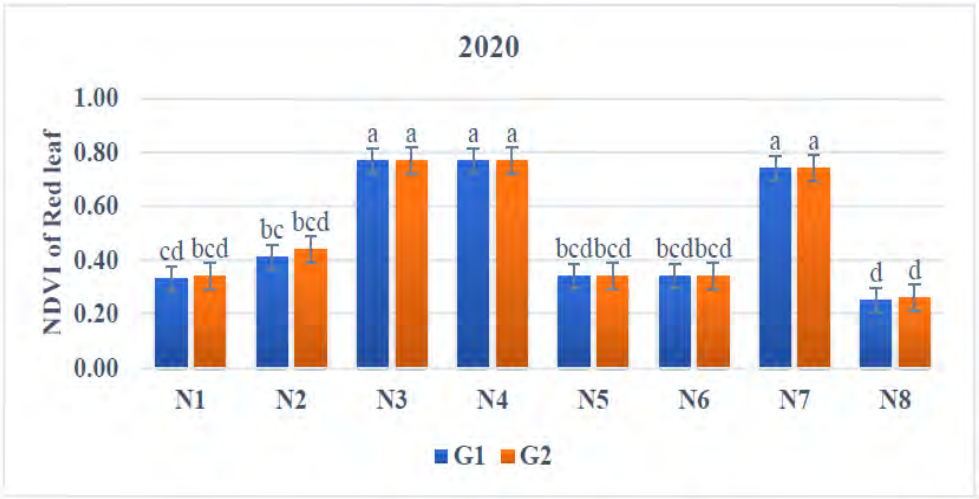
Statistical analysis: Statistical analysis of experimental data was carried out based on the mean value obtained. The level of significance used in 'F' and 'T' was P=.05. The treatment means were compared by Duncan's Multiple Range Test (DMRT) at 0.05 level of probability. Correlation matrix was worked out using R-Software.

Results and discussion

Spectral characterization of red leaf (130 DAS)

In the present study spectral characterization of red leaf was done through NDVI and SPAD value (Fig. 1 & 2). Genotypes show non-significant (P>.005) difference for NDVI and SPAD value of red leaf (NDVI of 0.52 and 0.50, SPAD value of 15.47 and 14.58 with First Class and Ajeet 155, respectively).Since there were some green leaves at the top of the plant due to its indeterminate character might have affected spectral properties of red cotton leaf resulted in non-significant difference. Non-significant difference with respect to genotypes to spectral properties was also reported by Gowramma (2017). Highest and comparable NDVI (0.78, 0.77 and 0.74, respectively) and SPAD value (31.70, 31.23 and 28.72, respectively) was recorded with N supplementation at 1.1 – 1.5 RI, 81 – 90 % SI and RDF as compared to rest of the treatments. Lowest NDVI and SPAD value was noticed with N omission (0.27 and 4.47). N deficiency caused reduction in chlorophyll, increased the anthocyanin pigmentation and was directly associated with variation in colour from dark green to light green and then to red. The interaction of genotypes and proximal sensor based N management practices were incomparable with respect to NDVI and SPAD value of red leaf. When subjected to DMRT, proximal sensor based N management practices of 1.1 - 1.5 RI and 81 – 90 % SI of both the genotypes recorded significantly higher NDVI and SPAD value as compared to all other treatment combinations except for RDF. The higher spectral properties at this stage might be due to lower red leaf index, higher leaf nitrogen; higher chlorophyll contents resulted in higher photosynthetic efficiency and resultant higher plant biomass. These results are at parity with Satyanarayan and Santhosh (2017). The intensity of reddening between different treatments is evident from Plate no. 1.





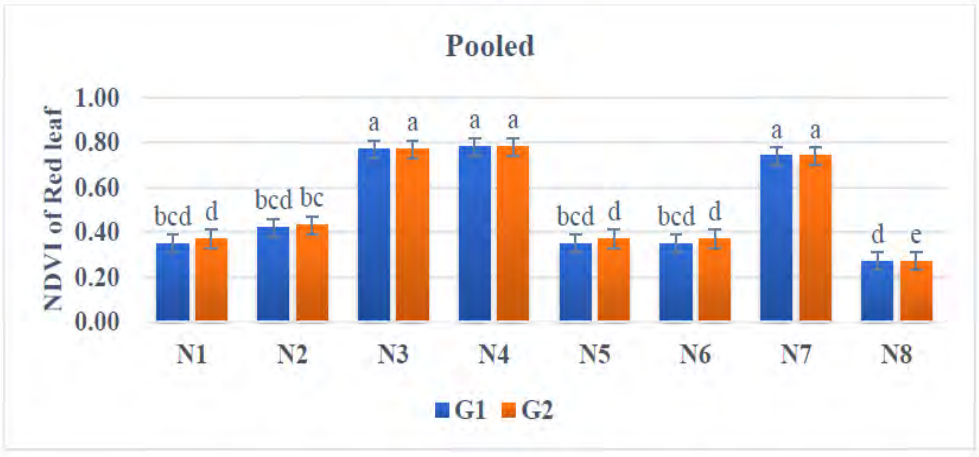
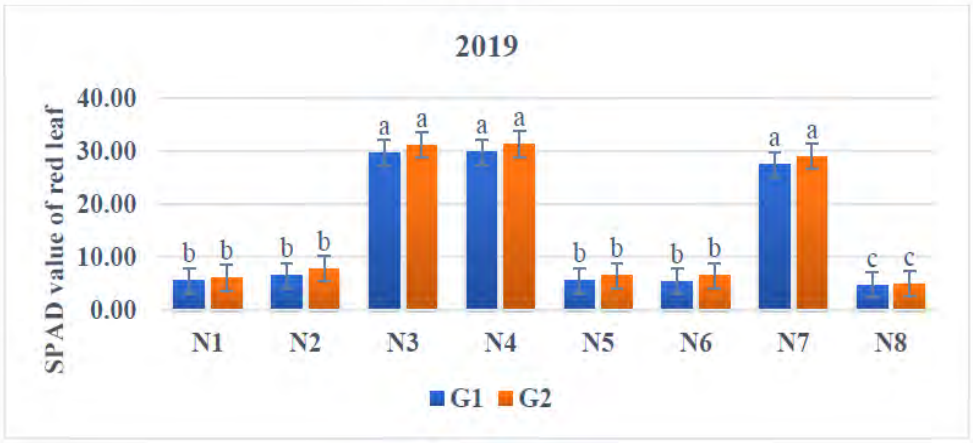


Fig. 1: NDVI of red leaf as influenced by proximal sensor based nitrogen management practices in Bt cotton (2019, 2020 and pooled). G1=Ajeet 155 and G2=First Class



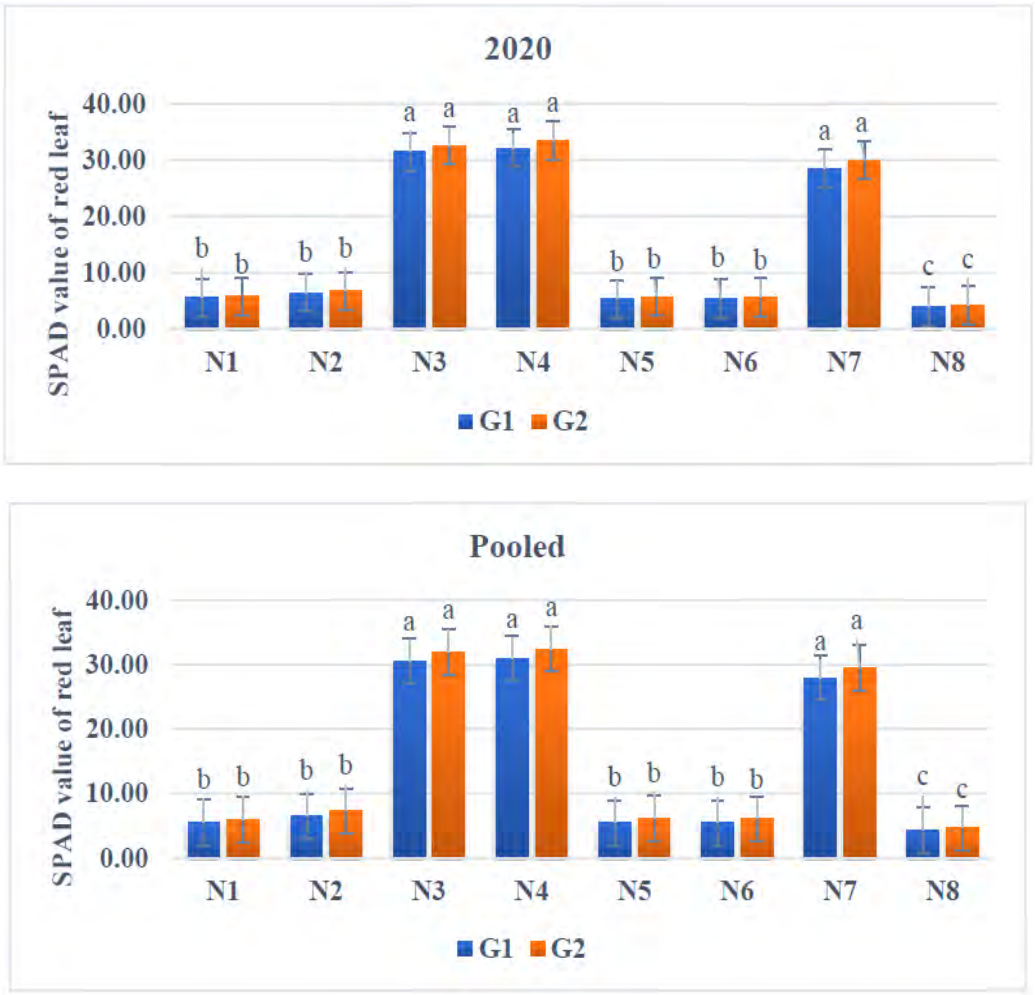


Fig.2: SPAD value of red leaf as influenced by proximal sensor based nitrogen management practices in Bt cotton (2019, 2020 and pooled). G1=Ajeet 155 and G2=First Class

Red leaf index (130 DAS)

The data (pooled) pertaining to red leaf index at 130 DAS (Fig. 3) revealed that; genotypes exhibited a significant difference (P < .005). Among the tested genotypes First Class (1.03) recorded significantly lower red leaf index as compared to Ajeet 155 (1.59). This might be due to significantly higher chlorophyll a, b and total chlorophyll in First Class resulted in healthy cotton leaves as compared to Ajeet 155 where reduction in chlorophyll content in red leaf was more. These results are in conformity with findings of Pagare and Durge (2011) and Hosmath et al. (2012).

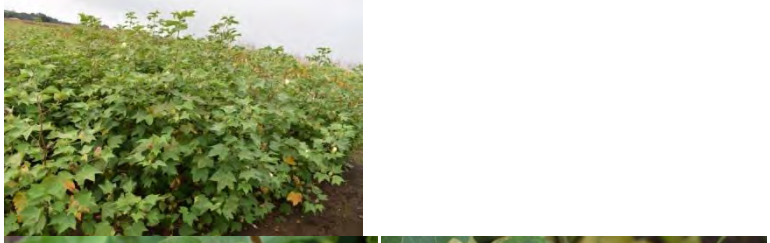
Red leaf index was significantly (P<.0001) influenced by different proximal sensor based N management practices, N supplementation at 1.1 – 1.5 RI and 81 – 90 % SI recorded significantly lower RLI (0.15 and 0.23, respectively) as compared to rest of the treatments, followed by RDF (1.12). Significant reduction of RLI in proximal sensor based N management practices might be due to synchrony of demand and supply of nitrogen p as per crop needs. Critical period for N demand is during pin head square stage and between early flowering to mid bloom stage and sensor based treatments received top dressing at these stages. Nitrogen supply coinciding with crop demand might have improved nitrogen uptake throughout growing period which enhanced photosynthetic rate and biomass aggregation. Because of this synchronization in demand and supply lower reddening was noticed. Whereas in conventional practice top dressing was done at fixed growth stages *i.e.* early squaring (30 DAS) and early

flowering stage (60 DAS).Significantly higher RLI was observed with N omission (2.23). Here, deficiency of N that enhanced anthocyanin pigmentation and reddening in leaf. These results were in conformity with Hallikeri et al. (2010).

Interaction of genotypes and proximal sensor based N management practices significantly (P<.005) influenced RLI. First Class with N supplementation at 1.1 – 1.5 RI and 81 – 90 % SI recorded significantly lower RLI (0.13 and 0.17, respectively) as compared to all other interaction treatments and found on par with Ajeet 155 with N supplementation at 1.1 – 1.5 RI and 81 – 90 % SI(0.23 and 0.30, respectively). Significantly highest RLI observed with Ajeet 155 with N omission (2.57). This might be due to real time application of N at critical growth stages reduced the reddening in cotton. Brar et al. (2004) and Hallikeri et al. (2010) indicated that status of chlorophyll content in leaf was affected by nitrogen.

Plate :1 Treatment wise intensity of leaf reddening in Ajeet 155 (G1) and First Class genotypes (G2)

G1 N7



G1 N4 & N3

Treatment wise Leaf reddening in Ajeet 155 (G1)

Treatment wise Leaf reddening in Ajeet 155 (G2)

Fig.3: Red leaf index (RLI) of Bt cotton as influenced by proximal sensor based nitrogen management practices (2019, 2020 and pooled)

Correlation of spectral values, N content in leaf, SCY with RLI

The correlation matrix (Fig. 4) indicates the association between NDVI, SPAD value, N content in leaf, SCY with red leaf index and found a perfect linear negative association. Strong negative association was observed between red leaf index v/s NDVI (-0.87), SPAD value (-0.85), N content in leaf (-0.85), seed cotton yield (-0.87).

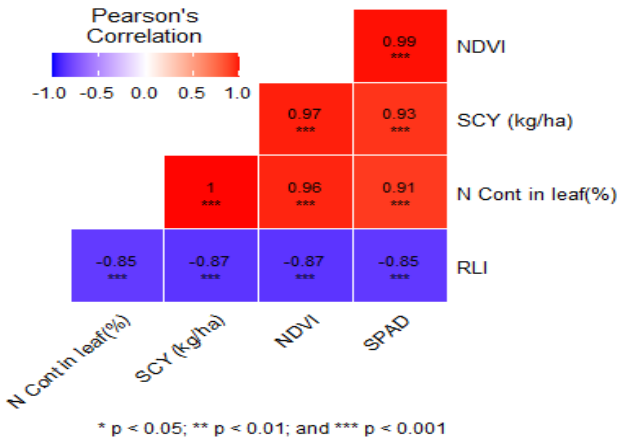


Fig.4: Correlation of spectral values, N content in leaf, seed cotton yield (SCY) with RLI as influenced by proximal sensor based nitrogen management practices in Bt cotton

Conclusion: Proximal sensor based nitrogen management *i.e.* top dressing between pin head square and mid flowering window through GreenSeeker (N supplementation at 1.1 – 1.5 RI) and SPAD meter (N supplementation at 81 – 90 % SI) significantly reduced the red leaf index in Bt cotton as compared to RDF with saving of 15 kg N ha^{-1} (10 per cent of recommended dose of nitrogen).

References

1. Atkinson GF **. Some diseases of cotton.** Alabama Polytechnical Institute of Agriculture-Experimental Station Bulletin, 41 (1892), pp. 61-65
2. Basavanneppa M A, Ajaykumar M Y, et al. Response of Bt cotton to foliar application of potassium nitrate in Tungha Bhadra project area. JCotton Res Dev. 2015;29(2):242-245.
3. Bhatt J G, Rao M R K, Appukuttan E, et al. Leaf reddening in hybrids of cotton. CommSoil SciPlant Analysis.1982; 13: 151-156.
4. Brar M S, Gill M S, et al. Effect of soil and foliar application of nutrients on yield and nutrient concentration in Bt cotton. Agricultural Res J.2009; 45(3&4): 126-131.
5. Dastur R H, Red leaf disease in American cottons (*Gossypium hirsutum*): Physiological studies on the cotton crop and their practical applications. 20-29, 1967; I.C.A.R. Pub. New Delhi, India.
6. Dastur R H, Singh K,et al.Investigation on the red leaf in American Cottons inMalwa and Bombay Karnataka. Indian Cotton Growing Review.1952; 6: 193-204.
7. Gowramma M D, Precision nutrient management in Bt cotton to achieve target yields. *M.Sc. Agri. Thesis*, Univ. Agric. Sci., Dharwad, Karnataka, India, 2017.
8. Hallikeri S S, Halemani H L, et al. Effect of nitrogen levels, split application of nitrogen and detopping on seed cotton yield and fibre quality in Bt-cotton. Karnataka J Agricultural Sci.2010;23(3): 418-422.
9. Hebbar K B, Rao R K, et al. Synchronized boll development of Bt cotton hybrids and their physiological consequences. Cur Sci.2007; 93: 693-695.
10. Honnali S N and Chittapur B M, Higher productivity and sustainability in cotton (*Gossypium hirsutum*) through management of leaf reddening by foliar nutrition. J Cotton Res Dev.2017; 30(2): 21-32.
11. Hosamani V, Chittapur B M, et al. Leaf reddening Enigma in Bt cotton: A review. IntJCurrent MicrobiolApplied Sci.2017; 6(11): 3764 – 3772.
12. Hosmath J A, Biradar D P, et al. Performance of Bt and non Bt cotton genotypes under leaf reddening malady situation. Karnataka J Agricultural Sci.2012; 25: 36-38.
13. Olga O S, Nitrogen management in field crops with reference strips and crop sensors. Idaho Crops and Soils News. Monthly newsletter, UI Extension serving Idaho crop producers. 2015; Pg. 1-6.
14. Pagare G A and Durge D V, Pigment analysis studies with reference to leaf reddening in Bt cotton. Int J Plant Sci.2011; 6(1): 42-44.
15. Pagare G A, Effect of leaf reddening on yield and yield attributes of cotton. Bionfolet,2011; 8(1): 25-30.
16. Sathyanarayanarao, Gante V K, et al. Management of leaf reddening in Bt-Cotton. Proceedings of ZREAC and ZREFC Meet, UAS Raichur, 2-4 June 2014, pp. 55.
17. Satyanarayan R and Santhosh U N, Management of leaf reddening through soil and foliar nutrition in irrigated Bt cotton (*Gossypium hirsutum* L.). Karnataka JAgril Sci.2017;31(2): 244-248.
18. Scharf P and Oliveira L F, Managing Nitrogen with sensors: some practical issues. Paper presented In: *Beltwide Cotton Conf.,* Nashville, Tennessee, January, 8-11, 2008; p. 1585-1587.
19. Subba Rao I V, Nutritional disorders of crops in Andhra Pradesh, Andhra Pradesh Agricultural University, Hyderabad, Technical Bull., 1975; pp. 42-43.
20. Wright P R, Premature senescence of cotton (*Gossypium hirsutum* L.)- Predominantly a potassium disorder caused by an imbalance of source and sink. Plant Soil.1999; 211: 231-239.
21. Zade V R and Dhopte A M, Anatomy and pigment analysis of red leaf in cotton. P K VRes J.1987*;* 11(2): 107-111.

Source-Sink Manipulation to Induce Reproductive Synchrony and Enhance Productivity in Cotton by Plant Growth Regulators

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Abstract

Background:
Cotton plant has to withstand myriads of biotic and abiotic stresses from germination to maturity. A good plant frame developed during the early vegetative phase of cotton would provide sufficient space for holding and catering to the needs of the reproductive parts during the later part of growth. Abiotic stresses like drought, water-logging, and high temperature cause considerable damage to the plant leading to stunted growth. As the cotton plant is photo-insensitive, they enter the reproductive stage and start producing the reproductive parts by 35-40 DAS, even before sufficient morph frame is available to hold the reproductive parts. This leads to reduced boll load or premature death. There are reports that mechanical removal of squares formed until 60-80 days, enhanced flowering in the later stages and had a positive effect on the seed cotton yield This paper summarizes the research initiated in the year 2000 to develop a foolproof method of mimicking the square removal through foliar application of Ethrel alone or in combination with Maleic hydrazide (MH) to induce the square shedding and compactness.

Results:
Field experiments were conducted at five locations (Faridkot and Sriganganagar in North Zone under irrigated condition, Surat under irrigated conditions in the central zone and Guntur & Dharwad in the rainfed condition in the south zone) across India under varied agroclimatic conditions through ICAR- All India Coordinated Research Project on Cotton (ICAR-AICRP on Cotton) to find out the effect of ethrel and Maleic hydrazide (MH) on growth, yield, and economics of cotton. The experiment was conducted in a randomized block design with three replications. The growth regulator - ethrel @ 8.5 µmoles (at 40 DAS) was tried alone and in combination with MH in 500, 750, 1000 ppm at 80 and 90 DAS. The results indicated that application of ethrel @8.5 µmoles at square initiation followed by MH @ 500 ppm at 95 DAS led to the production of higher seed cotton yield at Faridkot (2772 kg/ha), ethrel @8.5 µmoles at square initiation followed by MH@500 ppm at 80 DAS at Guntur (2050 kg/ha) and Dharwad (2266 kg/ha). In Sriganganagar, the application of ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95 DAS was found promising (1797 kg/ha). Among chemical treatments, highest values for Fertilizer use efficiency *(5.78 kg/kg) and water productivity (600.7 g/m^3) and B:C ratio (2.14) were observed under ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS at Faridkot. A significantly higher photosynthetic rate (27.7 µ mole/m^2/sec) was recorded in plants that received a foliar spray of Ethrel @ 8.5 µmoles at squaring and MH @ 500 ppm at 80 DAS when compared to control (22.9 µ mole/m2/sec) at Dharwad.*

Conclusion:
The foliar application of Ethrel @ 8.5µ moles at square initiation followed by MH@500 ppm at 80 or 95DAS and MH @ 750 ppm at 95 DAS were found effective in manipulating source-sink relationship and resulted in higher yield attributes, seed cotton yield, and indirectly influencing fertilizer use efficiency, water productivity and monetary returns. The highest B:C ratio (2.14) was exhibited with foliar spray of Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS.

Keywords: *Ethrel, Maleic* hydrazide, Seed cotton yield, Fertilizer use efficiency, morphoframe

Introduction:

Cotton, basically a perennial plant is domesticated and now grown as a forced annual. Through intensive breeding programmes many of the perennial characters are masked and the present cultivars are photo insensitive with determinate growth habits. But under unfavorable conditions, it reverts to its perennial nature.

About 60 percent of cotton cultivation in India is under rainfed conditions (Hebbar et al., 2013). Cotton suffers from various biotic and abiotic stresses right from germination to maturity. The growth during the seedling establishment phase has a role to play in yield realization. An ideal plant morphoframe would provide sufficient space for holding and catering the needs of the reproductive parts during the later part of growth. The environment, in which cotton is cultivated in India, is far from ideal. Even though over 95% of the crop in North India is grown under irrigated conditions, it grows in an environment characterized by long days and high temperatures since germination. In Central and South India, where it is predominantly grown as a rainfed crop, cloudy weather and water logging conditions during the early stages and drought or low soil moisture, or unseasonal rains at later stages adversely affect growth and yields.

Relatively little is known about hormonal control of cutout, but based on established effects of the hormones, it is speculated that auxin, cytokinins, and gibberellins promote growth and delay cutout. ABA, on the other hand, promotes cutout as it inhibits growth and prolongs bud dormancy (Davis and Curry 1991). Ethylene increases boll abscission and may restrict growth, but may not induce dormancy of buds (Kumari and Mridula, 2013). Various growth regulators have been tried in cotton to make it produce more bolls, limit vegetative growth or terminate fruiting. When boll load is limited by carbohydrate supply, exogenous modification of hormonal balance to increase boll set may be futile. More bolls may be set but will be of smaller size and plant growth is terminated prematurely.

As the cotton plant is relatively photo-insensitive, it starts producing the reproductive parts irrespective of the environmental and physical conditions by 40-45 DAS. Hence sufficient morpho-frame will not be available for the plant to hold the reproductive parts. This leads to reduced boll load and premature death of plants. There are reports that mechanical removal of squares till 60-80 days enhanced flowering in the later stages and had a positive effect on the seed cotton yield(Kumari and George.2012; Nawalkar et al., 2014). The drawback observed was that the reproductive process will not be terminated but only the flowers are removed mechanically. By this, the plant will be wasting its photosynthates on producing the squares which are mechanically removed. On the other hand, the availability of labor and cost of mechanical removal is quite expensive. This paper summarized the research initiated in the year 2000 to develop a foolproof method of mimicking the square removal through foliar application of Ethrel alone or in combination with Maleic hydrazide to induce the square shedding and compactness.

Materials and Methods

Field experiments were conducted at five locations (Faridkot and Sriganganagar in North Zone under irrigated condition, Surat under irrigated conditions in the central zone and Guntur & Dharwad in the rainfed conditions in the south zone) across India under varied agroclimatic conditions through ICAR-All India Coordinated Research Project on Cotton (ICAR-AICRP on Cotton) to find out the effect of ethrel and Maleic hydrazide (MH) on growth, yield, and economics of cotton. The experiment was conducted in Factorial RBD with three replications. The growth regulator ethrel @ 8.5µmoles (at 40 DAS) was tried alone and in combination with MH in 500, 750, 1000 ppm at 80 and 90 DAS. The selected growth regulators and its dose and time of application were imposed in two genotypes in Faridkot (G1.MRC 7017 BG II & G2.LHH 144), Surat (G1.G.cot Hy 8 BG II and G2.G.cot Hy 8), Sriganganagar (G1.Bioseed-6588 BG II & G2.F 2228), Guntur (G1.Jaddu BG II & G2.NDLH 1918) and Dharwad (G1.Mallika BG II & G2.RAHH-98). The biometric observations on growth and yield attributes and seed cotton yield were recorded and economics were also worked out. The recommended agronomical and plant protection measures were carried out in time to keep the crop in healthy condition. Five plants from each treatment were selected randomly and tagged for recording various observations on morpho physiological growth parameters and yield attributes periodically and at harvest. Seed cotton yield was worked out from the net plot basis and expressed as kg/ha. Statistical analysis was carried out.

Results and discussion

The cotton crop can accept considerable early damage and still make adequate yields due to mid and late-season growth. Certain kinds of mechanical damage to young cotton such as early hail damage to cotyledons, thrips feeding, and water stress prior to flowering have shown to cause little or no reduction in yield. Though 7-10 days of earliness may be lost by this damage, the delay has negligible effects on harvest in most seasons.

The foliar application of ethrel led to the shedding of pinhead size squares and further delay in reproductive activity by 15-20 days (**Plate 1**). During this period, the partitioning of the photosynthates was more towards root growth and stem girth in treated plants compared to control plants. The apical dominance was overcome leading to the sprouting of lateral buds. This leads to the production of more branches facilitating the production of a higher number of squares (**Plate 2**). Nitrate reductase activity increased significantly with the application of ethrel following the first week of application. The biochemical constituents like reducing sugars, amino acid and protein content in the leaf tissues also showed an

upward trend with foliar application of ethrel (Data not presented). The Photosynthetic rate was higher in treated plants over the control **(Table 1)**. The plant morpho-frame changed with luxurious growth which led to the production of more squares. This finally led to synchronous flowering and boll bursting (**Plate 3**). The overdose of the ethrel will lead to stem splitting and death of plant (**Plate 4**)

Biophysical parameters

The results in Dharwad revealed that the rate of photosynthesis was significantly higher in RAHH-98 over Mallika **(Table 1),** but other parameters *viz.,* stomatal conductance, transpiration and leaf temperature were non-significant. The effect of growth regulators, on biophysical parameters was significant except on leaf temperature. Significantly highest photosynthetic rate (27.7 µ mole/m^2/sec) recorded (T_3). Ethrel @ 45 ppm at 45 DAS and MH @ 500 ppm at 80 DAS over (T_1) control (22.9 µ mole /m^2/sec) stomatal conductance and transpiration rate were significantly higher in T8 over control. Foliar application of ethephon to spring barely caused an increase in penultimate leaf photosynthetic rate. (Pua & Chi, 1993).

The interaction effect of genotypes and growth regulators on biophysical parameters was significant except on leaf temperature. Maximum photosynthetic rate (28.91 µ mole/m^2/sec) recorded in RAHH-98 with T_8 followed by Mallika with T_8 (29.53) and least was in Mallika with T_2 (21.11). Maximum stomatal conductance was recorded in RAHH-98 with T_2 (0.24 µ mole/m^2/sec) followed by RAHH-98 with T_3 (0.23). The least stomatal conductance was in Mallika with T_4 (0.11). Leaf transpiration rate was maximum in Mallika with T_8 (5.83 µ mole/m^2/sec) and least was again in Mallika with T_4 (2.53).

Genotypes

Cotton genotypes are varying with indeterminacy. The understanding of degree of indeterminacy and the effect of growth retardant could help in the management of a cotton genotypes.The study has selected genotypes with varying background mainly Bt hybrids and isogenic line/pure line variety in all the centers. The pooled results found that a significantly higher number of bolls of 47.5 per plant and boll weight(g) of 4.0 and seed cotton yield of 2817 kg/ha was reported with MRC 7017 BG II at Faridkot. **(Table 2&3).** In Surat, a significantly higher number of bolls of 44.4 was registered which positively increased the seed cotton yield up to 2142 kg/ha in G.cot Hy 8 BG II (pooled basis). In Dharwad Mallika BG II recorded 26.3 % higher seed cotton yield over RAHH-98 in pooled results. The growth characters, yield attributes and seed cotton yield are varying amongst genotypes.

Growth Regulators

The pooled results revealed that none of the growth regulator treatments significantly influenced the plant height in the entire test centres (Table 2). Application of Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS (T_6) had recorded significantly higher number of bolls of 44.0 and boll weight of 3.7 g and higher seed cotton yield of 1797 kg/ha at Sriganganagar **(Fig.1).** The Sriganganagar comes under North zone, where ample irrigation facilities are available for cotton cultivation, facilitating vigorous rate of vegetative growth, hence positive response was noticed with growth regulator treatments. The pooled results of growth regulation treatments showed that, T_3 *i.e* application of Ethrel @ 8.5 µ moles at square initiation followed by MH@500 ppm at 80 DAS resulted

in significantly higher seed cotton yield (2050 kg/ha) at Guntur which were on par with all the treatments except control (T_1 1488 kg/ha) and combined application of Ethrel @8.5µ moles at square initiation followed by MH@ 1000 ppm at 95DAS (T_8, 1654 kg/ha).In Dharwad, application of 45 ppm Ethrel at 40 DAS followed by MH at 500 ppm at 95 DAS (T_4) recorded significantly higher yield (2266 kg/ha) might be due to registering of significantly higher boll weight (6.0 g) and highest number of bolls/plant (20.7) as compared to control (seed cotton yield (1911 kg/ha) and boll weight (5.7 g). However, it was on par with application of Ethrel @ 8.5µ moles at square initiation (T_2, 2176 kg/ha), combined application of 45 ppm Ethrel at 40 DAS follow by MH at 500 ppm at 80 DAS (T_3, 2230 kg/ha) and combined application of 45 ppm Ethrel at 40 DAS followed by MH at 750 ppm at 80 DAS (T_5, 2201 kg/ha).

The results of Guntur and Dharwad under rainfed condition interpreted that high and assured rainfall influenced the crop growth positively and modification of growth regulation favored higher yield attributes and seed cotton yield. However, the significant growth regulator effects were not observed in Surat. Veeraputhiran & Thiruvarasan (2021) found that application of ethrel @ 45 ppm on 40 DAS followed by mepiquat chloride @ 100 ppm on 90 DAS recorded the significantly higher growth and yield attributes and highest seed cotton yield. Earlier reports by Kataria *et al,* (2017) and Kataria and Valu (2018) revealed that significantly higher seed cotton yield (3923 kg/ha) was obtained with detopping at 75 DAS + application of MH@30 ppm at 90 DAS. They also reported that the increase in yield was primarily due to increased bolls/plant. Growth regulators increased the boll number and seed weight significantly in cotton (Shekar *et al*., 2015). Growth parameters registered remarkably maximum values of root length, monopodia, sympodia, open bolls at maturity and boll weight of cotton in plants detopped at 75 DAS followed by spraying of Ethrel @ 50 ppm at 90 DAS. (Vekaria *et al.,* 2021). Exogenous application of ethrel enhanced photosynthesis of mustard in irrigated and un irrigated conditions(Bhat *et al*., 2010). The interaction effect was significant with application of Ethrel @ 8.5micro moles at square initiation followed by MH@ 500ppm at 80 DAS in Jaddu BG II with higher seed cotton yield of 2050 kg/ha at Guntur. The variable response to treatment across the centres may be due to difference in genotypes with different degree of indeterminateness, varying environments and the interaction of both, as earlier reported by Sankaranarayanan et al., 2010. Genotypes with more indeterminate growth habits can sustain terminal growth longer than less indeterminate varieties.

Quality parameters

The fibre quality parameters were not affected due to treatments, this as reported by Kumari & Mridula, 2013 and Kataria et al., 2017.

Fertilizer Use Efficiency (FUE) and Water Productivity (WP)

The experimental results at Faridkot showed highest fertilizer use efficiency (5.43 kg/kg) and water productivity indices (565.1 g/m^3) for MRC 7017 BG II than LHH144 (Table 4). Among chemical treatments, highest values for FUE (5.78 kg/kg) and WP (600.7 g/m^3) were observed under T_4 *i.e* Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS as compared to all other treatments.

Economics

Among chemical treatments, highest B:C ratio (2.14) was exhibited under T_4 *i.e* Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS followed by T_6 *i.e* Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS (2.03) with the least B:C ratio with T_7 (Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm) at 80DAS (1.34) (Table 4). This was in line with findings of Veeraputhiran & Gunasekaran, (2020) where higher economic benefits were reported by the application of Mepiquat chloride under high-density planting systems

Conclusion:

The foliar application of Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 80/95DAS or MH@750 ppm at 95DAS were found effective in increasing the yield by manipulating source-sink relationship and indirectly influencing fertilizer use efficiency, water productivity and monetary return. The highest B:C ratio (2.14) was exhibited with foliar spray of Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95 DAS under Irrigated conditions in North India.

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References

1. Ashraf Bhat M., M.R. Mir, N.A. Khan, N.A. Lone, K.A. Bhat, S.M. Razvi, H. Ashraf, Nawsheeba Wani, Rizwan Rashid, S. Hayat ,W.A. Payne, Sabina Akhtar, and S.A. Wani .2010. Review article the role of ethrel in plant growth and development under different environmental conditions. *International Journal of Current Research* ,Vol. 4, pp. 004-013.
2. Constable, C.A. and H.W, Rawson, 1980. Effect of leaf position, expansion and age on photosynthesis, transpiration and WUE of Cotton. Aust. J. Plant Physiol., 7: 89-100.
3. Davis, T.D. and Curry, E.A. 1991. Chemical regulation of vegetative growth. *Critical Reviews in Plant Science* 10: 151-188.
4. Hebbar, K.B., Venugopalan, M.V., Prakash, A.H. and Aggarwal, P.K. (2013). Simulating the impacts of climate change on cotton production in India. Climate Change: 118: 3-15
5. Kataria, G.K and M.G.Valu .2018. Effect of detopping and growth retardants on physiological parameters and yield of *Bt* cotton (*Gossypium hirsutum* L.) *Journal of Cotton Research and Development* 32 (2) 226-230.
6. Kataria,G.K., M. G. Valu., V. L. Kikani and L. K. Dhaduk. 2017. Influence of modification of morphoframe through detopping, ethrel and maleic hydrazide on growth and yield of Bt cotton hybrid. *Journal of Cotton Research and Development*. 31 (1) 69-73 .
7. Kumari, S. R, and Mridula G (2013) Effect of early ethrel application in enhancing cotton production in vertisols of Andhra Pradesh, *India Agricultural Science Digest - A Research Journal* 33(3):193-197
8. Kumari, S.R. and M.George.2012. Physiological manipulation of plant morphoframes by MH and through nipping for enhancing cotton yield. *Green Farming*. 3 : 677-79.
9. Nawalkar, D., V.Kumar,Y. Ban and A.V.Narwade .2014. Influence of modification of morphoframe through Ethylene and Maleic hydrazide on growth and yield of cotton hybrids.*Ecology, Environment and Conservation* : 1241-46.
10. Pua, E.C., and G.L.Chi. 1993. De novo shoot morphogenesis and plant growth of mustard (Brassica juncea) in vitro in relation to ethylene. *Physiologia Plantarum* 88: 467-474.
11. Sankaranarayanan, K., C S Praharaj, P Nalayini, K K Bandyopadhyay and and N.Gopalakrishnan 2010. Climate change and its impact on cotton. Indian Journal of Agricultural Sciences 80 (7): 561-75
12. Shekar, K., M.Venkataramana, M. and Ratna Kumari, S. 2015. Response of hybrid cotton to Chloro mepiquat chloride and detopping under high density planting. *Journal of Cotton Research and Development*. 29 : 84-86.
13. Veeraputhiran, R., and S. Thituvarasan.2021. Effect of bio stimulant application on growth, yield and economics of cotton *Journal of Cotton Research and Development.* 35(1), 154-158.
14. Veeraputhiran, R., and M.Gunasekaran, M. 2020. Effect of time of growth retardant application on growth of cotton plant under high density planting system. *Journal of Cotton Research and Development* 34 : 67—71.
15. Vekaria, G.B., M. L. Patel, M. M. Talpada, V. D. Vora, T. J. Patel and D. S. Hirpara. 2021. Study on effect of growth regulators on growth parameters and detopping in Bt cotton (Gossypium hirsutum L.) under rainfed condition *Jounal of Cotton Research and Development* 35(1), 91-96.

Plate 1: Foliar application of Ethrel induced shedding of squares

Plate 2: Comparative growth of untreated and treated cotton plants with ethrel at 60 DAS

Plate 3: Ethrel treated cotton plant at Harvest with synchronous boll development

Plate 4: Symptom of stem splitting due to over dose of Ethrel spray

Table 1: Effect of growth regulators on Biophysical parameters (IRGA readings) in Bt and non Bt cotton at 100 DAS

	Photosynthesis (µ mol/m^2/sec)	Stomatal conductance (µ mol/m^2/sec)	Transpiration (m mol/m^2/sec)	Leaf temperature (^{0}C)
V1-Mallika Bt	24.72	0.16	3.75	30.79
V2- RAHH-98	26.97	0.20	4.32	31.14
CD @ 5%	1.03	NS	NS	NS
T1 : Control	22.90	0.18	4.12	31.47
T2 : Ethrel @8.5µmoles at square initiation(40 DAS)	23.35	0.18	3.96	30.79
T3 : Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 80DAS	27.70	0.20	4.13	30.92
T4: Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS	24.34	0.14	3.13	30.98
T5 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 80DAS	25.26	0.17	3.66	31.11
T6 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS	25.48	0.17	3.68	31.18
T7 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 80DAS	27.65	0.22	4.34	30.52
T8 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 95DAS	30.09	0.20	5.25	30.76
	2.28	0.03	0.72	NS

Table 2. Plant height (cm) and number of bolls/plant as influenced by ethrel and maleic hydrazide application in cotton

Genotypes	Plant height (cm)					Number of boll/plant				
	Faridkot	SNGR	Surat	Guntur	Dharwad	Faridkot	SNGR	Surat	Guntur	Dharwad
G1	159.1	125.1	135.1	124.5	119.0	47.5	41.0	44.4	36.2	20.8
G2	171.0	125.9	145.0	129.2	119.7	42.6	41.0	40.9	42.1	19.0
CD (5%)	5.3	NS	6.6	NS	NS	3.6	NS	NS	5.6	NS

Growth regulator										
T1 : Control	161.4	123.2	139.5	142.0	121.8	48.3	36.9	42.8	39.5	20.6
T2 : Ethrel @8.5µmoles at square initiation(40 DAS)	163.1	128.1	138.9	142.0	121.5	47.0	38.6	41.1	39.9	18.6
T3 : Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 80DAS	162.8	125.0	137.0	124.0	119.0	43.0	39.8	41.5	40.9	17.9
T4: Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS	168.8	124.6	141.7	124.0	119.1	50.6	42.1	41.5	41.0	20.7
T5 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 80DAS	167.0	125.4	139.5	122.0	121.3	39.3	39.2	45.1	40.4	20.2
T6 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS	166.7	126.6	143.7	120.5	117.0	48.5	44.0	45.2	40.2	20.6
T7 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 80DAS	166.5	125.1	140.8	119.5	118.0	37.7	43.7	43.1	36.8	20.2
T8 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 95DAS	167.2	126.1	139.8	120.5	116.7	44.5	43.4	44.8	34.8	20.4
CD (5 %)	NS	NS	NS	NS	NS	8.3	5.8	NS	5.3	NS

Faridkot (G1.MRC 7017 BG II & G2.LHH 144), Surat (G1.G.cot Hy 8 BG II and G2.G.cot Hy),Sriganganagar (G1.Bioseed-6588 BG II & G2.F 2228), Guntur(G1.Jaddu BG II & G2.NDLH 1918) and Dharwad (G1.Mallika Bt& G2.RAHH-98)

Table 3. Boll weight (g) and Seed Cotton Yield (kg/ha) as influenced by ethrel and maleic hydrazide application in cotton

Genotypes	Boll weight (g)					Seed Cotton Yield (kg/ha)				
	Faridkot	SNGR	Surat	Guntur	Dharwad	Faridkot	SNGR	Surat	Guntur	Dharwad
G1	4.0	3.2	3.0	4.7	6.1	2817	1494	2142	1972	2367
G2	3.7	3.1	2.9	4.0	5.9	2357	1499	1855	1769	1874
CD (5%)	0.1	NS	NS	0.4	NS	428	NS	172.6	NS	214.2
Growth regulator										
T1 : Control	4.0	2.9	3.1	4.2	5.7	2732	1289	2054	1488	1911
T2 : Ethrel @8.5µmoles at square initiation(40 DAS)	4.1	3.1	3.1	4.3	6.0	2629	1396	2012	1994	2176
T3 : Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 80DAS	3.7	3.1	3.0	4.2	6.1	2622	1439	1935	2050	2230
T4: Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS	4.1	3.4	2.9	4.5	6.0	2722	1708	2006	1932	2266
T5 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 80DAS	3.6	3.0	2.9	4.3	6.2	2397	1506	2140	1965	2201
T6 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS	3.9	3.7	2.8	4.3	5.9	2703	1797	2008	1887	1991
T7 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 80DAS	3.6	3.0	2.9	4.4	6.2	2423	1403	1880	1994	2117

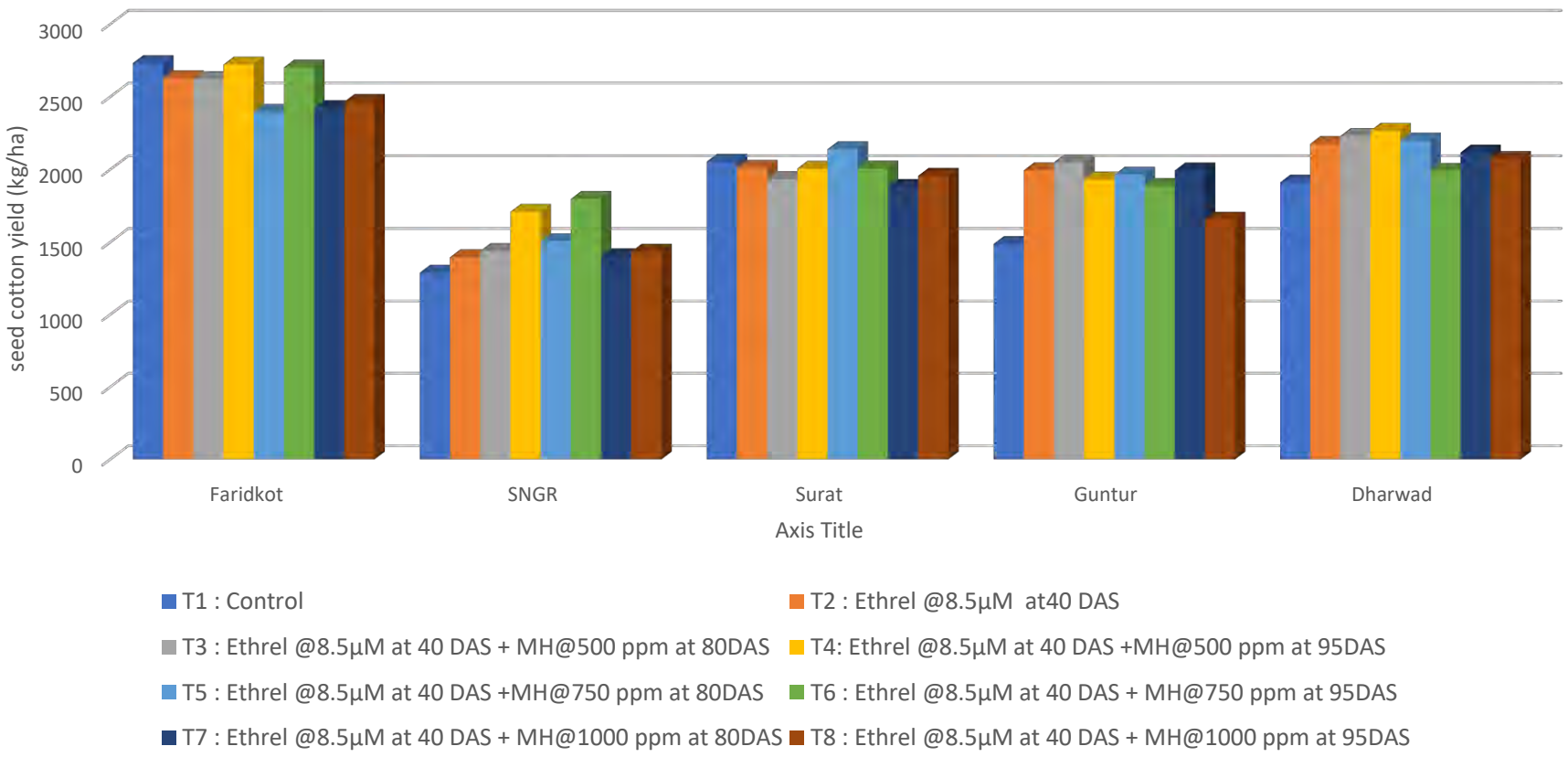
T8 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 95DAS	3.9	2.9	3.0	4.1	6.1	2466	1434	1957	1654	2073
CD (5 %)	0.3	0.2	NS	NS	0.2	261	99	NS	167	160.8

Faridkot (G1.MRC 7017 BG II & G2.LHH 144), Surat (G1.G.cot Hy 8 BG II and G2.G.cot Hy),Sriganganagar (G1.Bioseed-6588 BG II & G2.F 2228), Guntur(G1.Jaddu BG II & G2.NDLH 1918) and Dharwad (G1.Mallika Bt& G2.RAHH-98)

Table 4. Monetary parameters, FUE and water productivity under different treatments at Faridkot

Treatments	Cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio	FUE (kg seed cotton yield/ kg fert. applied)	Water productivity (g/m^3)
Main						
V_1: MRC7017	38696	114433	75736	1.95	5.43	565.1
Bt entry						
V_2 :LHH144,	34069	95478	61409	1.77	4.53	471.4
non Bt entry						
CD (0.05)	567	4255	3687	0.07	0.20	21.0
Sub						
T_1 : Control	37390	112510	75120	2.00	5.34	555.6
T_2 : Ethrel @8. 5µmoles (45 ppm) at square initiation (40 DAS)	37297	111811	74514	1.98	5.31	552.1
T_3 : MH@500 ppm at 80DAS	37179	110932	73552	1.97	5.27	547.8
T_4 : Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 80DAS	36015	102200	66184	1.82	4.85	504.6
T_5 : Ethrel @8.5µ moles at square initiation followed by MH@500 ppm at 95DAS	38608	121643	83035	2.14	5.78	600.7
T_6 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 80DAS	33900	86337	52437	1.52	4.10	426.3
T_7 : Ethrel @8.5µ moles at square initiation followed by MH@750 ppm at 95DAS	37604	114118	76513	2.03	5.42	563.5
T_8 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 80DAS	32753	77732	44979	1.34	3.69	383.8
T_9 : Ethrel @8.5µ moles at square initiation followed by MH@1000 ppm at 95DAS	36697	107316	70618	1.92	5.10	529.9
CD (0.05)	1203	9027	7823	0.15	0.42	44.5

Fig.1.Seed cotton Yield (kg/ha) by growth regulators



Direct sowing under plant cover: a possibility for the rapid establishment of cotton in a context of climate change in Mali

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Abstract

***Background**: In Sudano-Sahelian zone, crop productivity is limited by water availability and soil fertility. The cotton plant is particularly affected during the period of crop establishment. Sowing under cover crop (SCV) improves plant stand and yield. To achieve this objective, Split plot experimental design was usedwith 6 levels of cropping systems as main factor: T1 = maize residues (conservation of residues on the plot after harvest); T2 = maize residues + Brachiariaruziziensis; T3 = maize residues + Brachiariaruziziensis + Stylosantheshamata; T4 = maize residues + Brachiariaruziziensis + Cajanuscajan; T5 = maize residues + Brachiariaruziziensis + Mucunacochinchinensis; T6 = conventional system, export of residues after harvest. Two cotton varieties (NTA MS334 and BRS 293) as secondary factor were sown in conventional (CS) systems and compared to the four systems in SCV. The data collected concerned emergence, densities, height, number of bolls and yield. Analysis of variance was performed using StatBox 6.5 software and Newman and Keuls test for comparison of means at the 5% threshold.*

***Results**: The seedling emergence is faster, at least 3 days between SCV and conventional system.Density after emergence was higher in SCV, 38891 seedlings ha^{-1} compared to 36873 in CS The number of plants per hectare at harvest was greater than 65000. NTA MS334 cotton plants were taller than those of BRS 293. No significant difference was observed between the yields of the cropping systems, but a significant difference was observed between varieties BRS 293 (2720 $kg.ha^{-1}$) and NTA MS334 (2157 $kg.ha^{-1}$).*

***Conclusions**: The practice of direct sowing under cover crop allows rapid crops establishment in climate change context without any loss of yield.*

***Keywords:**cropping systems, residues, density, number of bolls, yield*

Le semis direct sous couverture végétale : une possibilité de mise en place rapide du cotonnier dans un contexte de changement climatique au Mali

Resume
Contexte : Dans la zone Soudano-sahélienne, la productivité des cultures est limitée par la disponibilité en eau et la fertilité des sols. Le cotonnier est particulièrement affecté pendant la période d'établissement des cultures. Le semis sous couvert végétal (SCV) améliore le peuplement et le rendement de la plante. Pour atteindre cet objectif, un plan expérimental de parcelles divisées a été utilisé avec 6 niveaux de systèmes de culture comme facteur principal : T1 = résidus de maïs (conservation des résidus sur la parcelle après la récolte) ; T2 = résidus de maïs + *Brachiariaruziziensis* ; T3 = résidus de maïs + *Brachiariaruziziensis* + *Stylosantheshamata* ; T4 = résidus de maïs + *Brachiariaruziziensis* + *Cajanuscajan* ; T5 = résidus de maïs + *Brachiariaruziziensis* + *Mucunacochinchinensi*; T6 = système conventionnel, exportation des résidus après la récolte. Deux variétés de coton (NTA MS334 et BRS 293) comme facteur secondaire ont été semées dans les systèmes conventionnels (CS) et comparées aux quatre systèmes dans le SCV. Les données recueillies concernaient la levée, les densités, la hauteur, le nombre de capsules et le rendement.

L'analyse de la variance a été effectuée à l'aide du logiciel StatBox 6.5 et du test de Newman et Keuls pour la comparaison des moyennes au seuil de 5 %.

Résultats : La levée des semis est plus rapide, au moins 3 jours entre le SCV et le système conventionnel.la densité après l'urgence était plus élevée dans le SCV, 38891 semis ha^{-1} contre 36873 dans le CS Le nombre de plants par hectare à la récolte était supérieur à 65 000. Les plants de coton NTA MS334 étaient plus grands que ceux de BRS 293. Aucune différence significative n'a été observée entre les rendements des systèmes de culture, mais une différence significative a été constatée entre les variétés BRS 293 (2720 kg ha^{-1}) et NTA MS334 (2157 $kgha^{-1}$).

Conclusions : La pratique du semis direct sous couvert permet un établissement rapide des cultures dans le contexte du changement climatique sans perte de rendement.

Mots clés : systèmes de culture, résidus, densité, nombre de capsules, rendement

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Introduction

Cotton cultivation is the engine of agriculture in cotton zone of Mali. It plays an important role in the country's economy (Camara, 2015). However, yields drop has been observed over the last few decades due to certain constraints (climatic hazards, pest damage and soil degradation) with an impact on food insecurity, reduced farm incomes, rural exodus and conflicts between farmers and herders (Deveze etHally des Fontaines, 2007).

Cotton cultivation area in Mali is located in Sudano-Sahelian zone, between 800 and 1200 mm isohyets (FAO, 2001). Cotton is grown without irrigation, in rotation with food crops (Bazile et al., 2000). The cotton plant consumes about 600 mm of water during its development cycle (Albergel et al., 1985). The cumulative rainfall recorded during year is very often higher than crop's water requirements. It is not a good indicator of water available for cotton cultivation. The spatio-temporal distribution of rainfall, which is an essential element for the growth and productivity of the crops, is often very poor.

In semi-arid regions, mulching has proven to be effective in reducing the risk of crop failure at field level through better rainfall capture and utilization (Scopel et al., 2004; Bationo et al., 2007). In the SCV practiced in Mali, the amount of biomass available in the plots varies between 4 and 9 tons/ha. This large biomass enriches the stock of soil organic matter (OM) and contributes to soil moisture retention (Turnel et al., 2015). The presence of cover crop residues before crop sowing stimulates soil biological activity by creating habitats for beneficials. Finally, cover crop residues protects soil against climatic hazards by limiting soil erosion, runoff and soil compaction (Wey et al., 2006 and Naudin et al., 2010). Thus, SCV is considered as an alternative to conventional agriculture using tillage (Gowing and Palmer, 2008). However, in a series of experiments conducted in sub-humid, semi-arid and dry sub-humid areas of Eastern and Southern Africa, Rockstrom et al. (2008) demonstrated that minimum tillage practices can increase water productivity and crop yields, even when there is little or no mulching of plant residues.

The major causes of soil degradation include high rate of population growth, which increases pressure on arable and marginal land, as well as annual expansion of cultivated areas and reduction of pasture and wasteland (Bationo et al., 1998). In the cotton-growing areas of Mali, area under cotton increased from 28360 ha in 1960 to 698158 ha in 2018, an annual increase of 40,72% (CMDT, 2018).

For soil fertility restoration, cropping systems in cotton-growing areas were characterized in the recent past by a cultivation period of three to five years, alternating with a fallow period that generally exceeded ten years, to allow natural soil fertility restoration (César etCoulibaly, 1991; Hien etSedogo, 1993; Segda et al., 1996). Nowadays, the system of long-term fallow is no longer possible with demographic pressure.

To meet the needs of a growing population, sustainable soil and water fertility management alternatives have been developed and introduced in the real world, including SCV. This technology

makes it possible to reduce the effect of wind and water erosion and to improve cotton and sorghum yields by 10 to 20% in areas with rainfall of 900 to 1000 mm.
The objective of this study is to evaluate the effect of direct seeding under cover crop residues on cotton yield.

Materials and Methods

The experiment was conducted at the Regional Centre for Agricultural Research (CRRA) of Sotuba located in commune I in the district of Bamako with a latitude of 12°66' 38" North and a longitude of 7°92' 22" West at an altitude of 320 m. The climate type is Sudano-Sahelian with two seasons (a rainy season from June to October and a dry season from November to May). The average rainfall over the last ten (10) years was 920 mm in seventy (70) rainy days.

Two cotton varieties with high yield potential were used for this experiment (BRS 293 and NTA MS334). BRS 293 is a variety originating from Brazil, it was introduced in Mali in 2009 and NTA MS334 is a cotton program of Institute of Rural Economy (IER) creation. The mineral fertilization was done with two types of fertilizers: cotton complex (14N-18P_2O_5-18K_2O+6S+1B_2O_3) and urea (46% N). Phytosanitary protection was done with two types of insecticides Emamectin Benzoate 19.2 g/l (Concentrated Emulsion EC)for the first two insecticide treatments and Cypermethrin 72 g/l + Acetamiprid 32 g/l. (EC)for the last four treatments.
The experimental design used was a Split plot with cultivation systems as main factor with 6 levels: T1 = maize residues; T2 = maize residues + *Brachiariaruziziensis*; T3 = maize residues + *Brachiariaruziziensis* + *Stylosantheshamata*; T4 = maize residues + *Brachiariaruziziensis* + *Cajanuscajan*; T5 = maize residues + *Brachiariaruziziensis* + *Mucunacochinchinensis;* T6 = conventional system, export of residues after harvest. Two varieties were used as a secondary factor: V1: NTA MS334 and V2: BRS 293. Each growing system was repeated 4 times. Cotton was sown in small holes of 3 cm at distances of 0.80 m between sowing lines and 0.30 m between bunches on the sowing line. De-marriage was carried out at 2 plants per pots. Three weeding's and one ridging were carried out during the vegetative development cycle of the cotton trees. Small stripes were drawn along the sowing line. The cotton complex was poured into continuous lines and then carefully closed. This operation was carried out fourteen (14) days after emergence (JAL) of the cotton plants. The cotton complex was applied at the rate of 200 kg.ha^{-1} in all the elementary plots. In the plots of cropping systems with cover crop residues, an additional 50 kg.ha^{-1} of urea was added at the time of application of cotton complex. At the 45th JAL, an additional 50 kg of urea ha^{-1} was added to all the elementary plots. The urea brought was also buried.
Soil pits were excavated for the description of the cultural profile. Soil samples were taken at depths of 0-20 cm and 20-40 cm for chemical and physical analyses. The date when 50% of theoretical pots were lifted was noted. This date was determined per elementary plot by passing every day after the appearance of the first seedling. Counting was carried out on 20 successive plots on the 4 central lines. Stand density estimates were made on the 4 central rows of each elementary plot after demarriage and at harvest. The height of twenty (20) plants was estimated in each elementary plot. The number of capsules was counted on 5 plants in each elementary plot. Yield was estimated by taking seed cotton harvested weight on the four (04) central rows of each elementary plot and extrapolating to hectare. The analysis of variance (ANOVA) was done using StatBox 6.5 software and Newman and Keuls test was used to determine the difference between the cropping systems at the 5% threshold.

Results

Soil analysis

Examination of soil profile revealed a succession of layers explaining the formation and evolution of soil, which was classified as a tropical ferruginous soil with a deep hydromorphy. The granulometric analysis carried out showed that on the surface (0 - 20 cm) the soil is sandy-silt with 14.6 g/100g of clay, 37.4 g/100g of silt and 47.9 g/100g of sand. At depth (20 - 40 cm) it is silty with 19.4 g/100g clay, 39.3 g/100g silt and 40.6 g/100g sand. The average water pH in the 0-20 cm layer was 6.1 while in the 20-40 cm layer it was 6.4 (Table 3). These pH values are slightly acidic (6 < pH < 6.6). Total nitrogen content was low both at the surface (0.03 g/100g) and at depth (0.02 g/100g). The cation exchange capacity was low 5.1 Cmol+ /kg in the 0-20 cm horizon and 5.7 Cmol+ /kg in the 20-40 cm horizon.
Table1: Chemical composition and pH of soil samples taken in Sotuba

Hori (cm)	pH eau	N %	P total ppm	P ass ppm	Ca méq/100 g	Mg méq/100 g	K méq/100 g	CEC méq/100 g

0 – 20	6,1	0,03	121,2	4,3	2,0	0,9	0,20	5,1
20-40	6,4	0,02	113,5	1,2	2,5	1,1	0,10	5,7

Legend: Hori: Horizon, N: Nitrogen; P ass: Assimilable phosphorus; Ca: Calcium; Mg: Magnesium; K: Potassium; CEC: Cation Exchange Capacity

Rainfall and sowing date

The cumulative average rainfall over the last 10 years was 920 mm in 70 days, while the experimental period (2014-2018) saw 838.6 mm in 71 rainy days (slightly less than the 10-year cumulative rainfall). It was very often higher than the water requirements of the cotton plant, which consumes about 600 mm of water during its development cycle (Albergel et al., 1985). Cumulation does not seem to be a good indicator of water availability, as its spatio-temporal distribution is very poor (Table 2). The analysis of rainfall in June shows a very high variability between decades of the same year with often rainless decades.

Seeding was very often carried out in the first or second decade of June depending on the rainfall pattern (Table 2). The optimum planting date was in the second decade of June in Sotuba. If the rainfall recorded during this period is low, sowing is done in the third decade of June.

Table2 : Date of sowing and quantities of rainfall recorded in mm during the month of June during the crop establishment period, annual total and number of rainy days

Year	Sowing date	Month of June			Annual cumulation	Number of days
		1e decade	2e decade	3e decade		
2014	19 June	117,1	35	60	881,6	69
2015	13 June	12.1	58.3	0	782,7	75
2016	23 June	49	15	103,5	833,5	76
2017	08 June	74,8	10,2	47,7	857,6	59
2018	01 June	19,1	31,1	42,2	837,8	78

Number of days between sowing and emergence

Sowing in insufficient moisture conditions can influence cotton seeds emergence speed. Variance analysis showed a significant difference (p=0.000) between cropping systems for the number of days between sowing and emergence. With the exception of the 2015-2016 season, the T1 and T6 cropping systems were statistically equivalent (Table 3). At the beginning of the wintering period the quantities of biomass present in these two plots were very low (less than 2 tons per hectare), the average number of days between sowing and emergence was 7 days. The other cropping systems (T2, T3, T4 and T5) were statistically equivalent, emergence was faster with an average of 4 days between sowing and emergence. The amount of biomass was higher between 4 and 9 tons per hectare.

During the 2014-2015 season, variance analysis showed a significant difference (p=0.008) between the two varieties for emergence speed. It was faster with BRS 293 variety (4.8 days) compared to 5.4 days for NTA MS334 variety. Interactions (Cropping Systems x Varieties) were not significant in all years.

Table3 : Nombre de jours entre le semis et la levée dans les parcelles des différents systèmes de culture et au cours des différentes campagnes agricoles à Sotuba

Cropping systems	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
T1	6,9 a	8,4 a	7,0 a	6,3 a	7,0 a
T2	4,3 b	5,3 c	4,0 b	4,0 b	4,0 b
T3	3,8 b	4,0 d	4,3 b	4,0 b	4,5 b
T4	4,6 b	4,0 d	4,0 b	4,0 b	4,3 b
T5	4,1 b	4,5 cd	4,3 b	4,3 b	4,8 b
T6	7,3 a	7,1 b	7,0 a	6,8 a	7,5 a
Probability	0,000	0,000	0,000	0,000	0,000
CV (%)	12,96	15,94	7,91	10,66	11,39
Varieties					
NTA MS334	5,4 b	5,8	5,1	4,9	5,5
BRS 293	4,8 a	5,3	5,0	4,8	5,2
Probability	0,008	0,056	0,484	0,589	0,160

Interaction					
Probability	0,402	0,456	0,764	0,904	0,464

Legend: T1 = maizeresidues (conservation of residues on the plot afterharvest); T2 = maizeresidues + Brachiariaruziziensis; T3 = maizeresidues + Brachiariaruziziensis + Stylosantheshamata; T4 = maizeresidues + Brachiariaruziziensis + Cajanuscajan; T5 = maizeresidues + Brachiariaruziziensis + Mucunacochinchinensis; T6 = conventional system, post-harvest export of residues.

Number of plants per hectare at harvest

The variance analysis showed a significant difference (p = 0.007) between treatments for the number of plants per hectare during the 2015-2016 season (Table 4). For the other seasons, no significant difference was observed between treatments.

During the campaigns 2014-2015, 2015-2016 and 2016-2017 significant differences were observed between varieties for the number of plants per hectare. In the last two campaigns, no significant differences were observed between them. The interaction was not significant in the different campaigns.

Table 4: Number of seedlings per hectare at harvest in the experimental plots in Sotuba over the five years

Cropping systems	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
T1	57 552	65 104 c	66 007	64 453	67159
T2	58 275	71 667 ab	61 709	63 580	75029
T3	62 645	73 125 a	63 449	61 903	74624
T4	63 976	67 760 bc	63 370	61 420	73119
T5	62 413	70 417 ab	65 849	62 477	72512
T6	55 527	67 187 bc	65 981	59 145	61603
Probability	0,163	0,007	0,731	0,396	0,005
CV (%)	12,30	4,60	10,62	7,98	9,09
Varieties					
NTA MS334	63 272 a	63 698 b	60 531 b	62 339	70 091
BRS 293	56 858 b	74 722 a	68 258 a	61 987	71 258
Probability	0,005	0,001	0,001	0,593	0,379
Interaction					
Probability	0,992	0,802	0,987	0,783	0,977

Légende : T1= résidus de maïs (conservation des résidus sur la parcelle après la récolte) ; T2 = résidus de maïs + Brachiariaruziziensis ; T3 = résidus de maïs + Brachiariaruziziensis + Stylosantheshamata ; T4 = résidus de maïs + Brachiariaruziziensis + Cajanuscajan ; T5 = résidus de maïs + Brachiariaruziziensis + Mucunacochinchinensis ; T6 = système conventionnel, exportation des résidus après la récolte.

Height of cotton plants

Only in 2016-2017 season, variance analysis showed a significant difference between treatments for cotton plant height (Table 5). No significant difference was observed during the other campaigns.

A highly significant difference (p = 0.000) was observed between cotton plants height of the two varieties. The NTA MS334 variety was very often taller than the BRS 293 variety. The BRS 293 variety is small in size, because in its area of origin (Brazil), harvesting is totally mechanized. Unlike the variety NTA MS334 which is harvested manually. Harvesting becomes easier if the cotton plants are of a certain size.

No significant interaction has been observed during the 5 campaigns.

Table 5: Average height of cotton plants in meters in the plots of the different cropping systems during the five years of experimentation.

Cropping systems	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
T1	112	139	111 b	136	129
T2	131	134	124 a	140	133
T3	124	137	106 b	141	130

T4	125	140	105 b	138	128
T5	117	132	104 b	128	126
T6	125	137	106 b	137	125
Probability	0,064	0,582	0,009	0,107	0,324
CV (%)	9,80	6,80	10,06	6,07	5,75
Varieties					
NTA MS334	129 a	138	116 a	144 a	132 b
BRS 293	116 b	135	103 b	130 b	125 a
Probability	0,001	0,216	0,000	0,000	0,005
Interaction					
Probability	0,824	0,939	0,183	0,936	0,865

Légende : T1= résidus de maïs (conservation des résidus sur la parcelle après la récolte) ; T2 = résidus de maïs + Brachiariaruziziensis ; T3 = résidus de maïs + Brachiariaruziziensis + Stylosantheshamata ; T4 = résidus de maïs + Brachiariaruziziensis + Cajanuscajan ; T5 = résidus de maïs + Brachiariaruziziensis + Mucunacochinchinensis ; T6 = système conventionnel, exportation des résidus après la récolte.

Number of bolls per plant

The variance analysis did not show a significant difference between growing systems for the number of bolls per plant (Table 6). However, significant differences were observed between varieties for the number of bolls per plant. The best numbers of bolls were observed with BRS 293variety. The interaction did not show a significant difference.

Table 6: Number of bolls per plant during the different campaigns.

Cropping systems	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
T1	18,2	10,8	16,2	13,2	12,8
T2	18,1	12,0	15,8	13,1	13,6
T3	18,2	11,4	15,9	11,2	13,7
T4	19,2	13,6	15,5	12,5	13,0
T5	19,3	12,9	15,5	13,1	13,5
T6	18,7	11,8	15,4	13,4	13,2
Probability	0,944	0,127	0,931	0,952	0,499
CV (%)	8,60	1,40	11,12	10,52	8,61
Varieties					
NTA MS334	15,7 b	11,0 b	14,1 b	11,9 b	12,7 b
BRS 293	22,6 a	13,1 a	17,3 a	13,1 4	13,9 a
Probability	0,001	0,002	0,000	0,000	0,007
Interaction					
Probability	0,840	0,084	0,809	0,812	0,554

Yield obtained

Variance analysis showed no significant difference between cropping systems for yield during the 5 years of experimentation (Table 7). The lowest yields were obtained in 2018-2019. This is mainly due to the pace of the season (rains came late, excess water was observed during August and September).

A highly significant difference was observed between the two varieties in terms of yield. The difference between the two varieties varied between 251 and 661 kg ha^{-1} depending on the year. BRS 293 was more productive than NTA MS334. The interaction (Cropping systems x Variety) was not significant.

Table 7: Yields obtained during the different campaigns.

Cropping systems	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019

T1	3 206	2 536	2 466	2 096	1 896
T2	3 571	2 479	2 563	2 043	1 707
T3	3 423	2 599	2 334	1 919	1 957
T4	3 471	2 630	2 281	2 148	1 766
T5	3 776	2 401	2 149	2 183	1 697
T6	3 299	2 469	2 242	2 148	1 706
Probability	0,112	0,677	0,690	0,359	0,526
CV (%)	13,1	12,2	23,4	12,02	18,98
Varieties					
NTA MS334	3 127 b	2 361 b	2 118 b	1 775 b	1 662 b
BRS 293	3 788 a	2 677 a	2 560 a	2 404 a	1 913 a
Probability	0,001	0,001	0,008	0,000	0,000
Interaction					
Probability	0,645	0,497	0,990	0,818	0,137

Discussion

No-till is a conservation agriculture (CA) practice based on integrated soil, water and biological resource management combined with external inputs FAO (2008b). It is based on three principles that are expected to improve biological processes above and below ground. These are: (1) minimal or no mechanical soil disturbance; (2) permanent organic soil cover (consisting of a growing crop or a dead mulch of crop residues); and (3) crop rotations.

In practice, not all farmers adopt CA principles for a variety of reasons, such as limited access to inputs (herbicides, cover crop seeds), ploughing constraints or resources to grow cash crops (Baudron et al., 2007). Under Malian conditions, it is mainly the management of crop residues that poses a problem with the raving of animals during the dry season. Naudin et al. (2015) have also shown that in Madagascar small producers have difficulties in maintaining soil cover because of its use for animal feed.

Soil samples are taken before the first rains. Thus, the values obtained after analysis are assumed to be the lowest values that can be observed during the year (Veldkamp et al., 1991; Sissoko, 2009). The pH values obtained are higher than 6 and do not have a negative effect on soil microbial life and the chemical forms of nutrients (Bertrand and Gigou, 2000). pH is certainly not an absolute value, but rather an indicator of soil condition.

Some authors have found that there is no clear relationship between the number of pots at harvest and the yield obtained in different cropping systems. Between 30000 and 40000 poquetsha^{-1}, yields vary greatly between 1-2.3 tons ha^{-1} depending on the cropping systems. Bednarz, et al. (2000) showed that yield is not influenced by densities ranging from 2.5 to 23 poquets/m². Rapidel, et al. (2006) obtained identical yields with densities ranging from 34000 to 48300 plants ha^{-1}. This is explained by the adjustment of the percentage of bollards in the different positions which cancels out the effect of the high density. Sekloka et al. (2016) found that at low densities, cotton plants were more floriferous and retained their bolls better, with average numbers of fruiting sites per plant and retention rates higher at 42000 plants ha^{-1} than at 125000 and 167000 plants ha^{-1}. Conversely, Smith et al. (1979) showed that seed cotton production is significantly different at high densities (170000 pots ha^{-1}) compared with low densities (34 000 pots ha^{-1}).
No significant differences were observed between cropping systems for yield. The same finding was made by Swanepoel et al, (2017) who showed that short-term studies in conservation agriculture do not show significant differences between improvements in soil organic matter content and yield. Conservation agriculture is presented as a panacea to the problems of low agricultural productivity and soil degradation in sub-Saharan Africa (Giller et al., 2009). However, there are prerequisites for the success of this innovation. The respect of the technical itinerary has made it possible to have yields that are twice as high as the average yield of the cotton production area in Mali, which is 984 kg ha^{-1}.

Conclusion

The positive effects of direct sowing under plant cover on the different agronomic parameters of the two cotton varieties used in this experiment clearly emerge from this work. Direct sowing under plant cover enabled rapid establishment of the cotton plants with low rainfall. The number of seedlings raised was improved in direct seeding systems (SCV) compared to the conventional system. The direct seeding under cover crop did not affect the growth of the cotton plants, which was overall normal. No significant difference was observed between yields obtained in the cropping systems. This indicates that the direct seeding cropping systems produce cotton well and save ploughing time. Therefore, direct seeding under cover crop could be an alternative for cotton cultivation, especially in the semi-arid zone where rainfall is increasingly erratic with great spatial and temporal variability.

The results obtained show that direct sowing under plant cover may well be an alternative for cotton growing, especially in the semi-arid zone where rainfall is increasingly erratic with great spatial and temporal variability.

References

1. Albergel, J., Carbonnel, J.P. et Vaugelade, J. Aléas climatiques et production agricole: le coton au Burkina. ActaOecolgicalaApplic, 1985; 3(3): 199-211.
2. Bationo A, Kihara J, Vanlauwe B, Waswa B, Kimetu J. Soil organic carbon dynamics, functions and management in West African agro-ecosystems. Agric. Syst., 2007; 94: 13–25. DOI: 10.1016 / j.agsy.2005.08.011
3. Bationo, A., F. Lompo and S. Koala. Research on nutrient flows and balances in West Africa: state-of-art. Agric. Ecosyst. andEvironm. 71: 19-35. Breman H and de Ridder, 1991. Manuel sur les pâturages des pays sahéliens. Eds Karthala, ACCT, CABO-DLO and CTA Paris ; Wageningen, 485 pp; CILSS, 2002, Etat de l'environnement au Sahel, Publications CILSS, Ouagadougou (BF), 1998; 56 p.
4. Baudron, F., Mwanza, H.M., Triomphe, B., Bwalya, M. Conservation Agricul-ture in Zambia: A Case Study of Southern Province. African Conservation Tillage Network, Centre de Coope´ ration Internationale de RechercheAgronomiquepour le De´ velopment, Food and Agricultural Organization of the United Nations, Nairobi, Kenya. 2007.
5. Bazile, D., Soumaré, M. et Dembelé, J. Conserver l'agro-biodiversité pour la stabilité de la production agricole – FAO. 2000.
6. Bednarz CW, Bridges DC, Brown SM. Analyse de la stabilité du rendement du coton à travers les densités de population. Agron. J. 2000; 92 : 128-135. DOI : 10.2134/agronj2000.921128x.
7. Bertrand, R., et Gigou, J. La fertilité des sols tropicaux. Paris, Maisonneuve et Larose, 2000; 397 pages.
8. Camara, M. Atouts et limites de la filière coton au Mali. Université de Toulon, 2015; 321 p., France.
9. Cesar J, Coulibaly Z. Le rôle de la jachère et des cultures fourragères dans le maintien de la fertilité des terres. Dans : Savanes d'Afrique, terres fertiles ?, Rencontres internationales CIRAD/Coopération Française, Montpellier, 1991; pp 271-290.
10. CMDT. Rapport bilan de la campagne 2017/2018 de la Compagnie Malienne pour le Développement des Textiles. 2018.
11. Deveze JC, Halley des Fontaines D. Débats, Echanges, Réflexions en Réseaux Développement Rural au Sud. Document AFD, Paris. 2005; DOI: https://doi.org/10.1111/j.1475-2743.2007.00137.x
12. FAO. Situation météorologique et état des cultures. Rapport Sahel n°2. 2001.
13. FAO. Conservation Agriculture http://www.fao.org/ag/ca/index.html. 23-10-2019. 2008b.
14. Giller KE, Witter E, Corbeels M, Tittonelle P. Conservation agriculture and smallholder farming in Africa: The heretics' view. Field Crops Research, 2009; 114 (1): 23-34. DOI: https://doi.org/10.1016/j.fcr.2009.06.017
15. Gowing JW, Palmer M. Sustainable agricultural development in sub- Saharan Africa: the case for a paradigm shift in land husbandry. Soil Use Manage. 2008; 24 (1), 92–99.
16. Hien V., Sedogo M. P. Étude des effets des jachères de courte durée sur la production et l'évolution du sol dans différents systèmes de culture du Burkina Faso, Dans C. Floret et G., Serpentié (Ed). La jachère en Afrique de l'Ouest, 1993; 221-232, Collection Colloques et Séminaires. ORSTOM, Paris.
17. Naudin K., Bruelle G., Salgado, P., Penot, E., Scopel, E., Lubbers, M., De Ridder, N., Giller K. E. Trade-offs around the use of biomass for livestoch feed ans soil cover in dairy farms in the Alaotra lake region of Madagascar. Agricultural Systems. 2015; Volume 134 Pages 36-47 https://doi.org/10.1016/j.agsy.2014.03.003
18. Naudin K., Gozé E., Balarabé O., Giller K. E., Scopel E. Impact of no-tillage and mulching practices on cotton production in North Cameroon: a multi-locational on-farm assessment. Soil and tillage research, 2010; 108(1-2): 68-76. DOI: 10.1016 / j.still.2010.03.002
19. Rapidel, B., Defeche, C., Traore, B., Lançon, J. and Wery, J. In-field development of a conceptual crop functioning and management model: A case study on cotton in southern Mali. European Journal of Agronomy, 2006; 24: 304-315. DOI: https://doi.org/10.1016/j.eja.2005.10.012
20. Rockstrom J, Kaumbutho P, Mwalley J, Nzabi AW, Temesgen M, Mawenya L, Barron J, Mutua J, Damgaard-Larsen S. Conservation farming strategies in East and Southern Africa: yields and rain water productivity from on-farm action research. Soil Till. Res. 2008; 103 : 23–32. DOI : https://doi.org/10.1016/j.still.2008.09.013
21. Scopel E, Macena F, Corbeels M, Affholder F, Maraux F. Modeling crop residue mulching effects on water use and production of maize under semi-arid and humid tropical conditions. Agronomy, 2004; 24: 383-395. DOI: 10.1051/agro: 2004029

22. Segda, Z., Hien V., F. Lompo, J., Bayala, etBecker M. Gestion améliorée de la jachère par utilisation de légumineuses de couverture. Dans C. Floret (Ed), La jachère, lieu de production, 1996; 133-139, CNRST/ORSTOM/CORAF/UE.
23. Sekloka E, Lançon J, Zinsou VA, Thomas G. Influence des conditions de culture sur la production de capsules chez le cotonnier (Gosssypium hirsutum L.) en conditions de culture pluviale au Bénin. Biotechnol. Agron. Soc. Environ. 2016; 20 (2): 161-170. DOI: 10.25518/1780-4507.12904
24. Sissoko, F. Analyse des flux d'eau dans les systèmes de culture sous couverture végétale en zone soudano sahélienne : cas du coton semé après une culture de sorgho/brachiaria au sud du Mali. Thèse de doctorat, Université SupAgro de Montpellier, France, 2009; p. 169.
25. Smith CW, Waddle BA, Ramey HH Jr. Plant Spacings with irrigated Cotton. Agronomy journal, 1979; 71 : 858-860. DOI : https://doi.org/10.2134/agronj1979.
26. Swanepoel, C.M., Swanepoel, L.H., Smith H.J. A review of conservation agriculture research in South Africa, South African Journal of Plant and Soil, 2017; DOI: 10.1080/0571862.2017.1390615
27. Turnel, M.S., Speratti, A., Baudrin, F., Verhulst, N., Govaerts, B. Crop residue management and soil health: S systems analysis. Agricultural systems. 2015; Volume 134 Pages 6-16 https://doi.org/10.1016/j-agsy2014.05.009.
28. Veldkamp, W.J., Traoré, A., N'Diaye, M.K., Keïta, M.K., Keïta, B. and Bagayoko, M. Fertilité des sols du Mali. Mali-Sud / Office du Niger. Interprétation des données analytiques des sols et des plantes, Institut d'Economie Rurale, Laboratoire Sol-Eau-Plante, Bamako, Mali. 1991.
29. Wey J., Dongmo A., Abakar M., Ibrahima S. – Simulation des effets du passage aux « Systèmes de culture sous couverture végétale » sur les transferts de biomasse à l'échelle des terroirs agropastoraux de Kilwo (Extrême-Nord) et Laindé Massa (Nord) au Cameroun. Nord-Cameroun : Garoua Projet ESA et Irad, Montpellier, 2006; 69 p.

Ginning traits, fiber and seed quality of cotton as influenced by foliar application of nitrogen, potassium and boron

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Abstract

Fertilizer management during reproductive stage of cotton is gaining attention because at this stage root activities decreases. To mitigate the problem, optimum foliar fertilizer combination of nitrogen (N), potassium (K) and boron (B) for foliar feeding, an experiment was conducted at Central Cotton Research Farm, Sreepur, Gazipur during 2009-2011with cotton variety cv. CB-10. Eight combinations of N, P and B viz.T0 (0.00 –0.00 – 0.00), T1 (7.50 – 40 – 0.75), T2 (7.50 – 40 – 1.00), T3 (7.50 – 50 - 0.75), T4 (7.50 – 50 – 1.00), T5 (9.00 – 40 – 0.75), T6 (9.00 – 40 – 1.00), T7 (9.00 – 50 – 0.75), T8 (9.00 – 50 – 1.00) g L^{-1} water were foliar sprayed at reproductive stage of cotton as treatment. The design of the experiment was randomized completely block design (RCBD) with three replications. The result show that, plant height (92.17 cm), Boll plant^{-1}(23.57), boll weight (5.27 g), seed cotton yield (1.39 t ha^{-1}), lint yield (0.49 t ha^{-1}), Seed yield (0.90 t ha^{-1}), 50% span length (12.19 mm), Oil (20.51%) and protein (20.11%), seed index (10.20), germination(88.09%), shoot and root length(11.78 cm, 10.91 cm) were maximum from T4 (7.50 – 50 – 1.00) foliar nitrogen, potassium and boron treatment combination. In case of all parameter without foliar fertilizer results was lowest except electric conductivity that is (149.57 µscm -1g-1). In future need to conduct research for pathways of nutrient absorption in cotton plant.

Key words: *cotton, foliar fertilizer, nitrogen, potassium, boron, seed cotton yield, fiberquality, seed quality, seed vigour.*

Introduction

Crop management including plant nutrition is the most important factor in cotton production. Therefore, research in this area is driven by the need to intensify production to obtain higher yields. Maintaining a balance of the vegetative and reproductive growth is the essence of managing cotton crop. It is well known from numerous fertilizer experiments that the yield of cotton has been strongly dependent on the supply of mineral nutrients to exploit the full genetic potential of the crop (Pettigrew *et al.,* 2000; Ullagaddi, 2001).Fertilizers may be applied either to the soil at or before planting or as a foliar application at or just prior to bloom. However, uptake of soil applied nutrients may be limited due to . reduced root activity and nutrient uptake that was not sufficient enough to realize optimum yield of cotton (Gormus, 2002; Snyder, 1991). Under such circumstances, foliar application of nutrients may be more efficient than soil application without involving the roots during this critical growth period of cotton (Sawan*et al*., 1988; Mohsen *et al*.2013).Karim *et. al.* (2016) reported that foliar application of NPK increased cotton growth, yield and quality traits as compared to control (basal dose only). Dordas (2006) reported that foliar B application can improve the lint and seed yield and seed quality of cotton. Howard et el. (1998) reported that combined application of foliar K and B increased seed cotton yield. In Bangladesh the combined effect of foliar applied NKB on cotton was not determined. The objective of this study was to determine appropriate concentration of NPB foliar fertilization for better growth, yield and fiber quality of cotton.

Materials and methods

The experiment was conducted at Central Cotton Research Farm, Sreepur, Gazipur (24.181911° N, 90.429238° E)during cotton growing season of 2009-2010. The status of the initial soil was given in Table-1.

Table 1. Soil properties of experiment site

Soil texture	Soil pH	Organic C (%)	N (%)	P (µg g^{-1})	K (meq 100 g^{-1})
Silty clay	6.7	0.45	0.06	38.6	0.16

Cotton variety Cv. CB-10 was used for experimental purpose. The cotton seed were planted on 13 July, 2011. A hand garden sprayer was used to spray foliar fertilizer solution. Foliar fertilizers were applied on leaves at evening. In this experiment eight treatment combinations was used for foliar spray in cotton (Table 2). Each combination contains N, P and B.

Table 2. Treatment combinations containing two best concentrations for foliar nitrogen, potassium and boron spray.

Treatments	Treatment combination (g L^{-1} water)		
	N	K	B
T0	0.0	0.0	0.00
T1	7.5	40	0.75
T2	7.5	40	1.00
T3	7.5	50	0.75
T4	7.5	50	1.00
T5	9.0	40	0.75
T6	9.0	40	1.00
T7	9.0	50	0.75
T8	9.0	50	1.00

The design of the experiment was arranged in RCBD design with three replications. The net plot size was 7m long and 3m wide. The distance between plots was 1.00 m. Monopodial branch was counted from five randomly tagged plants before the cotton picking. Similarly, sympodial branches arising on the main stem above the monopodial branches were counted. . The number of bolls per plant were counted from the eighteen randomly selected plants. About 10 bolls were selected at random from each treatment as per replication. The seed cotton was separated from each boll and weighed separately. The average boll weight (g) was computed. The seed cotton yield (t ha^{-1}) was calculated by using the seed cotton yield obtained from each plot area including the seed cotton of the five separately harvested plants. The seed cotton obtained from each plot was mixed thoroughly and 300 g of sample was drawn from all the treatments. This seed cotton wasginned separately with a hand ginning. Ginning percentage was calculated by using the following formula given by Santhanam (1976).

$$\text{Ginning percentage} = \frac{Weight\ of\ lint\ (g)}{Weight\ of\ seed\ cotton\ (g)} \times 100$$

Fiber lengths on individual seeds can be determined while the fibers are still attached to the seed, by hand stapling or by photoelectric measurement after ginning(Munro, 1987; Behery, 1993).

Lint of each treatment was collected after ginning and measured the length of fibre by using the fibrograph and average was made. In Fibrograph testing, fibers are randomly caught on combs and the beard formed by the captured fibers is scanned photoelectrically from base to tip. The amount of light passing through the beard is a measure of the number of fibers that extend various distances from the combs. Data were recorded as span length (the distance spanned by a specific percentage of fibers in the test beard). Span lengths are usually reported as 2.5 and 50%. The 2.5% span length is the basis for machine settings at various stages during fiber processing. The uniformity ratio was calculated by the ratio between the two span lengths expressed as a percentage of the longer length. Microniare value also known asfineness of cotton is associated with fibre diameter and fiber wall thickness. The microniare value represents the fibre diameter. Microniare value was determined by Microniare testing instrument. There are two instruments used to measure fibre strength; the Pressly and the Stelometer. In both these instruments, strength is measured by spading a bundle of parallel

fibre across two clamps. Forced is applied to clamps and gradually increased until the bundle breaks. Fibre strength was measured by the Presslyfibre strength tester. The oil percentage of seed was estimated from moisture free seed meal by solvent extraction using other petroleum either (boiling point 60°C to 80°C)in a Soxhlet apparatus for eight hours. The meal was pre-dried at 60°C for 24 hours. Two grams of meal was used for the estimation oil. No further oil was recovered from the residue after eight hours of refluxing. Nitrogen percentage of cotton seed was estimated by Kjeldahl method. The estimated result was multiplied by the factor 6.25 to obtained protein percent.

Statistical analysis

Analysis of variance and Duncan's Multiple Range Test (DMRT) were done by MSTAT-C (1988). Coefficient of variation was done by Microsoft Excel.

Results and discussion

Growth attributes

Cotton plants sprayed with NKB showed significant increase in plant height but it was not significant in producing branch per plant (Table 3).The shortest plants were observed in control treatment and the tallest plants, 15% increase over control treatment, were obtained by applying 7.50 – 50 – 1.00) g NKB L^{-1} water concentration. This agrees with the work of Odeleye *et al.* (2007) and Sawan *et al* (2001) that foliar application of NKB resulted in increase of cotton growth as these elements involve in hormone synthesis, translocation, carbohydrate metabolism and DNA synthesis which probably contributed to additional growth compared to control. However, effect of foliar NKB was not profound on branch per plant but it tended to increase although it was not consistent.

Seed cotton yield and yield contributing characters

The effect of foliar application of NKB on seed cotton yield and yield contributing characters were present in Table 4.

The effect of NKB rates on cotton yield parameters showed significant improvement and cotton yield increased with the increase of number of bolls per plants and boll weight. The highest number of bolls (23.57) and highest boll weight (5.27 g) were recorded from foliar application of 7.5–50–1.00 g NKB L^{-1} water.

Table 3. Effect of foliar application of N K B on growth attributes of cotton.

Foliar nutrient concentration	Plant height	Branch plant^{-1}	
(g L^{-1} water)	(cm)	Monopodial	Sympodial
T0	80.1 c	1.88	12.0
T1	84.2 bc	1.79	12.0
T2	85.2 bc	1.71	12.1
T3	89.8 ab	1.97	12.2
T4	92.2 a	2.01	12.5
T5	87.6 ab	1.87	12.2
T6	86.2 abc	2.18	12.3
T7	85.9 abc	2.04	12.7
T8	91.8 a	2.11	12.5
CV (%)	5.32	14.05	9.4

Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Similarly, Oosterhuis and Steger (1998) reported that the number of bolls and boll weight tended to be greatest in high NKB foliar application. Such increase in boll number and boll weight under foliar NKB application indicated that cotton plants were deficient for those elements at reproductive stage. This result further indicated that applied foliar B improved the utilization of N and K by cotton plants by increasing the translocation of different chemical compounds into the boll. Thus yield increase under foliar NKB was the consequence of enhanced boll setting and boll weight (Boquet*et al*., 1994: Sawan*et al* 2011).

Ginning characteristics

Ginning out turn, lint yield and seed yield of cotton increased significantly with the increase foliar NKB application at reproductive stage of cotton. Results of ginning characteristics revealed that ginning out turn did not influenced markedly by foliar NKB but lint and seed yield increased significantly by foliar

NKB application (Table 5). The highest lint (0.49 t ha^{-1}) and seed (0.90 t ha^{-1}) were recorded from treatment combination 7.50 – 50.00 – 1.00 g NKB L^{-1} water spraying to cotton plants. The concentration of foliar NKB beyond this treatment failed to increase lint and seed yields which suggested that foliar application of NKB at the rate of 7.50 – 50.00 – 1.00 g L^{-1} water is optimum for obtaining beneficial effect of foliar fertilization to cotton.

Table 4. Effect of foliar application of NKB on yield attributes and yield of cotton.

Foliar N-K-B Concentration (g L^{-1} water)	Boll $plant^{-1}$	Boll weight (g)	Seed cotton yield (t ha^{-1})
T0	15.8 d	4.79 c	0.92 c
T1	17.8 cd	4.42 cd	1.10 bc
T2	18.5 cd	4.17 cd	1.14 b
T3	19.3 bcd	3.82 d	1.23 ab
T4	23.6 a	5.27 a	1.39 a
T5	19.8 abc	4.99 b	1.26 ab
T6	20.2 abc	4.95 bc	1.28 ab
T7	19.0 bcd	4.82 bc	1.16 b
T8	22.7 ab	4.79 bc	1.32 ab
CV (%)	8.1	7.32	8.36

Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Table 5. Effect of foliar application of B ginning characteristics of cotton.

Foliar NKB Concentration (g L^{-1} water)	Ginning out turn (%)	Lint yield (t ha^{-1})	Seed yield (t ha^{-1})
T0	40.2 a	0.37 c	0.55 d
T1	38.2 ab	0.42 bc	0.68 cd
T2	37.7 ab	0.43 ab	0.71 bc
T3	35.8 b	0.44 ab	0.79 abc
T4	35.3 b	0.49 a	0.90 a
T6	35.9 ab	0.46 ab	0.82 abc
T5	36.5 ab	0.46 ab	0.80 abc
T7	37.1 ab	0.43 ab	0.73 bc
T8	40.2 a	0.48 ab	0.84 ab
CV (%)	6.25	7.88	6.18

Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Fibre quality

Fibre quality was improved by foliar NKB application over the control treatment (Table 6). Fibre length, uniformity ratio and fibre strength were greater at 7.5– 50 – 1.00 g NKB L^{-1} water foliar spray although micronaire value was not affected by foliar fertilization. Improvement in fibre quality was associated with mainly due to involvement in K of NKB mixture in the process of fibre development (Sawan*et al*., 2001). Other authors (Gormus, 2002; Pettigrew, 1999; Li *et al*., 1999) have reported similar effects of K on fibre properties of cotton. Oosterhuis (1994) found that fibre quality was improved by foliar NKB application with the increase occurring primarily in uniformity and strength which agreed well to our findings. Our results also consistent to the results of Gormus (2002) who reported lack of response of micronaire to different levels of NKB foliar application to cotton plants.

Seed quality

Seed quality in terms of protein and oil content of cotton seed differed significantly due to foliar NKB application. Both protein and oil content in cotton seed increased with the increase of NKB concentration and the highest protein (20.11%) and oil (20.51%) contend were found in seeds obtained from plants sprayed with 7.50 – 50.00 – 1.00 g $NKBL^{-1}$ water. Further increase in NKB concentration tended to decrease protein and oil content in cotton seed (Table 7). The increase in protein content at this treatment may be ascribed as better utilization of NKB in the process of protein synthesis. For example N is part of protein and K activates enzymes to metabolize to manufacture amino acids and proteins. While, B is required for reduction of nitrates for protein synthesis (Abady *et al*., 2008).

Seed vigour

Foliar application of NKB to cotton plants at reproductive phase had distinct impact on cotton seed vigpur. Improvement in seed quality due to foliar fertilization was ascertained by increase in seed index, germination, seedling growth and decrease in electrical conductivity values of seed leachates (Table 8). Seed quality was highest at 7.50 – 50.00 – 1.00 g NKB L^{-1} water foliar spraying plants. Seed vigour measured by electrical conductivity was also lowest (106.50 μScm^{-1} g^{-1}) in this treatment. Lowe and Ries (1972) reported that the lowest value of electrical conductivity associated with the highest seed vigour. High vigour seeds harvested from this treatment might be due to high accumulation of metabolites and nutrients in seeds harvested from well concentration of NKB foliar fertilization.

Table 6. Effect of foliar application of NKB on fibre properties of cotton.

Foliar N-K-B concentration (g L^{-1} water)	50% Span length (mm)	Uniformity ratio	Micronaire value	Pressly strength (PSI)
T0	11.2 d	41.01 e	4.04	80.16 d
T1	11.4 c	42.92 d	4.09	83.25 c
T2	11.7 c	43.27 cd	4.34	82.90 c
T3	11.7 c	44.61 bcd	4.39	83.25 c
T4	12.2 a	46.30 ab	4.65	84.76 ab
T5	11.9 ab	44.76 bcd	4.59	83.71 bc
T6	11.9 ab	45.10 bc	4.54	84.88 a
T7	11.7 c	43.93 cd	4.47	83.72 c
T8	11.7 c	47.50 a	4.66	84.76 ab
CV (%)	8.45	5.09	5.20	2.60

Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Table 7. Effect of foliar application of NKB on protein and oil content of cotton seed.

Foliar N-K-B concentration (g L^{-1} water)	Oil content (%)	Protein content (%)
T0	18.0 c	18.7 c
T1	19.3 b	19.2 bc
T2	19.6 ab	19.3 abc
T3	19.8 ab	19.3 abc
T4	20.5 a	20.1 a
T5	20.3 a	20.0 ab
T6	20.1 ab	19.6 abc
T7	19.7 ab	19.3 abc
T8	20.0 ab	19.4 abc
CV (%)	3.73	4.12

Means with common letter(s) within same column are not different significantly at 0.05 by DMRT.

Table 8. Effect of foliar NKB application on seed vigour attributes of cotton.

Foliar N-K-B concentration (g L-1 water)	Seed index (g)	Germination (%)	Shoot length (cm)	Root length (cm)	Electrical conductivity (µS cm -1g-1)
T0	9.14 b	68.99 c	9.120 c	10.33	149.57 a
T1	9.23 b	72.82 ab	10.36 b	10.40	125.86 b
T2	9.35 b	73.20 ab	10.74 b	10.54	122.45 bc
T3	9.92 a	72.82 ab	11.07 ab	10.61	119.36 bcd
T4	10.20 a	88.09 a	11.78 a	10.91	106.50 e
T5	10.02 a	76.94 ab	10.67 b	10.74	114.17 d
T6	10.11 a	74.94 ab	10.87 ab	10.26	112.58 de
T7	9.93 a	74.11 ab	10.57 b	10.13	117.77 cd
T8	10.24 a	68.99 c	11.78 a	10.91	105.65 e
CV (%)	2.05	3.25	6.22	7.93	5.26

Conclusion

Considering the above results, it may be summarized that morphological parameters, yield, fibre, grain and seed quality contributing parameters of cotton were positively correlated with foliar application of Nitrogen, Potassium and Boron.

References

1. Abady EMI, Seadh SE, Attia AN, Saidy AEAE. Impact of foliar fertilization and its time of application on yield and seed quality of soybean Processing. 2nd Field crops conference. FCAI. ARC. Egypt. 2008.
2. Behery, H.M. Short-fiber content and uniformity index in cotton. p. 40. International Cotton Advisory Committee review article on cotton production research No. 4, CAB Int., Wallingford, UK. 1993.
3. Boquet DJ, Moser EB, Breitenbeck GA. 1994. Boll weight and within plant distribution in field grown cotton given different levels of nitrogen. Agronomy Journal 86, 20-26. https://doi.org/10.2134/agronj1994.00021962008600010005.
4. Dordas, C. Foliar boron application affects lint and seed yield and improves seed quality of cotton grown on calcareous soils. Nutr Cycl Agroecosyst 2006; 76, 19–28.
5. Gormus O. Effect of rate and time of potassium application on cotton yield and quality in Turkey. Journal of Agronomy and Crop Science 2002; 188,382-388. https://doi.org/10.1046/j.1439-037X.2002.00583.
6. Gormus O. Effect of rate and time of potassium application on cotton yield and quality in Turkey. Journal of Agronomy and Crop Science 2002; 188,382-388. https://doi.org/10.1046/j.1439037X.2002.00583.
7. Howard, D. D., Gwathmey, C. O. and Carl E. Sams. Foliar Feeding of Cotton: Evaluating Potassium Sources, Potassium Solution Buffering, and Boron. Agron J., 1998; 90:740-746
8. Karim, F., J. Iqbal, M. Shabbir, S. Kaleem, M.Q. Waqar. Response of cotton to foliar and soil applied nutrients under Dera Ghazi Khan conditions. Journal of Environmental & Agricultural Sciences. 2016; 7:14-18.
9. Li FS, Xu YZ, Zhang C. Effects of nitrogen, phosphorus and potassium on the development of cotton bolls in summer. Archives of Agronomy and Soil Science 1999; 11, 24-30.https://doi.org/10.1080/03650340903222328
10. Lowe LB, Ries SK. Effects of environment onthe relation between seed protein and seedling vigorin wheat. Canadian Journal of Plant Science 1972; 52(2):157-164. https://doi.org/10.4141/cjps72-026.
11. Mohsen S, Majid R, Parisa YS. Influence of Different Application Rates of Boron on Biological Growth and Fiber Quality of Cotton. American-Eurasian Journal of Agricultural and Environmental Sciences 2013; 13(4), 548-552. https://doi.org/10.5829/idosi.aejaes. 2013.13.04.1981
12. MSTAT-C. MSTAT-C, a Microcomputer Program for the Design, Arrangement, and Analysis of Agronomic Research. Michigan State University East Lansing, East Lansing. 1988.
13. Munro, J.M. Cotton. 2nd ed. John Wiley & Sons, New York, NY. 1987.
14. Oosterhuis DM, Steger A. The influence of nitrogen and boron on the physiology and production of cotton. News & Views. In a regional newsletter published by the Potash & Phosphate Institute (PPI) and the Potash & Phosphate Ins. of Canada (Eds.Dr. C.S. Snyder) August 1998.
15. Odeleye FO, Odeleye OMO, Animashuan MO. Effects of Nutrient Foliar Spray on Soybean Growth and Yield (Glycine max (l.) merrill) in South West Nigeria. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2007; 35, 22-28.
16. Oosterhuis DM. Potassium nutrition of cotton in the USA, with particular reference to foliar fertilization. Challenging the Future: p. 133-146. In G. A. Constable and N. W. Forrester (ed.) Proc. World Cotton Res. Conf., 1st, Melbourne, Australia. 14-17 Feb. 1994. CSIRO, Melbourne, Australia.
17. Pettigrew WT, McCarty J C, Vaughn K. Leaf senencece-like characteristics contribute tocotton's premature photosynthetic decline. Photosynthesis Research 2000; 65, 187-195. https://doi.org/10.1007/s11120-019-00654-5.
18. Pettigrew WT. Potassium deficiency increases specific leaf weights and leaf glucose levels in field grown cotton. Agronomy Journal 1999; 91: 962-968. https://doi.org/10.2134/agronj 1999.916962.
19. Sawan ZM, Fahmy AH, Yousef SE. Effect of potassium, zinc and phosphorus on seed yield, seed viability and seedling vigor of cotton (*Gossypium barbadense* L.), Archives of Agronomy and Soil Science 2011;.57(1), 75-90. https://doi.org/10.1080 /03650340903222328
20. Snyder CS, Miley WN, Oosterhuis DM, Maples RL. Foliar application of potassium to cotton. University of Arkansas, Cooperative Extension Service. Cotton Comments. 1991; 4-91.
21. Santhanam V. Cotton Journal Edt. The Indian council of Agricultural Research. New Delhi. 1976
22. Sawan ZM, Hafez SA and Basyony AE. Effect of Nitrogen Fertilization and Foliar Application of Plant Growth Retardants and Zinc on Cottonseed, Protein and Oil Yields and Oil Properties of Cotton. Journal of Agronomy and Crop Science 2001. 186(3), 183– 191.
23. Sawan ZM, Hafez SA, Basyony AE. Effect of nitrogen and zinc fertilization and plant growth retardants on cottonseed, protein, oil yields, and oil properties. Journal of the American Oil Chemists' Society 2001; 78(11), 1087–1092.
24. Sawan ZM, El-Farra AA, Mohamed ZA. Effect of nitrogen fertilization, foliar application of calcium and some micro-elements on cottonseed, protein and oil yields and oil properties of Egyptian cotton. Annali di Botanica. Rome 1988; 46, 167–174.
25. Ullagaddi MS. Effect of dates of sowing and chemicals on seed yield and quality of Male parent SB (YF)-425 cotton (*Gossypium barbadonse* L.). M.Sc. (Agri.) Thesis. Univ. Agric. Sci. Dharwad. 2000.

Water deficit and salt stress tolerance: common identified quantitative trait loci in cotton (Gossypium hirsutum L.)

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Abstract

Background: *Cotton is the major source of natural fiber in the world. Gossypium hirsutum L. is the main species producing more than 90% cotton worldwide. This species is prune to a number of abiotic stresses. Among these abiotic stresses, water deficit and salt stress are the most damaging for cotton production. As a result of climate change, water deficit conditions have become frequent in major cotton producing countries of the world, and this has intensified already existing problem of salt stress. In view of this, it is imperative to find chromosomal regions associated with water deficit and salt stress tolerance simultaneously in cotton. A research project was planned to identify quantitative trait loci (QTLs) associated with water deficit tolerance in cotton through genome-wide association (GWA) approach and find out commonality in genetic architecture for water deficit and salt stress tolerance in cotton by comparing findings of present study with our already completed project of association mapping for salt stress tolerance in cotton. Plant material consisted of 76 cotton genotypes which originated from Pakistan, Australia, China, USA, and Uzbekistan.*

Results:

Cotton genotypes were phenotyped under normal and water deficit conditions in greenhouse and data were recorded at the seedling stage. Cotton genotypes showed significant differences for the morphological and physiological traits. Genotyping of the plant material was carried out with 95 polymorphic simple sequence repeats (SSRs) markers. Marker-trait associations were identified through TASSEL 3.0 software by using both general linear model (GLM) and mixed linear model (MLM). Some common QTLs for water deficit and salt stress tolerance were identified. Associated markers to these QTLs were NAU1167 (A3-Chr3), NAU3414 (A9-Chr9), NAU462 (A9-Chr9), and NAU1141 (A13-Chr13). These markers were located on chromosomes 3, 9 and 13. Phenotypic variance explained (R^2) value for these QTLs ranged from 9.39% to 20.38%.

Conclusion: *These findings suggest that there are important genes in the flanking regions of these markers involved in water deficit and salt stress TOLERANCE simultaneously. These findings also suggest that it is possible to pyramid water deficit and salt stress tolerance in an elite cotton genotype simultaneously.*

Keywords: Cotton, Abiotic stress, Water deficit, Salt stress, Genome-wide association mapping, Quantitative trait loci

Introduction

Cotton is the major source of natural fiber, an important raw material for textile industry. Four species of cotton are cultivated for fiber production-two tetraploid (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) and two diploid (*Gossypium arboreum* L. and *Gossypium thurberi* L.). However, more than 90% fiber worldwide is obtained from *Gossypium hirsutum* L. Major cotton producing countries of the world are India, USA, China, Brazil, and Pakistan.

Gossypium hirsutum L., the main source of cotton fiber, is prune to a number of abiotic and biotic stresses. Among the abiotic stresses, water deficit and salt stress are the most damaging to cotton production (Saeed et al. 2011). Due to climate change, water deficit conditions have become frequent in major cotton producing countries of the world. Water deficit conditions exaggerate already existing problem of salt stress for crop production. Water deficit and salt stress disturb normal plant growth and development. Due to these conditions, plant faces osmotic stress and its metabolic activities are hampered. Cotton is most sensitive to water deficit and salt stress at the seedling and reproductive stages. Cotton fiber yield and quality is badly affected due to water deficit and salt stress conditions. In view of this, it is imperative to characterize genetic diversity for water deficit and salt stress tolerance in cotton.

Molecular mapping approaches are helpful to identify quantitative trait loci (QTL) involved in traits of interest (Paterson et al. 2003; Saeed et al. 2014; Li et al. 2018). Genome-wide association studies (GWAS) are in common use to identify markers associated with various traits in humans, animals and plants (Ma et al. 2018). In the present study, a project was designed to identify markers associated with water deficit tolerance in cotton through GWAS approach and find out commonality of the markers/genes involved in water deficit and salt stress tolerance by comparing results of the present study with our already completed project of association mapping for salt stress tolerance in cotton (Saeed et al. 2014).

Material and methods

Plant material and phenotyping

In the present study, plant material consisted of a panel of 76 cotton (*Gossypium hirsutum* L.) genotypes. These genotypes originated from Pakistan, Australia, China, USA, and Uzbekistan. Experiment was conducted under the greenhouse conditions (25 ± 2 °C; 50% humidity; 13 h photoperiod). Seeds of the genotypes were sown in polythene bags containing compost soil at a depth of 2-3 cm. Polythene bags were arranged according to randomized complete block design (RCBD) with three replications and two treatments (control and limited irrigation). Seedlings were watered and fertilized till the appearance of first true leaf, and after that water was given daily to the control block, but to the treatment block water was withheld until the initial wilting stage and then water was given to relieve signs of wilting. Data was recorded for the morphological (shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight) and physiological (relative water content) at the 3rd main stem leaf stage (approximately 45 days post emergence).

Genotyping

Plant material was genotyped with simple sequence repeats (SSR) markers. A subset of 10 genotypes was screened for polymorphism using 500 SSR markers. Ninety-five SSR markers were found polymorphic and were used for genotypic data scoring of all 76 cotton genotypes. The details of genotyping procedure can be found at Saeed et al. (2014).

Assessment of sub-populations in the plant material

For the assessment of sub-populations in the plant material, STRUCTURE 2.0 software was used (Pritchard et al. 2000). In this software, burn-in time of 100000, 50000 MCMC repeats with allele frequencies independent and K ranging from 2 to 6 were used.

Identification of linkage disequilibrium and marker-trait associations

Extent of linkage disequilibrium and marker-trait associations were identified by using TASSEL version 2.1 (Bradbury et al. 2007).

Results and discussions

Plant phenotypic traits

Cotton genotypes showed great variation in all the studied traits-shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight and relative water content under both control and limited irrigation conditions (Table 1). A significant reduction in studied traits was noticed under limited irrigation condition.

Table 1. Descriptive statistics of the cotton genotypes

Trait/Statistics	Treatment	Mean	Sta.Dev	Minimum	Maximum
Shoot Length (cm)	Control	32.01	4.30	21.33	39.5
	Limited irrigation	18.60	3.22	10.33	28
Root Length (cm)	Control	20.88	4.05	11.33	35
	Limited irrigation	16.82	3.28	10	25
Fresh Shoot Weight (g)	Control	6.29	1.41	2.60	8.60
	Limited irrigation	1.78	0.55	0.71	3.6

Fresh Root Weight (g)	Control	0.77	0.19	0.35	1.27
	Limited irrigation	0.24	0.10	0.11	0.58
Dry Shoot Weight (g)	Control	1.21	0.34	0.37	1.91
	Limited irrigation	0.53	0.18	0.22	1.16
Dry Root Weight (g)	Control	0.16	0.05	0.05	0.26
	Limited irrigation	0.10	0.05	0.03	0.36
Relative water content	Control	50.16	10.46	28.39	91.53
	Limited irrigation	40.13	10.04	27.92	99.28

Sta.Dev *standard deviation Genotyping*

Plant material was genotyped with 95 polymorphic SSR markers. This genotyping revealed a total of 127 loci.

Assessment of sub-populations

STRUCTURE analysis revealed three sub-populations in the used plant material (Fig. 1). Sub-population 1 consisted of 6 genotypes. Sub-population 2 consisted of 29 genotypes. Sub-population 3 consisted of 34 genotypes. 7 genotypes were admixtures.

Identification of extent of linkage disequilibrium and marker-trait associations

The LD analysis revealed 1494 loci pairs in linkage disequilibrium out of a total of 8930 loci pairs ($P \leq 0.001$; $r^2 \geq 0.003$; $D' \geq 0.09$).

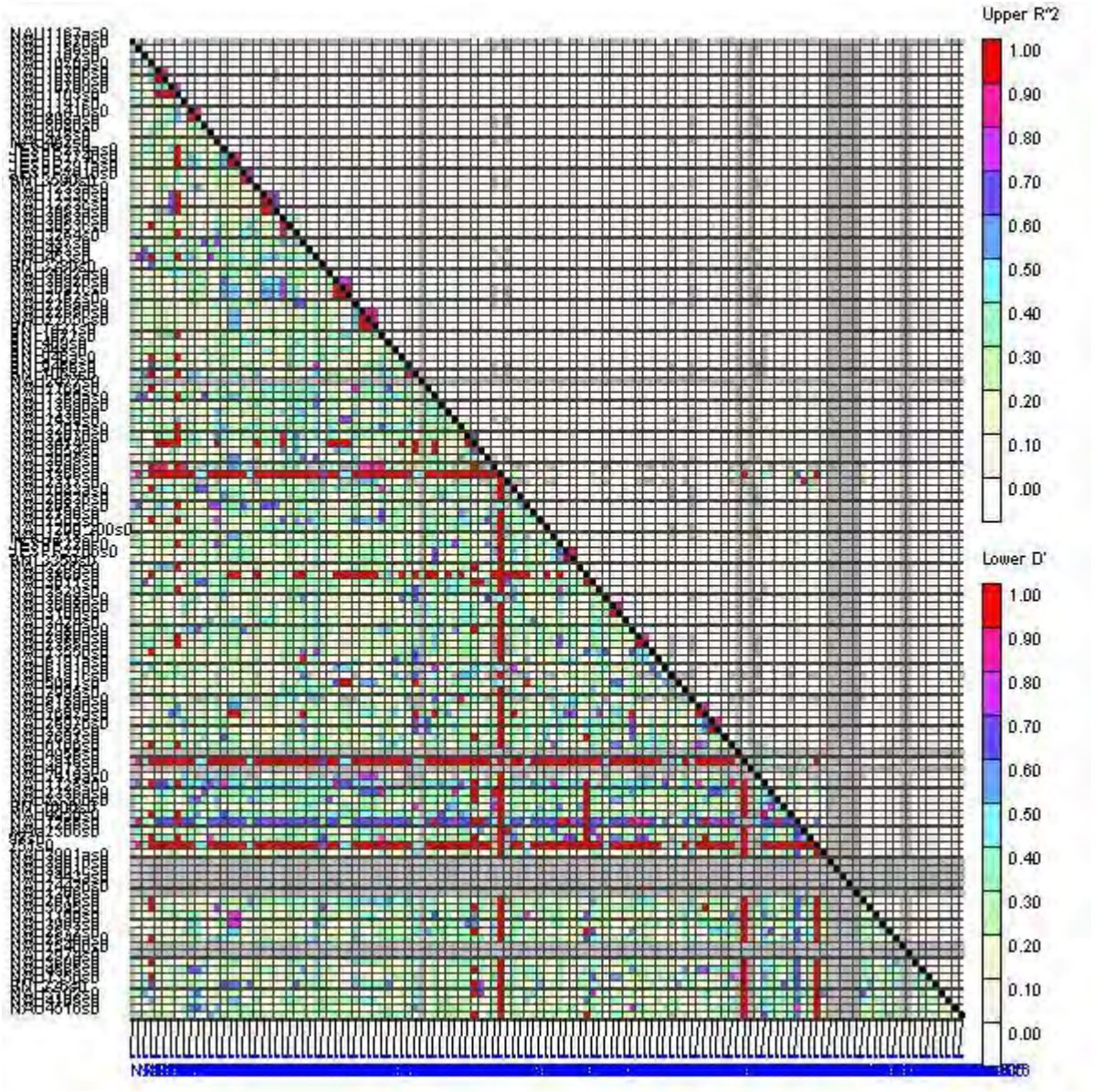

Figure 2. LD plot

A total of 7 highly significant marker-trait associations were identified in this study at $P \leq 0.005$ (Table 2). These marker-trait associations were for root length (5), fresh shoot weight (1), and fresh root weight (1). Marker-trait association for root length and fresh shoot weight were identified under limited irrigation conditions, and for fresh root weight were identified under control condition. These marker-traits associations were identified both under general linear model (GLM) and mixed linear model analyses. Associated markers for these associations were NAU5091, NAU4565, NAU2691, JESPr220, NAU3011, NAU437 and JESPR220. Phenotypic variance explained values (R^2) for these associations ranged from 14.86% to 25.81%. On comparison of the results of this study with our already completed project of association mapping for salt tolerance in cotton (Saeed et al. 2014), four markers were found to be common in water deficit and salt tolerance in cotton. These markers were NAU1167, NAU462, NAU1141, and NAU 3414. Three markers (NAU1167, NAU462, NAU1141) were found to be associated with root length and one marker (NAU3414) was found to be associated with fresh shoot weight. World cotton production is badly affected by water deficit and salt stress. Conventional breeding practices cannot cope the challenge to develop cotton cultivars having potential for sustained cotton production under both water deficit and salt stress conditions (found in most of the cotton producing countries of the world). Molecular mapping approaches are helping breeders to identify genomic regions associated with traits of interest and thus accelerating breeding programs through marker-assisted selection (MAS) approach.

In the present study, in addition to the 7 marker-trait associations for water deficit tolerance, 4 markers were found associated with water deficit tolerance, which were previously identified to be associated with salt tolerance in cotton (Saeed et al. 2014). It showed that it would be possible to pyramid both water deficit and salt tolerance in the same cotton cultivar through marker-assisted breeding (MAB) approach. It also highlighted the point that plants respond to the both type of stress conditions through common genetic entities.

Conclusion

Plants have the common genetic architecture to respond to the environmental conditions. The four markers associated with water deficit in the present study and with the salt tolerance in the previous study (Saeed et al. 2014) are a valuable resource of MAS to develop water deficit and salt tolerant cotton cultivars simultaneously.

Acknowledgement

Financial support for this research project was provided by the Higher Education Commission (HEC), Pakistan.

Table 2. Significant marker trait associations identified in this study ($P \leq 0.005$)

Trait	Treatment	Locus	Chr.	*P* Marker	R^2 Marker (%)	Heritability
Root Length (cm)	Limited irrigation	NAU5091	D11-Chr. 21	5.89E-05	25.81	1.00E-05
		NAU4565		1.85E-04	21.29	1.00E-05
		NAU2691	D3-Chr. 17	0.0013	16.88	1.00E-05
		JESPR220	D4-Chr. 22	0.0036	15.37	1.00E-05
		NAU3011	D13-Chr. 18	0.0049	21.21	0.0037
Fresh Shoot Weight (g)	Limited irrigation	NAU437	A2-Chr. 2	0.0037	14.86	0.2902
Fresh Root Weight (g)	Control	JESPR220	D4-Chr. 22	5.11E-04	16.03	0.1648

In the present study, in addition to the 7 marker-trait associations for water deficit tolerance, 4 markers were found associated with water deficit tolerance, which were previously identified to be associated with salt tolerance in cotton (Saeed et al. 2014). It showed that it would be possible to pyramid both water deficit and salt tolerance in the same cotton cultivar through marker-assisted breeding (MAB) approach. It also highlighted the point that plants respond to the both type of stress conditions through common genetic entities.

Conclusion

Plants have the common genetic architecture to respond to the environmental conditions. The four markers associated with water deficit in the present study and with the salt tolerance in the previous study (Saeed et al. 2014) are a valuable resource of MAS to develop water deficit and salt tolerant cotton cultivars simultaneously.

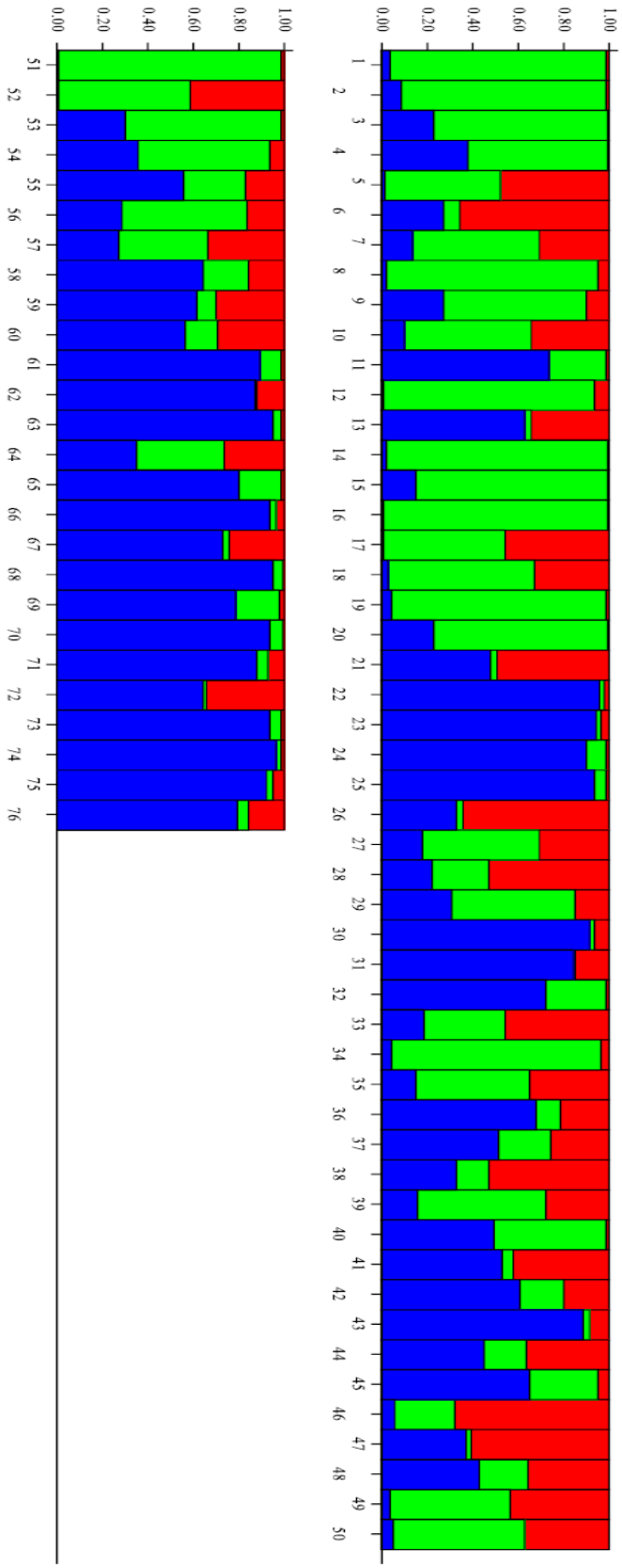

Figure 1. Sub-populations in the used plant material

Acknowledgement

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Table 2. Significant marker trait associations identified in this study ($P \leq 0.005$)

Trait	Treatment	Locus	Chr.	*P* Marker	R^2 Marker (%)	Heritability
Root Length (cm)	Limited irrigation	NAU5091	D11-Chr. 21	5.89E-05	25.81	1.00E-05
		NAU4565		1.85E-04	21.29	1.00E-05
		NAU2691	D3-Chr. 17	0.0013	16.88	1.00E-05
		JESPR220	D4-Chr. 22	0.0036	15.37	1.00E-05
		NAU3011	D13-Chr. 18	0.0049	21.21	0.0037
Fresh Shoot Weight (g)	Limited irrigation	NAU437	A2-Chr. 2	0.0037	14.86	0.2902
Fresh Root Weight (g)	Control	JESPR220	D4-Chr. 22	5.11E-04	16.03	0.1648

Table 3. Common markers for water deficit and salinity tolerance in cotton

Trait	**Treatment**	**Locus**	**Chr.**	***P* Marker**	**R^2 Marker (%)**	**Heritability**
Root length (cm)	Limited irrigation	NAU1167	A3-Chr3	0.006	20.38	0.0921
		NAU462	A9-Chr9	0.0183	11.62	0.0071
		NAU1141	A13-Chr13	0.0069	14.14	1.00E-05
Fresh shoot weight (g)	Limited irrigation	NAU3414	A9-Chr9	0.0086	9.39	0.221

References

1. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633-2635.
2. Li C, Fu Y, Sun R, Wang Y, Wang Q (2018) Single-locus and multi-locus genome-wide association studies in the genetic dissection of fiber quality traits in Upland cotton (*Gossypium hirsutum* L.). Frontiers in Plant Science 9:1083. DOI: 10.3389/fpls.2018.01083
3. Ma Z, He S, Wang X, Sun J, Zhang Y, Zhang G, Wu L, Li Z, Liu Z, Sun G, Yan Y, Jia Y, Yang J, Pan Z, Gu Q, Li X, Sun Z, Dai P, Liu Z, Gong W, Wu J, Wang M, Liu H, Feng K, Ke H, Wang J, Lan H, Wang G, Peng J, Wang N, Wang L, Pang B, Peng Z, Li R, Tian S, Du X (2018) Resequencing a core collection of upland cotton identifies genomic variation and loci influencing fiber quality and yield. Nature Genetics 50, 803–813.
4. Paterson AH, Saranga Y, Menz M, Jiang CX, Wright R (2003) QTL analysis of genotype × environment interactions affecting cotton fiber quality. Theoretical and Applied Genetics 106(3): 384-396.
5. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959. Saeed M, Guo WZ, Ullah I, Tabbasam N, Zafar Y, Rahman M, Zhang TZ (2011) QTL mapping for physiology, yield and plant architecture traits in cotton (*Gossypium hirsutum* L.) grown under well-watered versus water-stress conditions. Electronic Journal of Biotechnology 14 (3).
6. Saeed M, Guo WZ, Zhang TZ (2014) Association mapping for salinity tolerance in cotton (*Gossypium hirsutum* L.) germplasm from US and diverse regions of China. Australian Journal of Crop Science 8 (3): 338-346.

Changing Pattern of Cotton Whitefly Infestation Vis-à-Vis Climate Change in India

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Abstract:

***Background**: A retrospective study was conducted to determine whether climate change had any influence on the whitefly infestation patterns. Attempts were also made to understand which climatic factors triggered whitefly infestation.*

***Results**: The whitefly infestation level has changed significantly in all three locations. There was a shift in time when peak occurrence; advancing from two to seven weeks in these locations. Models that were used to assess what factors were causing these effects found that maximum temperature had a high influence in decreasing the whitefly infestations while minimum temperature had the highest influence in increasing whitefly infestations. Other factors (rainfall, morning and evening relative humidity) did not show any pattern or consistency over the years. While the present study was limited in its assessment of only five climatic factors, it has to be acknowledged that there are other influencing factors like the cultivation of susceptible varieties, sowing date, insecticide and fertiliser applications, irrigation schedules, proximity of other crops etc. have to be considered for more accurate assessments of the reasons for infestations.*

***Conclusion**: The study does suggest that temperature is playing some role and this could help to inform crop managers and researchers on appropriate management like less vegetative growth at the initial stages of the crop development as the whitefly advances its existence at this early stage. Other considerations could include early maturing and or okra leaf cultivars. Important appropriate IPM will need to be considered and revised as climate continues to change.*

***Methods**: For this study, the cotton survey data was collated from AICRP on for the locations Faridkot, Hisar, Sriganganagar (Northern India) on infestation of whitefly and weather data for the period 2005 to 2019. The study encompassed descriptive statistical analyses and statistical models to determine which factors influenced cotton whitefly infestations. Models assessed the maximum temperature, minimum temperature, rainfall, and both morning and evening relative humidity.*

***Keywords:** Whitefly, Climate Change, Cotton, Statistical Model, Temperature, Rain Fall, Humidity, North India*

Background

Change in weather factors due to climate change may have both positive as well as negative implications on agricultural production systems. Climate changes can specifically affect crop phenology and morphology, and alter the biotic as well as abiotic intrusions on plant growth. In India, it has been found that there are shifts in cotton pest infestation patterns, especially in cotton whitefly in recent years.

There have been numerous studies conducted to determine the effect of climatic change on agriculture crop production systems, especially on insect pest dynamics across the globe. Increasing warm and dry conditions are likely to induce more pest issues. Of all climate factors temperature is the most important factor influencing change in insect behaviour (Ali et al. 2018), distribution, development, life-cycle (Yamamura and Kiritani 1998; Jackson et al. 2011), survival rate (Williams et al. 2003), and reproduction (reference?). Changes in rainfall patterns especially delays in the onset of monsoons also tend to cause heavy damage to crops (Sharma, 2010). Roos et al. (2011) as these changes lead to different cultivation practices creating conducive environments for insect and pathogen attacks. During the 2015-16 cotton season in northern India, a severe outbreak of whitefly was witnessed in cotton growing zones. Some significant factors for outbreaks of whitefly were associated with scant rainfall, susceptible genotypes, delayed sowing, and abuse of chemical insecticides (Rishi Kumar et al. 2016). To assist this understanding and identify whether climate change is playing a role this paper, attempted to elicit whitefly scenarios vis-à-vis climate change with historical data collected in India.

Results

Whitefly infestation levels and associated climate factors changed significantly in all three locations. The study estimated the average whitefly population and climate factors for the entire study period (2005 to 2019); period 1: 2005-2010 (P1); and period 2: 2010-2019 (P2). Periods P1 and P2 were demarcated since there had been significant changes in whitefly populations and associated climate parameters in P2 in Northern India. The study also estimated average climate factors, which prevailed during peak occurrences of whitefly. This estimate could be otherwise stated as ideal climatic conditions for whitefly infestations.

There was a shift in peak timing of occurrences of whitefly infestations, advancing from two to seven weeks in all locations. In Faridkot, the peak occurrence was recorded in the 37th and 39th standard weeks during P1, whereas, it was recorded in the 30th and 37th standard weeks during P2. In Hisar, the peak occurrence was recorded in the 28th and 30th standard weeks during P1, whereas it was recorded in the 32nd and 37th standard weeks during P2. In Sriganganagar, the peak occurrence was recorded in the 31st and 36th standard weeks during P1, whereas it was recorded in the 29th and 33rd standard weeks during P2.

In Faridkot, during P1, the average infestation of whitefly was 2.63 per three leaves during the cropping season, whereas, it was 7.25 per three leaves with an increase of 176% during P2. (Table 1). Correspondingly, entire season analyses found that 5.75% decrease in minimum temperature; over 400 percent increase in rainfall; significantly 10% decrease in evening relative humidity in P2 compared to P1. Notably, a significant shift in all climatic factors in P2 compared to P1 during peak occurrences of whitefly in Faridkot (Table 1). In Hisar, during P1, the average infestation was 5.37 per three leaves, whereas, it was 23.34 per three leaves with an increase of 335% during P2. Again, a significant shift in all climatic factors except maximum temperature in P2 compared to P1 during peak occurrences of whitefly in Hisar. In Sriganganagar, during P1, the average infestation was 17.95 per three leaves, whereas, it was 18.43 per three leaves with an increase of just three percent during P2. The average infestation level ranged from 4.22 to 50.92 per three leaves during the entire period (2005 to 2019) at Sriganganagar. Notably, no significant change was found in the average whitefly population either in season analyses or in peak occurrence periods at Sriganganagar. Compared to Faridkot and Hisar, the maximum temperature at Sriganganagar was recorded higher, but, with a slight reduction in P2.

Table 1 Average climate factors and whitefly infestations

	Entire season				During peak occurrences			
	2005-2019	2005-10 (P1)	2010-19 (P2)	% change P2 over P1	2005-2019	2005-10 (P1)	2010-19 (P2)	% change P2 over P1
Faridkot								
Max Temp	34.26	34.23	34.28	0.15	33.77	32.72	34.12	4.28
Min Temp	25.29	26.26	24.75	-5.75	26.52	25.38	26.91	6.03
Rainfall	9.34	2.55	13.12	414.51	11.01	1.72	14.21	726.16
Morning	78.65	78.78	78.58	-0.25	82.48	80.00	83.33	4.16
Evening	56.92	60.94	54.66	-10.31	65.38	62.63	66.33	5.91
Whitefly	5.60	2.63	7.25	175.67	18.26	5.18	22.76	339.38
Hisar								
Max Temp	35.49	35.61	35.42	-0.53	35.23	35.47	35.14	-0.93
Min Temp	23.57	23.21	23.83	2.67	24.18	21.75	25.14	15.59
Rainfall	19.08	16.96	20.27	19.52	20.78	12.48	24.06	92.79
Morning	81.47	80.46	82.03	1.95	83.90	81.41	84.88	4.26
Evening	50.57	48.70	51.62	6.00	53.74	42.77	58.08	35.80
Whitefly	16.34	5.370	23.34	334.64	41.72	14.05	52.67	274.88
Srigangana								
Max Temp	37.97	38.31	37.78	-1.38	37.40	37.33	36.93	-1.07
Min Temp	24.87	24.73	24.95	0.89	25.55	25.68	25.50	-0.70
Rainfall	12.65	10.83	13.96	28.90	17.44	16.01	18.30	14.30
Morning	72.04	70.52	72.89	3.36	77.78	77.37	77.94	0.74
Evening	49.61	46.94	51.09	8.84	56.88	55.84	57.94	3.76
Whitefly	18.25	17.95	18.43	2.67	45.15	44.60	45.37	1.73

The model estimates at Faridkot found that maximum temperature had a high influence on decreasing whitefly infestations for the same week (SW) and previous weeks (PW) maximum temperature especially from the year 2015-16 onwards for the entire crop season. On the contrary, minimum temperature had a high influence on increasing whitefly infestations for the SW and PW minimum temperature especially from the year 2015-16 onwards for the entire crop season. The weather parameters - rainfall, morning relative humidity, and evening relative humidity, did not show any pattern over the years on whitefly infestation in Faridkot (Table 2).

In Hisar, the maximum temperature had a high influence in decreasing whitefly infestations for the SW and PW maximum temperature, and a significant change was found especially from 2013-14 onwards for the entire crop season. On the contrary, minimum temperature had a high influence on increasing whitefly infestations for the SW and PW minimum temperature, especially from 2013-14 onwards for the entire crop season. The morning relative humidity had some effect on triggering whitefly infestations, but did not show any consistent pattern over the years (Table 3).

In Sriganganagar, the maximum temperature of SW, PW, and WBPW had a high influence in decreasing whitefly infestations for the entire crop season. On the contrary, the minimum temperature of SW, PW, and WBPW had a high influence on increasing whitefly infestations (Table 4). The study found that the conducive climatic factor which increased the whitefly population in Faridkot have been 34.12, 26.91, 14.21, 83.33, and 66.33 respectively for Max Temp, Min Temp, RF, MRH, ERH. In Hisar, it was 35.14, 25.14, 24.06, 84.88, 58.08 and in Sriganganagar, it was 36.93, 25.50, 18.30, 77.94, 57.94 (Table 1).

Conclusion:

There have been changes in whitefly population dynamics due to climatic change in recent years even within the agroecological zone. The study suggests the agronomist/physiologist tailor the crop with appropriate canopy management especially less vegetative growth at the initial stage of the crop as the whitefly advances its existences at an early stage; suggests cotton breeders develop early maturing to escape the crop from later stage damage from whitefly or okra leaf varieties; and entomologist to refine the IPM strategies in the light of changing pattern of whitefly population dynamics due to climate change.

The present study is limited to finding the result with just five weather parameters. There are other influencing factors like the cultivation of susceptible varieties, date of sowing, insecticide and fertiliser applications, irrigation schedule, proximity crops etc. have to be included for a more accurate estimate. It requires a holistic study to collect data on all the factors mentioned for at least four to five years to make any comprehensive conclusion.

Table 2 Model estimation for whitefly infestation at Faridkot

	Maximum Temperature /°C			Minimum Temperature /°C			Rainfall /mm			Morning Relative Humidity /%			Evening Relative Humidity /%		
	SW	PW	WBP	SW	PW	WBP	SW	PW	WBP	SW	PW	WBP	SW	PW	WBP
2005-06	-2.23	-0.48	-2.54	-2.29	-4.08	-1.75	-0.08	-0.17	-0.08	3.49*	2.45	2.29	0.19	1.25	1.32
2006-07	1.52	0.71	0.59	-2.77*	-2.67*	-2.43*	0.07	-0.06	-0.01	-0.05	0.24	0.76	1.16	1.48	0.87
2007-08	-3.63	1.12	-2.05	0.87	-0.60	2.85	0.00	0.02	-0.12	2.90	-3.84	-0.36	-0.22	3.90	0.25
2008-09	-7.16*	-5.88*	-3.45	5.06*	5.68*	4.43	-0.30*	-0.45*	-0.29*	3.23	0.78	-1.07	-1.26	-0.16	0.76
2009-10	-6.87*	-3.39*	-2.53	7.03*	3.31	3.15	-0.02	-0.10	0.03	1.46*	-0.13	-0.98*	-1.11	0.66	0.94
2010-11	-5.99	-7.30	-8.68	3.75	6.00	7.99	-0.04	-0.01	-0.08	2.82	3.25	3.20	-0.46	-1.63	-2.00
2011-12	-4.94*	-5.40*	-6.71*	1.49	3.62*	7.04*	-0.17	-0.19	-0.06	2.77	1.07	-0.07	0.48	1.01	0.62
2012-13	-0.40	-1.00	-5.69	-0.65	0.83	3.96	-0.19	-0.20	-0.19	-0.90	-1.52	2.00	2.11	2.13	-0.10
2013-14	-4.00*	-5.68*	-4.48	4.91*	7.33*	8.83*	-0.07	-0.06	-0.05	-2.84	-1.12	-3.78	2.96	0.72	1.30
2014-15	-0.84	-1.53	-6.91*	-1.16	-0.57	3.06*	-0.06	0.03	-0.08	-0.85	-0.17	4.03*	3.04*	2.43*	-0.28
2015-16	-16.84*	-17.03*	-13.48*	12.92*	14.51*	13.03*	-0.41*	-0.31*	-0.21	6.53	5.73	2.63	-1.71	-2.00	-0.60*
2016-17	-5.53*	-1.13	8.27*	4.44*	1.32	-6.24*	-0.03	-0.03	-0.08	2.87	-0.36	-6.34*	-1.36	0.79	5.13*
2017-18	-11.68*	-8.66*	-2.39	10.01*	8.87*	4.84	0.04	0.02	0.15*	5.10	2.55	-1.87	-2.90	-1.83	0.65
2018-19	-6.35*	-6.31*	-2.36	6.53*	7.27*	4.37*	0.02	0.09*	0.08	2.28	2.34	-1.36	-1.83	-2.56	0.31

*: Significant at 5% level; Note: SW- same week weather data; PW-previous week weather data; WBPW- a week before the previous week

Table 3 Model Estimation for whitefly infestation at Hisar

	Maximum Temperature /°C			Minimum Temperature /°C			Rainfall /mm			Morning Relative Humidity /%			Evening Relative Humidity /%		
	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW
2005-06	-6.03*	-1.72	-0.49	3.44	0.33	0.20	-0.27*	-0.27*	-0.12	3.25	-0.44	-1.75	-0.60	2.13	2.54
2006-07	0.38	-8.24*	-0.88	0.12	7.94*	1.93	0.09	0.12	-0.06	-1.31	5.32*	-2.41	1.26	-4.63*	2.22
2007-08	-3.68	-3.10	-3.53	1.43	1.10	1.83	-0.36	-0.31	0.01	-0.37	-1.13	-0.17	3.14	3.73	2.34
2008-09	-5.66*	-3.81	-2.29	3.51	2.27	1.18	-0.02	-0.10	-0.12	4.08*	2.35	1.06	-2.18	-0.89	0.07
2009-10	-7.13*	-5.53*	-7.34*	4.74*	3.60*	6.23*	-0.19*	-0.18*	-0.02	4.14*	2.58*	3.04*	-1.35	-0.13	-1.20
2010-11	-1.78	2.07	4.19	2.80	-0.76	-1.54	-0.05	0.12	0.15	-0.50	-2.08	-3.23*	0.08	1.17	1.16
2011-12	0.44	-1.38	-1.19	-0.14	2.31	2.90	-0.28*	-0.07	-0.03	-3.13	-1.21	-1.58	3.54*	0.97	0.71
2012-13	-9.49	-8.96*	-10.24*	7.16	7.42*	9.52*	-0.01	-0.03	0.19	5.44	3.96	4.77	-2.94	-1.96	-3.51
2013-14	-14.64*	-19.28*	-9.69	11.06*	18.15*	11.26	-0.01	0.11	0.08	5.26	6.32*	-0.34	-1.04	-3.84	0.52
2014-15	-9.51	-14.33	-18.27*	5.27	12.62	17.17*	-0.26	-0.21	-0.14	4.67	6.06	7.65	-0.16	-3.35	-5.23
2015-16	-21.77*	-14.39*	-12.02	22.76*	14.54	12.27	-0.04	0.17	0.24	9.59*	5.24	3.17	-8.72*	-3.94	-1.94
2016-17	-8.74*	-1.03	2.70	6.51	3.33	0.71	-0.12	-0.39*	-0.27	1.95	-5.88*	-8.02*	0.93	5.34*	6.43*
2017-18	-16.29*	-6.24	-2.78	11.76*	-0.55	-2.97	-0.49*	-0.28	0.12	4.77	0.30	-0.79	0.64	6.47	6.33
2018-19	-13.09*	-13.69*	-7.00	8.74*	10.82*	6.66	-0.25	-0.08	-0.10	5.97*	5.85	-0.61	-1.23	-2.30	2.16

*: Significant at 5% level; Note: SW- same week weather data; PW-previous week weather data; WBPW- a week before the previous week

Table 4 Model Estimation for whitefly infestation at Sriganganagar

	Maximum Temperature /°C			Minimum Temperature /°C			Rainfall /mm			Morning Relative Humidity /%			Evening Relative Humidity /%		
	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW	SW	PW	WBPW
2005-06	-7.60	-3.29	-5.36	4.95	3.04	5.75	-0.16	-0.33	-0.23	4.46	-1.55	-0.83	-1.41	2.79	1.63
2006-07	-5.40	-5.21	-13.22*	6.38	6.75	11.99*	-0.41*	-0.33	-0.27	-3.83	-4.70	3.83	4.58	5.05	-1.23
2008-09	-15.48*	-15.90*	-17.41*	9.18*	10.60*	12.14*	-0.46*	-0.60*	-0.45	9.28*	6.65	7.30	-2.69	-0.56	-1.16
2009-10	-6.63*	-5.04*	-5.78*	3.97*	2.41*	2.23*	-0.35*	-0.29*	-0.20*	-0.19	-0.92*	1.64*	3.94*	4.55*	2.54*
2010-11	-10.82*	-11.51*	-10.36*	6.92*	8.64*	8.42*	-0.17	-0.28*	-0.17	6.38*	4.37*	3.81	-2.34	-0.91	-1.21
2011-12	-8.13*	-7.07	-7.12	6.42*	6.67*	8.34*	-0.16	0.16	-0.05	-1.28	-1.07	-4.15	4.00	2.50	4.57
2012-13	-6.24	-7.92*	-7.52*	6.02*	7.82*	9.27*	-0.12	-0.10	-0.13	-1.59	-0.33	-2.91	3.03	1.71	2.96
2013-14	-7.52*	-5.53	-6.54	6.46*	5.90	8.41	-0.48*	-0.30	-0.25	-2.65	-4.32	-4.31	5.05	5.44	4.26
2014-15	-17.23*	-14.03*	-13.28*	12.32*	11.10*	11.85*	-0.33*	-0.24*	-0.02*	10.97*	6.69*	4.93*	-5.52*	-2.77*	-2.19*
2015-16	-9.45*	-4.38	-10.85*	10.04*	7.57*	13.46*	-0.14	0.05	0.19	2.22	-2.53	3.51	-1.07	1.43	-4.12
2016-17	-2.72	-2.31	-1.04	-1.17	-1.56	-2.00	-0.30	-0.41	-0.50*	3.35	2.20	-0.46	0.41	1.68	3.82
2017-18	-0.64	-2.28	-3.12	-1.28	-1.38	-1.19	-0.38	-0.35	-0.26	0.86	2.20	3.69	1.35	1.56	0.57
2018-19	-9.39*	-4.82	0.70	7.33*	5.03*	2.58	-0.09	-0.01	0.02	3.55	-0.92	-5.28*	-0.78	1.77	3.41*

*: Significant at 5% level; Note: SW- same week; PW-previous week; WBPW- a week before the previous week

Methods

For this study, cotton survey data was collated from AICRP for the locations Faridkot, Hisar, and Sriganganagar (Northern India) on whitefly infestations and climate data for the period 2005 to 2019.

The study framed the methods by statistical means, piece by piece with descriptive statistical analyses; and finally framed a statistical model to find the weather factors influencing the cotton whitefly infestation.

Statistical models (log-linear) were developed for the whitefly population (dependent variable) against the climate factors (independent variables) - maximum temperature, minimum temperature, rainfall, morning relative humidity, and evening relative humidity. Models were then framed for three different scenarios – where climate factors were related to whitefly infestation based on the same week of the

infestation, the previous week, and two weeks before infestation.. This attempts to understand how whitefly production and distribution diverges for past and present climate..The models also adopt the transformation suggested by Yamamura (1999, 2006) that is, log(x+0.5) where x is the number of individuals.

$$\log(Y) = \alpha + \beta_1 \log (T_{max, t}) + \beta_2 \log (T_{min, t}) + \beta_3 \log (RF_{, t}) + \beta_4 \log (RH_{max, t}) + \beta_5 \log (RH_{min, t}) + u_i$$

$$\log(Y) = \alpha + \beta_1 \log (T_{max, t-1}) + \beta_2 \log (T_{min, t-1}) + \beta_3 \log (RF_{, t-1}) + \beta_4 \log (RH_{max, t-1}) + \beta_5 \log (RH_{min, t-1}) + u_i$$

$$\log(Y) = \alpha + \beta_1 \log (T_{max, t-2}) + \beta_2 \log (T_{min, t-2}) + \beta_3 \log (RF_{, t-2}) + \beta_4 \log (RH_{max, t-2}) + \beta_5 \log (RH_{min, t-2}) + u_i$$

Note: Equation (1) is to find the whitefly scenario based on the same week (SW) climatic factor; Equation (2) is to find the whitefly scenario based on the previous week (PW) climatic factor; Equation (3) is to find the whitefly scenario based on the weeks before the previous week (WBPW) climatic factor Where Y is Dependent Variable (Whitefly population); T_{max}, T_{min}, RF, RH_{max}, RH_{min} are independent variables, respectively, Maximum Temperature, Minimum Temperature, Rainfall, Morning Relative Humidity, Evening Relative Humidity; t is same week observation of climatic factor; t-1 is the previous week observation of climatic factor; t-2 week before previous week observation of climatic factor.

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References

1. Ali El-Sayed, Ahmed Nada and Said Moussa Abd El-Fattah. Impacts of Climate Change on Insect Pests of Main Crops in Egypt. 2018; DOI: 10.1007/698_2018_311.
2. Jackson, L., Wheeler, S., Hollander, A., O'Geen, A., Orlove, B., Six,J., et al. Case study on potential agricultural responses to climate change in a California landscape, Climatic Change, 2011; 109 (1): 407-427
3. Rishi Kumar, S. K. Sain, D. Monga, Sandhya Kranthi, V. S Nagrare and Kranthi 2016. Whitefly and Cotton Leaf Curl Disease Management Strategies for Cotton in North India. ICAR - CICR Technical Bulletin No. 1/2016. Page 12
4. Roos, J., Hopkins, R., Kvarnheden, and Dixelius, C. The impact of global warming on plant diseases and insect vectors in Sweden, European Journal of Plant Pathology, 2011;129(1):9-19
5. Sharma, H.C. 2010. Effect of climate change on IPM in grain legumes. In: *5th International Food Legumes Research Conference (IFLRC V), and the 7th European Conference on Grain Legumes (AEP VII),* 26-30th April 2010, Anatalaya, Turkey.
6. Williams, R.S., Lincoln, D.E. and Norby, R.J. Development of gypsy moth larvae feeding on red maple samplings at elevated CO_2 and temperature. *Oecologia*, 2003; 137: 114-122.
7. Yamamura, K. and Kiritani, K. A simple method to estimate the potential increase in the number of generations under global warming in temperate zones. *Applied Entomology and Zoology*. 1998; 33: 289-298.
8. Yamamura, K. Res Popul Ecol,1999; 41: 229. https://doi.org/10.1007/s101440050026
9. Yamamura. How to analyze long-term insect population dynamics under climate change: 50-year data of three insect pests in paddy fields. Popul Ecol. 2006; 48:31–48

Ecosystem level CO_2 exchanges from a rainfed cotton production system using eddy covariance technique

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Abstract

Background: *Ecosystem level carbon fluxes are key indicators of the functioning of crop production system and their quantification can help in understanding the sustainability of the production system under the current set of management practices. Eddy-covariance (EC) technique was used to quantify the CO_2 fluxes from a rainfed cotton production system in Central India during the 2021-22 crop season.*

Results: *Maximum height of the cotton crop was found to be 113.6±7.9 cm at 120 Days After Sowing (DAS) with peak (Leaf Area index) LAI of 2.4 ±0.3 at 90 DAS in the footprint area of the flux tower. The seed cotton yield was found to be 1526±316 kg ha^{-1}. The ecosystem carbon exchanges were found to be highly influenced by the crop growth stages and the prevailing weather condition. The ecosystem was found to be a net CO_2 source for about two weeks from the date of sowing, as the crop stand was meagre and ecosystem respiration superseded the photosynthetic processes. The ecosystem became net CO_2 sink thereafter with peak net CO_2 influx of 4-4.5 gC m^{-2} d^{-1} during flowering initiation to first boll opening stage during the month of September. The mean Gross Primary Production (GPP) was 1.5 gC m^{-2} d^{-1} during sowing to germination, 3.3 gC m^{-2} d^{-1} during germination to squaring, 5.2 gC m^{-2} d^{-1} during squaring to flowering initiation, 6.1 gC m^{-2} d^{-1} during flowering initiation to first boll opening. It reduced thereafter with value of 5.1 gC m^{-2} d^{-1} during first boll opening to first picking, 3.4 gC m^{-2} d^{-1} during first to second picking, 3.2 gC m^{-2} d^{-1} during second to last picking. Similarly, the mean Net Ecosystem CO_2 Exchange (NEE) was found to be 0.9 gC m^{-2} d^{-1} during sowing to germination, -0.4 gC m^{-2} d^{-1} germination to squaring, -1.8 gC m^{-2} d^{-1} during squaring to flowering initiation,-2.1 gC m^{-2} d^{-1} flowering initiation to first boll opening. It declined thereafter during the boll bursting stage and was -0.9 gC m^{-2} d^{-1} during the period from second to last picking. Throughout the season (180 days), the cumulative GPP was 803.4 gC m^{-2}, NEE was -213.8 gC m^{-2} and Ecosystem Respiration (R_{eco}) was 589.7 gC m^{-2}. Around 2.14 tonnes of C per ha was net assimilated by the cotton during the season of 2021-22.*

Conclusion: *The rainfed cotton crop is a strong net sink for capturing atmospheric CO_2. Around 2.14 tonnes C ha^{-1} was sequestered during the crop season. The C fluxes were found to be highly influenced by crop phenological stage and the prevailing weather condition. The EC datasets generated can be used to validate cotton crop simulation models and for up-scaling the carbon fluxes to regional scale using remote sensing proxies.*

Key Words: Gross Primary Production, Net Ecosystem CO_2 Exchange, Ecosystem Respiration

Introduction

Climate change and variability are impacting livelihood by accentuating the frequency and magnitude of extreme weather events and creating a huge stress on the cotton production system. It is increasing the likelihood of hazards occurring during the crop season (Shah et al. 2021) and the risk of partial or complete crop failure.

Like other agricultural ecosystems, the cotton production system is both, a source and a sink for atmospheric CO_2. Ecosystem level carbon fluxes are key indicators of the functioning of the production system. Quantifying these fluxes throughout the crop season would help in understanding whether the production system is a net source or sink for atmospheric carbon.

Eddy covariance (EC) based analysis is being widely adopted for measuring carbon and moisture fluxes between the atmosphere and terrestrial ecosystems including crop lands (Papale et al. 2006). The Net Ecosystem Exchange (NEE) is the difference between the Gross Primary Production (GPP) that represents the amount of carbon fixed through photosynthesis and Ecosystem Respiration (R_{eco}) that represents the net respiratory losses (Schulze et al. 2000). NEE is an instantaneous measurement of carbon flux and Eddy covariance (EC) techniques can be applied to estimate it (Baldochi et al. 2001). When NEE is integrated over the season it provides an estimate of sink strength of the production system.

Rainfed cotton production system represents a globally widespread and socio-economically important production system. In India, around 60% of the 13 million hectare area under cotton is rainfed. This system is prevalent over the central and peninsular India. It is imperative to understand and quantify the CO_2 fluxes in the rainfed cotton production system in order to understand its long term sustainability. EC based CO_2 fluxes have been reported under the cotton based production systems from North China (Bai et al. 2015, Li et al. 2018), South China (Ming et al. 2021), irrigated system of Arkansas, USA (Fang et al. 2020) and dryland cotton of Texas, USA (Menefee et al. 2020). We recently reported both moisture and CO_2 fluxes from rainfed cotton production system of Central India (Chakraborty et al. 2022). The results reported in the present paper are in continuation with this study. Our aim is to quantify the daily, phenophase-wise and season-long CO_2 fluxes from a rainfed cotton based cropping system raised on Vertisols and Vertic intergrades during the 2021-22 crop season.

Results

Meteorological conditions during the cotton growing season

Dekadal variations in temperature, rainfall and bright sun shine hours at the study site during the crop season 2021-22 are presented in Figure 1.

Sufficient rainfall in the second dekade of June facilitated preparation time, which facilitated the sowing of cotton crop in the last week of June month. The rainfall continued to be high with good distribution during July month, but lull periods were observed during first and last dekade of August month. Very high rainfall activities were observed during first and second week of September followed by diminishing rainfall thereafter. The duration of the crop from sowing to the third picking at the site of the EC tower was 180 days. The cumulative rainfall received from field preparation to harvest was 1190.7 mm. The rainfall received was above normal and rains continued till mid October, followed by a short spell (8.2 mm) on November 21. The dekadal mean maximum air temperature ranged from 29.4 to 34.3 ^{0}C and the mean minimum temperature ranged from 16.1 to 25.1 ^{0}C. The temperature decreased slowly down during the monsoon months due to cooling and thereafter a significant dip in the temperature was observed in the winter months of October - December. The mean sun shine hours were found to be in and around 5 h in the monsoon months with significant dips (as low as 2-3 h) due to cloudiness. Bright sun shine was observed in the post-monsoon months (October to December) with values ranging between 5.75 – 9.38 h.

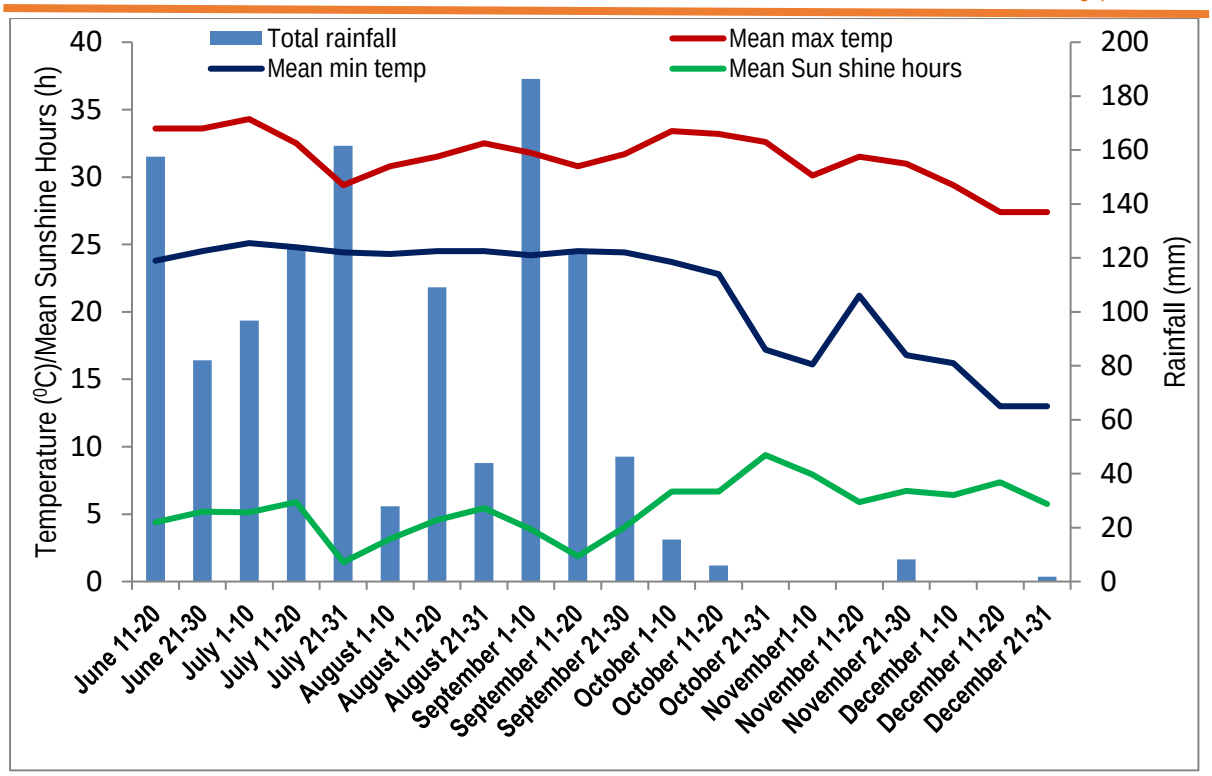

Fig. 1. Variations in meteorological parameters at the study site

Biophysical parameters of the cotton crop and yield

The mean data on biophysical attributes- plant height, LAI, dry matter of cotton crop along with seed cotton yield recorded in the footprint area of EC tower is summarized in Table 1.

Table 1. Biophysical parameters of the cotton crop during 2021-22 season

Parameter	DAS	Mean values (SD in parenthesis)
Height (cm)	30	27.7±3.3
	60	81.2±7.9
	90	110.7±10.1
	120	113.6±11.9
	150	113.4±10.3
LAI	30	0.32±0.03
	60	1.6±0.0.28
	90	2.4±0.39
	120	2.10±0.28
	150	1.70±0.25
Dry matter (kg/ha)	75	1907±298
	145	5540±915
Seed cotton yield (kg/ha)		1526±316

The height of cotton crop increased rapidly between 30 and 90 days and further increase in height ceased after 120 DAS. The LAI of the crop was low in the beginning and attained a value of 0.32 ± 0.03 at 30 days. It increased rapidly there after till 90 days and declined after 120 days as the crop senesced. The peak value of LAI was 2.4 ± 0.39 at 90 DAS. The above ground dry matter at 75 days corresponding to flowering stage was 1907 ± 298 kg/ha. It increased thereafter during the boll

formation stage and attained a value of 5540 ± 915 kg/ha at 145 days stage corresponding to first picking of open bolls. The mean seed cotton yield, from all the three pickings was 1526 ± 316 kg/ha.

Ecosystem CO_2 fluxes:

The NEE was partitioned into the GPP and R_{eco} using relationship between ecosystem respiration-temperature during the night-time. The data obtained at 30 minute interval was integrated at daily interval over the entire cotton growing season and depicted in Figure 2. The germination process of cotton seeds was completed in 5 days after sowing. During the first fortnight, soon after germination, the photosynthesis was negligible, and hence values of the GPP were found to be lower than R_{eco}. During this period, the cotton ecosystem acted as a net source for CO_2. Thereafter, the plants developed true leaves, started active photosynthesis and the values of GPP was found to be greater than R_{eco} leading to negative NEE values. Negative values of NEE indicated that more C was stored than is being released and the cotton ecosystem turned into a net sink of CO_2. The abrupt changes in NEE within the crop season was predominantly due to cloud cover that reduced incoming solar radiation and decreased photosynthesis. In general, the NEE was found to increase slowly as the crop growth progressed with peak value of nearly -4 gC m^{-2} day^{-1} during mid-September. It further decreased slowly till the last peaking. The GPP also followed similar pattern as it is proportional to the NEE. The Ecosystem respiration was found to be between 2- 4 gC m^{-2} day^{-1}, with significant variation as it is highly influenced by the minimum temperature.

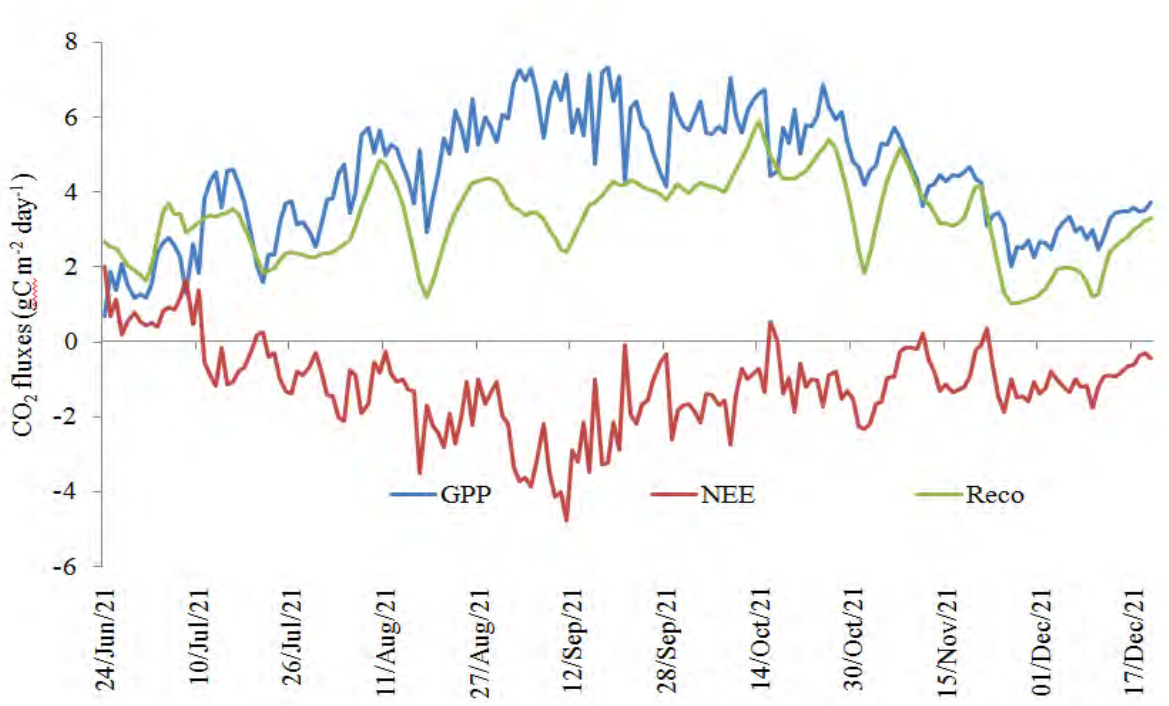

Figure 2: Temporal variations of daily ecosystem exchanges-NEE, Net Ecosystem CO2 Exchange; GPP, Gross Primary Production; R_{eco}, Ecosystem Respiration during the crop season 2021-22

Cumulative and mean carbon fluxes during each phenological phase of the cotton crop are presented in Table 3. The mean value of NEE was positive (0.9 g C m^{-2} day^{-1}) during the five day period from sowing to germination. The values turned negative thereafter, slowly increasing at a mean rate of -0.4 gC m^{-2} day^{-1} during the period to germination to squaring. High mean daily values of NEE were observed during the period from squaring to first boll opening (-1.8 to -2.1 gC m^{-2} day^{-1}). The mean values of NEE stabilized thereafter and declined during the phase between second and third picking to -0.9 gC m^{-2} day^{-1}. The mean daily values of GPP was above 5 gC m^{-2} day^{-1} during the period from

squaring to first picking when the crop was in its active growth phase. During the corresponding period the values of Reco also remained high in the range of 3.4 to 4.1 gC m^{-2} day^{-1}. The R_{eco} values later declined to 2.4 gC m^{-2} day^{-1} and 2.3 gC m^{-2} day^{-1} during the boll opening /picking period.

The highest cumulative values of GPP (278.2 gC m^{-2}), R_{eco} (184.1 gC m^{-2}) and NEE (-94.1 gC m^{-2}) were observed during the phenological phase from flowering to first boll opening. Incidentally, the values of crop height and LAI were also the highest during this period indicating a close relationship between C flux and crop growth. Through the entire growing season lasting 180 days, the cumulative GPP was 803.4 gC m^{-2}, NEE was -213.8 gC m^{-2} and Ecosystem Respiration (R_{eco}) was 589.7 gC m^{-2}.

Table 2: Quantitative estimates of CO_2 fluxes during different phenophases of cotton crop and for the entire crop season

Phenological stage			Carbon dioxide fluxes Cumulative (gC m^{-2})			Daily mean (gC m^{-2} day^{-1})		
	Date	DAS	GPP	NEE	R_{eco}	GPP	NEE	R_{eco}
Sowing-germination	24-28 June	1-5	7.5	4.5	12.1	1.5	0.9	2.4
Germination-squaring	29 June-10 August	6-48	139.8	-17.95	121.9	3.3	-0.4	2.8
Squaring –flowering initiation	11 August- 2 Sept.	49-71	119.7	-41.09	78.6	5.2	-1.8	3.4
Flowering initiation -First boll opening	3 Sept – 18 October	72-117	278.2	-94.1	184.1	6.1	-2.1	4.0
First boll opening to first picking	19 Oct-12 November	118-142	131.2	-27.44	103.7	5.1	-1.1	4.1
First picking–second picking	13 Nov-6 December	143-166	82.1	-25.5	56.6	3.4	-1.1	2.4
Second picking to third picking	7 Dec-20December	167-180	44.9	-12.3	32.7	3.2	-0.9	2.3
Crop season 2021-22	June 24 to Dec 20		803.4	-213.8	589.7			

Discussion

This study analyses the ecosystem exchange of CO_2 from a typical rainfed cotton production system in Central India during 2021-22 crop season using Eddy Covariance technique. The growth of the cotton plants in the footprint area of the EC tower followed a typical sigmoidal pattern, characterized by a slow initial growth phase, followed by a rapid grand growth phase from squaring to boll opening and a gradual decline during the boll bursting/harvesting period. The crop plants in the footprint area of the EC tower attained a maximum height of 113.6±11.9 cm at 120 days after sowing and a maximum LAI of 2.4±0.39. Similar values for crop height and LAI for rainfed cotton were reported earlier by several workers including Kumar et al. (2018). However the values were slightly lower than those reported at the same site (Chakraborty et al. 2022). LAI was the main driving force governing C fluxes (Guo et al. 2021).

The cumulative seasonal carbon dioxide exchange estimated in terms of GPP, R_{eco} and NEE were 803.4 gC m^{-2}, 589.7 gC m^{-2} and -213.8 gC m^{-2} respectively. The seasonal cumulative values of GPP for rained cotton obtained in the present investigation were comparable to the values reported recently by Sharma (2017) and Menefee et al. (2020) for dryland cotton from Texas, USA. Falge et al. (2002) reported that leaf-area index, physiological capacity, meteorological conditions and the length of the growing season are the main factors governing the GPP. The R_{eco} value obtained in the present study, 589.7 gC m^{-2}, was slightly lower than the corresponding values viz. 656.5 gC m^{-2} and 672.0 gC m^{-2} obtained during the crop seasons of 2018-19 and 2020-21 respectively at the same site (Chakraborty et al. 2022). Such inter-seasonal variations in the C fluxes under similar management practices could be attributed to the differences in the prevailing weather parameters, like air temperature, solar radiation (Fong et al. 2020) or its derivative photosynthetic photon flux density and soil moisture.

The mean daily values of GPP, R_{eco} and NEE varied during the different phenological phases of crop ontogeny (Table 2). The values of both GPP and R_{eco} were high during the phenological phase between flowering and first open boll and these values declined thereafter. The decline in GPP and R_{eco} during the months of November and December could be due to a combination of a decline in temperature due to the onset of winter and due to the senescence of the crop. A decline in LAI during the later stages would have also contributed to decline in C fluxes.

The ratio of NEE to GPP is the C use efficiency, representing the fraction of carbon absorbed by an ecosystem (Gough, 2011). In the present investigation this ratio was 26.6%. Negative values of NEE indicated that more C is being removed than is released by the production system. The NEE value obtained in the present investigation was -213.8 gC m^{-2} inferring that rainfed cotton production system in Central India is a sink for atmospheric CO_2 and around 2.14 tonnes of C was sequestered during the cotton cropping season 2021-22.

Materials and methods

CO_2 fluxes were measured from an EC flux tower located at the research farm of ICAR-Central Institute for Cotton Research, Nagpur, India (21.03^{0}N, 79.06^{0}E, 316 m above mean sea level). The climate of the study area is characterized as dry, sub-humid with an average precipitation of 1083 mm. The mean monthly climatic parameters of the study area (Nandankar 2011) are presented in Fig. 3.

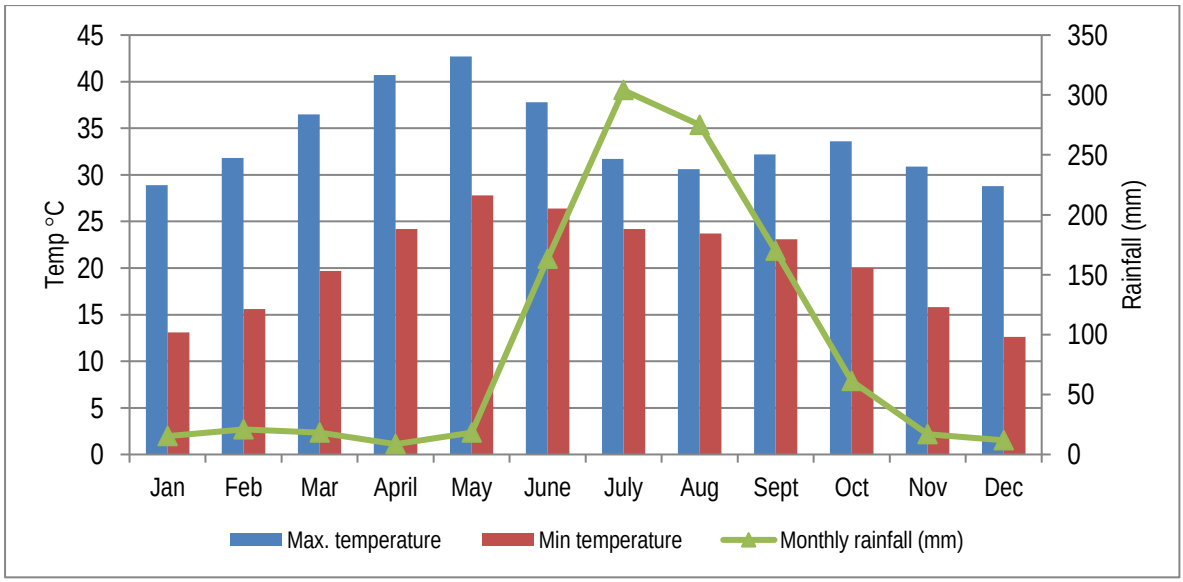

Fig. 3. Mean (1969-2010) monthly values of temperature and rainfall at the experimental location

The soil of the study area has silty clay texture (55% clay, 44% silt, 1% sand (Vertisol,Typic Haplusterts) with bulk density 1.35 Mg m^{-3}, pH 7.95 and organic carbon 0.69%. The topography of the experimental site is flat with around 0.3% slope from east to west.

Rainfed cotton crop was cultivated in a contiguous area of 60 hectares. BG II hybrid cotton was sown at the tower site on 24th June 2021 and the third (last) picking was done on 20th Dec 2021. Cotton was sown in the footprint area between the 22nd and 28th June and harvesting (manual picking) commenced in November and continued till the end of December. The plant population ranged from 25000-37000 plants/ha. The crop was raised using standard production and protection practices.

From each of the nine fields in the footprint area of the EC tower, five sampling blocks measuring 6m x 6m area, each containing around 90-130 plants were earmarked for recording biophysical data and

yield. Plant height and Leaf Area Index (LAI) were measured at 60, 90, 120 and 150 days after sowing (DAS). The LAI was measured using canopy analyzer (LI 2200, LICOR Inc, USA). Above ground dry biomass was estimated at 75 days and at first picking. Seed cotton yield was calculated on the basis of the total cotton harvested from the whole sampling block over three pickings.

The flux tower data was processed using EddyPro Software (version 6.1.LICOR, US) following standard procedures with all the correction and compensation, followed by gap filling and uncertainty analysis. Detailed methodology is available in (Chakraborty et al. 2022). The CO_2 flux was calculated using the equation by Baldocchi et al. (1988). The net ecosystem exchange (NEE) values obtained at 30 minutes internal were partitioned into GPP and R_{eco} using the relationship established by Reichstein et al. (2005). Thus seamless time-series of GPP, NEE and R_{eco} were further analyzed for their dynamics over the crop season.

Conclusions

During the cotton growing season 2021-22, spanning over 180 days, the cumulative GPP was 803.4 gC m^{-2}, NEE was -213.8 gC m^{-2} and Ecosystem Respiration (R_{eco}) was 589.7 gC m^{-2}. The ratio of R_{eco} to GPP was 73.4 percent and the ratio of NEE to GPP was 26.6%. The rainfed cotton crop is a strong net sink for capturing atmospheric CO_2. Around 2.14 tonnes C ha^{-1} was sequestered during the crop season. The C fluxes were found to be highly influenced by crop phenological stage, biophysical parameters and the prevailing weather condition. The EC datasets generated from this study can be used to validate cotton crop simulation models and for up-scaling the carbon fluxes to regional scale using remote sensing proxies.

Acknowledgement

The financial support provided by the Indian Space Research Organization- - Geosphere Biosphere Program under the soil-vegetation-atmosphere-flux (SVAF) project of National Carbon Project to undertake this investigation is gratefully acknowledged.

Abbreviations: DAS-Days After Sowing, EC- Eddy Covariance, LAI-Leaf Area Index, GPP- Gross primary productivity, NEE-Net Ecosystem Exchange, R_{eco}-Ecosystem Respiration, SD-Standard Deviation

References

1. Bai J, Wang J, Chen X, Luo G, Shi H, Li L, Li J. Seasonal and inter-annual variations in carbon fluxes and evapotranspiration over cotton field under drip irrigation with plastic mulch in an arid region of Northwest China. Journal of Arid Land. 2015; 7(2), 272-284. https://doi.org/10.1007/s40333-014-0012-x
2. Baldocchi D, Falge E, Gu L, Olson R, Hollinger D, et al. FLUXNET: A new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities, Bull. Amer. Meteor. Soc. 2001; 82, 2415-2434
3. Chakraborty A, Venugopalan MV, Mani JK, Bagadkar AJ, Manikandan A. Rainfed cotton crop in central India is a strong net CO2 sink: An eddy covariance-based analysis of ecosystem fluxes. Field Crops Research. 2022; Volume 286, 108595.
4. Falge E, Baldocchi D, Tenhunen J, Aubinet M, et al. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agricultural and Forest Meteorology. 2002; Vol 113, Issues 1–4, 2002, Pages 53-74, doi.org/10.1016/S0168-1923(02)00102-8
5. Fong BN, Reba ML, Teague TG, Runkle BR, Suvočarev K. Eddy covariance measurements of carbon dioxide and water fluxes in US mid-south cotton production. Agriculture, Ecosystems & Environment. 2020;292:106813. https://doi.org/10. 1016/j.agee.2019.106813
6. Gough CM. Terrestrial Primary Production: Fuel for Life. *Nature Education Knowledge.* 2013; (10):28
7. Guo H, Li S, Wong FL, Qin S, Wang Y, Yang D, Lam HM. Drivers of carbon flux in drip irrigation maize fields in northwest China. Carbon Balance Manag. 2021; 16:12. doi: 10.1186/s13021-021-00176-5. PMID: 33939031; PMCID: PMC8091713.
8. Kumar A, Karunakar AP, Nath A. Influences of Different Planting Density on Growth, Yield Attributes and Yields of hirsutum, arboreum and Bt Cotton. Int.J.Curr.Microbiol.App.Sci. 2018; 7(6): 1872-1888

9. Li X, Liu L, Yang H, Li Y. Relationships between carbon fluxes and environmental factors in a drip-irrigated, film-mulched cotton field in arid region. Plos one. 2018; 13(2), e0192467. https://doi.org/10.1371/journal.pone.0192467

10. Menefee D, Rajan N, Cui S, Bagavathiannan M, Schnell R, West J. Carbon exchange of a dryland cotton field and its relationship with Planet Scope remote sensing data. Agricultural and Forest Meteorology. 2020; 294, 108130. https://doi.org/10.1016/j.agrformet.2020.108130

11. Ming G, Hu H, Tian F, Khan MYA, Zhang Q. Carbon budget for a plastic-film mulched and drip-irrigated cotton field in an oasis of Northwest China. Agricultural and Forest Meteorology. 2021; 306:108447. https://doi.org/10.1016/j.agrformet.2021. 108447

12. Nandankar PK, Dewangan PLK, Surpam R. Climatic features of Nagpur, Regional Meteorological Centre, India Meteorological Department, Nagpur, India. 2011;p. 5.

13. Papale D, Reichstein M, Aubinet M, Canfora E, Bernhofer C, Kutsch W, Longdoz B, Rambal S, Valentini R, Vesala T, et al. Towards a standardized processing of Net Ecosystem Exchange measured with eddy covariance technique: Algorithms and uncertainty estimation. *Biogeosciences*. 2006; *3*:571–583.

14. Reichstein M, Falge E, Baldocchi D, Papale D, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Gilmanov T, Granier A, Grünwald T. On the separation of Net Ecosystem Exchange into assimilation and Ecosystem respiration: review and improved algorithm. Global change biology. 2005; 11(9):1424-1439. https://doi.org/10.1111/j.1365-2486.2005.001002.x

15. Schulze ED, Wirth C, Heimann M. Managing forests after Kyoto. Science. 2000; 289:2058–2059

16. Shah H, Hellegers P, Siderius C. Climate risk to agriculture: A synthesis to define different types of critical moments. Climate Risk Management. 2021;34:100378

17. Sharma S. Carbon, Evapotranspiration and Energy Balance Dynamics of Potential Bioenergy Crops Compared to Cotton in the Southern Great Plains. Doctoral dissertation, Texas A & M University, USA. 2017; Available electronically from https://hdl .handle.net /1969.1/161571.

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Comparative RNAi Efficiency in Cotton Sap Feeders through DSRNA Mediated Gene Knockdown

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Abstract

Background: *Cotton in India after the introduction of Bt-cotton has been facing the menace of sucking insect-pests such as Amrasca biguttulla biguttulla, Bemisia tabaci, Thrips tabaci and Phenacoccus solenopsis. The main stray control strategy of these insects rely only on synthetic insecticides due to non-availability of effective Bt-toxins or other alternative control strategies. Thus alternate novel pest management strategies are the need of hour and in this direction RNA interference (RNAi) has emerged as potential future tool for the management of insect-pests. To explore vital and potential genes in important sap-sucking insects of cotton, the transcriptome sequencing data was used to pull out the respective target genes sequences and the dsRNA against the respective genes was synthesized. Methods were developed for feeding/injecting dsRNA incorporated in diet against genes like Aquaporin (AQP) and Calcitonin (CAL), inhibitor of apoptosis (IAP), heat shock proteins (HSP20), vATPase, SNF (targeting transcription and expression).*

Results: *In case of cotton leafhopper, feeding of 500 ng/ul dsRNA targeting SNF7, IAP, AQP1 and vATPase genes caused 56.17 -77.12 per cent knockdown of targeted genes compared to control. In whitefly, the feeding of 400 ng/ul of dsRNA targeting AQP, CAL, hsp20 genes caused 1.25, 3.32 and 1.09-fold downregulation of targeted genes compared to control, respectively. In thrips, knockdown of SNF by feeding of 500 ng/ul of dsRNA resulted in 93 % knockdown of target gene compared to control. In case of mealybug 10 µg of dsRNA targeting CAL gene injected in 3rd instar nymph resulted in ~36-67% knockdown of the targeted gene.*

Conclusion: *The results clearly indicate possibility of gene knockdown in cotton pests through dsRNA feeding or injection, however variable response was observed towards RNAi among these insects. The results presented here shed light on the potential of RNAi in cotton sap feeders and which could be shaped into efficient pest management strategies to affect physiological and molecular pathways in these targeted insect pests.*

Keywords:
India, cotton, sucking pests, pest management, RNAi

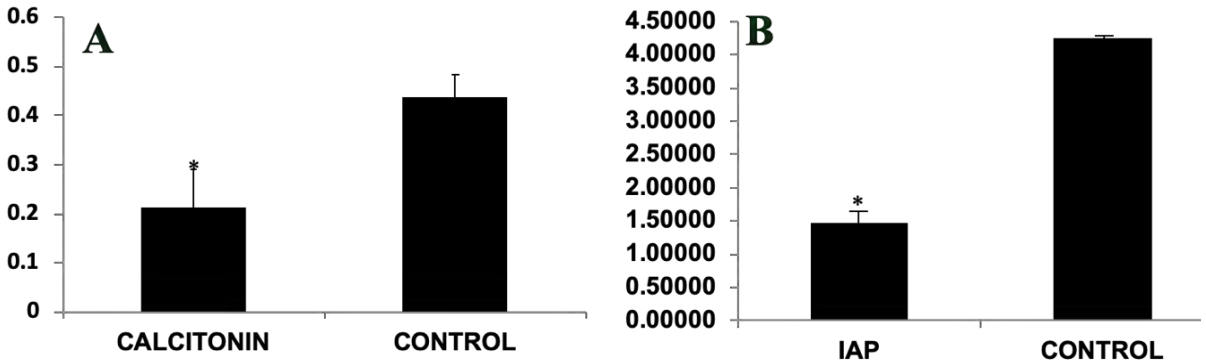
Background

Post Bt-cotton era, the cotton crop has witnessed major change in the insect-pest scenario in north India (Singh et al., 2016). Before the introduction of Bt-cotton in 2005 in Northern India, the cotton crop was under the serious threat of bollworms. The state of art pest management technology rightly addressed the problem of bollworms and was widely adopted by the cotton growers of the region (Kranthi, 2012). Post Bt cotton introduction, the cotton crop has witnessed the upsurge in one or the other sucking insect-pests of cotton. Just after the year of introduction in 2007, the north India witnessed unmanageable population of cotton mealybug Phenacoccus solenopsis, which caused huge yield losses to the cotton crop (Dhaliwal et al., 2010). The other major present-day threats to cotton production in north India from 2010 onwards are whitefly, thrips and leafhopper. Whitefly, Bemisia tabaci is a global pest capable of feeding on hundreds of plant species and transmits several major plant viruses (Brown, 2010). During 2015–2016, the massive infestation of whitefly in north India resulted in huge loss to cotton crop. Cotton leafhopper, Amrasca biguttula biguttula, commonly known as jassid, is among the most economically important sucking pests of cotton after whitefly. Leafhopper infested tender leaves become yellow, extremely small and show a mosaic pattern of yellowing which in the case of severe infestation turns brick red colour resulting in typical “hopper burn” symptom. The pest has potential of causing 25-45 % loss in seed cotton yield (Dhawan and Sidhu, 1986). Besides these two pests, since last few years Thrips tabaci is also gaining the status of major cotton pest in north India. The infestation of this pest was restricted only to seedling stage where it caused stunting of plant

and deformities in leaves. During previous few years the incidence has also been noticed in full grown plants where its damage results in brittle cotton leaves, which turn upright and have golden-silvery discoloration on the underside due to heavy thrips infestation. The present-day cotton has witnessed the upsurge in all these sap sucking insects with population levels above ETL throughout the cropping season in one or the other instances (Singh et al., 2016). The main stray control strategy for the management of these insects still relies on chemical insecticides. However, the indiscriminate use of insecticides has led to the development of high-level resistance against these chemicals in these sucking pests (Kranthi et al., 2002). Besides, the usage of synthetic insecticides is also associated with a number of environmental issues such as insecticide residues in soil and water and effects on non-targets, etc. In this direction, RNA interference (RNAi) has come up as a powerful tool for analysis of gene functions through down regulation of genes in vitro. RNAi has been successfully demonstrated in numerous insect species (Gu and Knipple, 2013; Kim et al., 2015). More significantly, expression of dsRNA directed against potent insect genes in transgenic plants has also given protection against insect-pests with a futuristic advantage that development of resistance in insects to gene specific dsRNA is very rare. Cotton at present is the only accepted transgenic in India so the scope of RNAi based transgenics or dsRNA based sprays hold good potential in this crop. Keeping in view all these facts and the potential of RNAi technology, the present study evaluates comparative knockdown efficiency of different genes in cotton sap feeders through dsRNA feeding or injection.

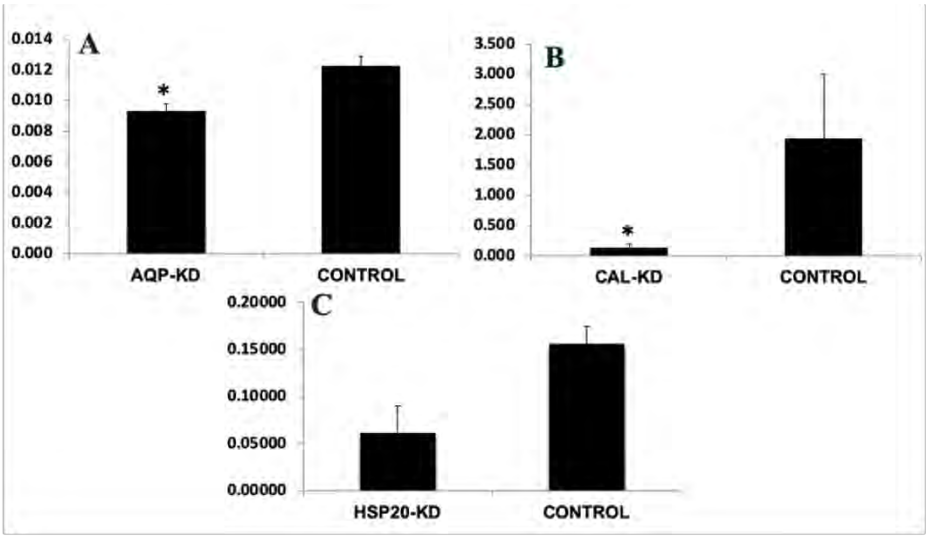
Results and Discussion

Injecting dsRNA (10µg) against Calcitonin and IAP (inhibitor of apoptosis) in P. solenopsis resulted in 50.9 and 65.5 % knockdown of these genes compared to the dsGFP control insects (Figure 1). The membrane feeding of dsRNA in sucrose diet didn't worked for mealybug, even addition of some food colour dyes (green, yellow) didn't affect the feeding preference of this insect to the artificial diet. Thus, injecting dsRNA directly into the haemolymph was preferred over feeding. In B. tabaci the knockdown of aquaporin (AQP), calcitonin (CAL) and heat shock protein 20 (HSP20) genes using 500 ng of respective dsRNA/ µl of sucrose diet caused 23.07, 87.6 and 61.02 % decrease in the mRNA transcripts of the respective genes as compared to the dsGFP fed whiteflies (Figure 2). The dsRNA (500 ng dsRNA/ µl of sucrose diet) mediated knockdown of IAP and AQP in A. biguttulla biguttulla resulted in significant reduction in the mRNA levels of these genes compared to dsGFP fed insects (Figure 3). RNAi in T. tabaci using membrane feeding assay (500 ng dsRNA/ µl of sucrose diet) resulted in 93.9 and 93.2 % knockdown in the expression of SNF7 and AQP as compared to the dsGFP control insects (Figure 4).



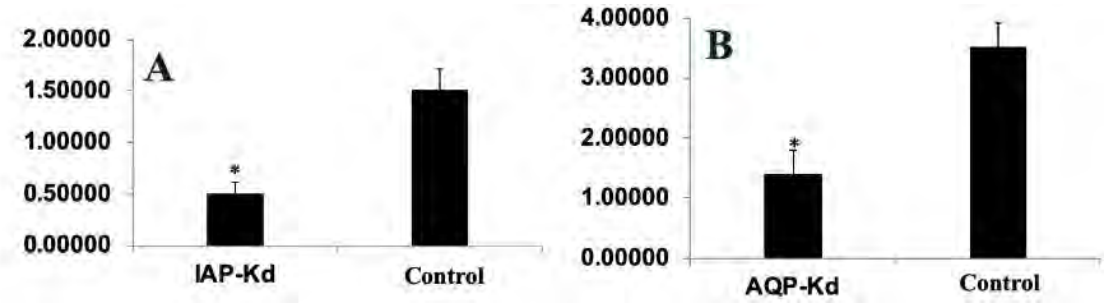
Notes: Expression normalized with 28S and compared to GFP control. * Significant differences (P ≤ 0.05, Student's t-test)

Figure 1: *Expression of candidate genes in P. solenopsis fed with dsRNA constructs injected (A) CAL (Calcitonin) knockdown (50.9%) (B) IAP (Inhibitor of apoptosis) knockdown (65.5%)*



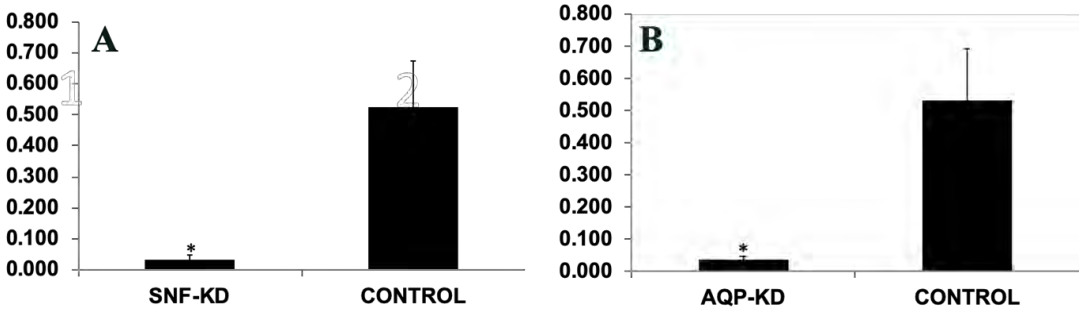
Notes: Expression normalized with Tubulin (Tub) and compared to GFP control. * Significant differences (P ≤ 0.05, Student's t-test).

Figure 2: *Expression of candidate genes in B. tabaci fed with dsRNA constructs incorporated liquid diet by membrane feeding assay (a) AQP (Aquaporin) knockdown (23.7%), (b) CAL (Calcitonin) knockdown (87.6%), (c) Hsp20 (Heat shock protein 20) knockdown (61.02%).*



Notes: Expression normalized with ribosomal protein 13 (RP13) and compared to GFP control. * Significant differences (P ≤ 0.05, Student's t-test)

Fig. 3. *Expression of candidate genes in A. biguttulla biguttulla fed with dsRNA constructs incorporated liquid diet by membrane feeding assay (A) Abb IAP (Inhibitor of Apoptosis) knockdown (66.5%) (B) Abb AQP (Aquaporin) knockdown (59.8%)*



Notes: Expression normalized with GSTD2 (Glutathione S transferase D2) and compared to GFP control. *Significant differences (P ≤ 0.05, Student's t-test).

Figure 4 *Expression of candidate genes in T. tabaci fed with dsRNA constructs (A) SNF7 (Multivesicular protein) knockdown (93.9%) (B) AQP (Aquaporin) knockdown (93.2%).*

RNAi response was variable among cotton sap feeders with thrips showing highly efficient processing of dsRNA compared to the other insect species. The RNAi response in insects is highly variable across

different insect orders (Shukla et al., 2016; Singh et al., 2017). Within the same insect the RNAi efficiency depends upon length of dsRNA, target gene, delivery method and even the response is variable with siRNA or dsRNA (Garbutt et al., 2013; Samarasinghe et al., 2011; Terenius et al., 2011). The selection of correct housekeeping genes for normalization also plays a key role in the relative expression in RNAi studies (Singh et al., 2018). Among the studied insects three belong to Hemiptera and one to Thysanoptera, the knockdown results indicate higher RNAi efficiency in thrips compared to the other insect species. Earlier studies suggest that coleopterans show highest RNAi efficiency followed by hemipterans and dipterans, on contrary the RNAi in lepidopterans showed poor response (Christiaens and Smagghe, 2014; Singh et al., 2017).

The hemipterans dsRNA specific gut nucleases are the major bottleneck for RNAi efficiency as these enzymes may partially or completely degrade the dsRNA in the gut of these insects (Christiaens and Smagghe, 2014). Our studies also indicate moderate level of knockdown of different genes in whitefly, cotton leafhopper and mealybug compared to thrips. This efficiency has been even variable among the various genes within the same insects. Like VATpase knockdown efficiency in cotton leaf hopper was 31.6 % and that of SNF7 was 77.1 % compared to the dsGFP control. The present studies gave in overview of scope RNAi in cotton pests which can be further studied in detailed to improve RNAi efficiency as well as the delivery method. Cotton is the only approved and widely acceptable transgenic in India, so the scope of further genetic manipulation in cotton is higher compared other food crops. Recent research with these insects suggest various ways for the enhancement of RNAi and its delivery in the insect system. However, dsRNA based transgenics or dsRNA-based sprays are the two major researchable issues, which need to be studied in details for bringing RNAi technology in field.

Methods

Maintenance of insect cultures: Insect cultures of mealybug, whitefly, cotton leafhopper and thrips were maintained in walk-in environmental chambers in insect proof cages on cotton plants.

RNA isolation, cDNA and dsRNA synthesis: The total RNA was isolated using Trizol reagent (Sigma) from different insects (mealybug- a adult female, whitefly-10-15 adults, cotton leaf hopper-5-late nymphs and thrips 15-20 adults) as per manufacturer's instructions. The cDNA was synthesized using First Strand cDNA Synthesis Kit (ThermoFisher Scientific) as per manufacturer's instructions**.** The gene specific template for dsRNA was amplified from the cDNA using PCR and gene specific primers (Table 1) and purified using Nucleospin Gel and PCR Cleanup kit (Macherey–Nagel) as per instruction manual. Gene specific dsRNA using MEGAscript™ RNAi Kit (Thermo Fisher Scientific) using with T7 sequences adapters at 5' of both reverse and forward primers (Table 1).

dsRNA feeding assays and injections: The double stranded RNA was of respective gene was fed to the respective insects in triplicates each comprising of five groups of ~50 adults for whiteflies, 10 groups of 5 nymphs for cotton leafhopper and 10 groups of ~15 adult thrips. The feeding as done as per earlier described methodologies for whitefly, cotton leafhopper and thrips (Brar et al., 2018; Singh et al., 2019; Thakur et al., 2014; Upadhyay et al., 2013). For mealybug the injection using Nanoject (Durmound Scientific, USA) in triplicates with each replicate comprising of 10 adult females as per earlier described methodology (Singh et al., 2019). Three days post-injection or feeding the live insects were processed for RNA isolation and cDNA synthesis as per methodologies described in the previous section. Gene known down was estimated through the quantitative expression of 100-150 bp of each gene carried out in RT-PCR (Roche Lightcyler) using 5µl of 5x SYBR Premix Ex Taq (Tli RNase H Plus) (Clontech takara), 0.2 µl of 10pmol primers (Table 1) and 1µl of 1/10 cDNA template in a 10µl reaction. The expression data in each knockdown assay was normalized using specific set of housekeeping genes for each insect (Table 1). The relative expression was estimated using delta CT method and data were analyzed using Student's t-test at 5% significance level.

Table 1 List of different primers used for dsRNA template amplification and synthesis, relative expression studies and normalization of expression data for each insect.

Primer name	Sequence 5'.....3'
	Phenacoccus solenopsis
dsPS_CAL_F	TAATACGACTCACTATACCTATGGTTGGTATGGTACA
dsPS_CAL_R	TAATACGACTCACTATAGATCGACGAATGAGGAGTAT
qPs_CAL_F:	CGGCGAAGTGATTTCAGCTATT
qPs_CAL_R	GTATATGTGGCTGCGTGCTATG
dsPS_IAP_F	TAATACGACTCACTATAGGTAGAGCATCGTCGTTATTC
dsPS_IAP_R	TAATACGACTCACTATAGCTTCCTCTCTGATCAAATC
qPS_IAP_F	AATAACGTATCCGGCCAAGG
qPS_IAP_R	CGACCGAGTTGGCAGAATTA
	Bemisia tabaci
qBt_Hsp20_F	GGAGAAAATGTTTCCAACCGTA
qBt_Hsp20_R	TCTCAGAGAGCACAGATAGCTAA
dsBt_Cal_F	TAATACGACTCACTATAATTTGATTGGTGAATGGAGCAG
dsBt_Cal_R	TAATACGACTCACTATACAAATGACAGTCGCAAATGAGT
qBt_Cal_F	ATTTGATTGGTGAATGGAGCAG
qBt_Cal_R	CAAATGACAGTCGCAAATGAGT
dsBt_Aqp_F	TAATACGACTCACTATATATGAACCCTGCTCGATCATTAG
dsBt_Aqp_R	TAATACGACTCACTATACTTTGAAAGTGATGGCGTAAAG
qBt_Aqp_F	TATGAACCCTGCTCGATCATTAG
qBt_Aqp_R	CTTTGAAAGTGATGGCGTAAAG
hBt_GST_F	TGGAGCAGTTGACTTGAGCA
hBt_GST_R	GCGTTGTTTAAAGGCGGCAA
hBt_UbiqT_F	CGACTCATCTTCTCAGGGAAAC
hBt_UbiqT_R	GCCTCCTCTCAATGCTAGAACA
	Amrasca biguttulla biguttulla
dsAbb_AQPD_F	TAATACGACTCACTATAGGGACTGCCAAACATGGATGGAT
dsAbb_AQP_F	TAATACGACTCACTATAGGGGGAGCAGTGATTGAAGGCATA
qAbb_Aqpd_F	CCAGTACAAGCTCCAATCCAGT
qAbb_Aqpd_R	GGTGGCTGCATTCAACTACTCT
dsAbb_Snf7_F	TAATACGACTCACTATAGGGGCTTTGGCAGTGGTCTTAGC
dsAbb_Snf7_R	TAATACGACTCACTATAGGGTAAAAGAGCGGCAATCCAAG
qAbb_Snf7_F	GAGCAGTGGAGAAACGAATGAC
qAbb_Snf7_R	ACGGGCGTACACAGGTTTACTT
hAbb_Ubiq_F1	CGATTCGACCATGCCTTACTT
hAbb_Ubiq_R1	GAGATTGACACGCTCCTGAAA
hAbb_Actin_F	CAGGCTGTGCTTTCTCTGTATG
hAbb_Actin_R	GATATGACTCGCTATCGGCATC
	Thrips tabaci
dsTb_AQP_F	TAATACGACTCACTATAGGGGAGATGAAGTACACGATGGC
dsTb_AQP_R	TAATACGACTCACTATAGGGCGCAGCACATCTGGATAA
qTb_AQP_F	GATGCACTGCGAGATGAAGTA
qTb_AQP_R	TGTTTCGCACACATCAGTGG
dsTb_SNF_F	TAATACGACTCACTATAGGGTTTGCGAGGAGAGCTTATGG
dsTb_SNF_R	TAATACGACTCACTATAGGG GAGGGCAGCAATTCCTACTT
qTb_SNF_F	AAGGCCTTGAGGAGGAATTAAG
qTb_SNF_R	TGAAGATCCCTGTCATCACTTTC
hTb_Actin_F	CCCTCCACCATCAAGATCAA
hTb_Actin_R	GAGATCCACATGGACTGGAA
hTb_18s_F	CTCGAAATGCTCGAGGAAAG
hTb_18s_R	GAATCAGGACGTGTCTCTAACC

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References

1. Brar, A.S., Gupta, M., Pandher, S. and Singh, S. (2018), Modified Diet Assay for Studying Feeding RNAi in Cotton Leafhopper , Amrasca Biguttula Biguttula, Protocols.Io, available at: https://www.protocols.io/view/modified-diet-assay-for-studying-feeding-rnai-in-c-ksgcwbw.pdf.
2. Brown, C.S., Goodwin, P.C. and Sorger, P.K. (2001), “Image metrics in the statistical analysis of DNA microarray data”, Proceedings of the National Academy of Sciences, Vol. 98 No. 16, pp. 8944–8949.
3. Brown, J.K. (2010), “Phylogenetic biology of the Bemisia tabaci sibling species group”, edited by Stansly, P.A. and Naranjo, S.E., Springer, New York.
4. Christiaens, O. and Smagghe, G. (2014), “The challenge of RNAi-mediated control of hemipterans”, Current Opinion in Insect Science, Elsevier Inc, Vol. 6, pp. 15–21.
5. Dhaliwal, G., Jindal, V. and Dhawan, A. (2010), “Insect pest problems and crop losses : changing trends”, Indian Journal of Ecology., Vol. 37, pp. 1-7.
6. Dhawan, A. and Sidhu, A. (1986), “Assessment of losses due to attack of cotton jassid on hirsutum cotton.”, Indian J Plant Prot., Vol. 14, pp. 45–50.
7. Garbutt, J.S., Bellés, X., Richards, E.H. and Reynolds, S.E. (2013), “Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: Evidence from Manduca sexta and Blattella germanica”, Journal of Insect Physiology, Vol. 59 No. 2, pp. 171–178.
8. Gu, L. and Knipple, D.C. (2013), “Recent advances in RNA interference research in insects: Implications for future insect pest management strategies”, Crop Protection, Vol. 45 No. 2013, pp. 36–40.
9. Kim, Y.H., Soumaila Issa, M., Cooper, A.M.W. and Zhu, K.Y. (2015), “RNA interference: Applications and advances in insect toxicology and insect pest management”, Pesticide Biochemistry and Physiology, Elsevier Inc., Vol. 120, pp. 109–117.
10. Kranthi, K.R. (2012), Bt Cotton Q & A, Indian Society for Cotton Improvement.
11. Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S. and Russell, D.A. (2002), “Insecticide resistance in five major insect pests of cotton in India”, Crop Protection, available at:https://doi.org/10.1016/S0261-2194(01)00131-4.
12. Samarasinghe, B., Knox, D.P. and Britton, C. (2011), “Factors affecting susceptibility to RNA interference in Haemonchus contortus and in vivo silencing of an H11 aminopeptidase gene”, International Journal for Parasitology, England, England, Vol. 41 No. 1, pp. 51–59.
13. Satnam Singh, Suneet Pandher, Pankaj Rathore, Aman Sharma, K.S. and R.K.G. (2016), “From conventional to Bt cotton and bollworms to whitefly: Cotton cultivation under threat in northern India.”, Proceedings of Beltwide Cotton Conferences, New Orleans, LA.
14. Shukla, J.N., Kalsi, M., Sethi, A., Narva, K.E., Fishilevich, E., Singh, S., Mogilicherla, K., et al. (2016), “Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects”, RNA Biology, Vol. 13 No. 7, pp. 656–669.
15. Singh, I.K., Singh, S., Mogilicherla, K., Shukla, J.N., Palli, S.R., Inderkant, S., Singh, S., et al. (2017), “Comparative analysis of double-stranded RNA degradation and processing in insects”, Scientific Reports, Vol. 7 No. 1, p. 17059.
16. Singh, S., Gupta, M., Pandher, S., Kaur, G., Goel, N. and Rathore, P. (2019), “Using de novo transcriptome assembly and analysis to study RNAi in Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae).”, Scientific Reports, Vol. 9 No. 1, p. 13710.
17. Singh, S., Gupta, M., Pandher, S., Kaur, G., Goel, N., Rathore, P. and Palli, S.R. (2019), “RNA sequencing, selection of reference genes and demonstration of feeding RNAi in Thrips tabaci (Lind.) (Thysanoptera: Thripidae)”, BMC Molecular Biology, Vol. 20 No. 1, available at:https://doi.org/10.1186/s12867-019-0123-1.
18. Singh, S., Pandher, S., Gupta, M., Kaur, G. and Rathore, P. (2018), “Reference Gene Selection in Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) and Their Normalization Impact on Gene Expression in RNAi Studies”, Journal of Economic Entomology, Vol. 112 No. X, pp. 1–11.
19. Suneet Pandher, Satnam Singh, Pankaj Rathore, M.G. and S.S. (2014), “Elucidating mechanism of tolerance in Gossypium hirsutum germplasm to cotton leaf hopper, Amrasca bigutulla (Ishida)”, Proceedings of National Symposium on Crop Improvement for Inclusive Sustainable Develeopemt, Punjab Agricultural University, Ludhiana-141004, pp. 487–488.
20. Terenius, O., Papanicolaou, A., Garbutt, J.S., Eleftherianos, I., Huvenne, H., Kanginakudru, S., Albrechtsen, M., et al. (2011), “RNA interference in Lepidoptera: An overview of successful and unsuccessful studies and implications for experimental design”, Journal of Insect Physiology, available at:https://doi.org/10.1016/j.jinsphys.2010.11.006.
21. Thakur, N., Upadhyay, S.K., Verma, P.C., Chandrashekar, K., Tuli, R. and Singh, P.K. (2014), “Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of v-ATPase a gene”, PLoS ONE, available at:https://doi.org/10.1371/journal.pone.0087235.
22. Upadhyay, S.K., Dixit, S., Sharma, S., Singh, H., Kumar, J., Verma, P.C. and Chandrashekar, K. (2013), “SiRNA machinery in whitefly (Bemisia tabaci)”, PLoS ONE, available at:https://doi.org/10.1371/journal.pone.0083692.

Detection and Distribution of Cotton Leafroll Dwarf Virus Infecting Cotton in the Mid-Southwestern United States

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Abstract

***Background**: Cotton leafroll dwarf disease is caused by the Cotton leafroll dwarf virus (CLRDV) which belongs to the genus Polerovirus and family Luteovridiae. CLRDV was first reported from Alabama in 2018 and poses a new challenge to cotton production in the United States (US). The purpose of this work was to detect and determine the distribution of CLRDV in three mid-southwestern states (Kansas, Oklahoma, and Texas) during the 2019 growing season.*

***Results:** During the first surveys, more than 104 leaf samples showing virus-like symptoms were collected from cotton plants in different areas located in the above three states. Symptoms observed on cotton plants included leaf yellowing, discoloration, reddening, and short internodes with reduced or small boll sets. Total RNA was extracted from individual samples and tested by reverse-transcription polymerase chain reaction (RT-PCR) using virus-specific primers of CLRDV. Nearly 50% of the samples were positive which confirmed the presence of CLRDV in samples collected from all three states. However, the disease incidence varied from field to field.*

***Conclusions:** These Results indicate the occurrence and distribution of CLRDV in different regions of Kansas, Oklahoma, and Texas and the virus could pose a threat to the production of cotton in the future.*

Keywords: *Cotton, Etiology, Cotton blue disease, Epidemiology, Polerovirus, Virus etiology, Cotton leafroll dwarf virus*

Background

Cotton is one of the most economical cash crops worldwide, which plays an important role in the textile industry. In the United States (U.S.) cotton is one of the leading cash crops which was grown in more than 17 states on an acreage of 13,720,000 acres during the 2019 growing season (Ali and Kater, 2017; NASS, 2019). A healthy crop of cotton is one of the priorities to sustain the productivity of cotton for various industries.

Several diseases caused by various pathogens have been reported to infect cotton worldwide. Among them, there are a few virus diseases, which cause a significant reduction in cotton yield. So far three RNA viruses: cotton bunchy top virus (CBTV) in Australia, cotton leaf roll dwarf virus (CLRDV) in Brazil/Argentina, and Tobacco streak virus (TSV) while DNA viruses: cotton leaf curl virus (CLCuV) in India and Pakistan and cotton leaf crumple virus (CLCrV) in the U.S. have been reported from cotton plants (King et al., 2009).

Recently, CLRDV has been reported for the first in 2017 in cotton fields in Alabama (Avelar et al., 2019). Subsequently, the virus has been reported in almost a dozen of states including Texas, Kansas, and Oklahoma (Ali and Samira, 2020a, Ali and Samira 2020b; Ali et al 2020).

More than 58% of cotton is cultivated in three mid-southwestern states (Kansas, Oklahoma, and Texas). Little information is available about the occurrence of CLRDV in these states before the initiation of this work. Therefore, field surveys were conducted during the 2019 growing season to determine the infection and distribution of CLRDV in cotton fields located in these mid- southwestern states (Fig. 2).

Figure.1 *Map of three mid- southwestern states where cotton samples were collected during 2019 growing season. Each black circle represents the the location where samples were collected.*

Figure.2 *Symptoms of CLRDV on cotton seedlings with yellowing and chlorosis on leaf edges and mottling collected during the 2019 growing season.*

Results

Field symptoms: A range of virus-like symptoms were observed in cotton fields that include: leaf yellowing, discoloration, reddening on the edges, upward leaf cupping and narrowing, stunting, and short internodes with reduced or small boll sets. The incidence of CLRDV varied from 1-20% in mid-southwestern states.

Detection of CLRDV: A total of 146 samples (104 symptomatic and 42 asymptomatic) samples were collected from cotton fields in three mid- southwestern states (Fig. 1). All samples were tested by RT-PCR. Analysis of the RT-PCR products obtained from each sample on 1% agarose gel showed that nearly 50% of samples were positive for CLRDV (Table 1). In all positive samples, the expected PCR product of 310 bp was observed on agarose gel, which was similar to the one obtained in the positive control used in the PCR reaction. Similarly, in all negative samples, no bands were obtained just like the PCR negative control that contained only water.

These results confirmed the occurrence of CLRDV in various locations in all three states. This was a limited survey for confirming the presence of the virus. However, in the future more detailed surveys will be conducted in all cotton-growing areas to determine the epidemiology of this virus including the transmission vector.

Table. 1 Number of cotton samples collected from cotton fields in mid-southern states during the 2019 growing seasons and tested by reverse transcription polymerase chain reaction (RT-PCR)

State	Locations	No of Symptomatic	No. of positive by RT-PCR	No. of Asymptomatic	No. of positive by RT-PCR
Kansas	Baber County	12	7	3	0
	Harper County	6	0	2	0
Oklahoma	Blaine County	5	5	3	0
	Caddo County	7	5	3	0
	Jackson County	2	0	1	0
Texas	College Station	4	4	3	0
	Corpus Christi	15	8	5	0
	Lubbock	15	8	6	0
	McAllen	10	0	6	0
	San Angelo	15	7	7	0
	Vernon	13	8	4	0
	Total	104	52	42	0

To further confirm the results, the PCR products of some positive samples from each location were sequenced by Sanger sequencing at the Department of Biological Science, the University of Tulsa. The nucleotide sequences from all positive samples were used in BLASTn search in the NCBI Genbank database.

Sequence comparison showed 97-99% identity of CLRDV isolates determined in this study with the CLRDV-Alabama isolate (MN071395) indicating that these CLRDV isolates are similar to the one reported in Alabama.

Distribution of CLRDV: Due to a limited number of samples in Kansa and Oklahoma, a distribution map of CLRDV cannot be made. However, in Texas, major cotton growing areas showed the presence of CLRDV inoculum, which could be a potential threat to cotton production in the future. In Oklahoma, CLRDV was found in cotton plants located in two out of three counties surveyed while in Kansas, one of the two counties contained cotton plants infected with CLRDV infection. Based on these results we can predict that the distribution and occurrence of CLRDV are widespread in cotton-growing counties in these three states. However, more systemic surveys will determine the actual incidence and distribution of CLRDV in the future.

Discussion

In Brazil and Argentina, CLRDV has been associated with cotton blue disease and the virus was isolated from symptomatic cotton plants. The symptoms caused by CLRDV observed in cotton plants in various states in the US were very variable and did not match those of cotton blue disease. In some cases, infected cotton plants showed very mild symptoms and were positive for CLRDV. However, in this work, asymptomatic plants were all negative to CLRDV. In addition, symptoms caused by nutrient deficiency in cotton fields will make it difficult to differentiate them from those caused by CLRDV. Therefore, symptomatology may not be a reliable diagnostic method in the field to determine the disease incidence of CLRDV in the US but will give a quick clue about the presence of virus diseases in the field. Lab diagnosis of the leaf samples by specific RT-PCR will be more reliable to confirm the infection of CLRDV in symptomatic cotton fields.

Based on our limited survey Results, the presence of CLRDV in the mid- southwestern states is very low. However, more surveys are needed in the future to determine the presence of CLRDV in both commercial cotton fields as well in varietal trial and experimental fields. At this stage, it is not quite clear whether CLRDV isolates in the US reported from various states are closely related to each other or not. Further biological characterization of CLRDV isolates from various states shall be characterized in future studies.

Nucleotide sequence comparison between the complete genome of CLRDV-AL isolate from Alabama and CLRDV isolates from Argentina and Brazil showed that the AL-isolate clustered on a separate branch in the phylogenetic tree (Avelar et al., 2020) which indicates that CLRDV isolates in the US may have some unique nucleotide differences that could play a role in the symptoms. However, further work is needed to confirm this hypothesis. CLRDV isolates in the US have been reported from cotton fields in several states and partial sequences of some genes are available, however more complete genome sequences are required for US CLRDV isolates to determine the phylogenetic relationship between CLRDV isolates from the US and the one reported from other countries.

A comparison of partial sequences (310 bp) of CLRDV isolates reported in this study from Kansas, Oklahoma, and Texas showed that these isolates are closely related to the CLRDV isolate reported from Alabama, which indicates that now there is one dominant strain of this virus. However, it might change once more sequences of CLRDV isolates are available from different states in the near future.

Conclusion

The presence of CLRDV in cotton fields has been confirmed in various locations in three states. However, further sampling is needed to have a more detailed epidemiological study of the CLRDV and its impact on cotton yield in the respective states. In addition, aphid vectors shall be regularly monitored and controlled to avoid further spread of the CLRDV to other locations within the states. In particular,

volunteer weeds and early aphid, infestation shall be effectively managed before the flowering stage of cotton and will significantly minimize the effects of CLRDV on cotton production.

Methods

The purpose of this study was to determine the presence and distribution of CLRDV in three mid-southwestern states including Kansas, Oklahoma, and Texas.

Field surveys: During the 2019 growing season, various surveys were made in the three mid-southern states to collect leaf samples from cotton plants that showed virus-like symptoms as well as asymptomatic cotton plants as well. All samples were placed in an individual Ziploc bag, labeled and brought to the Department of Biological Science, the University of Tulsa.

Total RNA extraction: Total RNA was extracted from all collected samples by the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO) and was used in two steps of reverse transcription polymerase chain reaction (RT-PCR). Total RNA was converted into complementary DNA (cDNA) using Superscript IV Reverse Transcriptase Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The polymerase chain reaction (PCR) was performed with primers CLRDV3675F and Pol3982R as described previously (Sharman et al. 2015) to amplify a specific PCR product of 310 bp of the ORF3-5 fragment of the CLRDV. PCR positive control for CLRDV for the specific fragment was obtained from the Plant Disease Diagnostic Lab at Auburn University, while water was used as a negative PCR control. All PCR products were analyzed on 1% agarose gels and the presence of expected bands was recorded in the respective samples.

Cloning and Sequencing: PCR products were purified from the gel and were either directly sequenced or cloned in the pGEM-T vector as reported previously (Ali et al., 2012) and then the recombinant DNA was sequenced by Sanger sequencing at the department of Biological Science, the University of Tulsa, Oklahoma. PCR. Sequences were analyzed using the Basic Local Alignment Search Tool (BLASTn) with queries in Gen-Bank databases. The top three hits against each database were reported for each sequence.

Acknowledgments

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References

1. Ali, A. M. Samira, Connor Ferguson. 2020. First report of Cotton leafroll dwarf virus from cotton (Gossypium hirsutum L.) in Oklahoma. Plant Disease 104: 2531.
2. Ali, A. and M. Samira. 2020a. First report of Cotton leafroll dwarf virus-infecting cotton (Gossypium hirsutum L.) in Kansas. Plant Disease 104:1880.
3. Ali, A. and M. Samira. 2020b. First complete genome sequence of Cotton leafroll dwarf virus from cotton in Texas, USA Microbiology Resources Announcement 9:e01587-19.
4. Ali, A., Mohammad, O., and Khattab, A. 2012. Distribution of viruses infecting cucurbit Crops and isolation of potential new virus-like sequences from weeds in Oklahoma. Plant Dis. 96:243-248.
5. Ali, A. and K. Hake. 2017. Early warning and remedies for emerging pests in cotton with emphasis on viruses. The ICAC Recorder., XXXV (1): 17-19.
6. Avelar S, Schrimsher DW, Lawrence K, Brown JK. 2019. First report of cotton leafroll dwarf virus associated with cotton blue disease symptoms in Alabama. Plant Dis., 103:592–592.
7. Avelar S, Sobrinho RR, Conner K, Nichols RL, Lawrence K, Brown JK. 2020. Characterization of the complete genome and P0 protein for a previously unreported genotype of cotton leafroll dwarf virus, an introduced polerovirus in the USA. Plant Dis., 104:780-786.
8. King, A. M. Q., Adams, M. J., Carstens, E. B. and Lefkowitz, E. J. 2012. Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses Elsevier Inc, page 1069.
9. NASS, 2019. USDA-National Agricultural Statistics Service, Washington, DC. Sharman, M., Lapbanjob, S., Sebunruang, P., Belot, J. L., Galbieri, R., Giband, M., and
10. Suassuna, N. 2015. First report of cotton leafroll dwarf virus in Thailand using a species-specific PCR validated with isolates from Brazil. Australas. Plant Dis. Notes. 10:24.

Development and Validation of an Adaptable IPM Module for Pink Bollworm in BG-II Bt Transgenic Cotton

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Abstract

Background: *Growing Bt cottons of Mon15985 (BG-II) event expressing Cry 1Ac and Cry 2Ab toxins to suppress bollworms paved way for suppression of bollworms and > 95 reduction in pesticide usage in India. With huge survival of pink bollworm Pectinophora gossypiella (Gelichidae : Lepidoptera) in Bt cotton fields of Gujarath during 2009 and widespread infestation in different states later in 2015, Bt technology lost its significance for this pest. Concurrently resistance to Cry toxins was eveident in Indian PBW populations. Insecticides, biocntrol and biorational tools were separately evaluated initially to for efficacy against PBW. Later an IPM module was developed and subjected for validation to conatin PBW. All the experimets were carriedout from 2016 to 1019 at ARS, Dharwad.*

Results: *Individual tools evaluated included insecticides, egg parasitoid Tricogramma bactrae, slieve traps models for mass trapping. Profenphos 50 EC @ 2.0 mL/ L and λ cyhalothrin 5 EC @ 0.5 mL/L with 5.40 and 5.73 % green boll damage by PBW respectively against 14.35 % in untreated check proved to be good insecticde compnants of IPM. Phero- sensor SP sleeve pheromone traps could show 23 to 24 adult PBW male moths per trap per night and included as mass trapping componant @20/ha. Futher,release of Tricogramma bactrae @ 2.5 lakhs /ha thrice consecutivelty from square formation at 15 DAS interval recorded only 3.15 % rosetted flowers, 9.30 % green boll damage and 6.75 % locule damage compared to 14.25 % rosetted flower and 13.10% boll damage in untreated check. This treatment was also included in IPM module. Further large scale evaluation of IPM module with these components was undertaken in comparison with farmers practice over two seasons. The cultivar was MRC-7383 BG-.II Bt cotton an intraspecific hybrid. Due to effective PBW management the mean green boll damage was 8.10 % in IPM blocks against 18.53% in farmers practices. The seed cotton yield was significantly high in IPM blocks (13.17 q/ha) as against 10.44 q/ha in non-IPM fields during 2018. There was 14.06 q/ha in IPM blocks during 2019 which was 3.27 q /ha enhancement over non-IPM.*

Conclusion: *The module comprising of profenfos as ovi-larvicidal insecticide, mass trapping action and consecutive release of egg parasitoid Tricogramma bactrae found to be adaptive for area-wide suppression of pink bollworm effectively. This module may combat resistance successfully.*

Keywords: *Pink bollworm Trichogramma, Mass trapping, Profenphos*

Background

Generally countries growing cotton profitably by successfully suppressing bollworm menace in 90's are cultivating Bt transgenic genotypes expressing Cry1Ac and Cry 2Ab. Management of insecticide resistance in cotton bollworm complex is a global issue that triggered the reliance on transgenic technology. Bt transgenic genotypes were commercialized, for the first time during 1996 and 2002 in the USA and India respectively. A decline of bollworm population since the introduction of Bt cotton has previously been reported by Kranthi, 2012. Growing Bt cotton genotypes of event Mon 15985 (BG-II) which produce two toxins viz., Cry 1Ac and Cry 2Abpaved the way for suppression of bollworms besides great reduction inbollworm specific pesticide usage, in India. That helped India to achieve top ranking in global cotton production from 9.52 million bales 2000-01 to 28.71 million bales in 2018-19. That helped to increase yields from 1.90 qt/ha to 3.66 gt/ha ignited the area under cotton from 8.53 million ha. to 12.66 million ha. during same period, respectively (Anonymous, 2019). Bollworms survival in GM cotton fields have been reported by many authors (Carriere et al., 2015; Ranjith et al., 2010) indicating compromise in Bt toxin efficacy as possible cause. The first field level failure case of Bt technology was

documented in India with the survival reports of pink bollworm (Pectinophora gossypiella Saunders) on BG-II cottons during 2009. By 2015, wide spread incidence of PBW attack on BG-II cotton has been noticed in Gujarat, Andhra Pradesh, Maharashtra and parts of Karnataka states. (Kranthi, 2015). Pink Bollworm infestation was 40 to 95% (damage)in Maharashtra, a major Btcotton growing state in India (Fand et al., 2019).The development of resistance in nationwide collected pink bollworm (PBW) population to Cry 1Ac and Cry 2Ab toxins was quite evident from a series of studies conducted by Central Institutefor Cotton Research (CICR), Nagpur., India(Naik et al, 2018)

The issue of PBW survival on BG-II has been persisting in South and Central India,making cotton cultivation difficult for growers. As an internal borer of Gelichidae family (Order: Lepidoptera) PBW leaves very little indication of its incidence and damage in fields. The neonates of PBW spend hardly few hours on plant/ bolls before entering into fruiting structures, particularly the bolls where it targets to feed on raw seeds. Unlike other insect pests it is not easily amenable for insecticidal control. Thus, holistic approach involving different tactics of IPM has been felt essential to manage PBW successfully. In the present study an IPM module has been developed and validated for management of PBW infesting Bt cottons under rainfed conditions.

Results

Evaluation of components for IPM

Insecticides: Among the insecticides tested at recommended doses in cotton fields, Profenophos 50 EC@ 2.0 ml/l (2.0 l /ha) appeared to be the better choice with least damage recorded (2.97 live larvae/50 bolls;5.40% green boll damage and 5.90% locule damage comparing with 14% greater damage in control treatment). This has lead to 6.80 q/ha yields advantage (Table 1). Other choice of insecticide was a pyrethroid (lambda cyhalothrin 5EC) @ 5.0ml/ha with 3.63 live larvae/50 bolls; 5.73 %green-boll damage and 6.23% locule damage with a yield advantage similar to Profenophos treatement (Table 1). These insecticides could be a better choice as IPM component and scheduled each for single use. Profenophos was used during boll formation stage (70 days after sowing) whereas Lambda Cyhalothrin was used after110 DAS, based on IPM principles.

Pheromones traps for mass trapping: Among six different PBW specific pheromone traps and lure modules readily available in the market.A sleeve trap model called Phero-Sensor RSP could register an edge over other models as it is including a sticky traps and trapping high number of moths. In 2016,Sleeve trap could catch 23.32 moths per night leading to significantly less rosette flowers (2.77%), greenboll damage(3.28%) and open boll damage(3.50%). All these observations found to be >12% (Fig.1). Further in 2017 also SP-Sleeve Trap found to be most efficient by recording highest moth catches (24.93/night) and significantly less rosette flowers (4.71%), green boll damage (2.74%) and open boll damage (3.70%) (Fig.2). Thus, this trap was used in a mass trapping exercise also @ 20 traps/ha in comparison with no trap to manage PBW on large scale area, during 2018. In treatments of mass trapping of PBW moths, Kuber BG-II Bt cotton hybrid could yield 16.68 q/ha seed cotton against 14.55 q/ha in check plot. Seasonlong mass trapping could restrict green boll damage to 12.05 % against 28.11% in the absence of trapping. Huge moth activity (upto 200/night) was evident in check field which was three folds higher than pheromone treatment plots (Table 2).

Table 1. Efficacy of selected insecticides on pink bollworm in BG-II Bt cotton

Treatments	Dosage (ml/litre)	*Larvae/50 bolls		** Green boll damage (%)		**Locule damage (%)	Yield	*Spiders/plant		*Coccinellids/plant	
		DBS	10 DAS	DBS	10 DAS			DBS	10 DAS	DBS	10 DAS
Profenophos 50% EC	2.00	8.07	2.97 (1.86)	12.18	5.40 (2.39)	5.90 (2.50)	17.73 a	2.60	2.20 (1.64)	2.00	1.60 (1.45)
Emamectin benzoate 5% SG	0.20	7.93	4.34 (2.19)	12.47	6.54 (2.64)	7.04 (2.74)	18.80 a	2.50	2.13 (1.62)	1.83	1.50 (1.41)
Thodicarb 75% WP	1.00	7.40	5.23 (2.39)	12.45	7.21 (2.76)	7.71 (2.85)	17.66 a	2.57	2.17 (1.63)	1.93	1.53 (1.42)
Flubendiamide 48% SC	0.10	7.73	4.73 (2.28)	12.16	6.88 (2.70)	7.38 (2.80)	18.63 a	2.53	2.23 (1.65)	1.87	1.47 (1.40)
Chlorantriniliprole 18.5% SC	0.25	7.80	3.92 (2.10)	11.82	6.21 (2.57)	6.71 (2.67)	19.40 a	2.47	2.07 (1.60)	1.73	1.33 (1.35)
Acepahte 75% SP	1.00	7.60	5.60 (2.47)	10.78	11.45 (3.44)	11.95 (3.51)	13.50 b	2.33	1.93 (1.56)	1.87	1.47 (1.40)
Lambda cyhalothrin 4.9% CS	0.50	7.93	3.63 (2.02)	11.14	5.73 (2.46)	6.23 (2.56)	17.39 a	2.67	2.27 (1.66)	1.90	1.70 (1.48)

Control (No insecticide spray)	--	8.47	8.65 (3.02)	11.33	14.35 (3.85)	14.85 (3.92)	10.93 b	2.73	2.70 (1.79)	1.83	1.90 (1.55)
S.Em. ±		0.09	0.13	0.80	0.12	0.11	0.11	0.11	0.04	0.11	0.11
C.D. (P=0.05)		NS	0.39	NS	0.35	0.34	0.34	0.34	NS	NS	NS
CV (%)		5.45	9.64	6.90	7.02	6.56	6.56	6.56	5.29	11.94	13.58

*Figures in the parenthesis are subjected to √x+0.5 transformations ** Figures in the parenthesis are subjected to arc sign transformation

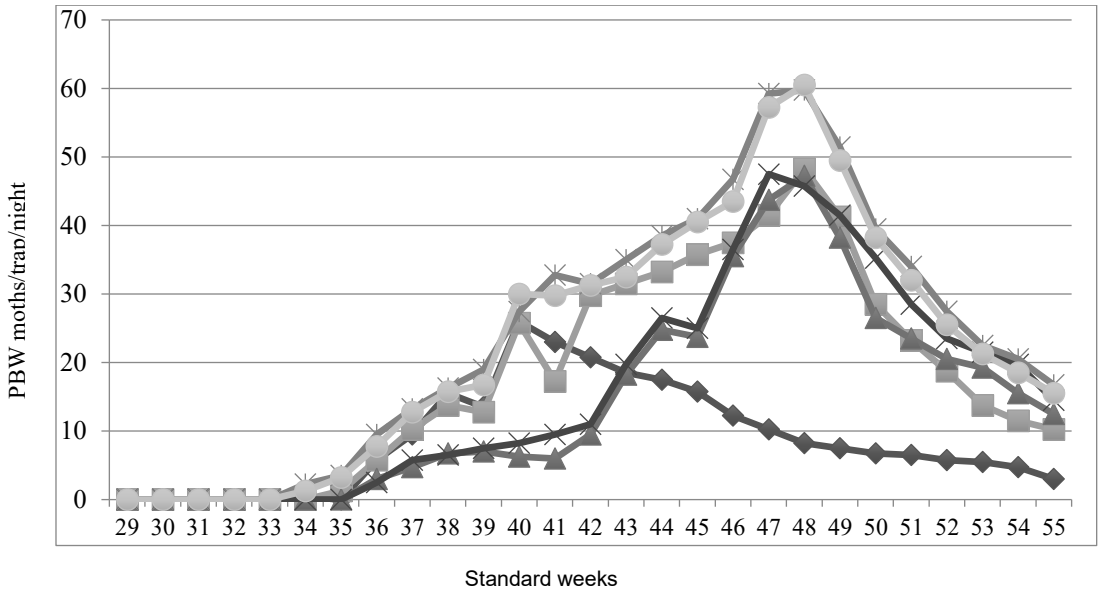

Figure: 1 *Pink bollworm adult moth catches in different pheromone traps (2016-17)*

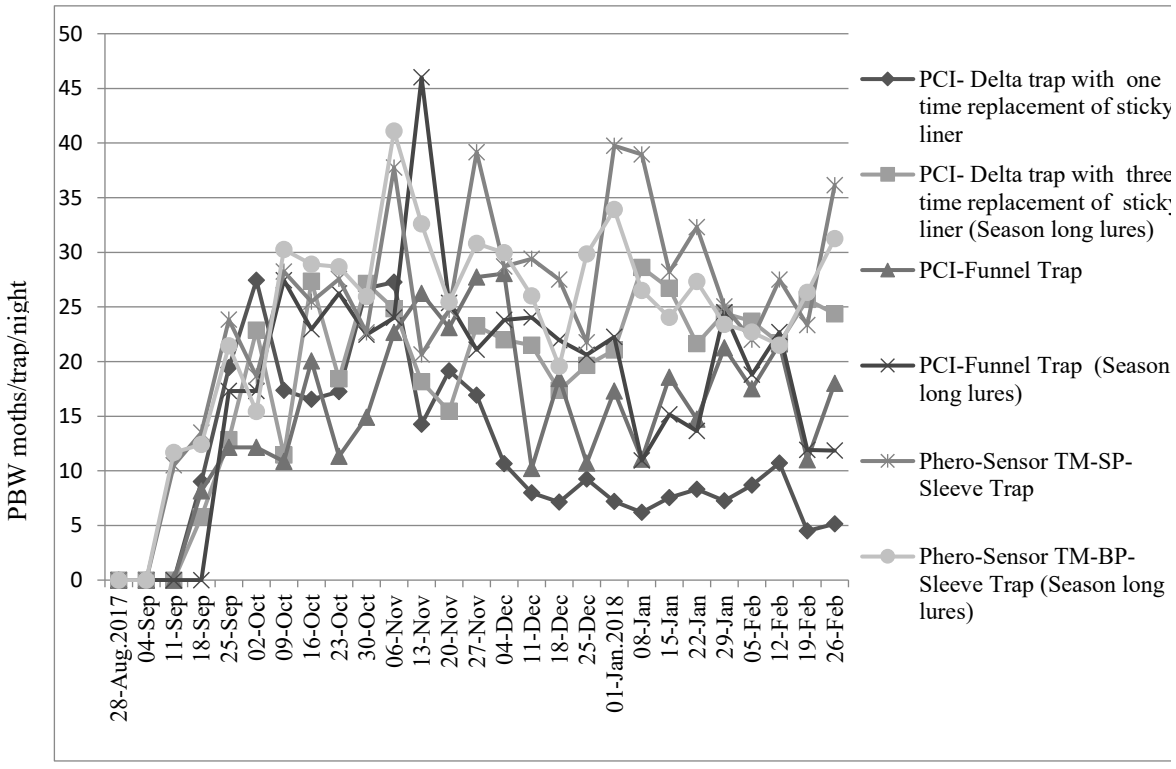

Figure 2 *Pink bollworm adult moth catches in different pheromone traps (2017-18)*

Table 2: Effect of PBW moths mass trapping in a large-scale exercises.

Observations	Mass trapping block*	Check block
PBW adult male moth catches /trap	35-70 /day	65-200 /day
Green boll damage (%)	12.05	28.11
Yield (q/ha)	16.68	14.55

*Phero sensor sleeve small plastic traps @ 20/ha

Bioefficacy of egg parasitoid Trichogrammatoidea bactraeNagaraj: During 2016 consecutive three release of T. bactrae @ 2.5 lakh/ha were made between 50 to 90 DAS. It was observed that PBW incidence significanlty reduced as rosettedflowers observedwere5.4%, green boll damage was(8.2%), livelarvaewere(3.5/50 bolls) and locule dmage was 5.9% (Table 3).

Table-3 Performance of Trichogramma bactrae against pink bollworms in cotton, 2016-17

Tr. No.	Treatment details	Rosette Flower (%)	Greenboll damage (%)	Pink bollworm larvae/ 50 bolls	Good opened bolls/pl	Bad opened bolls/pl	Locule damage (%)
T1	Release of T. bactrae @ 2.5 lakh/ha @ 45-55 DAS	9.53 (17.97)b	11.29 (19.62) b	5.53 (2.45)ab	12.93 (3.66)ab	7.60 (2.83)ab	10.06 (18.43)b
T2	Release of T. bactrae @ 2.5 lakh/ha @ 65-75 DAS	8.55 (16.99)bc	10.43 (18.84)b	5.10 (2.36)b	13.45 (3.72)ab	7.26 (2.78)ab	9.05 (17.48)bc
T3	Release of T. bactrae @ 2.5 lakh/ha @ 90-100 DAS	7.60 (16.00)bc	9.99 (18.42)bc	4.79 (2.29)b	13.74 (3.75)ab	6.93 (2.72)ab	8.10 (16.51)bc
T4	T1 followed by T2	7.18 (15.54)cd	9.63 (18.07)bc	4.54 (2.23)b	13.82 (3.76)ab	6.60 (2.66)ab	7.68 (16.08)c
T5	T2 followed by T3	6.83 (15.15) cd	9.54 (17.98)bc	4.05 (2.13)b	13.89 (3.77)ab	6.26 (2.60)ab	7.33 (15.53)cd
T6	Continous three releases (T1, T2 and T3)	5.46 (13.50) d	8.25 (16.69) c	3.51 (1.99)b	15.69 (4.00)b	4.93 (2.33) b	5.96 (13.63)d
T7	Chemical control (Profenophos @ 2.0 ml/L followed by Lambda cyhalothrin 0.5 ml/L based on ETL)	2.66 (9.39) e	5.67 (13.77)d	3.04 (1.88)b	18.82 (4.39)c	3.60 (2.02) c	3.16 (10.17)e
T8	No release	13.47 (21.52) a	14.55 (22.42)a	8.55 (3.01)a	12.56 (3.61)a	9.57 (3.17)a	13.93 (21.90)a
	S.Em. ±	1.42	1.35	0.12	0.14	0.12	1.37
	C.D. (P=0.05)	4.31	4.10	0.37	0.43	0.35	4.14
	CV (%)	11.75	11.26	9.21	6.46	7.65	14.59

The treatment gave highest yield (10.77q/ha) (Table 4).The findings were confirmed in 2017 trials,the efiicacy of egg parasitoid resulted in 3.15% rosetted flower, 9.30% green boll damage and 6.75% locule damage (Table 5). The treatment was quite appreciable in pest suppression when compared to insecticide applications and without any check. The incremental Benefit Cost Ratio (BCR) was 2.67 and 2.26, in, 2016-17 (Table 4) and 2017-18 (Table 5), respectively.Thus to target PBW at egg stage T.bactrae release could find a place in IPM schedule.

Table 4. Yield advantage and natural enemy safety through pink bollworm egg parasitism and insecticides, 2016-17

Tr. No.	Treatment details	Natural enemies /plant	Yield (q/ha)	Additional yield (T-C) (q/ha)	Avoidable yield loss (%)	IBC ratio
T1	Release of T. bactrae @ 2.5 lakh/ha @ 45-55 DAS	2.27 (1.66)a	9.56 bc	- -	- -	- -
T2	Release of T. bactrae @ 2.5 lakh/ha @ 65-75 DAS	2.60 (1.76) a	9.69bc	- -	- -	- -
T3	Release of T. bactrae @ 2.5 lakh/ha @ 90-100 DAS	2.40 (1.70) a	9.80bc	- -	- -	- -
T4	T1 followed by T2	2.90 (1.84) a	10.13bc	- -	- -	- -
T5	T2 followed by T3	2.40 (1.70) a	10.37bc	- -	- -	- -
T6	Continous three releases (T1, T2 and T3)	2.20 (1.64) a	10.77b	1.59	17.32	2.67
T7	Chemical control (Profenophos @ 2.0 ml/L followed by Lambda cyhalothrin 0.5 ml/L based on ETL)	0.80 (1.13) b	12.64a	3.46	37.69	2.30

T8	Check (No T. bactrae release or insecticide spray)	2.80 (1.80) a	9.18 c	- -	- -	- -
	S.Em. ±	0.07	0.45			
	C.D. (P=0.05)	0.22	1.36			
	CV (%)	7.42	7.57			

Table-5 Performance of Trichogramma bactrae against pink bollworm in cotton, 2017-18

Treatments	Flower rosetting (%)	Green boll damage (%)	Locule Damage (%)	Additional yield q/ha (T-C)	Avoidable yield loss (%)	IBC ratio
Trichogramma bactrae (Three releases)	3.15	9.30	6.5	1.35	15.42	2.26
Chemical control (Profenophos @ 2.0 ml/L followed by Lambda cyhalothrin 0.5 ml/L based on ETL)	6.50	5.75	4.75	2.75	31.42	1.97
Check (No T. bactrae release or insecticide spray)	14.25	13.10	12.10	-	-	-

Validation of IPM module for PBW management

The IPM module having various target specific components against PBW was evaluated in large scale plots in comparison with farmers practice or non-IPM plots. When rest of the management practices similar, the impact of IPM module appeared to be measureable difference in reduction in PBW damage. (explain the model, what was the timeing of application of each component, how much was the uniformity between IPM and non-IPM plots etc., What management practices were performed for other pests of cotton in both treatments, what impact has the managmeent of other pests of cotton on PBW in IPM and non IPM plots.). During 2018 under the IPM umbrella there was significantly lowest green boll damage (10.60 %), locule damage (5.74 %,), moth catches (27.56 /trap/week). In non-IPM fields green boll damage (18.00 %), locule damage (12.20 %,), moth catches (46.00 /trap/week) were higher side (Table 6). Thus due to effective PBW management the seed cotton yield was also significantly more in IPM blocks (13.17 q/ha) as against 10.44 q/ha in non-IPM fields. The confirmation trials conducted during 2019 also revealed better impact of IPM module over farmers practice (Table 6). Thus, when data was pooled for analyses of IPM efficacy suppression of PBW appeared to be quite impressive with 14.06 q/ha seed cotton yield which was nearly 3.27 q /ha enhanced yield over non-IPM (Table 6).

Table 6: Validation of pink bollworm IPM modules in Bt cotons

Year or season	Green boll damage (%)		Moth catches /trap/week		Locule damage (%)		Seed cotton Yield (q/ha)	
	IPM	Non IPM	IPM	Non-IPM	IPM	Non-IPM	IPM	Non-IPM
2018-19	10.60	18.00	27.56	46.00	5.74	12.20	13.17	10.44
2019-20	5.60	19.05	24.68	51.12	6.75	14.35	14.95	11.14
Average	8.10	18.53	26.12	48.56	6.25	13.28	14.06	10.79

Discussion

The efficacy of Bt transgenic traits in cotton against bollworm species is not appearing to be sustainable any more in India. Until survival of pink bollworms in BG-II cotton fields of Gujarath (India) the Cry 2Ab toxin resistance in Pink bollworms remained as an academic information. There has been a heavy loss due to PBW infestation in Bt cottons in India during 2013 and 2014 (Kranthi, 2015). The reported resistance in PBW to Cry 1Ac toxins (Ojha, 2014 : Dhurua and Gujar., 2011) or Cry 1Ac+ Cry 2Ab (Naik et al., 2018) super imposesthe use of other management options in addition to GMO seed technology. PWB, being an internal feeding insect and less exposed life stages are the limiting factors in exercising biocontrol or chemical control options for its management and and call for IPM approaches

(Henneberry and Naranjo., 1998). Further, reliance on IPM is convenient as growers in Karnataka to managing insect pest complex in Bt of non-Bt cottons for the last two decades through IRM practices. Hence, in the present studies, selective tools for the management PBW were evaluated to develop an adaptable IPM module. Though use of chemicals is a prominent tool in cotton IPM, but few PBW specific insecticides are available in market.. However, farmers apply on chemicals without knowing the efficacy or biological nature of the pest. In the present study profenphos 50 EC and lamda cyhalothrin 5 EC proved as highly effective products against PBW and included in the IPM module. The efficacy of profenophos was better in the past, (Patil et al., 2009) for managing PBW, mostly through its ovicidal and high larvicidal actions. Pyrethroides believed to act as quick knochdown and adulticide action against PBW(Gopala Swamy et al 2000). Hence they used the selective insecticide (no pyrethroides) in the later stage of crop growth with a precaution to avoid resurgence of aphids. Among biocontrol options egg parasitoids Trichogramma spp have been highly reliable for their species specificity, cheaper cost and easy mass production (Jalali,2016). Further,Mohamed et al., (2016) noticed thatT. bactrae can be successfully used as a biological control agent against both pink and spotted bollworms in cotton fields of Upper Egypt. Hence,in present IPM module its utilisation was highly justifiable. In different parts of the world lots of efforts have been made to manage PBW with different pheromone based tools/technologies viz., mating disruption (Lykouressis et al.,2005) and mass trapping (Agenor Mafra-Neto and Mohamed Habib., 1996).In present study we have selected a pheromone trap and lure model i.e. Phero sensor SP for its high moth trapping efficacy proved over two seasons. This has been used @ 20/ha as a mass trapping component after confirmation in a mass trapping exercise concurrently. Deployment of sleeve traps for PBW mass trapping was successful in our earlier studies (Patil et al., 2008). However,we could not rely upon any mating disruption tools due to non availability.All treatments selected for integration were safe to predominant arthropod natural enemies also as evident in the respective tables.

The IPM module thus having quite ideal, test verified, easily available and cheaper components was evaluated for two seasons under large scale trails in farmer's fields around ARS, Dharwad. Both years as well as from pooled data the IPM module appeared highly bio-effective in suppressing the pink bollworm. The reliable and measurable indicators of bio-efficacy viz., green boll and locule damage by PBW larvae were quite less in IPM module. On the contrary non-IPM fields suffered higher damage and compromised a yieldupto to 4.0q/ha. It was proved that farmers were relying arbitrarily on different insecticides rather than logical sequence of proven tools and/or have failed to recognise the pest activity. The IPM module of present study could be quite feasable for areawide adaptaion. Henneberry (2007) also explains about success of area-wide IPM programme in US in eradicating PBW menace.Field experiments conducted by EL –Kafez and Nada (2000) for three successive cotton seasons at El-Ebrahemea region of Egypt with Giza 85 cotton variety have shown better integration of T. bactrae with other control measures. A similar bio-intensive IPM module having T.bactrae found advantageous for PBW management in Gujarath according to Godhani et al., 2010. The authores experienced successful launch of nationwide implementation of IRM programme in India(Kranthi and Russell., 2009) mitigating insecticide /Bt toxin resistance issues in cotton.In a similar manner new initiatives have been taken under IRM-dissemination of PBW management strategies programme where in we have scope to popularise this module.

Conclusions

Use of sleeve traps (Phero-sensor-SP model) for mass trapping, egg parasitoid Trichogrammatoidea bactrae @2.5 lakh/ha and Profenphos 50EC found to effective in suppressing pink bollworm menace in Bt cottons. An IPM module having these componants along early sowing. Refugia and crop residue management would be ideal for areawide management of pink bollworm.

Methods

All the field experiments were conducted from 2016 to 2019 during kharif season under rainfed conditions at Agricultural Research Station, Dharwad (India) which is situated between 150 071 latitude, 760 06 l E longitude at 678m above MSL in Northern Transitional agroclimatic zone of Karnataka.The farm has deep black cotton soils.

Evaluation of different insecticides

A total of seven insecticides replicated thrice were evaluated along with a untreated in RBD having plot size of 5.4m X 5.4 m. The spacing was 90 cm X 60 cm.A BG-II Bt cotton hybrid Jadoo (KCK-1459) was sown on 10.7.2016 for the experiment. Application of insecticide was done three times between 45 to 110 days of crop growth using a knapsack sprayer at concentrations mentioned in the tables.

Evaluation of pheromone traps

In fairly large scale plots (500 m2) for each treatment six pheromone trap models were evaluated for their season long PBW moth trapping efficacy. A plot without any trap served as control. There was no interruption to natural activity of PBW in these plots. Each plot was replicated thrice. In each plot there were two traps of a particular types. Lures and sticky liners subjected for change in accordance with treatment definition. Moth trap catches in each trap was observed every day morning and data is presented as moths/trap/night after taking weekly average. The experiment was conducted for two seasons and BG-II Bt cotton Jadoo was sown on 10.7.2016 and 18.07.2017 for respective years. The mass trapping efficacy of phero sensor SP traps was assessed through a large scale trail in farmer's field adjacent to the research farm. Kuber BG-II Bt cotton hybrid was planted under 120 cm X 90 cm spacing on 30.7.2017. There were two treatments only (mass trapping v/s no mass trapping) speard over one acre plot each. Rest of the management was similar for both field. Traps were installed @ 20/ha before or during square formation stage and maintained upto 120 days of the crop growth. Each plot of one acre was divided into four quadrants (=replication) for observations on moths/trap/night and other parameters.

Trichogrammatoidea bactraeegg parasitoid efficacy

Field evaluation during 2016-17(season 1) was by sowing Jadoo (KCK-1459) cotton seeds on 10.7.2016 in an area of 18 m x 25 m (two rows of maize as around each treatment). Except two treatments (T7- chemical control-Profenophos @ 2.0 ml/L followed by Lambda cyhalothrin 0.5 ml/L based on ETL and T8-No release) all the other treatments includes release of Trichogrammatoidea bactrae(2.5 lakh/ha) at different time intervals at 45-55DAS (T1), 65-75DAS (T2), 90-100 DAS (T3), 45-55DAS followed by 65-75 DAS (T4), 75 DAS followed by 90-100 DAS (T5) and continous three release (T6). Observations of per cent rosette flower, green boll damage (%), pink bollworm larvae/ 50 bolls, good opened bolls, bad opened bolls, yield and Natural enemies/ plant were recorded at 15 days intervals. While during 2017-18 sowing was taken up on 20.07.2017 in a larger area of 36m x 50m (two rows of maize as around each treatment) per each treatment. Based on first season experimentation among the different treatments the best treatment (Continous three releases @ 45-55 DAS, 65-75DAS and 90-100 DAS) was selected for further studies in the prevailing season along with UTC (Untreated check) and chemical control (Profenophos @ 2.0 ml/L followed by Lambda cyhalothrin 0.5 ml/L based on ETL of 10 per cent damage .

Validation of IPM module

An IPM module was developed and subjected for validation throough large scale plot evaluation in farmers fields having black soils nearby ARS Dharwad research farm during 2018 and 2019. The BG-II cotton hybrid fields of cv MRC-7383 were selcted based on similar sowing dates, spacing and agronomic practices. The componants of IPM (T1) were i) release of T.bactrae @ 2.5 lakh/ha three times from square formation on wards at 15 days interval (to kill PBW eggs) ii) mass trapping through phero sensor SP sleve traps@ 20/ha (8 /ac) from 50days of sowing till futhers two months (change of lures @ 15 days) iii) application of profenphos 50EC@ 2.0mL/L at boll formation stage (70-80days) and lambda cy-halothrin 5EC @0.5 mL/L after 12º days of the crop. The farmers (T2) lack these target specific treatments. Howver had applications chlorpyrifos 20 EC @2.5mL/L , quinolphos 25 EC@2.0mL/L and fipronil 5EC@ 1.0mL/L and a combi product ampligo 150ZC (chlorantraniliprole+lambda cyhalothrin) @0.25mL/L., Rest of the management paractices espciellay tagetting sap feeders were common to both IPM and non-IPM fields. Particularly we relied on new molecules viz., flonicamid 50WDG and dinotefuron 20SG (both@0.3g/L) to avoid influence of leafhopper/thrips incdence in experimental fields. Each treatment was relicated five times (one acre each /treatment).

Observations and statistics

In all experiments the observations were made on number of rosetted flowers, numer of green bolls affected, locules damaged to reprent them as percent damage. In insecticide and trap models experiments observations recorded from ten randomly selcted plants in each treatments. In mass trapping trails these observations were made from 25 plants /treatment/replication and in IPM module evaluation 50 plants / treatment/replication. Trap catches were obserbved every day. The seed cotton yield in each treatmnet excluding orders row plants was harvested 2-3 times but presented as total yield in terms of q/ha. The data in insecticide and trap model experimets was subjected for one way ANOVA and f test. Means were sepertaed by DMRT. In large scale trails the data was subjected for students t test.

References

1. Agenor Mafra-Neto, Mohamed Habib, Evidence that mass trapping suppresses pink bollworm populations in cotton field. Entomologia Exp. Et Appl. 1996; 81(3): 315-323
2. Anonymous, Pocketbook of Agriculture Statistics. Govt. of India, Ministry of Agriculture & farmers welfare, Dept. of Agriculture, cooperation & farmers welfare,Directorate of Economics & Statisitics, New Delhi, 2019; pp22 - 34.
3. Carrière Y, Crickmore N, Tabashnik BE, Optimizing pyramided transgenic Bt crops for sustainable pest management. Nat. Biotechnol. 2015; 33, 161.
4. Dhurua S,Gujar GT, Field-evolved resistance to Bt toxin Cry1Acin the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manage Sci. 2011; 67(2): 898–903.
5. El-Hafez AA, Nada MA, Augmentation of Trichogrammatoidea bactrae Nagaraja in the IPM programme for control of pink bollworm, Pectinophora gossypiella (Saund.) in Egypt. Proceedings of Beltwide cotton conferences, San Antonio. USA.4-8, January, 2000. Vol 2 : 1009-1015
6. Fand BB, Nagrare V, Gawande S, Nagrale D, Naikwadi B, Deshmukh V, Gokte-Narkhedkar N, Waghmare V, Widespread infestation of pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechidae) on Bt cotton in Central India: A new threat and concerns for cotton production. Phytoparasitica. 2019 ; 47: 1–13.
7. Godhani RH, Patel RM, Patel BH, Korat DM, 2010.Evaluation of bio-intensive pest management module for the management of cotton pink bollworm, Pectinophora gossypiella (Saund.). Karnataka J. Agril. Sci.2010; 23(2) : 364-365
8. Gopala Swamy SVS, Rao NHP, Hanumanth Rao V, Insecticides in the control of pink bollworm in cotton. Pestology. 2000: 24 (7) : 7-11
9. Henneberry TJ, Integrated Systems for Control of the Pink Bollworm Pectinophora gossypiella in Cotton. In: Vreysen M.J.B., Robinson A.S., Hendrichs J. (eds) Area-Wide Control of Insect Pests. 2007; Springer, Dordrecht
10. Henneberry TJ, Naranjo SE, Integrated management approaches for pink bollworm in the southwestern United States. Integrated Pest Management Reviews. 1998; 3 : 31-52
11. Jalali SK, Prashant M, Lakshmi.BL, Trichogrammatids. Ecofriendly Pest Management for Food Security (Ed Omkar) Elsvier : 2016 : 139-181
12. Kranthi KR, Bt-Cotton-Question and Answer. Indian Society for Cotton Improvement; Central Institute for Cotton Research: Nagpur, India. , 2012; 68p
13. Kranthi KR, Pink bollworm strikes Bt cotton. Cotton Statistics and News. 2015; No 35 : 1-5
14. Kranthi KR, Russell D,. Changing trends in cotton pest management. In: Peshin, R., Dhawan, A.K. (Eds.), Integrated Pest Management: Innovation–Development Process,2009; Springer, Dordrecht pp. 499–541
15. Lykouressis D, Perdikis D, Samartzis D, Fantinou A , Toutouzas A, Management of the pink bollworm Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) by mating disruption in cotton fields. Crop Prot. 2005; 24(2) : 177-183
16. Mohamed HO, El-Heneidy AH, Ali GA, Azza AA, Non-chemical Control of the Pink and Spiny Boll worms in Cotton Fields at Assuit Governorate, Upper Egypt, II- Utilization of the Egg Parasitoid, Trichogrammatoidea bactrae Nagaraja. Egypt. J. Biol. Pest Control.2016; 26(4):807-813.
17. Naik CB,Kumbhare S,Kranthi S,Satija U,Kranthi KR, Field-evolved resistance of pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), to transgenic Bacillus thuringiensis (Bt) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. Pest Mange. Sci. 2018;74(11): 2544-2554
18. Ojha AA, Sowjanya SK, SachdevB, Rashmi MA, Ravi KC, Suresh PJ, Mohan KS, BhatnagarRK, Analysis of resistance to Cry1Ac in field collected pink bollworm, Pectinophora gossypiella (Lepidoptera:Gelechiidae), GM Crops & Food. 2014; 5(4): 280-286.
19. Patil SB, Udikeri SS,Hirekurubar RB, GuruprasadGS, Management of pink bollworm Pectinophoragossypiella (Saunders) through mass trapping in cotton.Pesticide Res J. 2008; 20(2) : 2010- 2013
20. Patil SB, Udikeri SS, Hirekurubar RB, Guruprasad GS,Abhilash C,Thiodicarb and Profenophos: promising insecticides for management of pink bollworm, Pectinophoragossypiella(Saunders) in cotton. Indian J. Ent.2009 ;71(2) :183-185
21. Ranjith MT, Prabhuraj A, Srinivasa YB, Survival and reproduction of natural populations of Helicoverpa armigera on Bt-cotton hybrids in Raichur, India. Curr. Sci. 2010 ; 99 :1602–1606.

Effectiveness of insecticide formulations of two essential oils against larvae of Dysdercus völkeri Schmidt (Hemiptera: Pyrrhocoridae), cotton pest in Burkina Faso.

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Abstract

Background: *To find alternatives to the use of synthetic chemical insecticides in the management of cotton pests, the effects of insecticidal oils and ashes based on soy oil and wood ash from Parkia biglobosa combined with pure essential oils of Ocimum americanum (OA) and Eucalyptus camaldulensis (EC) on larvae of Dysdercus völkeri Schmidt (1932) were evaluated. Adults of D. völkeri were collected from untreated cotton fields and reared. L2 larvae from the first generation of this breeding have been subjected to sensitivity tests.*

Results: *After 24 hours of exposure of these larvae, a variation in the mortality rate from 65.5% to (75.8%) for OA insecticide oil and from 62.5% to 79.5% for EC insecticide oil was recorded. More than 50% of the deaths of D. völkeri larvae were found for doses of 0.08 ml of EC insecticide oil and 0.09 ml of OA insecticide oil at concentrations of (7.7%) and (13.0%). Mortality rates ranged from (29.3%) to (88.8%) for OA insecticide ash and from 26.2% to 62.5% for EC insecticide ash. Amounts of 0.03 g of OA insecticide ash and 0.14 g of EC insecticide ash caused the mortality of 50% of the population of D. völkeri larvae at a concentration of 13.0%.*

Conclusion: *Determining the sensitivity of all stages of development of D. völkeri to ash and insecticide oils, as well as field trials, will be necessary to confirm the value of developing a biopesticide that promotes essential oil against this pest.*

Keywords: *Gossypium spp, Essential Oils, Cotton Insect Pests, Ocimum americanum, Eucalyptus camaldulensis, Dysdercus völkeri.*

Background

Cotton is an important cash crop and is by far the world's largest source of textile fiber (Prado and al., 2014). A key product of Burkina Faso's economy, its production was estimated at 683,000 tonnes of cotton seeds, harvested in 2016 over 740,379 ha (Commodafrica, 2017). Its cultivation contributes significantly to the fight against poverty (kabissa, 2019) by its ripple effect on agricultural development in general (Martin and al., 2000) and is an essential link in food security. However, since 2017, Burkina Faso has experienced a productivity decline of almost 30%, which now places it as the fourth largest producer in Africa (Commodafrica, 2019). This decline in productivity, mainly due to low yields (approximately 1 tonne/ha), is partly due to the lack of control of the pest complex (Badiane and al., 2015; Sarr and al., 2016; Brévault and al., 2019) including Dysdercus völkeri. Economic losses from this bug average 14.4% in West Africa (PR-PICA, 2014). It causes a decrease in capsular middleweight of around 19%, seed index of around 18% and germination rate of about 21.2%. Burkina Faso was the hardest hit with a 29.7% loss (Ochou and al., 2014). Cultural methods of control (Deguine and al., 2000), variety selection (Shahid and al., 2010), the action of entomophagy auxiliaries (Baraka and al., 2008) and entomopathogenic agents (Sahayaraj and al., 2016) reduce the impact of D. völkeri on cotton production. However, chemical control remains the most commonly used method of controlling this pest (Russel and al., 2006; Djagni and Fok, 2019). This struggle involving synthetic pesticides, however, has shown its limits with the emergence of resistance and resurgence (Brévault and al., 2003; Rohini and al., 2012; Kabissa, 2019). Managing pest resistance to insecticides is becoming more problematic (Luttrell and al. 1994) as global warming is accompanied by an increase in the number of annual generations of certain pests (Harrington and al., 2007; Brodeur and al., 2013; Barzman and al., 2015). In addition, the unintended effects of chemical control may be the cause of previously unpredictable pest outbreaks (Barzman and al., 2015; Kabissa, 2019). Further, the prolonged and inappropriate use of pesticides, in addition to negatively affecting the natural regulation of pests by their enemies (Ehler

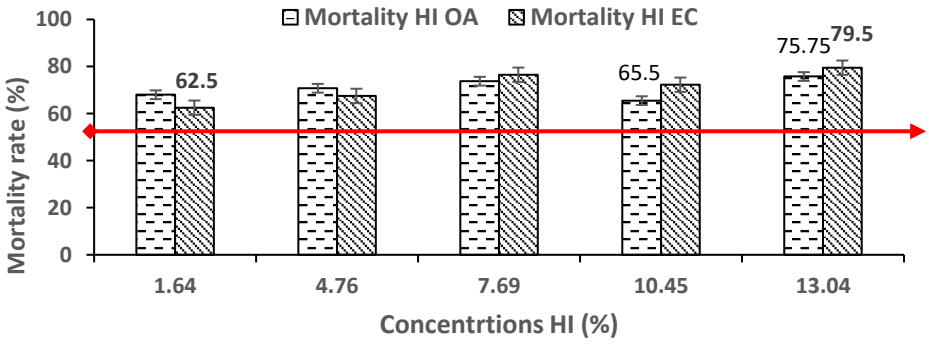
and al., 1973; Tsimbiri et al., 2015), is not without danger to human health and the environment (Tovignan and al., 2001; Kouser and al., 2011; Ferrigno and al., 2017). In the face of these findings, naturally occurring substances, particularly essential oils, represent an alternative solution to control plant predators. Their use has been the subject of several research studies and has generated strong scientific interest in the number of studies dealing with their effectiveness in plant protection (Sanon and al., 2005; Nyamador, 2009; Ndomo and al., 2009; Ilboudo and al., 2009a; 2010b; Bouchikhi and al., 2011; Nafadjara and al., 2013; Bokobana and al., 2014; Diabaté and al, 2014; Nadio and al., 2015; Boni and al., 2017; Abdoulaye and al., 2018). The overall objective of this study is to help reduce the impact of the species D. völkeri on cotton trees through the use of biopesticides based on essential oils. Specifically, the aim was to: (i) measure the toxicity level of insecticide formulations based on two essential oils on the larvae of D. völkeri; (ii) to identify promising biopesticide formulations against the larvae of this Dysdercus bug.

Results

Mortality by concentrations

Mortality of D. völkeri larvae under the influence of insecticide

Oils Fisher's PLSD variance analysis at the 5% threshold indicates a highly significant difference in different treatments after 24 hours of exposure of the larvae. Four different homogeneous groups stand out statistically (Table 1). However, it was recorded that there is no significant difference between OA and EC insecticide oil. In addition, Figure 1 provides for mortality rates for larvae of D. völkeri of different treatments based on concentrations of OA and EC insecticide oils. After 24 hours of testing, it was noticed that more than 50% mortality of larvae at every five (05) concentrations. Overall, mortality rates for larvae ranged from 65.5% to 75.75% for Ocimum americanum insecticide oil and from 62.5% to 79.5% for Eucalyptus camaldulensis insecticide oil. Indeed, OA and EC insecticide oils caused death of 68% and 62.5% respectively for the C1 (1.64 (v/v)), 70.75% and 67.5% for the C2 concentration (4.76% (v/v)), 73.75% and 76 (7.69% (v/v),65.5% and 72.25% for C4 (10.45% (v/v),), 75.75% and 79.5% for C5 (13.04% (v/v)). The highest mortality rates were found in concentrated insecticide oils at 13.04% for OA and EC.



HI: Insecticide Oil; HI OA: *O. americanum* Insecticide oil; HI EC: *E. camaldulensis* Insecticide oil

Figure 1: *Changes in mortality rates for larvae of D. völkeri based on concentrations of O. americanum and E. camaldulensis insecticide oils*

Table 1: Mortality of larvae of *D. völkeri* after 24 hours of exposure to OA and EC insecticide oils

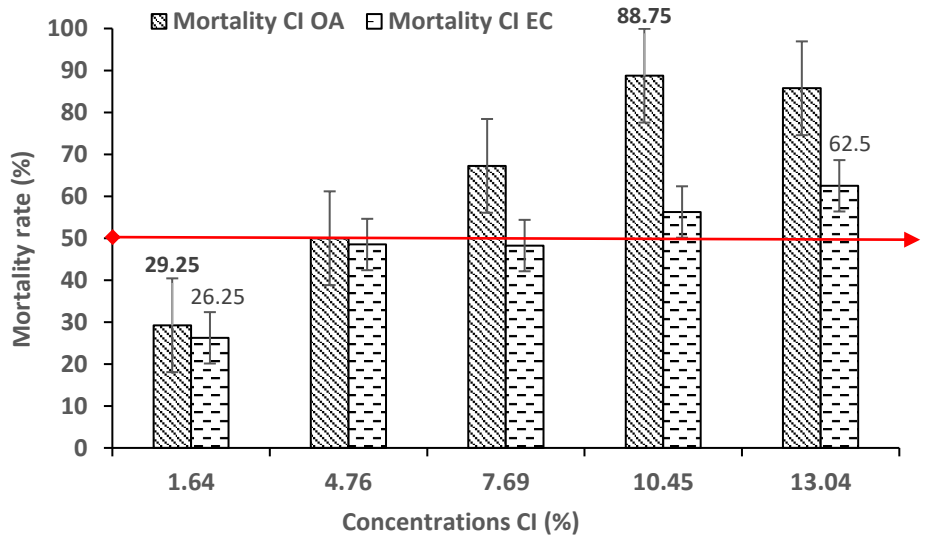
Treatments	%HI OA	%HI EC
THE	96.5a	100a
HI	70.5b	72.05b
THF	44.4c	48c
TA	1d	0d
F	251.475	282.144
Pr > F	< 0.0001	< 0.0001
	HS	HS

THE: Witness Pure Essential Oil; HI: Insecticide Oil; THF: Fixed Oil Witness; TA: Absolute Witness
Averages in the same line followed by identical alphabetical letters do not differ statistically
HS = high significant at p = 0.01.

Mortality of D. völkeri larvae under the influence of insecticide

Ash Fisher's PLSD variance analysis at the 5% threshold indicates a highly significant difference in larvae mortality in treatment after 24 hours of exposure. The analysis of Table 2 allows the distinction of three statistically different homogeneous groups. Also, observed that the activity of OA insecticide ash is identical to that of EC.

Figure 2 shows mortality rates of larvae of D. völkeri of different treatments based on concentrations of OA and EC insecticide ash. Mortality rates below 50% were recorded for OA insecticide ash at C1 (1.64%(v/m)) and EC insecticide ash at C1 (1.64%(v/m)), C2 (4.76%(v/m)) and C3 (7.69%(v/m)). Mortality rates after 24 hours of exposure ranged from 29.25% to 88.75% for OA insecticide ash and from 26.25% to 62.5% for EC insecticide ash. Insecticide ash from OA and EC caused death of 29.25% and 26.25% respectively for C1 (1.64%(v/m)), 50% and 48.5% for C2 (4.76%(v/m)), 67.25% and 48.5% for C2 concentration (4.76%(v/m)), 67.25% and 48.5% 25% for C3 (7.69% (v/m),88.75% and 56.25% for C4 (10.45%(v/m)) and finally 85.75% and 62.5% for C5 (13.04%(v/m)). The highest mortality rates were found in concentrated insecticide ash at 10.45% (v/m) for OA and 13.04% (v/m) for EC.



CI: Insecticide ash; CI OA: O. americanum Insecticide ash; CI EC: E. camaldulensis Insecticide ash

Figure 2: *Changes in mortality rates for larvae of D. völkeri based on concentrations of O. 3xcitatory and E. camaldulensis insecticide ash*

Determination of LC50 and LC90 of insecticide oils and ashes on larvae of D. völkeri

Determination of LC50 and LC90 of insecticide oils on larvae of D. völkeri

Perusal of the Table 2 indicates LC50 and LC90 insecticide oils of OA and EC on larvae of D. völkeri by concentrations. At the C1 concentration (1.64% (v/v)), 0.12 ml and 0.23 ml of OA insecticide oil resulted in mortality of 50% and 90% of the population of D. völkeri larvae respectively. At this same concentration, 50% and 90% of the population of the larvae of D. völkeri died in the presence of 0.15 ml and 0.35 ml of EC insecticide oil respectively. We can therefore say that the population of larvae of D. völkeri is sensitive to the same degree to these two insecticide oils for a concentration of 1.64% (v/v).

At C2 concentration (4.76% (v/v)); 0.11 ml and 0.28 ml of O. 3xcitatory cause mortality of 50% and 90% of the population of larvae of D. völkeri, respectively. On the other hand, it takes 0.12 ml and 0.24 ml of EC insecticide oil to observe the death of 50% and 90% to this same larva population respectively. However, the LC50 and LC90 of the OA and EC insecticide oils compared to each other do not differ statistically.

At the C3 concentration (7.69% (v/v)), 50% and 90% of the population of D. völkeri larvae died for doses of 0.10 ml and 0.20 ml of OA insecticide oil respectively. However, it would take 0.08 ml and 0.23 ml of EC insecticide oil to cause the mortality of 50% and 90% of the population of larvae of D. völkeri. Insecticide oils of OA and EC have the same toxic effect on larvae of D. völkeri at C3 concentration, as they are statistically identical.

At the C4 concentration (10.45% (v/v)), 50% and 90% of the population of the larvae of D. völkeri died for doses of 0.13 ml and 0.29 ml of OA insecticide oil, respectively. However, it would take 0.11 ml and 0.20 ml of EC insecticide oil for 50% and 90% of the same larvae population. These larvae respond identically to the toxic effects of these two insecticide oils at C4 concentration.

At the C5 concentration (13.04% (v/v)), 50% and 90% of the population of the larvae of D. völkeri died for doses of 0.09 ml and 0.19 ml of OA insecticide oil respectively. However, it would take 0.08 ml and 0.17 ml of EC insecticide oil to cause mortality of 50% and 90% of the same larvae population. The LC50 and LC90 of these two insecticide oils do not differ statistically, it can be deduced that at a concentration of 13.04% (v/v), the population of larvae of D. völkeri has the same sensitivity.

Table 2 : LC50 and LC90 of O. Americanum (OA) and E. Camaldulensis (EC) insecticide oils in relation to larvae of D. völkeri by concentrations

Concentrations		HI OA	HI EC
C1 (1,64% (v/v))	LC50 ± IC (ml)	0.12 a ± (0.11-0.14)	0.15 a ± (0.12-0.17)
	LC90 ± IC (ml)	0.23 a ± (0.21-0.26)	0.35 a ± (0.31-0.41)
	Slope	0.68	0.63
	Khi2	104.62	84.33
C2 (4,76% (v/v))	LC50 ± IC (ml)	0.11 a ± (0.09-0.13)	0.12 a ± (0.10-0.13)
	LC90 ± IC (ml)	0.28 a ± (0,25-0,32)	0.24 a ± (0.22-0.27)
	Slope	0.7	0.7
	Khi2	84	113.19
C3 (7,69%(v/v))	LC50 ± IC (ml)	0.10 a ± (0.08-0.11)	0.08 a ± (0.05-0.10)
	LC90 ± IC (ml)	0.20 a ± (0.18-0.23)	0.23 a ± (0.20-0.26)
	Slope	0.74	0.77
	Khi2	98.31	68.3
C4 (10,45% (v/v))	LC50 ± IC (ml)	0.13 a ± (0.11-0.15)	0,11 a ± (0,09-0,12)
	LC90 ± IC (ml)	0.29 a ± (0.26-0.33)	0,20 a ± (0,19-0,23)
	Slope	0.66	0.72
	Khi2	86.87	97.83
C5 (13,04% (v/v))	LC50 ± IC (ml)	0.09 a ± (0.07-0.10)	0.08 a ± (0.06-0.09)
	LC90 ± IC (ml)	0.19 a ± (0.17-0.22)	0.17 a ± (0.15-0.20)
	Slope	0.76	0.8
	Khi2	78.74	73.12

Averages in the same line followed by identical alphabetical letters do not differ statistically
HI OA: Ocimum 4xcitatory Insecticide oil; HI EC: Eucalyptus camaldulensis Insecticide oil
LC: Lethal concentration IC: confidence limit

Determination of LC50 and LC90 of insecticide ash on larvae of D. völkeri

Table 3 shows the LC50 and LC90 of the insecticide ash of OA and EC on the larvae of D. völkeri according to the concentrations.

At the C1 concentration (1.64% (v/m)), 0.47 g and 0.92 g of OA insecticide ash cause mortality of 50% and 90% of the population of D. völkeri larvae respectively. At the same concentration, 0.59 g and 1.24 g of EC insecticide ash cause 50% and 90% of the larvae of D. völkeri, respectively. The populations of larvae of D. völkeri are as sensitive to EC insecticide ash as that of OA without any statistical difference.

At the C2 concentration (4.76% (v/m),) 50% and 90% of the larvae of D. völkeri died for doses of 0.18 g and 0.35 g of OA insecticide ash respectively. We observed at the same concentration that 0.17 g and 0.62 g of ash insecticides EC caused mortality of 50% and 90% respectively of the L2 larvae of D. völkeri. However, larvae of D. völkeri are sensitive to both OA and EC insecticide ash to C2 concentration without any statistical difference.

At the C3 concentration (7.69% (v/m)), it takes 0.12 g and 0.20 g of OA insecticide ash to kill 50% and 90% of the larvae of D. völkeri, respectively. With this same concentration, it will take 0.23 g and 0.45 g of EC insecticide ash to cause the death of 50% and 90% respectively of a population of larvae of D. völkeri. However, there is a statistically significant sensitivity of these larvae to EC insecticide ash compared to that of OA for a population of 90% of individuals killed.

At the C4 concentration (10.45% (m/m)), 50% and 90% of the larvae of D. völkeri died for doses of 0.009 g and 0.14 g of OA insecticide ash respectively. For the same concentration, we note the mortality of 50% and 90% of larvae caused by 0.18 g and 0.42 g of EC insecticide ash respectively. Populations of larvae of D. völkeri are less susceptible to EC insecticide ash than those of OA, with a significant statistical difference.

At C concentration (13.04% (v/m),) 50% and 90% of the larvae of D. völkeri died for doses of 0.03 g and 0.16 g of OA insecticide ash respectively. At this same concentration, 0.14 g and 0.31 g of EC insecticide ash must be brought in to cause the death of 50% and 90% respectively of the larvae of D. völkeri. The population of larvae of D. völkeri is statically less sensitive to EC insecticide oil concentrated at 13.04% than that of OA at the same concentration.

Table 3 : LC50 and LC90 of O. 5xcitatory (OA) and E. Camaldulensis (EC) insecticide ash against larvae of D. völkeri based on concentrations

Concentrations		CI OA	CI EC
C1 (1,64% (v/m))	LC50 ± IC (g)	0.47 a ± (0.41-0.55)	0.59 a ± (0.49-0.78)
	LC90 ± IC (g)	0.92 a ± (0.79-1.14)	1.24 a ± (0.99-1.76)
	Slope	0.29	0.26
	Khi2	63	32.23
C2 (4,76% (v/m))	LC50 ± IC (g)	0.18 a ± (0.16-0.20)	0.17 a ± (0.12-0.22)
	LC90 ± IC (g)	0.35 a ± (0.31-0.41)	0.62 a ± (0.53-0.76)
	Slope	0.57	0.57
	Khi2	86.33	59.01
C3 (7,69% (v/m))	LC50 ± IC (g)	0.12 a ± (0.11-0.14)	0,23 b ± (0,21-0,26)
	LC90 ± IC (g)	0.20 a ± (0.18-0.22)	0,45 b ± (0,40-0,51)
	Slope	0.67	0.48
	Khi2	115.7	99.86
C4 (10,45% (v/m))	LC50 ± IC (g)	0.009 a ± (0.01-0.03)	0,18 b ± (0,16-0,21)
	LC90 ± IC (g)	0.14 a ± (0.12-0.17)	0,42 b ± (0,37-0,50)
	Slope	0.89	0.56
	Khi2	32.02	79.35
C5 (13,04% (v/m))	LC50 ± IC (g)	0.03 a ± (0.0004-0.0555)	0.14 b ± (0.12-0.16)
	LC90 ± IC (g)	0.16 a ± (0.13-0.19)	0.31 b ± (0.28-0.36)
	Slope	0.86	0.63
	Khi2	41.12	77.66

Averages in the same line followed by identical alphabetical letters do not differ statistically
CI OA: Ocimum 5xcitatory Insecticide ash; CI EC: Eucalyptus camaldulensis Insecticide ash
LC: Lethal concentration IC: confidence limit

Table 4: Experimental device of toxicity tests by insecticide ash concentration

Doses (g)	Treatments			
	CI	THE	TCF	TA
Dose 1	0.05	0.05	0.05	0
Dose 2	0.1	0.1	0.1	0
Dose 3	0.2	0.2	0.2	0
Dose 4	0.3	0.3	0.3	0
Dose 5	0.6	0.6	0.6	0

CI: Insecticide Ash; THE: Witness Pure Essential Oil; TCF: Fixed Oil Witness; TA: Absolute Witness

Discussion

Under the conditions of this study, insecticide formulations based on the essential oils of O. 5xcitatory and E. camaldulensis affected the survival of the larvae of D. völkeri. Results obtained from biological tests with both formulations (insecticide ash and insecticide oils) showed a direct relationship between the mortality rates of D. völkeri larvae, the concentration and dose of the insecticide on the one hand, and between the mortality rate and the duration of exposure on the other. Changes in mortality rates have shown that toxic effects depend on factors such as concentration, dose and duration of exposure (72 hours) of essential oils. Indeed, the comparison of each of the LC50 and LC90 between insecticide ash (OA and EC) shows a significant statistical difference for formulations concentrated at C3 (7.69% (v/m)); C4 (10.45% (v/m)) and C5 (13.04% (v/m)) while for concentrations of C1 (1.64% (v/m)) and C2 (4.76% (v/m)) no significant differences were noted. Therefore, it can be concluded that OA-based insecticide ash formulations are more effective than EC formulations at C3 (7.69% (v/m)); C4 (10.45%

(v/m)) and C5 (13.04% (v/m)) because it has the weakest and therefore most toxic LC50 and LC90. On the other hand, for C1 (1.64% (v/m)) and C2 (4.76% (v/m) concentration formulations), OA and EC have the same Insecticide activity. The work of Sanon et al. (2006), Yaka (2007), Ngamo (2007) ; Nafadjara et al. (2013) ; Ilboudo et al. (2010b; 2015c), have shown that the toxic or repellent effects of an essential oil or plant extract would depend on several factors, including its concentration, chemical composition and the sensitivity level of target insects; which confirms the results achieved. However, by comparing each of the LC50 and LC90 between insecticide oils (OA and EC), it is recorded that the insecticide activity of OA, is statistically identical to that of EC given the mortality rate of larvae of D. völkeri obtained in all concentrations. It can be presumed of equal effectiveness of these two types of formulations. In addition, by comparing the effectiveness of the insecticide ash formulation, the synthesis of the results analysis shows that the OA-based formulation was more toxic than that of EC. Indeed, ash insecticide OA holds the smallest value of the LC50 (0.009 g) at the C4 concentration (10.45% (v/m)) compared to that of EC which is 0.14 g at the C5 concentration (13.04% (v/m)). In terms of the formulation of oil insecticide, it was recorded that the EC-based LC50 (0.08 ml) at C3 (7.69% (v/v)) and C5 (13.04% (v/v)) compared to OA-based LC50 at C5 (13.04% (v/v)). Thus, EC's insecticide oil is more effective than that of OA. This action of these formulations on the mortality of larvae of D. völkeri could be due to their exposure to the toxic components of OA and EC contained in the various formulations. It may also be the result of a disturbance in the physiology of insects induced by pure essential oils.

Furthermore, during the mortality counting operation, it was found mainly at the level of high-concentration treatments that some individuals remained glued to the internal walls of petri dishes after their death. Spots were observed when these individuals came off the walls. Research by Nadio and al. (2015) have shown that Ocimum sanctum has insecticide properties in all larval stages and on adults of D. völkeri. However, its action varied with concentration. This could be explained by the presence in this essential oil of alcoholic and ketonic compounds that have the property of dissolving the protective teguments of insects (Agossou, 2001; Sanda and al. 2006). It was also found a marked decrease in motor skills in individuals who were still alive after 24 hours. These individuals barely move and have been stressed by insecticides. Recent work has shown that monoterpenes inhibit the enzyme cholinesterase responsible for acetylcholine hydrolysis (Ryan and al., 1988; Lopez and al., 2010) which is a most common 6xcitatory neurotransmitter in insects. Non-hydrolysis of acetylcholine causes an increase in its concentration, which leads to hyperactivity leading to the death of the insect. These compounds can also Furthermore, during the mortality counting operation, it was found mainly at the level of high-concentration treatments that some individuals remained glued to the internal walls of petri dishes after their death. Spots were observed when these individuals came off the walls. Research by Nadio and al. (2015) have shown that Ocimum sanctum has insecticide properties in all larval stages and on adults of D. völkeri. However, its action varied with concentration. This could be explained by the presence in this essential oil of alcoholic and ketonic compounds that have the property of dissolving the protective teguments of insects (Agossou, 2001; Sanda and al. 2006). It was also found a marked decrease in motor skills in individuals who were still alive after 24 hours. These individuals barely move and have been stressed by insecticides. Recent work has shown that monoterpenes inhibit the enzyme cholinesterase responsible for acetylcholine hydrolysis (Ryan and al., 1988; Lopez and al., 2010) which is a most increasing mortality of larvae caused by insecticide oils based on concentrations and doses could also be explained by the synergistic action of the fixed oil control and pure essential oil. On the other hand, the Fixed Ash Witnesses (TCF) behaved as absolute controls for which the mortality of the larvae was very insignificant or non-existent. The larvae of D. völkeri were not sensitive under the effect of the Fixed Ash Witness.

Conclusion

This study consisted of an evaluation of the effectiveness of soy oil and wood ash-based insecticide formulations combined separately with essential oils O. americanum (OA) and E. camaldulensis (EC) on the L2 larvae of D. vulkeri, a cotton pest. The tests led to the following conclusions:

- Insecticide oils from OA and EC showed an interesting biocide activity on the L2 larvae of D. völkeri. Mortality rates increased from 65.5% to 75.75% for O. 62.5% to 79.5% for E. camaldulensis. Fifty percent (50%) mortality of the L2 larvae of D. völkeri were found for doses of 0.08 ml of EC insecticide oil and 0.09 ml of OA insecticide oil at concentrations of 7.69% and 13.04%. EC's insecticide oil was therefore more effective than OA's.

- OA-based insecticide ash formulations were more effective than EC formulations. Mortality rates ranged from 29.25% to 88.75% for OA insecticide ash and from 26.25% to 62.50% for EC insecticide

ash. Amounts of 0.03 g of OA insecticide ash and 0.14 g of EC insecticide ash caused the mortality of 50% of the population of the L2 larvae of D. völkeri at the concentration of 13.04%.

Methods

Collection sites

Adult individuals of D. völkeri were collected, caged on a young cotton plant and transported for breeding to the entomology laboratory of the cotton program. This collection was conducted from 16 to 27 September 2018 in untreated cotton fields, in the South Central and Mouhoun Loop regions of Burkina Faso (Figure 3). In the first region, it was carried out in the localities of Tiakané (Site1: N: 11-12'24.2" W: 001-15'36.7"Altitude: 309 m; Site2: N: 11-11'51.8" W: 001-15'40.2" Altitude: 317 m) and Kombili (N: 11-18'26.0" W: 001-28'29.9"Altitude: 314 m) in Nahouri province. As for the second region, this collection was carried out in the locality of Massala (N: 12-33'14.6" W: 003-26'35."Altitude: 295 m) in the province of Mouhoun.

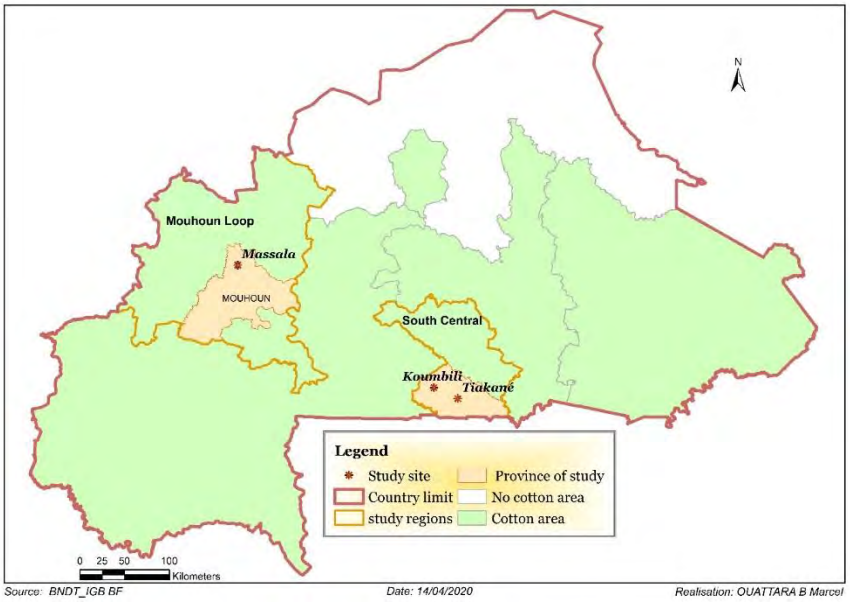


Figure 3: *Map indicating the collection sites*

Material

Biological material

First-generation L2 larvae from the laboratory breeding of Dysdercus völkeri formed the biological material for our study. The most vigorous larval individuals were selected for sensitivity tests.

Plant material The cotton leaf was used to carry out the various tests. The milky cotton seeds were used to feed the larvae before the tests were carried out.

Products used

The essential oils used for the various tests are derived from the plants of Ocimum americanum (OA) (Lamiaceae) and Eucalyptus camaldulensis (EC) (Myrtaceae). They were obtained from the Research Institute for Applied Science and Technology (IRSAT) in Ouagadougou. Samples of these harvested plants were dried in a greenhouse away from the sun for 72 hours before the essential oils were extracted. To obtain the essential oils, the plant samples were hydro distillated using a Clevenger-type device for 3 hours.

The fixed soybean oil used was granted to us by the program's entomology laboratory. The choice of this oil is justified by its excellent physical quality. The ash used comes from the wood of Parkia biglobosa used by the household. Precisely we had collected it from a single grill seller located in sector 20 of Bobo Dioulasso. It has been sifted to retain only fine powder.

Preparation of O. Americanum (OA) insecticide oils and ashes

Insecticide oils and ashes were prepared as follows: First, five (5) batches of five 100 ml capacity vials each containing 30 ml of vegetable soy oil (Table 5) and five (5) other batches of five jars already containing 30g of wood ash (Table 6I) were made. Then, using a micropipette, collected and introduced increasing volumes of 0.5 ml (OA) essential oil into the vials and jars respectively; 1.5 ml, 2.5 ml, 3.5 ml and 4.5 ml. Finally, each vial and jar was closed and shaken until a homogeneous mixture was obtained. Thus, homogeneous mixtures of oil and insecticide ash concentrations of 1.64% (v/v or v/m); 4.76% (v/v or v/m), 7.69% (v/v or v/m); 10.45% (v/v or v/m) and 13.04% (v/v or v/m) were obtained respectively. These homogeneous mixtures that are insecticide oils or ashes were kept in the refrigerator and were only removed during the experiments.

Table 5: Preparation of Insecticide Oils

N	Soy oil volume (ml)	Amount of HE brought (ml)	Concentration (%)
Lot 1	30	0.5	1.64
Lot 2	30	1.5	4.76
Lot 3	30	2.5	7.69
Lot 4	30	3.5	10.45
Lot 5	30	4.5	13.04

Essential Oil (HE)= OA (Ocimum americanum) or EC (Eucalyptus camaldulensis)

Table 6: Preparing insecticide ash

N	Amount of pure wood ash (g)	Amount of HE brought (ml)	Concentration (%)
Lot 1	30	0.5	1.64
Lot 2	30	1.5	4.76
Lot 3	30	2.5	7.69
Lot 4	30	3.5	10.45
Lot 5	30	4.5	13.04

Essential Oil (HE)= OA (Ocimum americanum) or EC (Eucalyptus camaldulensis)

Preparation of E. Camaldulensis (EC) insecticide oils and ashes

The process of preparing EC's insecticide oils and ashes is similar to that of OA.

Constitution of experimental lots

Insecticide oils Depending on the concentration (1.64% (v/v); 4.76% (v/v), 7.69% (v/v); 10.45% (v/v) and 13.04% (v/v)) of each insecticide solution, it was made up eight (08) batches of twenty larval individuals corresponding to the number of treatments. For each concentration of insecticide solution, the treatments consisted of five batches treated with insecticide oil at increasing doses (volumes) (0.05 ml; 0.1 ml; 0.2 ml; 0.3 ml and 0.6 ml) and three controls per dose. The controls consist of pure essential oil (THE) treatment, a fixed soy oil (THF) treatment and an absolute control (TA) without the introduction of insecticide oil. Depending on the type of insecticide treatment (OA, EC) and by given concentration, we have set up twenty (20) petri dishes each receiving twenty L2 larvae from D. völkeri for testing (Table 7). All five (5) concentrations required one hundred (100) petri dishes. For each type of oil treatment, four (04) repetitions were performed.

Table 7 : Experimental device of toxicity tests by concentration of insecticide oil

Doses (ml)	Treatments			
	HI	THE	THF	TA
Dose 1	0.05	0.05	0.05	0
Dose 2	0.1	0.1	0.1	0
Dose 3	0.2	0.2	0.2	0
Dose 4	0.3	0.3	0.3	0
Dose 5	0.6	0.6	0.6	0

HI: Insecticide Oil; THE: Witness Pure Essential Oil; THF: Fixed Oil Witness; TA: Absolute Witness

Insecticide ash The process of building experimental batches of insecticide ash is identical to that of insecticide oils (Table 8).

Table 8: Average mortality of larvae of D. völkeri after 24 hours of exposure to OA and EC insecticide ash

Treatments	%CI OA	%CI EC
THE	95.4a	100a
HI	65.65b	50.1b
TCF	2.4c	0c
TA	0.6c	0c
F	665.358	938.821
Pr > F	< 0.0001	< 0.0001
	HS	HS

THE: Witness Pure; Essential Oil CI: Insecticide ash; TCF: Fixed Oil Witness; TA: Absolute Witness
Averages in the same line followed by identical alphabetical letters do not differ statistically
HS = high significant at p = 0.01.

Conducting sensitivity tests

The fresh, green leaves of cotton trees were removed on the day of the test, thoroughly washed and dried in the shade to avoid contamination. After drying, they were sliced according to the size of the petri dishes in which they were deposited. Each washer leaf received insecticide treatment depending on the type of concentration and doses provided. Using soft clamps, twenty (20) stage L2 larvae were counted and put into contact with each treated leaf in a 90 mm diameter petri dish. The control lots were made up of petri dishes that had not received any insecticide treatment (Absolute Witness), containing a leaf treated with either pure essential oil (positive witness), pure soy oil (Fixed Oil Witness) or pure wood ash (Fixed Ash Witness). All petri dishes that received the individuals, were stored in a cage and placed in ambient laboratory conditions (T: 26.8 °C-31.9 °C and HR: 64%-78%) for the various observations. A total of 1,600 petri dishes and 32,000 L2 larvae collected from D. völkeri were used to carry out the entire study.

Parameters studied

Larvae mortality rate

The mortality rate of larvae is the ratio of the number of dead larvae to the number of larvae used for the trial. This rate is corrected using the Abbott formula (1925) and is determined by the following formula:

$$Mc\ (\%) = \frac{Me-Mt}{100-Mt} \times 100$$ with

Mc = corrected mortality

Me = mortality of the treated sample

Mt = mortality in the untreated witness

The observations took place 24 hours after the tests were carried out and recorded the number of dead individuals. We considered it dead, a larva that no longer moves. The test is rejected if the death rate in the control is greater than 10%.

Determining LC50 and LC90

LC50 or lethal dose 50 is the dose of a substance causing the death of 50% of a batch of animals experienced over a period of time. It is expressed in microlitre per litre (µl/l) or percentage (%). Similarly, LC90 or lethal dose 90 is the dose of a substance causing the death of 90% of a batch of animals experienced over a period of time. It is also expressed in microlitre per litre (µl/l) or percentage (%). The more efficient a product is, the lower its LC50 or LC90.

Statistical analysis

The analyses were carried out using the XLSTAT 2014 software. All data were subjected to a variance analysis (ANOVA). In the necessary cases, Fischer's PLSD test was used at the 5% probability threshold for the separation of averages.

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References

1. Abbott W. S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18, 265-267.
2. Abdoulaye M, Clement S, Haman K. T, Ahmed A, Mouhamadou B. A. and Elias N. N., 2018. Efficacy of Eucalyptus camaldulensis leaf extracts against the pea beetle Callosobruchus maculatus and their impact on biochemical and microbiological properties of the treated bambara groundnut grains. Journal of Entomology and Zoology Studies, 6(2) : 869-877.
3. Agossou A. B., 2001. Evaluation comparatives des effets pesticides de plantes locales : Cymbopogon schoenanthus. L., Jatrophas curcas L et Ocimum gratissimum L. sur des ravageurs du chou : Plutella Xylostella (Lepidoptera : Yponomeutidae) et Aphis Spiraecola (Homoptera : Aphididae). Mémoire de fin d'études agronomiques. ESAUL. Lomé, 52p.
4. Badiane D, Gueye M. T, Coly E.V, Faye O., 2015. Gestion intégrée des principaux ravageurs du cotonnier au Sénégal et en Afrique occidentale. Int. J. Biol. Chem. Sci., 9(5) : 2654-2667.
5. Baraka M.R, Bahira M.E, Alia M.A.E, Abd E.R, Imam A.I., 2008. Evaluation of the number of releases of the egg parasitoid, Trichogramma evanescens West, in suppressing the spiny bollworm, Earias insulana (Boisd.) infestation in El-Farafra cotton fields, New Valley Governorate, Egypt. Egyptian Journal of Biological Pest Control 18: 271–275.
6. Barzman M, Bàrberi P, Birch A.N.E, Boonekamp P, Dachbrodt- Saaydeh S, Graf B, et al. 2015. Eight principles of integrated pest management. Agronomy for Sustainable Development 35: 1199–1215.
7. Bokobana E. M., Koba K., Poutouli W. P., P. K., Nadio N. A., Laba B., Tozoou P., Raynaud C., Sanda K., 2014. Evaluation du potentiel insecticide et répulsif de l'huile essentielle de Cymbopogon schoenanthus (L) spreng. sur Aphis gossypii glover (Homoptera :aphididae), ravageur du cotonnier au Togo. REV. CAMES - VOL.02 NUM.02. 2014 * ISSN 2424-7235. 8p.
8. Boni B. Y, Pierre S, Komlan F. A, Mensah A, Alabi T, Verheggen F, Francis F.,2017. Plantes pesticides et protection des cultures maraichères en Afrique de l'Ouest (synthèse bibliographique). Biotechnol. Agron. Soc. Environ. 2017 21(4), 288-304
9. Bouchikhi T Z., 2011. Lutte contre la bruche du haricot Acanthoscelides obtectus (Coleoptera, Bruchidae) et la mite Tineola bisselliella (Lepidoptera, Tineidae) par des plantes aromatiques et leurs huiles essentielles. Thèse de Doctorat, Université Aboubakr Belkaïd – Tlemcen. Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre et de l'Univers, 169p.
10. Brévault T, Beyo J, Nibouche S, Vaissayre M., 2003. La résistance des insectes aux insecticides : problématique et enjeux en Afrique centrale. Savanes africaines : des espaces en mutation, des acteurs face à de nouveaux défis. Garoua, Cameroun. 6 p. ⟨hal-00142447⟩
11. Brévault T, Clouvel P., 2019. Pest management: Reconciling farming practices and natural regulations. Crop Protection 115 : 1–6.
12. Brodeur J, Boivin G, Bourgeois G, Cloutier C, Doyon J, Grenier P, Gagnon A E., 2013. Impact des changements climatiques sur le synchronisme entre les ravageurs et leurs ennemis naturels : conséquences sur la lutte biologique en milieu agricole au Québec. Rapport Ouranos. Rapport final projet Ouranos No 5500005-103.
13. Commodafrica 2017, http://www.commodafrica.com/24-04-2017-une-production-de-plus-de-800-000-tonnes-de-coton-au-burkina-en-2017-18.
14. Commodafrica 2019, http://www.commodafrica.com/16-04-2019-nouveau-rebond-anticipe-de-la-production-de-coton-en-afrique-de-louest.
15. De Oliveira A. C, Ribeiro-Pinto L. F, Paumgartten J. R., 1997. In vitro inhibition of CYP2B1 monooxygenase by betamyrcene and other monoterpenoid compounds. Toxicology Letters. 92 39-46.
16. Deguine J.P, Gozé E, Leclant F., 2000. The consequences of late outbreaks of the aphid Aphis gossypii in cotton growing in central Africa: Towards a possible method for the prevention of cotton stickiness. International Journal of Pest Management 46: 85–89.
17. Diabaté D., Gnago J.A., Koffi K. & Tano Y., 2014. The effect of pesticides and aqueous extracts of Azadirachta indica (A.Juss.) and Jatropha carcus L. on Bemisia tabaci (Gennadius) (Homoptera: Aleyrididae) and Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) found on tomato plants in Côte d'Ivoire. J. Appl. Biosci., 80, 7132- 7143.
18. Djagni K.K, Fok M., 2019. Dangers potentiels de l'utilisation des insecticides dans la culture cotonnière au Togo de 1990 à 2010. Cahiers Agricultures 28. DOI: 10.1051/cagri/2019023.
19. DOI 10.1007/s12600-017-0572-3
20. Ehler L. E, Eveleens K. G, Van Den Bosch R., 1973. An evaluation of some natural enemies of cabbage looper on cotton in California. Environ. Entomol. (2), p. 1009-1015.
21. Enan E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comparative Biochemisty and Physiology. 130C: 325-33.
22. Ferrigno S, Guadagnini R, and Tyre K., 2017. Is cotton conquering its chemical addiction. A review of pesticide use in global cotton production. Pesticide Action Network, UK. http://
23. Harrington R, Clark S.J, Welham S.J, Verrier P.J, Denholm C.H, Hulle M, Maurice D, Rounsevell M.D, et Cocu N., 2007. Environmental change and phenology of European aphids. Global Change Biol. 13: 1550-1564.
24. Ilboudo Z, Dabiré L.C.B, Nébié R.C.H, Dicko I.O, Dugravot S, Cortesero A.M, Sanon A., 2010. Biological activity and persistence of four essential oils towards the main pest of stored cowpeas, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research 46 (2) :124-128.
25. Ilboudo Z. Dabiré-Binso C.L., Sankara F., Nébié R.C.H & Sanon A., 2015. Optimizing the use of essential oils to protect stored cowpeas from Callosobruchus maculatus (Coleoptera: Bruchidae) damage. African Entomology, 23(1) :94-100.

26. Ilboudo Z., 2009. Activité Biologique de quatre huiles essentielles contre Callosobruchus maculatus Fab. (Coleoptera : Bruchidae), insecte ravageur des stocks de niébé au Burkina Faso. Thèse de Doctorat, 126p.
27. Kabissa J. C. B., 2019. Gestion des insecticides dans le domaine du coton : Progrès et perspectives. The ICAC Recorder, Mars 2019. Volume XXXVII, No 1 ISSN 1022-6303: 3-24.
28. Kouser S, Qaim M., 2011. Impact of Bt cotton on pesticide poisoning in smallholder agriculture: a panel data analysis. Ecol. Econ. 70, 2105–2113.
29. Lopez M.D, Pascual-Villalobos M.J., 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products. 31: 284–288.
30. Martin T, Ochou O.G, Hala N.F, Vassal J.M, Vaissayre M. 2000. Pyrethroid resistance in the cotton bollworm, Helicoverpa armigera (Hübner), in West Africa. Pest. Manag. Sci., 56: 549-554. DOI: 10.1002/(SICI)1526-4998 (200006)56 :6<549: AID-PS160>3.0.CO; 2-Y
31. Nadio N. A, Poutouli W. P, Akantetou P, Laba B.$, Tozoou P, Bokobana M. E, Koba K, Raynaud C, Sanda K., 2015. Propriétés insecticides et répulsives de l'huile essentielle d'ocimum sanctum l. envers dysdercus voelkeri schmidt (heteroptera : pyrrhocoridae), REV. CAMES - VOL.03 NUM.02. ISSN 2424-7235.
32. Nafadjara A, Koffi K, Poutouli W, Pikassele A, Bakouma L, Magnim E. B, Raynaud C., Komla S., 2013. Activités insecticides de l'huile essentielle de Cymbopogon schoenanthus (L.) Spreng sur les larves de stade II de Dysdercus völkeri Schmidt (Heteroptera : Pyrrhocoridae). Journal de la Société Ouest-Africaine de Chimie, 8p.
33. Ndomo A.F, Tapondjou A.L, Tendonkeng F, Tchouanguepf. M., 2009. Evaluation des propriétés insecticides des feuilles de Callistemon viminalis (Myrtaceae) contre les adultes d'Acanthoscelides obtectus (Say) (Coleoptera : Bruchidae). TROPICULTURA, 2009, 27, 3, 137-143.
34. Ngamo T.S.L, Ngatanko I, Ngassoum M. B, Mapongmestsem P. M, Hance T., 2007. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. African Journal of Agricultural Research Vol. 2(4), pp. 173-177.
35. Nyamador S.W., 2009. Influence des traitements à base d'huiles essentielles sur les capacités de reproduction de Callosobruchus subinnotatus Pic. et de Callosobruchus maculatus F. (Coleoptera: Bruchidae) : mécanisme d'action de l'huile essentielle de Cymbopogon giganteus Chiov. Thèse de Doctorat, Univ. Lomé, Togo, 174p.
36. Ochou O. G, Togola M. Hema O, Ayeva B, Bonni G, Badiane D, Ndour A, Cisse I., et Sawadogo F, 2014. Surveillance des infestations parasitaires en culture cotonnière en Afrique de l'Ouest. In 7èmeréunion Bilan PR-PICA, 45p.
37. Prado J.R, Segers G, Voelker T, Carson D, Dobert R, Phillips J, Cook K, Cornejo C, Monken J, Grapes L, Reynolds T, & Martino-Catt S., 2014, Genetically Engineered Crops: From Idea to Product. Annu. Rev. Plant Biol., 65, 769-790.
38. Price D.N, Berry M.S., 2006. Comparison of effects of octopamine and insecticidal essential oils on activity in the nerve cord, foregut, and dorsal unpaired median neurons of cockroaches. Journal of Insect Physiology. 52 : 309-319
39. PR-PICA, 2014. Bilan de la campagne agricole 2013-2014 au Burkina Faso, 7ème réunion bilan du PR-PICA, 27p.
40. Rohini A, Prasad N.V.V.S. D, and Chalam M.S.V, 2012. Management of major sucking pests in cotton by insecticides. J. Cotton Res. Dev. 20(1), 102-106.
41. Russell D, and Kranthi K, 2006. Cotton bollworm control in smallscale production systems Handbook. CFC Technical Paper 42. D. Russell and K. Kranthi (eds.): Common Fund for Commodities.
42. Ryan M.F. and Byrne O., 1988. Plant-insect coevolution and inhibition of acetylcholinesterase. Journal of Chemical Ecology.14: 1965-1975.
43. Sahayaraj K, & Tomson, M., 2010. Impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of Dysdercus cingulatus (fab.) (Hemiptera: Pyrrhocoridae). Journal of Biopesticides, 3: 163–167.
44. Sanda K., Koba K., Poutouli W., Idrissou N. and Agossou A.B., 2006. Pesticidal properties of Cymbopogon schoenanthus against the Diamonback Moth Plutella xylostella L. (Lepidoptera: Hyponomeutidae). Discov. Innov., 18(3), 212-217.
45. Sanon A, Dabiré L.C.B, Ouedraogo A.P, and Huignard J., 2005. Field occurrence of bruchid pests of cowpea and associated parasitoïds in sub humid zone of Burkina Faso: importance on the infestation of two cowpea varieties at harvest. Plant pathology Journal 4 (1), 14 - 20.
46. Sanon A, Ilboudo Z, Dabiré C. L. B, Nébié R C. H, Dicko I. O, & Monge J-P., 2OO6. Effects of Hyptis spicigera Lam. (Labiatae) on the behaviour and development of Callosobruchus maculatus F. (Coleoptera: Bruchidae), a pest of stored cowpeas International Journal of Pest Management; 52(2): 117 – 123.
47. Sarr M, Badiane D, Sane B, 2016. Evaluation de l'efficacité de nouveaux programmes de protection phytosanitaire contre les principaux ravageurs du cotonnier Gossypium hirsutum L. au Sénégal. Int. J. Biol. Chem. Sci. 10(5): 2163-2174.
48. Shahid R.M, Farooq M, Shakeel M, Mahmood A, Gogi D.M., 2017. Impact of Dysdercus koenigii Fabricius (Hemiptera: Pyrrhcoridae) density-dependent population on agronomic and qualitative characteristics of different transgenic cotton varieties. Phytoparasitica, 45 :125–133
49. Tovignan S, Vodouhè S, Dinham B., 2001. Cotton pesticides cause more deaths in Benin. Pestic. News 52, 12–14.
50. Tsimbiri P. F, Moturi W. N, Sawe J, Henley P, Bend J. R., 2015. Health Impact of Pesticides on Residents and Horticultural Workers in the Lake Naivasha Region, Kenya. Occupational Diseases and Environmental Medicine, Vol.3 No.2. PP. 24-34. wwwpan-uk.org.
51. Yaka P., 2007. Etude des propriétés insecticides de l'huile essentielle d'Aoellanthus pubescens Benth sur les chenilles de deux Lépidoptères sur Solanum macrocarpon L. (Gboma). Mémoire d'ingénieur agronome. Université de Lomé 57p.

Efficacy of Biological Control Agents on the Management of African Bollworm, *Helicoverpa armigera*, under Field Conditions

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Abstract

Background: *The study was conducted to evaluate the effect of different biological agents on the control of African bollworm, Helicoverpa armigera (Hübner, 1808) (Lepidoptera: Noctuidae). Field trials were conducted at the Agriculture Research Council - Industrial Crops, Rustenburg, North West Province of South Africa (25°39.0 S, 27°14.4 E), in 2017 and 2018. Four bio-pesticides (Eco-Bb®, Bb endophyte, Bolldex®, and Delfin®) were evaluated against H. armigera and compared with a pyrethroid, Karate®, and untreated control.*

Results: *In plots that were sprayed with Karate® and Bolldex® the number of H. armigera was significantly reduced compared to the untreated control. Plots that were treated with Eco-Bb® had the lowest number of damaged bolls when compared to the other treatments. The highest average seed cotton yield of 6 400 kg/ha was recorded in the plots that were treated with Bolldex®.*

Conclusion: *In summary, the efficacy of different bio-pesticides against H. armigera varied significantly; however, Karate® and Bolldex® resulted in better control of the pest.*

Keywords: *Cotton, pest control control, biopesticides, insecticides, South Africa*

Background

The African bollworm, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera), is an indigenous species considered to be a major pest of fibre crops in Africa (Cherry et al., 2003) and ranks as the most important lepidopteran pest in South Africa (Moran, 1983; Bell and McGeoch, 1996; Moore and Kirkman, 2010). Four heliothine species are reported as of economic importance in Africa but H. armigera is the only species of major economic importance (Greathead and Girling, 1989). The pest causes damage to crops estimated at greater than US$2 billion annually in Asia, Europe, Africa and Australasia (Tay et al., 2013). It has a very large range of host plants including cotton, pepper, corn, tomato, lucerne, soybean, sorghum and tobacco (Cunningham and Zalucki, 2014; Gu et al., 2018). H. armigera is perceived as a serious pest because of its polyphagy (Achaleke et al., 2005; Brévault et al., 2011), high fecundity (Noor-ul-Ane et al., 2018), high mobility (Fitt, 1989) and resistance to chemical insecticides (Chaturvedi, 2007; Pretorius, 2011; Yang et al., 2013).

In South Africa, cotton is a significant crop produced by both commercial and small-scale farmers (Van Jaarsveld, 2003) and it is mainly attacked by H. armigera (Li and Bouwer, 2012) in its larval stage, which causes high yield loss. Because the bollworm has a habit of entering the fruit, boll or pod, the plant affords it good protection against chemical sprays, making control almost impossible (Joubert, 2012). Low economic damage thresholds in cotton require a high level of control (Cherry et al., 2003), which results in reliance on synthetic insecticides (Mensah, 2002; Safna, 2018). Although chemical pest control is extensively used throughout the world, it has been generally regarded as environmentally unacceptable (Szewczyk et al., 2009). Excessive use of chemicals not only causes the economic restraint on farmers but also produces harmful side effects on the environment as well as mammals (Patel et al., 2015). Lately, many chemical pesticides in agriculture are under pressure to be eradicated due to their harmful effects and farmers are turning to biological pesticides (Vilas-Boas et al., 2007; Maghsoudi and Jalali 2017). Integrated control for H. armigera that seeks to minimize insecticide use and impact on non-target pests needs to be taken into account. One of the ways to overcome this situation is to use eco-friendly control measures, like biological agents. Hence, this study confines itself to the evaluation of the field efficacy of different biological agents on the control of H. armigera and other cotton pests.

Results and discussion

Plots that were treated with Karate® had the lowest significant number of H. armigera larvae compared to untreated control and were comparable to the plots that were treated with Bolldex® (Figure 1). The control had the highest number of H. armigera and the trend was similar for both the 2017 and 2018 seasons. All the treatments significantly reduced the number of H. armigera compared to the control during the 2018 season.

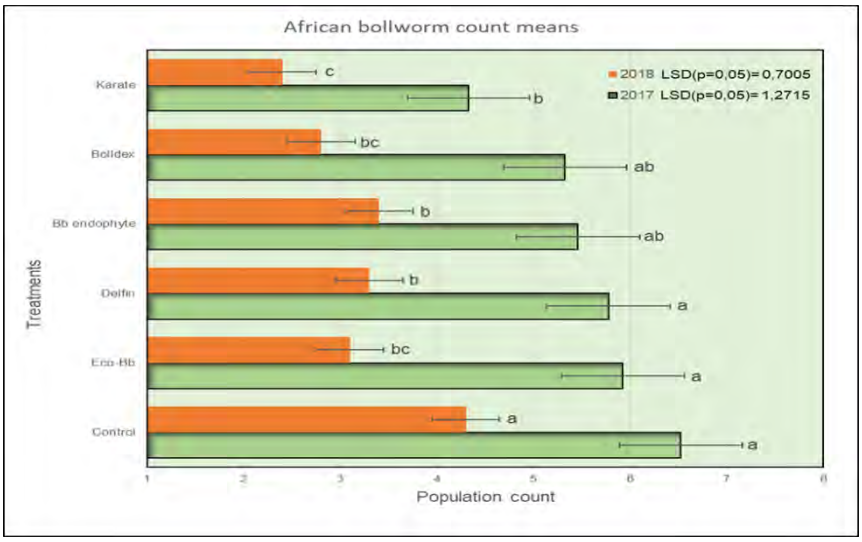

Figure 1 *The average numbers of H. armigera on different treatments during the 2017 and 2018 seasons*

The results shown in Figure 2 revealed that plots that were treated with Bolldex® had the lowest significant number of damaged bolls when compared to Bb endophyte and the control in 2017. In 2018, plots that were treated with Karate® had the lowest number of damaged bolls followed by Eco-Bb® and Bolldex®. In both seasons, all the treatments had a lower significant number of damaged bolls when compared to the untreated control. The highest seed cotton yield of 5 987 kg/ha (2017) and 6 818 kg/ha (2018) were recorded in the plots that were treated with Bolldex® followed by Karate®, which were higher than Eco-Bb®, Delfin® and Bb endophyte in 2017; and much higher than the untreated control in 2018 (Table 1). The average seed cotton yield was higher for all treatments in 2018 than in 2017. On average, plots that were treated with Bolldex® (45%) were found to be superior in increasing seed cotton yield compared to the untreated control, followed by Karate® (31%).

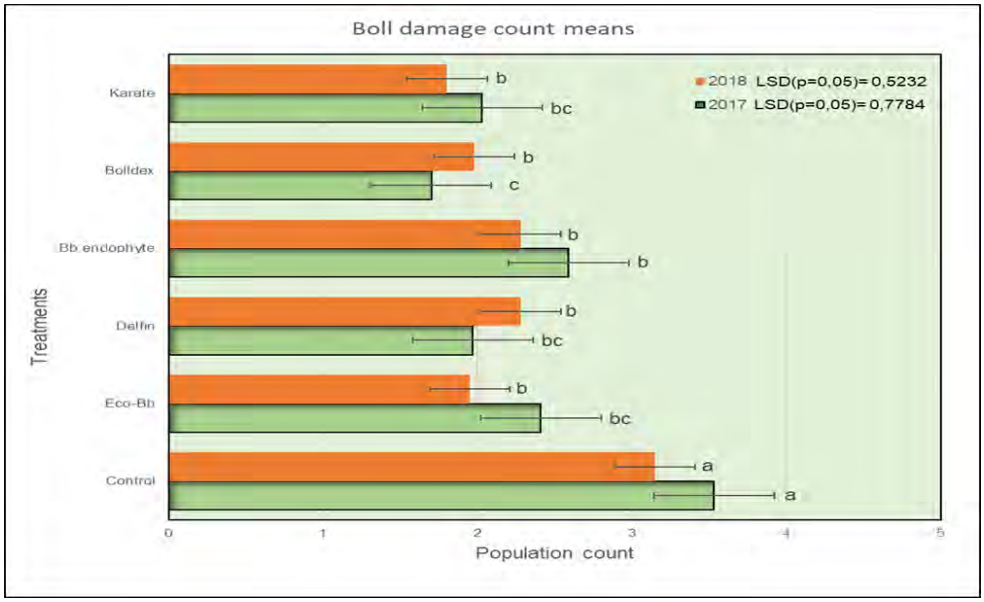

Figure 2 *The average number of boll damage on different treatments during the 2017 and 2018 seasons*

Table 1. Seed cotton yields obtained from the different treatments during the 2017 and 2018 seasons

Treatment	Seed cotton yield (kg/ha)	
	2017	2018
Eco-Bb®	3055 b	5961 ab
Bolldex®	5987 a	6818 a
Delfin®	3523 b	5755 ab
Bb endophyte	3100 b	6409 a
Karate®	5133 ab	6405 a
Untreated Control	4168 ab	4673 b
LSD (5%)	2373.8	1.6178
CV%	37.94516	17.88032
P value	0.1216	0.1436

In this study, considerable control of pests and consequent increases in yield were obtained with some of the biological agents. However, the chemical control had the best performance on the reduction of the cotton pests. Contrary to greenhouse trials, with controlled conditions, field trials are affected by different environmental factors (Tesfagiorgis, 2008), which may influence the effectiveness of the biological agents. From the study, it clearly can be realized that the insecticidal action of some of the tested biopesticides was comparable to the commercial synthetically insecticide. Bolldex® warrants more attention due to the fact that the reduction of H. armigera larvae was very close to the used standard Karate®. These results demonstrated the potential of biopesticides to reduce cotton pests and that they can be introduced as natural friendly pesticides in organic and commercial agriculture. This study demonstrated that both Bolldex® and Karate® significantly reduced the H. armigera populations during the two seasons. Khalique and Ahmed (2001) reported that the mortality response of H. armigera larvae to a combination of Karate® and B. thuringiensis subsp. kurstaki were highly compatible and synergistic. H. armigera nucleopolyhedrovirus (HaNPV) has been used in several countries and has been introduced in South Africa for use on several crops (Joubert, 2012; Madumbi Sustainable Agriculture, 2014).

Delfin® may have reduced the larval population of H. armigera compared to the control in 2018 but there were no significant differences in 2017. Biopesticides are effective in reducing the larval population of H. armigera when combined with nuclear polyhedrosis virus and parasitoids (Sharma et al., 2008). According to the ARC (2004), the economic injury levels of the different bollworms are five bollworms per plot. The results of this study showed that H. armigera larvae exceeded the threshold level of five bollworms for all the treatments except for the Karate® treatments in 2017, however, in 2018, less than 4 bollworms were recorded for all the treatments except for untreated control.

The least number of damaged bolls was observed in plots that were treated with Bolldex®, Karate®, and Eco-Bb®. The effect of Bolldex® and Karate® on the reduction of boll damage corresponded with the reduction of the bollworms on the plots where these treatments were applied. These results are also in concordance with the observation by Li et al. (2006) who found that more than 60% parasitism of H. armigera decreased boll damage by 80% compared with the control. Joubert (2012) reported that a trial was conducted for control of H. armigera on peaches and Bolldex® yielded 99% scar-free fruit. The data on yield revealed that significantly higher yield of seed cotton was recorded in the treatments with Bolldex® followed by Karate®. In 1997, Cole et al. reported that the Karate® evidently increased cotton yield by 12 % and provided good pest control whilst maintaining beneficial populations. This is contrary to the findings of Kumar and Stanley (2010) who reported that, although lambda-cyhalothrin enhanced seed cotton yields, it caused mortalities of both destructive and useful insect species.

Plots that were treated with Karate® had earlier boll opening than the other treatments in 2017. The additive effects were probably due to a combination of multiple mechanisms that affect the pathogens, as opposed to the fewer control mechanisms provided by a single antagonist. Ali (2016) stated that the average number of open bolls/plant is significantly influenced by spraying insecticides and salicylic acid.

The result of this study showed that biopesticides caused moderate to lower mortality of cotton pests and thus could be used within an integrated pest management programme. As a possible replacement or in conjunction with synthetic pesticides, the development of resistance could be delayed.

Methods

Trial site, layout, and planting

The trials were conducted at ARC – Industrial Crops (25°39.0S, 27°14.4E) in Rustenburg, North West Province. Each plot consisted of 6 rows, 5 m long, 1 m spacing between rows, 2 m path between replications and 20 cm spacing between plants. The treatments were replicated four times in a randomized block design. The non-GM cultivar, DeltaOPAL, was planted under irrigated conditions. Black soil (approximately 55% clay) was cultivated by tractor to obtain a fine tilth and the trials were hand planted. After emergence, weeds were controlled by means of hand hoeing and seedlings at the fourth true leaf stage were thinned out in order to obtain the plant population density of 5 plants/m. The trials were planted on 24 October 2016 and 17 October 2017.

Treatments and applications

Trade name	Active ingredient	Concentration
Eco-Bb®	Beauveria bassiana	300g/ha or 1 g/l
Bb endophyte	Beauveria bassiana	300g/ha or 1 g/l
Bolldex®	Nuclear polyhedrosis virus	200ml/ha
Delfin®	Bacillus thuringiensis	1kg/ha in 25l/ha water
Karate®	lambda-cyhalothrin	120ml/ha in 200l/ha water

The administration of treatments started 13 weeks after planting and weekly spray applications were done until 23 weeks after planting. Treatments were administered late afternoon due to the UV sensitivity of the biological agents (Zhang et al., 2016). The treatments were applied using a GARDENA® backpack pressure sprayer (chemical treatments) and Cooper Pegler CP15 Evolution® sprayer (biopesticide treatments).

Data collection

The efficacy of different treatments was assessed based on in situ counts of living H. armigera larvae. From 12 weeks after planting, twelve plants per plot were scouted weekly for the H. armigera and damaged bolls. The seed cotton yields were determined at the end of the season. The trials were harvested when over 90% of the bolls had opened and two middle rows were harvested per plot. Hand harvesting was done to ensure that the seed cotton was harvested and weighed accurately.

Analysis

The data were analyzed as a randomized block designed experiment. The data were subjected to appropriate analysis of variance. The Shapiro-Wilk test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where significant deviation from normality was observed and due to skewness, outliers were removed until it was normal or symmetrically distributed (Glass et.al. 1972). Student's t-LSDs were calculated at a 5% significance level to compare means of significant source effects (Snedecor and Cochran, 1967). The analysis was performed using Genstat Release 18 and SAS version 9.4 statistical software (SAS, 1999). The seed cotton yields were expressed in percentage (number of pests in treatment plots – number of pests in control plots ÷ number of pests in control plots X 100).

References

1. Achaleke, J., Brevault, T., Blondin L., Vassal, J. M., 2005. Helicoverpa armigera: truly polyphagous. https://agritrop.cirad.fr/549357/1/document_549357.pdf. Accessed 24 January 2019.
2. Ali, O. S. M., 2016. Effect of salicylic acid and its mixtures with three insecticides on some cotton insect pests. Doctoral dissertation, Faculty of Agriculture, Tanta University, Egypt.
3. ARC. 2004. Management guide for the cotton producer. Second Edition. Agricultural Research Council-Institute for Industrial Crops, Rustenburg, South Africa.
4. Bell, M.A., McGeoch, J.C., 1996. An evaluation of the pest status and research conducted on phytophagous Lepidoptera on cultivated plants in South Africa. African Entomology, 4(2), pp.161-170.
5. Brévault, T., Nibouche, S., Achaleke, J., Carrière, Y., 2011. Assessing the role of non-cotton refuges in delaying Helicoverpa armigera resistance to Bt cotton in West Africa. Evolutionary Applications, 5 (1), 53-65.
6. Chaturvedi, I., 2007. Status of insecticide resistance in the cotton bollworm, Helicoverpa armigera (Hubner). Journal of Central European Agriculture, 8 (2), 171-182.
7. Cherry, A., Cock, M., Van den Berg, H., Kfir, R., 2003. Biological control of Helicoverpa armigera in Africa. In: Biological Control in IPM Systems in Africa (Edited by Neuenschwander, P., Borgemeister, C. and Langewald, J.).

CABI Publishing, Wallingford, UK, p 329–346.
8. Cole, J. F. H., Pilling, E. D., Boykin, R., Ruberson, J.R., 1997. Effects of Karate® insecticide on beneficial arthropods in Bollgard® cotton. In: Proceedings Beltwide Cotton Conferences (1977), New Orleans, LA, USA. National Cotton Council, 2, 1118-1120.
9. Cunningham, J. P., Zalucki, M. P., 2014. Understanding heliothine (Lepidoptera: Heliothinae) pests: what is a host plant? Journal of Economic Entomology, 107, 881–896.
10. Fitt, G. P., 1989. The ecology of Heliothis species in relation to agroecosystems. The Annual Review of Entomology, 34, 17-52.
11. Glass, G. V., Peckham, P. D., Sanders, J. R., 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. Review of Educational Research, 42 (3), 237-288.
12. Greathead, D. J., Girling, D. J., 1989. Distribution and economic importance of Heliothis and of their natural enemies and host plants in southern and eastern Africa. In: Proceedings of the Workshop on Biological Control of Heliothis: Increasing the effectiveness of natural enemies, New Delhi, India, 11-15 November 1985. New Delhi, India: Office of International Cooperation and Development, USDA, 1989.
13. Gu, S., Han, P., Ye, Z., Perkins, L. E., Li, J., Wang, H., Zalucki, M. P., Lu, Z., 2018. Climate change favours a destructive agricultural pest in temperate regions: late spring cold matters. Journal of Pest Science, 91 (4), 1191-1198.
14. Jat, M. C., Jeyakumar, P., 2006. Bio-efficacy of botanicals and bio-agents on sucking pests of cotton. Annals of Plant Protection Sciences, 14 (1), 8-10.
15. Joubert, R., 2012. Biological bollworm control. Farmers Weekly: May 22, 2012. https://www.farmersweekly.co.za/agri-technology/farming-for-tomorrow/biological-bollworm-control/. Accessed 28 June 2019.
16. Khalique F., Ahmed K., 2001. Synergistic Interaction between Bacillus thuringiensis (Berliner) and lambda-cyhalothrin (Pyrethroid) against, chickpea pod borer, Helicoverpa armigera (Huebner). Pakistan Journal of Biological Sciences, 4 (9), 1120-1123. DOI: 10.3923/pjbs.2001.1120.1123.
17. Kumar, K. R., Stanley, S., 2010. Bio-efficacy of microbial and chemical insecticides on major lepidopterous pests of cotton and their (insect) natural enemies in cotton ecosystem in Tamil Nadu. Resistant Pest Management Newsletter, 20 (1), 4-7.
18. Leite, G. L. D., Picanço, M., Zanuncio, J. C., Ecole, C. C., 2006. Factors affecting herbivory of Thrips palmi (Thysanoptera: Thripidae) and Aphis gossypii (Homoptera: Aphididae) on the eggplant (Solanum melongena). Brazilian Archives of Biology and Technology, 49 (3), 361-369.
19. Li, H., Bouwer, G., 2012. Toxicity of Bacillus thuringiensis Cry proteins to Helicoverpa armigera (Lepidoptera: Noctuidae) in South Africa. Journal of Invertebrate Pathology, 109, 110–116.
20. Li, J., Yan, F., Coudron, T. A., Pan, W., Zhang, X., Liu, X., Zhang, Q., 2006. Field release of the parasitoid Microplitis mediator (Hymenoptera: Braconidae) for control of Helicoverpa armigera (Lepidoptera: Noctuidae) in cotton fields in Northwestern China's Xinjiang Province. Environmental Entomology, 35 (3), 694-699.
21. Madumbi Sustainable Agriculture, 2014. Bolldex® product label. http://www.madumbi.co.za/pdf/Bolldex/Bolldex%20Label.pdf. Accessed 04 June 2014.
22. Maghsoudi, S., Jalali, E., 2017. Noble UV protective agent for Bacillus thuringiensis based on a combination of graphene oxide and olive oil. Scientific Reports, 7 (1), 11019. doi:10.1038/s41598-017-11080-9.
23. Mensah, R. K., 2002. Development of an integrated pest management programme for cotton. Part 1: establishing and utililizing natural enemies. International Journal of Pest Management, 48 (2), 87-94.
24. Moore, S., Kirkman, W., 2010. HELICOVIRTM: a virus for the biological control of bollworm. SA Fruit Journal, 9 (4), 63-67.
25. Moran, V. C., 1983. The phytophagous insects and mites of cultivated plants in South Africa: patterns and pest status. Journal of Applied Ecology, 439-450.
26. Noor-ul-Ane, M., Kim, D., Zalucki, M. P., 2018. Fecundity and egg laying in Helicoverpa armigera (Lepidoptera: Noctuidae): model development and field validation. Journal of Economic Entomology, 111 (5), 2208–2216.
27. Patel, S. R., Patel, K. G., Ghetiya, L. V., 2015. Population dynamics of pod borer (Helicoverpa armigera Hubner) infesting chickpea in relation to abiotic factors. Agres – An International e-Journal, 4 (2), 163-170.
28. Pretorius, J.D., 2011. Status of resistance of Helicoverpa armigera (Lepidoptera: Noctuidae) and Diparopsis castanea (Lepidoptera: Noctuidae) to Bt cotton in South Africa (Master's thesis). North-West University, Potchefstroom, South Africa.
29. Safna, M., Naik K. V., Desai, V. S., Karmarkar, M. S., Shinde, B. D., Raut, P. P., 2018. Evaluation of the efficacy of some insecticides against fruit borer, Helicoverpa armigera (Hubner) infesting tomato. International Journal of Chemical Studies, 6 (2), 1158-1163.
30. SAS, 1999. SAS/STAT User's guide. 8 (2). SAS Institute Inc, SAS Campus Drive, Cary, North Carolina.
31. Shapiro, S. S., Wilk, M. B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 52, 591-611.
32. Sharma, G. D., Devi, N., Raj, D., Oberoi, C. K., 2008. Field efficacy of different insecticides and bio-pesticides against fruit borer (Helicoverpa armigera) and greenhouse whitefly (Trialeurodes vaporariorum). Himachal Journal of Agricultural Research, 34 (2), 40-46.
33. Snedecor, G. W., Cochran, W. G., 1967. Statistical methods. Sixth Edition, Ames, Lowa, the Lowa State University.
34. Szewczyk, B., Rabalski, L., Krol, E., Sihler, W., de Souza, M. L., 2009. Baculovirus biopesticides-safe alternative to chemical protection of plants. Journal of Biopesticides, 2 (2), 209-216.
35. Tay, W. T., Soria, M. F., Walsh, T., Thomazoni, D., Silvie, P., Behere, G. T., Anderson, C., Downes, S., 2013. A brave new world for an old world pest: Helicoverpa armigera (Lepidoptera: Noctuidae) in Brazil. PLOS One, 8 (11), e80134. doi:10.1371/journal.pone.0080134.
36. Tesfagiorgis, H. B., 2008. Studies on the use of biocontrol agents and soluble silicon against powdery mildew of zucchini and zinnia (Doctoral dissertation). University of KwaZulu-Natal, South Africa, p 108.
37. Van Jaarsveld, M. J., 2003. Geographic susceptibility of Helicoverpa armigera (Lepidoptera: Noctuidae) to insecticidal proteins in Bt-cotton in South Africa (Master's thesis). Department of Zoology and Entomology, Rhodes University, Grahamstown.
38. Vilas-Boas, G. T., Peruca, A. P. S., Arantes, O. M. N., 2007. Biology and taxonomy of Bacillus cereus, Bacillus anthracis, and Bacillus thuringiensis. Canadian Journal of Microbiology, 53, 673–687. doi: 10.1139/W07-029.
39. Xia, J., 1997. Biological control of cotton aphid (Aphis gossypii Glover) in cotton intercropping systems in China: a

simulation study. PhD Thesis. Wageningen University, Netherland, p173.
40. Yang Y., Li, Y., Wu, Y., 2013. Current status of insecticide resistance in Helicoverpa armigera after 15 years of Bt cotton planting in China. Journal of Economic Entomology, 106, 375–381.
41. Zhang, L., Zhang, X., Zhang, Y., Wu, S., Gelbič, I., Xu, L., Guan, X., 2016. A new formulation of Bacillus thuringiensis: UV protection and sustained release mosquito larvae studies. Scientific Reports, 6, 39425. doi:10.1038/srep39425.

Emerging Issues in Cotton Insect Pests Management in India and Way Forward

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Abstract

Cotton is attacked by several insect pests in India, among them whitefly, Bemisia tabaci Gennadius and the American bollworm, Helicoverpa armigera Hubner are two major production constrains occurring throughout the country. To mitigate the incidence of Helicoverpa armigera, Genetically Modified (GM) cotton (Bollgard containing Cry 1 Ac) was introduced in India during 2002 and Bollgard II (Cry1 Ac + Cry 2 Ab) during 2006. By 2010, around 35 seed producing companies engaged in supplying more than 600 Bt cotton hybrids in India. The cotton productivity increased from 300 kg to 560 kg per ha and the consumption of pesticides reduced by 50%. However, during last decade, new problems started emerging like severe incidence of leafhoppers throughout the crop growth period in central and southern states of India. In Andhra Pradesh, during 2011-12, leafhoppers caused a yield loss of 20% in a Bt cotton hybrid RCH 2 and average loss across Bt cotton hybrids was 16.3%. During 2018-19, on a susceptible cultivar (DCH 32) the hopper population remained above ETL (ETL is 6 nos. per three leaves) for 17 weeks (September to January) and reached a peak of 25.5 per three leaves during 39th Standard Week. Similarly, across the central cotton growing states, the leafhopper incidence was above ETL for more than two months during the crop growth period indicating the severity of the pest on cotton. Likewise, during 2015, in a northern state of Punjab, the whitefly population remained exceedingly above ETL (ETL is 8 adults per leaf) from July to September. In farmers' fields, the average population at different locations surveyed in July and August was around 190 whiteflies per leaf. From 2014-15 onwards, Pink bollworm started appearing in many states of India and the pest has developed many fold resistance to Cry 1 Ac and Cry 2 Ab and at present posing a serious threat to cotton cultivation in central and southern states. Now, cotton farmers across India, again resorted to application of chemical insecticides indiscriminately to manage the insect pests leading to the imbalance in cotton ecosystem.

Keywords: *Emerging issues, Bt cotton, insects pests, whiteflies, Helicoverpa, way forward and India*

Background

Cotton in India is attacked by two types of insect pests viz., sucking pests and bollworms. Among sucking insect's whitefly, Bemisia tabaci Gennadius and among bollworms, the American bollworm, Helicoverpa armigera Hubner were major insect pests with pan India appearance. Whitefly became major pest on cotton after 1984 and caused havoc in central and southern cotton growing states of India during mid-1980's. Predominent reason attributed was indiscriminate application of a class of insecticides namely "Synthetic pyrethroids" which were introduced during 1981 for use against the tobacco caterpillar, Spodoptera litura Fabricius on cotton. Sufficient experimental data available from across the globe indicating that insecticide induced resurgence is prevalent in whiteflies (Kranthi 2015). Likewise, severe incidence of Helicoverpa armigera on cotton threatened the cotton cultivation throughout India and created a social disturbance in many parts of India. To combat the problem, Bt cotton (Cry 1 Ac) was allowed for commercial cultivation in central and southern cotton growing states during 2002 and in Northern cotton growing states during 2005. Later, advanced version namely Bollgard II (Cry 1 Ac + Cry 2 Ab) was allowed during 2006. After the introduction of Bt cotton, the insect pest scenario changed completely and rightly addressed the prevailing issues on cotton (Dhawan 2008 and Kranthi 2012). By 2010, around 35 seed producing companies engaged in supplying more than 600 Bt cotton hybrids in India. The cotton productivity increased from 300 kg to 560 kg per ha and the consumption of pesticides reduced by 50% (Mayee 2016). However, a few years after introduction of Bt cotton, the incidence of sucking pests like leafhoppers, mirids started appearing in central and

southern cotton growing regions and mealybugs and whiteflies in Northern states of India, to manage the same farmers resorted to application of insecticides (Udikeri 2009, Prasada Rao et al., 2014 and Singh et al., 2016). Additional to the above, severe field incidence of Pink bollworm (PBW) was noticed in some central and Southern cotton growing regions (Kranthi 2015, Mohan 2017). Chinnababu Naik and co-workers (2018) conducted extensive resistance monitoring studies in PBW populations from 2010-2017 in 38 districts of 10 cotton growing states of India and concluded that the PBW populations have developed resistance to Cry I Ac and Cry2Ab in major cotton growing districts of Central and South India. Surprisingly, this problem is unique to India. No other Bt cotton growing country in the world is facing the situation. Again, farmers across India resorted to indiscriminate application of chemical pesticides to manage the cotton pests. This paper briefly discusses issues in cotton insect management focusing the happenings during the last decade; changes in dynamics of the leafhopper, Amrasca biguttula biguttula Ishida in central and south India, outbreak of Bemisia tabaci Gennadius in north India and resistance development in Pink bollworm Pectinophora gossypiella Saunders in India and way forward to mitigate the problems.

Changes in leafhopper dynamics

Prior to 1970s, cotton area was occupied by desi varieties mostly in dry land cotton growing tracts of central and south India, where the rainfall was low and distribution was erratic. For example, in Andhra Pradesh during 1970-71 the productivity was very low (45 kg/ha). Pest attacks were also less and farmers were not habituated to apply any chemical pesticides. However, the productivity went up to 362 kg/ha during 1983-84 season mainly due to the introduction of high yielding varieties/hybrids along with improved package of practices and extension of area under irrigated situation with long staple American cotton. Further, introduction of synthetic pyrethroids during 1980s also helped in achieving higher yields by effectively managing bollworms. But, cotton farmers faced severe challenges during mid-1980's due to epidemic outbreak of whitefly and from 1987 onwards due to Helicoverpa armigera. During 2000s introduction of novel chemistries like neonicotinoids as seed treatment against cotton sucking pests kept away leafhoppers on cotton up to 30-45 Days After Sowing (DAS) for few years. However, blanket seed treatment with neonicotinoids resulted in loss of efficacy and pest started appearing even at 20 DAS (Prasada Rao et al., 2011), repeated and indiscriminate use of the chemicals as foliar sprays resulted in several fold resistance in the pest against organoposphates and neonicotinoids (Prasada Rao et al., 2014). The Resistance Ratios were worked for different groups of insecticides. During 2010, the Guntur population exhibited highest RR of 1455 for acetamiprid, 766 for thiamethoxam, 262 for imidacloprid, 24 for acephate and 19 for monocrotophos. Introduction of huge number of sucking pests susceptible Bt cotton hybrids for commercial cultivation and subsequent shift in pesticide use pattern, changes in climate (frequent dry spells during South-West monsoon period) resulted again in severe incidence of leafhoppers on cotton was observed from 2007 onwards and reached sever form from 2010 onwards. During 2016, they remained above ETL throughout the crop growth period for 23 standard weeks (Figure 1). Similarly, many centres from Gujarath (Surath and Junagadh), Maharastra (Nanded and Rahuri), Madhya Pradesh (Khandwa) and Odissa (Bhavanipatna) also recorded severe incidence of leafhoppers during 2018-19 and incidence level were above ETL for more than two months during crop growth period (AICCIP, 2018). Ramalakshmi et al., 2015, reported that in Andhra Pradesh, estimated seed cotton yield loss due to leafhoppers was 20% in a Bt hybrid RCH 2 and average yield loss across Bt cotton hybrids was 16.3%.

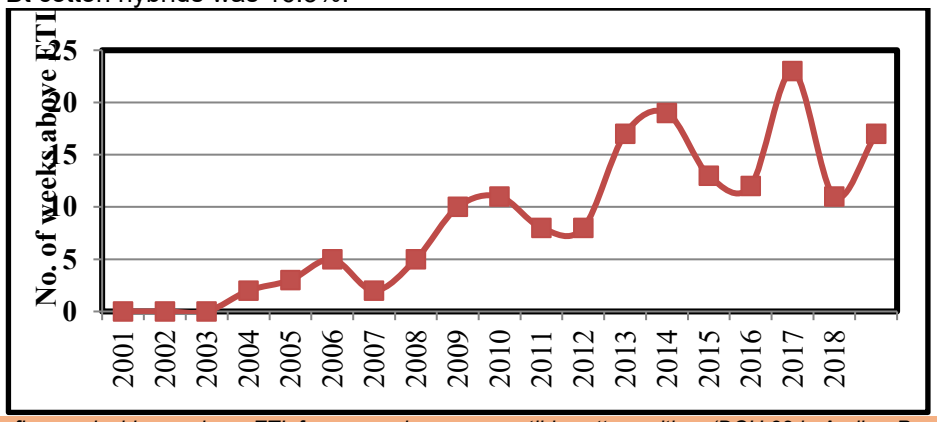

Fig. 1: *Leafhopper incidence above ETL for no. weeks on susceptible cotton cultivar (DCH 32 in Andhra Pradesh Source: AICCIP Annual reports, 2001 to 2018*

Whiteflies outbreak in North India

Historically, India witnessed several whitefly outbreaks starting from Bihar (1905), Punjab (1930), Andhra Pradesh (1984-87), Tamil Nadu, Maharastra and Karnataka (1985-87), Gujarath (186-87) and Punjab (1996). Further, whiteflies also transmit deadly Cotton Leaf Curl Disease (CLCuD) in hirsutam cotton (Rishi Kumar, 2016).

The Bt cotton was introduced during 2005 in northern states. Immediately after the introduction during 2007 season, mealybug incidence started appearing and attained severe form. Farmers applied chemicals indiscriminately. Nevertheless, the following years till 2010 saw the phenomenal decline in the mealybug population which may be attributed to comprehensive awareness and campaign programmes created across the state (Singh et al., 2012). Then, from 2010 onwards, whitefly upsurge was reported and reached to peak levels during 2015-16 season and whitefly incidence was above ETL during most part of the crop growth period and reached a peak of 190 per leaf in many farmers fields surveyed resulted in 40 - 60% yield loss across the state of Punjab. Failure to manage with insecticides and around 40-50% of the cotton area was ploughed during middle of the crop growth stage (Singh et al., 2016). It is evident from the fact that cotton productivity in Punjab has seen steep decline from 800 kg/ha during 2013 to 313 kg during 2015. Likewise, productivity of the North Zone was also dipped to 433 kg/ha during 2015 over a highest productivity of 729 kg/ha during 2013 (Table 1). It was also reported that high temperature with high humidity has a positive correlation with the population build-up of the whiteflies. Besides weather, inflow of susceptible genotypes, excessive use of nitrogenous fertilizers and insecticides or mixtures of insecticides and many other factors have played an important role in the population build-up of this notorious pest. Dr. Kranthi, 2015, the then Director. ICAR-CICR, Nagapur, India, opined that the whitefly on Bt cotton is a invited guest and an induced pest. Further, following factors responsible for the outbreak of whiteflies in North India during 2015-16.

a. Hairy and bushy genotypes
b. Late sowing
c. Indiscriminate application of nitrogenous fertilizers
d. Low availability of phosphorous and potassium fertlizers
e. Indiscriminate use of organoposphorous insectcides with pyrethroids
f. Whiteflies resistance to insecticides
g. Non implementation of IPM
h. Improper spray methods and
i. Favourable weather

Table 1. Productivity (kg lint/ha) of cotton in North zone of India (2010-11 to 2018-19)

State	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19*
Punjab	593	607	744	800	526	313	536	672	688
Haryana	587	690	719	761	603	401	611	571	690
Haryana	531	651	642	605	593	569	595	640	754
North Zone	571	651	704	729	579	433	590	617	712

Note: *Provisional, Source: AICCIP Annual report 2018-19.

Development of resistance in pink bollworm to Cry toxins

Chinnababu Naik and co-workers, 2018 reported nil larval incidence of PBW on Bt cotton in North India. But, in Central and South India larval recovery in Bt cotton ranged from 29 to 72% during 2014-2017. Likewise, mean Resistance Ratio (RR) for Cry1Ac was 47 (18-127) during 2013 that increased to 1387 (704-2060) during 2017. Similar increasing trend was observed for Cry2Ab also, mean RR increased from 5.4 (1-31) in 2013 to 4196 (1306-9366) in 2017.

Reasons for the incidence of PBW on Bt cotton in India (KR Kranthi 2015).

1. Almost all the cotton cultivating countries in the world cultivate Bt cotton varieties. India grows largely long duration Bt cotton hybrids, they serve as continuous hosts for PBW.
2. PBW develops resistance to hybrids quickly than varieties. As per Mendel's Law the seeds present in bolls of Bt cotton hybrids segregate 9:3:3:1 ratio. That means 6% of seeds contain no toxin, 56% of seeds contain Cry 1Ac and Cry 2 Ab, 19% of seeds contain Cry 1 Ac and

another 19% contain Cry 2 Ab. Thus, the seeds in a single boll at a time contain the toxins in different ratios that helps in development resistance very quickly because of selection pressure.
3. Large number of hybrids with varying flowering and fruiting periods that provide continuous food in an a overlapping manner
4. Early sowing of crop (April-May) which starts flowering that coincides with the minor seasonal peak pink bollworm that occurs in June -July.
5. Pink bollworm populations developed resistance to Cry1Ac and Cry 2Ab.
6. Squares, flowers and developing bolls that have less Bt toxin expression are more vulnerable.

Way forward

High level of incidence of leafhoppers far above ETL throughout the crop growth period, severe outbreak of whitefly and heavy field incidence and resistance levels in Pectinophora gossypiella against Bt cotton resulted again in repeated and indiscriminate use of insecticides on cotton. It is not a welcome sign and reminds us of pre Bt era. Hence, there is an urgent need to revisit and strict implantation of the time tested IRM/IPM practices, keeping in view of the complexity of the cotton pest management. Execution of community based location specific Integrated Pest Management strategies on a mission mode involving different stakeholders is the need of the day in achieving sustainable cotton production in India.

IPM strategies against sucking insect pests

1. Selection of moderately hairy sucking pest resistant varieties / hybrids.
2. Seed treatment with imidacloprid 48 FS @ 9 ml / kg protects against sucking pests during early stages of the crop.
3. Growing of intercrops like greengram or blackgram or soybean in 1:2 or 1:3 ratio will facilitate the buildup of native natural enemy populations that in turn keep sucking pests under check
4. Growing of cowpea as bund crop to encourage predacious insects like coccinellids, syrphids and chrysopids
5. Growing of barrier crops like maize and sorghum in the border rows prevents spread of sucking pests from neighbouring fields.
6. Stem application at 30 & 45 DAS with monocrotophos (1:4) and at 60 DAS with imidacloprid (1:20) for protecting the crop from early season sucking pests.
7. Setting up of yellow sticky traps @ 10 per acre for monitoring whitefly incidence.
8. ETL based application of insecticides as recommended by the state agricultural universities and research institutes.

Rishi Kumar and Co-workers 2016 proposed following IPM strategies for the management of whiteflies in North Zone.

1. Mass campaign - awareness campaigns and trainings helps in early detection.
2. Monitoring and management from February month on all alternate hosts-vegetables, ornamentals and weeds.
3. Cultivate recommended having tolerance to whitefly and CLCuD
4. Timely sowing: Upto May 15th for American cotton hybrids and upto April 30th for desi cotton varieties
5. Encourage the cultivation of desi cotton varieties since they are tolerant to whiteflies and immune to CLCuD
6. Apply only recommended dose of fertilizers as per package of practices of respective states
7. Promotion of scientific way of application of fertilizers. Apply first half dose of urea before square formation and remaining half dose between flowering to boll formation. Apply 2-4 foliar sprays of 2% potassium nitrate (13-0-45) starting from flower initiation onwards.
8. Irrigation- Apply first irrigation 4-6 weeks after sowing and afterwards based on necessity. Stop giving irrigation at 1/3rd of boll opening
9. Weed management- keep fields, bunds and surroundings weed free before sowing. Destroy volunteer plants/ratoon cotton near irrigationa channel or canal during off season
10. Barrier crop - grow two dense rows of sorghum, bajra or maize around the cotton field. Create ecological diversity by growing desi cotton or non-host crops between fields.
11. Yellow sticky traps- install 40-50 traps/acre as per the recommendation of respective SAUs during July-August. Adult suction traps during August when adult population is high.
12. Use of botanicals – initially spray 1.0% neem oil (0.03% or 300 ppm) + 0.5% laundry detergent emulsion followed by 2% castor oil emulsion + 0.5% laundry detergent

13. Use insect growth regulators (IGRs) - these are effective against whiteflies and relatively safe natural enemies. IGRs like diafenthiuron 50% WP @ 200 g/ac. or buprofezin 25% SC @ 320 ml or spiromesfin 22.9% SC @ 200 ml or pyriproxifen 10% EC @ 500 ml/ac. can be used after mid-August. To manage of second flush of whiteflies, ethion 50% EC @ 800 ml/ac. can be advised during the later part of the season in September. Against eggs and nymphs, spiromesfin or pyriproxifen is recommended.
14. In case of mixed infestation of whiteflies and leafhoppers, use flonicamid 50% WG @ 80 g / ac.
15. Never use synthetic pyrethroids, acephate and insecticide mixtures since they are known to aggravate the population if used indiscriminately.

IPM strategies for pink bollworm

Closed season and pheromone technology plays a crucial role in the management of cotton bollworms especially pink bollworm.

Closed Season

The closed season is legally enforced to stop pest carry over. Cotton plants must be destroyed to create a Dead period or Closed period, so as to prevent build-up of pests.

Application of pheromones

Lykouressis et al., 2005, evaluated the mating disruption of pink bollworm by monitoring its population with pheromone baited traps as well as sampling flowers and bolls to record damage levels in cotton fields during 1988 and 1989 in central Greece. It was concluded that mating disruption played a key role in reduction of pink bollworm catches in traps and lowering the damage. Jahnavi et al., 2019, reported that IPM module focusing on Mass trapping of PBW resulted in less (10%) open boll locule damage due to PBW in IPM module over 24% in farmers practice. Further, IPM module yielded 20% more seed cotton.

Comprehensive Integrated management strategies for the management of bollworms

a. Deep ploughing exposes bollworm pupae to birds and excessive sun heat
b. Crop rotation to break the pest cycle
c. Select sucking resistant/pest tolerant hybrids
d. Avoid spraying of insecticides like monocrotophos / acephate / neonicotinoids during the early crop growth period, these chemicals delays the maturity of the crop. Averting of these chemicals helps in synchronous early maturity of the bolls resulting in less incidence of PBW.
e. Practice balance application of NPK. Avoid excess use of nitrogenous fertilizers. Encourage the use of organic fertilizers.
f. Removal and destruction of rosette flowers, dropped squares and pre matured bolls to suppress the pest population
g. Avoid spraying of synthetic pyrethroids till November end. Spraying of synthetic pyrethroids or insecticide mixtures helps in buildup of sucking insect pests on cotton
h. Release of Trichogramma bractriae @ 60000/ac. at peak flowering period to enable the egg parasitization.
i. Promote short duration single pick varieties (150 days) under high density planting avoids PBW incidence.
j. After final picking, allow cattle, sheep and goat which will feed on immature green bolls and attacked bolls.
k. Encourage the practice of gin and field sanitation.
l. Follow ETL based sprayings (8 adults per trap per three consecutive nights).
m. Need based spray of thiodicarb 75% WP @ 300 g /ac or profenophos 50% EC @ 400 ml/ac. or quinalphos 25% EC @ 400 ml/ac. or chlorpyriphos 20% EC @ 500 ml/ac.
n. During final crop growth stages spray synthetic pyrethroids like cypermethrin 10% EC @ 250 ml/ac. or lambda cyhalothrin 5% EC @ 200 ml/ac.

References

1. AICCIP 2018-2019. Annual Report of All India Coordinated Cotton Improvement Project, Coimbatore, Tamil Nadu - 641 003. E1 - E155.
2. Chinnababu Naik, V., Sujit, K., Kranthi, S., Usha, S. and Kranthi, K. 2018. Field evolved resistance of pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechidae) to transgenic Bt-cotton expressing Cry 1Ac and Cry 2Ab in India. Pest Management Science, 74(11): 1-22.
3. Dhawan, A.K., Shera, P.S., Jindal, V., Agarwal, N. 2008. Changing scenario of cotton insect pest and their management strategies in the Punjab. p. 81-99. In: Cotton Research in Punjab. Cotton Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India.
4. Jahnavi, M., Prasad Rao, G. M.V. and Rajesh Chowdary, L. 2019. Assessment of Integrated Pest Management module for the management of Pink Bollworm in Cotton. Journal of Entomology and Zoology studies. 7 (3): 1126-1129.
5. Kranthi, K. R. 2012. Bt cotton Q&A. 1-72. Indian Society for Cotton Improvement, (ISCI). Central Institute for Research on Cotton Technology, Adenwala Road, Matunga, Mumbai, India. p. 1-72.
6. Kranthi, K. R. 2015. Pink Bollworm strikes Bt cotton. (Cotton Statistics and News published by the Cotton Association of India). 2015-16, No.35 (1st December, 2015), 1-5.
7. Kranthi, K.R. 2015. Whitefly-the black story, Cotton Statistics and News, published by the Cotton Association of India, September N0. 23, 8th Spetember 2015,1-6.
8. Lykouressis, D., Perdikis, D., Samartzis, D., Fantinou,S. and Toutouzos,S. 2005. Management of the Pink Bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechidae) by mating disruption in cotton fields. Crop Protection, 24, 177-183.
9. Mayee, C, D. 2016. Sustainable Cotton Production in India in the Past, Present and Future Supported by Technologies (Cotton Statistics and News published by the Cotton Association of India). 2016-17, No.6 (10th May, 2016), 1-9.
10. Mohan, Komaralingam, S. 2017. An area-wide approach to pink bollworm management on Bt cotton in India – a dire necessity with community participation, Current Science, Vol., 112 (10), 188-189.
11. Prasada Rao G. M. V., Kranthi, S, Prasad, N.V.V.S.D. ADG, Gurundipalli, Sujatha, P, Chenga Reddy, V. and Bhosle, B. 2014. Status of imidacloprid 70 WS (Gaucho) and thiamethoxam 70 WS (Cruiser) against the cotton leafhopper Amrasca devastans (Distant) on susceptible and tolerant btand non-bt cotton hybrids. Cotton Research Journal, Vol. 5., No.2, 96-101 pp, July-December 2014.
12. Prasada Rao, G.M.V., Prasad, N.V.V.S.D., Chenga Reddy, V. and Narayana, E. 2014. The cotton leafhopper, Amrasca devastans (Distant). ANGR Agricultural University, Lam, Guntur.1-80.
13. Ramalakshmi, V, Prasada Rao, G.M.V. and Madhumathi, T. 2015. Estimation of yield losses due to sucking pests in transgenic cotton. Indian Journal Plant Protection, 43 (1): 98-100.
14. Rishi Kumar, Sain, S. K., Monga, D., Kranthi, S., Nagare, V. S. and Kranthi, K. R. 2016. Whitefly and cotton leaf CurlDisease Management Strategies for cotton in North India. ICAR-CICR, Technical Bulletin No. 1/216, Page 12.
15. Singh, S., J.S Brar, S. Pandher, S. Kaur, P. JeyaKumar and O. M. Bambawale. 2012. Impact of area wide management of cotton mealybug Phenacoccus solenopsis (Tinsely) in Faridkot district of Punjab. Journal of Cotton Research and Devlopment, 26: 214-218
16. Singh, S., Pandheer, S., Rathore, P., Sharma, A., Kulvir, S. and gumber, R.K. 2016. From conventional to Bt Cotton and Bollworms to Whitefly: Cotton cultivation under threat in Punjab. Beltwide Cotton Conference, New Orleons, LA., January 5-7, 2016.
17. Udikeri, S.S. 2009. Incidence of mired bug, Creontiades biseretense on Bt cotton in Karnataka. Karnataka Journal of Agricultural Sciences. 22 (3 Spl. issue), 680-681.

Evaluation of a Biointensive IPM module for Management of Cotton Pink Bollworm, *Pectinophora gossypiella* (Saunders)

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Abstract

Background: *A field experiment was conducted during 2017-18 and 2018-19 at Main Agricultural Research Station, Raichur to evaluate the efficacy of egg parasitoid, Trichogrammatoidea bactrae against the cotton pink bollworm, Pectinophora gossypiella in a popular Bt cotton hybrid, Jadoo (KCH-14K59 BG-II).*

Results: *The results indicated that the inundative release of egg parasitoid, T. bactrae can significantly reduce the population of cotton pink bollworm, with a higher seed cotton yield.*

Keywords: *Cotton, egg parasitoid, Trichogrammatoidea bactrae, pink bollworm, Pectinophora gossypiella*

Background

Cotton (Gossypium spp.) is one of the most important fiber and cash crops in India and plays a predominant role in the industrial and agricultural economy of the country. It is being cultivated for domestic consumption and for export purposes in about 111 countries worldwide. Globally, cotton is cultivated in an area of 33.38 million hectares with a production of 121.37 million bales and the productivity is 792 kg per hectare (Johnson and MacDonald, 2018). India remains the leading country in cotton cultivation. In India the area under cotton crop is 124.44 lakh hectares with a production of 370 lakh bales and the productivity is 505.46 kg lint per hectare. Maharashtra, Gujarat and Telengana are the major cotton growing states of India covering 70.45 per cent of cotton area and 62.60 per cent of crop production. The area under cotton cultivation in Karnataka is about 5.46 lakh hectares with a production of 18 lakh bales and the productivity is 560.44 kg lint per hectare (Anon., 2018). More than 1326 species of insect pests have been reported on this crop throughout the world (Atwal, 2004) and nearly 130 different species of insect pests and mites are reported to cause damage to cotton crop in India which includes sucking pests and bollworm complex. Among the bollworms, the pink bollworm Pectinophora gossypiella (Saunders) is considered as the most devastating pest as it causes both qualitative and quantitative loss to the crop through its damage. Saunders (1843) reported this pest for the first time in India . It is known to have originated from Indo-Pakistan region (Cheema et al., 1980). After hatching, the early instar larva infests flowers, feeding on the floral parts by webbings inside the flower forming a rosette flower. Later, larvae bore into the bolls, tunneling in the lint and feeds on seed of cotton. In recent years, biological control method using natural enemies to manage pests has attracted more attention and has created awareness amongst all stakeholders. It is cost effective, ecofriendly and sustainable for a longer period (Babu, 1998). Many species of natural enemies are recorded on pink bollworm. The egg stage is more vulnerable to natural enemies as it is relatively more exposed when compared to larvae and pupae. The parasitization of the eggs by Trichogramma chilonis Ishii and Trichogrammatoidea bactrae Nagaraja were also noticed (Sarwar, 2017). Members of the family Trichogrammatidae (Hymenoptera) are minute egg parasitoids of several lepidopteran pests. They are 0.5 mm to 1.5 mm in size and found in terrestrial as well as aquatic habitats. Parasitoids of genus Trichogramma and Trichogrammatoidea are widely used as biocontrol agents due to their amenability to mass production in insectaries and voracious parasitizing habit on eggs of target pest hosts.

Results and Discussion

During 2017-18 the number of PBW larvae in T1 and T2 was 23.25 and 11.60 larvae per 10 bolls, respectively while in T3 maximum of 32.85 larvae per 10 bolls were noticed. Per cent rosette flowers in T1 was 7.82 per cent and it statistically differed from T2 which recorded 3.26 per cent, while in T3 it was 13.64 per cent. Highest locule damage of 60.18 per cent was noticed in T3 while in T1 and T2 the locule damage was 46.38 and 20.48 per cent. The highest seed cotton yield of 33.58 q/ha was recorded in T2 which was followed by T1 with a seed cotton yield of 29.88 q/ha and the lowest seed cotton yield of 16.78 q/ha in T3. Similarly during 2018-19 the results indicated that the number of PBW larvae in T1 and T2 was 11.62 and 8.32 larvae per 10 bolls, respectively while in T3 19.68 larvae per 10 bolls were recorded. Rosette flowers in T1 (4.36 %) and T2 (2.28 %) which differed statistically from each other and T3 recorded significantly highest rosette flower s (8.42 per cent). Highest locule damage of 40.16 per cent was noticed in T3 which was statistically inferior to T1 and T2 which recorded 12.64 and 7.84 per cent locule damage, respectively. Similarly, highest seed cotton yield of 32.56 q/ha was noticed in T2 which was followed by T1 (28.62 q/ha) and lowest seed cotton yield of 15.82 q/ha was recorded in T3. The present study is in line with Hutchison et al. (1990) who reported the effectiveness of T. bactrae on cotton pink bollworm eggs and Nadeem and Hamed (2008) also opined that T. bactrae is an important egg parasitoid to manage the pink bollworm.

Conclusion

The indiscriminate and excess use of chemical insecticides poses serious threat to non-target organisms and the environment. Hence, biological control can be adopted as a highly effective, target specific and ecofriendly insect management method. T. bactrae is a highly promising biological control agent, known to attack a number of lepidopteran pest species. In recent years, pink bollworm, P. gossypiella has emerged as a major threat to Bt cotton. To contain this pest use of biocontrol agents like egg parasitoids can be adopted. The present study indicated that the inundative releases of egg parasitoid, T. bactrae can successfully reduce the population of cotton pink bollworm, P. gossypiella, also resulting in higher seed cotton yield.

Methods

A field experiment was conducted to study the suitability and efficacy of egg parasitoid, Trichogrammatoidea bactrae Nagaraja as a component of IPM module for the management of cotton pink bollworm, P. gossypiella during two consecutive years 2017-18 and 2018-19 at Main Agricultural Research Station, Raichur. A popular cotton hybrid, Jadoo (KCH-14K59 BG-II) was cultivated with a spacing of 90 X 60 cm and the experiment composed of three treatments Viz., T1 (Erection of Pheromone Traps (Funnel type) @ 10/acre; Release of T. bactrae @ 1,00,000/ ha 6-8 releases from 35 DAS and application of azadiractin 1500 ppm), T2 (Spray of insecticides as per label claim for PBW) and T3 (Control). Each treatment was laid out in an area of one acre each and to record the observations each plot was divided into eight quadrants each of 500 sq mt size. To record the larval incidence of pink bollworm 10 bolls were randomly selected and dissected to record the number of PBW larvae in each block. Number of rosette flowers were counted in each quadrant and expressed as per cent rosette flowers. At harvest number of damaged locules was recorded in each quadrant and expressed in per cent and seed cotton yield was recorded and converted to hectare and expressed as q/ha and data was analysed statistically.

Table 1. Management of Pink bollworm by using Trichogrammatoidea bactrae in Bt cotton ecosystem during 2017-18 and 2018-19

Sl. No	Particulars	PBW larvae per 10 bolls*		Rosette flower (%) at 50% flowering #		Locule damage (%) #		Seed cotton yield (q/ha)	
		2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
1.	T1: 1. Erection of Pheromone Traps (Funnel type) @ 10/acre 2. Release of T. bactrae @ 100000/ ha 11 releases from 35 DAS	7.82 (16.24)	4.36 (12.05)	23.25 (4.87)	11.68 (3.49)	46.38 (42.92)	12.64 (20.83)	33.58	28.62

	3. Application of azadiractin 1500 ppm @ ETL								
2.	T2: 1. Profenophos 50 EC @ 2.0 ml/lt at 70 DAS 2. Thiodicarb 75 wp @ 1.0 gm/lt at 90 DAS 3. Lamda cyhalothrin 5 EC @ 0.5 ml/lt @ 110 DAS	3.26 (10.40)	2.28 (8.68)	11.60 (3.48)	8.32 (2.97)	20.48 (26.91)	7.84 (16.26)	29.88	32.56
3.	T3: Control	13.64 (21.67)	8.42 (16.87)	32.85 (5.77)	19.68 (4.49)	60.18 (50.87)	40.16 (39.33)	16.78	15.82
S Em +		0.43	0.31	0.11	0.13	1.32	0.65	1.16	1.08
CD (P=0.05)		1.29	1.04	0.34	0.40	3.96	1.96	3.58	3.25

*Figures in parentheses are square root transformed values
#Figures in parentheses are arcsine transformed values

References

1. Anonymous, 2018, Ministry of Agriculture and Farmers Welfare, Government of India, https://www.indiastat.com/table/agriculture -data/2/cotton-lint-kapas/1125690.data.aspx.
2. Atwal, A. S., 2004, Agricultural pests of South Asia and their management, Ludhiana, India, pp.221.
3. Babu, S. P. C., 1998, Biological control in India. National Training on Mass Multiplication of Biocontrol Agents, Coimbatore, 2-9 February, 1998
4. Cheema, M. A., Muzaffer, N. and Ghani, M. A., 1980, Investigations on phenology, distribution, host range and evaluation of predators of Pectinophora gossypiella (Saunders) in Pakistan. Pakistan cottons, 24: 139-176.
5. Hutchinson, W. D., Moratorio, M. and Martin J. M., 1990, Morphology and biology of Trichogrammatoidea bactrae (Hymenoptera: Trichogrammatidae), imported from Australia as a parasitoid of pink bollworm (Lepidoptera: Gelechiidae) eggs. Annals of the Entomological Society of America., 83: 46-54.
6. Johnson, J. and MacDonald, S., 2018, The World and United States Cotton Outlook. Paper presented In: Agricultural Outlook Forum 2018, 22-23, February, 2018, Virginia (USA), pp.1-16.
7. Nadeem, S. and Muhammad, H., 2008, Comparative development and parasitization of Trichogramma chilonis Ishii and Trichogrammatoidea bactrae Nagaraja under different temperature conditions. Pakistan Journal of Zoology, 40(6): 431-434.
8. Sarwar, M., 2017, Biological Parameters of Pink Bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera:Gelechiidae): A looming Threat for Cotton and its Eradication Opportunity. International Journal of Research in Agriculture and Forestry., 4(7): 25-36

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Management of Pink Bollworm Using Behavior Modifying Chemicals in Bt Cotton

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Abstract

Background: *Integrated pest management through mating disruption technique using SPLAT pheromone offers a practical and ideal approach to combat cotton pink bollworm, Pectinophora gossypiella (Saunders). Large-scale field trials were conducted during 2018-19 and 2019-20 in farmers fields to evaluate the bio efficacy and suitability of SPLAT pheromone (sex pheromone based commercial product) for management of pink bollworm. SPLAT pheromone was applied in three split dosages at 65-70, 95-100 and 125-130 days of crop growth using spoons hung on to leaf petioles of top shoots and its efficacy was compared with recommended plant protection (RPP) and untreated control for suppression of PBW incidence.*

Results: *The pink bollworm moth trap catches (2.60 and 2.40 moth/trap/night), average rosette flower (4.33 and 3.60%), green boll damage (6.22 and 7.13%), open boll damage (7.53 and 7.87%) and locule damage (8.75 and 8.42%) during 2018-19 and 2019-20, respectively was less in SPLAT pheromone treated block as compared to RPP and untreated control block. The effective reduction of pink bollworm in SPLAT pheromone treated block resulted in reduction in green boll damage which contributed for higher seed cotton yield of 28.85 and 26.50q/ha during 2018-19 and 2019-20, respectively with more net profit.*

Conclusion: *Considering the yield and advantage, SPLAT PBW pheromone found to be an economically viable and promising option for pink bollworm management in Bt cotton.*

Keywords**:** *SPLAT Pheromone, mating disruption, Pink bollworm (PBW), cotton.*

Background

Cotton is a premier commercial fiber crop and main stay of Indian economy subjected to ravages of many insect pests viz, sucking pests and bollworms right from seedling stage, their infestation impact cotton yield to an extent of 30-40 percent. In recent years, pink bollworm (PBW) has assumed major proportion even after the introduction of transgenic Bt cotton and jeopardizing the productivity. Its activity is observed in later part of cotton growing season. (Patil and Bheemanna, 1998). Soon after the emergence, the larvae enter into tender bolls. As a result farmers remain ignorant about the damaging potential of PBW and do not exercise target specific control measures. More ever, pink bollworm incidence aggravate with the advancement of cropping period when farmers stop the application of chemicals. Efforts to reduce the damage caused by this pest are made since a decade but as the early instars enters the bolls immediately after the emergence, period of exposure to insecticide is very short. Hence, insecticide application is not a viable option. In addition, regular use of insecticides resulted in many problems viz, pest resurgence, resistant development, secondary pest outbreak and upsetting of natural balance. These associated problems forced to follow integrated pest management practices comprising all suitable control strategies in close association with environment. As a possible means to reduce the pesticide load in the environment, behavior modifying chemicals are potentially viable alternative to the use of insecticides and are powerful pest management tools to integrate with other management strategies.

The synthetic sex pheromones have acquired a special role in IPM and reaches the stage of commercial production and herald a new era in management of PBW (Hummet et al.,1973). Recently, sex pheromone, in a slow release formulation reduces the pink bollworm adult population and consequent damage. Insect pest management through mating disruption with use of sex pheromones offers an ideal approach to combat the complex situation prevailing in cotton ecosystem.

Considering the need to find out suitable and practical measures to contain pink bollworm, large-scale field trials were under taken in farmers fields to evaluate the suitability and efficacy of SPLAT (Sex

pheromone formulation of pink bollworm) pheromone manufactured by ATGC-Biotech Pvt. Ltd, Hyderabad, India, during 2018-19 and 2019-20 cropping seasons.

Results

Moth trap catches

The moth trap catches was noticed from the month of August during conjugative seasons and increased gradually upto December thereafter the trap catches was subsided gradually and became negligible. Among the blocks lowest moth trap catches of 2.60 and 2.50 per trap per night was recorded in mating disruption blocks as compared to 4.00 and 3.59 moth per trap per night in recommended plant protection blocks (RPP) during the season. On contrary higher moth trap catches of 9.43 and 9.05 per trap per night was recorded in untreated control during the respective years and the moth trap catches was above the threshold level throughout the cropping season (Table 1)

Flowers damage (%)

Application of SPLAT pheromone influenced the pink bollworm damage on cotton flowers considerably. Initially PBW damage on flower was noticed during the first fortnight of August, during the consecutive season and increased gradually reaching its peak during September. There after declined after the formation of bolls. Comparative data on percent rosetted flower indicated that, SPLAT pheromone treated block registered low percentage of rosette flower (4.33% and 3.60% with a mean of 3.97%) compared to (7.69% and 7.60% with mean of 7.65%) recommended plant protection block. On the contrary flower damage in untreated block was on higher side (10.72% and 10.10% with a mean of 10.19%) during the consecutive seasons (Table 2).

Green boll damage (%)

The green boll damage (%) due to PBW was noticed from the second fortnight of the August and increased gradually with the advancement of cropping seasons. The lowest green boll damage of 6.22 and 7.13 per cent with a mean of 6.68 per cent with a reduction of 69.84 and 68.38 per cent over the control as compared to 11.52% and 11.54 per cent with a reduction of 44.15 per cent and 45.29 per cent in recommended plant protection block during the respective seasons however untreated control registered higher green boll damage of 21.63 per cent and 21.53 per cent during the consecutive seasons (Table 3)

Open boll damage (%)

SPLAT PBW pheromone treated block proved to be superior by recording lowest open boll damage of 7.53 and 7.87 per cent with a reduction of 70.98 and 71.63 per cent over control as compared to 21.43 and 22.27 per cent with reduction of 17.41 and 16.75 per cent reduction in open boll damage. On the contrary untreated block registered higher per cent open boll damage of 25.95 and 27.75 per cent, respectively during the cropping period (Table 4)

Locule damage (%)

The observation on locule damage revealed that SPLAT pheromone treated block registered lowest locule damage of 8.75 and 8.42 per cent with a reduction of 75.69 and 76.23 per cent over untreated control and found superior to recommended plant protection during both during season (Table 5).

Impact on natural enemies

The observations made during the respective years on the occurrence of natural enemies viz, coccinellids, chrysopids and syrphids in all the three blocks revealed no significant difference between untreated and SPLAT pheromone treated blocks. However, significantly, lowest natural fauna of coccinellids and chrysopids per plant were registered in recommended plant protection block (Table 6)

Yield and Economics

Behavioural modifying chemical influenced the incidence of PBW which lead to the contribution of higher seed cotton yield among the blocks SPLAT PBW pheromone treated block recorded higher seed cotton yield of 28.85 and 26.5 q/ha with higher profit of Rs 100450 and 137800 rupees during the respective years and found superior to recommended plant protection (Table 7)

Discussion

The influence of behavior modifying chemical on the incidence of pink bollworm was quite evident. Among the experimental blocks the data recorded over two seasons clearly indicated that, SPLAT pheromone treated block registered lowest moth trap catch (2.60 and 2.50 moths / trap / night) with a

reduction of 56.20 and 61.12 per cent and rosetted flower of 4.33 and 3.66 per cent with a reduction of 52.96 and 64.35 per cent, green boll damage of 6.22 and 7.13 per cent with a reduction of 69.84 and 66.92 per cent, open boll damage of 7.53 and 7.87 per cent with a reduction of 70.98 and 71.63 per cent, and locule damage of 8.75 and 8.42 per cent with a reduction of 75.69 and 76.23 per cent over untreated block. The effective suppression of pink bollworm in SPLAT pheromone treated blocks during the consecutive seasons, resulted in significant reduction in PBW incidence which contributed towards more number of good opened bolls per plant and higher seed cotton yield (28.85 and 26.50 q/ha). These results are in agreement with Qureshi et. al., (1988) who observed lower incidence of pink bollworm in flower (0.19%) and green boll damage (1.67%) using PB RopeL @ 200/ha. Similarly, Patil et. al., (2007) registered lower population of 9.76 and 8.40 larvae/50 bolls in PB Rope @ 200 dispensers per ha as compared to control block (20.48 and 19.40 larvae/50 bolls) during 2005 -06 and 2006-07 respectively. The effective suppression of pink bollworm in SPLAT pheromone treated block resulted in significant reduction in green boll damage contributed for higher seed cotton yield of 27.68 q/ha compared to recommend plant protection (18.75 q/ha) and untreated blocks (15.30 q/ha). These results corroborated with the findings of Shrinivas et.al. (2019) who reported higher seed cotton yield of 46.25 q/ha in SPLAT treated block compared to 24.55 q/ha in farmer's fields.

Conclusion

In the present investigation, there was marked enhancement in yield as well as net return in SPLAT pheromone treated block. Considering the yield advantage and net profit, application of SPLAT PBW @1250 g/ha found to be economically viable and promising options for pink bollworm management in Bt cotton.

Table 1: Comparative performance of different management practices on PBW moth trap catches

Month	Month wise mean PBW moth trap catch / night								
	2018-19			2019-20			Pooled Data		
	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)
Aug	0.58	0.36	4.00	1.75	1.20	4.75	1.17	0.78	4.38
Sept	3.80	1.38	5.75	2.15	1.15	6.15	2.98	1.27	5.95
Oct	4.26	2.13	7.15	2.97	2.15	7.22	3.62	2.14	7.19
Nov	5.48	3.71	13.75	3.95	3.01	11.50	4.72	3.36	12.63
Dec	6.91	5.70	16.50	5.11	4.25	13.75	6.01	4.98	15.13
Mean	4.00	2.60	9.43	3.18	2.40	8.67	3.59	2.50	9.05
% Reduction over control	31.10	56.20	-	26.10	61.12	-	28.6	58.7	-

Table 2: Comparative performance of different management practices on rosette flowers

Month		Rosette flowers (%)								
		2018-19			2019-20			Pooled data		
		Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)
Aug	I FN	3.50 (10.78)	1.25 (6.42)	7.25 (15.62)	3.95 (11.46)	1.95 (8.03)	6.71 (15.01)	3.73 (11.14)	1.60 (7.25)	6.98 (15.32)
	II FN	6.45 (14.71)	3.47 (10.74)	8.15 (16.59)	8.16 (16.60)	2.27 (8.67)	9.15 (17.61)	7.31 (15.69)	2.87 (9.75)	8.65 (17.10)
Sept	I FN	13.36 (21.44)	8.23 (16.67)	16.07 (23.63)	11.65 (19.96)	6.31 (14.55)	13.50 (21.56)	12.51 (20.71)	7.27 (15.64)	14.79 (22.62)
	II FN	14.21 (22.15)	9.23 (17.69)	14.17 (22.11)	12.30 (20.53)	7.35 (15.73)	16.75 (24.16)	13.26 (21.35)	8.29 (16.73)	15.46 (23.15)
Oct	I FN	12.85 (21.01)	7.14 (15.50)	13.95 (21.93)	10.31 (18.73)	6.70 (15.00)	17.00 (24.35)	11.58 (19.89)	6.92 (15.25)	15.48 (23.17)
	II FN	9.14 (17.60)	5.88 (14.03)	11.92 (20.20)	9.10 (17.56)	3.45 (10.70)	13.40 (21.47)	9.12 (17.58)	4.67 (12.48)	12.66 (20.84)
Nov	I FN	6.78 (15.09)	2.87 (9.75)	9.15 (17.61)	8.00 (16.43)	3.00 (9.97)	10.00 (18.43)	7.39 (15.77)	2.94 (9.87)	9.58 (18.03)
	II FN	4.36 (12.05)	2.64 (9.35)	7.18 (15.54)	7.05 (15.40)	1.35 (6.67)	6.70 (15.00)	5.71 (13.82)	2.00 (8.13)	6.94 (15.27)
Dec	I FN	3.84 (11.30)	2.00 (8.13)	6.71 (15.01)	4.45 (12.18)	1.21 (6.32)	5.11 (13.06)	4.15 (11.75)	1.61 (7.29)	5.91 (14.07)
	II FN	2.41 (8.93)	1.24 (6.39)	3.15 (10.22)	2.00 (8.13)	1.20 (6.29)	2.75 (9.55)	2.21 (8.55)	1.22 (6.34)	2.95 (9.87)
Mean %		7.69	4.33	10.27	7.60	3.60	10.10	7.65	3.97	10.19
Reduction over control		24.12	52.96	-	23.86	64.35	-	23.99	58.66	-

Tcal value (RPP vs MD) = 2.58 (P(T<=t)two-tail:0.018); Tcal value (RPP vs Control) =1.17 (P(T<=t)two-tail:0.25);
Tcal value (MD vs Control)= 3.78 (P(T<=t)two-tail:0.001)

Note: Figures in the parentheses are arcsine transformed values, Table t value=2.10

Table 3: Comparative performance of different management practices on green boll damage

Month		Green boll damage (%)								
		2018-19			2019-20			Pooled data		
		Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)
Aug	I FN	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	II FN	6.00 (14.18)	2.00 (8.13)	0.00 (0.00)	2.60 (9.28)	0.00 (0.00)	3.00 (9.97)	4.30 (11.97)	1.00 (5.74)	1.5 (7.03)
Sept	I FN	6.00 (14.18)	4.00 (11.54)	6.15 (14.36)	3.15 (10.22)	3.75 (11.17)	12.13 (20.38)	4.58 (12.36)	3.88 (11.36)	9.14 (17.60)
	II FN	10.00 (18.43)	7.00 (15.34)	22.33 (28.20)	7.70 (16.11)	8.90 (17.36)	18.75 (25.66)	8.85 (17.31)	7.95 (16.38)	20.54 (26.95)
Oct	I FN	12.50 (20.70)	8.00 (16.43)	25.10 (30.07)	2.50 (9.10)	4.90 (12.79)	26.65 (31.08)	7.50 (15.89)	6.45 (14.71)	25.88 (30.58)
	II FN	12.75 (20.92)	6.60 (14.89)	27.75 (31.79)	10.60 (19.00)	6.22 (14.44)	28.15 (32.04)	11.68 (19.98)	6.41 (14.67)	27.95 (31.92)
Nov	I FN	17.15 (24.46)	8.20 (16.64)	24.00 (29.33)	13.70 (21.72)	7.15 (15.51)	37.00 (37.46)	15.43 (23.13)	7.68 (16.09)	30.5 (33.52)
	II FN	19.25 (26.02)	9.11 (17.57)	28.95 (32.55)	18.90 (25.77)	9.95 (18.39)	27.00 (31.31)	19.08 (25.90)	9.53 (17.98)	27.98 (31.94)
Dec	I FN	16.45 (23.93)	10.10 (18.53)	38.06 (38.09)	27.00 (31.31)	12.11 (20.36)	32.00 (34.45)	21.73 (27.78)	11.11 (19.47)	35.03 (36.29)
	II FN	15.12 (22.88)	7.25 (15.62)	34.00 (35.67)	17.35 (24.62)	18.75 (25.66)	31.00 (33.83)	16.24 (23.77)	13.00 (21.13)	32.5 (34.76)
Mean %		11.52	6.22	20.63	11.55	7.13	21.53	11.54 (19.86)	6.68 (14.98)	21.08 (27.33)
Reduction over control		44.15	69.84	-	46.42	66.92	-	45.29	68.38	-
Tcal value (RPP vs MD)								1.21 (P(T<=t)two-tail:0.23)		
Tcal value (RPP vs Control)								1.51 (P(T<=t)two-tail:0.14)		
Tcal value (MD vs Control)								2.52 (P(T<=t)two-tail:0.02)		

Note: Figures in the parentheses are arcsine transformed values, Table t value=2.10

Table 4: Comparative performance of different management practices on Open boll damage

Pickings	Open boll damage (%)								
	2018-19			2019-20			Pooled Data		
	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)
I	15.68 (23.33)	6.23 (14.45)	21.70 (27.76)	14.17 (22.11)	7.19 (15.55)	23.60 (29.06)	14.93 (22.72)	6.71(15.01)	22.65 (28.41)
II	28.25 (32.11)	7.42 (15.81)	24.65 (29.77)	27.21 (31.44)	9.12 (17.58)	27.15 (31.40)	27.73 (31.76)	8.27 (16.71)	25.9 (30.58)
III	20.47 (26.90)	9.35 (17.80)	27.30 (31.50)	22.32 (28.19)	8.05 (16.48)	30.60 (33.58)	21.40 (27.40)	8.70 (17.15)	28.95 (32.54)

IX	21.34 (27.51)	7.12 (15.48)	30.15 (33.30)	28.72 (32.41)	7.15 (15.51)	29.65 (32.99)	25.03 (30.01)	7.14 (15.49)	29.9 (33.14)
Mean	21.43	7.53	25.95	23.10	7.87	27.75	22.27	7.70	26.85
% Reduction over control	17.41	70.98	-	16.75	71.63	-	17.08	71.31	
			T value (RPP vs MD)				5.88 (P(T<=t)two-tail:0.001)		
			T value (RPP vs Control)				1.41 (P(T<=t)two-tail:0.207)		
			T value (MD vs Control)				12.76 (P(T<=t)two-tail:1.419E-05)		

Note: Figures in the parentheses are arcsine transformed values, Table t value=2.44

Table 5: Comparative performance of different management practices on Locule damage

Pickings	Locule Damage (%)								
	2018-19			2019-20			Pooled Data		
	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)	Recommended plant protection	Mating disruption	Control (No spray for PBW)
I	16.50 (23.97)	7.50 (15.89)	30.50 (33.52)	17.00 (24.35)	9.00 (17.46)	27.00 (31.31)	16.75 (24.16)	8.25 (16.69)	28.75 (32.42)
II	18.50 (25.47)	8.50 (16.95)	37.75 (37.91)	22.15 (28.08)	7.80 (16.22)	35.00 (36.27)	20.33 (26.80)	8.15 (16.59)	36.38 (37.10)
III	22.00 (27.97)	10.00 (18.43)	31.00 (33.83)	25.00 (30.00)	9.75 (18.19)	38.75 (38.50)	23.50 (29.00)	9.88 (18.32)	34.88 (36.20)
IX	19.50 (26.21)	9.00 (17.46)	44.75 (41.99)	23.00 (28.66)	11.15 (19.51)	41.00 (39.82)	21.25 (27.45)	10.08 (18.51)	42.88 (40.91)
Mean	19.13	8.75	36.00	21.78	8.42	35.43	20.46	8.59	35.72
Percent Reduction over control	46.86	75.69	-	38.52	76.23	-	42.69	75.96	-
			T value (RPP vs MD)				8.23 (P(T<=t)two-tail:0.0001)		
			T value (RPP vs Control)				4.87 (P(T<=t)two-tail:0.002)		
			T value (MD vs Control)				10.53 (P(T<=t)two-tail:4.3E-05)		

Note: Figures in the parentheses are arcsine transformed values, Table t value=2.44

Table 6: Impact of different management practices on natural fauna (Coccinellids, Chrysoperla and Syrpids)

Months		Pooled Data (2018-19 and 2019-20)		
		Recommended plant protection	Mating disruption	Control (No spray for PBW)
Aug	I FN	0.75 (0.87)	1.37 (1.17)	1.40 (1.18)
	II FN	1.00 (1.00)	2.15 (1.47)	2.40 (1.55)
Sept	I FN	0.80 (0.89)	2.75 (1.66)	2.15 (1.47)
	II FN	0.63 (0.79)	1.95 (1.40)	2.17 (1.47)
Oct	I FN	1.23 (1.11)	2.13 (1.46)	1.97 (1.40)
	II FN	0.96 (0.98)	2.45 (1.57)	2.08 (1.44)
Nov	I FN	0.87 (0.93)	3.15 (1.77)	3.10 (1.76)
	II FN	0.92 (0.96)	3.05 (1.75)	2.95 (1.72)
Dec	I FN	1.37 (1.17)	2.75 (1.66)	3.00 (1.73)
	II FN	1.45 (1.20)	2.93 (1.71)	3.15 (1.77)
Mean		0.99	2.46	2.43
	Tcal value (RPP vs MD)		7.79 (P(T<=t)two-tail:3.53E-07)	
	Tcal value (RPP vs Control)		7.53 (P(T<=t)two-tail:5.75E-07)	
	Tcal value (MD vs Control)		0.12 (P(T<=t)two-tail:0.90)	

Note: : Figures in the parentheses are square root transformed values, Table t value: 2.10

Table 7:Comparative performance of different management practices on yield and economies

Particulars	Yield and economies								
	2018-19			2019-20			Pooled Data		
	Recommended plant protection	Mating disruption	Control (No treatment for PBW)	Recommended plant protection	Mating disruption	Control (No treatment for PBW)	Recommended plant protection	Mating disruption	Control (No treatment for PBW)
Seed Cotton yield (q/ha)	19.50	28.85	14.70	18.00	26.50	15.90	18.75	27.68	15.30
Cost of cultivation (Rs./ha)	37500	67500	37500	38000	38000	38000	37750.00	52750.00	37750.00
Cost of treatment (Rs./ha)	4600	6300	4600	4900	6300	4750	4750.00	6300.00	4675.00
Total Cost (Rs. /ha)	42100	43800	42100	42900	44300	42750	42500.00	44050.00	42425.00
Gross Return (Rs. / ha)	97500	144250	73500	93600	137800	82680	95550.00	141025.00	78090.00
Net Return (Rs./ha)	55400	100450	31400	50700	93500	89930	53050.00	96975.00	60665.00
Market value		Rs. 5000/qts			Rs.5200/ qts			Rs.5100.00/qts	

Methods

Large scale field trials were layout in farmers fields using Bt cotton hybrid, MRCH-7383 (BG-11) during June second week of 2018-19 and 2019-20 with a spacing of 90cm x 30cm under unprotected condition in three independent block of one hectare each, following recommended agronomical practices. The material and methods used for implementation of field experiments are as detailed below.

Block A: In this block, recommended insecticides for pink bollworm management based on the economic threshold level was taken up in addition to recommended plant protection measures for sucking pests control as and when the pests crossed threshold. The insecticidal intervention are as detailed below.

Target pests: Sucking pests

Sl.No	Name of insecticides
1	Fipronil 5%SC @ 1ml/l
2	Dinetofuron 20%SG @ 0.3g/l
3	Flonicamid 50%SG @ 0.3g/l
Sl.No	Target pest: Pink bollworm
1	Profenophos 50%EC @ 1.0 ml/l
2	Spinosad 45%SC @ 0.20 ml/l
3	Lamda cyhalothrin 5EC @ 0.5 ml/l

Block B: For mating disruption of PBW moths, SPLAT-PBW @ 1250 mg per hectare was applied in three split dosages. First application was made at 65-70 days after the sowing followed by 95-100 and 125-130 days of crop growth using spoons hung on to leaf petioles of top shoots. For sucking pests control, recommended plant protection measures were taken up as followed in block A based on threshold level.

Block C: In this block, similar agronomical practices and recommended plant protection for sucking pests' management were taken up and no chemical interventions were made for pink bollworm management.

Observations

In order to monitor the moth activity, two sleeve traps were installed in all the three blocks. The heights of the traps were kept 30 cm above the crop canopy and lures were replaced regularly at fortnightly intervals. The trapped moths of PBW were recorded at weekly interval till the end of trials during consecutive seasons. The moths were removed from the traps after counting and data has been presented in the farm of monthly average per trap catches /night basis.

The observations on rosette flowers due to PBW infestation was started on 25 randomly on selected plants from flowering stage and continued till the boll development. Based on the total number of flowers and number of rosette flowers per plant, percent flower resetting was worked out. Similarly for observation on the green boll damage, 50 bolls of two weeks old were collected randomly from each block and cut opened. Based on the total number and damaged bolls by pink bollworm, percent green boll damage was worked out. At the time of each picking, one hundred fully opened bolls were sampled randomly from each block. Based on number of locules and damaged locules due to PBW, percent locule damage was worked out. The population of predators viz, syrphids, coccinellids and chrysopids (Chrysoperla) were recorded on whole plant basis from ten plants and averaged to population per plant. Seed cotton yield was recorded over four picking. The data presented as cumulative yield (q/ha). Net profit was calculated by deducting the cost of cultivation from the gross income and expressed as net profit per hectare in rupees.

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References

1. Henneberry T. J., 1978, Pink bollworm: Chemical control in Arizona and relationship to infestation, lint yield, seed damage and aflotoxin in cotton seed J.Econ. entomol., 71,440-443
2. Patil, B.V., Bheemanna, M., 1998, Integrated pest management strategy for irrigated cotton. Proceedings of World Cotton Conference-2, Athens, Greece, Sept 6-12, 1998 pp. 801-805.
3. Patil, B.V., Bheemanna, M., Hanchinal, S.G. and Kengegowda, N., 2004, Developing IPM module for Bt cotton under irrigated ecosystem. In: Int. Symp.Strat. Sust Cotton Prodn, - A G Vis.3. Crop Protection, 23-25 November 2004, UAS, Dharwad, Karnataka (India),pp. 152-154.
4. Qureshi Z.A, M.D Arif, N Ahmed and Nazeebullah 1988 Control of pink bollworm by mating disruption technique. Pakistan. J.Sci.Ind.Res., 31,711-713.
5. Shrinivas., Sreenivas, A.G., Hanchinal, S.G., Sujay, H. and Beldhadi, R.V., 2009, Evaluation of different mass trapping mating disruption tools against pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) in Bt cotton ecosystem. J Entomol. Zoo. Stud., 7 (1): 1043-1048.

Molecular Characterization of Local Strains of the Causal Agent of Cotton Bacterial Blight in Argentina

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Abstract

Background: *Cotton Bacterial Blight is a disease caused by the pathogen Xanthomonas citri subsp. malvacearum. The disease causes losses up to 35% over final yield. There were identified 19 races of the bacterium, however there are no studies made about the pathogen variability in cotton growing areas in Argentina.*

Results*: In the present study, the isolation and molecular characterization of native isolates of the Cotton Bacterial Blight pathogen present in North of Argentina were carried out. Symptoms were identified in cotton cultivars grown in field and 36 isolates with Xanthomonas spp. morphological characteristics were obtained. Molecular analysis showed that two isolates belong to Xanthomonas citri subsp. malvacearum. Moreover, phylogenetic analysis using lepA housekeeping gene, showed that both isolates group within the races 1, 2, 3, 12 and 18, suggesting that these isolates could belong to any of these races. Additionally, pathogenicity assays carried out in Bacterial Blight susceptible cotton showed no differences between both isolates in relation with its growth.*

Conclusion*: Taken together, our preliminary data allowed to stablish basis in the knowledge about Xcm variability in cotton grow area in North of Argentina.*

Keywords: *Xanthomonas citri subsp. malvacearum, cotton bacterial blight, races, multilocus sequence analysis*

Background

Cotton Bacterial Blight (CBB) is a disease caused by the bacterium *Xanthomonas citri* subsp. *malvacearum* (ex Smith, 1901) (*Xcm*) (Schaad *et al*., 2005; 2006). The pathogen is distributed among all cotton growing areas of the world, and it has caused losses up to 35% of final yield in susceptible cultivars (Delannoy *et al*., 2005).

At present there have been identified 19 races of the pathogen in basis of their capacity of the induction of susceptibility or resistance in a defined panel of cotton varieties carrying different resistance genes. Among all races, race 18 is considered the most dangerous and it is supposed distributed in all cotton growing areas of the world (Jalloul *et al*., 2015; Delannoy *et al*., 2005; Hunter *et al*., 1968). *Xcm* is considered a high risk pathogen to cotton production because it can turn "*hypervirulent*" and cause infection in all commercial cotton varieties, becoming rapidly in a problem to cotton cost *effectiveness* (Akello & Hillocks, 2002; Maas, 2012).

In Argentina there are no studies about CBB pathogen variability in cotton growing areas. In order to contribute to the development of CBB management strategies in an efficiently and durable way, there is an interest in the knowledge of local strains of the pathogen.

The MLSA (Multilocus Sequence Analysis) has proved been a trustable way to classify bacterial species and subspecies of *Xanthomonas* genus. Almeida *et al.* (2010) have designed a bioinformatic platform that counts with a database specifically designed to plant pathogen microbes and other microbes associated with plants: Plant-Associated Microbes Database (PAMDB). The collection data of *Xanthomonas* gathers sequences of different housekeeping genes. Particularly to *Xcm*, PAMDB gathers data of isolations obtained from different countries.

The aim of the present work was to contribute to the understanding about the variability of *Xcm* in the cotton growing area of the North of Argentina by the isolation and molecular characterization of native strains.

Results

CBB symptoms were identified in cotton cultivars. Particularly, symptoms of CBB were identified in leaves and bolls of cotton DP 50 and Poraite INTA cultivars (Figure 1) grown in the experimental field of the Agronomics Experimental Station of the National Institute of Agricultural Technology in Reconquista-Santa Fe (Argentina), during 2018/19 growing season.

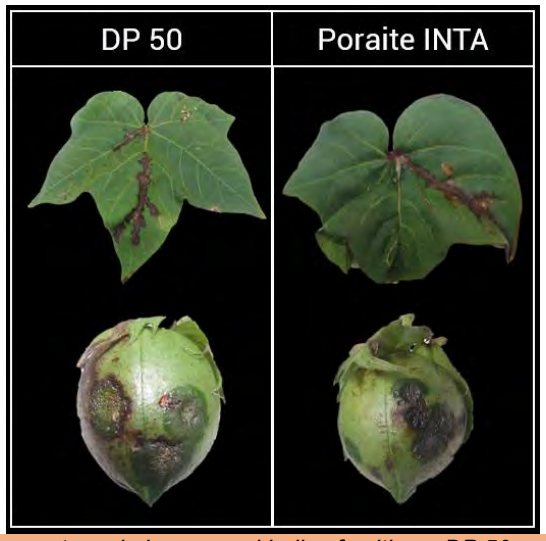

Figure 1. CBB symptoms in leaves and bolls of cultivars DP 50 and Poraite INTA

A collection of 36 isolates showing *Xanthomonas* spp. colony characteristics was obtained. To confirm the capacity of the isolates to induce disease, pathogenicity assays in cotton susceptible to CBB were made. Only 2 of the 36 isolates showed a water soaking like phenotype in response to inoculation (Figure 2A). To evaluate if there were differences in growth behavior between both isolates, *in planta* bacterial growth curves were made. The analysis of the curves determined that at 10 days post inoculation, the strains showed no statistical difference in relationship with its growth in susceptible cotton foliar tissue (Figure 2B).

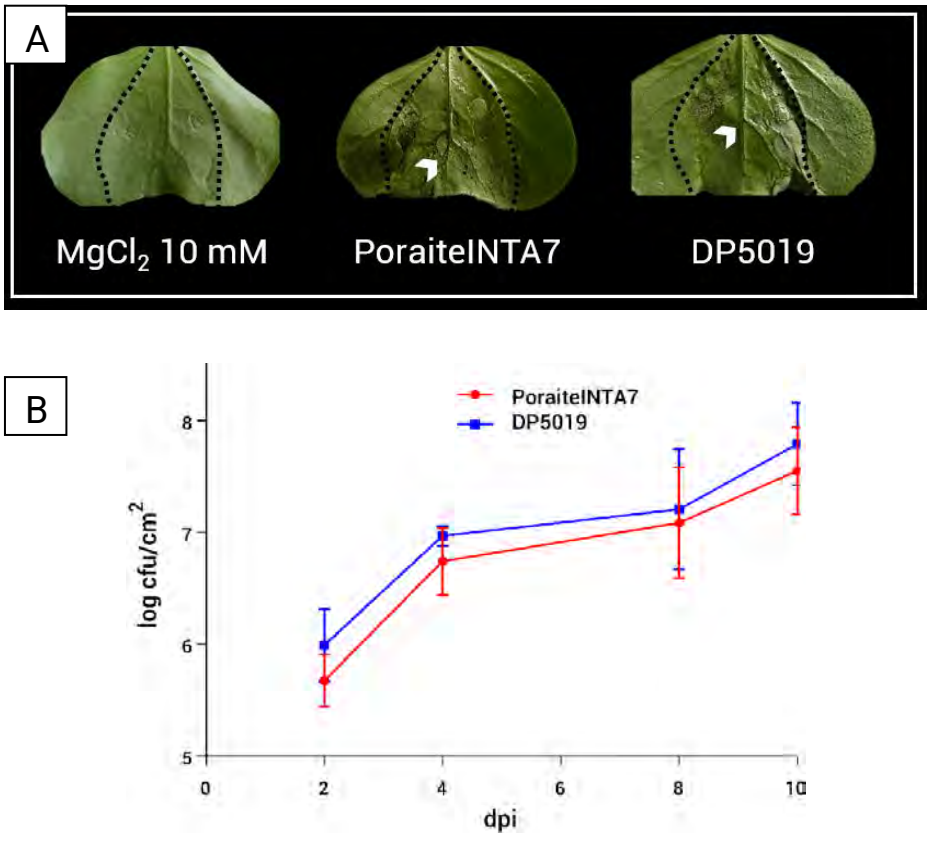

Figure 2. Growth behavior of two isolates capable of inducing disease

A: Water Soaking like phenotypes observed in cotyledons inoculated with bacterial suspensions of isolates “PoraiteINTA7” and “DP5019”
White arrows show water soaking symptoms. Spotted lines define inoculated areas. As negative control a sterile solution of $MgCl_2$ 10 Mm was used

B: *In planta* growth curves for isolates “PoraiteINTA7” and “DP5019” of *Xcm* obtained in the present work Values are expressed as media of three independent experiments ± standard deviation

Molecular analysis was performed on both isolates through amplification of *lepA* housekeeping gene. The phylogenetic tree was performed by comparing partial sequences of *lepA* gene obtained from 215 species of *Xanthomonas* (Almeida *et al*., 2010) and genomic sequences of *Xcm* races 18 and 20 (Cunnac *et al*., 2013; Phillips *et al*., 2017; Showmaker *et al*., 2017; Zhai *et al*., 2013). Phylogenetic tree was constructed based in partial sequences of *lepA* gene of 219 *Xanthomonas* strains and the sequences of the new isolates (“PoraiteINTA7” and “DP5019”). MAFFT and IQTree were used for the alignement and tree construction. Both isolates (“PoraiteINTA7 and DP5019”) grouped within *Xcm* cluster (Figure 3A). Interestingly, both isolates also grouped within *Xcm* reference races 18 bacteria (“XanthomonascitrimalvacearumX18” and “Xanthomonas citrimalvacearum strGSPB1386race18”) (Figure 3B).

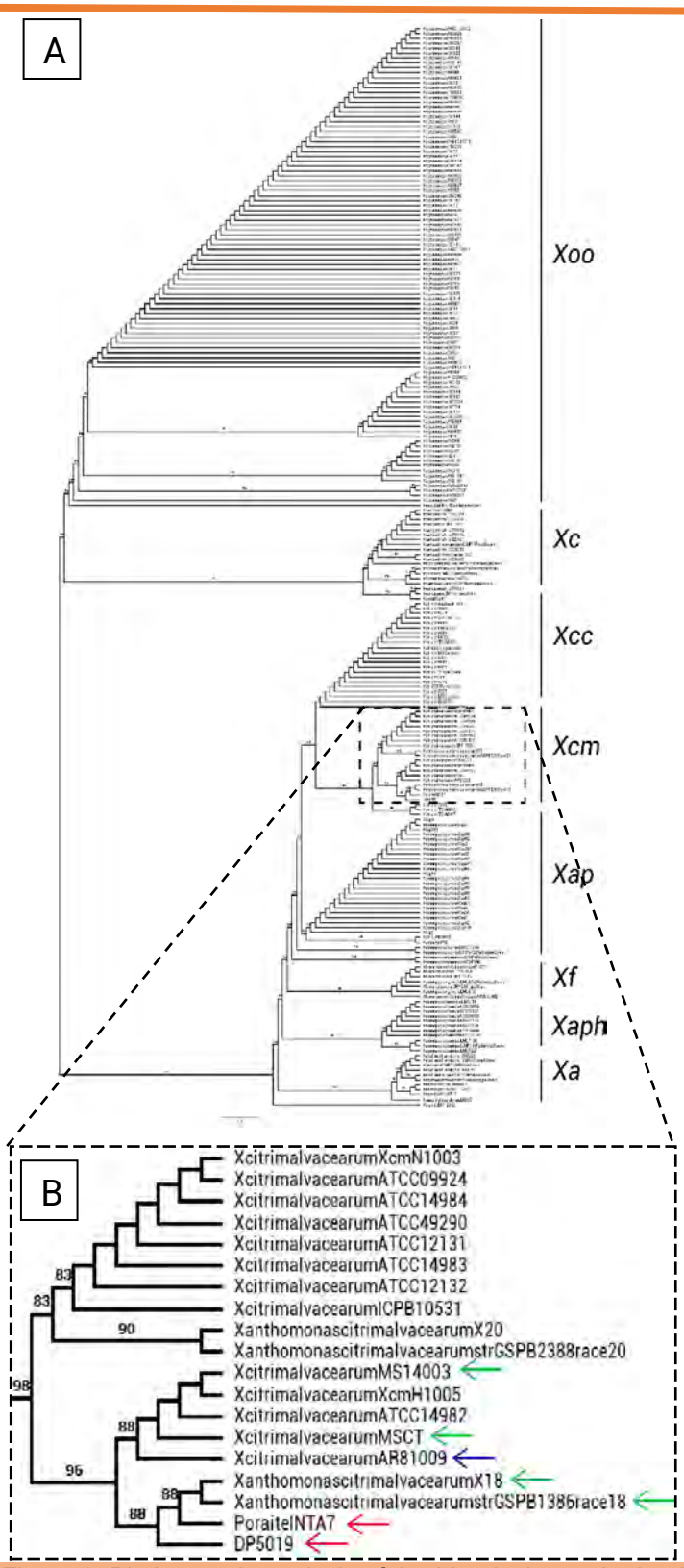

Figure 3. Phylogenetic analysis of the *Xcm* isolates obtained

A: full phylogenetic tree of total of *lepA* sequences analized. *Xa*: *Xanthomonas alfalfae*; *Xap*: *Xanthomonas axonopodis* pv. *punicae*; *Xaph*: *Xanthomonas axonopodis* pv. *phaseoli*; *Xc*: *Xanthomonas campestris*; *Xcc*: *Xanthomonas citri* subsp. *citri*; *Xcm*: *Xanthomonas citri* subsp. *malvacearum*; *Xf*: *Xanthomonas fuscans*; *Xoo*: *Xanthomonas oryzae* pv. *oryzae*.

B: amplified region of the tree corresponding to *Xcm* cluster.

Blue arrow shows the isolate original from Argentina. Green arrows show race 18 *Xcm* reference strain. Red arrows show the isolates obtained in the present work.

Although the isolates of the present work grouped within race 18 *Xcm* reference bacteria, in this group is also present an isolate of *Xcm* original from Argentina (AR81009), but there is no clear information about the race group in which this isolate belongs to.

Previously, Phillips *et al*., (2017), through a MLSA analysis using six housekeeping genes, were incapable of determining the race group in which AR81009 belongs to, suggesting that the isolate could belong to any of the races 1, 2, 3, 12 or 18.

Discussion

Two strains of *Xanthomonas citri* subsp *malvacearum* were isolated in cotton productive areas of Argentina. The phylogenetic analysis demonstrated that both isolates grouped in the same clade with the *Xcm* reference strains.

These new isolates grouped within other *Xcm*, some of them also belonging to race 18. Race 18 is considered one of the most aggressive and is supposed worldwide distributed. Currently, there is no knowledge about if this distribution is due to pathogen movement between world cotton areas or if the pathogens could come from independent origins (De Sousa Braga *et al*., 2016; Hillocks, 1992; Phillips *et al*., 2017).

Conclusions

In basis of the results obtained in this work, the new *Xcm* bacteria isolated could belong to any of races 1, 2, 3, 12 or 18 clade. In order to clarify the race group where the isolates belong, it will be necessary to incorporate in the MLSA analysis at least other two housekeeping genes, or analyze the new isolates through a Multiple Locus Variable Number of Tandem Repeats Analysis (MLVA) that allows the correct differentiation of the races of monomorphic bacteria (Zhao *et al.,* 2012; Pruvost *et al.,* 2014). The results of the present work allowed to stablish basis in the knowledge about *Xcm* variability in cotton grow area in North of Argentina.

Methods

Isolation of bacterial strains

The cotton varieties analyzed in this study were located in the experimental field of the Agronomics Experimental Station of the National Institute of Agricultural Technology in Reconquista-Santa Fe (Argentina), during 2018/19 growing season. Leaves and bolls of different cotton varieties grown in field conditions showing CBB symptoms were collected. Infected areas of collected tissue were cut in small pieces (4 x 2 mm), surface sterilized with sodium hypochlorite 1% (w/v) and washed with sterile distilled water twice. Bacterial cells were collected by homogenization of tissue with a plastic pestle. The suspensions were plated on nutrient agar medium (Britania, Buenos Aires). The plates were incubated at 28°C for 24-48 h. Yellow-colored colonies with typical morphological characteristics of *Xanthomonas* spp. colonies, were picked up again and streaked separately on agar plates to purify each strain.

Vegetal material, growth conditions and pathogenicity assays

For pathogenicity assays, Deltapine 50 (DP 50) seedlings, susceptible to local *Xcm*, with expanded cotyledons were used. The plants were grown and maintained after inoculation in growth chamber at 23 °C, a 12 h light-12 h dark photoperiod and 120-200 µE/sm^2 light.

Bacterial suspensions were prepared in a 10 Mm $MgCl_2$ sterile solution. The bacterial concentration was spectrophotometrically measured at λ=600 nm, using an UV-Vis Lambda 25 spectrophotometer (Perkin Elmer) and adjusted to 10^7 cfu/ml (OD_{600}=0,1).

The bacterial suspensions were surface inoculated in abaxial faces of cotyledons using the method of leaf infiltration described in Cox et al. (2017). As negative control cotyledons were inoculated with 10 Mm $MgCl_2$ sterile solution.

In planta bacterial growth curves

Disease progression was phenotypically monitored in three separate biological assays and the bacterial populations were quantified once a day for 10 days as described before. Statistical analysis of data was performed using the LSD Fischer test in the software INFOSTAT (Di Renzo et al., 2013).

Molecular characterization of the isolates

The amplification of a partial sequence of *lepA* (housekeeping gene of *Xanthomonas*) was made as described in Almeida *et al* (2010). Briefly, single fresh colonies of the isolates were picked with a sterile pestle and suspended in 30 µl of sterile distilled water. Bacterial lysis was made at 95 °C for 10 minutes. The solutions were centrifuged for 5 min at 5000 rpm, and 2 µl of supernatant was used for PCR amplifications. PCR products obtained were sequenced (Macrogen Sequencing Service, Corea). Data generated were analyzed using IQTREE software (http://www.iqtree.org/) with a maximum-likehood with 10000 repetitions of Ultrafast Bootstrap. Genetic relationships among the isolates were determined

by comparing with non-redundant sequences of *lepA* gene of different *Xanthomonas* species (PAMDB, http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl).

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References

1. Akello, B. & Hillocks, R. (2002). Distribution and Races of Xanthomonas axonopodis pv. malvacearum on Cotton (Gossypium hirsutum) in Uganda. Journal of Phytopathology. 150. 65 - 69. 10.1046/j.1439-0434.2002.00718.x.
2. Almeida, N. F., Yan, S., Cai, R., Clarke, C. R., Morris, C. E., Schaad, N. W., Schuenzel, E. L., Lacy, G. H., Sun, X., Jones, J. B., Castillo, J. A., Bull, C. T., Leman, S., Guttman, D. S., Setubal, J. C. & Vinatzer, B. A. (2010). PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. Phytopathol., 100(3): 208-215.
3. citri pv. citri Suggests a Worldwide Geographical Expansion of a Single Genetic Lineage. PLOS ONE, 9 (6): e98129. https://doi.org/10.1371/journal.pone.0098129
4. Cox, K. L., Meng, F., Wilkins, K. E., Li, F., Wang, P., Booher, N. J., Carpenter, S. C. D., Chen, L. Q., Zheng, H., Gao, X., Zheng, Y., Fei, Z., Yu, J. Z., Isakeit, T., Wheeler, T., Frommer, W. B., He, P., Bogdanove, A. J. & Shan, L. (2017). TAL effector driven induction of a SWEET gene confers susceptibility to bacterial blight of cotton. Nat. Commun., 8: 15588.
5. Cunnac, S., Bolot, S., Forero, Serna. N., Ortiz, E., Szurek, B., Noël, L. D., Arlat, M., Jacques, M-A., Gagnevin, L., Carrere, S., Nicole, M., Koebnik, R. (2013). High-quality draft genome sequences of two Xanthomonas citri pv. malvacearum strains. Genome Announc. 1(4): e00674-13. doi:10.1128/genomeA.00674-13.
6. De Sousa Braga, W., Silveira da Cunha, R. W., Suassuna, N. D., Coutinho, W. M. (2016).
7. Delannoy, E., Lyon, B., Marmey, P., Jalloul, A., Montillet, J. L., Daniel, J. F., Essenberg, M. & Nicole, M. (2005). Resistance of cotton to Xanthomonas campestris pv. malvacearum. Annu. Rev. Phytopathol., 43: 62-82.
8. Di Renzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. & Robledo, C. W. (2013). InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar.
9. Hillocks, R. J. (1992). Bacterial blight. In: Cotton Diseases, ed. Hillocks, R. J. Wallingford, UK: CAB Int. Redwood Press, 39-85.
10. Hunter, R. E., Brinkerhoff, L. A. & Bird, L. S. (1968). Development of a set of Upland cotton lines for differentiating races of Xanthomonas malvacearum. Phytopathology, 58: 830–832.
11. Jalloul, A., Sayegh, M., Champion, A. & Nicole, M. (2015). Bacterial blight of
12. cotton. Phytopathologia Mediterranea, 54 (1): 3-20.
13. Maas, S., Allen, S. & Weir, D. (2012). Cotton Symptoms Guide The guide to symptoms of diseases and disorders in Australian cotton. The Australian Cotton Industry Development & Delivery Team. Available in: https://www.cottoninfo.com.au/publications/cotton-symptomsguide
14. Phillips, A. Z., Berry, J. C., Wilson, M. C., Vijayaraghavan, A., Burke, J., Bunn, J. I., Allen, T. W., Wheeler, T. & Bart, R. S. (2017). Genomics-enabled analysis of the emergent disease cotton bacterial blight. PLoS Genetics, 13 (9): e1007003. https://doi.org/10.1371/journal.pgen.1007003.
15. Prevalence of race 18 of Xanthomonas citri subsp. malvacearum on cotton in Brazil. Trop. plant pathol., 41: 128-131. https://doi.org/10.1007/s40858-016-0073-9
16. Pruvost, O., Magne, M., Boyer, K., Leduc, A., Tourterel, C., Devret, C., Ravigné, V., Gagnevin, L., Guérin, F., Chiroleu, F., Koebnik, R., Verdier, V., Vernière, C. (2014). A MLVA Genotyping Scheme for Global Surveillance of the Citrus Pathogen Xanthomonas
17. Schaad, N. W., Postnikova, E., Lacy, G.H., Sechler, A., Agarkova, I., Stromberg, P. E., Stromberg, V. K. & Vidaver, A. K. (2005). Reclassification of Xanthomonas campestris pv. citri (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as X. smithii subsp. citri (ex Hasse) sp. nov. nom. rev. comb. nov., X. fuscans subsp. aurantifolii (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and X. alfalfae subsp. citrumelo (ex Riker and Jones) Gabriel et al., 1989 sp. nov. nom. rev. comb. nov.; X. campestris pv. malvacearum (ex smith 1901) Dye 1978 as X. smithii subsp. smithii nov. comb. nov. nom. nov.; X. campestris pv. alfalfae (ex Riker and Jones, 1935) dye 1978 as X. alfalfae subsp. alfalfae (ex Riker et al., 1935) sp. nov. nom. rev.; and "var. fuscans" of X. campestris pv. phaseoli (ex Smith, 1987) Dye 1978 as X. fuscans subsp. fuscans sp. nov. Syst Appl Microbiol, 28(6): 494-518.
18. Schaad, N. W., Postnikova, E., Lacy, G., Sechler, A., Agarkova, I., Stromberg, P. E.,
19. Stromberg, V. K. & Vidaver, A. K. (2006). Emended classification of xanthomonad pathogens on citrus. Syst. Appl. Microbiol., 29(8): 690-695.
20. Showmaker, K. C., Arick, M. A., 2nd, Hsu, C. Y., Martin, B. E., Wang, X., Jia, J., Wubben, M. J., Nichols, R. L., Allen, T. W., Peterson, D. G., & Lu, S. E. (2017). The genome of the cotton bacterial blight pathogen Xanthomonas citri pv. malvacearum strain MSCT1. Stand Genomic Sci., 12: 42. https://doi.org/10.1186/s40793-017-0253-3.
21. Zhai, J., Xia, Z., Liu, W., Jiang, X., & Huang, X. (2013). Genomic sequencing globally identifies functional genes and potential virulence-related effectors of Xanthomonas axonopodis pv. malvacearum. European Journal of Plant Pathology, 136 (4): 657–663.
22. Zhao, S., Poulin, L., Rodriguez, L. M., Serna, N. F., Lui, S. Y., Wonni, I., Szurek, B., Verdier, V., Leach, J. E., He, Y. Q., Feng, J. X., Koebnik, R. (2012). Development of a variable number of tandem repeats typing scheme for the bacterial rice pathogen Xanthomonas oryzae pv. oryzicola. Phytopathology., 102 (10): 948-956. doi: 10.1094/PHYTO-04-12-0078-R.

Radiation Biology of a Cotton Pink *Bollworm Pectinophora Gossypiella* (Saunders) and Potential of Irradiation Mediated Inherited (F1) Sterility Technique for the Pest Suppression

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Abstract

Background: *The influence of gamma radiation on the reproductive biology of the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) was determined to explore the prospective of the radio-genetic 'Inherited (F1) Sterility' technique to control this prominent pest of cotton.*

Results: *Radiobiological investigations involved dose response studies at a range of 0-200 Gy. For this one day old adults of P. gossypiella were subjected to 50, 100, 150 and 200 Gy of gamma radiation and moths were crossed with different combinations N♂× N♀ (Control), T♂× N♀, N♂× T♀ and T♂× T♀ in oviposition cage. Radiation-induced sterility in P1 and F1 generation had a positive correlation with gamma dose. The fecundity was significantly decreased with increasing irradiation dose and Normal females when mated with treated males, laid fewer eggs than other two crosses. The decrease in % egg hatch and increase in % sterility induced by gamma radiation were found to be positively correlated with the dose level in all the three crosses when compared to control. A higher dose of 200 Gy resulted in 15.95 % fertility in parent cross (normal ♀×treated ♂). A gamma dose of 150 Gy (administered to P1 males) could be considered as a suitable dose for F1 sterility, that induced 60.18 % corrected sterility in parent generation, followed by 82-85 % corrected sterility In F1 generation in all the three crosses F1♂× N♀, N♂× F1♀ and F1♂× F1♀. The incubation period of F1 eggs was significantly prolonged due to irradiation of male parent. There was a dose dependent increase in meta-morphic disruption up to the pupal and adult stage at 100–200 Gy dose range.*

Conclusion: *The present study indicated the potential use of a dose of 150 Gy to apply the 'F1 sterility' technique for ecofriendly suppression of the cotton pink bollworm. The present findings and reproductive performance of the irradiated moths in F2 generation along with other compatible methods (that are in progress) might help in formulating an effective integrated pest management module.*

Keywords: *Cotton, Pink bollworm, Pectinophora gossypiella, Radiation biology Inherited sterility*

Background

Cotton is the most important crop producing natural fiber. It has been under commercial cultivation for domestic consumption and export needs of about 111 countries in the world and hence called "King of fibers" or "White gold". India is an important grower of cotton on a global scale. In India, there are ten major cotton growing states which are divided into three zones, viz. north zone, central zone and south zone. North zone consists of Punjab, Haryana, and Rajasthan. Central zone includes Madhya Pradesh, Maharashtra and Gujarat. South zone comprises Andhra Pradesh, Telangana, Karnataka and Tamil Nadu. India has the largest area under cotton cultivation i.e.122.38 Lakh ha with production of 361 Lakh bales and productivity of 501 kg/ha (AICRP, 2018-19). In Karnataka, cotton is cultivated an area of 5.46 lakh ha with a total production of 12.24 lakh bales and productivity of 381 kg per ha. Cotton pest scenario has witnessed considerable changes in the cotton growing areas of India. Bollworms such as Helicoverpa armigera (Hub.), Earias vittella (F) and Pectinophora gossypiella (Saunders) cause major threat to cotton production.

The pink bollworm incidence in Bt cotton gradually increased and outbreak was observed in major cotton growing states of the country, Furthermore, the environmental risks associated with excessive use of chemical insecticides and development of insecticidal resistance impose serious additional limitations on controlling this insect pest. So, the investigation on pink bollworm management needs to be strengthened through ecofriendly, autocidal means of management such as sterile insect technique. This might be an ideal alternative to suppress this serious pest of cotton considering the excessive use of chemical pesticides in India.

The sterile insect technique (SIT) approach involves mass rearing target insects, then irradiating and releasing them into the crop environment to mate with their wild counterparts. This induces sterility in the wild population. Lepidoptera that receive sub sterilizing doses of radiation commonly produce progeny that are male biased with very low fertility. This is known as F1 sterility (North, 1975). The advantage of SIT is that it is species specific, environmentally friendly and easily integrated with biological control (Vreysen et al., 2006). Ionizing radiation is the primary method used to induce sterility in insects. Lepidopteron are resistant to radiation than Diptera, Hymenoptera and Coleoptera. They require higher radiation doses to induce full parental sterility. These high radiation doses induce unfavourable physiological and behavioural changes, resulting in released insects not being competitive with their wild counterparts for mates (František Marec, Marc J B Vreysen,2019).

Pink bollworm has exhibited typical characteristic lepidopteron response to gamma radiation. The purpose of the present study was to provide much needed information on reproductive behaviour, developmental profile and survival of F1 progeny of P. gossypiella at different gamma radiation doses.

The present study was carried out to establish a radiation mediated 'Inherited (F1) sterility technique' for suppression of pink bollworm in an ecofriendly approach by identifying an optimum dose which could impart required sterility with minimum adverse effect on reproductive parameters of irradiated insects (treated male parents and their F1 progeny).

Results

The effect of irradiation on reproductive behaviour of one day old P. gossypiella adults was evaluated by crossing in different mating combinations like N♀ × T♂, T♀ × N♂ and T♀ × T♂

N♀ × T♂: The reproductive behaviour of treated male adults was significantly affected by different irradiation doses of 50, 100, 150, and 200 Gy. The mean number of egg laid by normal female mated with treated male were recorded as 39.25 18.15, 7.57 and 3.12 at doses 50, 100, 150 and 200 Gy, respectively as compared to 75.25 eggs per female in control. Irradiation affected fertility, thus egg hatch greatly reduced from 78.97 % in control to 15.95% at 200 Gy and there was a reduction at 50, 100 and 150 Gy to be 61.27, 40.13 and 31.41 % , respectively. Radiation induced reduction in fertility led to 22.39-79.71 % sterility and 59.56-99.20 % control of reproduction at 50-200 Gy (Table 1).

T♀ × N♂: The total oviposition was reduced by 4.22–21.82 eggs per female at 50–250 Gy range when compared to the normal their it was (81.72 eggs/female). Radiation induced fertility was 63.35, 44.27, 36.89 and 21.48 % at 50, 100, 150 and 200 Gy, respectively when compared to normal (80.19 %). Radiation induced sterility significantly increased in all doses 18.43, 44.71, 53.91and 73.19 % at 50, 100, 150 and 200 Gy, respectively. Irradiation markedly reduced hatching of eggs there by causing 78.91-98.65 % control of reproduction at 50-200 Gy (Table2).

T♀ × T♂: The effect of gamma dose at a range of 50-200 Gy significantly affected the reproductive behavior of adults. When both sexes were exposed to radiation; fecundity was significantly reduced from 18.72 to 4.00 eggs per female at increasing irradiation doses of 50-200 Gy. The % egg hatching was significantly highest 78.64 % in control, which drastically reduced to 15.05 at 200 Gy dose. The % sterility induced by gamma irradiation was found to be positively correlated with the dose level, where it was recorded 22.68 - 93.61 % corrected sterility and 81.90 - 97.14 % control of reproduction at 50 200 Gy (Table 3).

Effect of parental (P1) irradiation on the metamorphic development and sex ratio of emerged adults in F1 progeny of pink bollworm treated as male parent and crossed with normal female

The incubation period of F1 eggs induced by 200 Gy of gamma radiation prolonged over other doses. There was a dose dependent increase in meta-morphic disruption up to the pupal and adult stage at 50 – 200 Gy dose range. The F1 pupa formation was reduced by 21 % at 50 Gy and by 40.50 at 200 Gy, as compared to the control (89.87 % pupae formation). The larval period was prolonged as the irradiation dose increased. The larval period was significantly increased at 150 and 200Gy which was 24.78 and 25.28 days respectively compared to the 23.50 days in control. Radiation induced

metamorphic disruption reduced the longevity of adult moths to 19.63 days in 200 Gy as compared to the control (20.83 days). Radiation was found to be more impacted on the F1 adult formation it was 78 % at 100 Gy, 68.46% at 150 Gy and 60.02 % at 200 Gy, indicating a significant reduction by 30 - 40 at 100 –200Gy as compared to the control their it was 91.86 %. The present findings suggested that the inherited (carry-over) effect of irradiated male parent was evidenced in terms of affected development and reduced survival of their F1 progeny (Table 4).

Discussion

Effect of irradiation on reproductive behaviour of P. gossypiella.

When one day old adults of P. gossypiella were irradiated with 50, 100, 150 and 200 Gy of gamma radiation and crossed in different mating combinations. The results revealed that the fecundity and % egg hatch was significantly decreased by increasing the irradiation dose and it was dose dependent (Qureshi et al, 1993).The increase in % corrected sterility and control of reproduction induced by gamma radiation were found to be positively correlated with the dose level in all three crosses. Females were more susceptible to gamma irradiation than males (Sallamet et al., 2003). It is evident that reduction in fecundity and egg fertility were dose dependent. F1 progeny were either fully or partially sterile depending on radiation doses received by the male parent. The F1 progeny was more sterile than the treated parent male, regardless of the dose to the male parent and the F1 males were usually more sterile than the F1 females (Knipling ,1955 and La Chance et al. 1973).

The number of eggs per female and the % hatched eggs was reduced by irradiation, with no eggs hatched when females were treated with 40 krad (Graham et al, (1972) and fecundity of irradiated females crossed with non-irradiated males was decreased by increasing the irradiation dose level (Salem et al, 2014). The decrease in hatchability percentage was dose dependent. The sterility probably occurred because of the lack of eupyrene sperm transferred by irradiated males during mating (Henneberry and Clayton, 1981). Sensitivity between males and females of pink bollworm, P. gossypiella might be an advantage, if a single dose of radiation would partially sterilize males and completely sterilize females. (Henneberry and Clayton,1988 and Qureshi et al. 1993).

Table 1. Effect of gamma radiation doses on reproductive behaviour of P. gossypiella adults as normal ♀ × treated ♂

Irradiation dose (Gy)	Eggs per female	Egg hatching %	Un hatched eggs %	Corrected sterility %	Control of reproduction (%)
0	75.25 (8.67)a #	78.97 (62.70)a**	21.03 (27.29)a**	0.00 (0.00)e**	0.00 (0.00)a**
50	39.25 (6.26)b	61.27 (51.53)b	38.79 (38.52)b	22.39 (28.11)d	59.56 (50.51)b
100	18.15 (4.26)c	40.13 (39.28)c	60.06 (50.80)c	49.14 (44.50)c	87.77 (69.54)c
150	7.57 (2.75)d	31.41 (34.06)d	63.46 (55.95)d	60.18 (50.89)b	96.04 (78.57)d
200	3.12 (1.76)e	15.95 (23.12)e	84.80 (67.03)e	79.71 (63.68)a	99.20 (84.91)e
F- value	F= 1.16* df= 4, 15	F= 90.57* df= 4, 15	F= 121.31* df= 4, 15	F= 144.66* df= 4, 15	F= 3.87* df= 4, 15

Figures in the parentheses are transformed values;** Figures in parentheses are arc sine transformed values;* indicates significant at P≤ 0.01 level and transformed data followed by same letter within a column are not significantly different at P≤ 0.01 level.

Table 2. Effect of gamma radiation doses on reproductive behaviour of P. gossypiella adults as treated ♀ × normal ♂

Irradiation dose (Gy)	Eggs per female	Egg hatching %	Un hatched eggs %	Corrected sterility %	Control of reproduction (%)
0	81.72 (8.90)a #	80.19 (63.63)a**	17.41 (24.66)a **	0.00 (0.00)e**	0.00 (0.00)a**
50	21.82 (4.39)b	63.35 (53.93)b	39.18 (38.75)b	18.43 (25.40)d	78.91 (62.68)b
100	15.57 (3.94)c	44.27 (41.70)c	62.28 (52.10)c	44.71 (41.95)c	89.78 (71.36)c
150	9.10 (3.01)d	36.89 (37.36)d	74.45 (59.63)d	53.91 (47.24)b	95.06 (77.17)d
200	4.22 (2.04)e	21.48 (27.53)e	83.43 (65.97)e	73.19 (58.88)a	98.65 (83.35)e
F- value	F= 489.14* df= 4, 15	F= 148.17* df= 4, 15	F= 246.13* df= 4, 15	F= 299.66* df= 4, 15	F= 5.81* df= 4, 15

Figures in the parentheses are transformed values;** Figures in parentheses are arc sine transformed values;* indicates significant at P≤ 0.01 level and transformed data followed by same letter within a column are not significantly different at P≤ 0.01 level.

Table 3. Effect of gamma radiation doses on reproductive behaviour of P. gossypiella adults as treated ♀ × treated ♂

Irradiation dose (Gy)	Eggs per female	Egg hatching %	Un hatched eggs %	Corrected sterility %	Control of reproduction %
0	80.32 (8.96)a #	78.64 (62.48)a**	21.22 (27.42)a**	0.00 (0.00)e**	0.00 (0.00)a**
50	18.72 (4.32)b	60.79 (51.23)b	39.12 (38.71)b	22.68 (28.42)d	81.90 (64.86)b
100	14.32 (3.78)c	40.95 (39.78)c	58.99 (50.17)c	47.88 (43.78)c	90.53 (72.09)c
150	6.77 (2.57)d	26.52 (30.97)d	73.43 (58.97)d	66.22 (54.49)b	93.13 (80.28)d
200	4.00 (1.00)e	15.05 (9.25)e	95.00 (77.07)e	93.61 (79.54)a	97.14 (84.52)e
F- value	F= 1.74* df= 4, 15	F= 68.38* df= 4, 15	F= 190.40* df= 4, 15	F= 111.78* df= 4, 15	F= 250.23* df= 4, 15

Figures in the parentheses are transformed values;** Figures in parentheses are arc sine transformed values;* indicates significant at P≤ 0.01 level and transformed data followed by same letter within a column are not significantly different at P≤ 0.01 level.

Table 4. Effect of gamma radiation on developmental profile and survival of F1 progeny of P. gossypiella adults as normal ♀ × treated♂

Gamma Dose (Gy)	Developmental period (days)				Larvae developed % **	Pupation % **	Adult eclosion % **
	Egg	Larva	Pupa	Adult			
0	3.98b ± 0.20	23.50b ± 0.45	7.33b ± 0.11	20.83b ± 0.23	86.95 (68.88)a	89.87 (71.76)a	91.86 (73.42)a
50	3.93b ± 0.20	24.18b ± 0.13	7.75b ± 0.12	20.46b ± 0.38	83.48 (60.05)a	79.00 (62.75)b	78.32 (62.26)b
100	4.20b ± 0.10	24.38b ± 0.33	7.85b ± 0.13	20.17b ± 0.59	73.38 (58.96)b	73.25 (58.86)c	78.00 (62.04)b
150	4.05b ± 0.10	24.78c ± 0.30	7.83b ± 0.15	20.24b ± 0.43	67.63 (53.33)c	66.40 (54.57)d	68.46 (55.84)c
200	4.23a ± 0.12	25.28a ± 0.33	8.15a ± 0.18	19.63a ± 0.63	62.58 (52.33)d	59.50 (50.47)e	60.02 (50.83)d
F- value	F=4.73* df= 4,15	F=4.5* df=4,15	F=4.6* df=4,15	F=4.6* D=4,15	F= 36.79* df= 4, 15	F= 66.89* df= 4, 15	F= 53.90* df= 4, 15

Means ± SE followed by same letter within a column are not significantly different at P≤0.01level; ** indicates figures in parentheses are arc sine transformed values; * indicates significant at P≤ 0.01 level and and transformed data followed by same letter within a column are not significantly different at P≤ 0.01 level.

Similar debilitating effects of gamma irradiation induces complete sterility without inducing pupal mortality and adult malformation was achieved by treating pupae of five and seven days old rather than first day old pupae of pink bollworm, since the sensitivity of pupae to gamma radiation decreased as they become older (Ouye et al, 1964). Similarly, pupal sensitivity in Sitotroga cerealella (Olivier) depended on age, the older the pupae, the less susceptible to gamma irradiation (Quershi et al.1968). Irradiation of pre pupae and one day old pupae resulted in reduced numbers, delayed and incomplete adult emergence and structural deformities including twisted wings and fusion of tarsal and antennal segments.

Developmental profile and survival of F1 progeny derived from irradiated adults of P. gossypiella.

The incubation period was slightly increased at all dosages, but significantly prolonged at 200 Gy and the mean duration of F1 larvae was prolonged at 200 Gy, as compared to the control. At 200 Gy the pupal period showed a marked increase versus control. There was a dose-dependent delay in the developmental period of the F1 eggs, larvae and pupae of pink bollworms. However the adult longevity of F1 progeny lived significantly shorter at higher dose (200 Gy) as compared to the control. Because of the unbalanced hormonal system due to irradiation might have led to prolonged developmental period of egg, larval and pupal period. F1 survival to adulthood decreased with increasing dose of radiation.

Similarly gamma radiation positively affected the developmental periods of Spodoptera littoralis where irradiation dose increased the developmental period of egg, larva and pupa (Hilmy et al., 1984). Rice moth, Corcyra cephalonica was irradiated as pupae, the irradiation increased the developmental periods of the F1 progeny (Hasaballa et al. (1985))

Conclusion

Among three crosses of one day adults of P. gossypiella, N♀ × T♂ mating combination has a significant effect on reproductive suppression of pink bollworm both in parent and F1 progeny. Reproductive performance of parental progeny of pink bollworm indicated that, the % corrected sterility positively correlated with increasing irradiation dose and at 150Gy the % corrected sterility was 53 to 66 % in all the three crosses. This dose gives better viability of F1 progeny with a higher inherited sterility effect. The present investigation has generated bench mark data for further research and paves path for area wide scale study for developing feasible irradiation technology.

Materials and Methods

Rearing of pink bollworm as referred by Dhara Jothi et al., 2016

Field collected pink bollworm larvae were maintained on the artificial semi synthetic diet in growth chamber (Bio Gene ISO 9001) by maintaining the temperature at 27±0.5°C, relative humidity of 60±10% and photoperiod of 14L:10D until pupation. Once pure culture started the individual larva was reared on artificial diet in bioassay trays having 158 cavities and covered with perforated cap. The fresh diet was replaced whenever necessary. After adult emergence, they were collected and released in oviposition jars of 45 x 30 x 30 cm (l x b x h) size containing cotton twigs having terminal leaves and squares inserted in a small plastic container with 10 % sucrose solution as adult food and also for egg laying. The bottom of the twigs was immersed in water to retain the turgidity of the tissue. Cotton twigs were changed once in two days in the oviposition jars and then transferred to a transparent plastic containers covered with black cloth and tightly fastened with a rubber band for egg hatching. The first instar larvae were transferred individually into rearing trays containing semisynthetic diet and covered with perforated cap. Later fourth instar larvae were transferred individually to plastic vial for pupation. Fresh diet was supplemented whenever necessary until pupation. This culture was utilized for further investigations on radiobiological studies under the laboratory conditions mentioned above.

Gamma irradiation

Gamma chamber was supplied and monitored by Board of Radiation & Isotope Technology (BRIT), Mumbai. Gamma chamber is installed in agricultural research station, Kalaburagi and monitored by trained technical person who has undergone training at BRIT, Mumbai. While irradiating pupae or adults, all the safety measures were taken as per BRIT guidelines.

Radiobiological studies on pink bollworm were conducted at insect rearing laboratory, University of Agricultural Sciences, Raichur (Raichur is situated in the North Eastern Dry Region (Zone -2) of Karnataka between 160 15' N latitude and 770 20' E longitude at 398.37m above mean sea level. The average rain of 692 mm mostly occurs between June and November, which coincides with the monsoon; there are scattered showers during pre- monsoon months of April and May. Mean maximum temperature is more than 300 C throughout the year except during December. Relative humidity is high during monsoon and low during summer. In the present study, the most commonly used radiation source was cobalt-60 (CO60) for the exposure of moths through Gamma Chamber-5000 and having maximum capacity of dose rate at 9kGy/hr. A dose range of 0–250 Gy was used for radio-biological investigations on pink bollworm.

Observation on reproductive performance of irradiated adults of P. gossypiella.

Various reproductive parameters were assessed by pairing irradiated insects with normal counterparts. For this one day old moths were exposed to different doses of gamma radiation viz., 0, 50, 100, 150 and 200 Gy. After irradiation, moths were paired with different combinations viz., N♀ × N♂ (Control), N♀ × T♂, T♀ × N♂ and T♀ × T♂. To study the reproductive behaviour each experiment was replicated ten times and each replicate constitutes 10 pairs of adults. These mating combination of moths were released into the oviposition cage kept in the growth chamber by maintaining temperature 27±0.5°C, relative humidity of 60±10% and photoperiod of 14L:10D for mating and oviposition.

The ovipositional behaviour was monitored in terms of pre-oviposition, oviposition period and total number of eggs laid per female. The % fertility was assessed by recording the number of eggs hatched out of total number of oviposited eggs for each mating combination. The daily profile of eggs being oviposited and their specific day-wise fertility were recorded. Radiation induced sterility was expressed as a percentage of eggs that failed to hatch. Corrected sterility and Control of reproduction were computed according to the methods mentioned below (Seth and Reynolds 1993).

Developmental profile and survival of F1 progeny derived from adults of P. gossypiella.

Development and metamorphosis of F1 larvae derived from sub sterilized male parents mated with unirradiated female moths from egg to adult emergence was observed. The developmental period of egg, larva, pupa and adult was recorded at a frequency of 24h. Also, the % survival of larvae, pupae and adults was computed. Also, the malformation of eclosed adults was examined.

Statistical analysis

Experiments were conducted using completely randomised design and data was analysed using Analysis of variance (ANOVA). The values were transformed to arcsine wherever required.

References

1. Abass A, Al Salem, H M, Abd El Hamid, NA I Gabarty, Embaby DM, Effects of gamma irradiation on the biological activity of the cotton leaf worm, Spodoptera littoralis (Boisd). J Nucl Tech Appl Sci. 201,5: 19- 26.
2. Abbott WS.A method of computing the effectiveness of insecticide. J Econ Entomol.1925: 18: 265.
3. Anonymous, ICAR-AICRP on cotton- Annual report. Cotton Advisory Board,
4. Ernest Miller R T, Staten EJ, and Pozzi J et al. Effect of 20krad of gamma radiation on reproduction of pink bollworm Pectinophora gossypiellea (Lepidoptera: Gelechiidae) and their F1 progeny: Potential impact on the identification of trap catches. J. Econ. Entomol. 1984,77: 304-307.
5. František Marec, Marc J B Vreysen. Advances and Challenges of Using the Sterile Insect Technique for the Management of Pest Lepidoptera. Insectsv.10(11); 2019 Nov PMC6921062
6. Graham HM, Sterile pink bollworm: Field releases for population suppression. J Econ Entomol. 1984:71(5): 233-236.
7. Hasaballa ZA, Ahmed MY, Rizk MM, et al. Effect of gamma radiation on the immature stages of Corcyra cephalonica Assuit. J Agric Sci. 1985:16:291-298.
8. Henneberry TJ, Clayton T E, Effects on reproduction of gamma irradiated laboratory reared pink bollworms and their F1 progeny after mating with untreated laboratory reared or native insects. J Econ Entomol.1981: 74: 19–23.
9. Hilmy N M, Waked AM, Abuldahab, FF, Effect of gamma radiation on the cotton leaf worm, S. littoralis (Boisd.) effect of different doses at two rates for each. Isotope and Rad. Res. 1984,11(2):129 -134.
10. Knipling E, , Possibilities of insect control or eradication through the use of sexually sterile males.J. Econ.Entomol.1955: 48: 459–469.
11. Lachance L E, Robert A B, Richard R D, Effect of low doses of gamma irradiation on reproduction of male pink bollworms and their F1 progeny. Environ. Entomol. 1973, 2(4): 653-658.

12. North DT, Inherited sterility in lepidoptera. Annual Review of Entomol. 1975,20: 167-182.
13. Ouye M T, Garcia R S, Martin DS et al. Determination of the optimum sterilizing dosage for pink bollworms treated as pupae with gamma radiation. J. Econ. Entomol. 1964:57: 387-90.
14. Qureshi Z A, Wilbur DA, Mills RB et al. Sub lethal gamma radiation effects on pre pupae, pupae and adults of angumois grain moth. J Econ Entomol. 1964, 61:1699-1705.
15. Qureshi ZA, Wilbur D A, Mills R B et al. Sublethal gamma radiation effects on pre pupae, pupae and adults of angumois grain moth. J Econ Entomol.1964: 61:1699-1705.
16. Sallam H A ,Mohamed H H, Inherited sterility progeny gamma irradiated male spiny bollworm, Earias insulana (Boisd). Arab J Nucl Sci Appli.1964, 36(1): 227-235.
17. Seth R K, Reynolds E S, Induction of inherited sterility in the tobacco hornworm, Manducasexta(Lepidoptera: Sphingidae) by substerilizing doses of ionizing radiation. Bull. Entomol. Res. 1993, 83: 227-235.
18. Suubiyakato, Wari-Astatiand, Suarto-Dwi. Sterilization of Helicoverpa armigera (Hubner) (Lepidopera: Noctuidae) by using gamma rays radiation. Peternakan Dan.Bio. 1998,240:195-201.
19. Vreysen M J B, Hendrichs J, Enkerlin W R. The sterile insect technique as a component of sustainable area-wide integrated pest management of selected horticultural insect pests. J Fruit and Ornamental Plant Res. 2006, 14: 107-130.

Toxicity of Synthetic Insecticides and Neem Oil against Arthropod Natural Enemies of mealybug, Phenacoccus solenopsis Tinsley (Sternorrhyncha : Pseudococcidae) Infesting Cotton

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Abstract

Background: *As an invasive dreaded pest into Pakistan mealbug Phenacoccus solanopsis needs better IPM strategies. The pest has niche of natural biocontrol agents for which safety has to be ensured before using insecticides in cotton ecosystem.*

Results: *A laboratory study was carried out to find out the residual contact toxicity of four chemical insecticides viz. Acephate 97 DF , Imidachloprid 20SL,Thaimethoxam 25 WG, combi product of Methomyl + Thioacetiimidate 40 SP and neem oil against arthropod natural enemies of mealybug Phenacoccus solanopsis the larvae of Chrysoperla carnea, adults of Brumus. suturalis and Agenesis Bambawalei. All the insecticides tested caused significant mortality of these beneficial bio-control agents of P. solenopsis. Methomyl + Thioacetiimidate 40 SP formulation found to be highly toxic to C. carnea (LC 50 0.064 ml.L-1) and A. bambawalei males (0.026 ml.L-1) at 24 h after exposure. For B.suturalis . Acephate appered to be highly toxic (LC 50 1.44 ml.L-1) at 24 h exposue. Imidachloprid and Thaimethoxam have also shown considerable toxicity to these natural enemies. Neem oil found to be harmless to larvae of C. carnea (LC50 = 247.06 ml.L-1) and adults of B. suturalis (LC50 =144.35 ml.L-1) but slightly harmful to adults of A. bambawalei at higher doses. Interestingly, A. bambawalei males (LC50 = 22.19 ml.L-1) were more susceptible to neem oil than females (LC50 =33.82 ml.L-1). Based on LC50 values.*

Conclussion: *Neem oil is safe to the bio-control agents of P. solenopsis and can be used in compatible with integrated pest management programs.*

Keywords: *Insecticides; neem oil; bio-control agents; P. solenopsis*

Background

Mealybugs are small, soft-bodied, plant sucking insects and their common name is due to the waxy material which covers the bodies of adult females (Downie and Gullan, 2004; McKenzie, 1967; Miller, 1991). A new menace to cotton in Pakistan since year 2005 was identified as Phenacoccus solenopsis Tinsley (Sternorrhyncha : Pseudococcidae) (Hodgson et al., 2008). It was first noticed area of Pakistan causing loss. Further this devastating polyphagous pest spread rapidly to entire cotton growing areas of the country and has become most damaging pest of cotton and many other economically important crops (Arif et al., 2009; Abbas et al., 2010).

Several species of parasitoids and predators attack P. solenopsis and under pesticide free conditions can effectively regulate mealybug populations (Tanwaret al., 2007; Gautamet al., 2010; Ram and Saini, 2010). Lady bird beetle Brumus suturalis Fabricius (Coleoptera:Coccinellidae) and green lacewing Chrysoperla carnea Stephens (Neuroptera : Chrysopidae) generalist predators are also important predators of cotton mealybug and have been found to consume all its nymphal instars (Sattar et al., 2007; Khuhro et al., 2002; Rashid et al., 2012). Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae) is a solitary endo parasitoid of P. solenopsis and have potential for use in augmentative release programs for its suppression (Hayat, 2009; Ram and Saini, 2010). These arthropod natural enemies contribute significantly to the biological control of cotton mealybug.

Control of cotton mealybug is relied upon the excessive use of broad spectrum synthetic insecticides but very little is known about the direct effects of these toxic chemicals used in cotton field on bio-control agents of cotton mealybug. On the contrary strategic use of bio-control agents and insecticides is integral in any IPM program. Hence safety of insecticides and/or botanicals has to be assured before going for management of mealybugs in cotton.

Hence an experiment was conducted to investigate the compatibility of these bio-control agents with the selected synthetic insecticides and neem oil and to check the resistance or tolerance if any for better IPM stratigies.

Results

Toxicity of Insecticides and Neem Oil to C. carnea larvae

The results of toxicity of four insecticides and neem oil to larvae of C. carnea are presented in Table 1.

Table 1: Toxicity of different synthetic insecticides and neem oil against C. carnea

Insecticides	Exposure time	Slope ± SE [Log 10 (dose)]	LC50 (ml. or gL-1) (95% FL)
Actara 25WG	24 Hrs	0.730 ± 0.093	0.507 (0.507 – 0.508)
(Thiamthoxam)	48 Hrs	0.547 ± 0.069	0.049 (0.025 – 0.099
Confidor 20% SL	24 Hrs	0.505 ± 0.602	0.16 (0.082 – 0.0340)
(Imidachloprid)	48 Hrs	0.370 ± 0.052	0.021 (0.008 – 0.056)
Commando plus 97% DF	24 Hrs	0.455 ± 0.057	0.064 (0.001 – 0.003)
(Acephate)	48 Hrs	0.474 ± 0.063	0.022 (0.010 – 0.048)
Lannate 40% SP	24 Hrs	0.565 ± 0.069	0.034 (0.178 – 0.676)
(Methomyl+ thioacet.)	48 Hrs	0.468 ± 0.065	0.010 (0.004 – 0.024)
Neem oil	24 Hrs	0.627 ± 0.276	247.06 (58.47 – 10438.5)
	48 Hrs	0.409 ± 0.090	231.93 (64.13 – 287.39)

Increase in concentration of insecticides linearly increased the proportion of mortality of C. carnea larvae. After 24 hrs commando plus (Acephate 97% DF) was the most toxic insecticide for C. carnea (LC50 = 0.064). The least toxic was the neem oil with LC50 = 247.06. Lannate (40% SP) was the most toxic after 48 hrs (LC50 = 0.010), whereas, neem oil was the least toxic having LC50 231.93 after 48 hrs of exposure time.

Toxicity of Insecticides and Neem Oil to Adults of B. suturalis

Commando plus proved to be the most toxic insecticide after 24 and 48 hrs of exposure periods having least LC50 values (1.44 and 0.16) as compared to other treatments (Table 2). Among the synthetic insecticides actara was moderately harmful (LC50=13.97 and 4.76) after 24 and 48 hrs of exposure periods. Neem oil was harmless to adults of B. suturalis. The LC50 values of neem oil (144.35 and 133.65) differed significantly as compared to other treatments.

Table 2: Toxicity of different synthetic insecticides and neem oil against B. suturalis

Insecticides	Exposure time	Slope ± SE [Log 10 (dose)]	LC50 (ml. or gL-1) (95% FL)
Actara 25WG	24 Hrs	0.1575 ± 0.043	13.97(17.31 – 112.71)
(Thiamthoxam)	48 Hrs	0.042 ± 0.034	4.76 (0.002 – 101.98)
Confidor 20% SL	24 Hrs	0.402 ± 0.063	2.91 (9.91 – 85.64)
(Imidachloprid)	48 Hrs	0.381 ± 0.050	1.11 (0.47 – 2.615)
Commando plus 97% DF	24 Hrs	0.341 ± 0.052	1.44 (4.64 – 45.107)
(Acephate)	48 Hrs	0.290 ± 0.043	0.16 (0.059 – 0.474)
Lannate 40% SP	24 Hrs	0.654 ± 0.117	6.16 (27.62 – 137.80)
(Methomyl+ thioaceti.)	48 Hrs	0.481 ± 0.062	2.41 (1.14 – 5.08)
Neem oil	24 Hrs	9.7 ± 18390.0	144.35 (Inf-0)
	48 Hrs	9.97 ± 17508.98	133.65 (Inf-0)

Toxicity of Insecticides and Neem Oil to A. bambawalei (Male)

The most toxic insecticides against adult A. bambawalei (male) were lannate and confidor (LC50 = 0.026 and LC50 = 0.027) compared to other insecticides (Table 3). The toxicity (LC50) of these compounds was almost 3.5 times higher than that of neem oil whereas, neem oil was the least toxic against the adults of A. bambawalei (LC50 = 22.19). After 48 hr confidor was the most toxic (LC50 = 0.001) however, there was no significant difference in LC50 against the A. bambawalei compared to other treatments. Neem oil was the least toxic (LC50 = 14.39).

Table 3: Toxicity of different synthetic insecticides and neem oil against A. bambawalei (Male)

Insecticides	Exposure time	Slope ± SE [Log 10 (dose)]	LC50 (ml.or gL-1) (95% FL)
Actara 25WG	24 Hrs	0.293 ± 0.043	0.23 (0.083 – 0.06)
(Thiamthoxam)	48 Hrs	0.277 ± 0.048	0.002 (0.006 – 0.013)
Confidor 20% SL	24 Hrs	0.139 ± 0.036	0.027 (0.002 – 0.250)
(Imidachloprid)	48 Hrs	0.258 ± 0.048	0.001 (0.003 – 0.011)
Commando plus 97% DF	24 Hrs	0.198 ± 0.038	0.258 (0.063 – 1.050)
(Acephate)	48 Hrs	0.303 ± 0.047	0.015 (0.004 – 0.048)
Lannate 40% SP	24 Hrs	0.355 ± 0.050	0.026 (0.010 – 0.068)
(Methomyl+ thioaceti.)	48 Hrs	0.274 ± 0.048	0.003 (0.0007 – 0.015)
Neem oil	24 Hrs	0.214 ± 0.047	22.19 (16.53 - 201.70)
	48 Hrs	0.151 ± 0.039	14.39 (5.22 - 196.79)

Toxicity of Insecticides and Neem Oil to A. bambawalei (Female)

Adults females of A. bambawalei were most susceptible to actara (LC50 = 0.006) which differed non significantly from other treatments except neem oil (LC50 = 33.82) as presented table 4. Confidor, actara and commando were consistently the most toxic insecticides when A. bambawalei was exposed after 48hrs by residual contact. Based on LC50 values, neem oil was the least toxic (LC50 = 25.63) compared to other treatments.

Table 4: Toxicity of different synthetic insecticides and neem oil against A. bambawalei (Female)

Insecticides	Exposure time	Slope ± SE [Log 10 (dose)]	LC50 (ml. org L-1) (95% FL)
Actara 25WG	24 Hrs	0.253 ± 0.041	0.006
(Thiamthoxam)	48 Hrs	0.305 ± 0.045	0.057 (0.02 – 0.160)
Confidor20% SL	24 Hrs	0.342 ± 0.048	2.90 (1.10 – 7.63)
(Imidachloprid)	48 Hrs	0.264 ± 0.044	0.012 (0.003 – 0.045)
Commando plus 97% DF	24 Hrs	0.188 ± 0.038	2.66 (0.52 – 13.42)
(Acephate)	48 Hrs	0.230 ± 0.039	0.219 (0.06 – 0.756)
Lannate 40% SP	24 Hrs	0.348 ± 0.047	0.316 (0.12 – 0.771)
	48 Hrs	0.390 ± 0.052	0.059 (0.02 – 0.139)
Neem oil	24 Hrs	0.213 ± 0.055	33.82 (0.056 – 204.14)
	48 Hrs	0.223 ± 0.053	25.63 (38.18 – 224.75)

Discussion and conclusion

Synthetic insecticides are used commonly against insect pest without considering their effects on the environment and bio-control agents. Present experiments were conducted to investigate the effects of insecticides that were determined to be toxic to the pest are likely to be harmless to the natural enemies of cotton mealybug. Botanical insecticides offer a better and much safer alternative for IPM systems compared to chemical insecticides (Copping and Menn, 2000). Neem products have been reported benign to parasitoids and predators and are compatible with integrated management programs (Lowery and Isman, 1995; Naumann and Isman, 1996).

In the present studies, neem oil proved safer to the larvae of C. carnea and adults of B. suturalis. Even at higher doses neem oil caused minimum mortality after residual contract as compared to synthetic insecticides. Aggarwal and Brar (2006) reported that the mortality of the first instar larvae of C. carnea was not affected by any of the azadirachtin enriched formulations. Neem oil was harmless to adult beetle B. suturalis. Adults of A. bambawalei were vulnerable to neem oil compared to C. carnea and B. suturalis. However, LC50 values for A. bambawalei (22.19 and 33.82 ml.L-1 for males and females) were significantly higher in comparison to synthetic insecticides. The lower mass of males might be a possible reason for their relatively higher susceptibility. A higher susceptibility of males to botanical insecticides was also reported by Charleston et al. (2005) who reported morphological differences (tibia length) in Cotesia plutellae males treated with extracts of Melia azedarach. The present results also agree with those reported by Aggarwal and Brar (2006) who observed that Neemazal at lower dose (200 mg.L-1) did not affect the emergence of Encarsia sofia adults, but at higher doses (800 mg.L-1) there was a significant reduction in adult emergence. Stark et al. (1992) found that low doses of neem seed extracts were safe to hymenopteran parasitoids.

All the tested insecticides were found to be toxic after 48 hrs exposure period except thiomethoxam

which was moderately toxic insecticide to bio-control agents of P. solenopsis. Thiomethoxam toxicity has also been reported by Naveed et al. (2010) as slightly harmful to beneficial insects and harmless to predatory mites. Similar findings were also reported by Nasreen et al. (2005) who found thiamethoxam as moderately toxic to larvae of C. carnea at lower concentration. Among the tested bio-control agents, C. carnea was found most tolerant to synthetic insecticides and neem oil as compared to other beneficial agents. Ishaaya and Casida (1981) reported that esterases present in the larvae of C. carnea have high hydrolyzing activity against synthetic insecticides which is co-related with the tolerance of these larvae to synthetic insecticides. These results proved that the increase of mealybug populations to pest status in Pakistan is mainly due to the disruption of beneficial bio-control agents of mealybug by the wide scale use of synthetic insecticides.

Laboratory experiments showed that neem oil was non-toxic to the adults of A. bambawalei, B. suturalis and larvae of C. carnea and may be incorporated into IPM programs for the control of P. solenopsis. In contrast, thiamethoxam, imidacloprid, acephate and methomyl + thioaceteimidate were highly toxic to bio-control agents under laboratory conditions, although, such high rates of mortality might not be projected under field conditions. Additional studies are needed to estimate the effects of insecticides and sub lethal effects of neem oil against selected bio-control agents under field conditions.

Materials

Studies were carried out in the laboratory of Entomology section, Agriculture Research Institute, Dera Ismail Khan Khyber Pakhtunkhwa, Pakistan.

Culture of Aenasius bambawalei

A stock colony of parasitoid, A. bambawalei was maintained in the bio-control laboratory of Entomology Section, Agricultural Research Institute, Dera Ismail Khan. Adults of A. bambawalei were kept in clear plastic cages with polyester mesh on one side for ventilation. Honey and sugar solution was provided on small cards as an artificial diet for adults. The fresh leaves of cotton crop infested with the adults of cotton mealybug (P. solenopsis) were placed daily in rearing cages of A. bambawalei and offered for parasitization. After 24 hrs the parasitized mealybugs were removed. After 4-6 days the mealybug adults turned into dark brown barrel shaped mummies confirming the parasitization of adult female mealybugs. Adults emerged from the parasitized adult mealybugs 7-12 days after the parasitization. Newly emerged adults (Male and Female) were used for bioassay experiments.

Culture of Chrysoperla carnea

Adults of C. carnea were collected from cotton fields in Dera Ismail Khan, Pakistan and reared in clear plastic jars of 30 x 30 x 30 cm3 with mesh on one side and were reared on artificial diet consisting of Yeast, honey and distilled water, mixed to a paste. The adults were offered diet every morning on a plastic sheet measuring 5×5 cm2. The back side of jar was sealed with net cloth for ventilation. The inside of the top of the cage was lined with black card sheet to facilitate the C. carnea females for egg laying. The newly laid fresh eggs were removed daily by replacing the top sheet by new one. Eggs were harvested daily with the help of razor blade and placed in plastic Petri dishes of 9 cm diameter. Frozen eggs of laboratory reared Sitotroga cerelella were provided as food to newly hatched larvae of the predator daily.

Culture of Brumus suturalis

Adults B.suturalis and their host P. solenopsis required for the study were collected from cotton fields and reared in plastic cages (13 x 13 x 13 inches) having polyester mesh on one side for ventilation. Fresh cotton leaves infested with P. solenopsis were provided as a food to newly hatched larvae of B.suturalis daily. The newly hatched larvae of predator were reared in single cells of a rearing tray until adult emergence. Larvae were reared individually to avoid competition and cannibalism. Newly emerged adults were used for bioassay experiments.

Bioassays

Surface treatment (thin film contact) bioassay method was used for the evaluation of toxicity levels of four synthetic insecticides viz. Acephate 97 DF (commando plus) , Imidachloprid 20SL (confidor),Thaimethoxam 25 WG (actara), combi product of Methomyl + Thioacetiimidate 40 SP(lannate) and neem oil against 3 day old larvae of C. carnea and newly emerged adults of B. suturalis and A. bambawalei. Neem oil for the experiment was prepared from seeds collected from neem plantation of Agricultural Research Institute, Dera Ismail Khan. Seed kernels were removed from the seed, shade dried and crude neem oil was extracted by crushing seeds in a double-screw oil expeller and stored in laboratory until used. The required concentrations (Table 5) were prepared from the stock

solution by adding calculated amount of distilled water using the method described by Musabyimana et al. (2001).

Table 5: Details of conventional insecticides and neem oil evaluated for bioassays against bio-control agents of cotton mealybug

Treatments	Active Ingredient	Recommended Dose	Test concentration (µl a.i.L-1)
ctara 25WG	Thiomethoxam	24 g.acre-1	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Confidor 20% SL	Imidachloprid	250 ml.acre-1	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Commando plus 97% DF	Acephate	300 g.acre-1	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Lannate 40% SP	Methomyl + thioacetiimidate	250 g.acre-1	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Neem oil	Azadirachtin		0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]

Values in [ ] represent concentrations evaluated for bioassays against C. carnea.

These pesticides were selected due to their frequent use for mealybug control in cotton growing regions. Six concentrations of each insecticide and neem oil were tested against the mentioned bio-control agents of P. solenopsis. Same concentrations of each insecticide and neem oil was tested at the start of trial against all bio-control agents of P. solenopsis but since mortality of C. carnea was negligible at the tested doses so another trial of higher doses for C. carnea was started. Glass Petri dishes (9 cm diameter) were used for C. cornea whereas; transparent plastic jars (5 × 3 cm2) were used for B. suturalis and A. bambawalei. The testing arenas were treated with different concentrations of each insecticide and neem oil then allowed for complete air drying for 30 minutes. Each treatment was replicated 5 times and contained eight larvae of C. carnea or adults of B. suturalis or A. bambawalei in each replication. The Petri dishes sprayed with distilled water alone served as untreated control. Larvae of C. carnea were introduced individually in the Petri dishes to prevent cannibalism between the larvae. The numbers of dead larvae or adults in each treatment were recorded after 24 and 48 hrs of exposure periods. During the trial, the larvae of C. carnea were fed on S. cerealella eggs and P. solenopsis were provided as food to adults of B. suturalis. Honey and water solution was provided as food on a plastic sheet for the parasitoids during the 48 hr exposure period. In this way a total of 2480 adults of A. bambawalei and 1240 of B. suturalis and 1240 larvae of C. carnea were tested for bioassay in five replications. The tested arenas were kept in an incubator maintained at 30± 2oC with 65% R.H. and a photoperiod of 12:12 h (L:D). Mortality of these bio-control agents was recorded 24 and 48 hrs after exposure.

Statistical Analysis

Lethal concentration (LC) estimates were estimated from insect mortality data using standard procedures (Pickett, 2009). The LC50 bioassay data were analyzed by specifying a generalized linear model with binomial errors (or quasibinomial if data were over dispersed) to estimate the slope and its standard error, with significance tested at the 5% level of probability. A function called “dose.p” from the MASS library that used logit regression analysis estimated LC50 values and their standard errors (se). Using these standard error values the 95% Confidence Intervals [CI; (LC50 ± (1.96 × se))] were calculated. Pair wise comparisons of LC50 values were significant at the 1% level of probability if their respective 95% CI’s did not overlap (Crawley, 2007).

References

1. Abbas, G., M.J. Arif, M. Ashfaq, M. Aslam and S. Saeed, Host plants distribution and overwintering of cotton mealybug (Phenacoccus solenopsis: Hemiptera: Pseudococcidae). Int. J. Agri. Biol., 2010. 12: 421-425.
2. Arif, M.I., M. Rafiq, and M.A. Ghaffar, Host plants of cotton mealybugPhenacoccus solenopsisa new menace to cotton agroecosystem of Punjab, Pakistan. Int. J. Agri. Biol., 2009. 11: 163-167.
3. Aggarwal N. and D.S. Brar, Effects of different neem preparations in comparison to synthetic insecticides on the whitefly parasitoid Encarsia sophia (Hymenoptera : Aphelinidae) and the predator Chrysoperla carnea (Neuroptera : Chrysopidae) on cotton under laboratory conditions.J. Pest Sci., 2006. 79: 201-207.
4. Charleston, D.S., R. Kfir, M. Dicke and L.E.M. Vet, Impact of botanical pesticides derived from Melia azedarach and Azadirachta indica on the biology of two parasitoid species of the diamondback moth. Biol. Cont., 2005.33: 131-142.
5. Copping, L.G. and J.J. Menn, Biopesticides: a review of their action, applications and efficacy. Pest Manag. Sci., 2000.

56: 651-676.
6. Crawley M.J. The R Book.John Wiley and Sons Ltd, West Sussex, U.K. 2007.
7. Downie, D.A. and P.J. Gullan, Phylogenetic analysis of mealybugs (Hemiptera : Coccoidea : Pseudococcidae) based on DNA sequences from three nuclear genes, and a review of the higher classification. Syst. Entomol., 2004.29: 238-259.
8. Gautam, S., A.K. Singh and R.D. Gautam, Olfactory responses of green lacewing, Chrysoperla sp. (carnea group) and Mallada desjardinsi on mealybug, Phenacoccus solenopsis (Homoptera: Pseudococcidae) fed on cotton. Acta Ento. Sinica, 2010. 53: 497-507.
9. Hayat, M., Description of a New Species of Aenasius (Walker) (Hymenoptera: Encytridae), a Parasitoid of Mealybug, Phenacoccus solenopsis(Tinsley) (Homoptera: Pseudococcidae) in India. Biosystematics.,2009. 3: 21-26.
10. Hodgson, C., G. Abbas, M.J. Arif, S. Saeed, and H. Karar, Phenacoccussolenopsis (Tinsley). (Sternorrhyncha: pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. Zootaxa, 2008. 1913: 1-35.
11. Ishaaya, I. and J.E. Casida, Pyrethroid esterase(s) may contribute to natural pyrethroid tolerance of larvae of the common green lacewing. Environ. Entomol., 1981. 10: 681-684.
12. Khuhro R.D., I.A. Nizamani and M.A. Talpur, Population abundance of predators in alfalfa and cotton fields at Tandojam. Pak. J. Appl. Sci., 2002.2: 300-303.
13. Lowery D.T. and M.B. Isman, Toxicity of neem to natural enemies of aphids. Phytoparasitica, 1995. 23: 297-306.
14. McKenzie H.L. (ed) Mealybugs of California with taxonomy, biology, and control of North American species (Homoptera: Coccoidea: Pseudococcidae),University of California Press, Berkeley. 1967.
15. Miller, D.R. The scales, scale insects or coccoids. Immature Insects(ed. by F. W. Stehr), pp. 90-107. Kendall/Hunt, Dubuque, Iowa. 1991.
16. Musabyimana, T., R.C. Saxena, E.W. Kairu, C. Ogol and Z.R. Khan. Effects of neem seed derivatives on behavioral and physiological responses of Cosmopolites sordidus (Coleoptera : Curculionidae). J. Econ. Entomol., 2001.94: 449-454.
17. Nasreen, A., G. Mustafa and M. Ashfaq, Mortality of Chrysoperla carnea Stephens (Neuroptera; Chrysopidae) after exposure to some insecticides; laboratory studies. South Pacific Stud., 2005. 26: 1–6.
18. Naumann, K. and M.B. Isman, Toxicity of neem (Azadirachta indica A. Juss) seed extracts to larval honeybees and estimation of dangers from field application. Amer. Bee J., 1996.136: 518-520.
19. Naveed, M., A. Salam, M.A. Saleem, M. Rafiq and A. Hamza, Toxicity of thiamethoxam and imidacloprid as seed treatments to parasitoids associated to control Bemisia tabaci. Pak .J. Zool., 2010. 42: 559-565.
20. Ram, P. and R.K. Saini, Biological control of solenopsis mealybug, Phenacoccus solenopsis Tinsley on cotton: a typical example of fortuitous biological control. J. Biolog. Cont., 2010.24: 104-109.
21. Rashid, M. M., M.K. Khattak, K. Abdullah, M. Amir, M. Tariq and S. Nawaz, Feeding potential of Chrysoperla carnea and Cryptolaemus montrouzieri on cotton mealybug, Phenacoccus solenopsis. J. Ani. Plant Sci., 2012. 22: 639-643.
22. Sattar, M., M. Hamed and S. Nadeem, Predatory potential of Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) against cotton mealy bug. Pak. Entomol., 2007. 29: 103-106.
23. Schuster, D.J. and P.A. Stansly, Response of two lacewing species to biorational and broad-spectrum insecticides. Phytoparasitica, 2000.28: 297-304.
24. Stansly, P.A. and T.X. Liu, Selectivity of insecticides to Encarsia pergandiella(Hymenoptera: Aphelinidae), an endoparasitoid of Bemisia argentifolii (Hemiptera: Aleyrodidae). Bull. Entomol. Res., 1997. 87: 525-531.
25. Stark, J.D., T.T.Y. Wong, R.I. Vargas and R.K. Thalman, Survival, longevity and reproduction of tephritid fruit fly parasitoids (Hymenoptera: Braconidae) reared from fruit flies exposed to azadirachtin. J. Econ. Entomol., 1992. 84: 1125-1129.
26. Tanwar, R.K., P. Jeykumar and D. Monga, Mealy bugs and their management, NCIPM Technical Bulletin, LBS Building, Pusa, Campus, New Delhi, India, 2007.pp. 16.

Within plant distribution, dynamics and ecocompatible management of thrips (Thripidae: Thysanoptera), an emerging pest of cotton in India

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Abstract

Background: *Studies were conducted to determine within plant distribution on different plant parts, population dynamics and management of thrips infesting hirsutum cotton. To study within plant distribution cotton plant parts viz. expanded leaves, squares, flowers and bolls were sampled between 45-133 days after sowing. Dynamics studies were conducted on Bt cotton and Non-Bt cotton under unprotected conditions. For eco-compatible management of thrips in cotton the efficacy of label claim and eco-friendly interventions were evaluated under field and laboratory conditions.*

Results: *Thrips population was significantly more abundant on leaves followed by flowers, squares and bolls, where the upper strata leaves harbour maximum thrips. Population dynamics indicated maximum incidence of thrips during earlier part of season. In Bt cotton genotype thrips population ranged from 0-41.92 in 2015-16, 0-32.1 in 2016-17, 0-81.2 in 2017-18 and 0-36.3/3 leaves in 2018-19, and the peak incidence was recorded during 29th -31st SMW. Similarly in Non Bt genotype, comparatively susceptible to thrips, population ranged between 0-37.36 in 2015-16, 0-26.9 in 2016-17, 0-106.8 in 2017-18 and 0-41.5/3 leaves in 2018-19.Beside several chemical insecticides, castor oil gave maximum average per cent mortality (48.17) followed by sesame oil, pongamia oil, neem oil 1500 ppm and neem oil 300 ppm during 2018-19 & 2019-20.*

Conclusion: *Based on within plant sampling and dynamics data, plant oils exhibiting moderate mortality both under field and laboratory conditions can be applied during earlier part of season when thrips incidence is higher and natural enemies are quite active.*

Keywords: *Thrips, sampling, population dynamics, castor, pongamia, sesame, eco-compatible management.*

Background

Thrips, an emerging sucking pest of cotton, not only in India but in many other countries (Panwar et al. 2015; Thrips Wiki, 2020) and generally appears in the early season (Sharma and Sharan 2016). Exact crop loss estimate due to thrips in India are not available but up to 50% yield reduction documented in United States (Cook et al. 2011).

Prophylactic treatments either in the form of seed treatment or systemic insecticides is recommended for its management (Cook et al. 2011) along with foliar applications of insecticides as preventive measure. In the absence of host resistance sources, thrips had acquired resistance against many neonicotinoids insecticides either used as seed treatments or foliar applications (Huseth 2016). Also to manage the higher incidence of whitefly during 2015 onwards (Rishi et al. 2019) exerted an additional selection pressure on thrips.

For management of thrips and other sucking pests several label claim insecticides have been recommended but they become ineffective with time due to development of resistance in pests. Similarly, their usage during earlier part of season have limitations due to the activity of predators. The biorationals and plant oil pressed out of seeds such as castor, pongamia and sesame have been found to be effective in controlling agricultural pests and also helps in conservation of natural enemies. So to deal with the increasing menance of thrips in cotton studies were conducted to assess its within plant distribution, dynamics during the cotton season and suitable intervention to manage it.

Results

Within plant distribution

Within plant distribution of thrips was studied during 2019-20 cotton crop season and it was observed that the thrips population was significantly more abundant on seedling and cotton leaves than all other parts sampled during 45-133 days after sowing. Among the different plant parts the leaves harbored maximum thrips ranging 0.53 to 6.93 followed by flowers 0.73 to 3.73 and square 0.00 to 0.93 while the bolls had negligible or nil thrips throughout the season. However, among the leaves, maximum population/leaf was recorded in upper strata leaves (8.24) followed by the middle (2.96) and lower (1.16) strata (Table 2 & 3).

Table 2. Average population of thrips on various plant parts of cotton plant at different stages of crop growth

DAS	Thrips population in each plant part based on three strata			
	Leaf*	Square*	Flower*	Boll*
45-52 DAS	5.83	0.00	2.13	0.00
56-70 DAS	6.27	0.93	3.73	0.00
74-87 DAS	6.93	0.33	3.47	0.00
91-104 DAS	0.60	0.53	1.73	0.00
108-133 DAS	0.53	0.20	0.73	0.00

*Average of upper middle lower canopy of 9 observations per 5 plants

Table 3. Within plant part distribution of thrips in different strata of cotton plant

Plants parts	Mean population/plant part between 45-133DAS*		
	Upper	Middle	Lower
Leaf	8.24(2.67)	2.96(1.88)	1.16(1.42)
Square	0.28(1.11)	0.60(1.22)	0.32(1.13)
Flower	3.04(1.99)	2.84(1.86)	1.16(1.44)
Boll	0.16(1.06)	0.00(1.00)	0.00(1.00)
CD	0.42	0.36	0.28
SE(m)	0.138	0.116	0.092
SE(d)	0.20	0.16	0.13
CV %	18.18	18.52	16.64

*Values in parenthesis are Square root transformed values ** Bolls were not available till 70 DAS

Population dynamics of thrips

The population of thrips/3leaves was recorded from the Bt cotton (RCH650BG-II) and non Bt cotton (HS-6) under unprotected conditions during 2015-16 to 2018-19 regularly at weekly intervals. In case of Bt cotton incidence of thrips started in 27th SMW and maximum population was recorded in 31st SMW (41.92) in 2015-16. In 2016-17, population range/3leaves was 0-32.1, incidence started in 23rd SMW whereas the maximum population was recorded in 29th SMW. In 2017-18, thrips population ranged between 0-81.2/3leaves. The incidence started in 25th SMW and the maximum population (81.2) was in 30th SMW. In 2018-19, thrips population ranged between 0-36.3/3leaves. The incidence started in 22nd SMW and maximum population (36.3) was found in 29th SMW. In non Bt cotton population range of thrips was 0-37.36/3leaves and incidence started in 27th SMW whereas maximum population (37.36/3leaves) was recorded in 29th SMW in 2015-16. In 2016-17, thrips population ranged between 0-26.9/3leaves. Incidence began in 23rd SMW and maximum population (26.9/3leaves) was during 32nd SMW. In 2017-18, thrips population range between 0-106.8/3leaves and incidence started in 25th SMW and maximum incidence (106.8/3leaves) was recorded in 30th SMW. In 2018-19, the population ranged between 0-41.5/3leaves and incidence began in 24th SMW. Maximum thrips (41.5/3leaves) populations were found in 25th SMW.

In both the genotype, among all the years peak incidence was recorded during 29th -31st SMW, however, highest thrips was recorded during 2017-18 (Fig1).

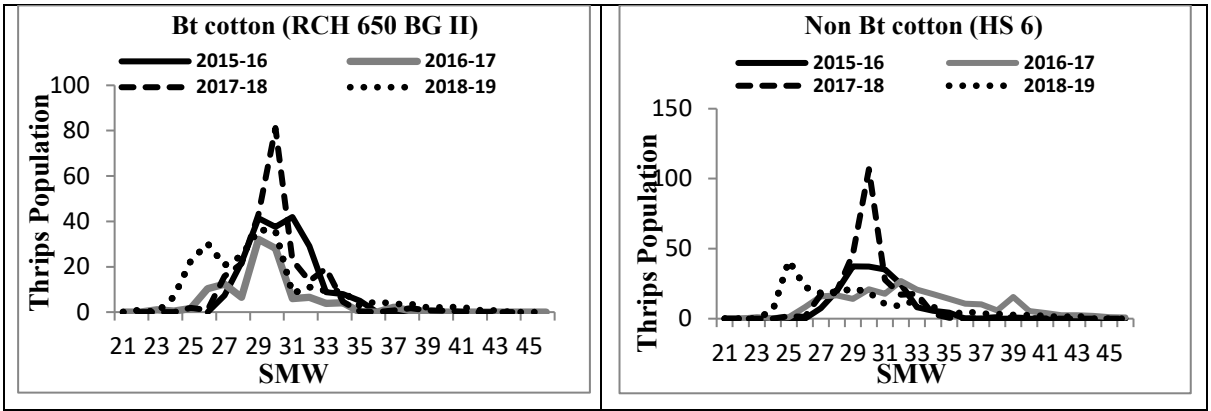

Figure 1: *Population dynamics of thrips during 2015-16 to 2018-19 cotton seasons in Bt and non Bt cotton*

Impact of insecticide treatments on thrips inhabiting cotton plant

Efficacy of label claimed insecticides was examined against thrips under laboratory conditions during 2017-18 & 2018-19 at recommended, below recommended and above recommended doses. Results obtained under recommended doses for different insecticides are given in Table 4. The average mortality ranged between 13.67-79.0 %. Maximum average was found in spinosad (79%) followed by fipronil (77.33%). Among plant extracts, castor oil gave maximum mortality (48.17%) followed by sesame oil (44.67%); pongamia oil (44.50%); neem oil 1500 ppm (35.33%) and neem oil 300 ppm (25.83%). Diafenthiuron, flonicamid, profenphos and buprofezin resulted into 63.50, 33.00, 67.33 and 49.50 per cent thrips mortality, respectively.

Under field evaluation, data recorded from treated plants at specific intervals during 2018-19 before and after 1st application, the thrips counts/3leaves before treatment ranged between38.10-44.10. After 7 days of spray, population ranged between 11.50-42.0/3 leaves. All the treatments were significantly superior over control. Similarly, during 2nd spray, the counts ranged between 31.80-51.30 before spray application and 11.96-49.89/3leaves after spray application. The maximum population recorded was in control whereas minimum in fipronil. Among the eco-friendly interventions, minimum population during both the years was recorded in neem oil followed by pongamia and castor oil. However, among 21 treatments maximum reduction in thrips population was recorded in Fipronil 5 % SC (76.03%) and minimum was in flonicamid 50 %WG (33.23%) among insecticides. Among plant oils, neem oil 300 ppm + detergent powder (0.1%), neem oil 1500 ppm+ detergent (0.1%), sesame oil+ detergent (0.1%), castor oil+ detergent (0.1%) and pongamia oil+ detergent (0.1%) reduced 38.52, 31.23, 49.43, 52.54 & 44.28 per cent thrips population, respectively. Reduction in thrips due to entomopathogens based biopesticides like Beauveria basiana, Metarhizium anisopliae & Verticillium lacanii range between 35.44 - 41.83 percent (Table 5).

Similarly during 2019-20, thrips population prior to spray initiation ranged between 23.33-35.33/3leaves. Maximum population after spray application was recorded in control (26.17/3leaves) and minimum (4.50/3leaves) in fipronil. Among all the 21 treatments maximum reduction in thrips population was recorded in profenphos 50% EC (82.11%) and minimum was recorded in flonicamid 50% WG (11.58 %). Among plant based oils, neem oil 300 ppm, neem oil 1500 ppm, sesame oil, castor oil and pongamia oil+ detergent (0.1%) recorded 35.79, 7.37, 37.89, 32.63 & 34.74 per cent reduction in thrips population, respectively. Entomopathogen based biopesticides like Beauveria basiana, Metarhizium anisopliae & Verticillium lacanii recorded mortality range from 26.32-32.63 per cent (Table 6). The populations of natural enemies were higher in plant based oils and entomopathogens as compared to insecticides based treatments.

Discussion

To develop environment friendly management tactics a greater understanding of pest distribution in the plant parts and population dynamics is required. Thrips are mainly considered as economic pest of cotton only at seedling stage (Gaines 1934; Watts 1937; Cook et al 2011) present study emphasized that though thrips are early season pest but remain present on cotton plant throughout the season with peak between 29-31st SMW. Over all throughout plant developmental stages, majority thrips were found on leaves in the middle part of plant primarily due to the preference for these leaves and only a few thrips inhabited the squares (Atakan et al. 1996). Studies have shown that leaves harbors higher number of thrips in the early season, squares and flowers in mid season and flowers and leaves in late

season with highest numbers on upper stratum (Parajulee et al. 2006). Similarly, adults were significantly more abundant on seedlings and flowers in 2014 and on flowers followed by seedlings and leaves from the middle canopy in 2015 (Jones et al. 2017). In our studies after leaves, thrips prefer flower which even depend upon the availability during activity period followed by squares but very negligible thrips were noticed on bolls. Population dynamics of thrips are known to vary among host plants, their plant parts, biotic condition and even transgenic and non transgenic hirsutum cotton (Akram et al. 2013; Ahmed et al. 2017). In current population dynamic studies under unprotected condition, prevalence of thrips reported one month after sowing with peak activity during 29-31st SMW and gradually population declined, rainfall pattern of the season also affect their dynamics. The earlier dynamic studies reported thrips incidence from 25th SMW onwards with peak activity during 31st SMW - 41st SMW (Janu et al. 2017; Meghana et al. 2018; Badgujar et al. 2018).

Application of insecticides is commonly used to manage thrips (Gore et al. 2012). As reported in North Indian cotton zone during earlier part of season thrips and whitefly occurred simultaneously in cotton fields and application of neonicotinoids and insecticides with new mode of action for whitefly control exert extra selection pressure on non target pest like thrips which may accelerate resistance development. The insecticides resulted into higher thrips mortality as compared to biopesticides (Ghelani et al. 2014). Neem based pesticides can be adopted on large scale as they are environment friendly and also IPM compatible. But neem based insecticides alone without any alternate option during early season for sucking pests in cotton is also a matter of concern. Among Bio-pesticides neem oil @ 1%, azadirachtin @0.0009% and V.lecanii @ 2.5 kg/ha was recorded most superior against all major sucking pests (Ghelani et al 2014). In our study besides neem based products, oils from castor (Ricinus communis), sesame (Sesamum indicum) and pongamia (Pongamia glabra) applied @ 10ml/liter water are good option for the management of thrips especially during earlier season when thrips as well as beneficial insect dynamic is at peak. These oils were not only a suitable alternate of neem based for early season but also equally effective during mid or late season.

Conclusion

An appropriate sampling in association with information on population dynamics will help in monitoring of thrips. Similarly using ecofriendly interventions especially plant based oils i.e. pongamia, castor and sesame in addition to neem, recorded significantly less population in comparison to control. These oils can be deployed during earlier part of the season to manage the thrips menace in cotton.

Table 4: Efficacy of different Insecticides against Thrips in Cotton under laboratory conditions during 2017-18 & 2018-19.

	Label Claim Insecticide	Below Recommended Dosage						Recommended/label Claim Dosage						Above Recommended Dosage					
		Dosage (g or ml/l)	Mortality (%)				Average mortality (%)	Dosage (g or ml/l)	Mortality (%)				Average mortality (%)	Dosage (g or ml/l)	Mortality (%)				Average mortality (%)
			2017-18		2018-19				2017-18		2018-19				2017-18		2018-19		
			O.V.	T.V.*	O.V.	T.V.*			O.V.	T.V.*	O.V.	T.V.*			O.V.	T.V.*	O.V.	T.V.*	
T1	Imidacloprid 17.8% SL	0.17	28	31.71	47	43.45	37.50	0.26	50	44.79	50	45.17	50.00	0.4	46	42.49	52	46.13	48.83
T2	Imidacloprid 70% WG	0.05	50	44.79	42	40.33	45.83	0.08	62	51.79	50	45.17	56.00	0.12	48	44.03	60	50.57	54.00
T3	Thiamethoxam 25% WG	0.05	24	29.06	40	39.37	32.00	0.26	36	36.85	41	39.79	38.50	0.4	40	39.37	51	45.75	45.83
T4	Dinortefuran 20% SG	0.2			46	42.88	46.33	0.33			50	45.17	50.33	0.6			57	49.01	57.00
T5	Thiacloprid 21.7% SC	0.14	32	34.21	50	45.17	41.00	0.26	32	34.57	54	47.09	43.00	0.4	42	40.56	60	50.57	51.00
T6	Clothiandin 25% WDG	0.3	44	41.72	42	40.56	43.33	0.5	58	49.40	47	43.07	52.17	1	54	47.28	58	49.39	55.83
T7	Fipronil 5% SC	3	58	49.40	61	51.57	59.50	4	72	58.05	83	65.62	77.33	6	76	60.50	85	67.55	80.50
T8	Diafenthiuron 50% WP	0.8	40	39.19	54	47.28	47.00	1.6	66	54.58	61	51.19	63.50	2.4	58	49.60	68	55.76	63.17
T9	Flonicamid 50%WG	0.3	24	28.93	32	34.61	28.00	0.4	30	32.97	36	37.03	33.00	0.6	30	32.97	42	40.37	35.83
T10	Profenophos 50 % EC	2	64	52.94	61	51.54	62.50	2.6	66	54.13	69	56.19	67.33	4	74	59.38	70	56.58	71.83
T11	Spinosad 45%SC	0.2	66	54.13	71	57.22	68.17	0.3	78	61.81	80	63.77	79.00	0.5	66	54.13	85	67.04	75.17
T12	Spinetoram 11.7% SC	0.8	71	57.29	58	49.40	64.17	1.12	68	55.35	72	58.28	70.00	1.7	76	60.47	78	62.05	76.83
T13	Buprofezin 25% SC	1.3	34	35.63	33	34.76	33.33	2.6	58	49.80	41	39.60	49.50	4	54	47.47	45	41.92	49.50
T14	Neem (300 ppm) + Detergent	3	32	34.56	43	40.76	37.50	5	36	36.63	46	42.68	40.83	8	34	35.84	53	46.51	43.50
T15	Neem (1500 ppm) + detergent	3	36	36.63	45	41.92	40.17	5	24	29.07	47	43.26	35.33	8	42	40.56	55	47.85	48.67
T16	Sesame oil + detergent (0.1%)	7	50	44.98	36	36.85	43.00	10	48	44.03	41	39.79	44.67	15	66	54.53	55	47.67	60.50
T17	Castor oil+ detergent (0.1%)	7	42	40.56	45	41.91	43.50	10	45	42.11	51	45.75	48.17	20	58	49.41	59	50.36	58.50
T18	Pongamia oil+ detergent (0.1%)	7	36	36.85	46	42.49	40.83	10	32	34.41	57	49.02	44.50	15	40	39.01	62	51.74	50.67
T19	Water + Detergent (0.1%)		22	28.15	21	27.48	21.83		22	27.83	21	27.47	21.67		22	27.95	21	27.01	21.33
T20	Water (Control)		14	22.14	13	21.37	13.83		14	21.86	13	21.30	13.67		14	21.65	13	20.82	13.17
	Range		14-71		13-71				14-78		13-83				14-76		13-85		
	C.D.			4.50		4.39				4.84		4.65				4.16		3.67	
	SE(m)			1.56		1.53				1.68		1.62				1.45		1.28	
	SE(d)			2.21		2.16				2.38		2.29				2.04		1.81	
	C.V.			16.92		16.37				16.75		16.19				15.61		14.54	

*Angular transformation O.V. - Original value T.V. – Transformed value

Table 5: Efficacy of different Insecticides against Thrips in Cotton under field conditions during 2018-2019

	Treatment	Dosage (g or ml)/liter	Average thrips/ 3 leaves								Population Reduction (%) over control	Natural enemies/3 leaves**
			1st Spray (14-July)				2nd Spray (14-Aug)					
			Pre		Post***		Pre		Post***			
			O.V	T.V*	O.V	T.V*	O.V	T.V*	O.V	T.V*		
1	Imidacloprid 70 % WG	0.1	43.10	6.64	34.11	5.92	42.40	6.58	20.50	4.63	58.91	0.33
2	Thiamethoxam 25 % WG	0.2	38.40	6.27	25.86	5.18	39.90	6.39	26.45	5.23	46.98	0.89
3	Thiacloprid 21.7 % SC	0.2	39.90	6.39	32.50	5.78	42.80	6.61	24.61	5.04	50.67	0.44
4	Clothiandin 25 % WDG	0.5	39.90	6.39	32.50	5.78	49.60	7.11	29.51	5.51	40.85	0.56
5	Fipronil 5 % SC	3	41.70	6.53	11.50	3.53	40.00	6.40	11.96	3.59	76.03	1.00
6	Diafenthiuron 50% WP	1.2	40.30	6.42	20.00	4.58	40.00	6.40	18.83	4.45	62.26	0.89
7	Flonicamid 50 %WG	0.3	41.80	6.53	33.50	5.86	43.50	6.67	33.31	5.85	33.23	0.56
8	Profenophos 50 % EC	2	39.10	6.32	19.00	4.46	42.20	6.57	16.40	4.15	67.13	0.56
9	Spinosad 45% SC	0.3	42.20	6.57	13.00	3.73	40.00	6.40	13.46	3.79	73.02	1.00
10	Spinetoram 11.7% SC	1.12	40.70	6.45	17.00	4.24	38.50	6.28	15.46	4.05	69.01	0.44
11	Buprofezin 25% SC	2	40.50	6.43	27.45	5.32	45.50	6.81	26.21	5.21	47.46	0.67
12	Beauveria basiana	5	41.20	6.49	30.80	5.63	44.40	6.73	32.21	5.75	35.44	1.11
13	Metarhizium anisopliae	5	39.30	6.34	31.41	5.69	39.20	6.33	29.12	5.48	41.63	0.89
14	Verticillium lacanii	5	38.80	6.30	33.80	5.89	46.90	6.92	29.02	5.46	41.83	1.00
15	Neem oil + Detergent (0.1%)	5	23.67	4.96	15.00	3.99	17.83	4.33	12.33	3.65	22.11	0.89
16	Neem (300 ppm) + Detergent	5	41.60	6.52	30.47	5.60	40.10	6.41	30.67	5.61	38.52	0.67
17	Neem (1500 ppm) + detergent	5	44.10	6.71	30.51	5.61	39.00	6.32	34.31	5.94	31.23	0.89
18	Sesame oil + detergent (0.1 %)	10	38.90	6.31	27.11	5.29	42.50	6.59	25.23	5.11	49.43	1.11
19	Castor oil+ detergent (0.1 %)	10	43.00	6.63	31.95	5.74	31.80	5.72	23.68	4.94	52.54	1.00
20	Pongamia oil+ detergent (0.1 %)	10	38.10	6.25	25.38	5.12	33.10	5.83	27.80	5.36	44.28	0.67
21	Water (Control)		40.70	6.45	42.00	6.55	51.30	7.23	49.89	7.13	0.00	1.44
	Range		38.10-44.10		11.50-42.00		31.80-51.30		11.96-49.89			
	C.D.			N/A		0.4		0.25		0.71		
	SE(m)			0.13		0.13		0.08		0.23		
	SE(d)			0.18		0.19		0.12		0.33		
	C.V.			2.85		3.64		1.84		6.57		

*Square root transformation O.V=original value T.V=Transformed value

**Natural Enemies include coccinellids, chrysopids, syrphids and Spider

***7 Days after spray

Table 6: Efficacy of different Insecticides against Thrips in Cotton under field conditions during 2019-2020.

	Treatment	Dosage (g or ml)/liter	Average thrips/ 3 leaves								Population Reduction (%) over control	Natural enemies/3 leaves**
			1st Spray (14-July)				2nd Spray (14-Aug)					
			Pre		Post***		Pre		Post***			
			O.V	T.V*	O.V	T.V*	O.V	T.V*	O.V	T.V*		
1	Imidacloprid 70 % WG	0.1	23.33	4.93	10.67	3.39	16.83	4.22	6.83	2.80	56.84	0.57
2	Thiamethoxam 25 % WG	0.2	29.83	5.55	17.00	4.24	16.67	4.11	8.67	2.91	45.26	1.00
3	Thiacloprid 21.7 % SC	0.2	29.83	5.55	15.50	4.06	15.67	4.07	6.33	2.71	60.00	0.47
4	Clothiandin 25 % WDG	0.5	35.33	6.02	25.50	5.14	17.17	4.26	11.00	3.46	30.53	0.67
5	Fipronil 5 % SC	3	29.17	5.48	4.50	2.34	23.50	4.87	3.50	2.12	77.89	0.36
6	Diafenthiuron 50% WP	1.2	24.00	5.00	9.00	3.16	22.50	4.84	8.83	3.13	44.21	0.56
7	Flonicamid 50 %WG	0.3	29.33	5.50	22.50	4.84	19.00	4.35	14.00	3.76	11.58	0.44
8	Profenophos 50 % EC	2	28.17	5.40	11.00	3.46	14.83	3.98	2.83	1.96	82.11	0.44
9	Spinosad 45%SC	0.3	34.50	5.96	9.00	3.15	18.67	4.44	3.50	2.11	77.89	0.67
10	Spinetoram 11.7% SC	1.12	29.17	5.49	8.67	3.11	20.33	4.62	5.67	2.58	64.21	1.11
11	Buprofezin 25% SC	2	25.83	5.18	16.50	4.18	22.67	4.85	12.17	3.60	23.16	0.89
12	Beauveria basiana	5	29.50	5.51	24.33	5.02	16.33	4.16	10.67	3.41	32.63	0.44
13	Metarhizium anisopliae	5	30.17	5.56	19.50	4.51	19.00	4.47	11.67	3.56	26.32	1.22
14	Verticillium lacanii	5	30.83	5.64	21.00	4.69	15.50	4.05	10.83	3.43	31.58	1.00
15	Neem oil + Detergent (0.1%)	5	23.67	4.96	15.00	3.99	17.83	4.33	12.33	3.65	22.11	0.89
16	Neem (300 ppm) + Detergent	5	32.17	5.73	20.83	4.67	15.50	4.05	10.17	3.33	35.79	1.00
17	Neem (1500 ppm) + detergent	5	34.67	5.97	21.50	4.74	22.17	4.81	14.67	3.95	7.37	1.11
18	Sesame oil + detergent (0.1 %)	10	27.17	5.29	16.33	4.14	15.00	3.97	9.83	3.26	37.89	1.00
19	Castor oil+ detergent (0.1 %)	10	31.33	5.68	17.83	4.34	20.00	4.57	10.67	3.41	32.63	1.00
20	Pongamia oil+ detergent (0.1 %)	10	29.67	5.52	23.67	4.96	18.67	4.43	10.33	3.36	34.74	0.89
21	Water (Control)		27.83	5.37	26.17	5.21	17.00	4.23	15.83	4.09	0.00	1.22
	Range		23.33-35.33		4.50-26.17		14.83-23.50		3.50-15.83			
	C.D.			N/A		0.74		N/A		1.14		
	SE(m)			0.29		0.25		0.43		0.38		
	SE(d)			0.41		0.35		0.61		0.54		
	C.V.			7.42		8.44		13.91		16.91		

*Square root transformation O.V=original value T.V=Transformed value

**Natural Enemies include coccinellids, chrysopids, syrphids and Spider

***7 Days after spray

Material and Methods

Within plant distribution

Within plant distribution was studied at different stages of crop growth. At each crop growth stage 10 plants were randomly selected and visual counts of thrips and its immature stages were taken from three leaves of each stratum i.e. upper, middle and lower canopy also similar observations were recorded from squares, flowers and bolls.

Population dynamics

The population dynamics studies were conducted under unprotected conditions on Bt cotton (RCH 650 BG-II) and Non- Bt cotton (HS-6) and the observation started from May onwards on 10 randomly selected tagged plants at weekly interval from upper, middle and lower canopy leaves.

Laboratory evaluation of biorationals and insecticides

Total 20 treatments were formulated that include 13 insecticides, five botanical oils, one detergent powder based and one untreated control with water (Table 7).The field spray solution of insecticides was prepared based on per liter water. However, castor, pongamia and neem oil emulsions were prepared following the procedure of Gahukar (1996). One gram detergent powder per litre of water was added to acts as surfactant and additionally 1 ml of triton-X-100/liter water was added to each oil formulation for ease in spray applications. Fresh leaves from unsprayed cotton plants were excised along with a petiole. Leaves were rinsed in normal water and air dried. The petioles of the leaves were secured with cotton swab immersed in 10% sucrose solution and agar. Stock solutions of insecticides and biopesticides were prepared. Air dried leaves were then dipped in insecticidal solutions for five seconds ensuring the complete leaf immersion. Treated leaves were allowed to air dry on blotting paper. Thereafter these leaves were laid adaxial side down on a layer of agar (2%) in insect breeding dish. Similarly for the neem based and other oils leaves were sprayed with insecticidal solution on both upper and lower side of leaves and carefully allowed to dry before bioassay. A group of 100 adult thrips was transferred on treated leaves and the dishes were kept at temperature 27 ± 2°C and relative humidity 60±10% with 16:8 (lights: dark) photoperiod regime. Mortality was estimated after 24 and 72 hours of treatment.

Table 7 Detail of the treatments applied

SN	Label claim insecticides and other plant based oils	Dose g or ml /litre water
1	Imidacloprid 17.8 % SL	0.20
2	Imidacloprid 70 % WG	0.08
3	Thiamethoxam 25%WG	0.29
4	Dinotefuran 20%SG	0.3
5	Thiacloprid 21.7 % SC	0.2
6	Clothianidin 25 % WDG	0.5
7	Diafenthiuron 50 % WP	1.6
8	Fipronil 5 % SC	3.0
9	Flonicamid 50 % WG	0.3
10	Profenophos50 % EC	2.0
11	Spinosad 45 % SC	0.8
12	Spinoteram 11.7 % SC	0.9
13	Buprofezin 25% SC	2.0
14	Neem oil 300 ppm + Detergent (0.1%)	5.0
15	Neem oil 1500 ppm+ Detergent (0.1%)	5.0
16	Sesame oil+ Detergent (0.1%)	10.0
17	Castor oil+ Detergent (0.1%)	20.0
18	Pongamia oil+ Detergent (0.1%)	10.0
19	Water + Detergent (0.1%)	
20	Water (Control)	

Field evaluation of biorationals and insecticides

To evaluate the efficacy of insecticides and botanicals oils under field conditions an experiment was laid out with 21 treatments each was replicate thrice in a Randomized Block Design. Bt-cotton (RCH773 BG II) was sown adopting recommended package of practices. Sprays were initiated when population of thrips reached ETL and two consecutive sprays were applied at fortnight interval. Observations were recorded from five plants/treatment, before and 3rd as well as 7th day after spray application.

Statistical Analysis

For statistical analysis of both laboratory and field evaluation, reduction (%) in thrips population was calculated based on pre and post treatment population and thereafter subjected to ANOVA test.

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References

1. Abd El-Razik MAA, Zayed GMM. Effectiveness of three plant oils in binary mixtures with pyridalyl, abamectin, spinosad and malathion against Callosobruchus maculatus (F.) adults. American J Biochem Mol Bio. 2014; 4:76-85.
2. Avidov Z, Harpaz I. Plant pests of Israel. Israel Universities Press, Jerusalem. 1969.
3. Akram M, Faisal H, Muhammad F, Muhammad A, Mussurrat H, Saghir A, Khuram Z, Hafiz AAK. A case to study population dynamics of Bemisia tabaci and Thrips tabaci on Bt and non-Bt cotton genotypes. Pak J Agril Sci. 2013;50(4); 617-623.
4. Ambarish S, Shashi KC, Somu G, Shivaray N. Studies on the Bio-efficacy of new insecticide molecules against insect pests in cotton aicrp on cotton. J Ento Zoo Studies. 2017;5(6);544-548.
5. Anonymous. Susceptibility test method series (method no.8). Insecticide Resistance Action Committee. 2009. Version:3.
6. Anonymous. Annual Report 2017-18. 2018a. http://agricoop.nic.in/sites /default/files/Krishi%20AR%202017-18-1%20for%20web.pdf
7. Anonymous. AICRP Project Coordinator's Report 2017-18. 2018b. http://aiccip.cicr.org.in/ CD_17-18/3_A1_A17_PC_report.pdf.
8. Badgujar AG, Bhosle BB, Sonkamble MM. (2018) Population Dynamics of Thrips, Thrips tabaci and Whitefly, Bemisia tabaci on Bt Cotton Hybrids. Inter J Cur Micro Appl Sci. 2018:6:614-625.
9. Bobade SN, Khyade VB. (2012) Detailed study on the properties of Pongamia pinnata (Karanja) for the production of biofuel. R J Chem Sci. 2012;2(7):16 - 20.
10. Boda V, Ilyas M.(2017) Evaluation of New Insecticides Against Sucking Pests Of Bt Cotton. Inter. J P A Envir Sci. 2017;7(2):66-72.
11. Cinthia PS, PatriciaVA, Roberto MB, Rodolfo FB, Alfredo JP. Source repellency of hydroethanolic extracts of Ricinus communis (Euphorbiaceae) to Scyphophorus acupunctatus (Coleoptera: Curculionidae) in the Laboratory. Florida Entomologist 2012;95(3):706-710.
12. Don C, Ames H, D S Akin, Jack R. Biology, Crop Injury, and Management of Thrips (Thysanoptera: Thripidae) Infesting Cotton Seedlings in the United States. J. Integ. Pest Mngmt. 2011;2(2).
13. Ekrem A, Moshe C, David R.1996Within-plant distribution of thrips and their predators:effects of cotton variety and developmental stage. Bulletin of Ento Res.1996;86: 641-646.
14. Daniel EF, Natarj K, Angus L C, Fred RM. Susceptibility to insecticides and activities of glutathione S-transferase and esterase in populations of Lygus lineolaris (Hemiptera: Miridae) in Mississippi. Pest Manag. Sci. 2016;72: 1595–1603.
15. Freidberg A, Susman I, Kaplan F. Cotton insects of Israel. The Cotton Production and Marketing Board.1989;126.
16. Gaines J C. A preliminary study of thrips on seedling cotton with special reference to the population, migration, and injury. J Econ Entomol.1934;27:740–743.
17. Gahukar RT. Formulation of neem-based products/pesticides. Pestology1996;20(9):44-45.
18. Ghelani MK, Kabaria BB, Chhodavadia SK. Field Efficacy of Various Insecticides Against Major Sucking Pests Of Bt Cotton. J Biopest. 2014;7:27-32.
19. Gore JA, Catchot FM, Greene J, Leonard BR, Cook DR, Snodgrass GL, Jackson R. Development of a plant-based threshold for tarnished plant bug (Hemiptera: Miridae) in cotton. J. Econ. Entomol. 2012;105: 2007–2014.
20. Huseth AS, Chappel TM, Langdon K, Morsello SC, Martin S, Greene JK, Herbert A, Jacobson AL, Reay-Jones FPF, Reed T. Frankliniella fusca resistance to neonicotinoid insecticides: An emerging challenge for cotton pest management in the eastern United States. Pest Manag. Sci. 2016;72: 1934–1945.
21. Meghana H, Jagginavar SB, Sunitha ND. (2018) Population dynamics of sucking insect pest complex on intra-specific and inter-specific Bt cotton under rainfed situation. Journal of Entomology and Zoology Studies. 2018;6(3): 466-471.
22. Madankar SS, Barkhade UP, Sangle PM. Population dynamics of jassid, Amrasca bigutulla bigutulla on Bt cotton. Trends in Biosci.2015;8(11):2809-2810.
23. Muhammad HA, Muhammad IH, Abu BM, Raza MA, Khaliq A, Yasir I, Hafiz Muhammad A. Population Dynamics of Thrips tabaci (Lindeman) in Relation to Abiotic Climate Factors on Bt and Non-Bt Cotton Cultivars. Zoological Soci. Paki. 2017;49(6):1937-2341.
24. Muhammad Hannan Ahmed, Muhammad Irfan Ullah, Abu Bakar M. Raza, M. Afzal, A. Khaliq, Yasir Iftikhar and Hafiz Muhammad Aatif, Muhammad Hannan Ahmed, Muhammad Irfan Ullah, Abu Bakar M. Raza, M. Afzal, A. Khaliq, Yasir Iftikhar and Hafiz Muhammad Aatif, Janu A, Dahiya KK, Jakhar P. Population Dynamics of Thrips, Thrips tabaci Lindemann in American Cotton (Gossypium hirsutum). Inter J Curr Microb Appl Sci. 2017;6(7):203-209.
25. Jones FPFR, Jeremy KG, Ames DH, Alana LJ, George GK, Dominic DR, Phillip MR. Within-Plant Distribution and Dynamics of Thrips Species (Thysanoptera: Thripidae) in Cotton. J Econ Entomol. 2017;1–13.
26. Knight I, Rains AGC, Culbreath AK, Toews MD. Conservation tillage and thiamethoxam seed treatments as tools to reduce thrips densities and disease in cotton and peanut. Crop Prot.2015; 76:92–99
27. Kolli GR, Balakrishnan V, Sundararajan R. Evaluation of larvicidal activity of Pongamia pinnata extracts against three mosquito vectors. Asian Pacific J Trop Biomed. 2013;3(11):853–858.

28. Nidhal S, Olfa B, Jazia S, Kamel M, Saber K, Majdi H, Saoussen S, Emna B, Souha K, Manef A, Brahim M, Férid L, Jouda MBJ. Fumigant and repellent potentials of Ricinus communis and Mentha pulegium essential oils against Tribolium castaneum and Lasioderma serricorne, Internl J Food Propertie. 2017;DOI: 10.1080/10942912.2017.1382508.
29. Panwar TS, Singh SB, Garg VK. Influence of meteorological parameters on population dynamics of thrips (Thrips tabaci Lindeman) and aphid (Aphis gossypii Glover) in Bt and non Bt cotton at Malwa region of Madhya Pradesh. J Agrometeorology. 2015;17(1);136-138
30. Parajulee MN, Shrestha R, Leser JF. Leser. Influence of tillage, planting date, and Bt cultivar on seasonal abundance and within-plant distribution patterns of thrips and cotton fleahoppers in cotton. Int. J. Pest Manage. 2006;52:249–260.
31. Patil PV, Bhamare VK. Persistance and relative toxicity of different insecticides against Thrips (Scirtothrips dorsalis) and whitefly (Bemisia tabaci) on cotton ecosystem. Interna J Chemical Studies. 2018; 6(5):735-740.
32. Patil PV, Bhamare VK, Pate PP. Bio-Efficacy of Different Insecticides against Thrips (Scritothrips dorsalis Hood) and Whitefly (Bemisia tabaci Gennadius) on BT Cotton. Bulletin of Environment, Pharmacology and Life Sci. 2018;7(4):10-14.
33. Rampadarath S, Puchooa D, Jeewon R. Jatropha curcas L: Phytochemical, antimicrobial and larvicidal properties. Asian Pacific Journal of Tropical Biomedicine.2016;6(10):858–865.
34. Rishi K, Sandhya K, Nagrare VS, Monga D, Keshav RK, Naveen R, Amarpreet S. Insecticidal activity of botanical oils and other neem-based derivatives against whitefly, Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) on cotton. International J Tropical Insect Sci. 2019;39:203–210.
35. Stewart S, Akin SD, Reed J, Bacheler J, Catchot A, Cook D, Gore J, Greene J, Herbert A, Jackson R. Survey of thrips species infesting cotton across the Southern U.S. Cotton Belt. J. Cotton Sci. 2013;17:1–7.
36. Sharma R, Sharan L. Evaluation of various Synthetic Insecticides against Thrips (Thrips tabaci) in Bt Cotton. International Journal of Environmental & Agriculture Research. 2016;2(8):35-37.
37. Sridhar S, Vijayalakshmi K. Neem: A User's Manual. CIKS, Chennai. 2002.
38. Stepanycheva EA, Petrova MO, Chermenskaya TD, Pavela R. Prospects for the use of Pongamia pinnata oil-based products against the Green Peach Aphid (Sulzer) (Hemiptera: Aphididae). Psyche: J Entomology. 2014;1–5.
39. Saif-ur-R, Jamil S,Waseem A,Waqas A, Muhammad A, Suhail A, Muhammad I. Population Dynamics of Thrips on Transgenic and Non-Transgenic Cultivars of Cotton. Advances in Zoology and Botany. 2013;1(4):71-77.
40. Swathi S, Muruananthan G, Ghosh SK. Oviposition deterrent activity from the ethanolic extract of Pongamia pinnata, Coleus forskohlii and Datura stramonium leaves against Aedes aegypti and Culex quinquefaciatus. Pharmacogn Mag. 2010;6(24):320-322.
41. ThripsW. ThripsWiki. ThripsWiki - providing information on the World's thrips. 2020; http://thrips.info/wiki.
42. Vasudevan P, Madan N, Sharma S. Ovicidal property of castor. Pesticides. 1989;2:36–39.
43. Vennila S, Biradar VK, Sabesh M, Bambawale OM. Know Your Cotton Insect Pests (Thrips). Crop Protection Folder Series CICR, Nagpur. 2007;http://www.cicr.org.in/pdf/kycp_thrips.pdf
44. Watts JG. Reduction of cotton yields by thrips. J Econ Entomology. 1937;6:860–863.

Effect of temperature on the bionomics of invasive pest *Phenacoccus solenopsis Tinsley* (Sternorrhyncha: *Pseudococcidae* and its native parasitoid

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Abstract

The present study used a mealybug-parasitoid cotton system to evaluate the effects of climate change on the bionomics of invasive pest (Phenacoccus solenopsis Tinsley (Sternorrhyncha: Pseudococcidae)) and its native parasitoid (Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae)) under a range of temperatures i.e., 20°C, 25°C, 30°C, 35°C and 40°C. High temperatures (30°C, 35°C, 40°C) significantly reduced nymphal development time and also reduced the overall longevity for both male and female of P. solenopsis, whereas, the percentage survival of all immature stages was higher at 30°C than that at other temperatures. The fecundity of P. solenopsis was higher at 30°C. Pre-oviposition duration was significantly shorter at 30°C but the post-oviposition period was longest at the same temperature. The oviposition period was shorter at higher temperatures (35°C and 40°C). Percentage parasitism by A. bambawalei was significantly greater both at 30°C and 35°C, whereas, percentage parasitoid emergence and number of female parasitoids were significantly higher at 30°C compared with other temperatures. The present study demonstrated that higher temperatures had negative effects on development and survival of the invasive pest, P. solenopsis and also had a negative impact on the efficacy of its native parasitoid, A. bambawalei, which may have profound effects on the community composition of terrestrial ecosystem.

***Keywords**: Aenasius bambawalei, Bionomics, Phenacoccus solenopsis, temperature*

Introduction

Direct climatic effects on the development, survival, reproductive physiology, spatial distribution and population dynamics show remarkable internal consistency in terrestrial ecosystem (Shaver et al. 2000; Bale et al. 2002; Dangles et al. 2008; Björkman et al. 2011). The global warming is a dominant variable of climate change that can influence the bionomic of organisms and consequently, drives population growth and abundance (Bale et al . 2002; Clarke 2003; Dangles et al. 2008). Bionomic of organism involves the life history parameters, development rate, survival, reproduction, generation time and survival of organism (Hespenheide 1991; Bonato et al. 2007).

The impact of temperature on life-history parameters has been considered for insect herbivores (Amarasekare et al., 2008b; Jandricic et al., 2010; Waterworth et al., 2011), predators (Logan et al. 2006; Logan 2008; Logan and Wolesensky 2007) and parasitoid species (Gould and Elkinton 1990; Chong and Oetting 2006; Hance et al. 2007). Life-history parameters of insect pest species responded differently to temperature than their natural enemies (Logan et al. 1976; Gilbert and Raworth 1996; Björkman et al. 2011). Temperature not only affects the insect development rate (Gilbert and Raworth 1996; Hou and Weng 2010) but also influences the trophic interactions between species with different thermal requirements (Rice and Allen 2009). Only a few studies have examined simultaneously the effects of temperature on the bionomic of both insect pest and their parasitoids (Hughes et al. 2010; Klapwijk et al. 2010; De Conti et al. 2011). However, data on the likely impact of temperature on bionomic of invasive insect pest and their native parasitoid species are currently lacking. The present investigations were carried out to examine how the bionomic of invasive insect pest and their native parasitoid species is influenced by temperature.

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (*Sterrhnorhycha: Pseudococcidae*) is a serious invasive pest of various ecological zones of the world (Wang et al. 2010; Abbas et al. 2010). Cotton mealybug is a highly polyphagous pest which attacks many plants including weeds, cotton, ornamental and medicinal plants (Abbas et al. 2005; Hodgson et al. 2008; Arif et al. 2009; Abbas et al. 2010; Wang et al. 2010). *Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae)* is a native solitary endoparasitoid of *P. solenopsis* (Hayat 2009; Bodlah et al. 2010) and plays an important role in controlling mealybug (Fand et al. 2011). Limited work has been carried out on the bionomics of *P. solenopsis* (Zhu et al. 2011) and no work has been carried out on the bionomic of A. bambawalei. In the present study, we investigated the influence of temperature on the bionomics of both P. solenopsis (invasive pest) and its native parasitoid *A. bambawalei*.

Materials and Methods

Insect culture

Phenacoccus solenopsis were available from long-standing culture maintained on pumpkins fruits, *Cucurbita moschata* Hindi (*Cucurbitales: Cucurbitaceae*). To minimize the maternal host plant effect (Tariq et al. 2010), the insects were established on *Gossypium hirsutum L. (Malvales: Malvaceae)* plants for two generations before using in the main experiment. *Phenacoccus solenopsis* were sub-cultured fortnightly and transferred to six week old *G. hirsutum* plants. *Aenasius bambawalei* mummies were collected from China rose plants, *Hibiscus rosasinensis* and reared on culture of P. solenopsis. Insect cultures were maintained at control environment facility (25 ± 2 °C; 65% RH; LD 16:8 h).

Experimental treatments

To access the influence of different temperature regimes on the bionomics of *P. solenopsis* and *A. bambawalei*, five temperatures, 20, 25, 30, 35 and 40 °C were selected with 65% RH and LD 10: 14 h.

Invasive pest performance

Newly emerged nymphs of cotton mealybug were separated from the main culture and transferred to clean and fresh cotton leaves in 9 cm Petri dishes. The duration of nymphal instars of newly hatched nymphs was recorded till their mortality. Parameters recorded for each individual at different temperature were development period (immature stages of male and female *P. solenopsis*), pre-reproductive, reproductive, post-reproductive, total male longevity and total female longevity (the duration from 1st day to mortality), (for male and female) Survival rate for 1st and 2nd instars (number of successful molted insects to next instar divided by total number of insects at the beginning of instar x 100).

The effect of tested temperatures on the fertility of test insects and the chances of parthenogenesis were investigated in fecundity experiments. 40 newly emerged females were chosen for this purpose. Fifty percent of the females were reared without a male whereas; remaining 50 percent females were reared with a newly emerged male for mating from the same treatment. Number of egg batches, eggs per batch and total eggs laid by single female were recorded.

Native parasitoid performance

A pair of newly emerged female P. solenopsis was introduced to a three week old *G. hirsutum* plant. Five G. hirsutum plants (replicates) were used to access the performance of *A. bambawalei* at each temperature. After two weeks (five-week-old plants) of *P. solenopsis* treatments, one hundred cotton mealybug were used to measure parasitoid performance. Newly emerged A. bambawalei females had been sexed into a 2.5 x 8 cm glass tubes and fed on 10% honey solution. Mixed instars of *P. solenopsis* were exposed to three paired parasitoids on a single treated plant (five replicates per treatments) under a bell cloche. Each bell cloches had five air vents to minimize the humidity inside the bell cloches. After 24 hours of parasitoid release, they were removed from the bell cloches and the remaining parasitoids were removed after twenty four hours of their release and the *P. solenopsis* were allowed to continue their development from parasitisation to mummy formation. Mummies were collected individually in a gelatin capsule and percentage parasitism, percentage emergence, immature and adult longevity for both sexes, total longevity for both sexes and sex ratio recorded.

Statistical analysis

Age specific life table was calculated by using generalized additive models as described by (Birch 1948; Rizvi et al. 2009). The effects of temperature on all studied parameters of *P. solenopsis* and A. bambawalei were analyzed using ANOVA. Data for male (1st and 2nd instar, prepupal, pupal and total immature longevity) and female *P. solenopsis* (1st, 2nd and 3rd instar and total immature longevity) were log transformed before analyses, whereas, the data for male total longevity and mated female total longevity of *P. solenopsis* were square root before analyses. Data for percent parasitism, percent emergence, female immature longevity, adult longevity and sex ratio were log transformed before analyses, whereas, the data for male immature longevity of A. bambawalei were square root transformed before analysis. Posthoc Tukey HSD tests were used to compare the means for all studied parameters of *P. solenopsis* and A. bambawalei (Crawley 2005; Crawley 2007). All analyses were performed with R, version 2.14.1 (R Development Core Team 2011).

Results

Developmental duration of male and female nymphal instars of *P. solenopsis*

All the temperatures had significant effects on all developmental parameters of male *P. solenopsis* (1st instar $F_{4, 145} = 375.12$; $P < 0.0001$, 2nd instar $F_{4, 145} = 119.14$; $P < 0.0001$, prepupal $F_{4, 145} = 235.48$; $P < 0.0001$, pupal $F_{4, 145} = 392.59$; $P < 0.0001$, total immature longevity $F_{4, 145} = 1032.90$; $P < 0.0001$ and total male longevity from birth to death $F_{4, 145} = 1076.30$; $P < 0.0001$) and female *P. solenopsis* (1st instar $F_{4, 145} = 232.18$; $P < 0.0001$, 2nd instar $F_{4, 145} = 127.04$; $P < 0.0001$, 3rd instar $F_{4, 145} = 289.78$; $P < 0.0001$, total immature longevity $F_{4, 145} = 622.47$; $P < 0.0001$, pre-oviposition duration $F_{4, 70} = 125.04$; $P < 0.0001$, oviposition duration $F_{4, 70} = 1.50$; $P > 0.0001$, post-oviposition duration $F_{4, 70} = 25.95$; $P > 0.0001$, total virgin female longevity from birth to death $F_{4, 145} = 189.91$; $P > 0.0001$ and total mated female longevity from birth to death $F_{4, 145} = 21.43$; $P > 0.0001$).

The effect of temperature was significant for the nymphal developmental time of male and female *P. solenopsis* (Fig. 1a & b; Tukey's HSD, $P < 0.05$). High temperatures (30 °C, 35 °C, 40 °C) significantly reduced the developmental duration of immature male and female longevity (Fig. 1a & b; Tukey's HSD, $P < 0.05$) and total nymphal longevity for both sexes (Fig. 1c; Tukey's HSD, $P < 0.05$). Total longevity was significantly greater for males, virgin and mated *P. solenopsis* at lowest temperatures (20 °C), and was statistically similar at higher temperature regimes (30 °C, 35 °C and 40 °C), whereas, it was recorded significantly lowest at higher temperatures (Fig. 2, Tukey's HSD, $P < 0.05$).

The pre-oviposition duration was significantly shorter and post-oviposition period was longer at 30 °C (Fig. 3, Tukey's HSD, $P < 0.05$). The oviposition duration was significantly shorter at higher temperatures (35 °C and 40 °C). Female *P. solenopsis* laid different number of egg batches at all temperatures ($F_{4, 70} = 4.26$; $P < 0.001$), and maximum number of egg batches were laid by single female at 30 °C (Fig. 4, Tukey's HSD, $P < 0.05$), whereas, number of eggs per batch were similar at all temperatures ($F_{4, 70} = 0.76$; $P > 0.05$). All temperatures had significant effect on total number of eggs laid by each female ($F_{4, 70} = 12.54$; $P < 0.0001$) and the total number of eggs laid by single female were significantly greater at 30 °C (Fig. 4, Tukey's HSD, $P < 0.05$).

Survival rate of *Phenaccous solenopsis*

The survival percentage of the 1st nymphal instar was similar on all temperature regimes ($F_{4, 10} = 2.21$; $P > 0.05$), whereas, the effect of temperature on survival rate was significant for 2nd nymphal instar ($F_{4, 10} = 8.11$; $P < 0.01$), 3rd female nymphal instar ($F_{4, 10} = 9.07$; $P < 0.01$), male prepupal stage ($F_{4, 10} = 6.45$; $P < 0.01$) and pupal stage ($F_{4, 10} = 5.05$; $P < 0.05$). The survival percentage for all the immature stages of male and female *P. solenopsis* was higher at 30 °C. High temperature (40 °C) significantly decreased the survival percentage of all the nymphal stages for both sexes (Fig. 5; Tukey's HSD, $P < 0.05$). The survival rate of male prepupal and pupal stages was significantly lower at 20 °C (Tukey's HSD, $P < 0.05$).

Age Specific Life Table of *Phenacoccus solenopsis*

Age specific life table of *P. solenopsis* were constructed at five different temperatures (Table 1). *Phenacoccus solenopsis* required 82 days (maximum) to complete one cohort at lowest temperature (20 °C), whereas the least duration of 55 days was recorded when it was reared at 40 °C. All temperatures directly affected the maximum mortality of *P. solenopsis*. Maximum mortality was recorded between 51-60 days, 41-50 days, 31-40 days, 41-50 days and 31-40 days for 20 °C, 25 °C,

30 °C, 35 °C and 40 °C, respectively. Fifty percent individuals survived during the first 45 days at 20 °C and 25 °C, 42 days, 40 days and 38 days at the remaining temperatures, respectively. It was noted that the life expectation of the test insect was steadily decreasing from the start of the investigation till the mortality at all the tested temperatures. Apparent mortality of *P. solenopsis* was affected by all temperatures; higher temperature (40 °C) had a greater influence on the apparent mortality. Life expectancy was observed better on certain days at different temperatures (Fig. 6). Life expectancy increased slightly on 63 and 83 day at 20 °C, on 53 day at 25 °C and 25 day at 35 °C, whereas, life expectancy increased sharply for a period of 15 days (36 to 50 days) and 8 days (38 to 46 days) at 30 °C and 40 °C, respectively.

Parasitism performance, longevity and sex ratio

All temperature had significant effect on percent parasitism ($F_{4, 195}$ = 96.67; P < 0.0001) and emergence ($F_{4, 195}$ = 28.03; P < 0.0001) of *A. bambawalei*. Both higher (40 °C) and lower (20 °C) temperatures negatively affected the percent parasitism and their emergence (Fig. 7a). Percentage parasitism was significantly greater at 30 °C and 35 °C, whereas, the percentage emergence of *A. bambawalei* was significantly greater at 30 °C (Tukey's HSD, P < 0.05) compared with other temperatures. All temperatures had significant effects on male longevity parameters (immature male, $F_{4, 145}$ = 420; P < 0.0001; adult male, $F_{4, 145}$ = 434.4; P < 0.0001 and total male longevity $F_{4, 145}$ = 556.3; P < 0.0001) and female longevity parameters of *A. bambawalei* (immature female, $F_{4, 145}$ = 452; P < 0.0001; adult female $F_{4, 145}$ = 526.4; P < 0.0001 and total female longevity $F_{4, 145}$ = 744; P < 0.0001). Same trend was observed for all the longevity parameters (Immature, adult and total longevity) of both sexes of *A. bambawalei* at all temperatures (Tukey's HSD, P < 0.05). Immature, adult and total longevity for both sexes was maximum at 20 °C compared with other temperatures (Fig. 7b &c; Tukey's HSD, P < 0.05). The main effects of temperature were significant for sex ratio of *A. bambawalei* ($F_{4, 195}$ = 98.21; P < 0.0001). The number of males of *A. bambawalei* was significantly greater at higher (40 °C) and lower (20 °C) temperatures compared with other temperatures. The number of males of *A. bambawalei* was significantly lowest at 30 °C (Fig. 7d; Tukey's HSD, P < 0.05).

Discussion

The global warming can affect the rate of development and life cycle duration of herbivorous insects and beneficial insects, but the responses may differ among species and guilds, fluctuating their interactions, community dynamics and structure of the ecosystem (Montoya and Raffaelli 2010; Robinet Roques 2010). In present investigations, temperature had marked effects on the growth, existence and fertility of invasive cotton pest (*P. solenopsis*) and its native parasitoid (*A. bambawalei*). Both invasive pest and its native parasitoid performed better at intermediate temperature (30 °C). Female *P. solenopsis* laid higher number of egg batches and total eggs at intermediate temperature; similarly the percent parasitism by *A. bambawalei* was higher at same temperature. Similarly, life expectancy of *P. solenopsis* increased sharply for a longer period at intermediate temperature (30 °C). Better performance of both insect species and sharp increase in life expectancy of *P. solenopsis* at intermediate temperature was may be due to the rate of many biological processes in relation to temperature typically peak at some intermediate temperature (Shaver et al. 2000).

Insects are expected to respond very quickly to change in temperature, as their physiological processes exhibit great sensitivity towards changing temperatures (Bale et al. 2002). Age specific table (see, Table 1) showed that the *P. solenopsis* required minimum duration (55 days) to complete a single generation at higher temperature (40 °C), which resulted in the production of additional yearly generation of alien species (Walther et al. 2009). Similarly, temperature strongly effects the development and survival of the insects, where, nymphal development slow down naturally at lower temperatures (20 °C) and the nymphal period shortened and advanced emergence of adults at higher temperatures (35 °C and 35 °C). Thus higher temperatures (35 °C and 40 °C) strongly accelerated the nymphal development of both invasive pests and its parasitoids, which will lead to production of extra generation (s) during a year (Bale et al. 2002; Walther et al. 2009) affecting population dynamics and its pest status (Musolin et al., 2010).

In the present study, temperature regimes had significant impacts on the fecundity of *P. solenopsis* and the total number of eggs and egg batches laid by single female was maximum at 30 °C, which was may be due to longer oviposition period at 30 °C (see, Fig. 3). These results are similar with the findings of Terblanche (2010), where invasive pest (Mediterranean fruit fly; *Ceratitis capitata*)

performed better at intermediate temperature range. In the present study, virgin female did not laid eggs at any temperature, which was unusual for mealybugs, further work should look on this aspect of mealybugs. Dillon et al. (2010) documented that slight changes in temperature lead to large changes in the metabolism of invertebrate tropical ectotherms. *Phenacoccus solenopsis* (males, virgin and mated females) developed faster at higher temperatures (35 °C and 40 °C) but the females had lower fecundity at higher temperatures which might be due to lower weight of insects (Bale et al., 2002) and/or due to large increase in metabolic rate in tropical ectotherm insects at higher temperatures, directly reduced discretionary energy for reproduction (Dillon et al. 2010).

In the present study, females had three immature instars whereas; male had two immature instars and a pupation period. Similarly, Hodgson et al. (2008) reported three nymphal stages for female *P. solenopsis* and two nymphal stages and a pre-pupal stage for male *P. solenopsis*. In the present study, nymphal survival rate for 1st instar was similar at all temperature, it decreased significantly at higher temperatures (35°C and 40 °C). Nymphal survival rate for male and female *P. solenopsis* instars was different, where survival rate decreased only at higher temperature (40°C) for 3rd instar female while it decreased significantly both at lower (20 °C) and higher temperatures (40°C) for male prepupal and pupal instars (see Fig. 5). This decrease in survival rate was may be due to the increase in VPD (vapor pressure deficient) at high temperature. The survival percentage for all the immature stages of male and female *P. solenopsis* was higher at 30 °C. High temperature (40 °C) significantly decreased the survival percentage of all the nymphal stages for both sexes. The apparent mortality of *P. solenopsis* was also greater at higher temperature (40 °C; Fig. 6), which was may be due to the heat stress caused by high temperature (Wang et al. 2010).

In insects, several factors experienced by females including temperature can influence the sex allocation (Ross et al. 2011). Temperature affects the sex allocation by female parasitoids, whereas, low temperature affects the reproduction, leading to production of more males (Hance et al. 2007). In the present study, temperature directly influenced the sex ratio of parasitoids, where female produced more males at extreme temperatures (20 °C and 40 °C) which was may be due to the direct impact of extreme temperatures on endosymbionts that influences sex ratio deposition in parasitoids (Hance et al. 2007). Similar results were reported for *Bracon brevicornis* reared on different hosts at different temperatures, where life cycle of parasitoid prolonged at lowest temperature (20 °C) and recorded shortened life cycle at higher temperature (35 °C). *Bracon brevicornis* female produced more males at lower (20 °C) and higher temperature (35 °C) (Thanavendan and Jeyarani 2010).

Temperature directly influenced the development rate, longevity and fecundity of different species of mealybugs including *P. solenopsis* (Lema and Herren 1985; Chong et al. 2003; Amarasekare et al. 2008; Kim et al. 2008; Lu et al. 2011) and mealybug parasitoid (Chong and Oetting 2006). In the present study, (*P. solenopsis*) invasive pest and its native parasitoid (*A. bambawalei*) developed faster at higher temperature range (35 to 40 °C) but their performance was lower on higher temperature range. With rapid development, enormous reproductive capacity and high survival rate of *P. solenopsis* at 30 °C could result in significant economic damage to the crops within a short period of time. Therefore, it is necessary to understand the life cycle of *P. solenopsis* and its parasitoid to combat the pest population before reaching to economic threshold level.

In the present study, invasive alien pest (*P. solenopsis*) and its parasitoid (*A. bambawalei*) were negatively affected by extreme temperatures. These results were similar with the findings of Hance et al. (2007), where extreme temperature had negative impact on insect pests and their parasitoids. The native parasitoid performed (percentage parasitism) better on a wider range of temperatures (30 °C and 35°C) as compared to the invasive insect pest. These differences in sensitivity may rise due to the better adaptation of native parasitoid (*A. bambawalei*) to a wider range of temperature than the invasive *P. solenopsis*. This better adaptation of parasitoid indicated the important role of *A. bambawalei* in the control of *P. solenopsis* during endemic period in Pakistan.

The present study demonstrates that the intermediate temperature had an important role in causing increases in population dynamics of native and invasive species, but the intermediate temperature range of native parasitoid species was greater compared with invasive species. Thus management practices regarding the existence of alien pest species need comprehensive estimation of fluctuating climatic conditions for native natural enemies in multitrophic interactions.

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References

1. Abbas G, Arif M.J, Saeed S (2005). Systematic status of new species of genus Phenacoccus cockerell (Pseudococcidae), a serious pest of cotton Gossypium hirsutum L. in Pakistan. Pak Entomol 27: 83-84.
2. Abbas G, Arif M.J, Ashfaq M., Aslam M, Saeed S (2010). Host plants distribution and overwintering of cotton mealybug (Phenacoccus solenopsis; Hemiptera: Pseudococcidae). Int J Agric Biol, 12: 421-425.
3. Amarasekare KG, Chong JH, Epsky ND, Mannion CM (2008). Effect of emperature on the life history of the mealybug Paracoccus marginatus (Hemiptera: Pseudococcidae). J Econ Entomol 101: 1798-1804.
4. Arif MI, Rafiq M Ghaffar A (2009). Host plants of cotton mealybug (Phenacoccus solenopsis): a new menace to cotton agroecosystem of Punjab, Pakistan. International J Agric Biol 11: 163-167.
5. Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biol 8: 1-16.
6. Birch LC (1948) The intrinsic rate of natural increase of insect population. Journal of Animal Ecol 17: 15-26
7. Bjorkman C, Kindvall O, Hoglund S, Lilja A, Barring L, Eklund K (2011). High temperature triggers latent variation among individuals: oviposition rate and probability for outbreaks. PLoS ONE 6.
8. Bodlah I, Ahmad M, Nasir MF, Naeem M. (2010). Record of Aenasius bambawalei Hayat, 2009 (Hymenoptera: Encyrtidae), a parasitoid of Phenacoccus solenopsis (Sternorrhyncha: Pseudococcidae) from Punjab, Pakistan. Pak J Zool 42: 533-536.
9. Bonato O, Lurette A, Vidal C, Fargues J (2007). Modelling temperature-dependent bionomics of Bemisia tabaci (Q-biotype). Physiolog Entomol 32: 50-55.
10. Chong JH, Oetting RD, van Iersel MW (2003). Temperature effects on the development, survival, and reproduction of the Madeira mealybug, Phenacoccus madeirensis Green (Hemiptera : Pseudococcidae), on chrysanthemum. Annals of the Entomol Society of America 96: 539-543.
11. Chong JH, Oetting RD (2006). Influence of temperature and mating status on the development and fecundity of the mealybug parasitoid, Anagyrus sp nov nr. sinope Noyes and Menezes (Hymenoptera : Encyrtidae). Environmen Entomol 35: 1188-1197.
12. Clarke A (2003). Costs and consequences of evolutionary temperature adaptation. Trends in Ecology & Evolution 18: 573-581.
13. Crawley MJ (2005). Statistics an Introduction Using R. John Wiley & Sons Ltd., West Sussex.
14. Crawley MJ. (2007). The R Book. John Wiley & Sons Ltd., West Sussex.
15. Dangles O, Carpio,C, Barragan AR, Zeddam JL, Silvain JF (2008). Temperature as a key driver of ecological sorting among invasive pest species in the tropical Andes. Ecolog Applications 18: 1795-1809.
16. De Conti BF., Bueno VHP, Sampaio MV, van Lenteren JC (2011). Biological parameters and thermal requirements of the parasitoid Praon volucre (Hymenoptera: Braconidae) with Macrosiphum euphorbiae (Hemiptera: Aphididae) as host. Biocontrol Sci Technol 21: 497-507.
17. Denlinger DL, Lee RE (1998). Physiology of cold sensitivity. Boulder, CO., Westwiew.
18. Dillon, M.E., Wang, G., and Huey, R.B. 2010. Global metabolic impacts of recent climate warming. Nature, 467: 704-706.
19. Fand BB, Gautam RD, Suroshe SS (2011). Suitability of various stages of mealybug, Phenacoccus solenopsis (Homoptera: Pseudococcidae) for development and survival of the solitary endoparasitoid, Aenasius bambawalei (Hymenoptera: Encyrtidae). Biocontrol Sci Technol 21: 51-55.
20. Gilbert N, Raworth DA (1996). Insects and temperature - A general theory. Canadian Entomol 128: 1-13.
21. Gould, JR, Elkinton JS (1990). Temperature-dependent growth of Cotesia melanoscela (Hymenoptera, Braconidae), a parasitoid of the gypsy moth (Lepidoptera, Lymantriidae). Environmental Entomol 19: 859-865.
22. Hance T, van Baaren J, Vernon P, Boivin G (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Rev Entomol 52: 107-126.
23. Hayat M (2009). Description of a new species of Aenasius Walker (Hymenoptera: Encyrtidae), parasitoid of the mealybug, Phenacoccus solenopsis Tinsley (Homoptera: Pseudococcidae) in India. Biosystematica 3.
24. Hespenheide HA (1991). Bionomics of leaf-mining insects. Ann Rev Entomol 36, 535-560.
25. Hodgson C, Abbas G, Arif MJ, Saeed S, Karar H (2008). Phenacoccus solenopsis Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. Zootaxa 1-35.
26. Hou YM, Weng ZQ (2010). Temperature-dependent development and life table parameters of Octodonta nipae (Coleoptera: Chrysomelidae). Environmental Entomol 39: 1676-1684.
27. Hughes GE, Owen E, Sterk G, Bale JS (2010). Thermal activity thresholds of the parasitic wasp Lysiphlebus testaceipes and its aphid prey: implications for the efficacy of biological control. Physiolog Entomol 35: 373-378.
28. Jandricic SE, Wraight SP, Bennett KC, Sanderson JP (2010). Developmental times and life table statistics of Aulacorthum solani (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models. Environment Entomol 39: 1631-1642.
29. Kim SC, Song J, Kim DS 2008). Effect of temperature on the development and fecundity of the Cryptic mealybug, Pseudococcus cryptus, in the laboratory. Journal of Asia-Pacific Entomol 11: 149-153.
30. Klapwijk MJ, Grobler BC, Ward K, Wheeler D, Lewis OT (2010). Influence of experimental warming and shading on host-parasitoid synchrony. Global Change Biol 16: 102-112.
31. Lee HS, Gillespie DR (2011). Life tables and development of Amblyseius swirskii (Acari: Phytoseiidae) at different temperatures. Experimen Appl Acarol 53: 17-27.
32. Lema KM, Herren HR (1985). The influence of constant temperature on population growth rates of the cassava mealybug, Phenacoccus manihoti. Entomologia Experimentalis et Applicata 38: 165-169.

33. Logan JA, Wollkind DJ, Hoyt SC, Tanigoshi LK (1976). Analytic model for description of temperature-dependent rate phenomena in arthropods. Environmen Entomol 5: 1133-1140.
34. Logan JD, Wolesensky W, Joern A (2006). Temperature-dependent phenology and predation in arthropod systems. Ecolog Model 196: 471-482.
35. Logan J.D, Wolesensky W (2007). Accounting for temperature in predator functional responses. Natural Res Model 20: 549-574.
36. Logan JD (2008). Phenologically-structured predator-prey dynamics with temperature dependence. Bull Math Biol 70: 1-20.
37. Lu Y, Guan X, Zeng L (2011). Effect of temperature on the development of the mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae). Sci Res Essays 6: 6459-6464.
38. Montoya JM, Raffaelli D (2010). Climate change, biotic interactions and ecosystem services. Philosophical Transactions of the Royal Society B: Biolog Sci, 365: 2013-2018.
39. Musolin DL, Tougou D, Fujisaki K (2010). Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug Nezara viridula (Heteroptera: Pentatomidae). Global Change Biol 16: 73-87.
40. Rice AD, Allen GR (2009). Temperature and developmental interactions in a multitrophic parasitoid guild. Australian J Entomol 48: 282-286.
41. Rizvi PQ, Ali A, Khalid S (2009). Age and stage specific life table of cabbage butterfly, Pieris brassicae L. (Lepidoptera: Pieridae) on various cole crops. J Plant Protec Res 48: 145-150.
42. Robinet C, Roques A (2010). Direct impacts of recent climate warming on insect populations. Integ Zool 5: 132-142.
43. Ross L, Dealey EJ, Beukeboom LW, Shuker DM (2011). Temperature, age of mating and starvation determine the role of maternal effects on sex allocation in the mealybug Planococcus citri. Behavioral Ecol Sociobiol 65: 909-919.
44. Shaver GR, Canadell J, Chapin FS, Gurevitch J, Harte J, Henry G, Ineson P, Jonasson S, Melillo J, Pitelka L, Rustad L (2000). Global warming and terrestrial ecosystems: A conceptual framework for analysis. Biosci 50: 871-882.
45. Tariq M, Staley JT, Wright DJ (2010). Maternal host plant effects on aphid performance: contrasts between a generalist and a specialist species on Brussels sprout cultivars. Agric Forest Entomol 12: 107-112.
46. Terblanche JS, Nyamukondiwa C, Kleynhans E (2010). Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (Ceratitis capitata). Entomolog Experiment et Applic 137: 304-315.
47. Thanavendan G, Jeyarani S (2010). Effect of different temperature regimes on the biology of Bracon brevicornis Wesmael (Braconidae: Hymenoptera) on different host larvae. J Biopesticides 3: 441-444.
48. Walther G, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobe, M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J (2009). Alien species in a warmer world: risks and opportunities. Trends in Ecology Evol 24: 686-693.
49. Wang YP, Watson GW, Zhang RZ (2010). The potential distribution of an invasive mealybug Phenacoccus solenopsis and its threat to cotton in Asia. Agricultural and Forest Entomol 12: 403-416.
50. Waterworth RA, Wright IM, Millar JG (2011). Reproductive biology of three cosmopolitan mealybug (Hemiptera: Pseudococcidae) species, Pseudococcus longispinus, Pseudococcus viburni, and Planococcus ficus. Annals of the Entomolog Soc Amer 104: 249-26.
51. Zhu Y, Huang F, Lu Y (2011). Bionomics of mealybug Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) on cotton. Acta Entomolog Sinica 54: 246-252.

Table 1. Age specific life table of *Phenacoccus solenopsis* at different temperatures reared on *Gossypium hirsutum*

Age (days) (x)	Individuals survive (l_x)	Individuals dying (d_x)	Percent mortality (100 q_x)	Age (days) (x)	Individuals survive (l_x)	Individuals dying (d_x)	Percent mortality (100 q_x)
20 °C				25 °C			
0	83	0	0.00	0	87	0	0.00
1-10	83	14	16.86	1-10	87	13	14.94
11-20	69	7	10.14	11-20	74	8	10.81
21-30	62	4	6.45	21-30	66	8	12.12
31-40	58	9	15.51	31-40	58	16	27.58
41-50	49	11	22.44	41-50	42	20	47.62
51-60	38	22	57.89	51-60	22	11	50
61-70	16	3	18.75	61-70	11	7	6.36
71-80	13	10	76.92	71-75	4	4	100
81-84	3	3	100				
30 °C				35 °C			

0	92	0	0.00	0	98	0	0.00
1-10	92	17	18.47	1-10	98	18	18.37
11-20	75	11	14.66	11-20	80	12	15
21-30	64	17	26.56	21-30	68	18	26.47
31-40	47	30	63.82	31-40	50	14	28
41-50	17	7	41.17	41-50	36	20	55.55
51-60	10	6	60.00	51-60	16	14	87.50
61-62	4	4	100.00	61	2	2	100
40 °C							
0	97	0	0				
1-10	97	21	21.65				
11-20	76	12	15.78				
21-30	64	10	15.62				
31-40	54	37	68.52				
41-50	17	12	70.59				
51-55	5	5	100.00				

Fig. 1. (a) immature male longevity (b) immature female longevity and (c) total nymphal longevity of *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

Fig. 2. Total longevity (birth to death) of male, virgin and mated female *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

Fig. 3. Pre-oviposition, oviposition and post-oviposition duration of *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

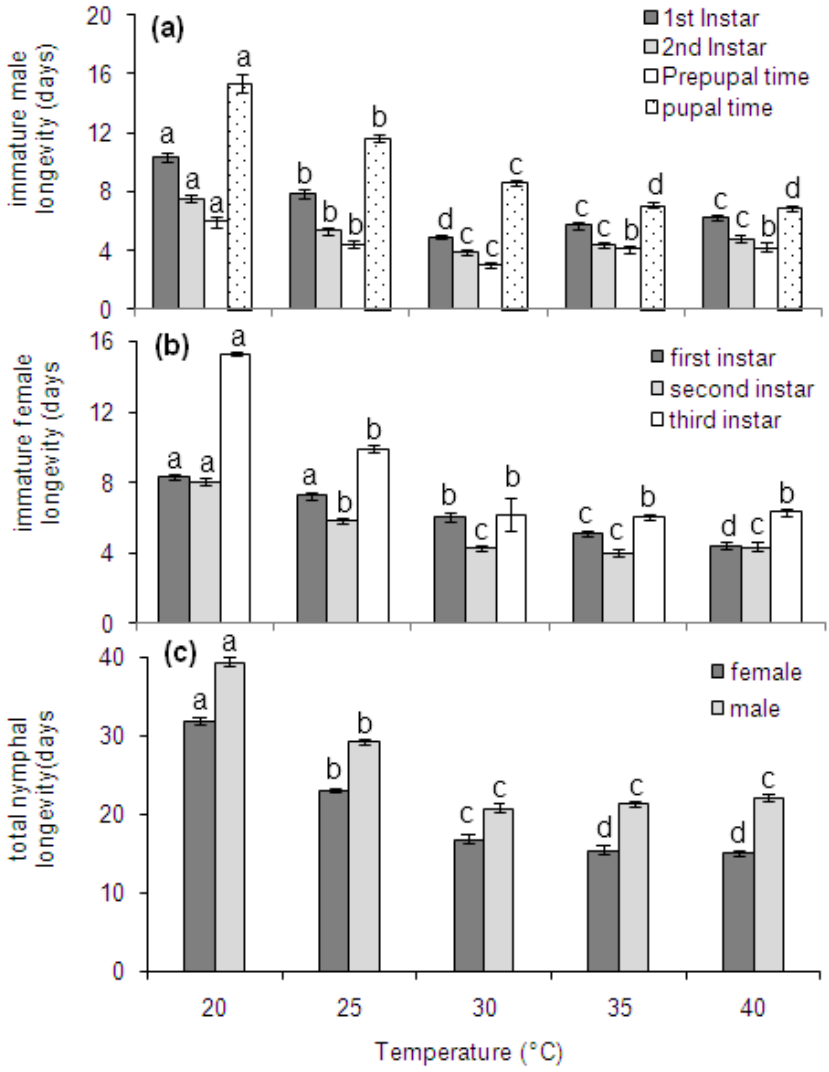
Fig. 4. Fecundity of *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

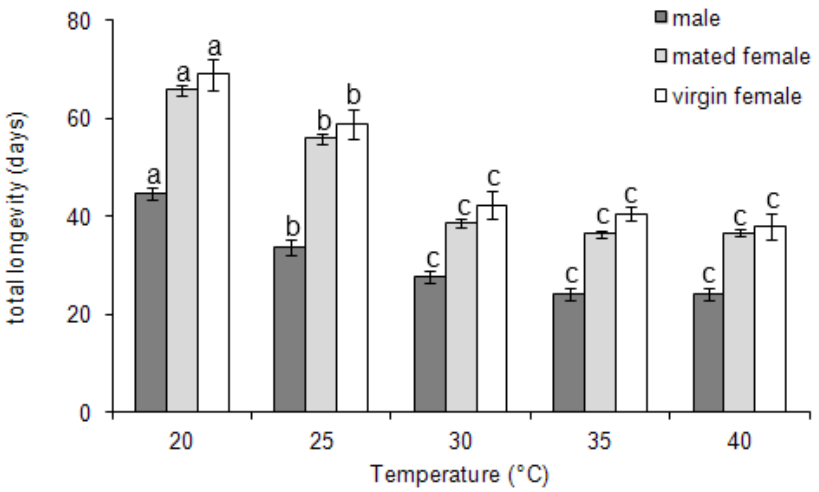
Fig. 5. Percent survival of immature stages of *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

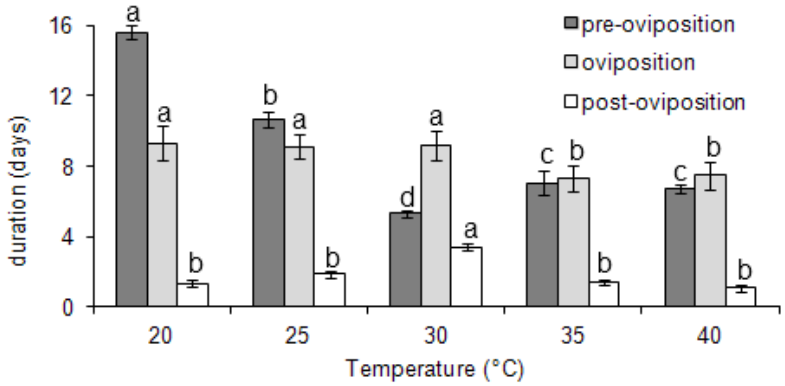
Fig. 6. Life expectancy of *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

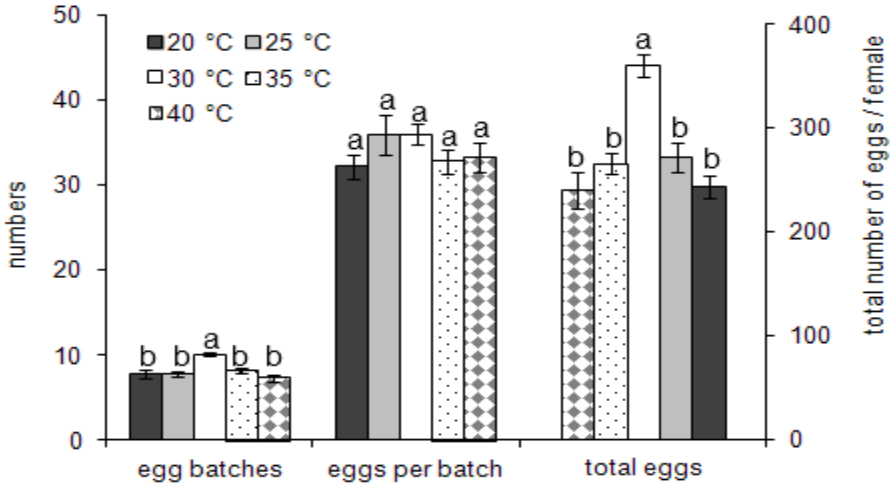
Fig. 7. (a) percent parasitism and percentage emergence (b) immature and adult longevity (days) of male and female *Aenasius bambawalei* (c) total longevity (days) of male and female *Aenasius bambawalei* and (d) sex ratio of *Aenasius bambawalei* (mean±S.E.M.) developed on *Phenacoccus solenopsis* (mean±S.E.M.) at different temperatures reared on *Gossypium hirsutum*. Within each

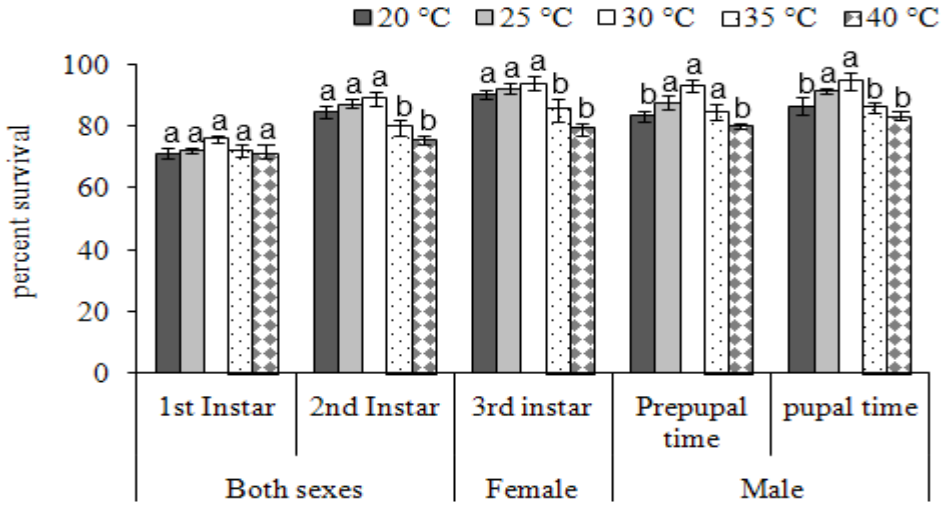
parameter, means sharing similar letters along the column are non-significantly different (ANOVA; Tukey's HSD; $P < 0.05$).

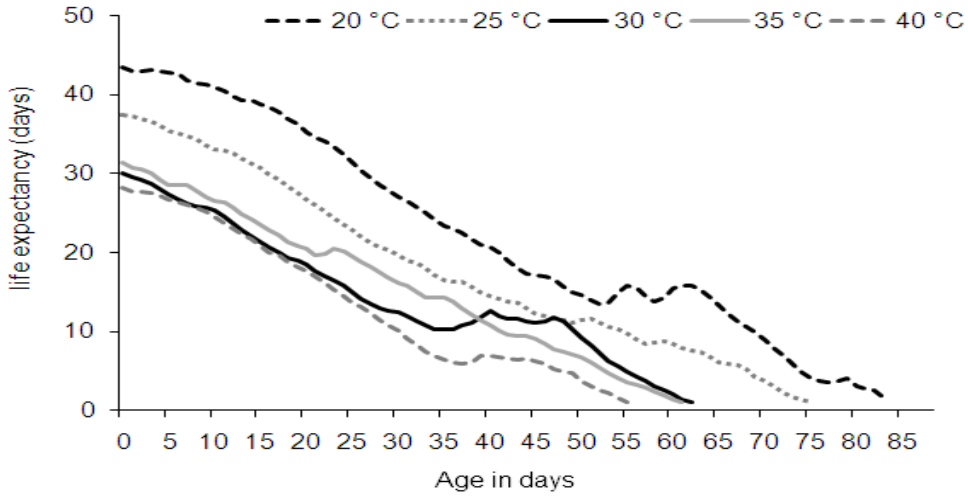


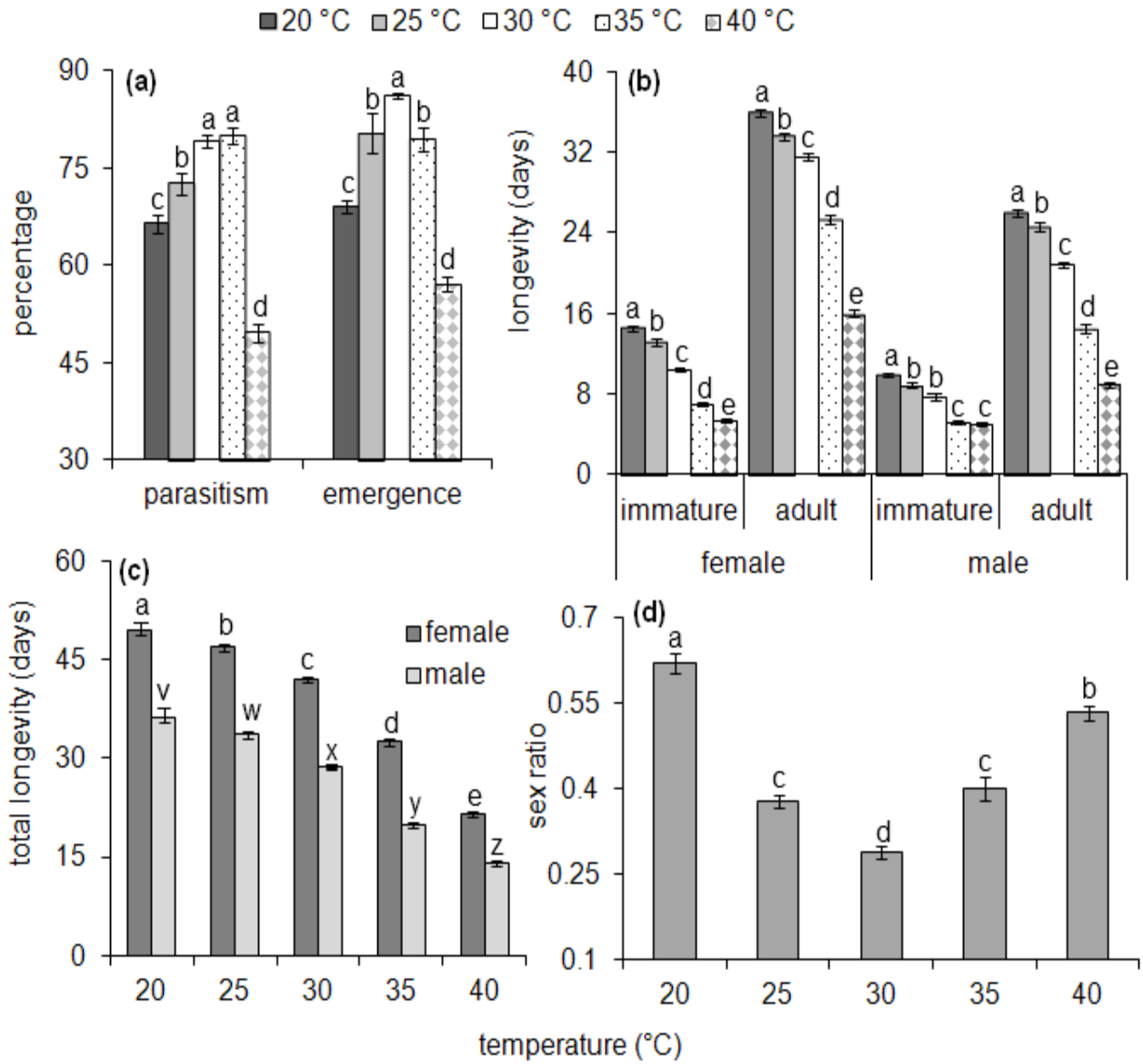

male
mated female
virgin female
total longevity (days)
Temperature (°C)
20
25
30
35
40


pre-oviposition
oviposition
post-oviposition
duration (days)
Temperature (°C)


20 °C
25 °C
30 °C
35 °C
40 °C
numbers
total number of eggs / female
egg batches
eggs per batch
total eggs


20 °C
25 °C
30 °C
35 °C
40 °C
percent survival
100
80
60
40
20
0
1st Instar
2nd Instar
3rd instar
Prepupal time
pupal time
Both sexes
Female
Male


20 °C
25 °C
30 °C
35 °C
40 °C
life expectancy (days)
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Age in days


20 °C
25 °C
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35 °C
40 °C
(a)
percentage
parasitism
emergence
(b)
longevity (days)
immature
adult
immature
adult
female
male
(c)
total longevity (days)
female
male
(d)
sex ratio
temperature (°C)

71

Is Cotton Leaf Curl Virus Vectored by *Bemisia tabaci* sex-biased?

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Abstract

***Background**: Bemisia tabaci is an important pest of numerous food and fibre crops across the globe causing direct damage through feeding as well as by transmitting a number of begomoviruses. Among the transmitted viruses Cotton Leaf Curl Virus is one of the most serious threats to cotton in northern India as well as neighboring countries Pakistan and Bangladesh. Due to high recombination efficiency, the virus has highly evolved over time into five major species such as Cotton leaf curl Kokharan virus (CLCuKoV), Cotton leaf curl Multan virus (CLCuMuV), Cotton leaf curl Allahabad virus (CLCuAlV); Cotton leaf curl Bangalore virus (CLCuBaV) and Cotton leaf curl Gezira virus (CLCuGeV). On the other hand, the vector B. tabaci also exists as a cryptic species complex consisting of many genetic groups also known as haplotypes, biotypes, strains etc. The vector competence of these genetic groups is variable with the one present in north India i.e. Asia-II-1 being a highly competent vector compared to other haplotypes and this has been attributed as one of the reasons for the prevalence of Cotton leaf Curl Disease (CLCuD) in north India compared to rest of cotton growing Indian region. It has been quoted many times in literature that female Bemisia tabaci is the efficient transmitter of compared to male whitefly, however the sex-biased transmission efficiency is still not clearly understood. The present studies report some of the possible reasons for sex-biased transmission of CLCuV vectored by B. tabaci*

***Results**: The present study with B. tabaci AsiaII-1 haplotype showed higher virus transmission efficiency of females compared to males. This variable begomovirus transmission efficiency has been related to previously identified key factors associated with B. tabaci. The higher density of endosymbiont Arsenophonus and variable expression of some midgut proteins genes i.e. Cyclophilin, Knottin, Hsp40, Hsp70 may be possibly imparting higher vector competency to the females compared to males. The present studies suggest low abundance of Arsenophonus spp.as well as lower expression of Cyclophilin gene in males as compared to females. This is further supplemented by overexpression of Knottin, Hsp40, and Hsp70 genes in males compared to females and thus collectively all these factors might be playing a key role in low virus transmission efficiency of males.*
Conclusions: The relative density of Arsenophonus spp. and expression of midgut proteins genes in male and female whitefly enriches our understanding of the sex-biased transmission efficiency of B. tabaci vectored begomovirus.

***Key words**: Bemisia tabaci, Asia II-1, CLCuV, sex-biased transmission, gut protein genes, endosymbionts*

Introduction

Insects are the most important biological vectors which account for the spread of more than 75% of plant viruses. Among insects sucking pests (Hemipterans) are the most common vectors for plant viruses which include whiteflies, aphids, plant hoppers, and leafhoppers. The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) emerged as the most important virus vector capable of transmitting more than four hundred plant viruses across the globe (1). The genera of plant viruses vectored by whiteflies include Begomoviruses (*Geminiviridae*), criniviruses (*Closteroviridae*), torrado viruses (*Secoviridae*), and ipomoviruses (*Potyviridae*). In recent years, the most substantial emergence of begomoviruses has been observed in cotton, cucurbits, okra, ornamental plants, and even weeds. Begomoviruses are economically important phytopathogens (Family Geminiviridae) comprising the largest group of plant viruses that cause damage to numerous crops across the globe. Cotton Leaf Curl Virus (CLCuV) belonging to genera Begomoviruses is highly destructive and poses a major threat to the fiber crops in north-Indian cotton-growing states by causing Cotton Leaf Curl Virus Disease (CLCuD) (2, 3) .

B. tabaci mediates the transmission of Begomoviruses in a persistent- circulative manner during which the virus breaches barriers in the digestive, hemolymph, and salivary systems, and interacts with insect proteins along the transmission pathway (4). After viral particles are released from the midgut to the hemolymph, they can be recognized, targeted and cleared by the host immune system. Therefore, the survival of virions within the hemolymph is vital for the systemic dissemination of persistent viruses before entry into salivary glands (5). The chaperone proteins of insect endosymbionts are involved in the maintenance of plant viruses in the hemolymph. Therefore, endosymbionts of whiteflies are involved in acquisition, retention, and transmission of whiteflies (6, 7).

Previous studies have evidenced that persistent transmission of Begomoviruses by *B. tabaci* vector involves the interaction of viral coat protein with insect-specific genes *Hsp70, Hsp40, Cyclophilin*, and *Knottin*. Research progress has been made for functional validation of these genes for regulating virus titer inside the whiteflies and their impact on virus transmission efficiency (8, 9). This piece of work has generated information on vector components that impart competence to female whiteflies for virus transmission. Future research needs to discover some more components associated with insect vectors to underpin the mechanism of the difference in CLCuV transmission by different sexes of *B. tabaci.*

Result and Discussion

Sex-biased differential transmission of (CLCuV) has been observed implicating females as efficient vectors compared to male whiteflies. Female whiteflies were observed to retain a high viral load (80%) when compared to males (Fig 1A). This is further supported by comparative viral transmission mediated by the release of female and male whiteflies. The results showed that female inoculated plants showed prominent cotton leaf curl disease (CLCuD) symptoms such as vein thickening and downward curling. However, no visible symptoms has been observed in male inoculated plants after 14 DPI (Days Post Inoculation) (Fig 1B). Quantification of viral load in cotton plants showed 74.3% higher viral inoculum in female inoculated plants compared to the male inoculated ones (Fig 1C). Our results clearly indicate that transmission of CLCuV occurs in a sex-dependant manner. Due to significantly higher retention and transmission of CLCuV female whiteflies are more efficient vectors than that of males. The high viral retention ability could be attributed to more feeding capacity of females than males (9).

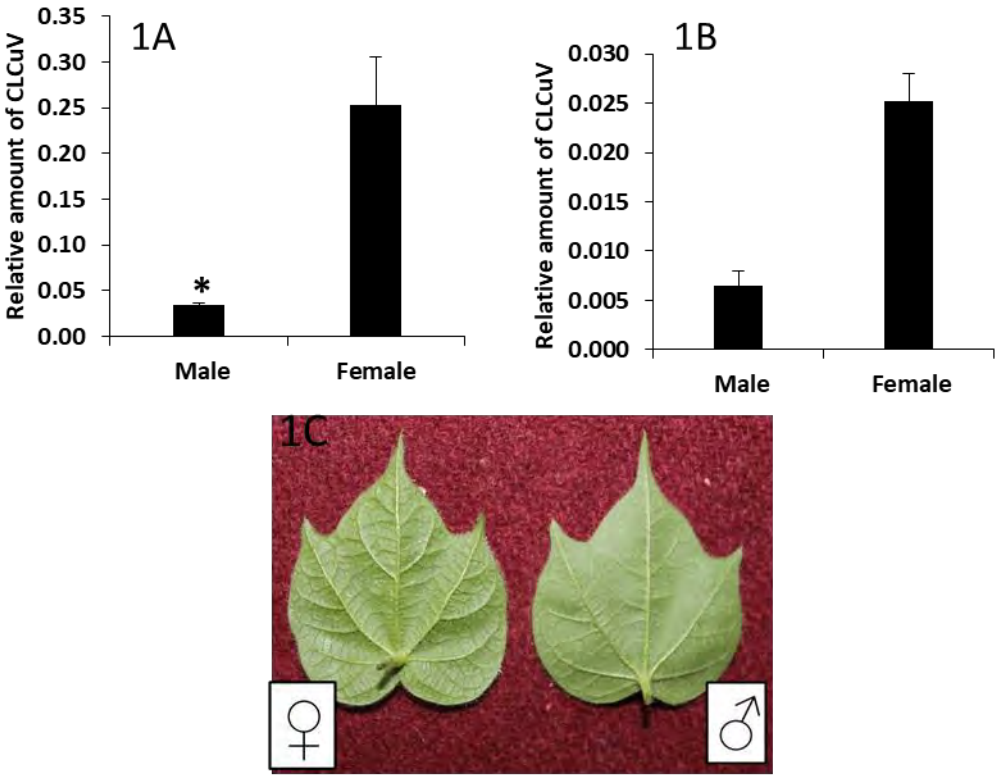

*Fig.1 Relative amount of CLCuV, A. Viruliferous B. tabaci male and 5 females, D. n=20 males and 20 females; C. Vein thickening in the cotton plant after inoculation with viruliferous male and female whiteflies. * Significant differences (P ≤ 0.05, Student's t-test).*

The high viral retention ability of females was further supported by the high infection status of endosymbiont Arsenophonus in non-viruliferous (55.9%) and viruliferous females (76.1%) when compared to the makes. Tetracycline-mediated (100 µg/ ml of diet) elimination of Arsenophonus in male and female whiteflies led to 74.9% and 54.3% reduction in viral load in Arsenophonus cured females and males. Arsenophonus cured females and males showed 76.7 % and 68.4 % downregulation in expression of GroEL when compared to control. Our previous study also implicated the role of Arsenophonus specific GroEL in regulating virus titer and its transmission by vector B. tabaci (10). GroEL belong to class of heat shock proteins which are involved in folding of damaged proteins. Previous studies has reported that endosymbiont associated GroEL safeguards the viral particles during its circulatory pathway to host plant from the enzymatic attack inside the haemolymph of whitefly (11). The validation of expression of midgut genes showed that viruliferous female whiteflies express more Cyclophilin when compared to males. The expression of Cyclophilin in viruliferous females was significantly higher (57.75%) compared to viruliferous males. The dsRNA (500 ng dsRNA/ µl of sucrose diet) mediated knockdown of Cyclophilin, led to a 48.3% and 19% reduction in female and male viral titer (Table 1). Cyclophilin are mainly involved in refolding of virus particle, facilitate vector-virus interaction, and aids in protective viral movement in whiteflies (12). The higher expression of cyclophilin in correlation with high retention of virus in female whiteflies as compared to males suggests that it also acts as a key regulator of CLCuV transmission in a sex-dependent manner.

In contrast to Cyclophilin relative expression of Hsp 40, Hsp70 and Knottin were significantly higher in males as compared to females. In viruliferous female whiteflies, silencing of Knottin resulted in a significant increase (80.7%) in viral load when compared to males. Knockdown of Knottin in males showed 20.7% more viral transcripts when compared to control GFP-fed males. Knottins belongs to structural family of proteins which are involved in regulating the acquisition and transmission of the Begomoviruses by limiting the amount of virus ingested by whiteflies (13). Thus the lower expression of Knottin in females may not restrain them from acquisition of virus from infected plants and they tend to acquire and transmit more viral particles and thus proven as more efficient vectors compared to males. In a similar scenario,non-viruliferous and viruliferous male whitefly adults express 82.05 and 61.61% significantly more Hsp40 compared to females. Similarly, the relative expression Hsp70 in the non-viruliferous and viruliferous males was 55.19% and 58.28% higher than females. Viruliferous female whiteflies with silenced Hsp70 and Hsp40 exhibited an increase in viral transcripts compared to control GFP-fed female flies. Heat shock proteins Hsp's are the constitutively expressed proteins, which are involved in maintaining the virus to a level that is not able to cause any harmful impact on longevity and fecundity of host whiteflies. Lower expression of Hsp 70 and Hsp40 in female suggest that large amount of virus particle may escape their binding with Hsp's causing enhanced CLCuv transmission rates. However, in males due to higher expression of these Hsp's may result in trapping of viral particles and causing their degradation in the host through Hsp-virus binding.

Table1. Comparative relative gene expression and impact of midgut genes and *Arsenophonus* in *Bemisia tabaci*

Whitefly associated Protein / genes	Fold change in expression in females comparison to males	Functional validation	Impact on viral load
Arsenophonus endosymbiont (23SrRNA)	3 fold increased	Sucrose infused - tetracycline (90ug/ml)	Decreased significantly in females
Hsp70	6 fold decreased	Sucrose fed-dsRNA	Increased significantly in females
Hsp40	5.7 fold decreased	Sucrose fed-dsRNA	Increased significantly in females
Knottin	12 fold decreased	Sucrose fed-dsRNA	Increased significantly in females
Cycloph	6.7 fold Increased	Sucrose fed-dsRNA	Decreased significantly in females

Conclusions:

The studies clearly suggest that the CLCuV virus vectored by Asia-II-1 B. tabaci is transmitted in a sex-biased manner with females being more efficient than the males. Some of the attributes which support this study are a large size and more feeding of females than males. Besides these, variable expression of some gut protein genes in males and females might be playing a key role in the differential transmission efficiency of the whitefly sexes. The higher density endosymbiont of Arsenophonus in females compared to male whiteflies may be playing a key role for the higher transmission efficiency of females than males. The higher expression of Arsenophonus produced GroEL protein gene in females implicates its role in protecting the virion particles in females compared to males and disrupting GroEL-virus interaction may be an element of sustainable control of whitefly as a vector in the future.

Materials and methods

Whitefly was maintained in walk-in environmental chambers on *Gossypium hirsutum* cultivar RST9. Total RNA was isolated from male and female whiteflies using TRI Reagent® RNA (Sigma-Aldrich, Inc.) as per the manufacturer's instructions which was further used (1µg) for reverse transcription using Primescript First-strand cDNA synthesis kit (Takara). Quantitative real-time PCR was used to study the differential retention of CLCuV in context to virus coat protein (CP) gene, relative expression of midgut protein genes such as *Cyclophilin, Hsp70, Hsp40,* and *Knottin,* and density of *Arsenophonus* spp. in male and female whiteflies on a Light cycler System (Roche Life Sciences, Mannheim, Germany) with SYBR® Premix Ex TaqTMII (Takara). The relative virus titer based on CP gene, expression of gut protein genes and *Arsenophonus* density was inferred using the $2^{-\Delta\Delta Ct}$ method. The virus coat protein-specific primers qCLCuV_ Forward-CGTCGACCTGTTGATAAACCTC and qCLCuV_Reverse-GCATATTGACCA CCGGTAACAG were used in the study for quantifying viral copies.

For understanding sex-biased transmission of CLCuV equal males and females were released separately on virus-free cotton plants for 48h followed by removal of whiteflies and observation of symptoms. At the appearance of early symptoms, total RNA was extracted using TRI Reagent® RNA Isolation Reagent (Sigma-Aldrich, Inc.) from the male and female inoculated plants and was reverse transcribed to cDNA as per the earlier described methodology. The expression of the virus coat protein gene in the host plant was quantified using RT-PCR and the expression was normalized by the *histone 3* gene of cotton.

For relative expression of midgut protein genes (*Cyclophilin, Hsp70, Hsp40,* and *Knottin*) and *Arsenophonus* spp. density, ten individual viruliferous and non-viruliferous male and female whiteflies were processed for total RNA isolation followed by RT-PCR as described in an earlier section. For *Arsenophonus* density based on the relative expression of the *23SrRNA* gene of the bacterium was quantified using quantitative RT-PCR in both viruliferous and non-viruliferous males and females. The expression data in all cases were normalized using *B. tabaci -β tubulin* as a housekeeping gene.

For functional validation of midgut genes the dsRNA-mediated gene silencing was carried out followed by an estimation of the impact of each gene on viral titer in each sex. The viruliferous adult male and female whiteflies were given feeding access to dsRNA (400 ng/ ul of diet) incorporated in 20% sucrose diet against gut protein genes along with GFP control. Total RNA was isolated after 48 h post-feeding and relative expression of *Hsp70, Hsp40, Knottin* and *Cyclophilin* was estimated in both viruliferous and non-viruliferous male and female whiteflies followed by relative gene expression of viral coat protein in both male and female whiteflies. *Arsenophonus*. was eliminated using tetracycline 90 µg/ml incorporated in sucrose diet solution followed by comparative estimation of viral load in both female *Arsenophonus-* and male adults *(Arsenophonus-)* using qRT-PCR detection as per earlier description.

Acknowledgments

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References

1. Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S. Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol. 2011; 49: 219–48.
2. Singh D, Gill JS, Gumber RK, Singh R, Singh S. Yield and fibre quality associated with cotton leaf curl disease of Bt-cotton in Punjab. Journal of Environmental Biology. 2013; 34:113–6.
3. Gupta VK, Sharma R, Singh S, Jindal J, Dilawari VK. Efficiency of Bemisia tabaci (Gennadius) populations from different plant-hosts for acquisition and transmission of cotton leaf curl virus. Indian Journal of Biotechnology. 2010; 9: 271–5.

4. Rosen R, Kanakala S, Kliot A, Cathrin Pakkianathan B, Farich BA, Santana-Magal N, et al. Persistent, circulative transmission of begomoviruses by whitefly vectors. Curr Opin Virol. 2015; 15:1–8.
5. Ghanim M. A review of the mechanisms and components that determine the transmission efficiency of Tomato yellow leaf curl virus (Geminiviridae; Begomovirus) by its whitefly vector. Virus Res. 2014 Jun 24; 186:47–54.
6. Kliot A, Cilia M, Czosnek H, Ghanim M. Implication of the bacterial endosymbiont Rickettsia spp. in interactions of the whitefly Bemisia tabaci with tomato yellow leaf curl virus. J Virol. 2014; 88:5652–60.
7. Su Q, Pan H, Liu B, Chu D, Xie W, Wu Q, et al. Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus. Sci Rep. 2013; 3:1367.
8. Kaur R, Gupta M, Singh S, Joshi N, Sharma A. Enhancing RNAi Efficiency to Decipher the Functional Response of Potential Genes in Bemisia tabaci AsiaII-1 (Gennadius) Through dsRNA Feeding Assays. Front Physiol. 2020 Mar 2; 11:123.
9. Singh I, Kaur R, Kumar A, Singh S, Sharma A. Differential expression of gut protein genes and population density of Arsenophonus contributes to sex-biased transmission of Bemisia tabaci vectored Cotton leaf curl virus. PLoS ONE. 2021; 16: e0259374.
10. Kaur R, Singh S, Joshi N. Pervasive Endosymbiont Arsenophonus Plays a Key Role in the Transmission of Cotton Leaf Curl Virus Vectored by Asia II-1 Genetic Group of Bemisia tabaci. Environ Entomol. 2022; 51 (3):564–77.
11. Rana VS, Singh ST, Priya NG, Kumar J, Rajagopal R. Arsenophonus GroEL interacts with CLCuV and is localized in midgut and salivary gland of whitefly B. tabaci. PLoS ONE. 2012; 7(8):e42168.
12. Kanakala S, Ghanim M. Implication of the Whitefly Bemisia tabaci Cyclophilin B Protein in the Transmission of Tomato yellow leaf curl virus. Front Plant Sci. 2016; 7:1702.
13. Hariton Shalev A, Sobol I, Ghanim M, Liu S-S, Czosnek H. The Whitefly Bemisia tabaci Knottin-1 Gene Is Implicated in Regulating the Quantity of Tomato Yellow Leaf Curl Virus Ingested and Transmitted by the Insect. Viruses. 2016; 8: 205.

Boeremia exigua leaf spot; a new emerging threat to *Gossypium hirsutum L.*

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Abstract

***Background**: Cotton (*Gossypium hirsutum L.*) is a cash crop all over the world including Pakistan. Severe foliar disease symptoms were observed on cotton fields from different districts of cotton zones of province Punjab, Pakistan. Infected plant leaves were sampled from different area and processed for the isolation of causal organism. Fungal isolates identified morphologically and conidia of the fungus observed oblong to ellipsoid, hyaline, aseptate, but occasionally 1-septate with dimensions ranging from 2.7 to 7.8 × 1.6 to 3.5 µm.*

***Results**: Both isolates showed moderate reactions to the NaOH test for E metabolite. The pathogen is morphologically identified as Boeremia exigua. Further, internal transcribed spacer region (ITS) and TUB gene of the fungal isolates were amplified. The sequence of partial ITS and TUB showed 99% homology with isolates of B. exigua. The pathogenicity test was performed at three months old cotton plants by using two isolates of B. exigua. After 15 days of inoculation, necrotic spots start developing on the leaves that were similar to those observed in the field. The fungal pathogen was re-isolated from the leaves of all inoculated plants, identified morphologically and satisfying Koch's postulates.*

***Conclusion**: This disease might be a potential threat to cotton production in Pakistan. However further studies required to know the behavior, alternate hosts and spreading nature of the pathogen.*

***Keywords**: Leafspot, Cotton, Fungal Pathogen, Fungal characterization, Phylogenetic analysis*

Introduction

Cotton (*Gossypium hirsutum* L.) is the world's second most important oil seed crop which belongs to genus *Gossypium* species malvacae. (Farooq et al., 2014; Azhar et al., 2013; Ali et al., 2010). It is a prime source of fiber and plays important role in socio-economic progress of many countries as more than 20 million farmers cultivate it as their main cash crop globally (Khan et al., 2022). It is also the major cash crop of Pakistan with second most important in terms of area after wheat and being a cash crop, plays significant role in country's exports and foreign exchange (SMEDA, 2010). Annually, about 3 million hectares has been under-cultivation throughout Pakistan (Cororaton et al., 2008). It stands vital in agriculture as well as textile sector of the economy. It contributes around 0.6 percent to GDP and 3.1 percent of the value added in agriculture. During 2020-21, the crop was cultivated on 2,079 thousand hectares (GoP, 2021-22). Pakistan ranked fourth in cotton production after China, USA and India. Cotton is the chief source like fiber, feed and edible oil (Paytas and Tarrago, 2012; Brown et al., 2022; Abdullah et al., 2022). It is hot area crop required water after succession of time for all stages of growth (Revathi. 2012). Cotton is attacked by many insects and abiotic factors as well as viral, fungal and bacterial diseases which ultimately affect the yield. (Kumar et al., 2021). The most affected part of cotton is the leaf which includes about 80% of disease and the most destructive disease on foliar is leaf spots caused by fungi (Revathi, P & Hemalatha 2014).

Boeremia exigua (Desm.) Aveskamp, Gruyter &Verkley (basionym: Phoma exigua Desm.) (Aveskamp et al. 2010) has a worldwide distribution in agriculture, horticulture and forestry (Boerema et al. 2004). Taxonomically B. exigua is considered to be an assemblage of over ten varieties (Aveskamp et al. 2010; Berner et al. 2015). Thus, morphological identification is often supported by molecular methods (Kowalski et al., 2019) Several identification methods of Boeremia/Phoma species and varieties, are

based on gene or sets of gene sequencing such as ITS and β-tubulin gene (Aveskamp et al. 2009, 2010; Berner et al. 2015).

In the recent study, irregular wide ranged leafspot on cotton leaves were observed in different cotton growing areas. Further, morphological and molecular characterization of causal organism were studied to know the potential causal organism and its epidemiology. It is initial study regarding this new disease and required further deep study to know the nature and epidemic potential of this disease.

Materials and Methods

Field Survey and Sample collection

During June, 2018 a field survey was conducted in the fields of cotton zone. Total six districts of Punjab were scouted for leaf spot. It was observed that a leaf spot of different pattern (Fig 1) is present in Layyah, Muzaffargarh and Multan districts in Punjab province of Pakistan. However, disease incidence was zero in the areas of Vehari, Sahiwal and Bahawalpur. Disease incidence percentage (Noordzij et al., 2010) was calculated by using following formula;

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of plants examined}} \times 100$$

The cotton fields in affected areas were severely affected by foliar disease. The infected leaves were collected and processed for the isolation and identification of fungal pathogen causing cotton leaf spot. Infected leaf samples were placed into sampling bags and were properly labeled. After that, all the collected samples were carried to the laboratory at Institute of Agricultural Sciences (IAGS), University of the Punjab, Lahore, Pakistan and stored in refrigerator at 4 0C till the next procedure.

Isolation, purification and molecular characterization of fungi

Infected leaf samples were cut into small pieces and infected parts were taken. Excised leaf samples were surface sterilized with 1 % sodium hypochlorite for one minute, followed by washing with sterilized autoclaved distilled water (Topuz et al., 2016). Pieces of necrotic leaves were inoculated on Malt Extract Agar (MEA) media and incubated at 25±2ºC for one week. Fungal isolates were identified by recording the colony characters and the presence of E metabolites (Aveskamp et al., 2010). Total genomic DNA of B. exigua isolates was extracted using modified CTAB method to amplify the ITS region of ribosomal DNA (including ITS1, 5.8S and ITS2) (White et al., 1990) and the β-tubulin (TUB) genes of the fungal isolates, using the following PCR primers: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3′), TUB: BT2A (5'-GGTAACCAAATCGGTGCTGCTTTC-3′) and BT2B (5'-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Samson et al., 2004). PCR reactions were performed in 50 µL of reaction mixture consisting of the following composition: and 50 ng of template DNA. The PCR reactions were performed in 50 µL of a reaction mixture consisting of the following composition: master mixture 25 µL (PCR buffer 1×, MgCl2 2 mM, dNTP 0.2 mM each, DNA Taq polymerase 1 U (Thermo Fisher Scientific, Waltham, MA, USA), primers 1 µL each, and 50 ng of template DNA. Amplification of DNA was done in a thermal cycler (T100TM Thermal Cycler, BIO-RAD, Singapore) by using the following temperature profile: initial denaturation for 5 min at 95 °C, followed by 40 cycles of denaturation for 45 s at 95 °C, annealing at 60 °C (ITS) and 62 °C (TUB) for 45 s, with 10 min final extension at 72 °C and tempered at 4 °C for sample restoration. The length of amplified DNA fragments and efficacy of PCR was verified by 1% agarose gel electrophoresis. The purification and sequencing of amplified PCR products using ITS1 and BT2A primers was carried out by Molecular and Cellular Imaging Center (MCIC), Illumina MiSeq Sequencing, USA. Sequences were edited and identified by searching the most similar sequences in NCBI GenBank and by using Mega-X the phylogenetic trees were constructed.

Pathogenicity Test

The pathogenicity test was performed at three months old cotton plants by using two isolates of B. exigua. Aqueous conidial suspension (1.5 × 105 conidia mL-1) was harvested from 15 day-old cultures grown on MEA and the leaves were inoculated with conidial suspension. The control plants were inoculated with sterile distilled water. Three replicates for each isolate were used in a duplicated experiment. All inoculated plants were covered with plastic bags to maintain the humidity for 48 hours and placed in a greenhouse at 25 to 30 °C. After 15 days of inoculation.

Results and discussion

Disease Incidence

In different cotton fields; surveys were carried out to observe disease incidence, including Layyah, Muzaffargarh, Multan, Vehari, Sahiwal and Bahawalpur. Disease incidence recorded in these areas given in the following Table 1.

Table 1. Various areas and their recorded disease incidence.

No of Obs	Area Observed	Disease Incidence(%)
1	Layyah	34
2	Muzaffargarh	28
3	Multan	30
4	Vehari	10
5	Sahiwal	16
6	Bahawalpur	22

Morphological characterization of *Boeremia exigua*

Small necrotic spots surrounded by chlorotic haloes were observed on the upper side of infected leaves of cotton field. The necrotic spots were expanded into large areas of 3 to 8 cm in diameter which was distinguished by a brown margin and severely infected leaves became chlorotic and abscessed (Plate 1). After 7 days of incubation on malt extract agar, fungal cultures varied in color with olivaceous black in centers with soft and aerial mycelia. Ascomata was pseudothecial, sub globose. Asci were 8-spored, biseriate cylindrical or subclavate. *Conidiomata* pycnidial, variable in shape and size, mostly globose to sub globose, superficial or immersed into agar, solitary or confluent; *ostiole* non-papillate or papillate, lined internally with hyaline cells when mature; conidiomatal wall pseudoparenchymatous, multi-layered, outer wall brown pigmented. *Conidiophores* reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform. The conidia were oblong to ellipsoid, hyaline, aseptate, but occasionally 1-septate with dimensions ranging from 2.7 to 7.8 × 1.6 to 3.5 µm (Plate 1 A & B). Isolates showed moderate reactions to the NaOH test for E metabolite. On the bases of these morphological characters, the pathogen was identified as Boeremia exigua (Boerema et al., 2004; Aveskamp *et al.* 2010).

Pathogenicity test

The fungal pathogen was re-isolated from the leaves of all inoculated plants, identified morphologically considering Koch's postulates. Necrotic spots developed on the leaves that were similar to those observed in the field. Necrotic lesions on inoculated plants were visible after 15 days of inoculation resulting in disease incidence. Disease symptoms were same on inoculated plants in pathogenicity test as in cotton fields of different districts.

Molecular Characterization

Genomic DNA of B. exigua was amplified by using PCR with universal primer pairs. These included ITS1/ITS4 and BT2A/BT2B, primers that gave the amplifications of an approximately 475 bp and 215 bp rDNA fragments respectively (Figure 2). The unique partial sequences of ITS and β-tubulin of Boeremia exigua were submitted to NCBI database acquiring the gene bank numbers of ITS sequences (LT838276 and LT838277) and β-tubulin (LT838278 and LT838279). Using the Basic Local Alignment Search tool (BLAST) (Altschul et al. 1997) the homologous sequences of ITS and β-tubulin sequences were extracted from NCBI database showing more than 98% homology with the isolates of B. exigua. Alignment of sequences was done using Mega-X (Kumar et al., 2018).

Phylogenetic analysis was performed using neighbor-joining method in MEGA 6 version 6.0 (Tamura et al., 2012). The phylogenetic tree developed on the base of ITS region (Figure 3) showed that the sequence (LT838276) isolated from G. hirsutum have 100% homology with these two isolates MK333925 and MK333929 of B. exigua from China which may be due to similarity in their ecology, the weather conditions and similar host species. While the sequence (LT838277) from the same host did not show much homology with any of the clade members. It may be due to difference in its genetics and host. For the β-TUB1 and 2 phylogenetic analyses revealed that the sequences LT838278 and LT838279 showed 99% homology with B. exigua isolate # KY273917 and KY273918 isolated from Russia and MH732951 and KR653201 from Chaina respectively. The similarity reflects the close

relationship in soil conditions, weather and host species from which they were extracted. Studies done by Chen et al. (2015) revealed that PCR performed with ITS regions (GenBank MT397284) and tub2 (MT414712) also showed similarity with the sequences present in the GenBank. Similarly, Colman et al (2020) also reported that ITS and tub2 sequences yielded 99.8% and 99.5% homology with sequences of the type species of B. exigua var. exigua available in GenBank isolated from sweet potato in Brazil.

Earlier, Farr and Rossman (2019) reported Boeremia exigua on various plants worldwide, but mostly in connection to rots of various organs, and particularly associated with post-harvest diseases. B. exigua was also reported to cause leaf spot disease from different parts of word on various plants and trees such as zonate leaf spot of A. altissima in Korea (Jung et al., 2022) leaf spot of walnut trees (Juglans regia) in China (Wang et al., 2022), white clover in China (Wang 2020), branch blight on walnut in China (Cai et al., 2021), black spot-like symptoms on soybean in Germany (Schaffrath et al., 2020), leaf spots on sweet potato in Brazil (Colmán et al., 2020), leaf spots on Hydrangea paniculata in Italy (Garibaldi et al., 2018) and stem and leaf spot on common speedwell in Switzerland (Michel et al., 2017).

According to our knowledge and by observing disease incidence and phylogenetic analysis it showed that Phoma leaf spot of cotton caused by B. exigua is new disease in Pakistan. This disease might be a potential threat to cotton production in Pakistan. *B. exigua* has been demonstrated the causal agent of black spot of pea and Boeremia Blight on Pyrethum in Australia. It has been already reported on different plants in the United States, Italy, Russia and China (Daughtrey et al., 1995; Zhao et al., 2016).

Reference

1. Brown, Judith K., and Zulqurnain Khan. "Breeding Cotton for Cotton Leaf Curl Disease Resistance." In Cotton Breeding and Biotechnology, pp. 171-197. CRC Press, 2022.
2. Abdullah, Khalid, and Zahid Khan. "Breeding Cotton for International Trade." In Cotton Breeding and Biotechnology, pp. 323-338. CRC Press, 2022.
3. Khan, Z., Ali, Z., & Khan, A. A. (2022). Role of Breeding and Biotechnology in Sustainable Cotton Production. In Cotton Breeding and Biotechnology (pp. 1-14). CRC Press.
4. Kumar, D., Kumar, V., Kumar, A., & Mann, S. S. (2021). Chapter-6 Root-Knot Nematode Problem and Their Management in Cotton Crop. ENTOMOLOGY, 105.
5. Revathi, P., & Hemalatha, M. (2014). Cotton leaf spot diseases detection utilizing feature selection with skew divergence method. International Journal of scientific engineering and technology, 3(1), 22-30.
6. Kowalski, T., Kraj, W., Bednarz, B., & Rossa, R. (2019). The association of Boeremia lilacis with necrotic lesions on shoots and leaf petioles and its pathogenicity towards Fraxinus excelsior. European Journal of Plant Pathology, 154(4), 961-974.
7. Jung, B. N., Park, J. H., Lee, C. K., & Shin, H. D. (2022). Zonate leaf spot of Ailanthus altissima in Korea is caused by Boeremia exigua. Forest Pathology, e12735.
8. Wang, F., Dun, C., Tang, T., Duan, Y., Guo, X., & You, J. (2022). Boeremia exigua Causes Leaf Spot of Walnut Trees (Juglans regia) in China. Plant Disease, PDIS-10.
9. Wang, J. H., Tang, M. M., Zhou, W., Bai, Y. J., & Han, Y. Z. (2021). First Report of Leaf Spot Associated with Boeremia exigua on White Clover in China. Plant Disease, 105(2), 504-504.
10. Colmán, A., Lima, I. M., Costa, H., & Barreto, R. W. (2020). Boeremia exigua causing leaf spots on sweet potato in Brazil. Australasian Plant Disease Notes, 15(1), 1-2.
11. Garibaldi, A., Gilardi, G., Matic, S., & Gullino, M. L. (2018). First Report of a Leaf Spot Caused by Boeremia exigua var. exigua on Hydrangea paniculata in Italy.
12. Michel, V. V., Daepp, M., Woudenberg, J. H. C., de Gruyter, J., & de Wit, P. J. G. M. (2018). First report of Boeremia exigua var. exigua causing stem and leaf spot on common speedwell in Switzerland. Plant Disease, 102(2), 440-440.
13. Cai, F., Yang, C., Ma, T., Jin, M., & Cui, L. (2021). First Report of Boeremia exigua var. exigua Causing Branch Blight on Walnut in China. Plant Disease, 105(10), 3291.

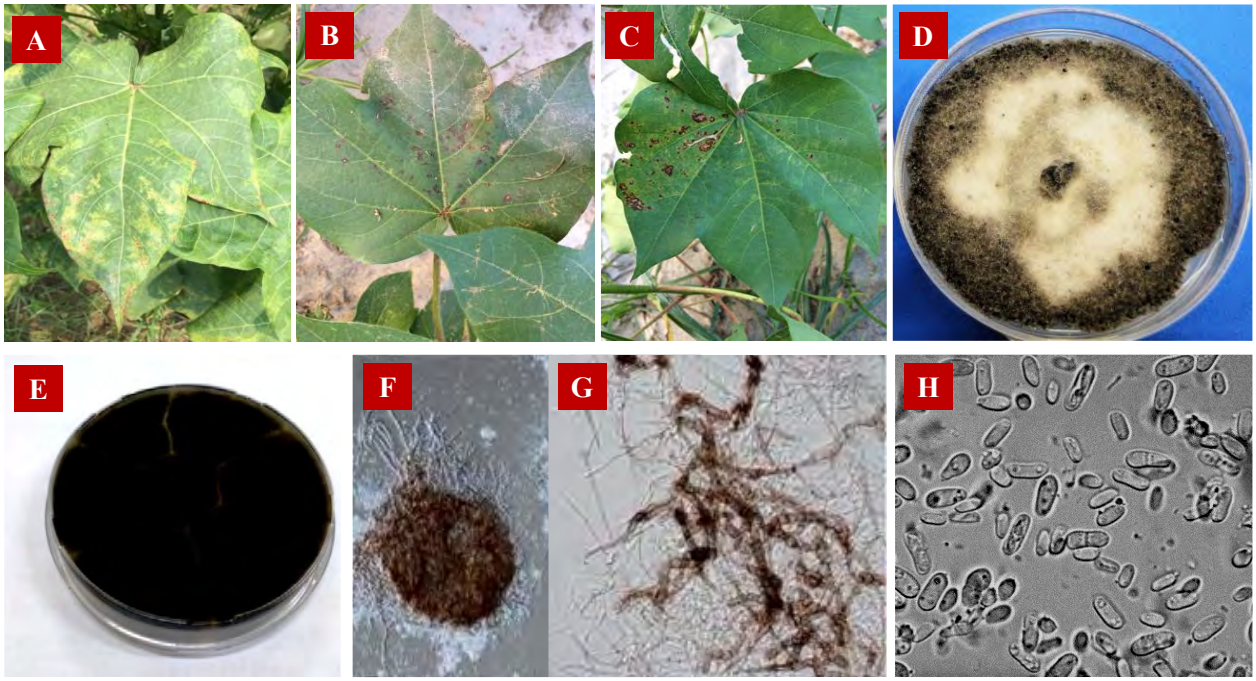

Figure 1. Cotton plant showing disease symptoms Morphological characterization of *Boeremia exigua*. (A)- Colony morphology on malt extract agar (MEA); (B)- Colony reverse on MEA; (C)- Pycnidia at 10X; (D)- Hyphae at 10X; (E & F) matured single celled hyaline, oval conidia at 100X.

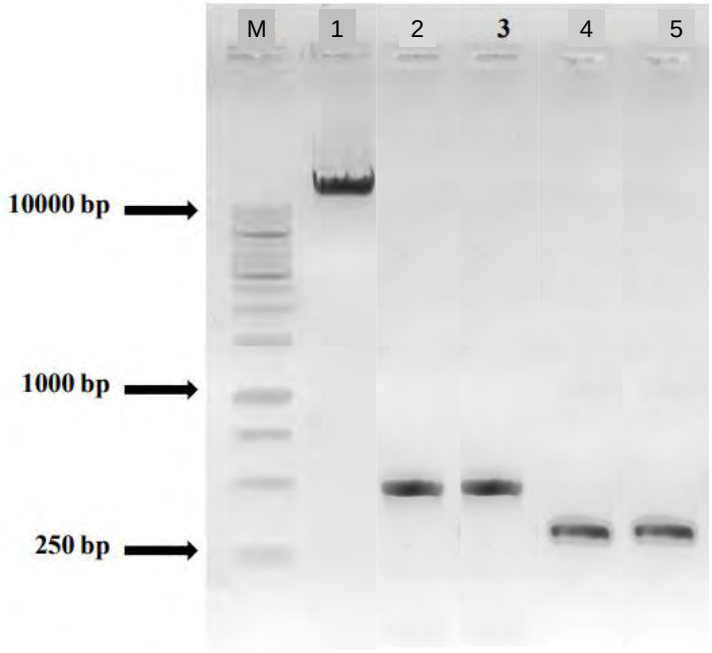

Figure 2. Agarose gel electrophoresis. **1.** Total genomic DNA isolated from cotton leaves. **2&3.** ITS1/ITS4 amplified PCR product Amplified PCR product of approximately 475 bp. **4&5.** PCR product of 215 bp using β-tubulin primers. **M**: DNA size marker.

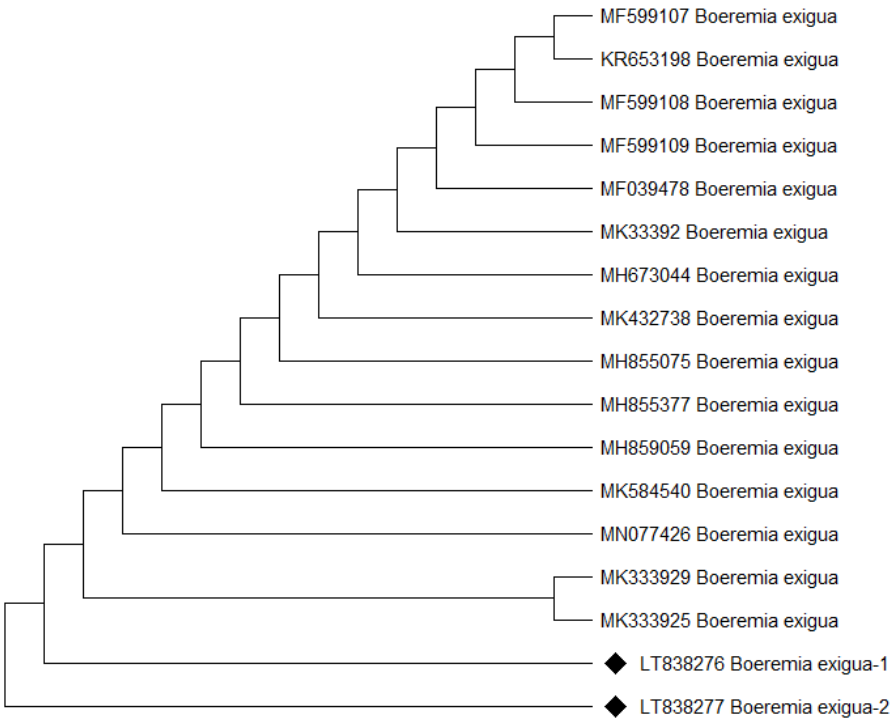

Figure 3. Phylogenetic tree showing the relationships between different clades of *Boeremia exigua* on the base of ITS region.

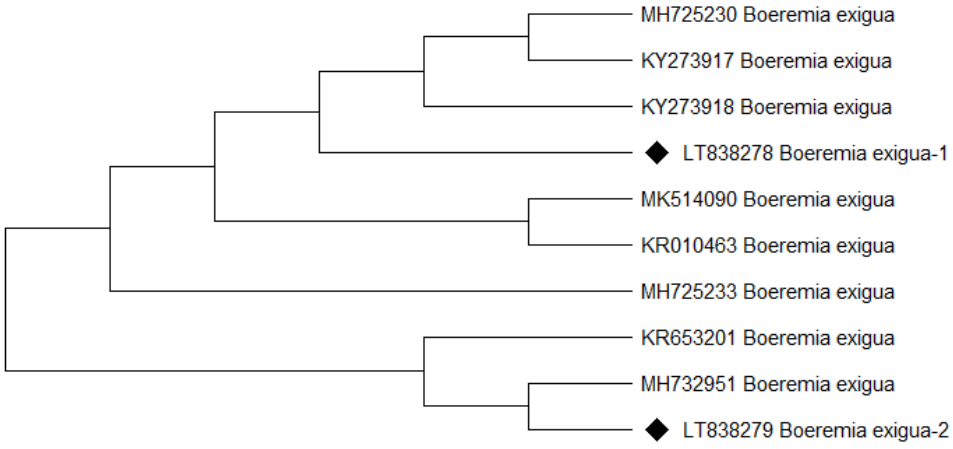

Figure 4. Phylogenetic tree showing the relationship between different clades of *Boeremia exigua* on the base of β-tublin 1&2

Interactive effects of abiotic factors on abundance of sucking pests on Bt cotton

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Abstract

Background: *Crop productivity primarily gets highly influenced by biotic and abiotic stress. Several biotic fauna influence the growth of which insects are the most limiting factor to obtain the desired yield. On the other hand, abiotic factors play an important role for the biotic stress abundance. Cotton (Gossypium hirsutum L.) is an important commercial crop and globally acclaimed as white gold which is attacked by various type of insect pests like sucking and bollworms which results in significant reduction in productivity as they ravage the crop throughout cropping period. Though genetically engineered Bt cotton provides effective management of bollworm complex, sucking pests still pose a great threat in cultivation of Bt cotton. Different abiotic factors influence the seasonal activity and population dynamics of sucking pests in cotton agroecosystem. Hence, the attempt is made here to ascertain the role of weather parameters on incidence of sucking pest complex viz., leaf hopper, aphids and thrips at ARS Dharwad, Karnataka, India during 2020-21 and 2021-22*

Results: *Among sucking insect pests, the incidence of a leaf hopper, thrips and aphids remained active through the cropping season in varying population density. Thrips (Thrips tabaci L.) population was only observed in early stage of the crop growth and peak activity of thrips notices from 40-55 days onwards and the average population ranged from 31.80 - 41.70 thrips per 3 leaves. The peak incidence of 41.20-47.30 no. of thrips population per three leaves was observed from August 2nd FN- September months which coincides with maximum temperature of 27.80 - 29.19 oC and minimum temperature of 20.37-20.60 oC. Correlation of thrips population with minimum temperature revealed significant positive (r=0.322*) relationship. Cumulative effect of both maximum and minimum temperature designated 23.30 per cent (R2 = 0.233) role on population abundance. Multiple regression analysis with thrips population revealed that every increase in 1oC temperature will lead to increase in 3.97 thrips per 3 leaves along with decrease in 1oC temperature will also lead to increase in 4.41 thrips per 3 leaves. Leaf hopper attained peak during 2nd FN of September and was maximum of 11.25 average nymphs per 3 leaves in consecutive years (2020-21 and 2021-22). The population of leaf hopper, A. biguttula showed highly significant positive correlation (r= 0.478**) with maximum temperature (29.50-30.61oC). Effect of maximum temperature by multiple regression analysis showed that 22.80 per cent influence on population (R2 = 0.228), which indicates that every increase in 1oC temperature will lead to increase in 1.11 nymphs per 3 leaves. Further peak activity of aphids was noticed from 120-130 days after sowing and with maximum of 37.54 average aphids per 3 leaves in 2nd FN of November in 2020 and 1st FN of December in 2021. The peak average incidence of 37.54 aphids population per three leaves was observed in November and December months of two consecutive years which coincides with maximum (27.37-30.50 oC) and minimum temperature (16.19-17.35 oC). Correlation studies revealed that minimum temperature (r= -0.687**) exhibited negative and highly significant relationship with no. of aphids per 3 leaves. However, aphid population showed positive and highly significant relation with maximum temperature (r=0.378*), but negative and highly significant relationship with minimum temperature (r=-0.687**), maximum relative humidity (-0.607**) and minimum relative humidity (r=-0.669**). Whereas, rainfall exhibited negative relationship with aphid population (r=-0.272). Among all factors, influence of minimum temperature showed that (R2 = 0.472) 47.20 per cent on the population, which indicates every decrease in 1oC temperature will lead to increase in 6.05 no. of aphids per 3 leaves.*

Conclusion *Considering the role of weather factors which influences population buildup of sucking pests in transgenic Bt Cotton, aids in forewarning and timely action before actual infestation in conducive weather.*

Key words: *Correlation, Regression, Leaf hopper, Thrips, aphids, Bt cotton, abundance*

Introduction

Crop productivity primarily gets highly influenced by biotic and abiotic stress. Several biotic fauna influence the growth of which insects are the most limiting factor to obtain the desired yield. On the other hand abiotic factors play an important role for the biotic stress abundance. Survival and thriving at extreme physical conditions require peculiar adaptations and plastic responses. Among abiotic factors, temperature and humidity stand out as the most important ones constraining abundance and distribution of insect. Furthermore, it is well documented that abiotic factors, regulate the ecology of insect communities. Although effects of temperature on survival, development, and reproduction of insects have been exhaustively explored over several decades, there is still a lot of interest on how temperature and other abiotic factors set the limits of distribution and define abundance of insect species. Cotton, (*Gossypium hirsutum* L.) is the important cash crop in India due to its high industrial demand. Despite of huge share in areas the productivity of cotton (290 kg/ha) is still very lower than even the world average productivity. It is anticipated that this low production is mainly attributed due to infestation of pest problem. An array of insect pests has been reported to infest the crop rendering the low yield. About 162 species of insects has been known to occur in cotton at various stages of growth, of which 8 are key pests (Dhawan, 2000).

Cultivation of cotton under diversified agro climatic situations makes the crop to suffer a lot by different kinds of pests and diseases. Large area under rainfed situations and extensive replacement of conventional varieties with superior hybrids made the crop easily vulnerable to insect pests. The major reason for the low productivity in cotton is damage caused by insect pests. In India, as many as 162 species of insect-pests are known to attack cotton from sowing to maturity which cause up to 50-60 per cent loss (Agarwal et al., 1984). Cotton pests can be primarily divided into bollworms and sucking pests. Among sucking pests, aphid, *Aphis gossypii* (Glover), leafhoppers, *Amrasca biguttula biguttula* (Ishida), thrips, *Thrips tabaci* (Lind.) and whitefly, *Bemisia tabaci* (Genn.) are of major importance. These sucking pests occur at all the stages of crop growth and responsible for indirect yield losses. A reduction of 22.85 per cent in seed cotton yield due to sucking pests has been reported by Satpute et al. (1990).

Materials and Methods

A field experiment was conducted at the Agricultural Research Station, Dharwad under unprotected conditions (for sucking pests) to study the seasonal abundance pattern of sucking insect pests in popularly grown Jaadoo BGII cotton genotype and was kept unsprayed throughout the cropping season. The ARS Dharwad is located between15 0 N latitude and 76.46 0 E longitude at an altitude of 678 meters above mean sea level with annual average rainfall 922.7 mm. The plot size was 8.1 x 5.4 m with 10 rows of 10 plants for each genotype under 90 x 60 cm spacing replicated four times. All the recommended agronomical practices were followed to raise the crop successfully as per package of practices prescribed for the region (Anon., 2020).The population of sucking pests were estimated from 15 days after sowing (DAS) on population of adults as well as nymphs of thrips, aphids and nymphs of leafhoppers at weekly intervals on three leaves (top, middle and bottom) in ten plants selected randomly. Later the population was averaged to present as number per three leaves.

Results and Discussion

Seasonal incidence of sucking insect pests

Among sucking insect pests, the Thrips (*Thrips tabaci* L.), Leaf hopper (*A. biguttula*) and Aphids (*Aphis gossypi*) were key pests and remained active through the cropping season. *T. tabaci* population was only observed in early stages of the crop growth.

Thrips (*Thrips tabaci* L.): Similarly peak activity of thrips notices from 40-55 days onwards with average population ranged from 31.80-47.30 thrips per three leaves during both the consecutive year. The peak incidence of 41.20-47.30 no. of thrips population per three leaves was observed from August 2nd FN-September months which coincides with maximum (27.80 - 29.19 °C) and minimum temperature (20.37-20.60 °C). A population of *Thrips tabaci* L showed significant positive relationship with minimum temperature (r= 0.322*). However, the correlation with maximum temperature (r= 0.264), maximum relative humidity (r= 0.201) and minimum relative humidity (r=0.185) was positive relationship during all the years. Based on correlation was negative relationship with rainfall (r=-0.047). Cumulative effect of both maximum and minimum temperature designated that 23.30 per cent population (R^2 = 0.233). Multiple regression analysis revealed that every increase in 1°C temperature will lead to increase in 3.97 no. of thrips per 3 leaves along with decrease in 1°C temperature will also lead to increase in 4.41 no. of thrips per 3 leaves (Table 1 and 2).

Leaf hopper (*A. biguttula*): The population build up of sucking pests in relation to the abiotic factors were ascertained through the correlation studies along with multiple regression analysis. The results revealed that the peak activity of leaf hopper starts from 50 days after sowing and the average nymphal population ranged from 6.40 -11.70 per three leaves. The peak incidence of 10.80-11.70 nymphs per 3 leaves was observed from September-October months which coincide with maximum temperature of 29.50-30.61°C and minimum temperature of 19.9-20.96 °C during two consecutive years. The multiple regression analysis between abundance of *A. biguttula* as a dependent variable and weather parameters revealed 22.80 per cent (R^2= 0.228) influence on population. Among the parameters maximum temperature has great role in activity of leaf hopper that every increase in 1°C temperature will lead to increase in 1.11 leaf hopper per 3 leaves (Table 1 and 2).

Aphids (*Aphis gossypi*): Further peak activity of aphids notices from 120-130 days after sowing and the average population ranged from 30.40-41.50 per three leaves. The peak incidence of 30.40 - 41.50 aphids population per three leaves was observed in November month which coincides with maximum (27.37-30.50 °C) and minimum temperature (16.19-17.35 °C) during two consecutive years. However correlation showed that the mean temperature also showed positive correlation with incidence of *Aphis gossypi* and it was significant (r=0.387**). The regression analysis incidence of aphid showed negative and highly significant relationship (r=-0.687**) with minimum temperature and 47.20 per cent influence on aphid population. Which indicates that every decrease in 1°C temperature will lead to increase in 6.05 aphids per 3 leaves (Table 1 and 2).

The understanding of the ecological factors (either biotic or abiotic) affecting the population of the insect pest is a prerequisite for planning the effective and more precise management strategy for a particular pest. The increased incidence of aphid towards tail end of the season irrespective of genotypes might be due to positive correlation of aphids with maximum temperature as disclosed by Mohapatra (2008). Sitaramaraju et al. (2010) who also observed population of *A. biguttula* throughout the cropping season. However, Gosalwad et al. (2009) reported the peak population of *A. biguttula* during 2nd week of October and the end of October at Parbhani (Maharashtra). In Guntur (Andhra Pradesh), Sitaramaraju et al. (2010) and Soujanya et al. (2010) observed the peak abundance of leafhoppers in 2nd week of October to 3rd week of November. Mohapatra (2008) also reported its peak in 2nd week of October in Western Orissa.

The leafhopper incidence was recorded in two peaks *i.e.* during second fortnights of August and November in all Bt genotypes, which recorded peak leafhopper incidence during second fortnight of September (Phulse and Udikeri., 2014). Selvaraj et al. (2011) and Prasad et al. (2008) reported abundance of *A. biguttula* with maximum and minimum temperature ranging from 30.5 to 33 °C and 20 to 24 °C, respectively. According to Selvaraj *et al*. (2011), morning relative humidity from 82 to 90 percent and evening relative humidity from 55 to 66 percent favours the multiplication of *A. biguttula* which corroborates

with the present findings. Mean peak population of aphid [*Aphis gossypii* (Glover)] and jassid [*Amrasca biguttula biguttula* (*Ishida*)] was recorded at 34 and 37 meteorological week, respectively coinciding with abiotic factors 30–32°C maximum temperature, 21–22°C minimum temperature and more than 70% mean relative humidity in rainfed *Bt* cotton. The population dynamics of pest reaches above ETL (Economical Threshold Level) or to lower and higher vital limit are associated with changing abiotic factors (Pralhad and Shinde, 2021). Seasonal abundance and crucial role of different weather parameters on the population fluctuation of *A. biguttula* and *B. tabaci* in cotton agroecosystem which can be helpful in forecasting and formulating effective management strategies for these insect pests.

Table1: Seasonal Incidence of Sucking pests in transgenic Bt Cotton, 2020-22

Fort Night (FN)	2020-21			2021-22		
	Leaf hopper	Thrips	Aphids	Leaf hopper	Thrips	Aphids
			August			
1st FN	0.00	0.00	0.00	0.00	0.00	0.00
2nd FN	2.20	18.33	2.20	1.60	11.80	1.20
			September			
1st FN	4.00	32.90	3.40	3.80	31.80	4.50
2nd FN	6.70	41.20	8.9	8.10	47.30	6.80
			October			
1st FN	10.80	38.10	16.50	11.70	36.70	14.20
2nd FN	6.70	22.62	20.20	9.40	28.62	20.20
			November			
1st FN	6.20	17.10	30.40	7.10	16.20	24.60
2nd FN	3.90	9.20	38.89	6.00	8.20	31.40
			December			
1st FN	5.90	1.30	36.30	3.80	2.20	36.20
2nd FN	5.70	1.10	13.60	4.60	1.00	14.20

Table 2: Population of sucking pests on Bt Cotton vs. Climatic factors

S. No.	Climatic factors	Correlation Coefficients		
		Leaf hopper	Thrips	Aphids
1.	Maximum Temperature	0.478**	0.264	0.378**
2	Minimum Temperature	-0.170	0.322*	-0.687**
3	Relative Humidity (Max)	-0.253	0.201	-0.607**
4	Relative Humidity (Min)	-0.277	0.185	-0.669**
5	Rainfall (mm)	-0.226	-0.047	-0.272

Table 3: Correlations and Regression analysis with weather factors

Insect pests	MaxT (X_1)	MinT (X_2)	RHmax (X_3)	RHmin (X_4)	Rainfall (X_5)	Regression equation	R^2 (%)
Thrips	0.264	0.322*	0.201	0.185	-0.047	Y=-181.699+4.41 X_2+3.97X_1	23.30
Leafhopper	0.478**	-0.170	-0.253	-0.277	-0.226	Y=-26.633+1.11X_1	22.80
Aphids	0.378**	-0.687**	-0.607**	-0.669**	-0.272	Y=132.22-6.01 X_2	47.20

References:

1. Agarwal, R. A., Gupta, G. P. and Garg, D. O., 1984, Cotton pest management in India. *Res. Publn.*, Azadnagar, Delhi, pp. 1-19.
2. Anonymous, 2019, Improved Package of Practices for Higher Yields.UAS, Dharwad.
3. Dhawan AK. Cotton pest scenario in India: Current status of insecticides and future perspectives. Agrolook. 2000;**1**(1):75-85
4. Gosalwad, S. S., Kamble, S. K., Wandnerkar, D. W. and Awaz, B. H. 2009, Population dynamics of major insect pests of cotton and their natural enemies. J. Cotton Res. Dev. 23, 117–125.
5. Mohapatra, L. N., 2008, Population dynamics of sucking pests in *hirsutum* cotton and influence of weather parameters on its incidence in western Orissa. *J. Cotton Res. Dev*., 22 (2): 92-194
6. Phulse V. B. and S. S. Udikeri, 2014, Seasonal incidence of sucking insect pests and predatory arthropods in desi and Bt transgenic cotton. *Karnataka J. Agric. Sci.,* 27 (1): 28-31.
7. Prasad, N. V. V. S. D., Rao, N. H. P. and Mahalakshmi, M. S. (2008): Population dynamics of major sucking pests infesting cotton and their relation to weather parameters. J. Cotton Res. Dev. 22, 85–90.
8. Pralhad J. and Shinde P.B., 2021, Prediction of aphid and jassid in rainfed Bt cotton using regression models based on abiotic factors. Indian Journal of Dryland Agricultural Research and Development. 35(1) : 43-49
9. Satpute, U. S., Patil, V. N., Katole, S. R., Men, V. D. and Thakare, A. V., 1990, Avoidable field losses due to sucking pests and bollworms in cotton. *J. Appl. Zool. Res.*, 1 (2): 67-72.
10. Selvaraj, S., Adiroubane, D. and Ramesh, V. (2011): Population dynamics of important insect pests of bhindi inrelation to weather parameters. Pestology 34, 35–39.
11. Sitaramaraju, S., Parsad, N. V. V. S. D. and Krishnaiah, P. V. (2010): Seasonal incidence of sucking insect pests on Bt cotton in relation to weather parameters. Ann. Pl. Prot. Sci. 18, 49–52.
12. Soujanya, P. L., Prasad, N. V. V. S. D. and Rao, P. A. (2010): Population dynamics of sucking pests and their relation to weather parameters in *Bt*, stacked *Bt* and non *Bt* cotton hybrids. Trends in Biosciences 3, 15–18.
13. Udikeri, S. S., Patil, B. V., Basavanagoud, K., Khadi, B. M., Kulkarni, K. A. and Vamadevaiah, H. M., 2012, Impact of Bt transgenic cotton on population dynamics of aphids and natural enemies. *Indian J. Agri. Sci.,* 82(6): 555-60.

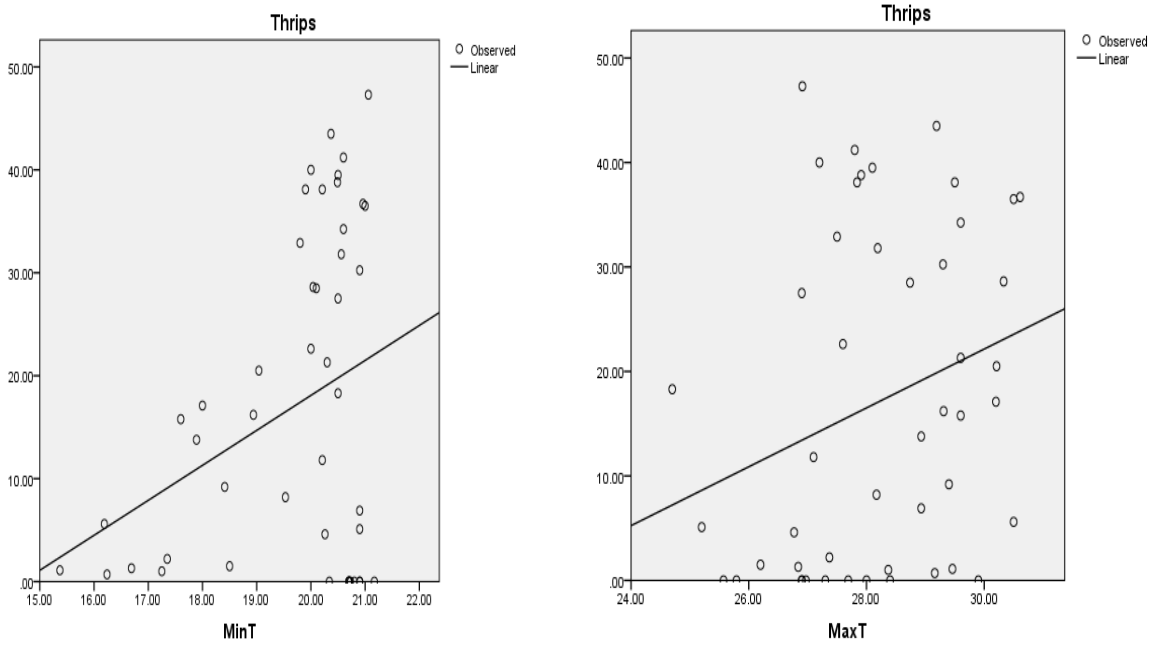


Fig 1: Incidence of thrips in relation to temperature

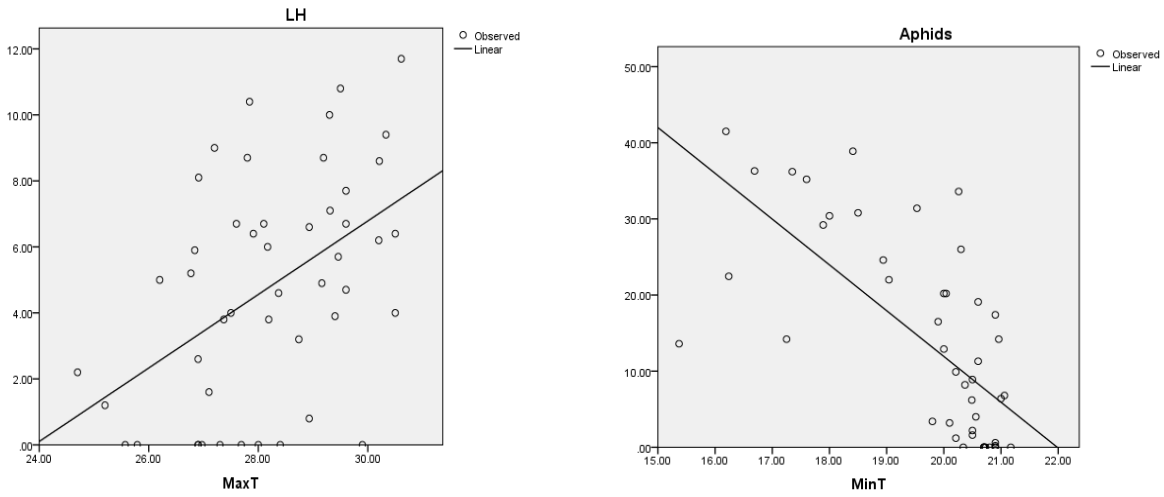


Fig 2: Incidence of Leaf hoppers and aphids in relation to temperature

Fig 3: Incidence of sucking pests in Bt cotton in relation to abiotic factors during 2020-21

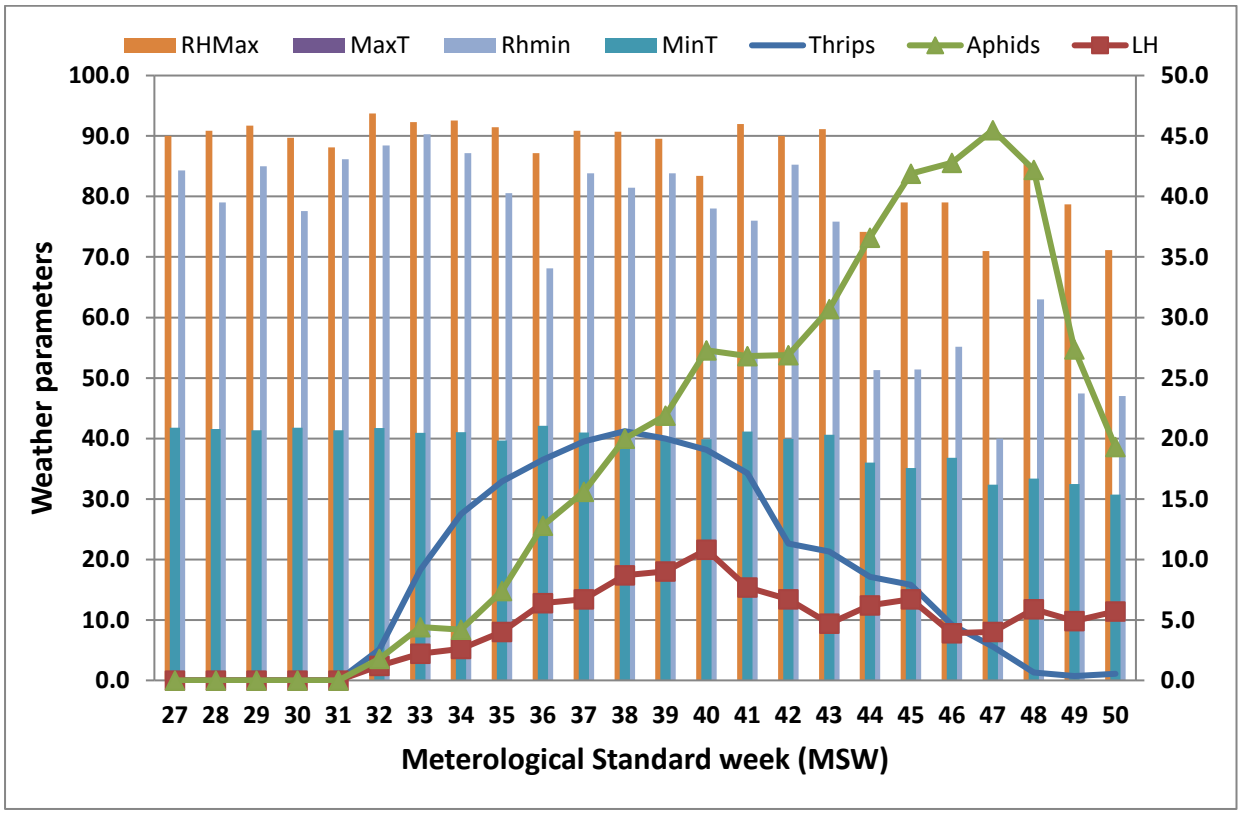
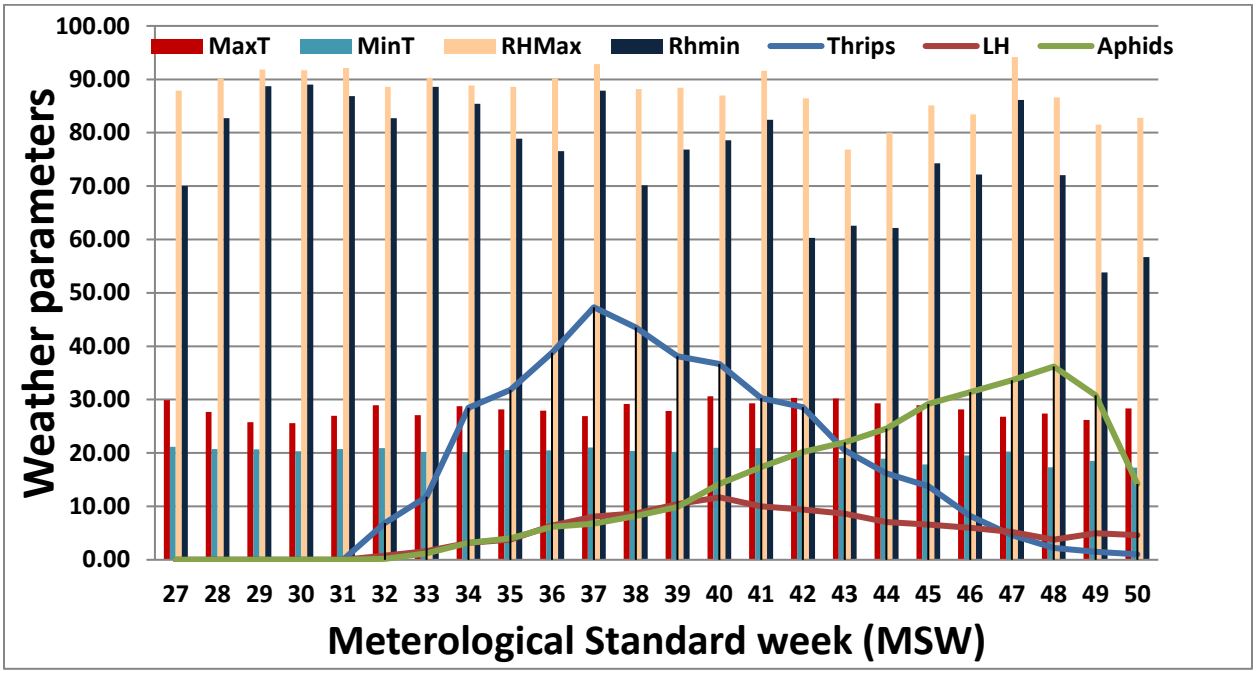


Fig 4: Incidence of sucking pests in Bt cotton in relation to abiotic factors during 2021-22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Meterological Standard week (MSW)

CPE-23: ECO-FRIENDLY MANAGEMENT OF PINK BOLLWORM (PECTINOPHORA GOSSYPIELLA) ON COTTON CROP

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Abstract

Background:
Cotton is a significant commercial crop playing a spectacular role in country social, financial and political undertakings in Pakistan. The Pink bollworm (Pectinophora gossypiella) is a key pest which attack the cotton crop and it is distributed all over the world where cotton has grown. The incidence of pink bollworm has been reported in nearly every cotton producing country in the world. In the present study, two experiment was conducted.

Results:
In 1st experiment four different treatments PB ropes, Delta traps, Light traps and Capsule lures were evaluated to check the pertage infestation reduction of P. gossypiella under field conditions. In 2nd experiment two treatments Beauveria bassiana and a noval insecticide Radiant 120 SC (Spintoram) were evaluated. The research was designed to assess the effect of Mating disruption techniques (PB Rope), Pheromones trap, Delta trap,Light trap, Entomopathogenic fungi and a noval insecticide Spinetoram against pink bollworm in cotton crop under filed condition. Result revealed that highest 96.33% infestation reduction of P. gossypiella was recorded in PB ropes while in case of insecticides Radiant (Spintoram) perform well gave 79.16 % infestation reduction.

Conclusion:
Results showed Pb ropes gave highest infestation reduction that is 96.33%. So, the Pink bollworm can be controlled by using all these eco-friendly techniques along with chemical control

Keywords: *Entomopathogenic fungi, Pectinophora gossypiella, Pb ropes, Radiant, Spinetoram*

Introduction

Agriculture is the backbone of Pakistan's economy and majority of population attach with them through farming. Some of them are involved directly or indirectly in framing through manufacturing, proceeding and distributing of major agriculture commodities. In the region of South Asia, Pakistan is emerging as developed state comprising the 207 million world population (Radio Free Europe, 2017). A distinctive role is played by agriculture in economic system holding 19.2% of GDP. Agriculture is providing an indispensable job in making sure food secure, producing overall monetary growth, diminishing poverty and transforming in direction of mechanization (Economic Survey of Pakistan, 2011-12). Cotton (*Gossypium hirsutum* L.) is an important crop in agriculture as well as in the textile industry. It contributes around 0.6 percent to GDP and 3.1 percent to agricultural value addition (Pakistan economic survey, 2021-22). Cotton is one of the most important economic crop of Pakistan. It is extremely important to Pakistan's economy (Amin *et al.,* 2017).

Cotton is a significant commercial crop playing a spectacular role in country's social, financial and political undertakings and it is gifted to human civilization from Indian sub-continent (Atwal, 2002).

It plays a supreme role in the economy of Pakistan. *Gossypium hirsutum* being the Pakistan's main cash crop is also populous as "Silver Fiber" (Gill and Dhawan, 2006). The Gossypium genus contain almost 50 species among which 45 are diploids and remaining 5 are tetraploids. *Gossypium hirsutum* the tetraploid specie is among the most cultivated species (Waghmare *et al*., 2005). In Pakistan cultivation of cotton is mainly done in the province of Punjab and Sindh. About 77% production of cotton in Pakistan comes from Punjab and 23% production from other provinces. The main Punjab districts that contribute in huge cotton production are (Faisalabad, Jhang, Raheem Yar Khan, Multan, Vehari, Khanewal, Lodhran, Bahawalpur, Ranipur and Bahawalnagar). The Sindh regions for cotton cultivation are (Nawab shah, Ghotki, Khairpur, Ahmed, Kazi and Naushero-feroze). The desired conditions required for cotton are hot and dry climate therefore cotton is cultivated best in the above-mentioned regions (Shuli *et al*., 2018).

Cotton shows a variety of insect pest spectrum and about 1326 species of insect pest have been reported worldwide (Parmar and Patel, 2016). Cotton is highly exposed to pest attacks, which may causes up to 87% losses in the yield of cotton (Talley et al., 2009). Due to the insect attack, its growth period undergoes to wreck (Gangadhar et al., 2007). The average yield of cotton in Pakistan is less as compare with other countries due pest attack. There are various reasons which cause the loss in yield from the beginning of its growth and to maturity the insect pest attacks cause the 5-10% losses. Due to lint quality and deterioration losses of cotton is 10-40%, that cause about 30-40% average yield loss (Khan et al.,2009).

Pink bollworm (Pectinophora gossypiella) is considered one of the most harmful cotton pests because it is harder to control it with insecticides (Lykouressis et al., 2005). The incidence of pink bollworm has been reported in nearly every cotton producing country in the world (Salama et al., 2013). Surveys in the United States and Mexico show that pink bollworm has 46 plant species as the preferred hosts in these two countries. Okra (Hibiscus esculentus L.), cotton and ornamentals plants are considered as the most favorite host of the pink bollworm (Saleh et al., 2013). Pink bollworm (Pectinophora gossiypiella) cause most severe damage to the cotton crop worldwide on the basis of damage it was declared the most injurious pest of cotton crop (Rajput, 2017). The present study was planned to explore the maximum reduction potential eco-friendly techniques against pink bollworm.

Material and Methods

The present research was conducted in field area of Entomological Research Institute, AARI, Faisalabad. The research study was conducted from May 2021 to November 2021. The study was planned to check the impact of Eco-friendly techniques against Pink Bollworm (*P. gossypiella*) on Cotton crop. Crop was sown on 20^{lst} May, 2021 under Randomized Complete Block Design (RCBD) having three replications with plant to having plot size 10 x 30 m^2. Pre-irrigated field for the experiment was prepared by ploughing. Cotton seeds were sown in each block manually. Common cultural practices were adopted to maintain a good crop. Study field and surrounding areas were made weed free by removal of weeds regularly with manual operations. Meteorological data was obtained from Department of Crop Physiology, Ayub Agriculture Research Institute, Faisalabad. The meteorological data contained daily temperature and relative humidity.

Study Area

Cotton crop was sown in RCBD design with three replications having plot size 10 x 30 m^2 in the research area of Entomological Research Institute, AARI Faisalabad during the kharif season 2021.

Experimental Layout and Treatments

Six different treatments was used to manage the Pink bollworm infestation. The treatments and their dose rates are given below.
This experiment will consist of six treatments

Table 1: Treatment detail with dose rate

Sr. No.	Treatments	Dose rate
1	PB Ropes	120/Acre
2	Delta Traps	8/Acre
3	Capsule Lures	5/Acre
4	*Beauveria bassiana*	1×10^8 CFU/ml
5	Light traps	1 per plot
6	Radiant 120 SC (Spintoram)	100 ml/acre

Experiment 1: In this experiment used five treatments including control plot.

The data for adult catches and for PBW % infestation was recorded at 10 day intervals. PB ropes was installed at 10-meter distance, length and width wise, Delta Traps was installed @ 8 traps per acre at equal distance, PBW capsule lures was used @ 5 lures/acre at a uniform distance,. Light traps was installed @ 1/ plot.

Data Collection:: The data regarding (%) infestation reduction of *P. gossypiella* recorded at 10 days intervals by the time of pest appearance up to crop maturity. Infestation of *P. gossypiella* started in the 3rd week of july and the first data was collected on 16th july, 2021 just after the pest incidence. Five plants were selected randomly from each replication of each replications and the (%) infestation of *P. gossypiella* on each plant was recorded. Data was collected from 6th July 2021 to 27th October, 2021

Experiment 2: In this experiment used three treatments including control plot. The *Beauveria bassiana* 1×10^8 CFU/ml was sprayed @ 200 gm/ acre. Radiant 120 SC (Spintoram) was sprayed. Before application take pre-treatment data and then take data after 7 days of first application and then take 14 days data of 2nd application

Data Collection:

The data regarding (%)infestation of *P. gossypiella* recorded at before treatment on 18th August, 2021. The first data was collected on 25th August, 2021 after 7 days of first application. Five plants were selected randomly from each replication of each replications and the (%)infestation of *P. gossypiella* on each plant was recorded. 2nd Data was collected on 01lst September, 2021 after 7 days of 2nd application.

Statistical Analysis

Data regarding the (%) Infestation of *P. gossypiella* and (%)Infestation reduction of *P. gossypiella* was recorded at 10 intervals from the time of pest appearance up to crop maturity. The data was collected on data sheets and arranged that data on excel sheets for statistical analysis. The data collected from all the replications was analyzed by analysis of variance (ANOVA) and mean values between the genotypes were compared by using Tukey HSD Test at P≤0.05.

Results and discussion

Table 2: Mean Comparison of (%) infestation reduction of *P. gossypiella* treated with Pb ropes, delta traps, light traps and capsule lures after 10 days interval

Treatments	after 10 day	after 20 day	after 30 day	after 40 day	after 50 day	after 60 day
Pb ropes	96.30 a	96.10 a	96.20 a	96.33 a	89.43 a	87.06 a
Delta traps	85.03 b	74.76 b	72.73 b	63.76 b	59.00 b	53.00 b
Light traps	75.13 c	61.00 c	56.16 c	43.60 d	41.83 c	38.10 c
Capsule lures	81.33 bc	71.93 b	68.50 b	54.70 c	47.96 c	46.60 b
Control	0.00 d	0.00 d	0.00 d	0.00 e	0.00 d	0.00 d
Treatments	after 70 day	after 80 day	after 90 day	after 100 day	after 110 day	after 120 day
Pb ropes	85.30 a	82.96 a	77.53 a	72.76 a	71.73 a	73.96 a
Delta traps	56..20 b	58.40 b	52.30 b	52.23 b	52.66 b	54.33 b
Light traps	45.20 c	38.96 c	36.40 c	39.86 c	37.60 d	42.23 c
Capsule lures	52.06 b	49.80 b	42.83 c	43.10 c	43.70 c	46.93 bc
Control	0.00 d	0.00 d	0.00 d	0.00 d	0.00 e	0.00 d

The data was recorded at 10 days interval. The results revealed significant difference among the treatments. The Tukey HSD Test was used to compare the mean between treatments at P≤0.05. The findings revealed that besides untreated check the treatment 3 (Light trap) had the lowest (%) infestation reduction in all 10 days intervals. Minimum infestation reduction (36.40%) was found in light traps after 90 days data. The highest (%) infestation reduction was recorded (96.33%) in Pb rope in first data after 10 days.

According to these results, maximum (%) infestation reduction was recorded in Pb ropes. While all others treatments gave satisfactory results and showed a significant control against pink bollworm.

Table 3: Mean Comparison of (%) Infestation and (%) Infestation reduction of *P. gossypiella* against *Beauveria bassiana* and radiant after 7 and 14 days

Treatments	Pre-treatment	% infestation (PBW)		% infestation reduction		[c] Bio-control /plant	Survival %
		7-DAA	14-DAA	7-DAA	14-DAA	7-DAA	7-DAA
Beauveria bassiana	17.24	12.07	9.06	44.15 b	57.02 b	1.72	52.46 bcd
Radiant (Spintoram)	17.97	6.54	4.07	69.71 a	79.16 a	1.87	57.11 bcd
Check	17.63	21.62	22.05	0.00 c	0.00 c	3.27	100.0 a
Tukey HSD at 5%				6.751	5.196		4.092

***DAA: Days after application**

Significant variations were indicated in the (%) infestation of *P. gossypiella* treated with *Beauveria bassiana* at same concentration with three replications, compared with untreated check

The results revealed that besides untreated checks highest (%) Infestation of *P. gossypiella* was recorded in treatment 1 (*Beauveria bassiana)* (12.07%) and (9.6%) after 7 and 14 days respectively and lowest (%) Infestation of *P. gossypiella* was recorded in treatment 2 (Radiant*)* (6.54%) and (4.07%) after 7 and 14 days respectively

While, lowest (%) Infestaion reduction of *P. gossypiella* was recorded in treatment 1 (*Beauveria bassiana)* (44.15%) and (57.02%) after 7 and 14 days respectively and highest (%) Infestation reduction of *P. gossypiella* was recorded in treatment 2 (Radiant*)* (69.71%) and (79.16%) after 7 and 14 days respectively.

Conclusion

Eco- friendly techniques have effective results against pink bollworm. All treatments have shown good results to reduce infestation of this pest on cotton crop. Pb ropes gave highest infestation reduction that is 96.33%. So, the Pink bollworm can be controlled by using all these eco-friendly techniques along with chemical control.

Author contributions

Imran Nadeem and Qurban Ali conceived and designed the experiment. Imran Nadeem, Qurban Ali, Muhammad Kamil Malik and Aqsa Abbas performed the experiment. Muhammad Kamil Malik and Aqsa Abbas analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

References

1. Amin, A., W. Nasim, M. Mubeen, M. Nadeem, L. Ali, H.M. Hammad, S.R. Sultana, K. Jabran, M.H. Rehman, S. Ahmad, M. Awais, A. Rasool, S. Fahad, S. Saud, A.N. Shah, Z. Ihsan, S. Ali, A.A. Bajwa, K.R. Hakeem, A. Ameen, Amanullah, H.U. Rehman, F. Alghabar, G.H. Jatoi, M. Akram, A. Khan, F. Islam, S.T. Ata-Ul-Karim, M.I.A. Rehmani, S. Hussain, M. Razaq and A. Fathi. 2017. Optimizing the phosphorus use in cotton by using CSM-CROPGRO-cotton model for semi-arid climate of Vehari-Punjab, Pakistan. Environ. Sci. Pollut. Res. 24:5811-5823. https://doi.org/10.2166/wcc.2019.179.
2. Atwal, A. S. 2002. Agricultural pests of South Asia and their management. Kalyani Publ., Ludhiana, India. 221.
3. Economic Survey of Pakistan, 2011-12.
4. Economic Survey of Pakistan, 2021-22.
5. Gangadhar B, K.K. Dahiya and B.L. Takar. 2007. Impact of abiotic factors on population dynamics of sucking pests in transgenic cotton. J. Cotton. Res. 21:103-105. . https://doi.org/10.18805/ijare.v49i5.5806
6. Gill, H.K. and A.K. Dhawan. 2006 Global status of Insectcides resistance in Helicoverpa armigea on cotton. J. Cott. Res .Dev., 20: 226-231. https://doi.org/10.1603/EC12286

7. Khan, R., S. Ahmad, M. Saleem and M.K Nadeem. 2009. Field evaluation of different insecticides against spotted bollworms *Earias spp*. at district Sahiwal. Pak. Entomol. 29(2):129-134.
8. Lykouressis, D., D. Perdikis, C. Michalis and A. Fantinou. 2004. Mating disruption of the pink bollworm *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) using gossyplure PB-rope dispensers in cotton fields. J. Pest Sci. 77:205-210. https://doi.org/10.1007/s10340-004-0055-4
9. Parmar, V.R., and C.C. Patel. 2016. Pink bollworm: a notorious pest of cotton: a review. Agr. Res. Int. J., 5:88-97.
10. Radio Free Europe/Radio Liberty (2017). Census shows Pakistans Population has reached over 207 million
11. Rajput. 2017. Effect of Different Synthetic Pesticides Against Pink Bollworm *Pectinophora gossypiella* (Saund.) on Bt. and non-Bt. Cotton Crop. J. Basic Appl. Sci. 13:454-458. https://doi.org/10.6000/1927-5129.2017.13.75
12. Salama, M.A., M.A. Abd El-Baki, J.B.A. El-Naggar and E.Y. El-Naggar. 2013. Efficiency of some insecticides sequence on cotton bollworms and histopathological effects of some biocides on pink bollworm larvae. Egypt. J. Agric. Res. 91:429-448.
13. Saleh, A. A., L. R. Elgohary, W. M. Watson and A. S. Elabasy. 2013. Efficiency of some new insecticides on cotton bollworms, *Pectinophora gossypiella* (Saund.) and *Earias insulana* (boisd.). J. Plant Prot. Res.,4: 617-624. https://doi.org/ 10.21608/JPPP.2013.87410
14. Shuli, F., A.H. Jarwar, X. Wang, L. Wang and Q. Ma. 2018 Overview of the cotton in Pakistan and its future prospectics. Pak. J. Agric. Res., 31: 396. http://dx.doi.org/10.17582/journal.pjar/2018/31.4.396.407
15. Talley, Y.M., R.I. Thote, S.A. Nimbekar. 2009. Assessment of losses due to insect pest of cotton and benefit of protection schedule. J. Plant Prot. Res. 12:88-91.
16. Waghmare, V.N., J. Rong, C.J. Rogers, G.J. Pierce, J.F. Wendel and A.H. Paterson. 2005. Genetic mapping of a cross between Gossiypium hirustum and the Hawaiian endemic, Gossipiyum tomentosum. Thero. Appl. Genet., 111: 665-676. https://doi.org/10.1007/s00122-005-2032-6.

Management of Bt. resistance in *Pectinophora gossypiella* against Bt. Cultivar through non-Bt refugia

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Abstract

Transgenic cotton provides eco-friendly management of cotton bollworms, enhances pest control by increasing the natural enemies, reduces the reliance on insecticide, and increases farmer profits. However, due to the extensively farming of Bt cotton, all its benefits are affected due to evolution of resistance in bollworms. Refugia can be a source for decreased infestation of Pectinophora gossypiella (Saunders). The present study evaluates the different methods of refuge (i.e. row, border, block, and seed mix) in cotton. Infestation of P. gossypiella in Bt cotton with refuge was high as compared to Bt cotton with no refuge. Results showed that the average range of P. gossypiella infestation to flowers, green bolls, and open bolls on Bt cotton plants having refuge was 1-2, 4 to 58, and 9-22, respectively, then Bt cotton plants having no refuge, during 2020 and 2021. Infestation of P. gossypiella to the flower of Bt genotype with refuge was observed in September, to green bolls in October as compared to Bt genotypes having no refuge where infestation was observed from August to September. Refugia cultivation approaches (row, border, block, and seed mix) exhibited significantly minimum occurrence of P. gossypiella. An average yield of all refuge treatments ranged from 16 to 17 kg per plot to Bt (mean of 4-7). The observed outcomes suggest that the refuge strategy delays the evolution of resistance in P. gossypiella This strategy can be used as managing approaches against P. gossypiella in Bt cotton.

Keywords: *Border, block, green boll infestation, open boll infestation, Pectinophora gossypiella, row, refuge crop, seed mixture, transgenic cotton*

Highlights

Infestation of Pectinophora gossypiella on Bt Cotton without refuge ranged between 40- 100% as compared to Bt cotton having refuge. Intensification of pink bollworm damage to green bolls, and open bolls, was observed increasing with crop season. Pectinophora gossypiella has continued its pestilence against Bt cotton without refuge as compared to Bt cotton with refuge which benefits the country cotton production and also maximizes the grower profit.

Introduction

Pectinophora gossypiella (Saunders) Lepidoptera: Gelechiidae, is destructive pest of cotton (Sarwar, 2017)and causes 20-30% loss in the yield (Ahmed *et al.,* 2005). Repetitive use of conventional insecticides has given rise to resistance evolution in bollworms (Forrester *et al.,* 1993; McCaffery, 1998; Yang *et al*., 2000; Kranthi *et al*., 2002). For bollworms management, Bt cotton was developed as an alternate. (Wu and Guo, 2005). Cultivation of Bt cotton decreased insecticide use along with management of target pest, increased biodiversity and farmer's profits (Wu and Guo, 2005; Hutchison *et al*., 2010; Tabashnik *et al.,* 2010; Lu *et al*., 2012; Downes *et al.,* 2016). It

was approved as insecticidal crop in the USA in 19961996 and thereafter in other countries including Pakistan (2010) (James, 2006)

Upon infestation of Bt cotton Cry1Ac is produced which act as insecticidal agent (Wilson *et al.*, 1992; Flint *et al.*, 1995; Flint and Parks, 1999; Wu *et al.,* 2000; Shelton *et al.*, 2002; Mendelsohn *et al.*, 2003) (Wilson *et al*., 1992; Flint *et al*., 1995; Flint and Parks, 1999; Wu *et al*., 2000; Shelton *et al*., 2002; Mendelsohn *et al*., 2003) (Roush, R.T. 1994). Due to the extensive cultivation of Bt cotton, insect pests developed resistance against Cry1AC (Dhurua and Gujar, 2011; Tabashnik *et al.*, 2013; Gassmann *et al.*, 2014). Monophagous lepidopteran pests like *P. gossypiella* became resistance too against Cry1Ac (Tabashnik *et al.*, 2013; Jin *et al.*, 2015; Sansinenea, 2019).

The effectiveness and intensity of Cry1Ac protein depend upon the potential of genotypes to produced Bt protein, plants parts, and growth stage (Kranthi *et al.,* 2005; Olsen *et al.*, 2005; Wan *et al.*, 2012). It also affects the biological factors of target insect species due to the life parameters of insect's relay on larval stage, and the quantity of ingested Cry1Ac toxin (Stewart *et al.*, 2001). Kranthi *et al.,* (2005) reported that an expression level of 1.9 µg/g has a lethal effect against *Helicoverpa armigera,* below this level can cause the evolution of a high degree of resistance in insect against Cry1Ac. Tabashnik *et al.,* (2013) also documented that pink bollworm selected from the laboratory exhibited cross-resistance of up to 420-fold against Cry1Ac and resistance of 240-fold to Cry2Ab.

To have the good management of bollworms in cotton, Cry1AC must be in higher concentration. For the management of resistance against the crop, the insect pest population should be converted from resistant to susceptible. This conversion is possible through cultivation of refugia among the Bt cotton plants (Gould, 1998; Tabashnik *et al.,* 2005; Tabashnik *et al.,* 2008; Tabashnik *et al.,* 2009; Carrière *et al.*, 2010; Hutchison *et al.*, 2010; Tabashnik *et al.*, 2013; Jin *et al.*, 2015; Downes *et al.,* 2016). Refugia cultivation limits the selection pressure on target insect pest and improves the life span of transgenic cotton (Onstad, 1986; Grettenberger and Tooker, 2015; Jin *et al.*, 2015). Cultivation of refuge (non-Bt) in Pakistan is not practiced and *P. gossypiella* populations are highly resistant against Bt cotton. This study is designed to evaluate the effect of different refugia approaches in Bt cotton. The refugia cultivation could be a source of managing resistance in *P. gossypiella*.

Materials and Methods

Collection of cotton genotypes

Cotton varities (Bt) Tassco-115 and (non Bt) CRID-644 were obtained from Pakistan Central Cotton Committee (PCCC).

Cultivation of cotton genotypes

Seed bed was prepared by tillage and DAP application. Cotton seeds were treated with Thiamoethoxam and Azoxystrobin one day prior to sowing. Before germination of the crop, Glyphosate was applied. Tassco 115 was intercropped with CRIS-644 by row, boarder, block and seed mixed approaches (Fig. 1). No insecticide was applied for management of bollworms. The crop was observed for different parameters explained below.

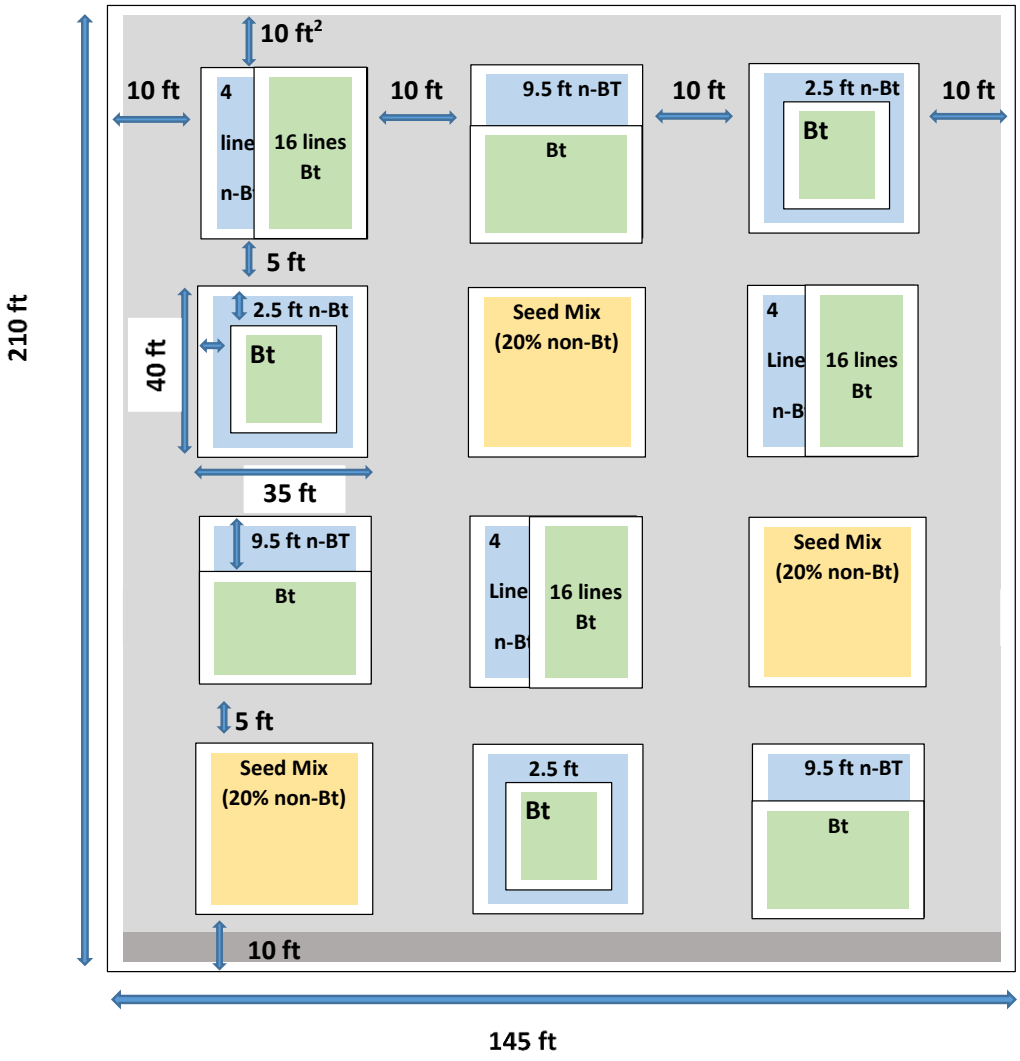

Fig. 1: Layout of Refuge trial

Rosette flower infestation:

At the onset of flowering, *P. gossypiella* infestation to rosette flower was observed. Tested cotton field was randomly selected for nine places (having three cotton plants of Bt and non Bt strains). Total number of infested and healthy flowers were calculated at selected sites. Percent infestation was calculated by the equation given below. (Shrilakshmi and Udikeri, 2021);

$$\text{Flower Infestation } (\%) = \frac{\text{Infested/Rosette flower}}{\text{Total flower observed}} \times 100 \quad \text{.....(1)}$$

Green bolls infestation:

To observe the green boll infestation, green bolls from Bt cotton and non Bt cotton plants were collected from (selected sites) monthly (till harvesting). Collected bolls were placed in laboratory and dissected after four days for presence of *P. gossypiella* larvae. The infestation was calculated by the equation given below.(Shrilakshmi and Udikeri, 2021);

$$\text{Closed Green Boll Infestation (\%)} = \frac{\text{Infested bolls}}{\text{Total bolls collected}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Open bolls infestation per plant:

At the picking season, five cotton plants from each treatment were selected randomly and observed for open boll infestation. The infestation was calculated by the equation given below.

$$\text{Open Boll Infestation (\%)} = \frac{\text{Infested open bolls}}{\text{Total open bolls}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Seed Cotton Yield:

From all treatments, the total seed cotton yield was documented in kg after harvesting each year in 2020 and 2021.

Statistical Analysis:

Infestation percentage to rosette flowers, green bolls, and open bolls per plant on all treatments during 2020 and 2021 were analyzed by using ANOVA (analysis of variance) and means were compared by using Tukey's honestly significant difference test (Tukey's HSD) (Statistics 8.1 software). Similarly, data on seed cotton yield was also analyzed by ANOVA and Tukey's HSD.

Results

Pink bollworm infestation to rosette flowers, green bolls, and open bolls was described below on different layouts of refuge at different plant growth stages (i.e. 60 to 130 days).

Flower Infestation to Non-Bt and Bt genotypes due to Pink bollworm:

Pink bollworms infestation of cotton flowers started in June and end in October in 2020 and 2021. But the incidence of pink bollworms (%) on flowers was started in August on Bt genotype plants (used as control) without no refuge as compared to Bt plants having refuge during 2020 and 2021 (Table. 1). In September 2020 and 2021, pink bollworm flower infestation (%) was observed on all method of refuge cultivation with highly significant difference (F=35.16; df=4,8; P=0.000) and (F=963.18; df=4,8; P=0.000) respectively. Pink bollworm infestation was declined on all tested refuge cultivation layouts except the Bt genotype in October 2020. While in October 2021, its incidence was also noted on all tested designs of refuge with a highly significant difference (F=166.08; df=4,8; P=0.000) as shown in Table (1).

Incidence of flower infestation (%) to Non Bt plants from all treatments were observed highly significant in September 2020 (F=65.66; df=3,6; P=0.000), September 2021 (F=58.71; df=3,6; P=0.000) and October 2021 (F=20.35; df=3,6; P=0.000) as shown in Table (2). But its infestation to flower was found non-significant in October 2020 (F=1.26; df=3,6; P=0.369). The average range of pink bollworm flower infestation to non-Bt plant (control plot) was noted from 2.24 to 10.95 in 2020 and 5.78 to 30.31 in 2021 as compared to non-Bt plants of other treatments (Table 2).

The data of pink bollworm flower infestation (%) on non-Bt plants from all treatments (having refuge and not having refuge) presented in Table (2), indicated that higher infestation was observed on non-Bt plants by comparing means of all treatments of both tables (1 and 2). The same pink bollworm flower infestation behavior was detected in August, September, and October during 2020 and 2021 on Bt plants (Table 1) and non-Bt plants (Table 2).

Pink bollworm Infestation (%) to Green Bolls on Non-Bt and Bt genotypes:

Pink bollworm infestation (%) to Green bolls of Bt genotype

The data presented in figure 2a displayed the incidence of pink bollworm to green bolls from Bt plants of all refuge treatments and Bt genotype during 2020 and 2021. Infestation was recorded highly significant on Bt plants of all treatments in August (F=2791.00; df=4,8; P=0.000), September (F=75.02; df=4,8; P=0.000) and October (F=21.64; df=4,8; P=0.000) during 2020. In 20201, extremely significant difference was also access on Bt plants of all Bt genotype across the season as in August (F=52.12; df=4,8; P=0.000), September (F=1010.91; df=4,8; P=0.000) and October (F=9.53; df=4,8; P=0.003) as showed in fig. (2a).

Pink bollworm larval presence in green bolls of Bt plants from all treatments was presented in figure 2b. This data showed that the incidence of larvae on green bolls picked from Bt plants of all refuge and Bt genotype was found to increase from August to October in 2020 and 2021. A highly significant occurrence of larvae/green bolls of Bt plants was detected on all tested treatments from August to October in 2020 (August (F=95.91; df=4,8; P=0.000), September (F=81.05; df=4,8; P=0.000) and October (F=24.30; df=4,8; P=0.000)) as well as in August (F=15.09; df=4,8; P=0.000), September (F=17.74; df=4,8; P=0.000), and October (F=28.79; df=4,8; P=0.000) in 2021 (Fig. 2b). The average of larvae per boll was detected from 0.67-3.18 larvae/boll.

Table 1: Pink bollworm infestation (%) to flower from Bt plants of different layouts of refuge cultivation and Bt genotype

Treat.	2020				2021			
	July[a]	August	September	October	July	August	September	October
RO	00.00± 00.00[b]	00.00 ± 00.00b	00.51 ± 00.51b	00.00 ± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	00.58 ± 00.36b	00.00 ± 00.00b
BO	00.00 ± 00.00	00.00 ± 00.00b	01.28 ± 00.36b	00.00 ± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	00.28 ± 00.28b	00.57 ± 00.57b
BL	00.00 ± 00.00	00.00 ± 00.00b	01.37 ± 00.41b	00.00± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	00.82 ± 00.53b	00.68 ± 00.34 b
SM	00.00 ± 00.00	00.00 ± 00.00b	1.11 ± 00.55b	00.00 ± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	00.19 ± 00.10b	00.39 ± 00.19b
COBt	00.00 ± 00.00	02.46 ± 00.67a	08.54 ± 00.89a	05.32 ± 05.32a	00.00 ± 00.00	08.32 ± 00.32a	25.61 ± 00.49a	28.56 ± 01.92a
P		0.001	0.000	0.460		0.000	0.000	0.000
F		13.61	35.16	1.00		652.90	963.18	166.08
df	4,8	4,8	4,8	4,8	4,8	4,8	4,8	4,8

[a] Data was recorded from July to October during 2020-2021.

[b] Entries in the same column, for Pink bollworm infestation (%) to flower, followed by different letters are significantly different (P < 0.05) and the same letter show not significantly different (P > 0.05). Means were separated using Tukey's HSD test. Data shown are means of three replications; values are means ± standard errors.

Table 2: Pink bollworm flower infestation (%) from non-Bt plants of different designs of refuge cultivation and non-Bt genotype

Treat.	2020				2021			
	July[a]	August	September	October	July	August	September	October
RO	00.00± 00.00[b]	00.00 ± 00.00b	03.90 ± 00.29b	00.00 ± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	02.40 ± 00.97b	05.70 ± 01.93b
BO	00.00 ± 00.00	00.00 ± 00.00b	03.16 ± 00.46b	00.46 ± 00.46a	00.00 ± 00.00	00.00 ± 00.00b	02.06 ± 00.73b	06.83 ± 02.65 b
BL	00.00 ± 00.00	00.00 ± 00.00b	03.70 ± 00.57b	00.00 ± 00.00a	00.00 ± 00.00	00.00 ± 00.00b	01.68 ± 00.13b	07.26 ± 03.76 b
CONBt	00.00 ± 00.00	02.24 ± 00.19a	10.95 ± 00.55a	06.47 ± 05.54a	00.00 ± 00.00	05.78 ± 00.64a	12.91 ± 00.67a	30.31 ± 02.13a
P		0.000	0.000	0.369		0.000	0.000	0.001
F		128.87	65.66	1.26		81.08	58.71	20.35
df	3,6	3,6	3,6	3,6	3,6	3,6	3,6	3,6

[a]Data was recorded from July to October during 2020-2021.

[b] Entries in the same column, for Pink bollworm flower infestation (%), followed by different letters are significantly different ($P < 0.05$) and the same letter show not significantly different ($P > 0.05$). Means were separated using Tukey's HSD test. Data shown are means of three replications; values are means ± standard errors.

Percent Pink bollworm infestation to Green bolls of non-Bt genotype

The incidence of pink bollworm to green bolls of non-Bt plants of all treatments was recorded extremely significant in in August (*F*=89.48; df=3,6; *P*=0.000), September (*F*=17.81; df=3,6; *P*=0.002), and October (*F*=12.00; df=3,6; *P*=0.006) in 2020. Similar infestation to green bolls was examined from August to October ((*F*=85.53; df=3,6; *P*=0.000), (*F*=431.74; df=3,6; *P*=0.002), (*F*=5.86; df=3,6; *P*=0.032)) in 2021. Maximum green bolls damage to non Bt plants of non-Bt genotype (without refuge) was detected across season during 2020 and 2021 (Fig. 3a). The numbers of pink bollworm larvae in green bolls of non-Bt plants of all investigated treatments was recorded significant in August (*F*=17.72; df=3,6; *P*=0.002), September (*F*=05.07; df=3,6; *P*=0.043) and October (*F*=8.88; df=3,6; *P*=0.01) during 2020 as well as in August (*F*=07.95; df=3,6; *P*=0.016), September (*F*=186.71; df=3,6; *P*=0.000) and October (*F*=17.53; df=3,6; *P*=0.002) during 2021 (fig. 3b).

Pink bollworm percent infestation to Green bolls of different tested layout of refuge

The pink bollworm damage to green bolls from all refuge treatments and non-refuge treatments was noted highly significant in August (*F*=199.35; df=5,10; *P*=0.000), September (*F*=88.15; df=5,10; *P*=0.000), and October (*F*=15.85; df=5,10; *P*=0.000) in 2020. Pink bollworm infestation to green bolls of all tested treatments in 2021 was also examined extremely significant in August (*F*=47.62; df=5,10; *P*=0.000), September (*F*=1086.11; df=5,10; *P*=0.000), and October (*F*=115.29; df=5,10; *P*=0.000). Greater green bolls infestation means was detected to non Bt and Bt genotype (without refuge) range from 70-100 than other genotype having refuge across season during 2020 and 2021(Fig. 4a).

Larval presence of pink bollworm in green bolls of all investigated treatments with refuge and without refuge was presented in figure (4b). Extremely significant difference was observed in August (*F*=19.13; df=5,10; *P*=0.001), September (*F*=08.21; df=5,10; *P*=0.002) and October (*F*=49.64; df=5,10; *P*=0.000) in 2020. Similar presence of larvae to green bolls on refuge treatments and Bt and non-Bt genotype without no refuge was also detected greatly significant in August (*F*=05.87; df=5,10; *P*=0.008), September (*F*=72.90; df=5,10; *P*=0.000) and October (*F*=28.74; df=5,10; *P*=0.000) in 2021 (fig. 4b).

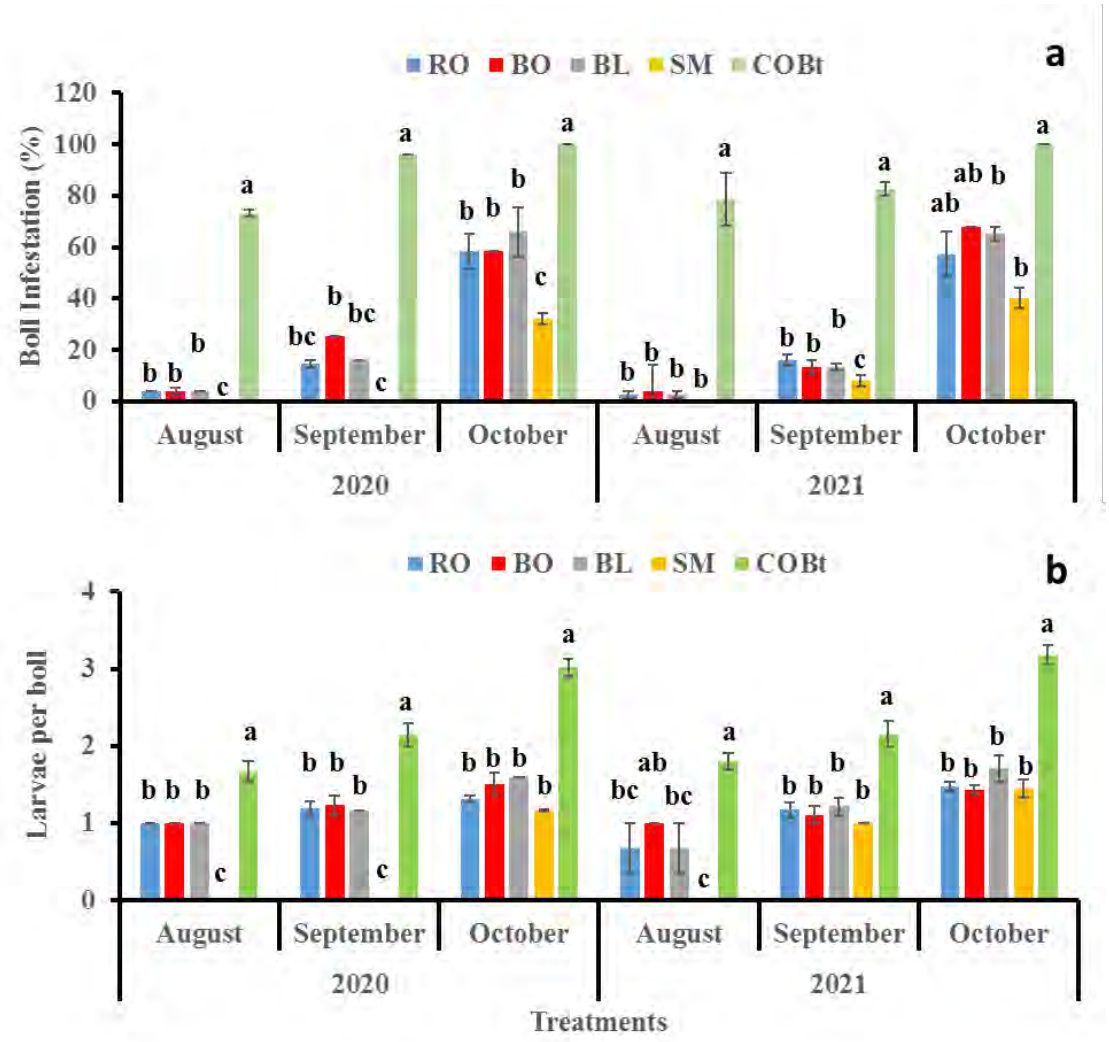

Figure 2: Percent Infestation of Pink bollworm to Bt plants on different tested refuge layout during different months of 2020 and 2021 (a) Green bolls Infestation (b) Presence of larvae per boll

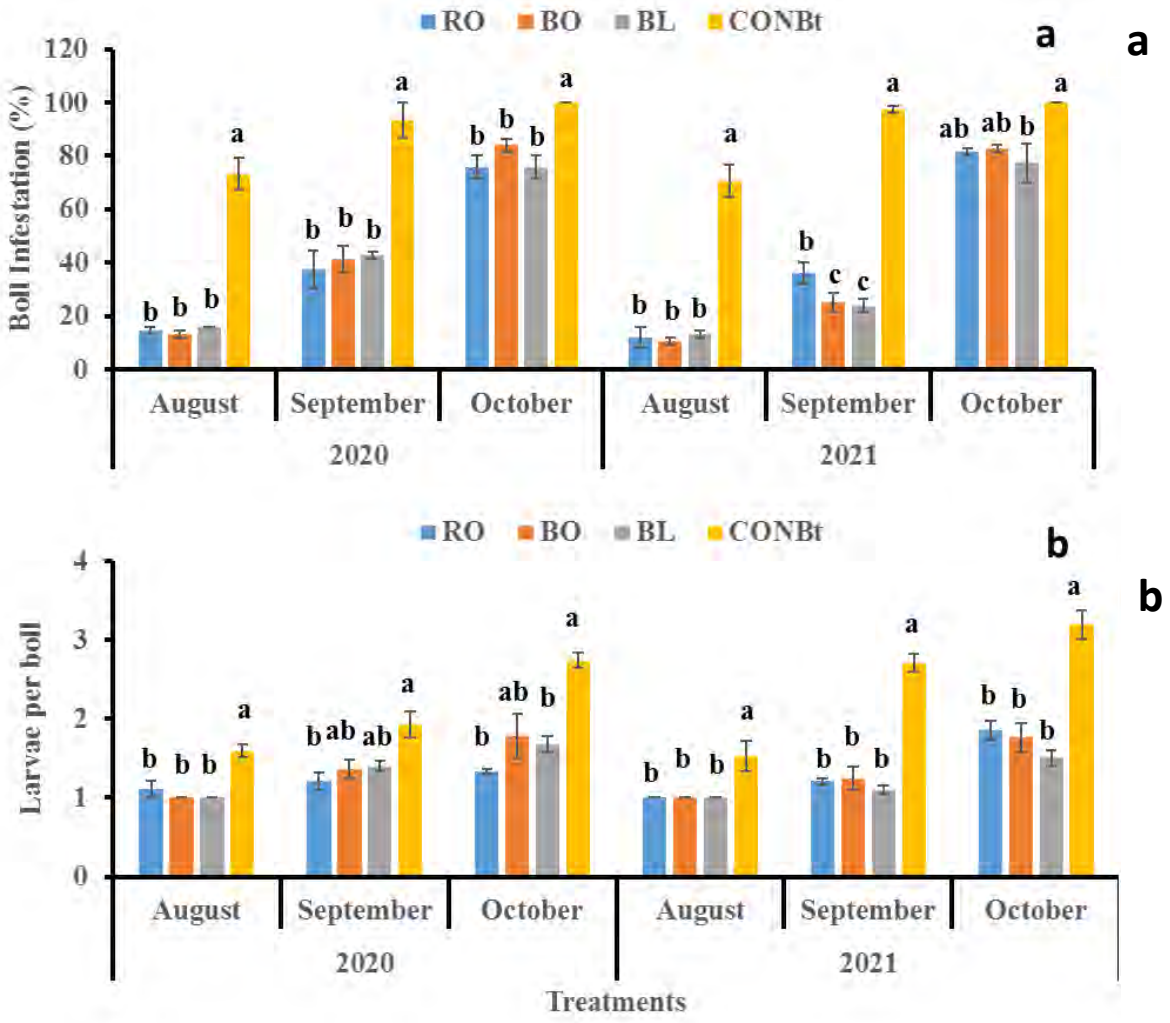

Figure 3: Pink bollworm Percent Infestation to non-Bt plants on all tested layout of refuge across different months during 2020 and 2021 (a) Green bolls Infestation (b) Presence of larvae per boll

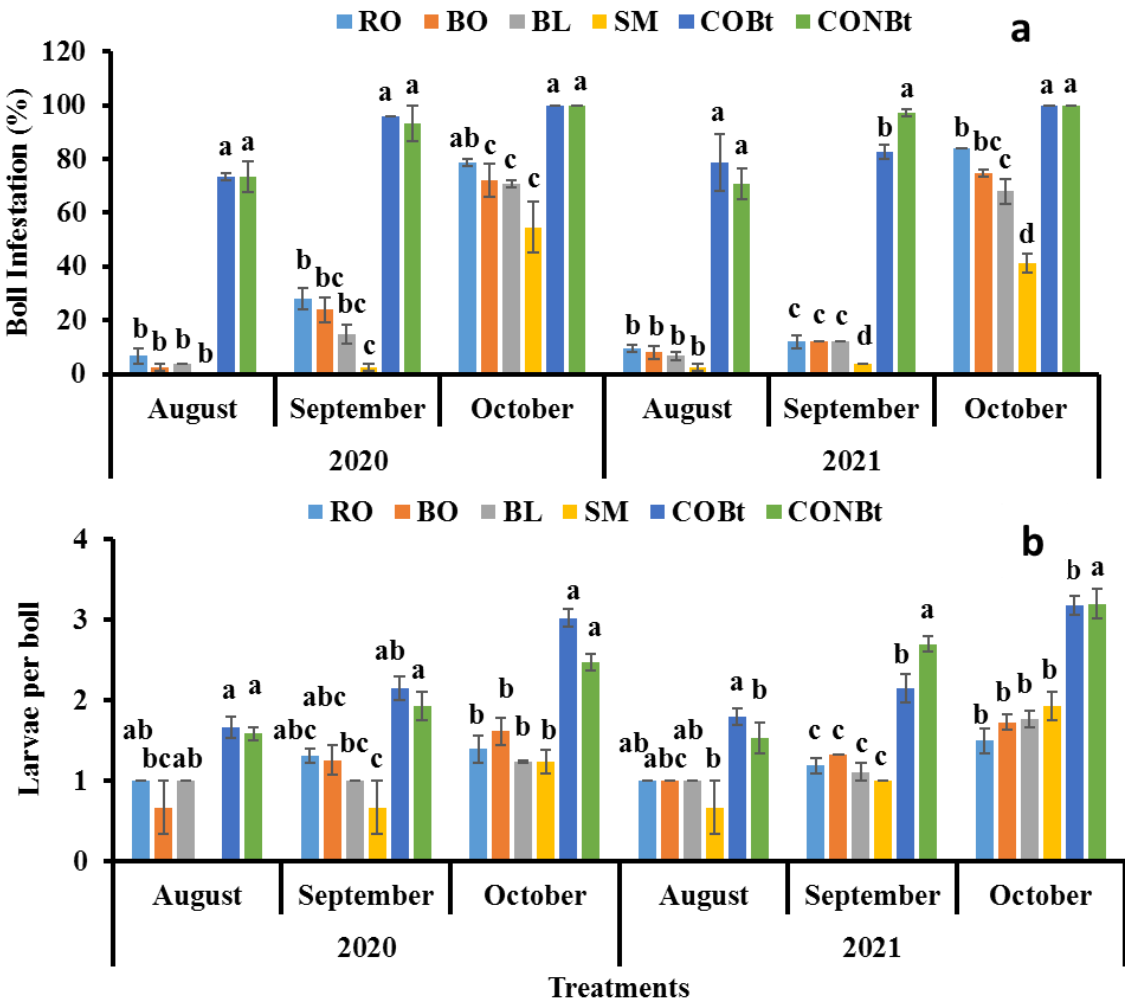

Figure 4: Pink bollworm Infestation (%) to all treatments on all tested refuge layout with different months during 2020 and 2021 (a) Green bolls Infestation (b) Presence of larvae per boll

Open bolls infestation per plant on Bt and Non-Bt genotypes:

Presented data in fig (5a) showed that pink bollworm infestation to open bolls on Bt plants of all investigated treatments was recorded highly significantly different during 2020 (F=11.98; df=4,8; P=0.001) and (F=11.70; df=4,8; P=0.002) 2021. Maximum open bolls/ Bt plant damage was detected in the Bt genotype (without no refuge) and the minimum infestation was observed on all Bt plants of the Bt genotype (with refuge) as shown in Fig (5a). Pink bollworm open bolls infestation to Non-Bt plants of all tested treatments was observed non-significant in 2020 (F=2.30; df=3,6; P=0.177) as well as 2021 (F=1.21; df=3,6; P=0.383) as presented in fig. (5b). Open bolls damage was recorded the same between Non-Bt plants of tested treatments and also the same in both years from 2020 to 2021 (Fig. 5b).

Seed Cotton Yield:

The seed cotton yield data (kg/plot) presented that highly significant difference was exist between treatments during 2020 (F=14.32; df=5,10; P=0.000) and 2021 (F=45.17; df=5,10; P=0.000) as displayed in Fig. (6a). This yield was higher and the same in refuge layouts as

compared to Bt and non-Bt genotype (used as control and was without refuge). Seed cotton yield in kg/ plot was recorded the same in both years 2020 and 2021. No significant difference (F=1.71; df=5,22; P=0.204) was observed in yield during 2020-2021 (Fig. 6b).

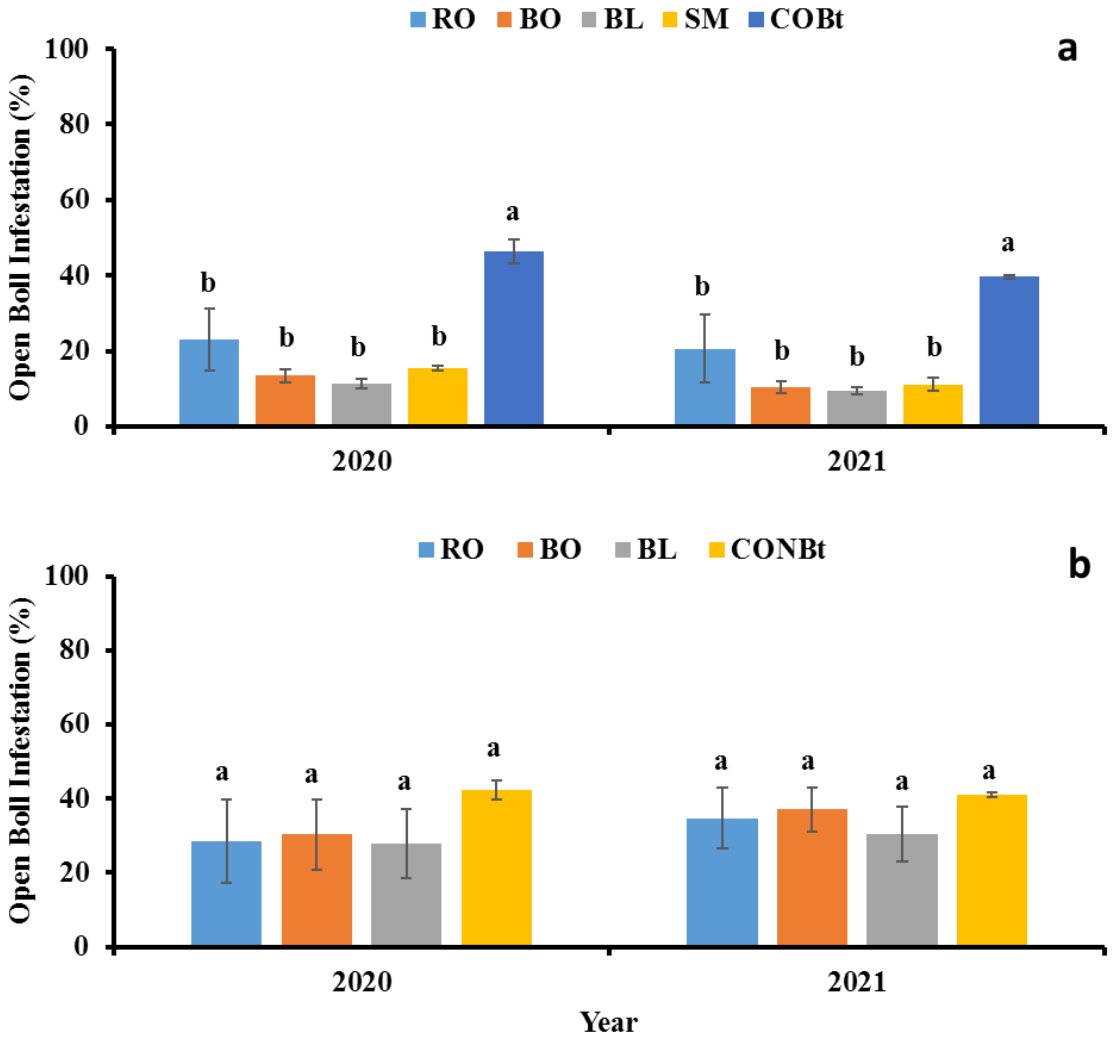

Figure 5: Percent Infestation of Pink bollworm to open bolls all treatments during 2020 and 2021 (a) Percent Infestation to Bt plants (b) Infestation percentage to non-Bt plants

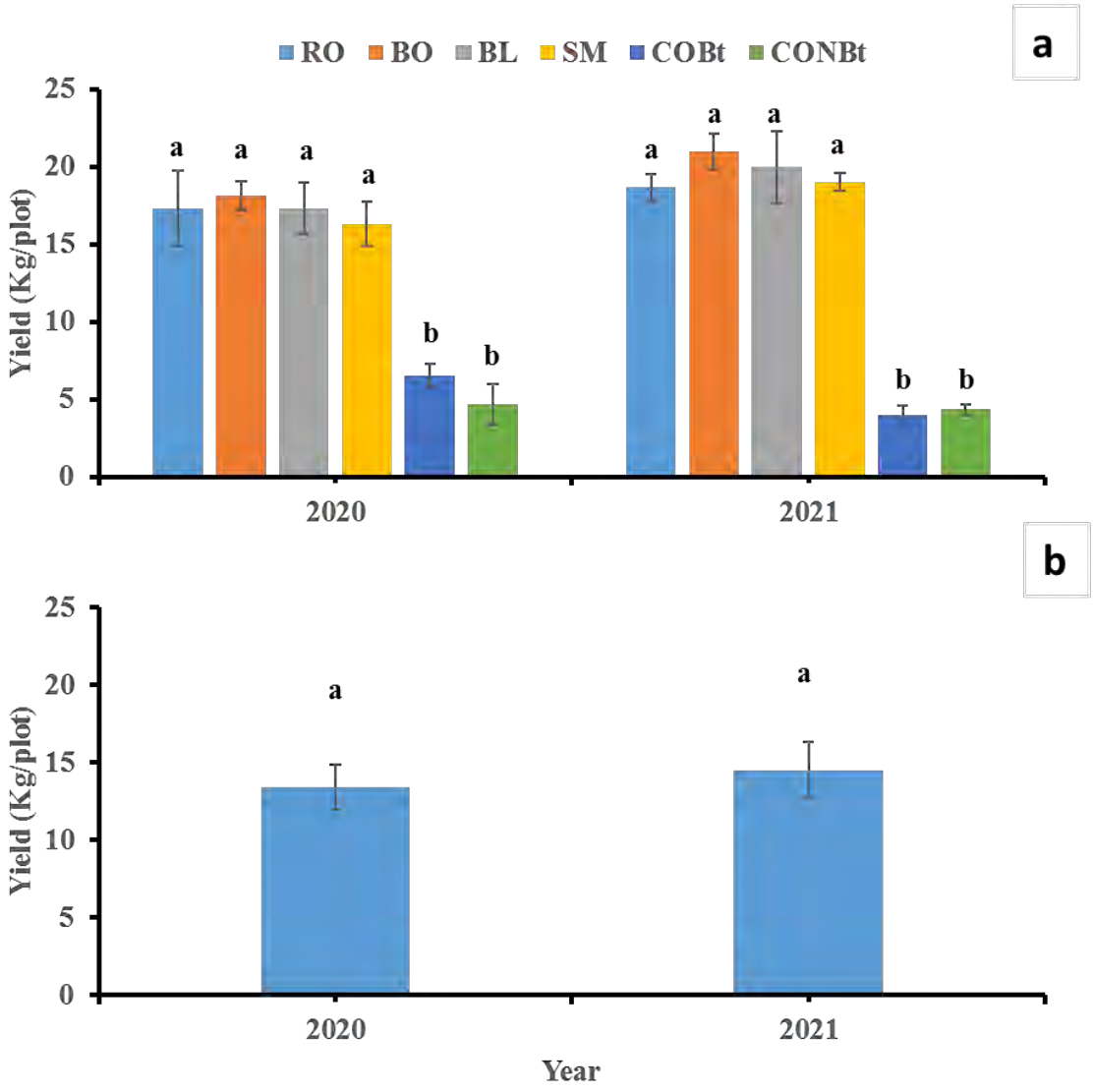

Figure 6: Seed cotton yield (kg/plot) during 2020 and 2021 (a) from all layouts of refuge cultivation with Bt and non-Bt genotypes (b) Overall Seed cotton yield (kg/plot) from all treatments during 2020 and 2021

Discussion:

The study explained the effect of refugia in Bt cotton for the management of *P. gossypiella* at flower, green boll and open boll stage. At flower stage the occurrence of *P. gossypiella* was found less in Bt cotton with refuge as compared to non-refuge experimental units. Related studies exhibited the high occurrence of *P. gossypiella* in (Bt cotton) in flower and open boll stage (Udikeri, 2006; Onkaramurthy *et al.,* 2016). Infestation of pink bollworm to green bolls was recorded high on Bt cotton having no refuge (Naik *et al.*, 2021; Shrilakshmi and Udikeri, 2021).

Infestation of *P. gossypiella* on rosette flower was recorded maximum in Bt cotton without any refugia, which is supported by the results of similar studies (Naik *et al.*, 2021). Pink bollworm damage to green bolls was observed maximum (average range of from 73-100) on treatment (CoBt and CoNBt) having no refuge as compared to others having refuge across the season from August to October during 2020.

Similarly, its infestation to open bolls of refuge treatments (row, border, block, and seed mix) was lowest (mean range from 9 to 22) than open bolls of non-refugee treatment (average of 39 to 46) during 2020 and 2021. Studies also supported these results by exhibiting less infested bolls on Bt as compared to non-Bt cotton (refuge) (Carrière *et al.,* 2001). Green boll infestation, in another study, was recorded low on Bt cotton having non-Bt cotton intercropped as refuge (Gujar *et al.*, 2010).

Yield was observed significantly different among all treatments during 2020 and 2021. Maximum yield was obtained from refuge treatment compared to the treatment without refuge in 2020 and 2021. Yield in Kg per acre was the same for all treatments in 2020 while significantly different in 2021. During 2020 and 2021, the yield was found same. (Cotton hybrid (F2) was developed in the Yangtze River Valley of China, which has in-built refuge of non-Bt plants and expressed higher yield. (Wan *et al.,* 2017). Other reports of non Bt cotton as a refuge treatments also proved to have impact on *P. gossypiella* infestation and yield(Gujar *et al.*, 2010).

This study showed that non-Bt (as a refuge) helps maintain the resistance level of Bt genotypes against pink bollworm by lowering the selection pressure (Huang *et al.,* 2006). Refuge delay the resistance by increasing the number of susceptible individuals that mate with resistant individuals and delaying the resistance by diluting the population (Gould, 1998; Tabashnik *et al.*, 2004; Hagerty *et al.*, 2005; Tabashnik *et al.,* 2005; Tabashnik *et al.,* 2008; Carrière *et al.*, 2010). Wan *et al.,* (2012) also suggested that non-Bt cotton (as a refuge) must be sowed with transgenic Bt cotton on a large scale for the management of pink bollworm.

Many simulation models and research experiments were conducted to evaluate the influence of block and seed blend refuge on the evolution of resistance (Crowder and Onstad, 2005; Onstad *et al.*, 2006, 2011; Grettenberger and Tooker, 2015). Limited empirical suggestion and simulation models accessed that the efficiency of the refuge tactic depends intensely on refuge size, genetic basis and alleles frequency of resistance, costs of life-history related to resistance, level of mating between the susceptible and resistant adults, and level of mortality due to transgenic crop (Tabashnik, 1990; Roush, 1994; Liu and Tabashnik, 1997; Gould, 1998; Peck *et al.*, 1999; Shelton *et al.*, 2000, 2002).

Results of some simulated modeling showed that seed blend accelerates the evolution of resistance by increasing the dominance of resistance or reducing the susceptible insect survival because in this tactic target insect feed on both Bt and non-Bt plants (Mallet and Porter, 1992; Tabashnik, 1994; Tabashnik *et al.*, 2004; Onstad *et al.*, 2006; Heuberger *et al.*, 2011; Ives *et al.*, 2011). The zero refuge strategy was followed in China because there is extensive cultivation of many crops with transgenic cotton that crops support the *Helicoverpa armigera* population as an alternate host to Bt cotton (Wu *et al.*, 2002; Wu, K. M. Guo, 2005; Gao *et al.*, 2009). So its population from these hosts produces a susceptible population that dilutes the resistance surviving population of *H. armigera* from Bt cotton (Huang *et al.*, 2010).

An extensive outbreak of pink bollworm infestation on Bt cotton genotypes existed in Pakistan. However, any published research article has not yet proved yet pink bollworm resistance. We cannot ignore that the enormous incidence of pests in one part of the country might threaten the other parts. Hereafter, it is time to devise appropriate policies and strategies (by implementing non-Bt cotton as a refuge) for the eco-friendly pink bollworm resistance management against the Bt cotton genotype.

Conclusion

Resistance evolution decrease the Based on the results, it is determined that the highest infestation of pink bollworm was recorded on all Bt genotypes without refuge than in Bt genotype with refuge. It is also concluded that the damage of pink bollworm to flower, green bolls, and open bolls, was observed maximum in late season as increasing from August to October Besides, further studies were compulsory for the deployment of transgenic cotton having Bt levels high as lethal for bollworm and also approved refuge as resistance management strategies in Pakistan.

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References:

1. Ahmed, G., M.J., A. and S.M.I.W.S. 2005. Studies regarding resistance in different genotypes of cotton against bollworm complex. Int. J. Agric. Biol. 5:196-198.
2. Brévault, Thierry Tabashnik, Bruce E. Carrière, Y. 2015. A seed mixture increases dominance of resistance to Bt cotton in Helicoverpa zea. Sci. Rep. 5.
3. Brévault, T., S. Heuberger, M. Zhang, C. Ellers-Kirk, X. Ni, L. Masson, X. Li, B.E. Tabashnik and Y. Carrière. 2013. Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc. Natl. Acad. Sci. U. S. A. 110.
4. Carrière, Yves, Dennehy, Timothy J., Pedersen, Brent Haller, Shirley Ellers-Kirk, Christa Antilla, Larry Liu, Yong Biao Willott, Elizabeth, Tabashnik, B.E. 2001. Large-scale management of insect resistance to transgenic cotton in Arizona: Can transgenic insecticidal crops be sustained? J. Econ. Entomol. 94.
5. Carrière, Y., D.W. Crowder and B.E. Tabashnik. 2010. Evolutionary ecology of insect adaptation to Bt crops. Evol. Appl. 3.
6. Crowder, D.W. and D.W. Onstad. 2005. Using a generational time-step model to simulate dynamics of adaptation to transgenic corn and crop rotation by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98.
7. David W. Onstad. 1986. Insect Resistance Management Biology, Economics, and Prediction, 2nd edition.
8. Dhurua, S. and G.T. Gujar. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag. Sci. 67.
9. Downes, Sharon Walsh, T. and W.T. Tay. 2016. Bt resistance in Australian insect pest species.
10. Downes, S., T. Parker and R. Mahon. 2010. Incipient resistance of Helicoverpa punctigerato the Cry2AB Bt toxin in bollgard II® cotton. PLoS One 5.
11. Flint, H. M. Parks, N.J. 1999. Seasonal infestation by pink bollworm, Pectinophora gossypiella (Saunders), of transgenic and non-transgenic cultivars of cotton, Gossypium hirsutum L., in central Arizona. Southwest. Entomol. 24:13–20.
12. Flint, H.M., T.J. Henneberry, F.D. Wilson, E. Holguin, N. Parks and R.E. Buehler. 1995. The effects of transgenic cotton, Gossypium hirsutum L., containing Bacillus thuringiensis toxin genes for the control of the pink bollworm, Pectinophora gossypiella (Saunders) and other arthropods. Southwest. Entomol. 20:281–292.
13. Forrester et al. 1993. Management of pyrethroid and endosulfan resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) in Australia. Bull. Entomol. Res. Suppl. Ser.
14. Gao, Y., K. Wu and F. Gould. 2009. Frequency of bt resistance alleles in H. armigera during 2006-2008 in Northern China. Environ. Entomol. 38.
15. Gassmann, A.J., J.L. Petzold-Maxwell, E.H. Clifton, M.W. Dunbar, A.M. Hoffmann, D.A. Ingber and R.S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple Bacillus thuringiensis toxins in transgenic maize. Proc. Natl. Acad. Sci. U. S. A. 111.
16. Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology.
17. Grettenberger, I.M. and J.F. Tooker. 2015. Moving beyond resistance management toward an expanded role for seed mixtures in agriculture.
18. Gujar, G.T., V. Kalia, G.K. Bunker and S. Dhurua. 2010. Impact of different levels of non-Bt cotton refuges on pest populations, bollworm damage, and Bt cotton production. J. Asia. Pac. Entomol. 13.

19. Hagerty, A.M., A.L. Kilpatrick, S.G. Turnipseed, M.J. Sullivan and W.C. Bridges. 2005. Predaceous arthropods and lepidopteran pests on conventional, bollgard, and bollgard II cotton under untreated and disrupted conditions. Environ. Entomol. 34.
20. Heuberger, S., D.W. Crowder, T. Brévault, B.E. Tabashnik and Y. Carrière. 2011. Modeling the effects of plant-to-plant gene flow, larval behavior, and refuge size on pest resistance to bt cotton. Environ. Entomol. 40.
21. Huang, J.K., L. Hai, R.F. Hu, S.D. Rozelle, and C.P. 2006. Eight years of Bt cotton in farmer fields in China: has the bollworm population developed resistance. 10th Annu. Int. Consort. Agric. Biotechnol. Res. Conf. 29.
22. Huang, J.K., J.W. Mi, H. Lin, Z.J. Wang, R.J. Chen, R.F. Hu, S. Rozelle and C. Pray. 2010. A decade of Bt cotton in Chinese fields: Assessing the direct effects and indirect externalities of Bt cotton adoption in China. Sci. China Life Sci. 53.
23. Hutchison, W.D., E.C. Burkness, P.D. Mitchell, R.D. Moon, T.W. Leslie, S.J. Fleischer, M. Abrahamson, K.L. Hamilton, K.L. Steffey, M.E. Gray, R.L. Hellmich, L. V. Kaster, T.E. Hunt, R.J. Wright, K. Pecinovsky, T.L. Rabaey, B.R. Flood and E.S. Raun. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science (80-.). 330.
24. Ives, A.R., P.R. Glaum, N.L. Ziebarth and D.A. Andow. 2011. The evolution of resistance to two-toxin pyramid transgenic crops. Ecol. Appl. 21.
25. James, C. 2006. A PPLICATIONS ISAAA Briefs BRIEF 35 Global Status of Commercialized Biotech / GM Crops : 2006 BRIEF 35 Global Status of Commercialized Biotech / GM Crops : 2006.
26. Jin, L., H. Zhang, Y. Lu, Y. Yang, K. Wu, B.E. Tabashnik and Y. Wu. 2015. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. Nat. Biotechnol. 33.
27. Kranthi, K. R., Naidu, S., Dhawad, C. S., Tatwawadi, A. and S. Mate, K., Patil, E., Bharose, A. A., Behere, G. T., Wadaskar, R. M., Kranthi. 2005. Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera). Curr. Sci. 89.
28. Kranthi, K.R., D.R. Jadhav, S. Kranthi, R.R. Wanjari, S.S. Ali and D.A. Russell. 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 21.
29. Liu, Y.B. and B.E. Tabashnik. 1997. Experimental evidence that refuges delay insect adaptation to Bacillus thuringiensis. Proc. R. Soc. B Biol. Sci. 264.
30. Lu, Y., K. Wu, Y. Jiang, Y. Guo and N. Desneux. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487.
31. Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: Are seed mixtures or refugia the best strategy? Proc. R. Soc. B Biol. Sci. 250.
32. McCaffery, A.R. 1998. Resistance to insecticides in heliothine Lepidoptera: A global view. Philos. Trans. R. Soc. B Biol. Sci. 353.
33. Mendelsohn, M., J. Kough, Z. Vaituzis and K. Matthews. 2003. Are Bt crops safe?
34. Mohan, K.S., K.C. Ravi, P.J. Suresh, D. Sumerford and G.P. Head. 2016. Field resistance to the Bacillus thuringiensis protein Cry1Ac expressed in Bollgard® hybrid cotton in pink bollworm, Pectinophora gossypiella (Saunders), populations in India. Pest Manag. Sci. 72:738–746.
35. Naik, V.C.B., S. Kb, S. Kranthi, V.S. Nagrare, S. Kumbhare, N. Gokte-Narkhedkar and V.N. Waghmare. 2021. Pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) survival on transgenic cotton in India. Egypt. J. Biol. Pest Control 31.
36. Olsen, K.M., J.C. Daly, H.E. Holt and E.J. Finnegan. 2005. Season-long variation in expression of Cry1Ac gene and efficacy of Bacillus thuringiensis toxin in transgenic cotton against Helicoverpa armigera (Lepidoptera: Noctuidae). J. Econ. Entomol. 98.
37. Onkaramurthy, S.G., Goud, K.B. and Udikeri, S.S. 2016. Field performance of second generation (BG-II) Bt cotton genotypes against bollworm complex under rainfed conditions. J. Phytopathol. Pest Manag. 12-20.
38. Onstad, David W., Mitchell, Paul D. Hurley, Terrance M. Lundgren, Jonathan G., Patrick Porter, R. Krupke, Christian H. Spencer, Joseph L., Difonzo, Christine D., Baute, Tracey S. Hellmich, Richard L., Buschman, L.L., W.D. Hutchison and J.F. Tooker. 2011. Seeds of change: Corn seed mixtures for resistance management and integrated pest management. J. Econ. Entomol. 104.
39. Onstad, D.W., B.E. Hibbard, T.L. Clark, D.W. Crowder and K.G. Carter. 2006. Analysis of density-dependent survival of Diabrotica (Coleoptera: Chrysomelidae) in cornfields. Environ. Entomol. 35.
40. Peck, S.L., F. Gould and S.P. Ellner. 1999. Spread of resistance in spatially extended regions of transgenic cotton: Implications for management of Heliothis virescens (Lepidoptera: Noctuidae).
41. Roush, R.T. 1994. Managing Pests and Their Resistance to Bacillus thuringiensis: Can Transgenic Crops Be Better Than Sprays? Biocontrol Sci. Technol. 4.
42. Sansinenea, E. 2019. Applications and Patents of Bacillus spp. in Agriculture. Intellectual Property Issues in Microbiology.
43. Sarwar, M. 2017. Pink Bollworm Pectinophora gossypiella (Saunders) [Lepidoptera: Gelechiidae] Practices of Its Integrated Management in Cotton. Int. J. Plant Sci. Ecol 3:1–6.
44. Shelton, A.M., J.D. Tang, R.T. Roush, T.D. Metz and E.D. Earle. 2000. Field tests on managing resistance to Bt-engineered plants. Nat. Biotechnol. 18.
45. Shelton, A.M., J.Z. Zhao and R.T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants.
46. Shrilakshmi and Udikeri. 2021. Incidence of pink bollworm Pectinophora gossypiella Sanders in different agro-ecological zones of Karnataka. J. Entomol. Zool. Stud. 9:607–612.

47. Stewart, S.D., J.J. Adamczyk, K.S. Knighten and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of Bacillus thuringiensis berliner on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94.
48. Tabashnik, B.E., M.S. Sisterson,P.C. Ellsworth, T.J. Dennehy, L. Antilla, L. Liesner, M. Whitlow, R.T. Staten, J.A. Fabrick, and G.C.U. 2010. Suppressing resistance to Bt cotton with sterile insect releases. Nat. Biotechnol. 28:1304–1307.
49. Tabashnik, Bruce E., Gassmann, Aaron J. Crowder, David W., Carrière, Y. 2008. Insect resistance to Bt crops: Evidence versus theory.
50. Tabashnik, Bruce E., Van Rensburg, J. B.J., Carríre, Y. 2009. Field-evolved insect resistance to Bt crops: Definition, theory, and data. J. Econ. Entomol. 102.
51. Tabashnik, Bruce E.. Dennehy, Timothy J., Carrière, Y. 2005. Delayed resistance to transgenic cotton in pink bollworm. Proc. Natl. Acad. Sci. U. S. A. 102.
52. Tabashnik, B.E. 1990. Modeling and Evaluation of Resistance Management Tactics. Pesticide Resistance in Arthropods.
53. Tabashnik, B.E. 1994. Delaying insect adaptation to transgenic plants: Seed mixtures and refugia reconsidered. Proc. R. Soc. B Biol. Sci. 255.
54. Tabashnik, B.E., T. Brévault and Y. Carrière. 2013. Insect resistance to Bt crops: Lessons from the first billion acres.
55. Tabashnik, B.E., F. Gould and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J. Evol. Biol. 17.
56. Tabashnik, B.E., G.C. Unnithan, L. Masson, D.W. Crowder, X. Li and Y. Carrière. 2009. Asymmetrical cross-resistance between Bacillus thuringiensis toxins Cry1Ac and Cry2Ab in pink bollworm. Proc. Natl. Acad. Sci. U. S. A. 106.
57. Udikeri, S.S. 2006. Evaluation of new generation Bt cotton genotypes, Sustainability of Cry protein expression, computation of ETL, Effect on aphid predators and development of IPM module for Bt Cotton under rainfed conditions. Ph.D. Thesis, Univ. Agric. Sci. Dharwad, Karnataka, India.
58. Wan, P., D. Xu, S. Cong, Y. Jiang, Y. Huang, J. Wang, H. Wu, L. Wang, K. Wu, Y.C. and A.M. 2017. Hybridizing transgenic Bt cotton with non-Bt cotton counters resistance in pink bollworm. Proc. Natl. Acad. Sci. U. S. A. 114:5413–5418.
59. Wan, P., Y. Huang, H. Wu, M. Huang, S. Cong, B.E. Tabashnik and K. Wu. 2012. Increased frequency of pink bollworm resistance to Bt toxin Cry1Ac in China. PLoS One 7.
60. Wilson, D.F., H.M. Flint, R.W. Deaton, D.A. Fischhoff, F.J. Perlak, T.A. Armstrong, R.L. Fuchs, S.A. Berberich, N.J. Parks and B.R. Stapp. 1992. Resistance of Cotton Lines Containing a Bacillus thuringiensis Toxin to Pink Bollworm (Lepidoptera: Gelechiidae) and other Insects. J. Econ. Entomol. 85:1516–1521.
61. Wu, K., Guo, Y., Wang, W. 2000. Field resistance evaluations of Bt transgenic cotton GK series to cotton bollworm. Acta Phytophyl. Sin. 27:317–321.
62. Wu, K. M. Guo, Y.Y. 2005. The evolution of cotton pest management practices in China.
63. Wu, K., Y. Guo and S. Gao. 2002. Evaluation of the natural refuge function for Helicoverpa arnigera (Lepidoptera: Noctuidae) within Bacillus thuringiensis transgenic cotton growing areas in north China. J. Econ. Entomol. 95.
64. Yang, Y., Zhu, M., Wang, D. 2000. Yearly changes of the control effects of pyrethrums insecticides on Pectinophora gosspiella saunders. Acta Agr. Jiangsu. 16:92–96.

Effects of Ecological Approach on the Management of Cotton aphid, *Aphis gossypii* (Glov) in Selected Cotton Genotypes, Gezira State, Sudan

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Abstract

Background: *Cotton is grown in Sudan as rainfed crop, as well as grown by permanent irrigation. Usually insecticides were used to control cotton insect pests, but in Gezira scheme cotton produced for the first three decades (1911-1945) without chemical control. That might be due to the balanced nature. This research aims to reduce the cost of production and mitigating the environmental pollution through managing cotton aphid, Aphis gossypii (Glov) via ecological approach at Gezira State, Sudan.*

Methodology: *Two experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan during season 2013/14 and 2014/15. I each season a factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni, Brazili (LL) and Hamid local variety. The sowing dates were third week of June and July, whereas the spaces were 25 and 50 cm intra row. Treatments were arranged in a completely randomized block design with three replicates. The cultural practices were followed as recommended by the Agricultural Research Corporation, Wad Medani, Sudan. The insect pest was recorded either for their presence or their damage that inflicted on cotton plants. Insect predators were monitored as eggs, larvae and adults of Chrysoperala sp., Chielomense spp. and Exochomus spp. Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure.*

Results: *Regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of aphid (0.3-9.6%) compared to Hamid cultivar (3-12.8%). Hamid cultivar lately sown significantly harbored low number of aphid (3-6.8%) compared to Hamid cultivar early sown (3-12.8). There were no significant differences in percentages aphid infestation (3.5%) between narrow spacing and wide spacing. Seni 1 and Brazili (LL) cultivars harbored low number of Chrysopa spp., Cheiolomens spp. and Exochomus nigromaculatus compared to Hamid cultivar.*

Conclusions: *Hamid cultivar lately sown significantly harbored low number of the insect predators compared to Hamid cultivar early sown. In conclusion, cotton aphid was checked during September to October to the end of the season without insecticide use. Insect pests and natural enemies reflected density dependent pattern. The interaction between the cultivar, sowing date and spacing has positive effect on the insect control.*

Keywords: *Aphid, Cheiolomens, Chrysopa, Cotton and Exochomus.*

Introduction

Cotton (*Gosypium* spp.) is a soft stable fibre of shrub plant native to tropical and subtropical regions of the world. Cotton was cultivated since 7000 years ago by the inhabitants of the Indus valley civilization. However, Egyptian cotton (*G. barbadense* L.) planting in Sudan went back to the 19th century when it was grown for the first time in eastern Sudan (Tokar area), driven by the interest and the initiative of the Turkish – Egyptian rule (Faki, 2006). Short staple cotton was grown in Gezira area as rainfed crop before the establishment of the Gezira scheme (Mudawi, 2007).

However, the long staple cotton was commercially grown in 1905 at Zeidab scheme in northern Sudan. Nevertheless, 1925 was a land mark for its commercial production in Sudan, following the establishment of Sennar dam. Since then cotton has played a leading role as a cash crop. The organization of cotton production in Sudan started through establishing a large governmental administration with the participation of tenants. Since then cotton is the most important cash crop in Sudan where the annual revenue from cotton often constitutes 40-60% of the total national income (Mursal *et al.,* 1997). In the Sudan about 900.000 feddans were used to be groawn annually to cotton with an average yield of 2.9 - 4.9 Quintal/fed in the Gezira (Omer, 2002, Seid Ahmed, 2002; Nimer and Saboh, 1996) (1.0 Quintal = 100 pounds, lint cotton).

Despite the historical position of Sudan on cotton market, over years, Sudan's cotton production assumed a declining trend, mainly due to declining in area and crop yield. Production average was 930 thousand bales (420 lb) of lint during 1970s; it declined to an average of 432 thousand bales during the 1990s and was drop further during the 2000s (Faki, 2006). Yield have been fluctuating between a minimum of 779 to a maximum of 1949 kg/ha in the irrigated sector compared to between 94 and 952 kg/ha in the rainfed sector (Faki, 2006). After the enforcement of the Gezira Scheme act for the year 2005, the production relation started to change and the major features of this change were the relaxation of the strong government grip on agricultural scheme and as a result the tenants were not committed to the crop rotation where cotton was the backbone.

Sudan produces five types of cotton, namely the extra-fine, fine, higher account, medium and course account was represented by the varieties Barakat 90 (EFC), Shambat-B (FC), Nour (HCA), Barac (67) B (MC), Albar (57) 12 (CC) and Acrain (CC) Elfadil (2007). Some of these cultivars were out of production such as Shamabat-B, because of its ginning problems and low turn of ginning outcome (Mursal, 1994). New varieties have been released such as Hamid, Abdin, Knigt, Khairalla (Mursal *et al.*, 2004). Burhan, Khalifa and Wagar (Mustafa *et al.,* 2007) to replace some of long grown varieties such as Shambat-B. Bt cotton which was introduced from China (Seni-1) has been approached (Ali *et al.,* 2012) and was cultivated in the irrigated and rainfed sectors. Elhassan (2015) reported that the optimum sowing date of cotton varies with variety, from period of late July to early August for Egytian long stable cotton. Planting date management was not only having a longer effect on crop growth, development and yield, but it also had impact on insect pest management (Brown *et al.,*1998). An early crop can be produced by selecting early maturing cotton cultivars, narrow spacing or combination of both (Elhassan, 2015). Reduced season management on which early planting plays a major role has become increasingly important with increased late seasons insect pressure.

Cotton aphid, *Aphis gossypii* (Glov), (Homoptera: Aphididae) is very widely distributed in the world (Elahmadi and Kais, 1986). In Sudan it was reported in all the cotton growing areas (Ripper and George, 1965). The cotton aphid is an extremely polyphagous, it was recorded on cotton cucurbits, citrus, coffee, egg- plants, potato and okra (Schumtterer, 1977; Ibrahim, 1993). No sexual forms are known in tropics, so that reproduction is most probably exclusively parthenogenic (Caresche, 1986). The female giving birth to living nymph, green olive or verging to reddish brown in colour. The nymph is similar to the old female but have pads instead of wings (Schumtterer, 1969). However, in Sudan, the nymphs pass through four developmental stages (Sharf Eldin, 1978). The development for nymphal instars take place in 4 days at 28-30^{0}C and 10-12 days at 20-28^{0}C. The number of nymph produced by one female under favourable condition may reach up to 156 (Schumtterer, 1977; Ibrahim, 1993). Cotton aphid gives about fifty generations per year (Elahmadi and Kais, 1986; Ibrahim, 1993). Colonies of aphids were found on the young shoots, lower surfaces of the leaves, fruiting bodies and green bolls (Ripper and George, 1965). Cotton aphids inflict damage by sucking the plant sap and the injection of toxic saliva, and indirect damage through secretion of honey dew on the upper surface of the leaves. Drops of honey dew upon cotton lint lower its value and increase the cost of genning (Peeter *et al.,* 2001). Early heavy infestation renders the plant to be stunted, reduce the leaf area index, showing symptoms of leaf curl and plant with dead top (Nimer and Saboh, 1996). Late infestation shown as leaves shedding and bolls open prematurely (Ibrahim, 1993). Seeds are low in viability and weight. In the Sudan, cotton aphid has ability to transmit the pea mosaic virus and Lucerne mosaic virus to different legume (Schumtterer,

1969). Aphid infestation has an overall debilitating effect on the plant reduce both yield and quality (Ripper and George, 1965). It was reported in the Sudan Gezira that 11% of the unsprayed field, the aphid, is parasitized with *Aphelinus sudanensis* (Munir, 1989). On the other hand, a number of predators were reported to kill aphids. These predators were including cocinellids, chyrsopids, syrphids, mirids and spiders (Ripper and George, 1965; Schumtterer, 1969; Herrera, 1986; Beije and Ahmed, 1997).

General objective

To adopt plant population, sowing date and genetically modified cotton as tools for pest control under integrated pest management (IPM).

Specific objectives

- To grow cotton under various intra row spacing.
- To test different cotton genotypes.
- To grow cotton at a set of sowing dates.
- To monitor major insect pests and their predators.

Materials and Methods

Experimental site

Experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan, latitude 14° 22' N, longitude 33° 39' E and Altitude 407 meter above sea level, during the seasons 2013/2014 and 2014/2015. The climate of the study area is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season extended from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12°C in January and 42°C in May, respectively. The soil of the area is characterized by heavy clay soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous (Elbasher, 2016).

Treatments

A factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni I designated as (SC), Brazili (LL) designated as (BC) and Hamid local variety designated as (HC). The sowing dates were third week of June (S_1) and July (S_2). The spaces were 25 and 50 cm intra row for narrow space (NS) and wide specs (WS), respectively.

The experiment layout and cultural practices

The experiment was composed of 12 treatments (3×2×2) replicated 4 times to give 48 plots. The plot size was 6 rows each of 10.0 m length to give a plot size of 48.0 m^2. The experiment was laid out as a completely randomized block design (CRBD). The experimental area was deep plowed, harrowed and prepared in ridges of 0.8 m apart. The distance between each two plots was two ridges and between each two replicates was 3 m. Planting was done manually and each hole received 5-6 cotton seeds treated with Gaucho 70% WS at a rate of 3.0 g kg^{-1} seed. For weed control pre-emergence herbicides, Stomp 500 EC + Gezagard 500 FW were used as a product mixture at a dose of 0.60 + 0.20 kg ai. per feddan (4200 m^2). Hand weeding was done twice. The plots were irrigated after herbicides application, then followed by another watering 72 hours after the first irrigation. After that irrigation was practiced periodically every two weeks, till harvesting. Plants were thinned to 3 plants after 4 weeks of the effective watering. Fertilization was done after thinning using Urea at 2N dose. The first 1N was done immediately after thinning and the second 1N dose was applied a month later.

Data Collection

The insects were recorded either for their presence or their damage that inflicted on cotton plants. The monitored pest was cotton aphid, *Aphis gossypii* Glov. However, insect predators were the lacewing, *Chrysoperala carnea*, the *Chielomense propinqua* Vicina and *Exochomus nigromaculatus,* were also monitored with insect pests count.

Insect count

Sucking insect on leaves

For monitoring the insect pest 10 plants were checked in each plot. Five leaves were chosen, two leaves from the bottom of the plant, one in the middle and two at the top of the plant. The lower leaf surface was gently exposed by thumb, and hand palm being on the lower surface of the leaf while the upper surface of the leaf leaned and supported by hand palm early in the monitoring (6.00 am-7.00 am). In each leaf aphid was counted and recorded.

Plant sucking insect

Each of the 10 plants/plot considered for aphid was examined. For presence of cotton aphid, data were recorded as percentage infestation.

Insect predators

These were monitored as eggs, larvae and adults *Chrysoperala* sp., *Chielomense* spp. and *Exochomus* spp.

Data analysis

Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure. Significant ($P \leq 0.05$) means were separated using Duncan's Multiple Range test (DMRT). The statistical analysis was done using the Software MSTAT.

Result

Effects of cultivar, sowing date and spacing on percentages aphid infestation

In general, regardless of sowing date and spacing, the results showed that aphid appeared on September, increased on October and sharply decreased on November throughout season 2013/14 and season 2014/15 (Table 1 and 2). The results also showed that there were significant ($P \leq 0.05$) interaction effects between cultivar, sowing date and spacing on percentages aphid infestation. There were significant interaction effects between cultivar and sowing date, between cultivar and spacing, between sowing date and spacing on the percentages aphid infestation. Cultivar and sowing had significant on the percentages aphid infestation. However, spacing had no effect on the percentages aphid infestation.

Season 2013/14

The results showed that during September Hamid cultivar lately sown significantly ($P \leq 0.05$) harbored the lowest number of aphid and there were no significant difference in percentages aphid infestation between narrow spacing (2.7 %) and wide spacing (0.8%) (Table 1). However, Hamid cultivar early sown significantly harbored the highest number of aphid and there were no significant differences in percentages aphid infestation between narrow spacing (9.8 %) and wide spacing (13.8%). On the other hand, Seni 1 and Brazili (LL) cultivars lately sown with wide spacing significantly harbored low number of aphid compared with lately with narrow spacing. During October Brazili (LL) cultivar lately sown with narrow spacing significantly ($P \leq 0.05$) harbored the lowest number of aphid (7.7%), while Hamid cultivar lately sown with narrow spacing significantly ($P \leq 0.05$) harbored the highest number of aphid (13.1%). There were no significant differences in

percentages aphid infestation between Seni 1 and Brazili (LL) cultivars, between early and late sowing, between narrow and wide spacing. During November, although there were no significant differences in percentages aphid infestation between treatments. Hamid and Seni 1 cultivars lately sown harbored the lowest number of aphid (1.4%), while Hamid cultivar early sown with wide spacing harbored the highest number of aphid (4.4%).

Table (1): Effects of cultivar, sowing date and spacing on percentage aphid infestation, season 2013/14

Treatment			Percentage aphid infestation			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	General performance
SC	S_1	NS	7.0 bc	7.7 c	3.1	9.6 bc
		WS	4.8 bcd	9.4 abc	1.8	7.8 cd
	S_2	NS	4.8 bcd	9.1 abc	2.4	7.8 cd
		WS	3.5 cd	8.2 bc	1.4	7.5 cd
BC	S_1	NS	4.4 bcd	11.1 abc	2.4	8.2 bcd
		WS	5.3 bcd	9.1 abc	1.9	8.3 bcd
	S_2	NS	4.5 bcd	9.1 abc	1.6	7.9 bcd
		WS	3.5 cd	9.1 abc	1.5	8.4 bcd
HC	S_1	NS	9.8 ab	12.1 ab	2.4	10.8 ab
		WS	13.8 a	12.1 ab	4.4	12.8 a
	S_2	NS	2.7 e	13.1a	1.9	6.3 d
		WS	0.8 de	12.1 ab	1.4	6.8 cd
SE±			0.96	0.73	0.69	0.50
CV (%)			39.02	62.77	62.77	14.41

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

The general performance showed that Hamid cultivar lately sown significantly harbored the lowest number of aphid and there were no significant differences in percentages aphid infestation between narrow spacing (6.3 %) and wide spacing (6.8%) (Table 1). However, Hamid cultivar early sown significantly harbored the highest number of aphid and there were no significant differences in percentages aphid infestation between narrow spacing (10.8 %) and wide spacing (12.8%). On the other hand, Seni 1 and Brazili (LL) cultivars lately sown with wide spacing significantly harbored low number of aphid compared with lately with narrow spacing.

Season 2014/15

The results showed that during September aphid only appeared only on Hamid cultivar early sown with wide spacing (0.8 %) and on lately sown with narrow spacing (1.0 %) (Table 2). There were no significant differences (P ≤ 0.05) in percentages aphid infestation between treatments. During October aphid did not appeared on Seni 1 early sown with narrow and wide spacing and on Brazili (LL) cultivar early sown with narrow spacing. Hamid cultivar lately sown significantly harbored low number of aphid and there were no significant differences in percentages aphid infestation between narrow spacing (3.5 %) and wide spacing (3.8%). During November, irrespective of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of aphid compared to Hamid cultivar. Hamid cultivar lately sown significantly harbored low number of aphid compared to Hamid cultivar early sown. There were no significant differences in percentages aphid infestation between narrow spacing (9.3 %) and wide spacing (9.0%).

The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of aphid compared to Hamid cultivar (Table 2). Hamid cultivar lately sown significantly harbored low number of aphid compared to Hamid cultivar early sown.

There were no significant differences in percentages aphid infestation (3.5%) between narrow spacing and wide spacing.

Table (2): Effects of cultivar, sowing date and spacing on percentage aphid infestation, season 2014/15

Treatment			Percentage aphid infestation			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	General performance
SC	S_1	NS	0.7 (0.0)	0.7 b (0.0)	1.1 c (1.0)	1.0 c (0.5)
		WS	0.7 (0.0)	1.1 ab (0.0)	1.4 bc (2.3)	1.1 abc (1.0)
	S_2	NS	0.7 (0.0)	1.3 ab (1.3)	1.1 c (1.0)	1.0 c (0.5)
		WS	0.7 (0.0)	1.1ab (1.0)	1.1 c (1.0)	1.1 abc (0.8)
BC	S_1	NS	0.7 (0.0)	0.7 b (0.0)	1.0 c (0.8)	0.8 c (0.3)
		WS	0.7 (0.0)	1.0 ab (0.5)	0.7 c (0.0)	0.7 c (0.0)
	S_2	NS	0.7 (0.0)	1.1 ab (1.0)	1.1 c (1.0)	1.0 c (0.5)
		WS	0.7 (0.0)	1.5 ab (2.0)	1.7 c (2.8)	1.4 abc (2.8)
HC	S_1	NS	0.7 (0.0)	2.2 a (4.3)	3.0 ab (8.8)	2.0 ab (3.5)
		WS	1.0 (0.8)	2.2 a (4.5)	2.8 ab (7.8)	1.9 ab (3.0)
	S_2	NS	1.1 (1.0)	1.9 ab (3.5)	3.1 ab (9.3)	2.0 ab (3.5)
		WS	0.7 (0.0)	1.9 ab (3.8)	3.0 ab (9.0)	2.0 ab (3.5)
SE±			0.19	0.37	0.44	0.24
CV (%)			35.02	37.34	34.97	25.19

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Effects of cultivar, sowing date and spacing on *Chrysopa* spp. mean number, season 2013/14

The results showed that *Chrysopa* spp. only appeared in season 2013/14. During September and October there was no significant differences in the mean number of *Chrysopa* spp. between the two spaces on the two sowing dates on the three cultivars (Table 3). During November and December *Chrysopa* mean number increased compared to previous months with no significant differences between the two spaces on the two sowing dates on Seni I cultivar. Brazili (LL) and Hamid cultivars showed no significant differences between the two spaces on early sowing, while, on late sowing, wide spacing was significantly higher on *Chrysopa* mean number. *Chrysopa* started at low level on September and reached the peak in November and then lowered in number during December. During November the number of *Chrysopa* was significantly higher among Hamid plants grown late at narrow spacing (10.5) compared to Brazili (LL) grown late at narrow spacing (5.3). The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Chrysopa* spp. (5.3-6.8 *Chrysopa* mean number per 100 plants) compared to Hamid cultivar (9-10.5 *Chrysopa* mean number per 100 plants). Hamid cultivar

lately sown significantly harbored low number of *Chrysopa* spp. (9-9.3 *Chrysopa* mean number per 100 plants) compared to Hamid cultivar early sown (9.5-10.5 *Chrysopa* mean number per 100 plants).

Effects of cultivar, sowing date and spacing on mean number of adult *Cheiolomens* spp., season 2013/14

The results showed that *Cheiolomens* spp. only appeared in season 2013/14. There were no significant differences on *Cheiolomens* spp. adult mean number between the two spaces on the two sowing dates on all cultivars during September and October (Table 4). During November Seni I cultivar showed no significant differences on *Cheiolomens* spp. between the two spaces on early sowing, while, on late sowing narrow spacing was significantly higher on *Cheiolomens* spp. adult mean number (7). Brazili (LL) and Hamid cultivars with narrow spacing were significantly higher on *Cheiolomens* spp. on early sowing. While, they were significantly lower on late sowing. The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Cheiolomens* spp. adult (1-1.6 *Cheiolomens* spp. adult mean number per 100 plants) compared to Hamid cultivar (2-2.9 *Chrysopa* mean number per 100 plants).

Table (3): Effect of cultivar, sowing date and spacing on *Chrysopa* mean number per 100 plants, season 2013/14

Treatment			*Chrysopa* mean number / 100 plants				
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.	General performance
SC	S_1	NS	1.6 (2.8)	2.7 (7.0)	3.4 ab (11.8)	2.7 b (7.5)	2.6ab (6.5)
		WS	1.4 (1.8)	2.6 (6.5)	3.2 ab (10.0)	3.0 ab (9.0)	2.5ab (5.8)
	S_2	NS	0.8 (0.3)	2.8 (7.5)	3.4 ab (11.8)	2.7 b (7.0)	2.5ab (5.8)
		WS	0.8 (0.3)	2.8 (7.3)	3.3 ab (10.8)	2.7 b (7.0)	2.5ab (5.8)
BC	S_1	NS	1.1 (1.3)	2.6 (6.3)	3.3 ab (10.5)	2.8 b (7.5)	2.7ab (6.8)
		WS	1.2 (1.5)	2.6 (6.5)	3.3 ab (10.8)	3.1 ab (9.5)	2.5ab (6.0)
	S_2	NS	0.8 (0.3)	2.9 (8.3)	2.6 b (6.8)	3.0 ab (8.5)	2.4 b (5.3)
		WS	0.7 (0.0)	2.8 (7.8)	3.3 ab (10.5)	3.7 ab (13.5)	2.5ab (6.0)
HC	S_1	NS	0.7 (0.0)	2.9 (8.3)	4.4 ab (19.0)	4.8 ab (24.0)	3.1ab (9.0)
		WS	1.5 (2.3)	3.1 (9.0)	4.1 ab (16.3)	3.5 ab (13.0)	3.1ab (9.3)
	S_2	NS	1.1 (0.8)	2.9 (7.8)	4.8 a (23.0)	5.2 a (27.8)	3.3 a (10.5)
		WS	0.8 (0.3)	3.1 (9.3)	4.3 ab (18.3)	5.1 a (26.5)	3.2 ab (9.5)
SE±			0.33	0.25	0.52	0.60	0.23
CV (%)			43.94	12.53	20.38	23.89	11.81

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

Table (4): Effects of cultivar, sowing date and spacing on *Cheiolomens* spp. adult mean number per 100 plants, season 2013/14

Treatment			*Cheiolomens* spp. adult mean number per 100 plants			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	General performance
SC	S_1	NS	(2.0)	1.5 (1.8)	2.3 ab (5.0)	1.4 ab (1.4)
		WS	(0.5)	1.4 (1.8)	2.3ab (5.0)	1.4 ab (1.4)
	S_2	NS	(0.0)	1.5 (2.0)	2.3 ab (5.3)	1.4 ab (1.4)
		WS	(0.0)	1.5 (1.8)	1.9 b (3.5)	1.2 b (1.0)
BC	S_1	NS	(2.5)	1.8 (2.8)	2.6 ab (6.8)	1.4 ab (1.6)
		WS	(3.8)	1.6 (2.3)	1.9 b (3.8)	1.4 ab (1.4)
	S_2	NS	(0.8)	1.4 (1.5)	2.0 b (3.5)	1.2 b (1.0)
		WS	(1.3)	1.6 (2.0)	2.5 ab (5.8)	1.3 b (1.3)
HC	S_1	NS	(4.0)	1.7 (2.5)	3.5 a (11.8)	1.8 a (2.9)
		WS	(0.8)	1.7 (2.8)	3.0 ab (8.5)	1.6 ab (2.0)
	S_2	NS	(0.0)	1.6 (2.3)	3.0 ab (8.5)	1.7 ab (2.3)
		WS	(0.0)	1.8 (3.0)	3.3 a (10.3)	1.7 ab (2.3)
SE±				0.21	0.33	0.13
CV (%)				18.36	18.56	13.07

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

Effects of cultivar, sowing date and spacing on the *Exochomus nigromaculatus* mean number per 100 plants

The results showed that there were no significant differences between the two spaces on the two sowing dates on the three cultivars on the number of *Exochomus* spp. during October on the two seasons (Table 5 and 6). The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Exochomus nigromaculatus* (6.8-10.3 *Exochomus* spp. adult mean number per 100 plants) compared to Hamid cultivar (15.5-24.5 *Exochomus* spp. adult mean number per 100 plants).

Season 2013/14

During November there was no significant differences between the two spaces on the two sowing dates on the *Excchomus* spp. on Seni I cultivar (Table 5). While, Hamid showed no significant differences between the two spaces on early sowing. On late sowing date, narrow spacing is significantly higher on *Exochomus* spp. mean number. During November Seni I and Brazili (LL) showed higher significant differences on *Exochomus* spp. mean number on narrow spacing on early sowing dates and significantly higher on wide spacing on late sowing. Hamid cultivar showed no significant differences between the two spaces on late sowing, while, on early sowing wide

spacing was significantly higher. General performance throughout the season, Seni I cultivar showed no significant differences between the two spaces on early sowing, while, on late sowing, narrow spacing, was significantly higher. Brazili (LL) cultivar and Hamid at wide spacing was significantly higher compared to narrow spacing on the two sowing dates.

Season 2014/15

Exochomus spp. mean number in Seni I cultivar was significantly higher on narrow spacing on the two sowing dates (Table 6). Brazili (LL) cultivar showed no significant differences between the two spaces on the two sowing dates. Hamid cultivar at wide spacing is significantly higher on *Exochomus* spp. on early sowing date, while, there was no significant differences between the two spaces on late sowing. *Exochomus* spp. started at low level in October and reached the peak on November and got lower in number during December. However Hamid cotton plants grown late at narrow spacing harbored significantly high number of *Exochomus* spp. ($P \leq 0.05$) compared to Brazili (LL) cotton plants grown late and narrow spacing. As general performance the number of *Exochomus* spp. was significantly greater on Hamid cotton plants grown late at narrow spacing compared to Seni 1 and Brazili (LL) cultivar grown early or late at narrow or wider spacing.

Table (5): Effects of cultivar, sowing date and spacing on *Exochomus* spp. adult mean number per 100 plants, 2013/14

Treatment			*Exochomus* spp. adult mean number per 100 plants			
Cultivar	Sowing date	Spacing	Oct.	Nov.	Dec.	General performance
SC	S_1	NS	1.1 (1.0)	3.2 ab (10.5)	2.7 ab (7.5)	3.0 d (9.0)
		WS	1.5 (1.8)	2.9 ab (8.5)	3.2 ab (10.0)	2.6 d (6.8)
	S_2	NS	1.1 (0.8)	3.4 ab (11.8)	2.7 b (7.0)	3.2bcd (10.3)
		WS	1.4 (1.5)	3.3 ab (10.8)	2.7 b (7.0)	3.1 cd (9.3)
BC	S_1	NS	1.4 (1.5)	3.1 ab (9.3)	2.8 ab (7.5)	3.0 d (8.5)
		WS	1.1 (0.8)	3.3 ab (10.8)	3.2 ab (9.5)	3.2bcd (10.0)
	S_2	NS	1.1 (1.0)	2.6 b (6.8)	3.0 ab (8.5)	2.7 d (7.3)
		WS	1.3 (1.3)	3.3 ab (10.8)	3.2 ab (11.0)	3.3bcd (10.3)
HC	S_1	NS	1.6 (2.0)	4.4 ab (19.0)	4.8 ab (24.0)	4.5abc (20.5)
		WS	1.5 (2.0)	4.1 ab (16.3)	4.0 ab (18.0)	4.0 abcd (15.5)
	S_2	NS	1.3 (1.3)	4.8 a (23.0)	5.2 a (27.8)	5.0 a (24.5)
		WS	1.3 (1.23)	4.3 ab (18.3)	5.1ab (26.5)	4.6 ab (20.8)
SE±			0.28	0.53	0.66	0.40
CV (%)			30.68	21.06	26.15	15.89

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

Table (6): Effects of cultivar, sowing date and spacing on *Exochomus* spp. adult mean number per 100 plants, 2014/15

Treatment			*Exochomus* spp. adult mean number per 100 plants				
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.	General performance
SC	S_1	NS	1.1 (0.8)	1.0 (0.5)	2.4 ab (5.3)	1.5 ab (2.0)	1.6 ab (2.3)
		WS	1.0 (0.5)	1.1 (0.8)	1.7 b (2.8)	1.0 b (0.8)	1.2 b (1.0)
	S_2	NS	0.7 (0.0)	1.1 (0.8)	1.7 b (2.5)	0.8 b (0.3)	1.2 b (1.0)
		WS	0.8 (0.3)	1.1 (1.0)	2.1 ab (4.0)	1.0 b (0.5)	1.4 ab (0.8)
BC	S_1	NS	1.0 (0.5)	1.3 (1.3)	2.0 ab (3.5)	0.7 b (0.0)	1.3 b (1.3)
		WS	1.0 (0.5)	1.1 (0.8)	1.6 b (2.3)	0.7 b (0.0)	1.1 b (0.8)
	S_2	NS	0.7 (0.0)	1.1 (0.8)	1.9 ab (3.0)	1.1 ab (1.0)	1.3 b (1.3)
		WS	1.0 (0.5)	1.3 (1.3)	2.0 ab (4.5)	0.9 b (0.5)	1.3 b (1.5)
HC	S_1	NS	1.0 (0.5)	1.3 (1.3)	2.9 ab (8.0)	1.5 ab (1.8)	1.6 ab (2.3)
		WS	1.3 (1.3)	1.2 (1.0)	3.1 a (9.8)	2.3 a (5.3)	2.0 a (3.8)
	S_2	NS	0.8 (0.3)	1.0 (0.5)	2.6 ab (6.3)	1.6 ab (2.5)	1.7ab (2.3)
		WS	0.8 (0.3)	1.1 (0.8)	2.6ab (6.5)	1.6 ab (2.5)	1.7 ab (2.3)
SE±				0.25	0.36	0.36	0.18
CV (%)			27.92	30.89	22.95	40.87	16.97

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

Discussions

Insects inflicted a loss on cotton yield estimated as 30 – 50% (Peeter *et al.,* 2001). Pest control, high yield and good quality of cotton production can be practiced by cultural methods specially plant population (Smith and Falcon, 1973). Planting date management not only has a longer on crop growth, development and yield, but, also has impact effect on insect pest management (Brown *et al.,* 1998). Cotton aphid is extremely polyphagus recorded on cotton and other plants from different families (Schumuterer, 1969). Cotton aphid recorded up to 50 generations per season (Elahmadi and Kais, 1986 and Ibrahim, 1993). The unused of pesticides lead to overcome of the aphid through the natural enemies and this was reported by Ripper and George (1965); Schumuterer (1969) and Ibrahim (1993).

The finding of this study, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of aphid (0.3-9.6%) compared to Hamid cultivar (3-12.8%). Hamid cultivar lately sown significantly harbored low number of aphid (3-6.8%) compared to Hamid cultivar early sown (3-12.8). There were no significant differences in percentages aphid infestation (3.5%) between narrow spacing and wide spacing. Seni 1 and Brazili (LL) cultivars harbored low number of *Chrysopa* spp., *Cheiolomens* spp. and *Exochomus nigromaculatus* compared to Hamid cultivar. Hamid cultivar lately sown significantly harbored low number of the insect predators compared to Hamid cultivar early sown.

Elhassan (2015) reported that the optimum sowing date of cotton varies with variety, from period of late July to early August for Egytian long stable cotton. Planting date management was not only having a longer effect on crop growth, development and yield, but it also had impact on insect pest management (Brown *et al.,*1998). An early crop can be produced by selecting early maturing cotton cultivars, narrow spacing or combination of both (Elhassan, 2015). Reduced season management on which early planting plays a major role has become increasingly important with increased late seasons insect pressure.

The *Chrysopa* larvae are sometime called aphid lion and have been reported to eat between 100 – 600 aphids, although they may have difficulty finding prey in crops with hairy or sticky leaves (Rosenheim and Wilhoit, 1993). Mass production of *Chrysoperla carnea* in a Texas cotton field trial reduced bollworm infestation by 96% although more recent studies show that *C. carnea* predation on other predators can disrupt cotton aphid control (Rosenheim and Wilhoit, 1993). *Chrysoperla carnea* is considered an important aphid predator in Russian among Egyptian cotton crops, German sugar beets and European vine yards (Rosenheim and Wilhoit, 1993).

Cheiolomens spp. is important predatory insect that contribute to biocontrol programs of some major economic pests. It is polyphagus predator feeds mostly on aphids, although it feeding on soft scale, mealybug, whiteflies and psyllids was also recorded. It is often reaches high population so that it can be used as biocontrol agent in greenhouses (Trunck *et al.,* 2007). *Exochomus nigromaculatus* is cheap and natural form of biocontrol for Aphid, whitefly, scale insect and the pest way to attract this insect into the green spaces is to steer clear of pesticides for as long as possible during the warm season (Najajrah *et al.,* 2019).

Conclusion

Genetically modified cotton in addition of controlling bollworm through possessing toxic protein that kill bollworm larvae, it also controls other insect pest through keeping the natural enemies and spare them to do their role in insect control when the infestation is complex without insecticide use. Cotton aphid was checked during September to October to the end of the season without insecticide use. Natural enemies increased in number and reached the peak during September to October. Insect pests and natural enemies reflected density dependent pattern. The interaction between the cultivar, sowing date and spacing has positive effect on the insect control.

References

1. Beije, C.M. and Ahmed, M.A. (1997). Natural Enemies and Cotton Pest Control in Sudan. In: Integrated pest management in vegetables wheat and cotton in the Sudan, A participatory Approach (Edited by Dabrowski, Z.T.) FAO/Government of the Sudan Cooperative Project. GCP/SUD/025/NET, Wad Medani, Sudan. Published by ICIPE Science Press, Nairobi, Kenya, pp. 177-190.
2. Brown, P.W., B. Russel, J. Silvertooth, P. Ellsworth, S. Husman, T. Knowles, L. Clark, D. Dunn and M. Schneider. (1998). The Arizona cotton advisory program p. 5-12. Cotton, Univ. of Arizona Rep. P-112.
3. Caresche, L. (1986). Lsinsecctes nivibles ala culture de cotonnier dans le. Sudaouest de Madaga scar. Institute de Recherces Agronomi Quede Medgascar. Bull. No. 3.
4. Elbasher, O.A. (2016). Vermination of climate changes using rainfall and temperature as indicators and its impacts on agricultural production in the arid zone of Sudan. Ph.D. Thesis, University of Gezira, Sudan.
5. Elfadil, A.B. (2007). Sudan cotton research and production scenarios; challenges, achievement and prospect country report ICAC Washington, www.icac. orgtisregional_networksdocumentsafrica…sudan
6. Elhassan, O.A. (2015). The Review of Scientific Research on Cotton Sudan. China-aid Agricultural Technology Demonstration Center. Pp. 115-126.
7. Faki, H. (2006). Sustainable cotton production from Sudan's Report ICAC, Washington, 4-13.
8. Herrera, J.M. (1986). Natural enemies of cotton pests. In: Workshop on integrated pest control for cotton, 22-26, June 1986, Wad Medani, FAO/ARC, pp 22-48.

9. Munir, B. (1989). Semi-annual technical report, September 1985-February 1986, GCP/SUD/025/NET. Wad Medani, Sudan. Pp 3.
10. Mursal, I.E. (1994). Cotton varieties for IPM and rainfed area. ARC variety release meeting, Khartoum, Sudan.
11. Mustafa, A.M., Babiker, E.A. and Elhassan, O.M. (2007). Proposal for the release of new high yielding and highly resistance to bacterial blight cotton varieties. ARS. Sudan.
12. Najajrah, M.H.; Swaileh, K.M.; Mazin, B. (2019). Systematic list geographic distribution and ecological significance of ladybeetles (Coleoptera: Coccinellidae) from the West Bank (Central Palestine) Zootaxa 4664(1): 1-46, DoI.Mediated http://doi.org/10.11646/zootaxa4661.1plazi.orgtaxonomictreatmentdatabase
13. Omer, B.M. (2002). Benefits and efficacy of two groups of insecticides in controlling the major cotton insect pests at the Rahad Agricultural Scheme, Ph.D. Thesis, University of Gezira, Sudan.
14. Peeter, M.C.; Van Langenhove, L.; Lowwagie, J.; Water Keyn, L. and Mergeai, G. (2001). Fibre crops cotton *Gossympium hirstum* L. In: Crop Production in Tropical Africa. Edited by Romain, H. Raemeakers. Pp 1040-1071.
15. Ripper, W.E. and George, L. (1965). Cotton pests of the Sudan: Their habits and control (1st ed.), Blackwell Scientific Publication, Oxford. Pp. 90-113.
16. Rosenheim, J.A. and Wilhoit, R.R. (1993). Predators that eat other predators disrupt cotton aphid control. Cal. Agric. (47): 7-9.
17. Sharaf El-Din, N. (1978). Investigations on the biology, ecology and control of the cotton aphids in the Sudan. Proceedings of a symposium held in Khartoum, Feb. 6-8. Ministry of Agriculture and University of Khartoum, Sudan. Pp. 369-373.
18. Schumtterer, H. (1977). Pest in tropical crops. In: Diseases, Pests and weeds in tropical crops. Edited by Dri turgen kranz, Dr. Heinz Schumtterer and Dr. Werner Koch vering paul parey printed in W. Germany by Saldruck stein kopf and Sohn, D-1000 Berlin 36. Binding by lvderitz and bauer, D. 1000 Berlin 61(74): 309-381.
19. Schumutterer, H. (1969). Pests of crops in Northeast and Central Africa (1st ed.), Gustav, Fisher, Germany, pp. 70-90.
20. Smith, R.F. and Falcon, L.A. (1973). Insect control for cotton in California, Cotton Grow, Rev. 50: 15-27.
21. Trunck, W., Wise, I. and Malheson, F. (2007). Abundance of some native coccinellines (Coccinellidae: Coleoptera) before and after appearance of the Lana *Coccinella septempunctat*, 135: 391-404-dian Enomologest.
22. إبراهيم، جمعة نخيل **(1993):** الحشرات الاقتصادية. منشورات جامعة حلب.
23. **إبراهيم الحاج مرسال، ميرغني فيصل، الحمدي عبدالله أبابكر، إبراهيم عثمان العبيد، أبو الحسن صالح إبراهيم، مختار نوري عثمان (1997):** دليل المدرب (8) أساسيات المحاصيل الحقلية، السودان، وزارة الزراعة والغابات، هيئة البحوث الزراعية TCP/SUN/6616.
24. **الحمدي أحمد زياد، قيس وجيه (1986):** حشرات المحاصيل الحقلية والإرشادات الدالة على التعرف عليها في الوطن العربي. منشورات جامعة دمشق، دار المستقبل للطباعة.
25. **السيد، سليمان السيد أحمد (2002):** مشروع الجزيرة الأسطورة الحية (دراسة أولية)، الخرطوم.
26. **نمري يوسف، صبوح محمود (1996):** محاصيل الألياف (الجزء النظري) – منشورات جامعة دمشق، مطبعة ابن حيان، دمشق.
27. **يوسف عبد القادر عبد الفتاح ضوي (2007):** الشيخ المجاهد عبد القادر ود حبوبة، شركة الجزيرة للطباعة والنشر المحدودة، ود مدني ص 134-138.

Effects of Ecological Approach on the Management of Cotton Mealybug, *Phenacoccus solenopsis* (Tinsley), in Selected Cotton Genotypes, Gezira State, Sudan

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Abstract

Background: *Cotton is grown in Sudan as rainfed crop, as well as grown by permanent irrigation. Usually insecticides were used to control cotton insect pests, but in Gezira scheme cotton produced for the first three decades (1911-1945) without chemical control. That might be due to the balanced nature. This research aims to reduce the cost of production and mitigating the environmental pollution through managing cotton mealybug, Phenacoccus solenopsis (Tinsley) via ecological approach at Gezira State, Sudan.*

Methodology: *Two experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan during season 2013/14 and 2014/15. I each season a factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni, Brazili (LL) and Hamid local variety. The sowing dates were third week of June and July, whereas the spaces were 25 and 50 cm intra row. Treatments were arranged in a completely randomized block design with three replicates. The cultural practices were followed as recommended by the Agricultural Research Corporation, Wad Medani, Sudan. The insect pest was recorded either for their presence or their damage that inflicted on cotton plants. Insect predators were monitored as eggs, larvae and adults of Chrysoperala sp., Chielomense spp. and Exochomus spp. Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure.*

Results: *Regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of mealybug (2.3-8%) compared to Hamid cultivar (9.5-95.5%). Seni 1 and Brazili (LL) cultivars harbored low number of Chrysopa spp., Cheiolomens spp. and Exochomus nigromaculatus compared to Hamid cultivar. Hamid cultivar lately sown significantly harbored low number of the insect predators compared to Hamid cultivar early sown.*

Conclusions: *In conclusion, cotton mealybug was checked during September to October to the end of the season without insecticide use. Insect pests and natural enemies reflected density dependent pattern. The interaction between the cultivar, sowing date and spacing has positive effect on the insect control.*

Keywords: *Cheiolomens, Chrysopa, Cotton and Exochomus, mealybug and Phenacoccus.*

Introduction

Cotton (*Gosypium* spp.) is a soft stable fibre of shrub plant native to tropical and subtropical regions of the world. Cotton was cultivated since 7000 years ago by the inhabitants of the Indus valley civilization. However, Egyptian cotton (*G. barbadense* L.) planting in Sudan went back to the 19th century when it was grown for the first time in eastern Sudan (Tokar area), driven by the interest and the initiative of the Turkish – Egyptian rule (Faki, 2006). Short staple cotton was grown in Gezira area as rainfed crop before the establishment of the Gezira scheme (Mudawi, 2007). However, the long staple cotton was commercially grown in 1905 at Zeidab scheme in northern Sudan. Nevertheless, 1925 was a land mark for its commercial production in Sudan, following the establishment of Sennar dam. Since then cotton has played a leading role as a cash crop. The organization of cotton production in Sudan started through establishing a large governmental

administration with the participation of tenants. Since then cotton is the most important cash crop in Sudan where the annual revenue from cotton often constitutes 40-60% of the total national income (Mursal *et al.,* 1997). In the Sudan about 900.000 feddans were used to be grown annually to cotton with an average yield of 2.9 - 4.9 Quintal/fed in the Gezira (Omer, 2002, Seid Ahmed, 2002; Nimer and Saboh, 1996) (1.0 Quintal = 100 pounds, lint cotton).

Despite the historical position of Sudan on cotton market, over years, Sudan's cotton production assumed a declining trend, mainly due to declining in area and crop yield. Production average was 930 thousand bales (420 lb) of lint during 1970s; it declined to an average of 432 thousand bales during the 1990s and was drop further during the 2000s (Faki, 2006). Yield have been fluctuating between a minimum of 779 to a maximum of 1949 kg/ha in the irrigated sector compared to between 94 and 952 kg/ha in the rainfed sector (Faki, 2006). After the enforcement of the Gezira Scheme act for the year 2005, the production relation started to change and the major features of this change were the relaxation of the strong government grip on agricultural scheme and as a result the tenants were not committed to the crop rotation where cotton was the backbone.

Sudan produces five types of cotton, namely the extra-fine, fine, higher account, medium and course account was represented by the varieties Barakat 90 (EFC), Shambat-B (FC), Nour (HCA), Barac (67) B (MC), Albar (57) 12 (CC) and Acrain (CC) (Elfadil, 2007). Some of these cultivars were out of production such as Shamabat-B, because of its ginning problems and low turn of ginning outcome (Mursal, 1994). New varieties have been released such as Hamid, Abdin, Knigt, Khairalla (Mursal *et al.*, 2004), Burhan, Khalifa and Wagar (Mustafa *et al.,* 2007) to replace some of long grown varieties such as Shambat-B. Bt cotton which was introduced from China (Seni-1) has been approached (Ali *et al.,* 2012) and was cultivated in the irrigated and rainfed sectors. Elhassan (2015) reported that the optimum sowing date of cotton varies with variety, from period of late July to early August for Egytian long stable cotton. Planting date management was not only having a longer effect on crop growth, development and yield, but it also had impact on insect pest management (Brown *et al.,*1998). An early crop can be produced by selecting early maturing cotton cultivars, narrow spacing or combination of both (Elhassan, 2015). Reduced season management on which early planting plays a major role has become increasingly important with increased late seasons insect pressure.

Cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae), is native to USA and introduced to several countries in all the continents (Cabi, 2015; Nagrare *et al.*, 2011). It was reported in Egypt (Abd- Rabon *et al.,* 2010) and Ethiopa (Hemba *et al.,* 2012). However, it was reported in China in 2009 (Nagrare *et al.,* 2011). In 2010, Sudan imported cotton seeds from China and the mealy bug was possibly introduced. Joshi (2015) reported that the cotton mealy bug entered cotton fields in 2008 in Vidarbha region, India, through Bt cotton seeds imported from USA. International dispersal of the species over vast areas is by transporting infested plant materials. Local and regional movement is by wind, water, farm animals, insects, birds and human beings (Cabi, 2015 and Nagrare *et al.,* 2011). During the 2-3 years, infestations with mealybug have spread in different cultivated and wild plant species and reported from different states in Sudan (Mohamed, 2015). A survey conducted in the Gezira and Khartoum State during 2015, showed that at least 26 host plant species that belong to 16 plant families are host plants.

The cotton mealybug has a morphological diversity, biological adaptations and ecological adjustments that give it a high capacity to feed on different host plants (Hodgson *et al.,* 2008). It has been recorded from more than two hundred plant species including field crops, vegetables, ornamentals, weeds, bushes and trees (Hodgson *et al.,* 2008; Nagrare *et al.,* 2011; Cabi, 2015). The mealybug sucks the plant sap, excretes honey dew and while feeding it injects a toxic substance into the plant parts. The insect feeding results in chlorosis, defoliation and deformation. Reduced plant vigor, reduced yield for each infested plant. The excretion of honey dew results in development of black sooty mould which contaminate plant products (Cabi, 2015 and Nagrare *et al.,* 2011). This pest is also suspected as a vector of some plant diseases (Culik and Gullan, 2005).

General objective

To adopt plant population, sowing date and genetically modified cotton as tools for pest control under integrated pest management (IPM).

Specific objectives

- To grow cotton under various intra row spacing.
- To test different cotton genotypes.
- To grow cotton at a set of sowing dates.
- To monitor major insect pests and their predators.

Materials and Methods

2.1. Experimental site

Experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan, latitude 14° 22' N, longitude 33° 39' E and Altitude 407 meter above sea level, during the seasons 2013/2014 and 2014/2015. The climate of the study area is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season extended from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12°C in January and 42°C in May, respectively. The soil of the area is characterized by heavy clay soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous (Elbasher, 2016).

2.2. Treatments

A factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni I designated as (SC), Brazili (LL) designated as (BC) and Hamid local variety designated as (HC). The sowing dates were third week of June (S_1) and July (S_2). The spaces were 25 and 50 cm intra row for narrow space (NS) and wide specs (WS), respectively.

2.3. The experiment layout and cultural practices

The experiment was composed of 12 treatments (3×2×2) replicated 4 times to give 48 plots. The plot size was 6 rows each of 10.0 m length to give a plot size of 48.0 m^2. The experiment was laid out as a completely randomized block design (CRBD). The experimental area was deep plowed, harrowed and prepared in ridges of 0.8 m apart. The distance between each two plots was two ridges and between each two replicates was 3 m. Planting was done manually and each hole received 5-6 cotton seeds treated with Gaucho 70% WS at a rate of 3.0 g kg^{-1} seed. For weed control pre-emergence herbicides, Stomp 500 EC + Gezagard 500 FW were used as a product mixture at a dose of 0.60 + 0.20 kg ai. per feddan (4200 m^2). Hand weeding was done twice. The plots were irrigated after herbicides application, then followed by another watering 72 hours after the first irrigation. After that irrigation was practiced periodically every two weeks, till harvesting. Plants were thinned to 3 plants after 4 weeks of the effective watering. Fertilization was done after thinning using Urea at 2N dose. The first 1N was done immediately after thinning and the second 1N dose was applied a month later.

2.4. Data Collection

The insects were recorded either for their presence or their damage that inflicted on cotton plants. The monitored pest was cotton mealybug, *Phenacoccus solenopsis* (Tinsley). However, insect predators were the lacewing, *Chrysoperala carnea*, the *Chielomense propinqua* Vicina and *Exochomus nigromaculatus,* were also monitored with insect pests count.

2.4.1. Insect count

Sucking insect on leaves

For monitoring the insect pest 10 plants were checked in each plot. Five leaves were chosen, two leaves from the bottom of the plant, one in the middle and two at the top of the plant. The lower leaf surface was gently exposed by thumb, and hand palm being on the lower surface of the leaf while the upper surface of the leaf leaned and supported by hand palm early in the monitoring (6.00 am-7.00 am). In each leaf mealybug was counted and recorded.

Plant sucking insect

Each of the 10 plants/plot considered for cotton mealybug was examined. For presence of cotton mealybug, data were recorded as percentage infestation.

2.4.2. Insect predators

These were monitored as eggs, larvae and adults *Chrysoperala* sp., *Chielomense* spp. and *Exochomus* spp.

2.5. Data analysis

Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure. Significant ($P \leq 0.05$) means were separated using Duncan's Multiple Range test (DMRT). The statistical analysis was done using the Software MSTAT.

Result

In general, regardless of sowing date and spacing, the results showed that mealybug was started to appear during November in season 2013/14 in a high number and during September in a small number in season 2014/15 (Table 1 and 2). The results also showed that there were significant ($P \leq 0.05$) interaction effects between cultivar, sowing date and spacing on percentages mealybug infestation. There were significant interaction effects between cultivar and sowing date, between cultivar and spacing, between sowing date and spacing on the percentages mealybug infestation. Cultivar and sowing had significant on the percentages mealybug infestation. However, spacing had no effect on the percentages mealybug infestation.

3.1. Effect of cultivar, sowing date and spacing on percentages mealybug infestation (2013/14 - 2014/15)

Season 2013/14

The results showed that cotton mealybug infestation was at range of 18 -98% (Table 1). Hamid cultivar was reflecting significant percentage infestation ($P \leq 0.05$) compared to Seni1 and Brazili (LL) cultivars, during November. Similar results were persisting during December and in the general performance. Again the percentage infestation was in the range of 13-100% during December and 14-96% in the general performance (Fig. 1).

Season 2014/15

Mealybug appeared early, during September at a range of 1-10% of cotton plants showing its presence (Table 2). However, the infestation increased to 5-20% in October and dropped to 1-17% in November. It was further dropped to 0-8% in December. The general performance showed a range of 3 – 15%. In all situation stated above Hamid cultivars harboured more mealybug in term of percentage infestation compared to Seni 1 and Brazili (LL) cultivars (Fig. 1).

Table (1): Effect cultivar, sowing date and spacing on percentage mealybug infestation (2013/14)

Treatments			Percentage mealybug infestation			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.
SC	S_1	NS	0.0	0.0	27.8 c (21.5)	26.6 bc (20.0)
		WS	0.0	0.0	25.4 c (18.8)	28.3 bc (22.5)
	S_2	NS	0.0	0.0	25.2 c (18.8)	24.8 bc (17.5)
		WS	0.0	0.0	18.7 c (18.8)	20.7 c (12.5)
BC	S_1	NS	0.0	0.0	44.4 bc (48.3)	32.7 bc (29.2)
		WS	0.0	0.0	28.6 c (23.8)	35.1 b (33.5)
	S_2	NS	0.0	0.0	32.6 c (30.8)	26.7 bc (20.0)
		WS	0.0	0.0	31.6 c (28.8)	27.5 bc (21.8)
HC	S_1	NS	0.0	0.0	76.4 ab (88.8)	90.5 a (100.0)
		WS	0.0	0.0	85.8 a (97.5)	90.5 a (100.0)
	S_2	NS	0.0	0.0	79.8 ab (91.5)	85.5 a (98.5)
		WS	0.0	0.0	70.0 ab (78.78)	85.8 a (97.5)
SE±					9.58	3.40
CV (%)					29.77	10.03

* Data are transformed to arc sign.
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Table (2): Effect of cultivar, sowing date and spacing on percentage mealybug infestation (2014/15)

Treatments			Percentage mealybug infestation			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.
SC	S_1	NS	1.9 (4.0)	3.5 abc (12.0)	2.8 abc (8.3)	1.2 ab (1.75)
		WS	2.7 (8.0)	3.1 abc (9.3)	1.4 c (2.3)	1.1 ab (1.3)
	S_2	NS	1.1 (1.0)	2.3 c (6.3)	1.2 c (1.3)	1.2 ab (1.8)
		WS	1.1 (1.0)	2.3 c (5.5)	1.9 bc (4.3)	0.7 b (0.0)
BC	S_1	NS	1.5 (2.5)	3.3 abc (10.5)	1.9 bc (4.3)	1.1 ab (1.0)
		WS	2.2 (7.0)	3.0 abc (9.0)	2.1 abc (4.3)	1.1 ab (1.0)
	S_2	NS	1.9 (4.5)	2.7 abc (7.0)	1.9 bc (3.5)	1.1 ab (1.0)
		WS	1.5	2.6 bc	2.1 abc	1.4 ab

			(2.5)	(6.5)	(5.8)		(2.0)
		NS	3.3	4.7 a	3.9	ab	2.9 a
	S_1		(11.0)	(22.0)	(15.0)		(7.8)
		WS	1.5	4.5 ab	4.2 a		3.1 a
HC			(4.0)	(20.5)	(17.0)		(9.0)
		NS	1.9	3.7 abc	3.8	ab	1.6 ab
	S_2		(4.5)	(13.8)	(14.3)		(3.0)
		WS	0.9	4.1 abc	4.1 a		2.1 ab
			(0.5)	(16.0)	(16.8)		(4.5)
SE±			0.86	0.54	0.60		0.54
CV (%)			67.85	23.10	32.49		49.94

* Data are transformed to arc sign.
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

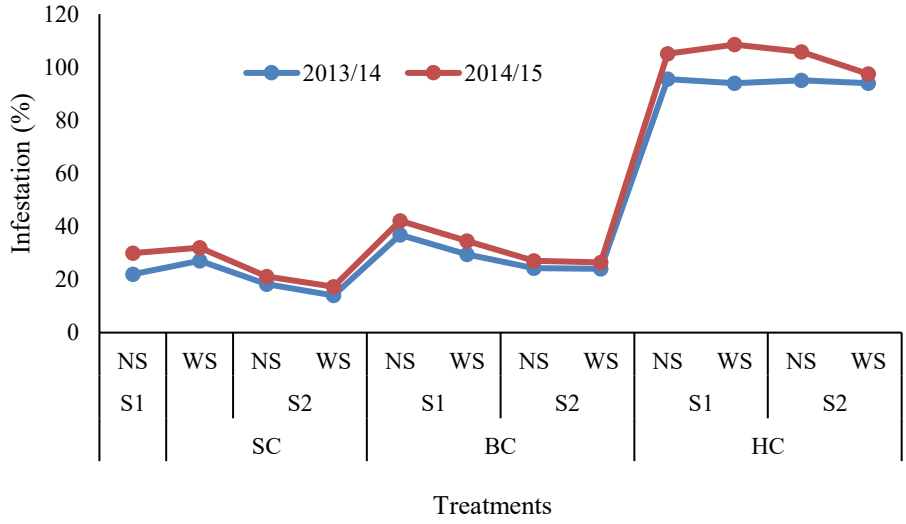

Fig. (1): Effect cultivar, sowing date and spacing on percentage mealybug infestation (season 2013/14 and 2014/15)

3.2. Effects of cultivar, sowing date and spacing on *Chrysopa* spp. mean number, season 2013/14

The results showed that *Chrysopa* spp. only appeared in season 2013/14 cultivars (Table 3). During September and October there was no significant differences in the mean number of *Chrysopa* spp. between the two spaces on the two sowing dates on the three. During November and December *Chrysopa* mean number increased compared to previous months with no significant differences between the two spaces on the two sowing dates on Seni I cultivar. Brazili (LL) and Hamid cultivars showed no significant differences between the two spaces on early sowing, while, on late sowing, wide spacing was significantly higher on *Chrysopa* mean number. *Chrysopa* started at low level on September and reached the peak in November and then lowered in number during December. During November the number of *Chrysopa* was significantly higher among Hamid plants grown late at narrow spacing (10.5) compared to Brazili (LL) grown late at narrow spacing (5.3). The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Chrysopa* spp. (5.3-6.8 *Chrysopa* mean number per 100 plants) compared to Hamid cultivar (9-10.5 *Chrysopa* mean number per 100 plants). Hamid cultivar

lately sown significantly harbored low number of *Chrysopa* spp. (9-9.3 *Chrysopa* mean number per 100 plants) compared to Hamid cultivar early sown (9.5-10.5 *Chrysopa* mean number per 100 plants) (Fig. 2).

Table (3): Effect of cultivar, sowing date and spacing on *Chrysopa* mean number per 100 plants, season 2013/14

Treatment			***Chrysopa* mean number / 100 plants**			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.
SC	S_1	NS	1.6 (2.8)	2.7 (7.0)	3.4 ab (11.8)	2.7 b (7.5)
		WS	1.4 (1.8)	2.6 (6.5)	3.2 ab (10.0)	3.0 ab (9.0)
	S_2	NS	0.8 (0.3)	2.8 (7.5)	3.4 ab (11.8)	2.7 b (7.0)
		WS	0.8 (0.3)	2.8 (7.3)	3.3 ab (10.8)	2.7 b (7.0)
BC	S_1	NS	1.1 (1.3)	2.6 (6.3)	3.3 ab (10.5)	2.8 b (7.5)
		WS	1.2 (1.5)	2.6 (6.5)	3.3 ab (10.8)	3.1 ab (9.5)
	S_2	NS	0.8 (0.3)	2.9 (8.3)	2.6 b (6.8)	3.0 ab (8.5)
		WS	0.7 (0.0)	2.8 (7.8)	3.3 ab (10.5)	3.7 ab (13.5)
HC	S_1	NS	0.7 (0.0)	2.9 (8.3)	4.4 ab (19.0)	4.8 ab (24.0)
		WS	1.5 (2.3)	3.1 (9.0)	4.1 ab (16.3)	3.5 ab (13.0)
	S_2	NS	1.1 (0.8)	2.9 (7.8)	4.8 a (23.0)	5.2 a (27.8)
		WS	0.8 (0.3)	3.1 (9.3)	4.3 ab (18.3)	5.1 a (26.5)
SE±			0.33	0.25	0.52	0.60
CV (%)			43.94	12.53	20.38	23.89

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

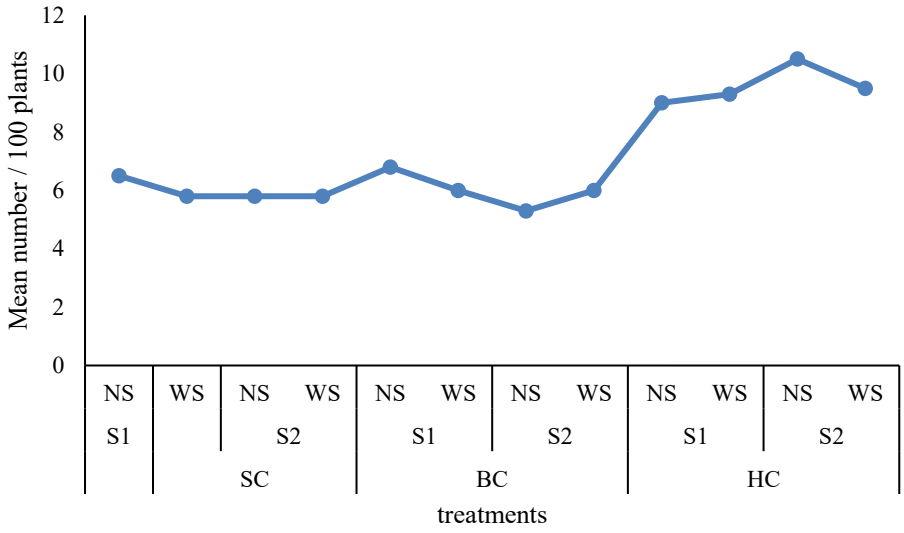

Fig. (2): Effect of cultivar, sowing date and spacing on Chrysopa mean number per 100 plants, season 2013/14

3.3. Effects of cultivar, sowing date and spacing on mean number of adult *Cheiolomens* spp., season 2013/14

The results showed that *Cheiolomens* spp. only appeared in season 2013/14. There were no significant differences on *Cheiolomens* spp. adult mean number between the two spaces on the two sowing dates on all cultivars during September and October (Table 4). During November Seni I cultivar showed no significant differences on *Cheiolomens* spp. between the two spaces on early sowing, while, on late sowing narrow spacing was significantly higher on *Cheiolomens* spp. adult mean number (7). Brazili (LL) and Hamid cultivars with narrow spacing were significantly higher on *Cheiolomens* spp. on early sowing. While, they were significantly lower on late sowing. The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Cheiolomens* spp. adult (1-1.6 *Cheiolomens* spp. adult mean number per 100 plants) compared to Hamid cultivar (2-2.9 *Chrysopa* mean number per 100 plants) (Fig. 3).

Table (4): Effects of cultivar, sowing date and spacing on *Cheiolomens* spp. adult mean number per 100 plants, season 2013/14

Treatment			*Cheiolomens* spp. adult mean number per 100 plants		
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.
SC	S_1	NS	1.5 (2.0)	1.5 (1.8)	2.3 ab (5.0)
		WS	0.9 (0.5)	1.4 (1.8)	2.3ab (5.0)
	S_2	NS	0.7 (0.0)	1.5 (2.0)	2.3 ab (5.3)
		WS	0.7 (0.0)	1.5 (1.8)	1.9 b (3.5)
BC	S_1	NS	1.5 (2.5)	1.8 (2.8)	2.6 ab (6.8)
		WS	1.9 (3.8)	1.6 (2.3)	1.9 b (3.8)

		NS	1.1 (0.8)	1.4 (1.5)	2.0 b (3.5)
	S_2	WS	1.2 (1.3)	1.6 (2.0)	2.5 ab (5.8)
		NS	1.9 (4.0)	1.7 (2.5)	3.5 a (11.8)
	S_1	WS	1.1 (0.8)	1.7 (2.8)	3.0 ab (8.5)
HC		NS	0.7 (0.0)	1.6 (2.3)	3.0 ab (8.5)
	S_2	WS	0.7 (0.0)	1.8 (3.0)	3.3 a (10.3)
SE±			0.42	0.21	0.33
CV (%)			50.95	18.36	18.56

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

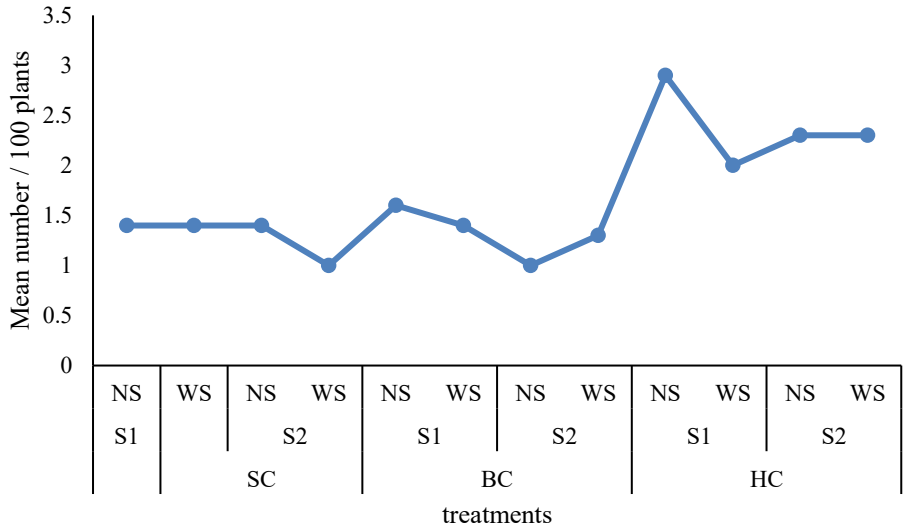

Fig. (3): Effects of cultivar, sowing date and spacing on Cheiolomens spp. adult mean number per 100 plants, season 2013/14

3.4. Effects of cultivar, sowing date and spacing on the *Exochomus nigromaculatus* mean number per 100 plants

The results showed that there were no significant differences between the two spaces on the two sowing dates on the three cultivars on the number of *Exochomus* spp. during October on the two seasons (Table 5 and 6). The general performance showed that, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of *Exochomus nigromaculatus* (6.8-10.3 *Exochomus* spp. adult mean number per 100 plants) compared to Hamid cultivar (15.5-24.5 *Exochomus* spp. adult mean number per 100 plants) (Fig. 4).

Season 2013/14

During November there was no significant differences between the two spaces on the two sowing dates on the *Excchomus* spp. on Seni I cultivar (Table 5). While, Hamid showed no significant differences between the two spaces on early sowing. On late sowing date, narrow spacing is significantly higher on *Exochomus* spp. mean number. During November Seni I and Brazili (LL) showed higher significant differences on *Exochomus* spp. mean number on narrow spacing on early sowing dates and significantly higher on wide spacing on late sowing. Hamid cultivar showed no significant differences between the two spaces on late sowing, while, on early sowing wide spacing was significantly higher. General performance throughout the season, Seni I cultivar showed no significant differences between the two spaces on early sowing, while, on late sowing, narrow spacing, was significantly higher. Brazili (LL) cultivar and Hamid at wide spacing was significantly higher compared to narrow spacing on the two sowing dates (Fig. 4).

Table (5): Effects of cultivar, sowing date and spacing on *Exochomus* spp. adult mean number per 100 plants, 2013/14

Treatment			*Exochomus* spp. adult mean number per 100 plants		
Cultivar	Sowing date	Spacing	Oct.	Nov.	Dec.
SC	S_1	NS	1.1 (1.0)	3.2 ab (10.5)	2.7 ab (7.5)
		WS	1.5 (1.8)	2.9 ab (8.5)	3.2 ab (10.0)
	S_2	NS	1.1 (0.8)	3.4 ab (11.8)	2.7 b (7.0)
		WS	1.4 (1.5)	3.3 ab (10.8)	2.7 b (7.0)
BC	S_1	NS	1.4 (1.5)	3.1 ab (9.3)	2.8 ab (7.5)
		WS	1.1 (0.8)	3.3 ab (10.8)	3.2 ab (9.5)
	S_2	NS	1.1 (1.0)	2.6 b (6.8)	3.0 ab (8.5)
		WS	1.3 (1.3)	3.3 ab (10.8)	3.2 ab (11.0)
HC	S_1	NS	1.6 (2.0)	4.4 ab (19.0)	4.8 ab (24.0)
		WS	1.5 (2.0)	4.1 ab (16.3)	4.0 ab (18.0)
	S_2	NS	1.3 (1.3)	4.8 a (23.0)	5.2 a (27.8)
		WS	1.3 (1.23)	4.3 ab (18.3)	5.1ab (26.5)
SE±			0.28	0.53	0.66
CV (%)			30.68	21.06	26.15

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Season 2014/15

Exochomus spp. mean number in Seni I cultivar was significantly higher on narrow spacing on the two sowing dates (Table 6). Brazili (LL) cultivar showed no significant differences between the two spaces on the two sowing dates. Hamid cultivar at wide spacing is significantly higher on

Exochomus spp. on early sowing date, while, there was no significant differences between the two spaces on late sowing. *Exochomus* spp. started at low level in October and reached the peak on November and got lower in number during December. However Hamid cotton plants grown late at narrow spacing harbored significantly high number of *Exochomus* spp. ($P \leq 0.05$) compared to Brazili (LL) cotton plants grown late and narrow spacing. As general performance the number of *Exochomus* spp. was significantly greater on Hamid cotton plants grown late at narrow spacing compared to Seni 1 and Brazili (LL) cultivar grown early or late at narrow or wider spacing (Fig. 4).

Table (6): Effects of cultivar, sowing date and spacing on *Exochomus* spp. adult mean number per 100 plants, 2014/15

Treatment			***Exochomus* spp. adult mean number per 100 plants**			
Cultivar	Sowing date	Spacing	Sep.	Oct.	Nov.	Dec.
SC	S_1	NS	1.1 (0.8)	1.0 (0.5)	2.4 ab (5.3)	1.5 ab (2.0)
		WS	1.0 (0.5)	1.1 (0.8)	1.7 b (2.8)	1.0 b (0.8)
	S_2	NS	0.7 (0.0)	1.1 (0.8)	1.7 b (2.5)	0.8 b (0.3)
		WS	0.8 (0.3)	1.1 (1.0)	2.1 ab (4.0)	1.0 b (0.5)
BC	S_1	NS	1.0 (0.5)	1.3 (1.3)	2.0 ab (3.5)	0.7 b (0.0)
		WS	1.0 (0.5)	1.1 (0.8)	1.6 b (2.3)	0.7 b (0.0)
	S_2	NS	0.7 (0.0)	1.1 (0.8)	1.9 ab (3.0)	1.1 ab (1.0)
		WS	1.0 (0.5)	1.3 (1.3)	2.0 ab (4.5)	0.9 b (0.5)
HC	S_1	NS	1.0 (0.5)	1.3 (1.3)	2.9 ab (8.0)	1.5 ab (1.8)
		WS	1.3 (1.3)	1.2 (1.0)	3.1 a (9.8)	2.3 a (5.3)
	S_2	NS	0.8 (0.3)	1.0 (0.5)	2.6 ab (6.3)	1.6 ab (2.5)
		WS	0.8 (0.3)	1.1 (0.8)	2.6ab (6.5)	1.6 ab (2.5)
SE±			0.26	0.25	0.36	0.36
CV (%)			27.92	30.89	22.95	40.87

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

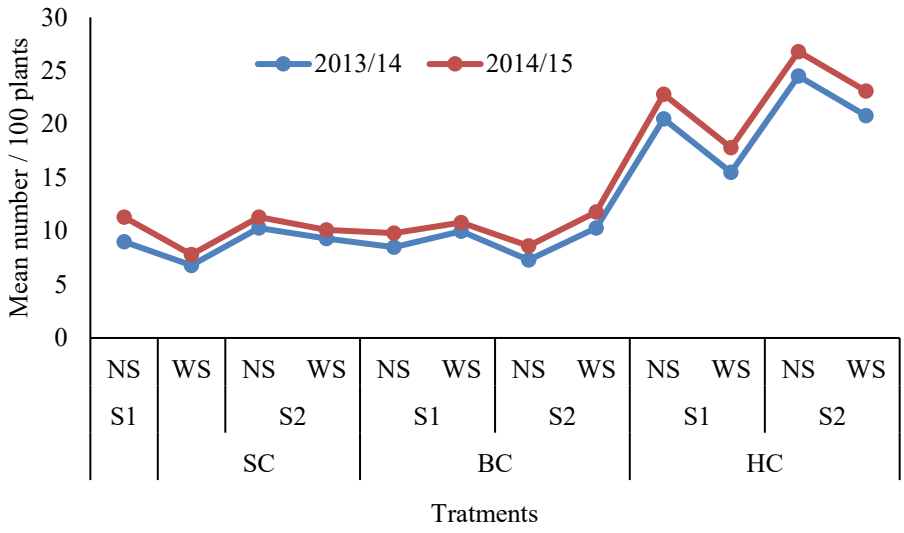

Fig. (4): Effects of cultivar, sowing date and spacing on Exochomus spp. adult mean number per 100 plants, 2013/14

Discussions

Insects inflicted a loss on cotton yield estimated as 30 – 50% (Peeter *et al.,* 2001). Pest control, high yield and good quality of cotton production can be practiced by cultural methods specially plant population (Smith and Falcon, 1973). Planting date management not only has a longer on crop growth, development and yield, but, also has impact effect on insect pest management (Brown *et al.,* 1998). Mealybug is a newly invading insect in cotton fields during this work in a drastic infestation correlated with coccinellides *Exochomus nigromaculatus* which appeared during November 2013/14 and it had obvious effect on mealybug.

The finding of this study, regardless of sowing date and spacing, Seni 1 and Brazili (LL) cultivars harbored low number of mealybug (2.3-8%) compared to Hamid cultivar (9.5-95.5%). Seni 1 and Brazili (LL) cultivars harbored low number of *Chrysopa* spp., *Cheiolomens* spp. and *Exochomus nigromaculatus* compared to Hamid cultivar. Hamid cultivar lately sown significantly harbored low number of the insect predators compared to Hamid cultivar early sown.

Bt cotton such as Seni 1 and Brazili is widely adopted, because it required substantially less pesticide than conventional cotton varieties, produce higher yields and incomes for poor farmers and better for health of small holder by reducing the number of insecticides spray (Fok and Xu, 2011). Elhassan (2015) reported that the optimum sowing date of cotton varies with variety, from period of late July to early August for Egytian long stable cotton. Planting date management was not only having a longer effect on crop growth, development and yield, but it also had impact on insect pest management (Brown *et al.,*1998). An early crop can be produced by selecting early maturing cotton cultivars, narrow spacing or combination of both (Elhassan, 2015). Reduced season management on which early planting plays a major role has become increasingly important with increased late seasons insect pressure. Moreover, the interaction effects between cultivar, sowing date and spacing might affect the seasonal temperature and relative humidity which were the most important factors that influence that the biology of cotton mealybug, hence the infestation will be affected (Elobeid *et al*, 2018 and 2019).

Chrysopa larvae are considered an important predators of long tailed mealybug in greenhouses and interior land escapes (Kapissa *et al.,* 1996). *Cheiolomens* spp. is important predatory insect that contribute to biocontrol programs of some major economic pests. It is polyphagus predator feeds mostly on aphids, although it feeding on soft scale, mealybug, whiteflies and psyllids was also recorded. It is often reaches high population so that it can be used as biocontrol agent in

greenhouses (Trunck *et al.,* 2007). *Exochomus nigromaculatus* is cheap and natural form of biocontrol for Aphid, whitefly, scale insect and the pest way to attract this insect into your green spaces is to steer clear of pesticides for as long as possible during the warm season (Najajrah *et al.,* 2019).

Conclusion

Genetically modified cotton in addition of controlling bollworm through possessing toxic protein that kill bollworm larvae, it also controls other insect pest through keeping the natural enemies and spare them to do their role in insect control when the infestation is complex without insecticide use. Cotton mealybug was checked during September to October to the end of the season without insecticide use. Natural enemies increased in number and reached the peak during September to December. Insect pests and natural enemies reflected density dependent pattern. The interaction between the cultivar, sowing date and spacing has positive effect on the insect control.

References

1. Abdel Rahman, A.A. (1967). Annual Report of the Entomology Section Khashm Elgirba research Substation for season 1966/67 Rep. Agric. Res. Corp. Sudan.
2. Brown, P.W., B. Russel, J. Silvertooth, P. Ellsworth, S. Husman, T. Knowles, L. Clark, D. Dunn and M. Schneider. (1998). The Arizona cotton advisory program p. 5-12. Cotton, Univ. of Arizona Rep. P-112.
3. Cabi. (2015). *Phenoacoccus solenopsis* (Cotton mealy bug). Invasive species Compendium. www.cabi.org. P. 40.
4. Culik, M.P. and Gullan, P.J. (2005). A new pest of tomato and other records of mealy bugs (Hemiptera: Pseudoccidae) from Espirito Santo, Brazil. Zootaxa 964: 1-8.
5. Elbasher, O.A. (2016). Vermination of climate changes using rainfall and temperature as indicators and its impacts on agricultural production in the arid zone of Sudan. Ph.D. Thesis, University of Gezira, Sudan.
6. Elfadil, A.B. (2007). Sudan cotton research and production scenarios; challenges, achievement and prospect country report ICAC Washington, www.icac. orgtisregional_networksdocumentsafrica…sudan
7. Elhassan, O.A. (2015). The Review of Scientific Research on Cotton Sudan. China-aid Agricultural Technology Demonstration Center. Pp. 115-126.
8. Elobeid, A E., Ali E. A., Elmubark, E. E., Dafaallah, A. B and Abdellah, AG. M. (2019). The Effect of Seasonal Temperature and Relative Humidity on the Infestation of Cotton Mealybug, *Phenacoccus solenopsis* Tinsley, on Tomato in Gezira State, Sudan (2016). *International journal of Horticulture, Agriculture and Food Science*, 3(3): 127-131.
9. Elobeid, A E., Elmubark, E. E., Abdellah, AG. M. and Dafaallah, A. B. (2019). Biology of Cotton Mealybug, *Phenacoccus solenopsis* (Tinsley) on some Vegetable Crops in the Gezira State, Sudan. *International Journal of Environment, Agriculture and Biotechnology*, 4(2): 246-249.
10. Faki, H. (2006). Sustainable cotton production from Sudan's Report ICAC, Washington, 4-13.
11. Fox, M. and Xu, N. (2011). Variety market development: A Bt cotton cropping factor and constrain in China. AgBioForum 14(2): 47-60. Available pn thr World Wide Web: http://www.agbioforum.org.
12. Hemba, M., Malatu, B. and Lemma, H. (2012). Cotton mealy bug *Phenacoccus solenopsis* (Hemiptera: Pseudoccidae) a newly introduced pest threatening cotton production in Ethiopia. Pest Management Journal of Ethiopia 16: 60-67.
13. Hodgson, C.B., Abass, G., Arif, M.J., Saeed, S. and Karar, H. (2008). *Phenacoccus solenopsis* Tinslru (Sternorrhyncha : Coccinoidea: Pseudococcidae), an invasive mealy bug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. Zootaxa, 1913: 1-35.
14. Joshi, C. (2015). Mealybugs damage crops. http://termirepel.com/beloy/12.5
15. Kabissa, J.C.B., Kayumbo, H.V. and Yarro, J.G. (1996). Seasonal abundance of chrysopids (Neuroptera: Chrysopidae) and Aphis gossypiii (Glover) (Homoptera: Aphidudace) on cotton in eastern Tanzania crop protection 15(1): 5-8.
16. Mursal, I.E. (1994). Cotton varieties for IPM and rainfed area. ARC variety release meeting, Khartoum, Sudan.
17. Mustafa, A.M., Babiker, E.A. and Elhassan, O.M. (2007). Proposal for the release of new high yielding and highly resistance to bacterial blight cotton varieties. ARS. Sudan.
18. Nagrare, V.S., Rishi, Kumar, Dhara Jothi, B. Amutha, M. Deshumkh, A.J., Bisane, K.D. and Kranthi, K.R. (2011). Compendium of Cotton Mealy bugs. Central Institute for Cotton research, Nagpur, pp. 42.
19. Najajrah, M.H.; Swaileh, K.M.; Mazin, B. (2019). Systematic list geographic distribution and ecological significance of ladybeetles (Coleoptera: Coccinellidae) from the West Bank (Central Palestine) Zootaxa 4664(1): 1-46, Dol.Mediated http://doi.org/10.11646/zootaxa4661.1plazi.orgtaxonomictreatmentdatabase
20. Omer, B.M. (2002). Benefits and efficacy of two groups of insecticides in controlling the major cotton insect pests at the Rahad Agricultural Scheme, Ph.D. Thesis, University of Gezira, Sudan.
21. Peeter, M.C.; Van Langenhove, L.; Lowwagie, J.; Water Keyn, L. and Mergeai, G. (2001). Fibre crops cotton *Gossympium hirstum* L. In: Crop Production in Tropical Africa. Edited by Romain, H. Raemeakers. Pp 1040-1071.
22. Smith, R.F. and Falcon, L.A. (1973). Insect control for cotton in California, Cotton Grow, Rev. 50: 15-27.

23. Trunck, W., Wise, I. and Malheson, F. (2007). Abundance of some native coccinellines (Coccinellidae: Coleoptera) before and after appearance of the Lana *Coccinella septempunctat*, 135: 391-404-dian Enomologest.

24. إبراهيم، جمعة خليل (1993): الحشرات الاقتصادية. منشورات جامعة حلب.

25. إبراهيم الحاج مرسال، يوسف فيصل، الحمدي عبد الله البكر، إبراهيم عثمان العبيد، أبو الحسن صالح إبراهيم، مختار نوري عثمان (1997): دليل المدرب (8) لمشروعات المحاصيل الحقلية، السودان، وزارة الزراعة والغابات، هيئة البحوث الزراعية TCP/SUN/6616.

26. السيد سليمان سيد أحمد (2002): مشروع الجزيرة الأسطورة الحية (دراسة أولية)، الخرطوم.

27. نمر يوسف، صبوح محمود (1996): محاصيل الألياف (الجزء النظري) – منشورات جامعة دمشق، مطبعة ابن حيان، دمشق.

28. يوسف عبد القادر عبد الفتاح ضوي (2007): الشيخ المجاهد عبد القادر ود حبوبة، شركة الجزيرة للطباعة والنشر المحدودة، ود مدني ص 134-138.

Effects of some Cultural Practices on the Management of the African Bollworm, *Helicoverpa armigera* Hun, Gezira State, Sudan

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Abstract

Cotton is grown in Sudan as rainfed crop, as well as grown by permanent irrigation. Usually insecticides were used to control cotton insect pests, but in Gezira scheme cotton produced for the first three decades (1911-1945) without chemical control. That might be due to the balanced nature. This research aims to reduce the cost of production and mitigating the environmental pollution through managing the African bollworm, *Helicoverpa armigera* Hun. *via* cultural practices at Gezira State, Sudan. Two experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan during season 2013/14 and 2014/15. In each season a factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni 1, Brazili (LL) and Hamid local variety. The sowing dates were third week of June and July, whereas the spaces were 25 and 50 cm intra row. Treatments were arranged in a completely randomized block design with three replicates. The insect pest was recorded either for their presence or their damage that inflicted on cotton plants. Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure. In general, the results showed that there were significant ($P \leq 0.05$) interaction effects between cultivar, sowing date and spacing on the number of eggs, larvae and on percentage of shedding of flowers and buds caused by African bollworm. Significantly, the lowest number of eggs (0 eggs per 100 plants) was recorded in Seni 1 cultivar lately sown with wide spacing, while the highest number of eggs (2.8 eggs per 100 plants) was recorded in Hamid cultivar early sown with narrow spacing. Significantly, the lowest number of larvae (0-0.8 larvae per 100 plants) was recorded in Seni 1 cultivar early sown with narrow spacing, while the highest number of larvae (4.5 -6.3 larvae per 100 plants) was recorded in Hamid cultivar early sown. Significantly, the percentage of shedding of flowers and buds (1.2-9 %) was recorded in Seni 1 cultivar early sown with either narrow or wide spacing, while the highest percentage of shedding of flowers and buds (26-31.2%) was recorded in Hamid cultivar lately sown. The highest weight was achieved by Seni 1 cultivar followed by Brazili (LL) cultivar and then by Hamid cultivar. In conclusion, to achieve the lowest incidence and damage to cotton the Gezira State, Sudan Seni 1 cultivar could be early sown (third week of June) with either narrow spacing (25 cm a part between holes).

Keywords: African bollworm, Cotton, Cultivar, Sowing date, Spacing.

Introduction

Cotton (*Gosypium* spp.) is a soft stable fibre of shrub plant native to tropical and subtropical regions of the world. Cotton was cultivated since 7000 years ago by the inhabitants of the Indus valley civilization. However, Egyptian cotton (*G. barbadense* L.) planting in Sudan went back to the 19th century when it was grown for the first time in eastern Sudan (Tokar area), driven by the interest and the initiative of the Turkish – Egyptian rule (Faki, 2006). Short staple cotton was grown in Gezira area as rainfed crop before the establishment of the Gezira scheme (Mudawi, 2007). However, the long staple cotton was commercially grown in 1905 at Zeidab scheme in northern Sudan. Nevertheless, 1925 was a land mark for its commercial production in Sudan, following the establishment of Sennar dam. Since then cotton has played a leading role as a cash crop. The organization of cotton production in Sudan started through establishing a large governmental administration with the participation of tenants. Since then cotton is the most important cash crop in Sudan where the annual revenue from cotton often constitutes 40-60% of the total national income (Mursal *et al.,* 1997). In the Sudan about 900.000 feddans were used to be groawn annually to cotton with an average yield of 2.9 - 4.9 Quintal/fed in the Gezira (Omer, 2002, Seid Ahmed, 2002; Nimer and Saboh, 1996) (1.0 Quintal = 100 pounds, lint cotton).

Despite the historical position of Sudan on cotton market, over years, Sudan's cotton production assumed a declining trend, mainly due to declining in area and crop yield. Production average was 930 thousand bales (420 lb) of lint during 1970s; it declined to an average of 432 thousand bales during the 1990s and was drop further during the 2000s (Faki, 2006). Yield have been fluctuating between a minimum of 779 to a maximum of 1949 kg/ha in the irrigated sector compared to between 94 and 952 kg/ha in the rainfed sector (Faki, 2006). After the enforcement of the Gezira Scheme act for the year 2005, the production relation started to change and the major features of this change were the relaxation of the strong government grip on agricultural scheme and as a result the tenants were not committed to the crop rotation where cotton was the backbone.

Sudan produces five types of cotton, namely the extra-fine, fine, higher account, medium and course account was represented by the varieties Barakat 90 (EFC), Shambat-B (FC), Nour (HCA), Barac (67) B (MC), Albar (57) 12 (CC) and Acrain (CC) Elfadil (2007). Some of these cultivars were out of production such as Shamabat-B, because of its ginning problems and low turn of ginning outcome (Mursal, 1994). New varieties have been released such as Hamid, Abdin, Knigt, Khairalla (Mursal *et al.*, 2004). Burhan, Khalifa and Wagar (Mustafa *et al.,* 2007) to replace some of long grown varieties such as Shambat-B. Bt cotton which was introduced from China (Seni-1) has been approached (Ali *et al.,* 2012) and was cultivated in the irrigated and rainfed sectors. Elhassan (2015) reported that the optimum sowing date of cotton varies with variety, from period of late July to early August for Egytian long stable cotton. Planting date management was not only having a longer effect on crop growth, development and yield, but it also had impact on insect pest management (Brown *et al.,* 1998). An early crop can be produced by selecting early maturing cotton cultivars, narrow spacing or combination of both (Elhassan, 2015). Reduced season management on which early planting plays a major role has become increasingly important with increased late seasons insect pressure.

The African bollworm, *Helicoverpa armigera* Hun (Lepidoptera: Noctuidae), was reported in Africa, southern Europe, Asia, Australia, and Newzealand (Ibrahim, 1993, Elahmadi and Kais (1986). In Sudan, it was reported with wide range hosts (Schumtterer, 1969). The African bollworm is polyphagous. It was reported on a number of plants including field crops such as cotton, groundnut, maize and sorghum, and on vegetables crops such as tomatoes, cucurbit, peas and peppers as well as fruit trees such as citrus (Joyce, 1959 and Ibrahim, 1993).

Eggs are laid singly on the primordial leaves at the top of plants or end of the branches as well as on fruiting bodies (Anonymous, 1984). The incubation period is usually 3-5 days (Caresche, 1986). The larval period is governed by temperature and availability of food (Pearson, 1958; Elahmadi and Kais, 1986), it was ranging between 18-25 days (Schumtterer, 1969, Balla, 1975), to give 5-6 instars (Lazarevic, 1965; Ibrahim 1993, Elahmadi and Kais, 1986). The pupal period was 8-13 days and can be extended for several weeks (Schumtterer, 1969). Adult can be lived for 10-

14 days during summer time and the period is more during winter to give several hundreds of eggs (Anonymous, 1984). However, 3-4 generations per season were reported on cotton (Balla, 1978).

African bollworm is the most important pest on cotton that is because it attacks the fruiting bodies such as buds, flowers and bolls (Joyce, 1959). A single larva can inflict damage on more than one fruiting body on the same plant (Peeter *et al.,* 2001). Young fruiting bodies usually shed off, while, large bolls getting rottened (Ibrahim, 1993). Hence, it affects both quality and quantity (Schumtterer, 1977). The African bollworm is more serious when infestation takes place at fruiting stage. It was proved that 7 larvae/100 plants reduced the yield by 24% (Balla, 1978). The insect is more damaging on shorter growing cycle cultivars under rainfed areas (Ripper and George, 1965).

One of the major constraints of cotton production in Sudan is the insect pests. These pests include African bollworm (*Helicoverpa armigera* Hun (Mathew, 1989). Insect pests control depends mainly on insecticides use. However chemical control is expensive and it constitutes 20-40% of the total cost of production (FAO, 1973). The misuse of insecticides creates chemical resistance to target pest, appearance of secondary pests and pest resurgence. Nevertheless, the impact of insecticides upon natural enemies is drastic (Loaota, 2001). However, insecticide misuse has a negative impact on human being, animals and environment which could be shown in food and feed contamination as well as pollution (OTA, 1990; Joffee, 1995).

Cotton is produced in many countries under integrated pest management (IPM) one of the major components of IPM is the cultural practices including plant population. Better design of plant population is a good tool for pest control, high yield and good quality of cotton production (Smith and Falcon, 1973). Genetically modified (GM) cotton was developed to reduce the heavy reliance on pesticides. In many regions, the main pest in commercial cotton are Leipdopterus larvae which were killed by the (Bt) protein in transgenic cotton they eat. This eliminates the need to use large amount of broad spectrum insecticides to kill Leipdopterus larvae and at the same time it spares natural insect predators in the farm, thus contributing to no-insecticides pest management.

General objective

To adopt plant population, sowing date and genetically modified cotton as tools for pest control under integrated pest management (IPM).

Specific objectives

i) To grow cotton under various intra row spacing.
ii) To test different cotton genotypes.
iii) To grow cotton at a set of sowing dates.

Materials and Methods

2.1. Experimental site

Experiments were conducted at the Experimental Farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan, latitude 14° 22' N, longitude 33° 39' E and Altitude 407 meter above sea level, during the seasons 2013/2014 and 2014/2015. The climate of the study area is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season extended from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12°C in January and 42°C in May, respectively. The soil of the area is characterized by heavy clay soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous (Elbasher, 2016).

2.2. Treatments

A factorial experiment was conducted with three cotton varieties, two sowing dates and two spaces. The varieties were Seni I designated as (SC), Brazili (LL) designated as (BC) and Hamid local variety designated as (HC). The sowing dates were third week of June (S_1) and July (S_2). The spaces were 25 and 50 cm intra row for narrow space (NS) and wide specs (WS), respectively.

3.3 The experiment layout and cultural practices

The experiment was composed of 12 treatments (3×2×2) replicated 4 times to give 48 plots. The plot size was 6 rows each of 10.0 m length to give a plot size of 48.0 m^2. The experiment was laid out as a completely randomized block design (CRBD). The experimental area was deep plowed, harrowed and prepared in ridges of 0.8 m apart. The distance between each two plots was two ridges and between each two replicates was 3 m. Planting was done manually and each hole received 5-6 cotton seeds treated with Gaucho 70% WS at a rate of 3.0 g kg^{-1} seed. For weed control pre-emergence herbicides, Stomp 500 EC + Gezagard 500 FW were used as a product mixture at a dose of 0.60 + 0.20 kg ai. per feddan (4200 m^2). Hand weeding was done twice. The plots were irrigated after herbicides application, then followed by another watering 72 hours after the first irrigation. After that irrigation was practiced periodically every two weeks, till harvesting. Plants were thinned to 3 plants after 4 weeks of the effective watering. Fertilization was done after thinning using Urea at 2N dose. The first 1N was done immediately after thinning and the second 1N dose was applied a month later.

3.4. Data Collection

The insects were recorded either for their presence or their damage that inflicted on cotton plants. The monitored pest was African bollworm, *Helicoverpa armigera* Hun. Eggs and larvae were examined on each of the 10 plants per plot. The examined area was the top third part of the plant where egg is laid. Also, the percentage shedding of flowers and buds caused by ABW was recorded. The seed cotton weight in kg/fed was recorded for 4 picks.

3.6. Data analysis

Data were transformed as needed and subjected to analysis of variance (ANOVA) procedure. Significant ($P \leq 0.05$) means were separated using Duncan's Multiple Range test (DMRT). The statistical analysis was done using the Software MSTAT.

Result

African bollworm was started to appear during August in season 2013/14, during September in season 2014/15 and then decreased during October on both seasons. On season 2013/14, it was found to appear in August at low level at both plant spaces on Hamid cultivar only, whereas no incidence on Seni 1 and Brazili (LL) cultivars was reported. The results were monitored as evaluation of the count of eggs, larvae and the shedding of reproductive organs (buds + flowers). Generally, the ABW on the three cultivars was under check.

3.1. Effects of interaction of cultivar, sowing date and spacing on incidence of ABW eggs

Season 2013/14

The results showed that the African bollworm was started to appear during August and then decreased during October (Table 1). In August, the insect eggs were found to appear at low level (2.5 eggs/ 100 plants) at both plant spaces on Hamid cultivar only, whereas no incidence on Seni 1 and Brazili (LL) cultivars was reported. In September, there were significant ($P \leq 0.05$) interaction effects between cultivar, sowing date and spacing on the number of eggs per 100 plants. Significantly, the lowest number of eggs (0 eggs per 100 plants) was recorded in Seni 1 cultivar lately sown with wide spacing, while the highest number of eggs (5 eggs per 100 plants) was

recorded in Hamid cultivar early sown with narrow spacing. Moreover, the were no significant differences in numbers of eggs between the rest of the treatments. In October, there were significant interaction effects between cultivar, sowing date and spacing on the number of eggs per 100 plants. Regardless, sowing date and spacing, the insect eggs were not found on Seni 1cultivar. The insect eggs were not found on Brazili (LL) cultivar early sown with wide spacing and lately sown with narrow spacing. Also, the insect eggs were not found on Hamid cultivar early sown with wide spacing.

Table (1): Effect of cultivar, sowing date and spacing on ABW Eggs mean number/100 plants (2013/14)

Treatment			ABW Eggs mean number/100 plants			
Cultivar	Sowing date	Spacing	Aug.	Sep.	Oct.	General performance
SC	S_1	NS	0.7 (0.0)	1.2 ab (1.0)	0.7 (0.0)	0.9 bc (0.3)
		WS	0.7 (0.0)	1.1 ab (0.8)	0.7 (0.0)	0.8 bc (0.3)
	S_2	NS	0.7 (0.0)	1.1 ab (1.0)	0.7 (0.0)	0.8 bc (0.3)
		WS	0.7 (0.0)	0.7 b (0.0)	0.7 (0.0)	0.7 c (0.0)
BC	S_1	NS	0.7 (0.0)	1.1 ab (1.0)	1.4 (2.0)	1.1 abc (1.0)
		WS	0.7 (0.0)	1.0 ab (0.8)	0.7 (0.0)	0.8 bc (0.3)
	S_2	NS	0.7 (0.0)	1.2 ab (1.5)	0.7 (0.0)	0.9 bc (0.3)
		WS	0.7 (0.0)	1.1 ab (1.0)	1.4 (2.0)	1.1 abc (1.0)
HC	S_1	NS	1.5 (2.5)	2.0 ab (3.4)	1.4 (2.0)	1.7 a (2.8)
		WS	1.5 (2.5)	2.3 a (5.0)	0.7 (0.0)	1.6 ab (2.3)
	S_2	NS	0.7 (0.0)	2.0 ab (3.4)	1.4 (2.0)	1.5 ab (2.0)
		WS	0.7 (0.0)	1.6 ab (2.5)	1.5 (2.0)	1.4 abc (1.8)
SE±			0.19	0.26	0.22	0.16
CV (%)			45.92	37.68	44.99	28.52

* Data are transformed to $\sqrt{x + 0.5}$

** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.

*** Actual data between parentheses.

The general performance showed that there were significant interaction effects between cultivar, sowing date and spacing on the number of eggs per 100 plants. Significantly, the lowest number of eggs (0 eggs per 100 plants) was recorded in Seni 1 cultivar lately sown with wide spacing, while

the highest number of eggs (2.8 eggs per 100 plants) was recorded in Hamid cultivar early sown with narrow spacing.

Season 2014/15

The results showed that the African bollworm was started to appear during October (Table 2). In October, the number of eggs was low (0 – 1.8 eggs per 100 plants) and there were no significant interaction effects between cultivar, sowing date and spacing on the number of eggs per 100 plants. The general performance showed that there were no significant interaction effects between cultivar, sowing date and spacing on the number of eggs per 100 plants. Significantly, the lowest number of eggs (0 eggs per 100 plants) was recorded in Seni 1 cultivar early sown with narrow spacing and lately sown with narrow spacing. While the highest number of eggs (1.3 eggs per 100 plants) was recorded in Hamid cultivar early sown with wide spacing.

Table (2): Effect of cultivar and sowing date and spacing on ABW eggs mean number/100 plants (2014/15)

Treatment			ABW eggs mean number/100 plants		
Cultivar	Sowing date	Spacing	Sep.	Oct.	General performance
SC	S_1	NS	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)
		WS	0.7 (0.0)	0.9 (0.0)	0.9 (0.4)
	S_2	NS	0.7 (0.0)	0.7 (0.5)	0.7 (0.0)
		WS	0.7 (0.0)	1.0 (1.0)	0.8 (0.3)
BC	S_1	NS	0.7 (0.0)	1.0 (0.8)	1.0 (0.5)
		WS	0.7 (0.0)	0.9 (0.5)	1.0 (0.3)
	S_2	NS	0.7 (0.0)	1.0 (1.0)	0.9 (0.5)
		WS	0.7 (0.0)	0.8 (0.3)	0.8 (0.3)
HC	S_1	NS	0.7 (0.0)	1.3 (1.3)	1.3 (1.3)
		WS	0.7 (0.0)	1.5 (1.8)	1.3 (1.3)
	S_2	NS	0.7 (0.0)	1.2 (1.0)	1.1 (0.8)
		WS	0.7 (0.0)	1.2 (1.3)	1.1 (0.8)
SE±			0.10	0.30	0.20
CV (%)			19.43	40.95	29.51

* Data are transformed to $\sqrt{x + 0.5}$
** Actual data between parentheses.

3.2. Effects of interaction of cultivar, sowing date and spacing on incidence of ABW larvae

Season 2013/14

The results showed that the incidence of ABW larvae was started to appear during September and then decreased during October (Table 3). In August, the insect larvae were found to appear at low level (2.5 larvae/ 100 plants) on Hamid cultivar early sown with narrow and wide spaces,

whereas no incidence on Seni 1 and Brazili (LL) cultivars was reported. In September, there were significant (P ≤ 0.05) interaction effects between cultivar, sowing date and spacing on the number of larvae per 100 plants. Significantly, the lowest number of larvae (0.3 larvae per 100 plants) was recorded in Seni 1 cultivar lately sown with narrow and wide spaces, while the highest number of larvae (9.3 larvae per 100 plants) was recorded in Hamid cultivar early sown with wide spacing. In October, there were no significant interaction effects between cultivar, sowing date and spacing on the number of larvae per 100 plants. The general performance showed that there were significant interaction effects between cultivar, sowing date and spacing on the number of larvae per 100 plants. Significantly, the lowest number of larvae (0 larvae per 100 plants) was recorded in Seni 1 cultivar early sown with narrow spacing, while the highest number of larvae (4.5 larvae per 100 plants) was recorded in Hamid cultivar early sown with wide spacing.

Season 2014/15

The incidence of ABW larvae was started to appear during September and then decreased during September (Table 4). In September, the number of larvae was low (0 – 2.5 larvae/ 100 plants) and there were no significant interaction effects between cultivar, sowing date and spacing on the number of larvae per 100 plants. The general performance showed that there were significant interaction effects between cultivar, sowing date and spacing on the number of larvae per 100 plants. Significantly, the lowest number of larvae (0.8 larvae per 100 plants) was recorded in Seni 1 cultivar early sown with narrow spacing. While the highest number of larvae (6.3 larvae per 100 plants) was recorded in Hamid cultivar early sown.

3.3. Effects of interaction of cultivar, sowing date and spacing on incidence of ABW (eggs + larvae)

Hamid cultivar at early sowing was vulnerable to egg laying and larvae of ABW at significant level (P ≤ 0.05) compared to cultivar Seni 1 at both sowing dates and Brazili (LL) at late sowing date only (Table 5 and 6). On the other hand, Hamid cultivar at late sowing date for the two spaces under test gave similar results to Brazili (LL) at early sowing date at the two spaces used.

Table (3): Effect of cultivar, sowing date and spacing on ABW larvae mean number/100 plants (2013/14)

Treatment			ABW larvae mean number/100 plants			
Cultivar	Sowing date	Spacing	Aug.	Sep.	Oct.	General performance
SC	S_1	NS	0.7 (0.0)	1.0 c (0.5)	0.7 (0.0)	0.7 c (0.0)
		WS	0.7 (0.0)	1.4 bc (1.8)	0.7 (0.0)	1.0 bc (0.5)
	S_2	NS	0.7 (0.0)	0.8 c (0.3)	1.5 (2.5)	1.1 bc (1.0)
		WS	0.7 (0.0)	0.8 c (0.3)	0.7 (0.0)	0.8 bc (0.3)
BC	S_1	NS	0.7 (0.0)	1.9 abc (3.3)	0.9 (0.5)	1.0 abc (2.3)
		WS	0.7 (0.0)	2.2 abc (4.8)	0.7 (0.0)	1.0 bc (0.5)
	S_2	NS	0.7 (0.0)	1.2 bc (1.8)	0.7 (0.0)	0.8 bc (0.3)
		WS	0.7 (0.0)	1.1 c (1.0)	0.9 (0.5)	1.0 bc (0.5)
HC	S_1	NS	1.5 (2.5)	2.7 ab (7.0)	1.6 (3.0)	2.1 ab (4.0)
		WS	1.5	3.1 a	1.3	2.3 a

			(2.5)	(9.3)	(2.0)	(4.8)
	S_2	NS	0.7	2.0 abc	1.3	1.5 abc
			(0.0)	(3.8)	(2.5)	(2.0)
		WS	0.7	1.8 abc	1.2	1.4 abc
			(0.0)	(3.3)	(1.5)	(1.8)
SE±			0.27	0.41	0.45	0.31
CV (%)			45.66	35.08	61.86	33.21

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Table (4): Effect of cultivar and sowing date on ABW larvae mean number/100 plants (2014/15)

Treatment			**ABW larvae mean number/100 plants**		
Cultivar	Sowing date	Spacing	Sep.	Oct.	General performance
SC	S_1	NS	0.7 (0.0)	1.3 cd (3.0)	1.1 d (0.8)
		WS	0.7 (0.0)	1.6 bcd (5.0)	1.3 bcd (2.3)
	S_2	NS	0.7 (0.0)	1.0 d (2.8)	1.2 cd (1.5)
		WS	0.7 (0.0)	2.7 ab (3.8)	1.2 cd (1.3)
BC	S_1	NS	1.3 (1.5)	2.4 abc (13.3)	2.4 a (4.5)
		WS	1.3 (1.5)	2.3 abc (7.5)	2.2 ab (2.3)
	S_2	NS	0.9 (0.8)	2.2 abcd (10.)	1.7 abcd (3.3)
		WS	0.7 (0.0)	3.2 a (11.5)	1.9 abcd (4.0)
HC	S_1	NS	0.9 (0.5)	2.9 a (18.5)	2.4 a (6.0)
		WS	1.6 (2.5)	2.9 a (21.5)	2.4 a (6.3)
	S_2	NS	1.1 (1.0)	2.5 abc (20.8)	2.2 a (7.0)
		WS	0.7 (0.0)	1.3 cd (15.3)	2.1 abc (4.3)
SE±			0.29	0.36	0.27
CV (%)			44.29	23.42	20.83

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Table (5): Effect of cultivar, sowing date and spacing on ABW (eggs +larvae) mean number/100 plants (2013/14)

Treatment			ABW (eggs +larvae) mean number/100 plants		
Cultivar	Sowing date	Spacing	Sep.	Oct.	General performance
SC	S_1	NS	1.8 b (3.3)	0.7 (0.0)	1.5 b (2.0)
		WS	1.9 b (4.5)	0.7 (0.0)	1.4 b (2.3)
	S_2	NS	1.1 b (1.3)	1.2 (6.0)	1.1 b (3.3)
		WS	0.7 b (0.0)	1.2 (1.5)	0.9 b (0.5)
BC	S_1	NS	3.7 ab (15.3)	1.34 (2.5)	3.1 ab (10.3)
		WS	4.1 ab (17.0)	0.7 (0.0)	3.2 ab (10.3)
	S_2	NS	2.0 b (9.0)	0.7 (0.0)	1.1 b (1.0)
		WS	1.7 b (5.3)	1.3 (2.0)	1.5 b (4.0)
HC	S_1	NS	6.1 a (37.3)	2.6 (10.0)	5.1 a (26.3)
		WS	6.1 a (40.5)	1.8 (6.0)	5.2 a (26.8)
	S_2	NS	3.5 ab (15.8)	1.9 (5.5)	3.2 ab (13.0)
		WS	3.8 ab (12.3)	2.4 (8.5)	2.3 ab (7.8)
SE±			1.0104	0.87	0.77
CV (%)			47.45	86.09	43.31

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Table (6): Effect of cultivar, sowing date and spacing on ABW (eggs + larvae) mean number /100 plants (2014/15)

Treatment			ABW (eggs + larvae) mean number /100 plants		
Cultivar	Sowing date	Spacing	Sep.	Oct.	General performance
SC	S_1	NS	1.8 a (3.0)	1.7 c (3.0)	1.3 b (1.3)
		WS	2.5 a (6.0)	2.2 bc (5.0)	1.6 b (2.3)
	S_2	NS	1.8 a (3.0)	1.7 c (2.8)	1.3 b (1.5)
		WS	2.5 a (3.0)	1.9 c (3.8)	1.3 b (1.3)
BC	S_1	NS	2.2 ab (6.0)	3.7abc (13.3)	2.2 ab (4.5)
		WS	2.7 a (7.0)	2.7 abc (7.5)	1.6 ab (2.3)

HC	S_2	NS	2.1 a (4.0)	3.2 abc (10.5)	1.3 b (3. 3)
		WS	2.3 a (6.0)	3.4 abc (11.5)	2.1 b (4.0)
	S_1	NS	3.2 a (10.3)	4.3 ab (18.5)	2.5 ab (6.0)
		WS	3.4 a (12.0)	4.5 ab (21.5)	2.5ab (6.3)
	S_2	NS	3.3 a (11.0)	4.6 a (20.8)	2.7 ab (7.0)
		WS	2.5 a (7.0)	3.8 abc (15.3)	2.1 b (4.3)
SE±			0.8	0.64	0.37
CV (%)			33.18	28.57	27.11

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

3.3. Effects of interaction of cultivar, sowing date and spacing on shedding of flowers and buds caused by ABW

Season 2013/14

Seni 1 cultivar showed that no significant difference between the two spaces tested on the early sowing, while in late sowing, wide spacing the shedding was significantly high (Table 7). In early sowing, shedding was significantly low on the two spaces compared to late sowing. Brazili (LL) cultivar showed no significant differences between the two sowing dates and the two spaces under test. Nevertheless, Hamid cultivar reflected no significance differences on the two spaces during the two sowing dates.

Table (7): Effect of cultivar, sowing date and spacing on percentage shedding of flowers and buds caused by ABW (2013/14)

Treatment			Percentage shedding of flowers and buds			
Cultivar	Sowing date	Spacing	1st reading	2nd reading	3rd reading	General performance
SC	S_1	NS	0.7 b (0.0)	2.3 c (5.0)	0.7 (0.0)	1.5 d (1.7)
		WS	0.7 b (0.0)	2.3 c (5.6)	0.7 (0.0)	1.5 d (1.9)
	S_2	NS	0.7 b (0.0)	3.5 bc (11.8)	0.7 (0.0)	2.1 cd (3.9)
		WS	0.7 b (0.0)	4.8 abc (28.2)	0.7 (0.0)	2.8 bcd (9.0)
BC	S_1	NS	3.0 ab (10.3)	5.0 abc (27.2)	0.7 (0.0)	4.3 abc (19.2)
		WS	3.2 a (9.8)	5.3 abc (31.6)	1.5 (3.6)	4.2 abc (17.5)
	S_2	NS	4.1 a (16.5)	6.0 abc (39.5)	1.6 (4.2)	4.5 abc (20.1)
		WS	3.6 a (15.3)	5.0 abc (29.8)	2.5 (8.9)	4.0 abc (18.0)
HC	S_1	NS	3.2 a (10.0)	6.8 ab (46.9)	1.7 (5.0)	5.2 ab (26.3)
		WS	4.2 a	6.5 abc	0.7	4.5 abc

			(17.3)	(43.0)	(0.0)	(20.1)
	S_2	NS	2.8 ab (8.7)	6.8 ab (50.9)	3.1 (14.6)	5.0 ab (26.0)
		WS	4.3 a (18.3)	8.5 a (72.7)	2.2 (6.7)	5.6 ab (31.2)
SE±			0.66	1.18	0.99	0.67
CV (%)			35.79	31.90	100.48	25.00

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Season 2014/15

The results showed that on Seni 1 cultivar no significant difference between the two spaces on early sowing, while on late sowing wide spacing was reflecting significantly high level of shedding of reproductive organs (Table 8). However, Brazili (LL) cultivar under wide spacing was reflected no significant differences during the two sowing dates, while under narrow spacing the cultivar was reflecting significantly higher level of shedding than the wide spacing on both sowing dates.

Table (8): Effect of cultivar, sowing date and spacing on percentage shedding of flowers and buds caused by ABW (2014/15)

Treatment			Percentage shedding of flowers and buds			
Cultivar	Sowing date	Spacing	1st reading	2nd reading	3rd reading	General performance
SC	S_1	NS	2.0 bc (1.2)	0.7 b (0.0)	0.7 (0.0)	1.3 c (1.2)
		WS	1.8 c (1.3)	0.7 b (0.0)	0.7 (0.0)	1.2 c (1.3)
	S_2	NS	2.5 abc (2.4)	0.7 b (0.0)	0.7 (0.0)	1.6 bc (2.4)
		WS	5.2 abc (11.2)	0.7 b (0.0)	0.7 (0.0)	3.1 abc (11.2)
BC	S_1	NS	7.7 abc (25.0)	3.6 a (13.6)	0.7 (0.0)	5.0 a (25.0)
		WS	5.7 abc (17.4)	3.4 a (11.3	0.7 (0.0)	4.0 abc (17.4)
	S_2	NS	6.2 abc (21.6)	4.2 a (17.1)	2.8 (12.2)	4.5 ab (21.6)
		WS	5.3 abc (17.7)	3.8 a (17.6)	2.2 (6.3)	3.9 abc (17.7)
HC	S_1	NS	7.6 a (26.2)	4.2 a (17.9)	1.7 (5.0)	5.1 a (26.2)
		WS	6.6 abc (18.5)	3.2 ab (11.7)	0.7 (0.0)	4.3 ab (18.5)
	S_2	NS	7.ab (26.2)	3.4 ab (12.0)	2.8 (11.7)	5.0 a (26.2)
		WS	8.5 a (30.1)	4.2 a (18.2)	1.3 (2.1)	5.5 a (30.1)
SE±			1.64	0.72	0.93	0.80
CV (%)			41.55	37.20	100.31	30.61

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

3.4. Effects of interaction cultivar, sowing date and spacing on seed cotton weight in kg/fed (2013/14 – 2014/15)

Seni 1 cultivar is significantly high in weight than Hamid cultivar (Table 9). The early sown cotton in the three cultivars gained more yield compared late sown cotton cultivars. On the Other hand, narrow spacing yielded much better than yield on wide spacing among all cotton cultivars. However, as a general rule narrow spacing and early sowing proved to have much yield than other alternative among all cotton cultivars.

Table (9): Effect of cultivar, sowing date and spacing on percentage shedding of flowers and buds caused by ABW (2014/15)

Treatment			Seed cotton weight in kg/fed	
Cultivar	Sowing date	Spacing	2013/14	2014/15
SC	S_1	NS	(1494.0) 38.4 a	(2232.4) 47.2 abc
		WS	(1464.3) 38.2 a	(2288.7 47.8a
	S_2	NS	(1129.3) 33.5 ab	(2266.0 47.5 ab
		WS	(1045.8) 38.2 a	(2411.8) 49.0 a
BC	S_1	NS	(618.5) 24.8 abcd	(1870.3) 43.0 abcd
		WS	(752.6) 27.4 bcd	2184.0) 46.7 abc
	S_2	NS	(1110.8) 33.2 abc	(1650.9) 40.4 abcd
		WS	(700.6) 26.3 bcde	(1265.4) 35.6 d
HC	S_1	NS	(661.8) 25.5 bcde	(1728.4) 41.5 abcd
		WS	(615.4) 24.7 cde	(1405.1) 37.2 cd
	S_2	NS	(357.6) 18.9 de	(1410.8) 37.2 bcd
		WS	(332.8) 18.2 e	(1279.6) 35.7 d
SE±			1.64	0.72
CV (%)			41.55	37.20

* Data are transformed to $\sqrt{x + 0.5}$
** Means in the same column followed by the same letter(s) are not significantly (P ≤ 0.05) different according to Duncan's Multiple Range Test.
*** Actual data between parentheses.

Discussions

Insects inflicted a loss on cotton yield estimated as 30 – 50% (Peeter *et al.,* 2001). Pest control, high yield and good quality of cotton production can be practiced by cultural methods specially plant population (Smith and Falcon, 1973). Planting date management not only has a longer on crop growth, development and yield, but, also has impact effect on insect pest management (Brown *et al.,* 1998). The African bollworm is serious because it shows several generations on cotton (Balla, 1978) and feed on fruiting bodies including buds, flowers and bolls (Joyce, 1959) where a single larva can inflict damage on more than one fruiting body on the same plant (Peeter *et al.,* 2001). Although, the total number shedding of fruiting bodies due to the damage of (ABW) was in the range of 30-70% in August and 20-40% in September, but, yet no larvae were detected. It was

known that the females of (ABW) were highly production and if left unchecked in, severe crop damage will be done due to feeding of larvae on fruiting bodies, buds, flowers and bolls (Ibrahiem *et al.,* 1993; Anon., 1990b). Herrera (1986) proved that efficacy of general predators such as Chrysopa and members of Spider families as a mean of African bollworm control was due to the fact that, they feed on eggs and young larvae, that means before damage is taking place. Narrow spacing induced early flowering and short maturity period (El Hassan, 2015). In the Sudan, cotton was grown under a population of less than 30000 plants per feddan. However, in USA this number of plants per feddan was increased 7-10 times (Smith and Falcon, 1973). In this study when the number of plants per feddan was increased (narrow spacing) the yield increased (spacing 25 cm) the yield is more than the traditional spacing, 50 cm. Similar trend of increasing in yield due to narrow spacing was observed when Hamid and Brazili (LL) cultivar was grown at 25 cm spacing compared to 50 cm spacing. Cotton varieties are different in term of their response to insect pests. It was stated that long staple cotton planted on July are subject to infestation of Sudan bollworm, pink bollworm and flea beetle (Ripper and George, 1965; Schullz *et al.,* 1967; Schumtterer, 1969 and Jackson, 1970).

Conclusion

There were significant ($P \leq 0.05$) interaction effects between cultivar, sowing date and spacing on the number of eggs, larvae and on percentage of shedding of flowers and buds caused by African bollworm. In conclusion, to achieve the lowest incidence and damage to cotton the Gezira State, Sudan Seni 1 cultivar could be early sown (third week of June) with either narrow spacing (25 cm a part between holes).

References

1. Ali, A.M., Hengyong, Xu, Kamal, N.M., Elhassan, O.M., Mustafa, A.M., Mohamed, Y.S., Suliman, E.H. Assar, A.H., Elturabi, S., Abdelatif, A.H., Elsheikh, A., Elagib, T.Y., Yousif, L.A., Adlan, M.A., Hadad, M., Qingliang Yin and Yuqiu, G. (2012). Release of a hybrid and a variety of Bt-Cotton (*G. hirsutum* L.) for Production in the Sudan. ARC, Variety Release Committee Meeting, Khartoum, Sudan.
2. Anonymous. (1984). Managing pests in cotton, insects and mites. In: Integrated Pest Management for Cotton in Western Region of the United States. University of California, Division of Agricultural and Natural Resources. Edited by Seney, H. Printed 91984 in the United States of America. Publication 3305, pp. 73: 18-91.
3. Balla, A.N. (1975). Some aspects of the control of an American bollworm in Sudan Gezira and Managil. Proceeding of Ciba-Geigy symposium on the strategy control of cotton pests in the Gezira. Pp. 37-39.
4. Balla, A.N. (1978). Studies on the American bollworm *H. armigera* (Hut.) in the Sudan Gezira. Symposium on Crop Pest Management in the Sudan. Feb. 1978. Pp. 335-366.
5. Brown, P.W., B. Russel, J. Silvertooth, P. Ellsworth, S. Husman, T. Knowles, L. Clark, D. Dunn and M. Schneider. (1998). The Arizona cotton advisory program p. 5-12. Cotton, Univ. of Arizona Rep. P-112.
6. Bushara, A.G. and Kanan, H.O. (1995). Pesticides Recommended by the National Pests and Diseases Committee 1970-2013. Second Edition. Danitol 20% EC +Lannate 90 % SP.
7. Caresche, L. (1986). Lsinsecctes nivibles ala culture de cotonnier dans le. Sudaouest de Madaga scar. Institute de Recherces Agronomi Quede Medgascar. Bull. No. 3.
8. Elbasher, O.A. (2016). Vermination of climate changes using rainfall and temperature as indicators and its impacts on agricultural production in the arid zone of Sudan. Ph.D. Thesis, University of Gezira, Sudan.
9. Elfadil, A.B. (2007). Sudan cotton research and production scenarios; challenges, achievement and prospect country report ICAC Washington, www.icac. orgtisregional_networksdocumentsafrica…sudan
10. Elhassan, O.A. (2015). The Review of Scientific Research on Cotton Sudan. China-aid Agricultural Technology Demonstration Center. Pp. 115-126.
11. Faki, H. (2006). Sustainable cotton production from Sudan's Report ICAC, Washington, 4-13.
12. Herrera, J.M. (1986). Natural enemies of cotton pests. In: Workshop on integrated pest control for cotton, 22-26, June 1986, Wad Medani, FAO/ARC, pp 22-48.
13. Jackson, J.E. (1970). The influence of environment on cotton growth in the Gezira. In : Cotton growth in the Gezira environment (Edited by Siddig, M.A. and Hughes, L.C.) pp. 78-104, W. Heffers and Sons Ltd., Cambridge, England.
14. Joffee, S.R. (1995). Desert locust management and time for change world bank discussion papers, No. 284. Washington, D.C.: The World Bank.
15. Joyce, R.J.V. (1959). Cotton spraying in the Sudan Gezira II. Entomological problems arising from spraying. Plant Prot. Bull. FAO. Pp. 79-103.
16. Lazarevic, B.M. (1965). Ann. Rep. Agric. Res. Div. Sudan, 1964-65.
17. Mathews, G.A. (1989). Cotton insect pests and their management. University of London. Longman. Group U.K. Limited.
18. Mursal, I.E. (1994). Cotton varieties for IPM and rainfed area. ARC variety release meeting, Khartoum, Sudan.

19. Mustafa, A.M., Babiker, E.A. and Elhassan, O.M. (2007). Proposal for the release of new high yielding and highly resistance to bacterial blight cotton varieties. ARS. Sudan.
20. Omer, B.M. (2002). Benefits and efficacy of two groups of insecticides in controlling the major cotton insect pests at the Rahad Agricultural Scheme, Ph.D. Thesis, University of Gezira, Sudan.
21. OTA. (1990). A plague of locusts OTAF. 450 Washington, DE: Government Printing Office, Special Report, pp 129.
22. Peeter, M.C.; Van Langenhove, L.; Lowwagie, J.; Water Keyn, L. and Mergeai, G. (2001). Fibre crops cotton *Gossympium hirstum* L. In: Crop Production in Tropical Africa. Edited by Romain, H. Raemeakers. Pp 1040-1071.
23. Ripper, W.E. and George, L. (1965). Cotton pests of the Sudan: Their habits and control (1st ed.), Blackwell Scientific Publication, Oxford. Pp. 90-113.
24. Schultz, D.R.; Jackson, J.E. and Faulkner, R.C. (1967). Studies on the sowing dates of cotton in the Sudan Gezira II. The relationship between sowing date of cotton and the incidence of insect pests. J. Agric. Sci. Camb. 69: 317-327.
25. Schumtterer, H. (1977). Pest in tropical crops. In: Diseases, Pests and weeds in tropical crops. Edited by Dri turgen kranz, Dr. Heinz Schumtterer and Dr. Werner Koch vering paul parey printed in W. Germany by Saldruck stein kopf and Sohn, D-1000 Berlin 36. Binding by lvderitz and bauer, D. 1000 Berlin 61(74): 309-381.
26. Schumutterer, H. (1969). Pests of crops in Northeast and Central Africa (1st ed.), Gustav, Fisher, Germany, pp. 70-90.
27. Smith, R.F. and Falcon, L.A. (1973). Insect control for cotton in California, Cotton Grow, Rev. 50: 15-27.
28. إبراهيم، جمعة جليل (1993): الحشرات الاقتصادية. منشورات جامعة حلب.
29. إبراهيم الحاج مرسال، يحيى عيسى فيصل، الحمدي عبد الله البكر، إبراهيم عثمان العبيد، أبو الحسن صالح إبراهيم، مختار نوري عثمان (1997): دليل المدرب (8) لسلسلة آفات المحاصيل الحقلية، السودان، وزارة الزراعة والغابات، هيئة البحوث الزراعية TCP/SUN/6616.
30. الحمدي أحمد زياد، قيس وجيه (1986): حشرات المحاصيل الحقلية والإرشادات العلمية للتعرف عليها في البلاد العربية. منشورات جامعة دمشق، دار المستقبل للطباعة.
31. السيد سليمان سيد أحمد (2002): مشروع الجزيرة الأسطورة الحية (دراسة أولية)، الخرطوم.
32. لعوتة تاج السر بشير محمد (2001): تعداد الآفات والحشرات، أساليب أخذ العينات والطرق الإحصائية، جامعة سنار، دار عزة للنشر، الخرطوم.
33. منظمة الأغذية والزراعة للأمم المتحدة (FAO) (1973): توجيهات عن المكافحة المتكاملة للآفات الحشرية التي تصيب القطن، شعبة خبراء المكافحة المتكاملة - إعداد لويس أ. فالكون وري ف. سميث، روما.
34. نمر يوسف، صبوح محمود (1996): محاصيل الألياف (الجزء النظري) – منشورات جامعة دمشق، مطبعة ابن حيان، دمشق.
35. يوسف عبد القادر عبد الفتاح حضوي (2007): التاريخ المجاهد عبد القادر ود حبوبة، شركة الجزيرة للطباعة والنشر المحدودة، ود مدني ص 134-138.

Essential oils used as bioinsecticides against two main biting-sucking insects (*Bemisia tabaci* Gennadius and *Jacobiella fascialis* Jacobi) and for the improvement of seed and fiber quality of cotton plants in Ivory Coast

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Abstract

Bacground: *The objective of the study was to evaluate, the insecticidal efficacy of essential oils extracted from plants on the key biting-sucking pests such as whitefly (Bemisia tabaci) and jassidae (Jacobiella fascialis) and, its effect on the quality parameters of the seed and fiber of cotton. A field experiment was conducted wherein essential oils of Ocimum gratissimum and Cymbopogon citratus were applied to cotton plants every two weeks from day 45 to 115 after emergence. The effects of these extracts were compared with synthetic-chemical insecticides i.e. acetamiprid and cypermethrin (positive control) and an untreated control (negative control).*

Results: *After 160 days of cultivation during which insect population data were collected, the seed cotton samples were picked, ginned and fiber quality tests were conducted. The results obtained revealed that the essential oils of O. gratissimum at concentrations of 1 and 5 %, gave the cotton plants better protection against whiteflies and jassids than untreated control. These cotton plants obtained, in the same way as the synthetic chemical, a better rate of healthy seeds (52.00; 51.50 and 56.50 % respectively), of fatty matter (10.84; 11.35 and 14 % respectively) and a fiber of very good reflectance (76.40; 76.30 and 75.20 % respectively). On the other hand, the average number of biting-sucking insects was positively correlated with the rate of rotten seeds, the yellow index of the fiber and the acidity rate of the fat.*

Conclusions : *This clearly demonstrates that the improvement of seed and fiber quality of cotton is closely related to adequate plant protection. The essential oil of O. gratissimum could therefore be integrated in the plant protection programs of cotton in Ivory Coast.*

Key words: *Essential oils, Ocimum gratissimum, Cymbopogon citratus, cotton, white fly, jassid, Ivory Coast.*

Introduction

Cotton belongs to the family Malvaceae in which the genus Gossypium includes about fifty species. It is a perennial shrub, but is cultivated as an annual in all sub-humid and semi-arid zones (Hussein *et al.,* 2005). In Ivory Coast, the development of cotonculture dates back to the early 1900s (Cavana, 2002). It remains the driving force of the economy of the savanna zone thanks to the cash income it provides. Indeed, in the North, this crop has become established with a contribution of 1.7 % to national GDP and 7 % as a share of exports (Markus and Francis, 2010). Before the military-political crisis of September 2002, cotton cultivation employed about 200,000 producers. In recent years, the total area planted to the crop is 392,131 ha. It employs more than 150,000 producers and feeds nearly 3.5 million people (Koffi, 2013).

Although cotton cultivation has enabled several countries and the populations that have adopted it to make undeniable socio-economic progress, parasitism, however, remains one of the main constraints to production (Ferron *et al.,* 2006). In Ivory Coast and in all West African countries, cotonculture is subject to particularly high and diversified parasitism. Indeed, the main pests of this crop are represented by several large groups, namely arthropods (the mite *Polyphagotarsonemus latus*, the aphid *Aphis gossypii*, the jassid *Jacobiella facialis*, the whitefly *Bemisia tabaci*), phyllophagous lepidopterans (*Haritalodes derogata*, *Spodoptera littoralis*, *Anomis flava*) and carpophagous lepidopterans (*Helicoverpa armigera*, *Earias spp*,

Pectinophora gossypiella, *Thaumatotibia leucotreta*, *Diparopsis watersi* (Andriambololona *et al.,* 1997). Carpophagous are generally the most devastating to cotton (Dhara *et al.,* 2015). Average crop losses, due to these pests, vary between 24.7 and 88.9 % depending on the year and growing area in the absence of phytosanitary treatment (Vaissayre *et al.,* 1984; Cauquil and Vaissayre, 1994).

Thus, strategies such as early sowing to avoid outbreaks of carpophagous caterpillars, particularly *Helicoverpa*, the use of varieties that are tolerant to attacks (varieties with good hairiness to deter jassids), chemical treatment on thresholds during the first 45 days, and systematic chemical treatment (basic program) every 14 days starting on the 45th day after sowing, have been put in place by the actors in the cotton sector for the protection of cotton crops in Ivory Coast. Moreover, the number of insecticide treatments amounts to six per campaign, including two or three in the vegetative phase and three or four in the fruiting phase of the plant. With this strategy, cotton alone absorbs over 60 % of the chemicals sold on the Ivorian market (Fleischer *et al.,* 1998). The pesticides commonly used are divided into several groups, including organochlorines (5.5 %), carbamates (9.7 %), organophosphates (20.8 %), pyrethroids (25.8 %), organophosphate-pyrethroid combinations (32.2 %), and other types of pesticides (5.9 %).

The practice of chemical pest control is one of the success factors that has accompanied the development of cotton sectors in West and Central Africa (Badiane *et al.,* 2002; FARM, 2005; Hussein *et al.,* 2005; Popp *et al.,* 2013). However, it threatens the sustainability of production systems. The use of chemical pesticides on both food and export crops has always represented a real danger for farmers and populations. The risks incurred by farmers in the treatment of crops, such as cotton, which requires several applications of pesticides before harvest, can be deadly (Thiam and Mamadou, 2009). According to the World Health Organization (WHO), chemical use is responsible for approximately 220,000 cases of death each year worldwide (Cherin *et al.,* 2012). A study in the northern region of Ivory Coast showed that 65 % of the diseases suffered by vegetable, cotton and mango farmers and consumers are linked to synthetic pesticides (Thiam and Mamadou, 2009).

Chemical pesticides can also have adverse effects on natural resources. In this regard, analyses have shown the presence of not only numerous organochlorines and organophosphates in several soil matrices and in samples of aquatic organisms, but also hexachlorocyclohexane (HCH), endosulfan, heptachlor and dichlorodiphenyltrichloroethane (DDT) in cow's milk from northern cotton-producing areas (Fleischer *et al.,* 1998; Tunç *et al.,* 2000; Traoré *et al.,* 2008). Furthermore, the lack of selectivity of chemical pesticides towards their target causes harmful effects on beneficial insects (EPPO, 1994; Sigrist *et al.,* 1994; Hayo, 1997). This can lead to an increase in pest populations in the following period of the cotton crop (Martin *et al.,* 1997). Beneficial insects are formidable predators of pests, so reducing their populations through chemical use could indirectly lead to an increase in pest populations. In addition, the overuse of synthetic pyrethroids has led to the development of insect pests resistant to these biocides (Moshi and Matoju, 2017). In addition, in recent years, there has been an emergence of pests such as whitefly (*Bemisia tabaci*) and jassid (*Jacobiella fascialis*) in the pest facies of cotton (Koné *et al.,* 2017 ; Didi *et al.,* 2018).

In an integrated protection concept, the use of biological insecticides (plant extracts, kairomones, growth regulators) or semibiological insecticides (oils, detergents) against pests, represent possible methods (Deguine and Vaissayre, 2000). Several studies have focused on bioactive natural products (Karima, 2015). Among the products with high added values, but present in small quantities and with biological and olfactory activity, essential oils are worth mentioning (Jouault, 2012).
Apart from these, plant extracts in aqueous, hydroalcoholic or oily preparation, also give good results in crop protection (Marchand *et al.,* 2014). For example neem, obtained from the Meliaceae, *Azadirachta indica* and *Melia azedarach*, is the subject of significant research in Africa and Asia (Saxena *et al.,* 1989; Biao *et al.,* 2018). In Mali, several plants are traditionally used for sorghum preservation (Anjarwalla *et al.,* 2016). In Burkina Faso, various essences from chili pepper, *Cassia sp.* and lemon grass (*Cymbopogon sp.*), are used for cowpea grain protection (Tiendrebeogo *et al.,* 2017). In Benin, the insecticidal effect of *Hyptis suaveolens* was successfully evaluated on *Sesamia calamistis* (Lepidoptera: Noctuidae), a major maize

pest (Adda *et al.,* 2011). In Togo, the essential oil of *Ocimum canum* and its major compound terpineol-4 were evaluated on adults of *Aphis gossypii*, a cotton aphid (Akantetou *et al.,* 2011). In Ivory Coast, studies of this type are very few or almost non-existent in cotton crops. However, the country has a very important floristic heritage. The exploration and development of this heritage, which is rich in bioactive substances derived from the secondary metabolism of local plants, could allow the development of biopesticides.

In view of the disadvantages associated with the use of synthetic pesticides and the possibilities of exploiting plant extracts, the research questions addressed in this document are as follows: what alternative solutions, more respectful of the environment and socio-economically acceptable, could be used for the sustainable production of cotton in Ivory Coast? Can biopesticides be used in Ivorian cotton production? Is it possible to use plant extracts to limit the use of synthetic pesticides?

It is in this context that the present work is inscribed, the general objective of which is to reduce the use of synthetic pesticides by evaluating the insecticidal efficacy of essential oils extracted from nine aromatic plants (*Ocimum gratissimum*, *Ocimum canum*, *Melaleuca leucadendron*, *Hyptis suaveolens*, *Lippia multiflora*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus globulus* and *Citrus sp.*) on three important cotton pests (*Helicoverpa armigera*, *Pectinophora gossypiella* and *Thaumatotibia leucotreta*). The specific objectives were to: (i) to determine the chemical composition of the essential oils of the local aromatic plants used; (ii) to determine, under controlled conditions, the insecticidal potential of the essential oils on the main carpophagous pests of cotton.

Materials

2.1. Plant material

2.1.1. Aromatic plants used

Nine species of local aromatic plants belonging to five families were selected. They are *Ocimum canum, Ocimum gratissimum* and *Hyptis suaveolens* (Lamiaceae) ; *Cymbopogon citratus* and *Cymbopogon* nardus (Poaceae) ; *Melaleuca leucadendron* (Myrtaceae) ; *Eucalyptus globulus* (Myrtaceae) ; *Lippia multiflora* (Verbenaceae) ; and Citrus sp (Rutaceae). The choice was guided by their endemic presence in all localities of the country and particularly in the North and Center, with the exception of *M. leucadendron* (Soro *et al.,* 2006; Diomandé *et al.,* 2015). In addition, a rich bibliography exists on the antibacterial, antiviral, antifungal (Camara *et al.,* 2010) and insecticidal (Yarou *et al.,* 2017) activities of their essential oils.

The collections of aromatic plants were carried out in the Korhogo area for *O. canum*, *H. suaveolens* and *Citrus sp.* The species *L. multiflora*, *M. leucadendron* and *E. globulus* were collected in the Yamoussoukro locality, while *O. gratissimum*, *C. citratus* and *C. nardus* were collected in Bouaké **(Figure 1)**. These plants were formally identified by a Botanist of the National Center of Floristics (CNF) at the University Félix Houphouët-Boigny of Abidjan (Ivory Coast).

- ***Ocimum gratissimum* L.**

Ocimum gratissimum L. (Lamiaceae) is an erect, sublinear herbaceous plant 1 to 2 m high, with numerous branches. It is a bushy shrub, with opposite, oval leaves from 1,5 to 2,5 cm long and from 0,6 to 1,2 cm wide, coarsely toothed and very fragrant. The inflorescences are in spikes of small flowers with greenish corollas. In central Ivory Coast, among the Baule people, this plant is called "aromangni**" (Figure 1A)**.

- ***Cymbopogon citratus (DC.) Stapf***

C. citratus (DC.) Stapf is a perennial aromatic herb belonging to the Poaceae family **(Figure 1B)**. It is native to southern India and Sri Lanka (Karunamoorthi *et al.,* 2010). These leaves can reach 90 cm in length and 1.25 cm in width. *C. citratus* reproduces by rhizomes (Iwu, 2014). Common names for *C. citratus* are citronella or lemon grass (French), lemon grass or West Indian lemongrass in English (Kouamé *et al.,* 2015).

- ***Cymbopogon nardus L.***

C. nardus is an evergreen herbaceous perennial, forming dense cespitose clumps 1 to 1.8 m tall **(Figure 1C)**. The long, narrow, ribbon-like, tapered-tipped, glaucous-green leaves are

about 1 m long and 0.5 to 1.6 cm wide. The leaves are emitted by creeping rhizomes forming at the base of them false russet stems of 1 to 2 cm in diameter.

- ***Lippia multiflora Moldenke***

L. multiflora Moldenke also known as L. adoensis Hochst is an herbaceous plant in the genus Lippia **(Figure 1D)** that belongs to the family Verbanaceae (Jigam *et al.,* 2009). L. multiflora is mainly distributed throughout tropical Africa, South and Central American countries (Pascual *et al.,* 2001). The plant can reach a height of 2.7-4.0 m and has large bluish-green oblong-lanceolate leaves (Ameyaw, 2009).

- ***Hyptis suaveolens L. (Poit.)***

This is an annual, aromatic, erect herb up to 2 meters **(Figure 1E)**. It is a Lamiaceae. Its root is white or brown taproot. Its stem is quadrangular, hollow, with glandular hairs. Its leaves are pubescent on both sides (Ahoton *et al.,* 2010).

- ***Melaleuca leucadendron L.***

The plant is exploited for its essential oil, wood and bark (Chevalier, 1927). The species is recognizable by its leathery leaves placed vertically. It is also differentiated by its sessile flowers in long spikes, all showing five phalanxes of long stamens usually whitish-yellow **(Figure 1F)**.

Ocimum gratissimum

Cymbopogon citratus

Cymbopogon nardus

Lippia multiflora

Hyptis suaveolens

Melaleuca leucadendron

Figure 1. Local aromatic plants

Ocimum canum

Citrus sp.

Eucalyptus globulus

Figure 1. Cont.

- ***Ocimum canum Sims***

O. canum is an erect, branched herbaceous plant, about 0.5 to 0.6 m tall **(Figure 1G)**. It is a Lamiaceae with elliptical to lanceolate leaves, punctuated with glands. Its fruit consists of four achenes each containing one seed. Its seeds are small, fine, oblong and of dark brown color. It is used as an ornamental plant and for its medicinal properties.

- ***Citrus sp.***

The Citrus genus consists of Angiosperm plants of the Rutaceae family **(Figure 1H)**. This genus contains the trees yielding the fruits commonly known as "citrus": orange, lemon, grapefruit, mandarin. The leaves and flowers bear glands that produce a sweet-smelling essential oil.

- ***Eucalyptus globulus Labill.***

From the Myrtaceae family, Eucalyptus are among the largest deciduous trees in the plant world **(Figure 1I)**. These native Australian plants account for 95% of Australia's forest. The leaves are bluish, evergreen and covered with oil glands. The flowers are white, cream, yellow, pink or red in color and are a source of nectar for honey production in Australia.

2.2. Animal material

The insect strains used **(Figure 2)** during the study consisted of larvae and adults of *P. gossypiella* Saunders **(Figure 2A)**, *T. leucotreta* Meyrick **(Figure 2B)**, *H. armigera* Hübner **(Figure 2C)**. Indeed, these carpophagous insects are generally the most devastating to cotton (Dhara *et al.,* 2015).

2.3. Technical materials

2.3.1. Rearing material

Rearing of insects (*T. leucotreta*, *P. gossypiella* and *H. armigera*) took place in a climatic room (25 °C temperature, 70 ± 5% relative humidity). This rearing room was equipped with two humidifiers, one dehumidifier, one hygrometer and two ultraviolet lamps **(Figure 3)**. Well plates, plastic dishes, test tubes, Petri dishes, forceps, brushes, sterile square gauze pads and absorbent cotton were used in addition to the usual laboratory equipment for successful rearing.

2.3.2. Observation equipment

A binocular magnifying glass was used to sex chrysalises during rearing and a hand-held magnifying glass was used to better appreciate the condition of the butterflies or caterpillars during post-test observations. A follow-up sheet was used to record the results after each observation.

2.3.3. Weighing and measuring equipment

An "aeADAM" scale with a capacity of 450g and a precision of 0.001g was used to weigh the products, butterflies (adults) and larvae. Eppendorf" type syringes were used to take precise samples of the solutions to be applied. For the bioassays, an Arnold Micro applicator (Burkard, UK) with a calibration syringe and needle was used **(Figure 4)**.

2.4. Chemical materials

Chemicals were used in the laboratory for sensitivity testing, such as pure acetone for dilutions and control tests. Carbon dioxide (CO_2) was used to sedate the butterflies before contact application of the essential oils.

Methods

3.1. General information on the study area

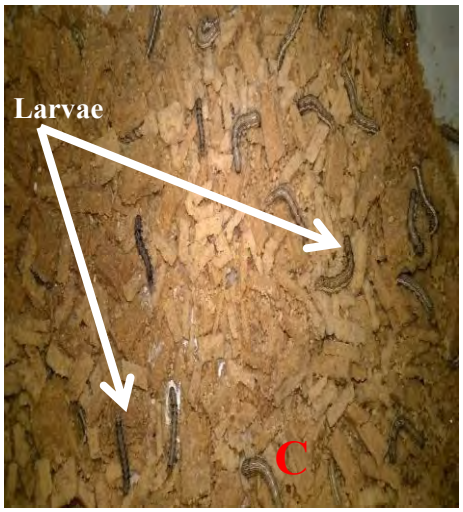
This chapter provides information on the geographical location, climate, vegetation, relief, hydrographic network, geological and soil formations of the study area.

3.1.1. Geographic location and climate

Bouaké is the city where the experiments took place. It is the capital of the Gbêkê region, located in the center of Ivory Coast, about 367 km from Abidjan, the economic capital, with geographic coordinates of 7°41 north latitude, 5°01 west longitude, and 339 m in altitude. The study was conducted at the cotton research station of the National Centre for Agronomic Research (CNRA). It is located at latitude 7°44' North and longitude 5°04' West, with an altitude of 376 m **(Figure 5)**.

Pectinophora gossypiella adult (Lepidoptera)

Thaumatotibia leucotreta adult (Lepidoptera)



Helicoverpa armigera larvae (Lepidoptera)

Figure 2. The cotton pests used in the insecticide tests

The Bouaké region belongs to the transition zone between the savanna in the north and the forest in the south. As a result, it is under the influence of a fairly nuanced climate where the division of the year into two dry seasons and two rainy seasons is not clear. The climate of the region is Sudanian (Ouattara, 2001). This locality is influenced by a humid tropical climate. According to Aubreville (1949), the Bouaké region is located in the Guinean Forest climate zone and particularly in the Bauleen-Dahomean sub-climate. Sunshine is constant and humidity is relatively low. Relative humidity, which fluctuates between 70 and 80% during rainy periods, can drop to 55% in January (N'goran, 2008). The temperature varies between 25 and 38 °C, with an annual rainfall that varies from 1000 mm to 1700 mm.

3.1.2. Vegetation, relief and hydrography

The vegetation of the Bouaké region is characterized by a sub-Sudanese savanna in the north, with patches of dense dry forest and a pre-forest zone in the south. There are also forest galleries along the waterways. The relief is not very uneven with an average altitude of 200 m. There are no very marked summits. However, some hills are present. The terrain also has several low-lying areas. Bouaké is an intermediate zone between the northern plateaus and the southern plains (Avenard *et al.,* 1971). The hydrographic network is dominated by the Bandama, which is the most important river in the region. It is essentially made up of the tributaries of the Bandama and the N'Zi, which are the Kan and the Pklara (N'goran, 2008).

Figure 3. *Insect rearing equipment in a climatic room*
NB : nesting box, PB : plastic boxes, ULV : ultraviolet light lamp, Hr : humidifier

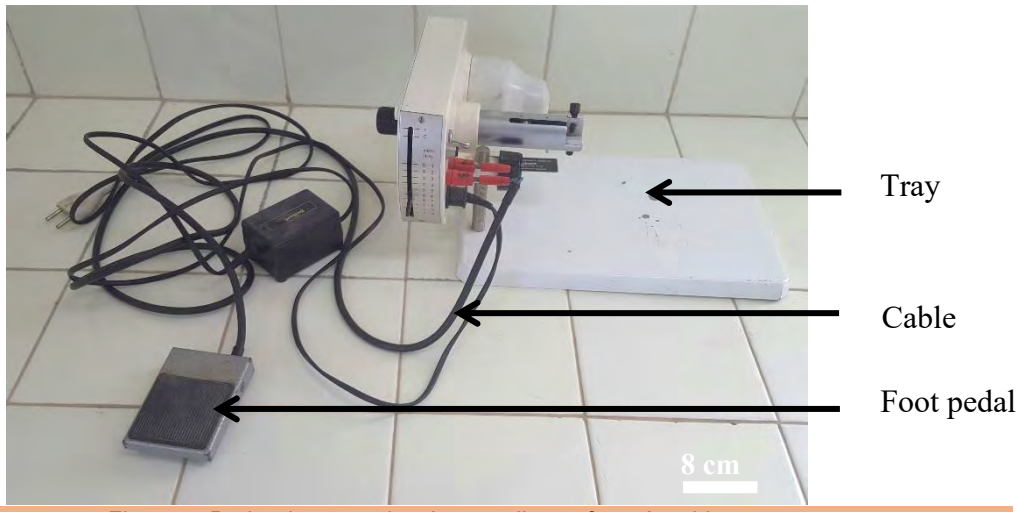


Figure 4. Burkard automatic micro applicator from Arnold

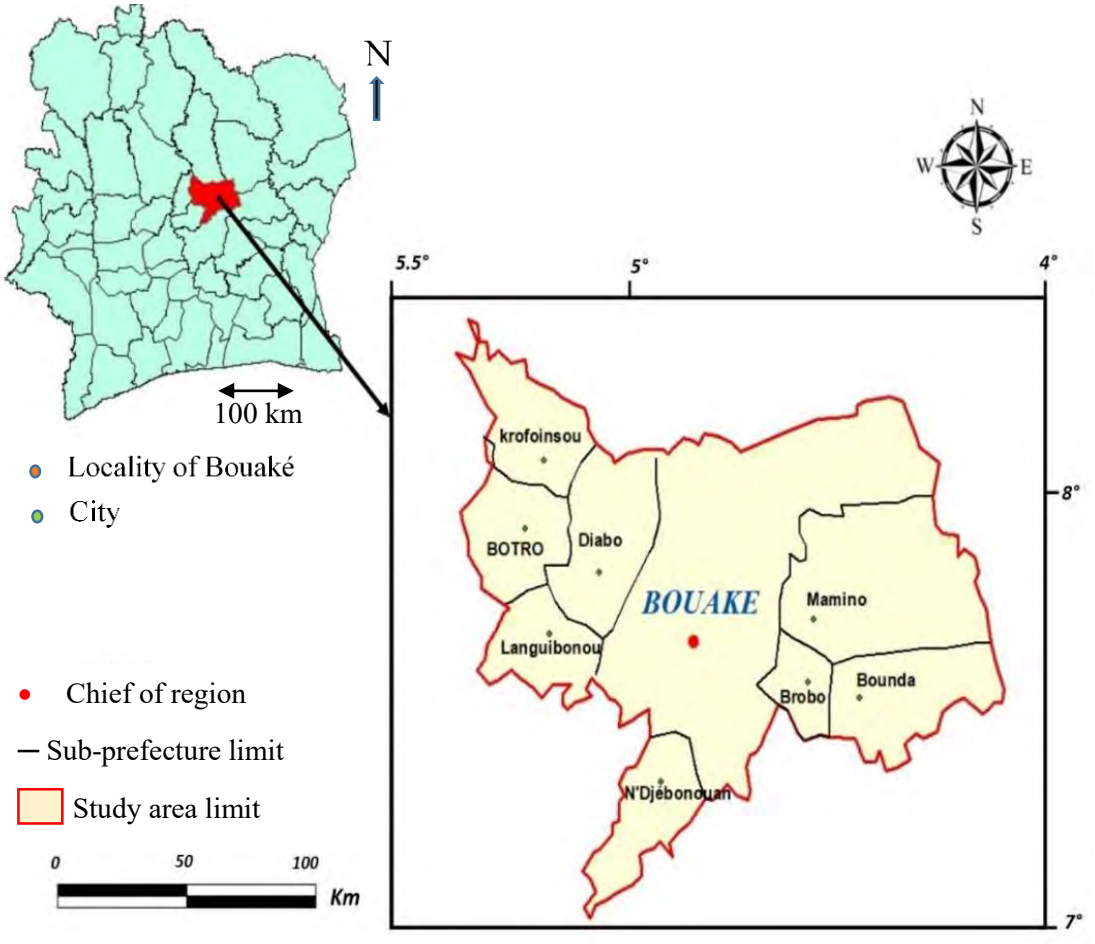


Figure 15. Localization of the study area in Bouaké, central Ivory Coast (Kobenan et al., 2022)

3.1.3. Geological and pedological contexts

The geology of the region is dominated by metamorphic formations of sedimentary, volcanic, volcano-sedimentary origin and Eburnian granitoids. Homogeneous and heterogeneous biotite granitoids are found east of Bouaké as well as unconformable granitoids (granodiorite), subalkaline two-mica and migmatites, migmatite granites (Ouattara *et al.,* 2012). The Bandama-N'Zi interfluve corresponds to the central step, characterized by an inclination of several meters, compared to the reliefs of the north of the country where it is distinguished the granitic horst of Bouaké.

The soils of the region are generally shallow and light. They are reworked ferralitic soils that, in the morphological landscape, occupy the high positions that are the summits, upper slopes and mid-slopes (N'cho, 1991). The lower slopes are occupied by sandy soils (Yoro, 2000). Finally, the lowlands present a variety of hydromorphic soils with a sandy texture alternating with those with a sandy-clay texture or even frankly clay. The water reserve of these soils is on average low (50-80 mm). The permeability is generally high. However, these soils often present a film of battance which favors runoff.

3.2. Determination of the chemical composition of essential oils

3.2.1. Extraction of essential oils

The leaves of the aromatic plant species were dried at room temperature in the laboratory (28 ± 2 °C) for one week before extraction. With respect to the plant species Citrus sp, we used the zest instead of the leaves. The essential oils were extracted by steam distillation for 3 h using a stainless steel Clevenger apparatus with a 250 kg capacity still **(Figure 6)**. At each operation, 10 kg of leaves or peel (case of *Citrus sp.*) are used. The extraction was carried out in the biochemistry laboratory of the University Péléfero Gon Coulibaly in Korhogo.

It consists of a classical distillation in a boiling still in which the plant material is not in contact with water. The water vapor, as it passes through the plant material, is loaded with volatile

compounds and condenses inside a refrigerant. The essential oils, which are less dense than water, are collected by simply settling on its surface (Tia *et al.,* 2013).
The collected essential oils are left to rest in a separating funnel for one hour in the dark to remove any trace of water. They are then stored in colored glass vials and kept at 4°C in a refrigerator protected from light until use.

3.2.2. Gas chromatographic analysis

The essential oils were analyzed by gas chromatography (GC) coupled with selective mass spectrometry (GC/MS) at the Department of Chemical and Food Engineering (GCAA) of the National Polytechnic Institute HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro. The analysis of chemical constituents was performed using an Agilent technologies 6890 N Network GC System version N.04.07 chromatograph equipped with a fused silica HP- 1 (phenyl-methyl polysiloxane) capillary column of volume 25 m x 200 µm x 0.33 µm. The oven was programmed at a temperature ranging from 50 to 150 °C at a gradient of 5 °C/min. The carrier gas was helium with a flow rate of 0.8 ml/min. The temperatures of the injector (Agilent Technologies 7683) and detector (Agilent 5973 Network) were 250 and 280 °C, respectively. A volume of 1 µl of essential oil sample diluted in diethyl ether was injected in split mode.
The individual volatile constituents were identified by their mass spectra and retention times in comparison with those of existing compounds in the database. Confirmation of the compounds was done by comparison (Tia *et al.,* 2013) to the standard reference data existing in the Nist 98.l and wiley275 database. The relative percentages of the different constituents were obtained by integrating their peaks on the chromatograms.

3.3. *In-vitro* determination of the insecticidal potential of essential oils on the main cotton pests

3.3.1. Breeding of insect pests in the laboratory

- **Obtaining *H. armigera* larvae**

Larvae were collected from the plants or from the green bolls of cotton (in the case of *H. armigera*). These collections were made in untreated plots set up for this purpose at the experimental station. These larvae were reared on an artificial nutrient medium based on Agarose, cornmeal and wheat germ (Couilloud and Giret, 1980; Doffou, 2013) under the following conditions: temperature of 26 ± 2 °C, relative humidity 70 ± 5 % and photoperiod of 12 h (Patana, 1977). After about 10 days, the resulting chrysalids were separated by sex and placed in plastic jars. Upon emergence of the first adults (butterflies) constituting the first generation (G0), pairs were formed and stored in boxes covered with a sterile film of hydrophilic cotton gauze serving as "nesting boxes". This gauze film represents, in fact, the support for the oviposition of the females. After the females have mated and laid their eggs, the gauze carrying the eggs is placed on the underside of the lids of a rectangular box containing artificial nutrient medium of known composition **(Table I)**. After the eggs hatch, the neonate larvae have direct access to their food, which they easily reach. They constitute the first generation (G1) from the rearing; they are the ones that will be the object of toxicological tests.

- **Obtaining adults of *P. gossypiella* and *T. leucotreta***

For *P. gossypiella* and *T. leucotreta*, the moths used are derived from larvae collected from untreated cotton plots in the Bouaké area. Those that have reached the last larval stage are transferred to the laboratory, in plastic boxes (50 cm x 20 cm), containing cotton. This medium is devoid of food, a living condition that will favor the pupation of the larvae and thus the formation of the chrysalis (Doffou, 2013). They were subjected to a photoperiod of 12 hours, a temperature of 26 ± 2 °C, and a relative humidity of 70 ± 5% (Patana, 1977). After one week, incubated larvae pupate and pupation lasts an average of 13 days before butterfly emergence. Butterflies of P. gossypiella and T. leucotreta, freshly emerged from their cocoons, one day old after emergence, were used for toxicological tests.

Figure 6. Clevenger apparatus (stainless steel) for the extraction of essential oils

Table 1. Composition of the artificial nutrient substrate for the rearing of *Helicoverpa armigera*

Composition	quantity
Cornmeal	336 g
Wheat germ	84 g
Brewer's yeast	90 g
Ascorbic acid	16 g
Methyl-paraben	12 g
Salt wesson	24 g
Antibiotic	02 g
Distilled water	2800 ml
Agar-agar	60 g
Formol (35 %)	2 ml
Choline chloride	2.4 g

Source: Couilloud and Giret, 1980 modified by Ochou (2019)

3.3.2. Constitution of experimental batches of butterflies or larvae

Day-old butterflies of *P. gossypiella* and *T. leucotreta* and first instar larvae (L1-L2) of *H. armigera* were selected for the in vitro tests. In order to have a homogeneous weight distribution, larvae and moths were weighed individually using a precision balance. Butterflies weighing between 10 and 20 mg and larvae weighing 15 to 20 mg were selected. Thirty individuals were used for each of the essential oil concentrations.

3.3.3. Insect anesthesia technique

Only the butterflies were anesthetized. Thus, after the constitution of the batches, all the butterflies of the same batch were put to sleep with carbon dioxide at a fixed flow rate of 20 l/min. In general, the butterflies were put to sleep for 10 seconds to facilitate their processing.

3.3.4. Preparation of the solutions

The essential oils of each of the aromatic plants were diluted in acetone. Indeed, acetone is a good solvent for essential oils and is not toxic to insects (Doffou, 2013). The concentration range was determined based on several preliminary laboratory tests. Thus, for each of the essential oils, the extract diluted 1/80th with acetone (80 % solution) was used to prepare the different concentrations tested: 0.25; 0.50; 1; 2; 4; 8; 16; 32; and 64 %. No chemical-based solutions were prepared for the bioassays. The purpose of the bioassays was to determine the lethal concentrations of each essential oil in order to select the most effective ones for the field trials.

3.3.5. Conduct of bioassays

- **Treatment by topical application**

The applications of the essential oils covered the period from 2016 to 2018, i.e., the 2016-2017 and 2017-2018 cotton seasons. During bioassays, each individual (moth or larva) received 1 µl of solution on the dorsal thorax using an Arnold Burkard-type micro applicator (Kaan *et al.,* 2016). Application of the essential oils was done in ascending order of the prepared concentrations. The zero concentration, consisting only of the diluting solvent (acetone), served as an absolute control. Indeed, the controls tested with the dilution solvent will allow to judge the vigor of the insects used during the bioassays and the validity of the results obtained. Each batch of 10 treated insects was immediately transferred into a cellophane bagged egg-laying chamber (12 cm × 16 cm) in the case of butterflies or into well plates (one larva per well) for larvae. They were fed with a 10% (v:v) honey solution, as recommended by Wu *et al.* (2006), during their storage at the temperature of 26 ± 2 °C and 70 % relative humidity for the different observations. In sum, the experiments involved a total of 5400 butterflies and 5400 larvae divided into batches of 10 insects per concentration. Each concentration applied was repeated three times.

3.3.6. Data collection

Mortality was measured 48 h after topical application. Immobile and visibly moribund insects, i.e., a larvae or moth that was no longer feeding and, when placed on its side or back, could no longer stand up, was considered dead. Any individual that moves with great difficulty and is unable to coordinate its movements, in particular, that of getting back on its stomach, was considered dead. Any individual that moves and moves normally was considered alive (Doffou, 2013). If mortality in the control batch was greater than or equal to 10%, the test was rejected or when dead or moribund insects were observed among the controls, mortality rates were corrected by the Abbott (1925) formula :

$$\mathbf{Mc}\ (\%) = \frac{\mathbf{M_t} - \mathbf{M_0}}{100 - \mathbf{M_0}} \times 100$$

Mc : Corrected mortality rate
M0 : Mortality rate in the control group
Mt : Mortality rate in the treated group

3.4. Statistical analysis of the data

All data collected during the experiments were entered using Excel 2016 software. Using WinDL 32.0 software (CIRAD, Montpellier, version 1998), the lethal concentrations causing 50 % (LC_{50}) and 90 % (LC_{90}) death of the tested insects were determined for each of the essential oils, at two days after exposure of the moths or larvae. The comparison between the essential oils with respect

to their toxicities on each of the pests was done using a descriptive analysis. Thus, the extracts with the lowest lethal concentration values (LC_{50} or LC_{90}) were the most toxic (Tchoumbougnang *et al.,* 2009).
In addition, a hierarchical ascending classification (HAC) was performed in order to determine the similarities between the essential oils according to their toxicity levels. This statistical analysis resulted in a dendrogram that clearly shows the different groups of essential oils. This multivariate analysis was performed using R software version 4.0.5 with packages such as FactomineR and Factoextra.

Results

4.1. Chemical composition of essential oils

4.1.1. Chemical composition of the essential oil of *Ocimum gratissimum*

The chromatogram analysis of the essential oil of *O. gratissimum* **(Figure 7)** revealed compounds presented in **Table II**. According to the results obtained from the chemical analysis, the essential oil of *O. gratissimum* consists of eighteen compounds representing 100% of the identified components, of which 92.26 % are monoterpenes (60.19% hydrocarbon and 32.07 % oxygen) and 7.74% are hydrocarbon sesquiterpenes only. In the group of hydrocarbon monoterpenes, p-cymene with a content of 37.79 % and sabinene (6.60 %) were the most represented terpene elements. Thymol (24.57 %) and camphor (5.53 %) occupied a preponderant place among the oxygenated monoterpenes that are present in this essence. The essential oil of *O. gratissimum* is also composed of sesquiterpenes such as β-cis-caryophyllene (4.42 %), selinene (1.55 %), copaene (1.14 %) and α- caryophyllene (0.63 %).

Table II. Chemical composition of the essential oil of *Ocimum gratissimum**

Terpene groups	N° (peak)	Constituents	%
Hydrocarbon monoterpenes	1	sabinene	6.60
	2	α-pinene	1.86
	3	β-phellandrene	0.89
	4	β-pinene	0.49
	5	β-myrcene	3.97
	6	α-terpinene	1.66
	7	p- cymene	37.79
	8	d-limonene	1.26
	9	γ-terpinene	3.62
	10	2-nitro-p-cymene	2.05
Oxygenated monoterpenes	11	terpinene-4-ol	1.38
	12	thymol methyl ether	0.59
	13	thymol	24.57
	14	camphor	5.53
Hydrocarbon sesquiterpenes	15	copaene	1.14
	16	β-cis-caryophyllene	4.42
	17	α- caryophyllene	0.63
	18	selinene	1.55
		Total	100

*Kobenan *et al.,* 2018a

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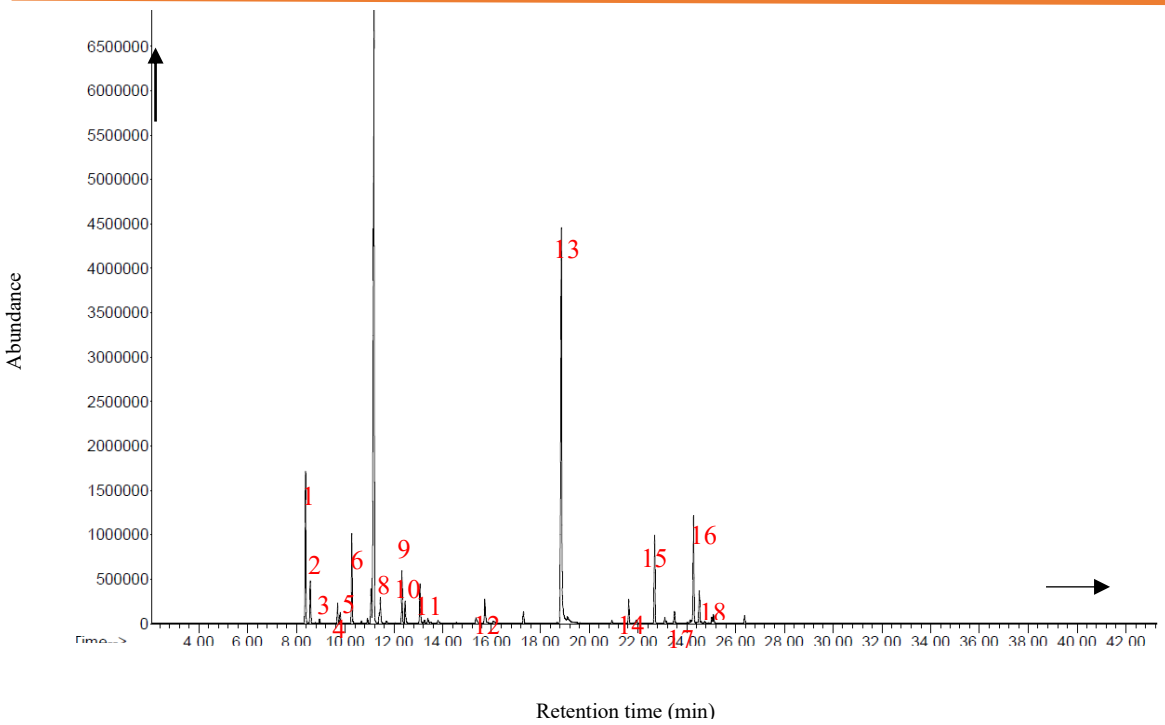

Figure 7. Analytical chromatogram of the essential oil of Ocimum gratissimum

4.1.2. Chemical composition of the essential oil of *Cymbopogon citratus*

The chromatogram of the essential oil of *C. citratus* revealed four major constituents **(Figure 8)** presented in **Table III**. These are neral (39.33 %), α-citral (31.89 %) and sulcatone (2.49 %), oxygenated monoterpenes that form the bulk of the constituents extracted from the plant (73.71 %). Hydrocarbon monoterpenes are represented by β-myrcene (26.29 %). No sesquiterpene molecules were detected in the essential oil of *C. citratus*.

Table III. Chemical composition of the essential oil of *Cymbopogon citratus**

Terpene groups	N° (Peak)	Constituents	%
Hydrocarbon monoterpenes	1	β-myrcene	26.29
Oxygenated monoterpenes	2	α-citral	31.89
	3	neral	39.33
	4	sulcatone	2.49
		Total	100

*Kobenan *et al.*, 2018b

4.1.3. Chemical composition of the essential oil of *Cymbopogon nardus*

The essential oil of *C. nardus* consists of eight compounds materialized by eight peaks on the analytical chromatogram **(Figure 9)**. In **Table IV**, the levels of the constituents are presented. This oil contains 91.57 % of monoterpenes including hydrocarbon (44.98 %) and oxygenates (46.59 %). The constituents belonging to the hydrocarbon monoterpenes group are β-myrcene (11.19 %), d-limonene (4.70 %) and carene (29.09 %). Linalool, neral and α-citronellol are the majority oxygenated monoterpenes in this essential oil with 23.38 %, 20.23 % and 23.38 % respectively. Sesquiterpenes are represented at a percentage of 8.43 % with β-elemene (1.77 %) and guaia-10 (6.66 %).

4.1.4. Chemical composition of the essential oil of *Lippia multiflora*

The essential oil of *L. multiflora* is composed of monoterpenes and sesquiterpenes with respective percentages of 87.10 and 12.90 % presented by the analytical chromatogram **(Figure 10)** and **Table V**. The main monoterpene components are α-phellandrene (39.01 %), β-phellandrene (8.11 %) and p-cymene (7.70 %) which are mainly hydrocarbon monoterpenes. The proportion of total oxygenated monoterpenes was 27.75 % represented by d-limonene (4.53 %), β-geranial (11.86 %) and α-citral (15.89 %). This essential oil presented hydrocarbon-only sesquiterpenes such as α- caryophyllene and β-farnesene at 4.87 % and 8.03 % respectively.

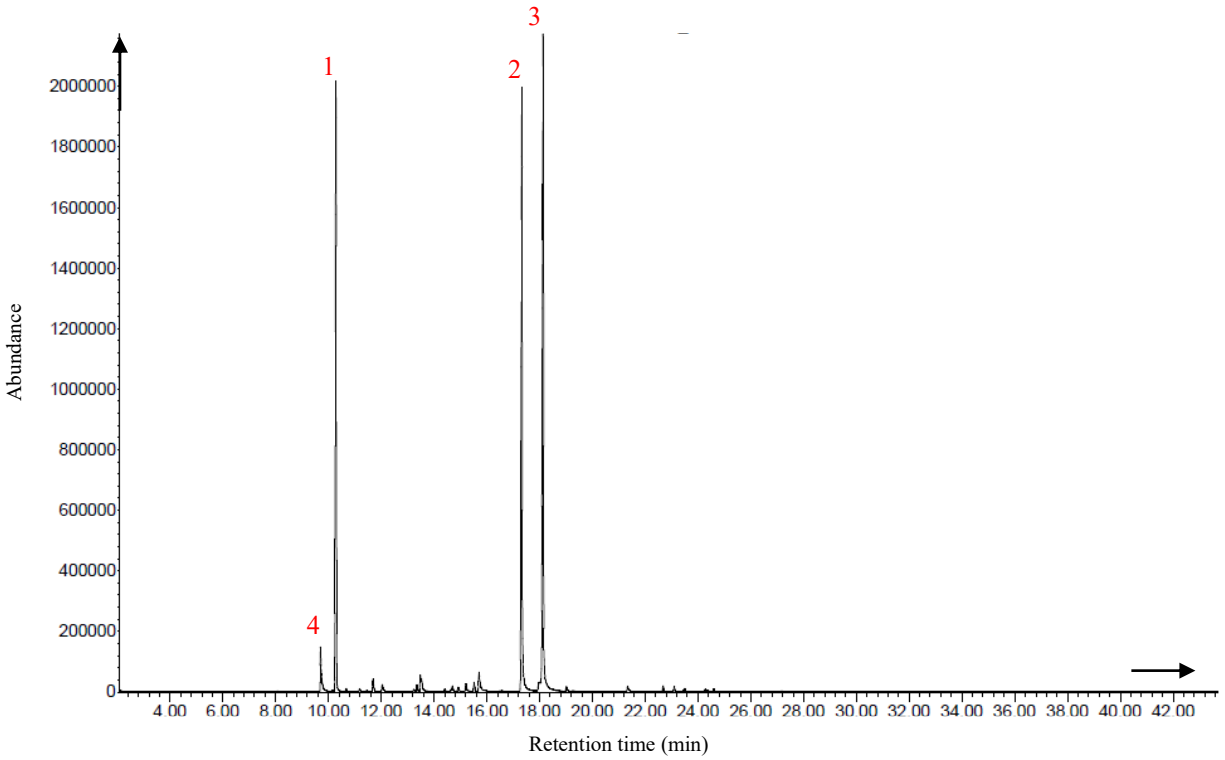

Figure 8. Analytical chromatogram of the essential oil of Cymbopogon citratus

Table IV. Chemical composition of the essential oil of *Cymbopogon nardus**

Terpene groups	N° (Peak)	Constituents	%
Hydrocarbon monoterpenes	1	β-myrcene	11.19
	2	d-limonene	4.70
	3	carene	29.09
Oxygenated monoterpenes	4	α-citronellol	2.98
	5	linalol	23.38
	6	neral	20.23
Hydrocarbon sesquiterpenes	7	β-elemene	1.77
	8	guaia-10(14)	6.66
		Total	100

Table V. Chemical composition of the essential oil of *Lippia multiflora*

Terpene groups	**N° (Peak)**	**Constituents**	**%**
Hydrocarbon monoterpenes	1	α-phellandrene	39.01
	2	p-cymene	7.70
	3	β-phellandrene	8.11
	4	d-limonene	4.53
Oxygenated monoterpenes	5	β-geranial	11.86
	6	α-citral	15.89
Hydrocarbon sesquiterpenes	7	α- caryophyllene	4.87
	8	β-farnesene	8.03
		Total	100

*Kobenan *et al.*, 2018b

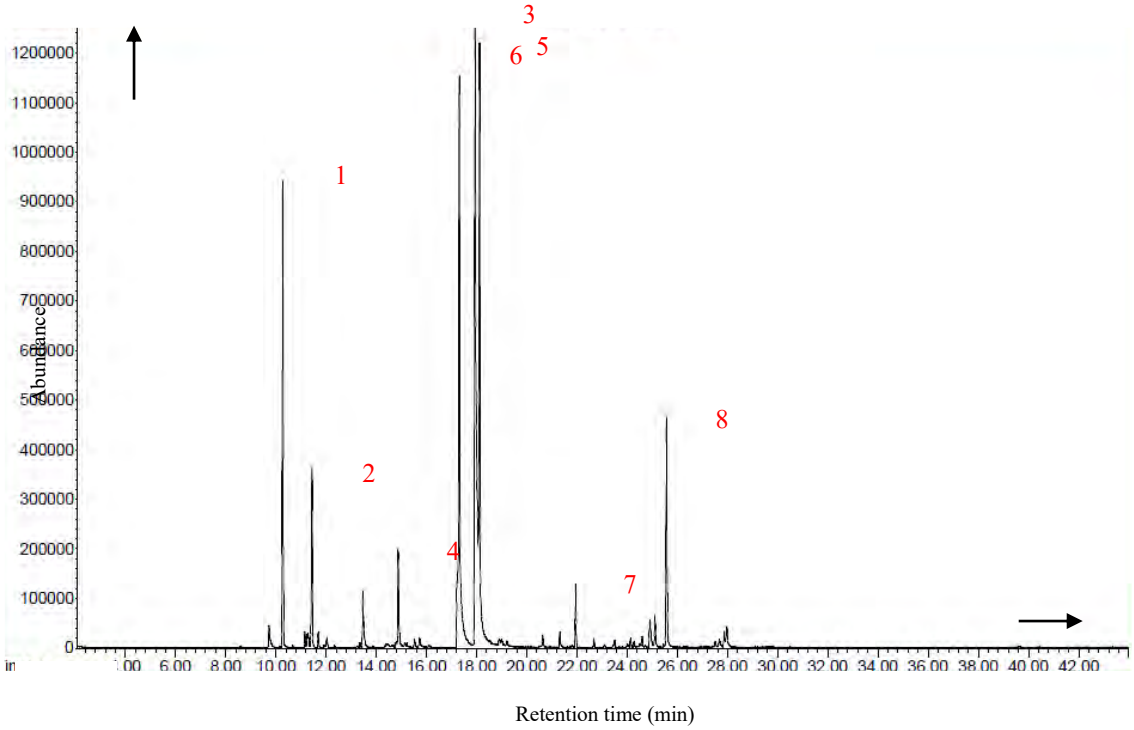

Figure 9. Analytical chromatogram of the essential oil of Cymbopogon nardus

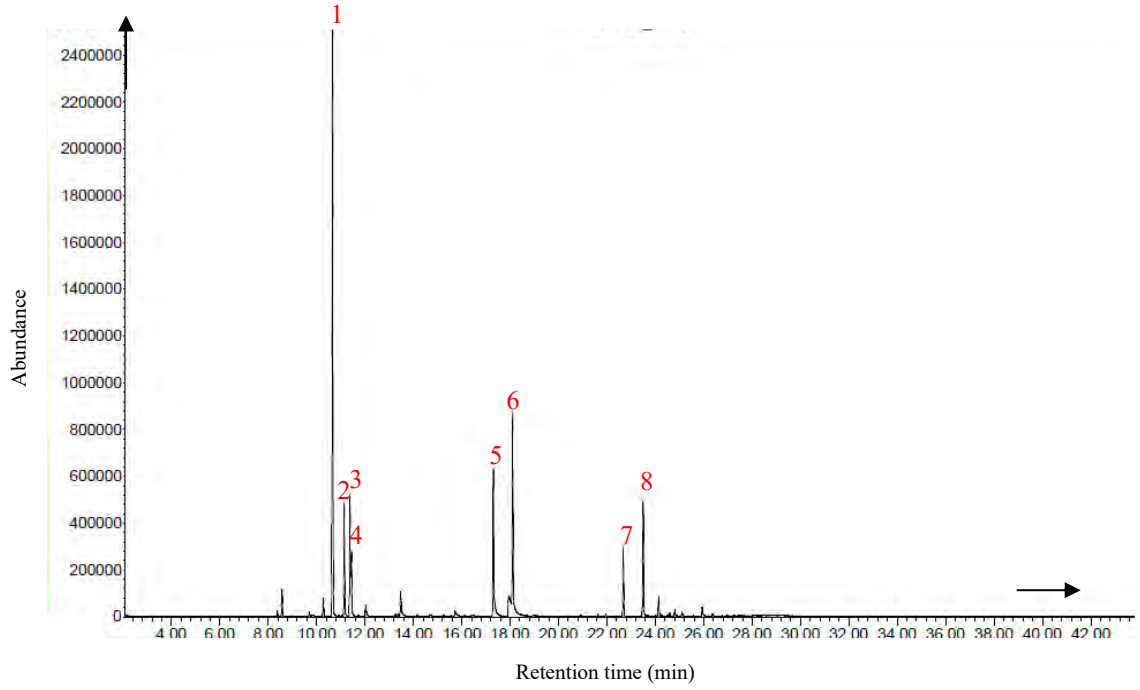

Figure 10. Analytical chromatogram of the essential oil of Lippia multiflora

4.1.5. Chemical composition of the essential oil of *Hyptis suaveolens*

The chromatogram of the essential oil of *H. suaveolens* revealed 18 peaks **(Figure 11)** whose constituents are presented in **Table VI**. The extract is composed of 70.80 % monoterpenes of which 26.87 % are hydrocarbon and 43.93 % are oxygenated. The hydrocarbon monoterpenes detected were β-pinene (7.68 %), p-cymene (5.22 %), β-phellandrene (4.69 %) and α-pinene (2.98 %). As for oxygenated monoterpenes, the majority compound was eucalyptol (1,8 cineol) at 39.41 % and the majorities, with levels of 2.52 and 1.14 %, were Borneo camphor and camphor, respectively. Sesquiterpenes such as β-elemene, β-caryophyllene, α-bergamotene, α-caryophyllene, β-selinene, β-gurjunene, isolongifolene, β-caryophyllene oxide were identified and the most represented was β-caryophyllene.

Table VI. Chemical composition of the essential oil of *Hyptis suaveolens*

Terpene groups	N° (Peak)	Constituents	%
Hydrocarbon monoterpenes	1	α-pinene	2.98
	2	β-phellandrene	4.69
	3	β-pinene	7.68
	4	p- cymene	5.22
	5	4-carene	0.86
Oxygenated monoterpenes	6	eucalyptol	39.41
	7	camphor	1.14
	8	Borneo camphor	2.52
Hydrocarbon sesquiterpenes	9	dimethyl-p-styrene	2.89
	10	copaene	1.72
	11	β-elemene	0.68
	12	β-caryophyllene	18.39
	13	α-bergamotene	2.47
	14	α- caryophyllene	1.27
	15	β-selinene	0.94
	16	β-gurjunene	0.70
	17	isolongifolene	2.07
Oxygenated sesquiterpenes	18	β -caryophyllene oxyde	4.38
		Total	100

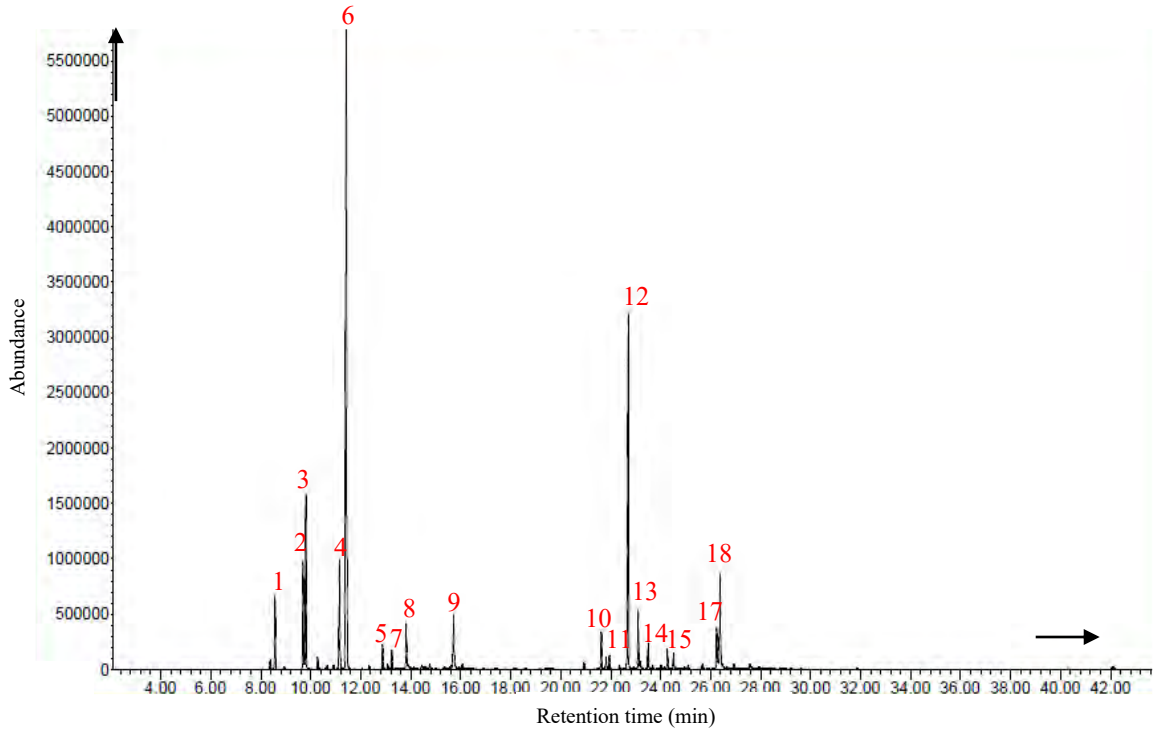

Figure 11. Analytical chromatogram of the essential oil of Hyptis suaveolens

4.1.6. Chemical composition of the essential oil of *Melaleuca leucadendron*

The chromatogram of the essential oil of *M. leucadendron* presented seven peaks **(Figure 12)** corresponding to the compounds recorded in **Table VII**. In the analyzed extract, monoterpenes were detected at 87.57 % and sesquiterpenes at 12.44 %. Eucalyptol or 1,8-cineole, an oxygenated monoterpene, with a rate of 62.73 %, was the most represented in the sample. Apart from this constituent, α-terpinol, another element of the same family was identified at a rate of 12.34 %. The hydrocarbon monoterpenes as a whole showed a rate of 12.50 % with α-pinene and β-pinene (9.22 and 3.28 %, respectively). Virdiflorene (10.09 %) was the only oxygenated sesquiterpene in this sample. Other compounds belonging to the hydrocarbon sesquiterpene family were identified: β-cis-caryophyllene (1.41 %) and azulene (0.94 %).

Table VII. Chemical composition of the essential oil of *Melaleuca leucadendron*

Terpene groups	N° (peak)	Constituents	%
Hydrocarbon monoterpenes	1	α-pinene	9.22
	2	β-pinene	3.28
Oxygenated monoterpenes	3	eucalyptol	62.73
	4	α-terpinol	12.34
Hydrocarbon sesquiterpenes	5	β-cis-caryophyllene	1.41
	6	azulene	0.94
Oxygenated sesquiterpenes	7	virdiflorene	10.09
		Total	100

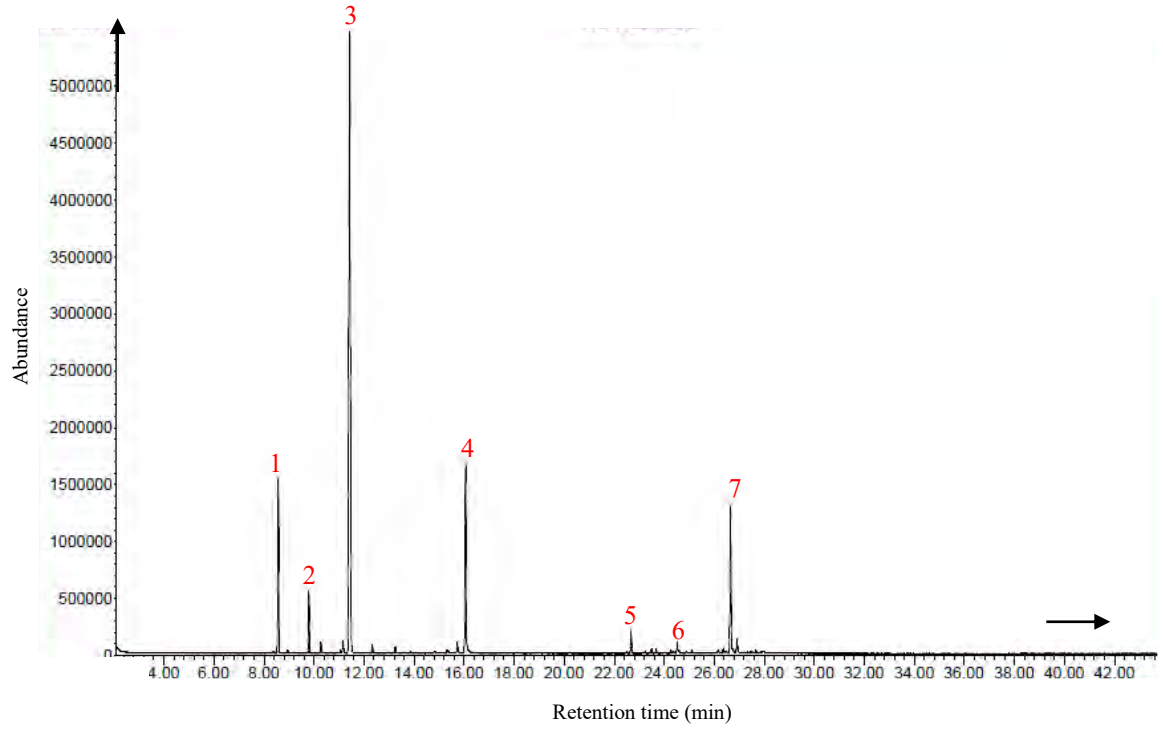

Figure 12. Analytical chromatogram of the essential oil of Melaleuca leucadendron

4.1.7. Chemical composition of the essential oil of *Citrus sp.*

Chemical analysis of *Citrus sp.* essential oil showed that it contains mostly d-limonene at a level of 59.36 %, followed by β-pinene (19.42 %) and γ terpinolene (10.85 %). These three compounds belong to the hydrocarbon monoterpene group. A low level of 10.37 % was recorded in oxygenated monoterpenes with terpene-4-ol (3.63 %) and α-terpinol (6.74 %). All these terpene elements are represented on the analytical chromatogram by their peaks **(Figure 13)** and their percentage contents were shown in **Table VIII**.

Table VIII. Chemical composition of the essential oil of *Citrus sp**

Terpene groups	N° (peak)	Constituents	%
Hydrocarbon monoterpenes	1	β-pinene	19.42
	2	d-limonene	59.36
	3	γ-terpinolene	10.85
Oxygenated monoterpenes	4	terpene-4-ol	3.63
	5	α-terpinol	6.74
		Total	100

*Kobenan *et al.*, 2018b

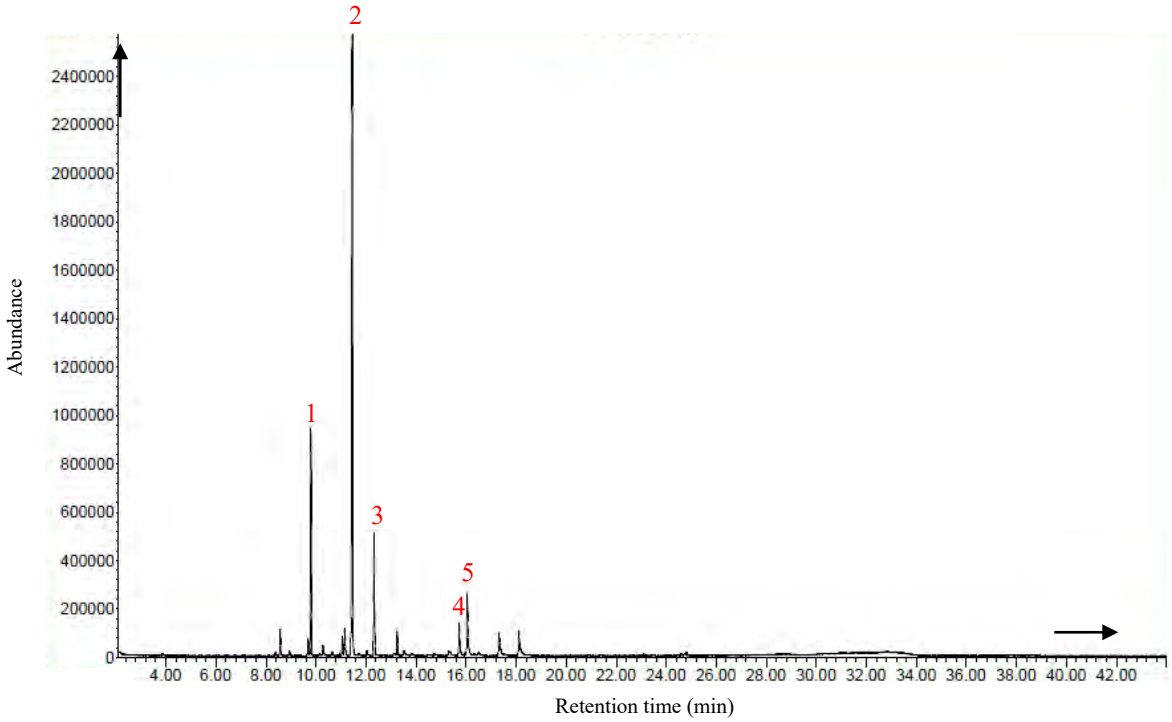

Figure 13. Analytical chromatogram of the essential oil of Citrus sp.

4.1.8. Chemical composition of the essential oil of *Ocimum canum*

Twelve peaks presented in the chromatogram **(Figure 14)**, appeared following the analysis of the essential oil of *O. canum*. The chemical analysis revealed a predominance of oxygenated monoterpene compounds (76.81 %) such as eucalyptol or 1,8 cineol (41.75 %), camphor (16.94 %) and myrtenal (13.50 %). However, it has relatively few hydrocarbon monoterpenes and sesquiterpenes **(Table IX)**. α-pinene (5.29 %), 1S-α-pinene (3.59%), β-pinene (3.29 %), p-cymene (1.49 %), camphene (2.11 %), and α-caryophyllene (5.92 %) were the only hydrocarbon components identified in the analyzed sample. In total, twelve compounds representing 99.98 % and divided into three major terpene groups were identified in this oil.

4.1.9. Chemical composition of the essential oil of *Eucalyptus globulus*

The chromatogram of the essential oil of *E. globulus* revealed only four peaks **(Figure 15)** representative of the constituents whose percentages are presented in Table X. The analyzed extract is mainly composed of oxygenated monoterpene with only eucalyptol at 80.16 %. The hydrocarbon monoterpenes are composed of α-pinene, p-cimene and γ-terpinene at 5.62 %, 10.33 % and 3.39 %, respectively.

Table IX. Chemical composition of the essential oil of *Ocimum canum**

Terpene groups	N° (peak)	Constituents	%
Hydrocarbon monoterpenes	1	α-pinene	5.29
	2	camphene	2.11
	3	β-pinene	3.29
	4	p- cymene	1.49
	5	1S-alpha pinene	3.59
Oxygenated monoterpenes	6	polyphenol ether	1.48
	7	1.8 cineol	41.75
	8	camphre	16.94
	9	terpinene-4-ol	1.99
	10	borneo camphor	2.63
	12	myrtenal	13.50
Sesquiterpènes hydrocarbonés	11	α- caryophyllène	5.92
		Total	99.98

*Kobenan *et al.*, 2018a

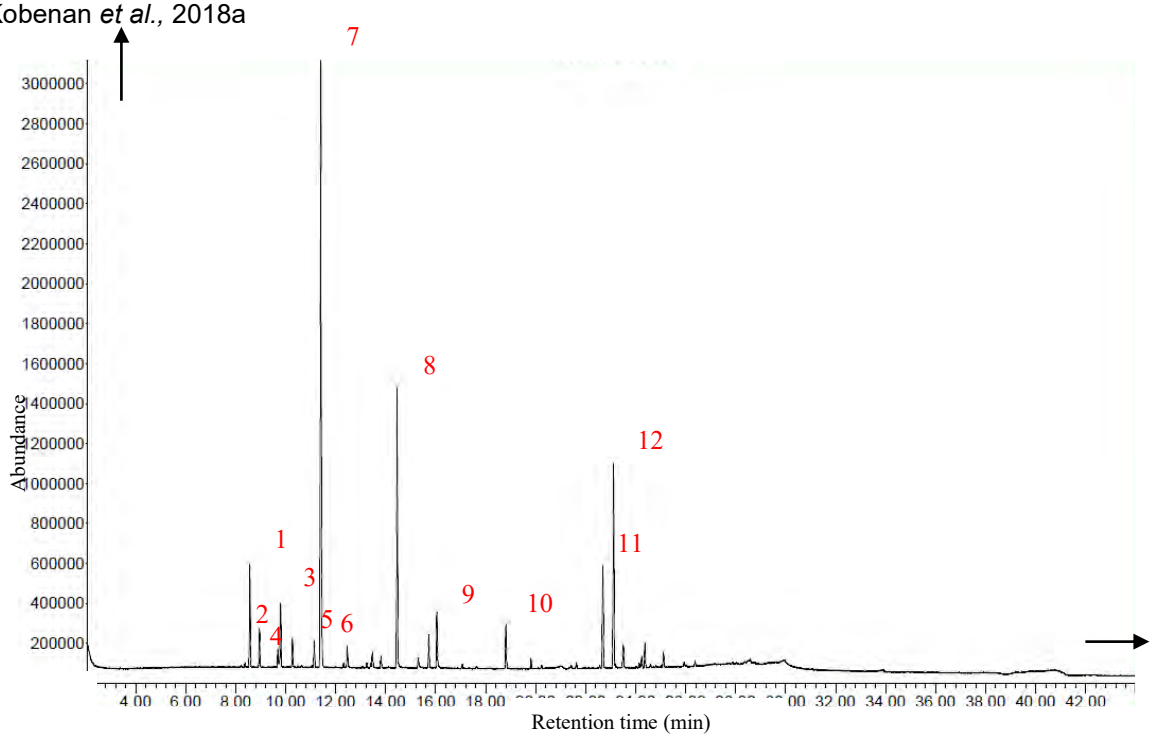

Figure 14. Analytical chromatogram of the essential oil of Ocimum canum

Table X. Chemical composition of the essential oil of *Eucalyptus globulus*

Terpene groups	N° (peak)	Constituents	%
Hydrocarbon monoterpenes	1	α-pinene	5.62
	2	p-cimene	10.33
	3	γ-terpinene	3.89
Oxygenated monoterpenes	4	eucalyptol	80.16
		Total	100

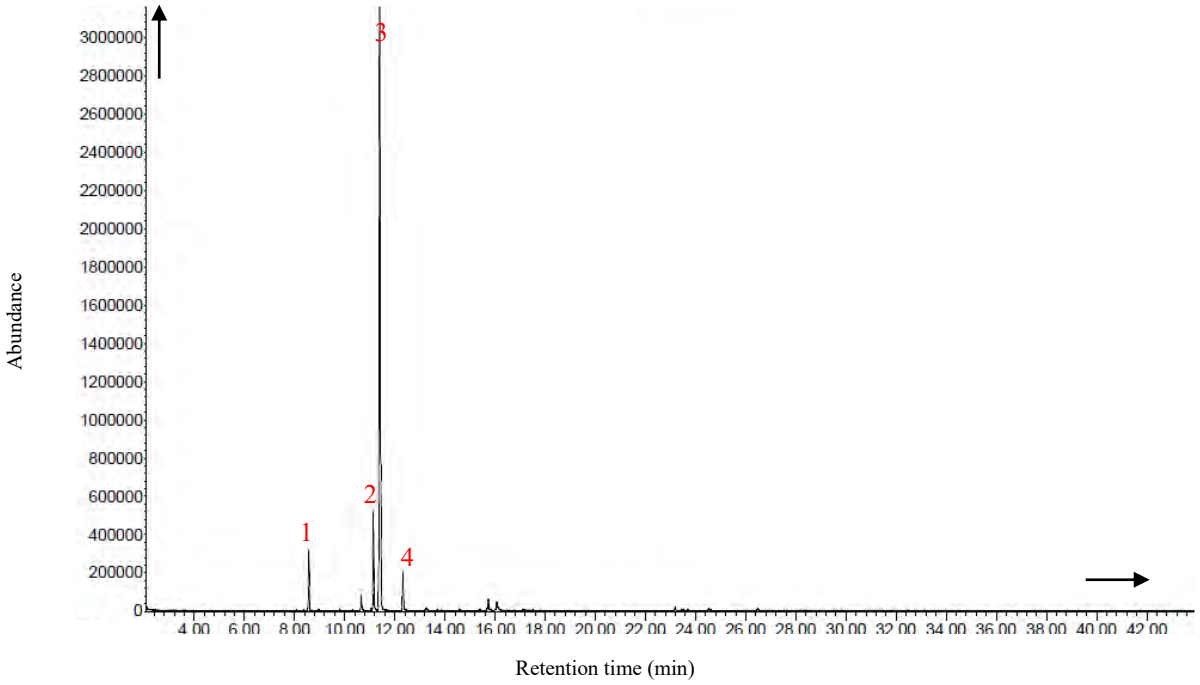

Figure 15. Analytical chromatogram of the essential oil of Eucalyptus globulus

4.2. Lethal concentrations of essential oils after two days of exposure of insects

4.2.1. Case of *Pectinophora gossypiella* adults

Lethal concentrations causing death of 50 % (LC_{50}) and 90 % (LC_{90}) of the populations tested were determined for each of the essential oils. From the results recorded in **Figure 16**, the extracts of *O. gratissimum*, *C. citratus*, *C. nardus* and *L. multiflora* showed the lowest LC_{50} (1.01; 1.67; 1.71 and 1.74 % respectively). In contrast, 11- to 15-fold higher values were obtained with extracts of *E. globulus* (16.05 %), *Citrus sp.* (12.80 %) and *O. canum* (11.33 %). Relative to LC_{90} **(Figure 17)**, the highest values among the nine essential oils tested on P. gossypiella were from plants such as *M. leucadendron* (145.79 %), *H. suaveolens* (59.71 %) and *O. canum* (30.41 %). However, the lowest values were obtained with the extracts of *C. citratus*, *O. gratissimum* and *C. nardus* with LC_{90} of 4.57 %, 5.05 % and 9.51 %, respectively.

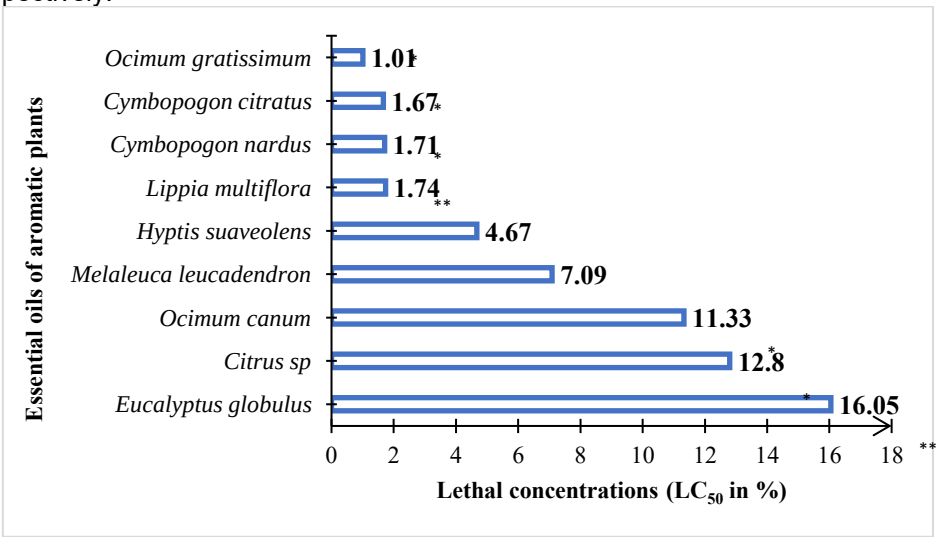

Figure 16. Lethal concentrations (LC50) of essential oils for adults of Pectinophora gossypiella after two days of exposure

*Kobenan *et al.*, 2018 ; **Kobenan *et al.*, 2022

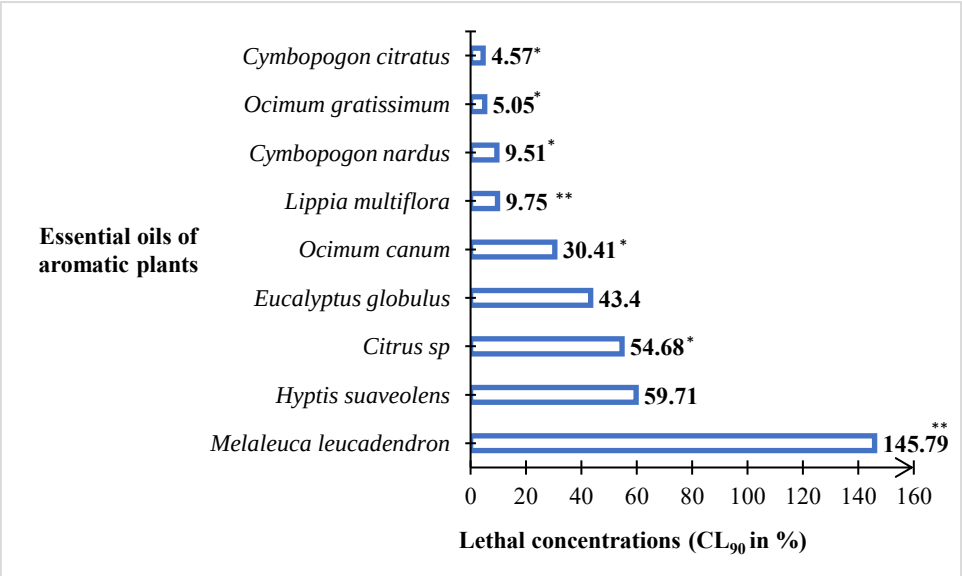

Figure 17. Lethal concentrations (LC90) of essential oils for adults of Pectinophora gossypiella after two days of exposure

*Kobenan *et al.*, 2018 ; **Kobenan *et al.*, 2022

4.2.2. Case of adults of *Thaumatotibia leucotreta*

The most effective essential oils on *T. leucotreta* were those of *O. gratissimum*, *C. citratus* and *L. multiflora* **(Figure 18)**. The most toxic to this pest was *O. gratissimum* with an LC_{50} of 0.93%. That of *C. citratus* and *L. multiflora* gave LC_{50} values of 1.39 %. Thus, these two plants have near-similar toxicities to this pest. However, *E. globulus* with a high LC_{50} value was the least toxic of all the essential oils tested (10.23 %). Concerning LC_{90} **(Figure 19)**, *O. gratissimum* presented the lowest value (5.56 %).

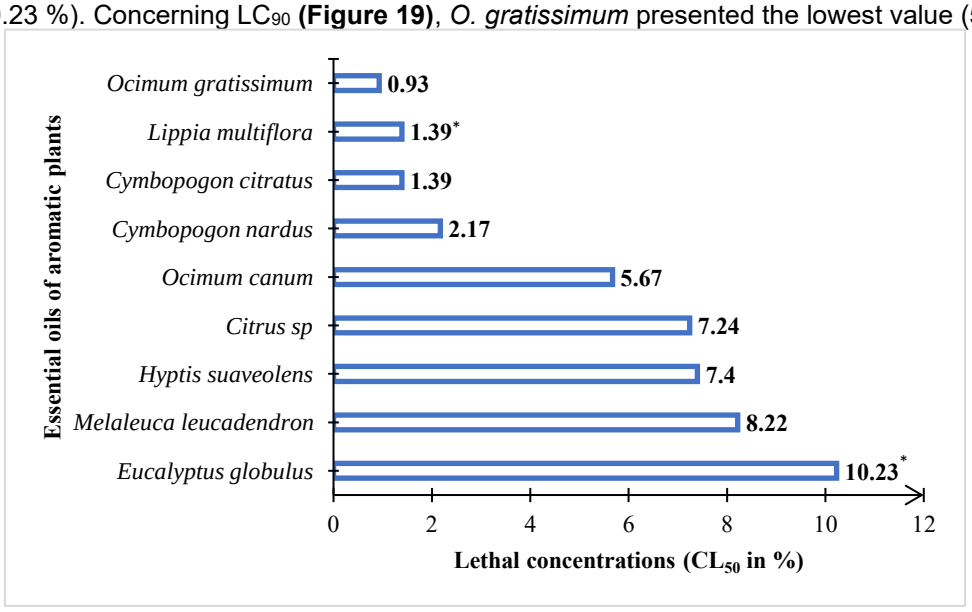

Figure 18. Lethal concentrations (LC50) of essential oils for adults of Thaumatotibia leucotreta after two days of exposure

*Kobenan *et al.*, 2022

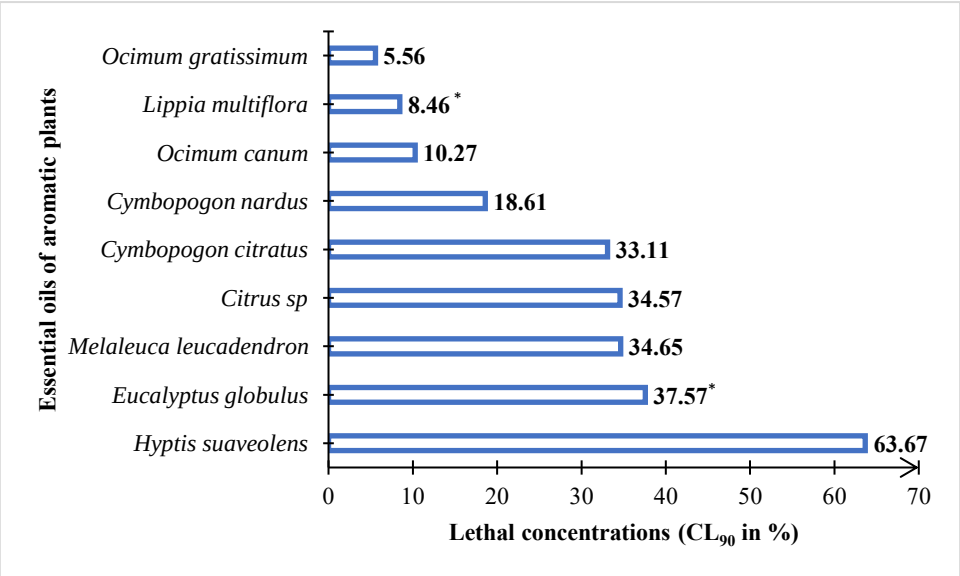

Figure 19. Lethal concentrations (LC90) of essential oils for adults of Thaumatotibia leucotreta after two days of exposure

*Kobenan *et al.*, 2022

4.2.3. Case of *Helicoverpa armigera* larvae

The LC_{50} and LC_{90} of the tested populations were calculated for each of the essential oils on *H. armigera*. According to the results recorded in **figures 20 and 21**, the most toxic extracts on *H. armigera* are those of *O. gratissimum* and *L. multiflora*. Indeed, the latter presented the lowest LC_{50} values (6.92 and 7.20 % respectively). On the other hand, much higher LC_{50} values were obtained with the essential oils of *Citrus sp.* (38.42 %), *C. citratus* (27.73 %) and O. *canum* (19.12 %) reflecting their low toxicity towards the pest. With the essential oil of *O. gratissimum*, the LC_{90} was 17.14 %. It is the most toxic. With *Citrus sp.* extract, a concentration four times higher than that of *O. gratissimum* would have to be applied to obtain 90 % mortality. This essential oil is the least toxic and the larvae of *H. armigera* are not very sensitive to it. This low sensitivity of the pest was also observed with essential oils of *C. citratus* (LC_{90} = 51.08 %) and *O. canum* (LC_{90} = 44.57 %) in particular.

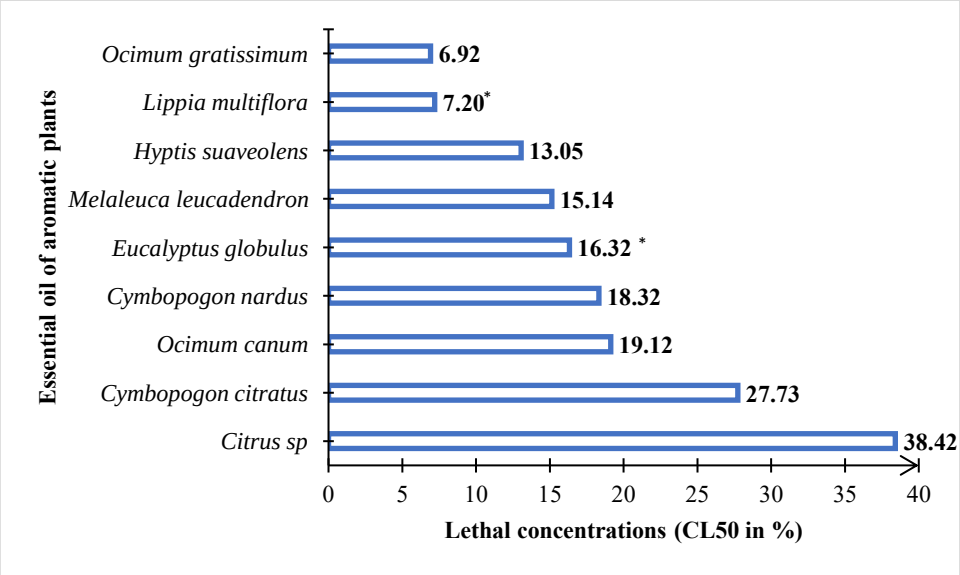

Figure 20. Lethal concentrations (LC50) of essential oils for Helicoverpa armigera larvae after two days of exposure

*Kobenan *et al.*, 2022

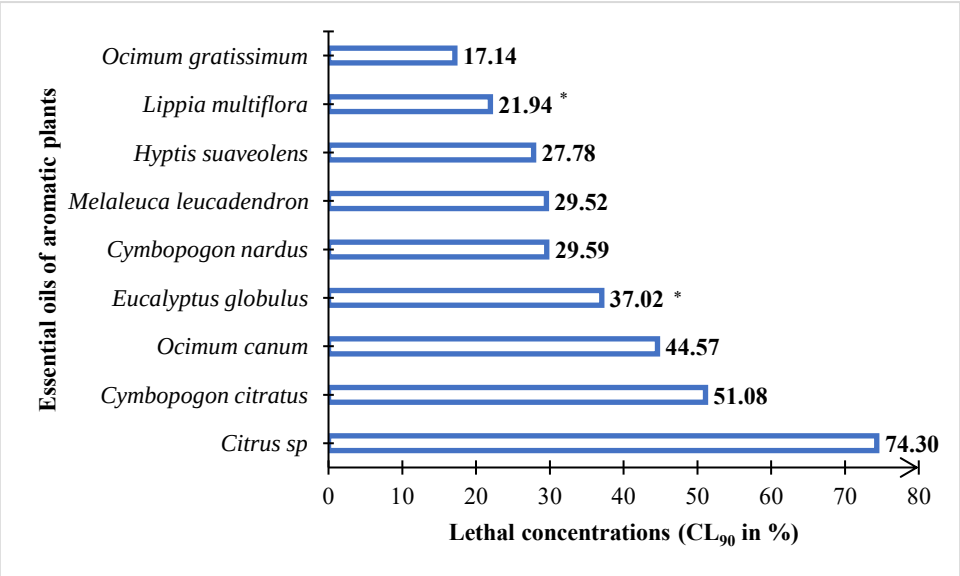

Figure 21. Lethal concentrations (LC90) of essential oils for Helicoverpa armigera larvae after two days of exposure

*Kobenan *et al.*, 2022

4.2.4. Classification of essential oils according to their toxicity on all three tested pests (*P. gossypiella*, *T. leucotreta* and *H. armigera*)

A hierarchical ascending classification (HAC) was carried out in order to determine the similarities between the essential oils according to their toxicity levels towards all the pests tested in the laboratory. This analysis was done with the LC_{50} and LC_{90} values of each of the essential oils obtained on all the pests tested. The analysis of the graph shows that the essential oils tested are divided into four groups **(Figure 22)**. Group 1 is made up exclusively of the essential oil of *Citrus sp.* The second group was made up of the essential oils of *O. canum* and *E. globulus*. The third group is composed of essential oils of *H. suaveolens* and *M. leucadendron*. Finally, the last group characterized by the same level of toxicity, included the essential oils of *O. gratissimum*, *L. multiflora*, *C. nardus* and *C. citratus*. Based on the analyses carried out previously, it can be noted that this last group of essential oils, is not only characterized by the lowest lethal concentrations, but by high levels of toxicity against all the pests.

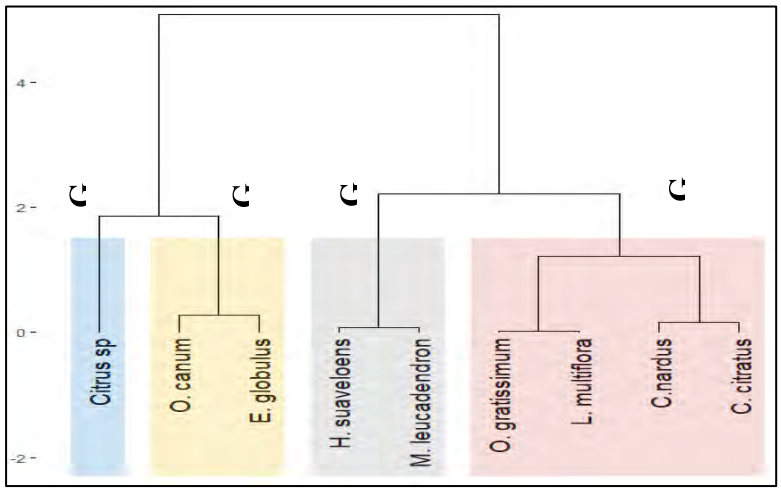

Figure 22. Dendrogram of essential oils according to their toxicity levels for all the pests tested The four different colors indicate the groups of essential oils formed.

Discussion

The results of the analysis of the chemical composition of essential oils revealed that the one extracted from *O. gratissimum* is rich in hydrocarbon monoterpenes. These results corroborate those obtained from the work of Tchoumbougnang *et al.* (2009), Akantetou *et al.* (2011) and Ouédraogo *et al.* (2016). Indeed, in the chemotypes studied by these authors, a high composition of hydrocarbon elements was detected in the essences of *O. gratissimum*. Para-cymene and thymol, were the majority molecules in the essential oil of *O. gratissimum*. Koffi *et al.* (2013) made these same findings on a chemotype from southern Ivory Coast. The remarkable presence of thymol in the present chemotype studied, is generally singular to essential oils of *O. gratissimum* as shown by several authors (Ngamo *et al.,* 2001; Guèye *et al.,* 2011; Nguemtchouin, 2012).

Relative to the essential oil of *C. citratus*, it is rich in oxygenated monoterpenes with a predominance of compounds such as α-citral (or geranium) and neral. Several authors have noted this in many countries (Tchoumbougnang *et al.,* 2009; Ntonga *et al.,* 2014; Kpoviessi *et al.,* 2013). In Ivory Coast, Kouamé *et al.* (2015) worked on chemotypes characterized by a high percentage of citral and neral. However, only one hydrocarbon molecule, myrcene, was detected in this essential oil. This finding was also made by de Chisowa *et al.* (1998) in Zambia. Indeed, the chemotype used by these authors was characterized by the presence of this molecule as the only monoterpene hydrocarbon compound.

Regarding the essential oil of *C. nardus*, its chromatographic analysis mainly highlighted the pronounced presence of neral, linalool and a low content of citronellol. These results confirm those of Wei and Wee (2013) who revealed a low proportion of citronellol in the essential oil of the same species. On the other hand, the results on the Togolese chemotype analyzed by Koba *et al.* (2009) was rather strongly composed of citronellol and geraniol with a low content of neral. Also in the same vein, recently in Burkina Faso, the essential oil extracted from *C. nardus* and analyzed during the work of Ouédraogo *et al.* (2016) had essentially the same composition as previously reported.

GPC identified α-phellandrene, β-phellandrene and p-cymene, d-limonene, β-geranial, α-citral, α-caryophyllene, and β-farnesene as the main compounds in the *L. multiflora* essential oil studied in this work. In the work carried out in Ivory Coast on the same essential oil by Kanko *et al.* (2004), a substantially identical profile had been obtained. However, the difference between the chemotype studied and that of the authors previously cited, is in the percentage of phellandrene (47.12 % against 1.1 %), α-Citral (15.89 % against 0 %) and cineol (0.00 % against 43.2 %). This variation would be due to the difference in localities where the plants were collected. Our chemotype comes from the North of the country and theirs was from Central Ivory Coast. Also, aromatic compounds such as carvacrol and thymol were completely absent from our chemotype, yet highly represented in the essential oils of plants from Eastern, Southern and Central Ivory Coast (Soro, 2016). The presence of carvacrol in the extract of *L. multiflora* has been reported by many authors in several countries (Viana *et al.,* 2000; Abena *et al.,* 2003; González-Trujano *et al.,* 2017). This compound had been considered characteristic of the essential oil of this aromatic plant. The *H. suaveolens* extract was monoterpenoid in nature (~71% of the oil was monoterpenes). The main chemical constituents identified were β -phellandrene, β-pinene, eucalyptol or 1,8-cineole, β-caryophyllene and p-cymene. Our results are consistent with previous studies that reported the presence of monoterpene hydrocarbons such as α-phellandrene, α-pinene, limonene, β-thujene, γ-terpinene, allo-ocimene and o-cymene in the essential oil of *H. suaveolens* leaves (Conti *et al.,* 2012; Ngom *et al.,* 2012; Sharma *et al.,* 2019). In general, the qualitative variation of the extract of this plant between the studied chemotype and those reported in the literature, is not significant with however the accentuated presence of 1,8 cineol and caryophyllene.

Chromatographic analysis of the essential oil of *M. leucadendron* revealed only seven compounds in the chemotype while the same species grown in Senegal contained 43 (Fall *et al.,* 2017). However, between the main compounds of the two chemotypes, quantitative differences were observed for chemical elements such as 1,8 cineol (62.73 % versus 28.87 %), α-terpineol (12.34 % versus 7.06 %). On the other hand, the essential oil of *M. leucadendron* of Egyptian origin was also characterized by the presence of 1,8 cineol with a rate of 64.30 % (Farag *et al.,* 2004) and relatively similar to that of Ivory Coast (62.73 %).

The essential oil of *O. canum* used was composed mainly of 1,8 cineol (eucalyptol) and camphor. Boland *et al.* (1991) had shown that these terpene compounds were found in plants such as basil and especially eucalyptus, at levels of up to 90 %. Twenty years later, the work of Hassane *et al.* (2011),

confirmed the presence of 1,8 cineol and camphor as the majority compounds in their chemotype extracts that came from two distinct localities in Morocco. In Benin, on the other hand, Akantetou *et al.* (2011) revealed low levels of these terpene elements.
The essence obtained from lemon peels was very rich in hydrocarbon monoterpenes (89.63 %) with limonene (59.36 %) and pinene (19.42 %) as the majority compounds. Boukabache and Boudjefdjouf (2016) found that lemon peel essential oils contained high levels of limonene and pinene. However, their levels were relatively lower than those obtained in our work. In Algeria, Ammad *et al.* (2018) also obtained an essential oil extracted from lemon as rich in limonene (61.68 %) with a considerable proportion of neral (21.66 %).

Four compounds were identified in the extract of *E. globulus* including eucalyptol or 1,8-cineole (80.16 %), α-pinene (5.62 %), p-cimene (10.33 %) and γ-terpinene (3.39 %). The composition of this essential oil is consistent with the work that showed that monoterpenes are the predominant constituents and 1,8-cineole is the main one. Indeed, among the different components of eucalyptus oils, 1,8 cineol is the most important. In the extract of *E. globulus* analyzed in this present work, not only was 1,8 cineol the main component, its level was practically the same regardless of the origin of the species (Maciel *et al.,* 2010; Ait-Ouazzou *et al.,* 2011 and Merghnia *et al.,* 2018). However, our results are in contradiction with those obtained in Tunisia by Hafsa *et al.* (2016). The latter determined a very low level of 1,8 cineol (3.16%) in their chemotype.

In relation to the study on the insecticidal efficacy of essential oils of nine aromatic plant species from Ivory Coast, the bioassays conducted highlighted the lethal concentrations (LC_{50} and LC_{90}) by contact or topical application of each of the essential oils on the three carpophagous pests (*P. gossypiella*, *T. leucotreta* and *H. armigera*) of cotton. This allowed us to know that all the evaluated aromatic plants possess insecticidal effects and that insect mortality was strongly dependent on the concentration of the applied essential oils, as mentioned by Bokobana *et al.* (2014), Elidrissi *et al.* (2014) and Desramaux (2018). This first finding confirms their insecticidal properties in addition to their antifungal and antimicrobial activities highlighted by previous works (Camara *et al.,* 2010; Gonzalez *et al.,* 2013).

Apart from this aspect, the toxicity of essential oils would be related to the high presence of monoterpene hydrocarbon and oxygenated compounds in the extracts of the nine local aromatic plants. Indeed, Konstantopoulou *et al.* (1992) revealed that monoterpenes in essential oils are toxic to many insects. They could inhibit acetylcholinesterase, octopamine or cytochrome P450 mono-oxygenases causing the death of insects exposed to them (De-Oliveira *et al.,* 1997).

In addition, Boutabia *et al.* (2016), indicated that the insecticidal efficacy of an essential oil would be due to the nature and chemical structure of its terpene constituents. Moreover, referring to LC_{50}, the essential oil of *O. gratissimum* is the most toxic on the three tested pests (*P. gossypiella*, *T. leucotreta* and *H. armigera*). This efficacy on insects would be related to the presence of thymol in the extracts (Johnson *et al.,* 2006 and 2018; Tchoumbougnang *et al.,* 2009; Ouédraogo *et al.,* 2016). The same would be true for bacteria (Chabrier *et al.,* 2007) and fungi (Elajjouri *et al.,* 2008). Other authors such as Cloyd and Chiasson (2007) have hypothesized that this oxygenated compound would act directly on the cuticle of insects and mites, especially those with soft bodies by causing its degradation. Thymol would also interfere with the activity of synapses, which would prevent respiration by suffocation and lead to the death of the insect (Priestley *et al.,* 2003; Gonzalez *et al.,* 2013).

Besides this plant, extracts of *C. citratus*, *C. nardus*, *L. multiflora* were also effective on adults of *P. gossypiella* and *T. leucotreta*. On the first pest, the respective LC_{50} determined with these plants, were lower than 2 %, but higher than that obtained with the essential oil of *O. gratissimum*.
The toxicities of *C. citratus* and *C. nardus* extracts were essentially identical, but higher than that of *L. multiflora*. The insecticidal potentials would be justified by the richness of the essential oils in oxygenated monoterpenes compared to non-oxygenated or hydrocarbon compounds (Pavela, 2008). This assertion could also explain the low toxicity of lemon (*Citrus sp.*) peel extract, due to its poverty in oxygenated compounds. However, it is insufficient to understand the low efficacy of the essential oils of *H. suaveolens*, *M. leucadendron*, *O. canum* and *E. globulus*, which are very rich in them. Related to this finding, it would probably be appropriate, as Boutabia *et al.* (2016), to relate the insecticidal efficacy of essential oils to the richness and quality of their oxygenated terpene constituents such as α-citral and neral (Tchoumbougnang *et al.,* 2009; Hernandez *et al.,* 2015). In *L. multiflora*, the biocidal potential of the essential oil could be due to the presence of citral, as suggested by Soro's (2016) work on varieties of the species rich in this constituent. However, the presence of citral alone cannot explain the good

efficacy of the essential oil of *L. multiflora* insofar as that of *C. citratus* has a high proportion of it (31.89 %) yet with low toxicity on *H. armigera* larvae. Under these conditions, the larvicidal efficacy of *L. multiflora* relative to *C. citratus*, would be attributed to its richness in terpene elements (8 against 4), with certainly more synergistic interactions (Boughendjioua, 2017).

The essential oils of *O. canum*, *E. globulus* and *Citrus sp.*, were generally the least effective on most of the pests tested. The last two mentioned have a poor chemical profile with four and five terpene compounds, respectively, which could justify their low toxicity. Moreover, the limonene predominant in the extract of *Citrus sp.*, would be very little toxic for the insects. Moreover, the insecticidal efficacy of essential oils could be a function of the weight of the target subjects. In this hypothesis, larvae that are heavier than moths would require relatively higher doses of extracts for their eradication.

Conclusion

The essential oils extracted from the nine local aromatic plants (*O gratissimum*, *C. citratus*, *C. nardus*, *L. multiflora*, *H. suaveolens*, *Ocimum canum*, *M. leucadendron*, *E. globulus* and *Citrus sp.*) have high monoterpene compositions (oxygenated and hydrocarbon). However, they were relatively low in sesquiterpenes. On the other hand, they all showed insecticidal properties on adults of *P. gossypiella* and *T. leucotreta* on the one hand, and on larvae of *H. armigera* on the other hand. Referring to the values of lethal concentrations, the study revealed that the essential oil of *O. gratissimum* was the most toxic on the moths of *P. gossypiella* and *T. leucotreta*; and on the larvae of *H. armigera*. Based on LC_{50} and LC_{90} lethal concentrations, the essential oils of *O. gratissimum*, *L. multiflora*, *C. citratus* and *C. nardus* were the most effective on all pests tested. The phytosanitary protection of cotton, in the context of environmental protection, could be rethought by using essential oils for the development of bioinsecticide.

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References

1. Abbott W. S., 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265 - 267.
2. Abena A. A., Diatewa M., Gakosso G., Gbeassor M., Hondi-Assah T. & Ouamba J. M., 2003. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia, 74: 231 - 236.
3. Adda C., Atachi P., Hell K. & Tamò M., 2011. Potential use of the bushmint, *Hyptis suaveolens*, for the control of infestation by the pink stalk borer, *Sesamia calamistis* on maize in southern Benin, West Africa. Journal of Insect Science, 11 (33): 1-14.
4. Ahoton L. E., Alavo T. B. C, Ahomadegbe M. A, Ahanhanzo C. & Agbangla C., 2010. Domestication du gros baume (*Hyptis suaveolens* (L.) Poit.): techniques de production et potentiels insectes ravageurs au Sud du Bénin. International Journal of Biological and Chemical Sciences, 4 (3): 608 - 614.
5. Ait-Ouazzou, Lorán S., Bakkali M., Laglaoui A., Rota C., Herrera A., Pagán R. & Conchello P., 2011. Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and Rosmarinus officinalis from Morocco, Journal of the Science of Food and Agriculture, 91: 2643 - 2651.
6. Akantetou P. K., Koba K., Nenonene A. Y., Poutouli W. P., Raynaud C. & Sanda K., 2011. Evaluation du potentiel insecticide de l'huile essentielle d'*Ocimum canum* Sims sur *Aphis gossypii* Glover (Homoptera : Aphididae) au Togo. International Journal of Biological and Chemical Sciences, 5 (4): 1491 - 1500.
7. Ameyaw Y., 2009. A growth regulator for the propagation of *Lippia multiflora* Moldenke, an herbal for the management of mild hypertension in Ghana. Journal of Medicinal Plants Research, 3 (9): 681 - 685.
8. Ammad F., Moumen O., Gasem A., Othmane S., Hisashi K. N., Zebib B. & Merah O., 2018. The potency of lemon (Citrus limon L.) essential oil to control some fungal diseases of grapevine wood, Comptes Rendus Biologies, 341 (2) : 97 - 101.
9. Anjarwalla P., Belmain S., Sola P., Jamnadass R. & Stevenson P. C., 2016. Guide des plantes pesticides. World Agroforestry Centre (ICRAF), Nairobi, Kenya, 74 p.
10. Andriambololona L., Rasolonjatovo E. & Mong-Gine T., 1997. Comptes rendus des activités et expérimentations de prévulgarisation. HASYMA Toliara, Documents internes, 25 p.
11. Aubreville A., 1949. Climat, foret et désertification de l'Afrique tropical, Société d'édition géographique, maritime et coloniale, 116 p.
12. Avenard J. M., Eldin M. & Perreau A., 1971. Le milieu naturel de la Ivory Coast. Mémoire ORSTOM, n°50, Paris, 391 p.
13. Badiane D., 1995. Situation parasitaire entomologique du cotonnier au Senegal et méthodes de contrôle. Mémoire de titularisation, Centre de Recherches Agricoles (C.R.A.), Tambacounda, Sénégal, 92 p.
14. Badiane O., Ghura D., Goreux L. M. & Masson P. R., 2002. Evolution des filières cotonnières en Afrique de l'Ouest et du Centre., DC: Banque Mondiale, Policy Research Working Paper Washington, USA, n° 2867-B, 24 p.
15. Biao F., Afouda L. & Koné D., 2018. Effet des extraits aqueux à base d'ail (*Allium sativum*), de neem (*Azadirachta indica*), d'hyptis (*Hyptis spp.*) et d'huile d'arachide sur les pucerons, vecteurs du virus de la panachure du piment vert (*Capsicum chinense*) au Nord-Bénin. Journal of Animal & Plant Sciences, 3 (38): 6336 - 6348.

16. Bokobana E. M., Koba K., Poutouli W. P., Akantetou P. K., Nadio N. A., Laba B., Tozoou P., Raynaud C. & Sanda K., 2014. Evaluation du potentiel insecticide et répulsif de l'huile essentielle de *Cymbopogon schoenanthus* (L.) Spreng. sur *Aphis gossypii* Glover (Homoptera : Aphididae), ravageur du cotonnier au Togo. Revue Cames, 2 (2): 48 - 55.
17. Boland D. J., Brophy J. J. & House A. P. N., 1991. Eucalyptus Leaf Oils: Use, Chemistry, Distillation and Marketing. Inkata Press, Melbourne, Australie, 252 p.
18. Boughendjioua H., 2017. Composition chimique et activité antibactérienne de l'huile essentielle de *Lavandula officinalis* cultivées dans la région de Skikda en Algérie. Bulletin de la Société Royale des Sciences de Liège, 86: 88 - 95.
19. Boukabache M. & Boudjefdjouf F. Z., 2016. Extraction, identification de l'huile essentielle par CPG-SM de l'espèce Citrus limon et mise en evidence de son activité antibacterienne. Memoire de fin de cycle pour l'obtention de Diplome de Master en Métabolisme secondaire et molecules bioactives, Université des Frères Constantine, Département: Biologie et Ecologie Végétale, Algérie, 72 p.
20. Boutabia L., Telailia S., Bougueto F. I., Guenadil F. & Chefrour A., 2016. Composition chimique et activité antibactérienne des huiles essentielles de *Rosmarinus officinalis* L. de la région de Hammamet (Tébessa-Algérie). Bulletin de la Société Royale des Sciences de Liège, 85: 174 - 189.
21. Camara B., Dick E., Sako A., Koné D., Kanko C., Boye M. A. D., Aké S. & Anno A., 2010. Lutte biologique contre *Deightoniella torulosa* (Syd.) Ellis par l'application des huiles essentielles d'*Eucalyptus platyphylla* F. Muell. et de *Melaleuca quinquenervia* L. Phytothérapie et Ecologie, 8: 240 - 244.
22. Cauquil J., 1993. Maladies et ravageurs du cotonnier en Afrique au Sud du Sahara. Montpellier, France, Edition CIRAD, 92 p.
23. Cauquil J. & Guillaumont M., 1979. Etude de la fructification du cotonnier sans protection et par deux pyréthrinoïdes. In: Compte rendu du Congrès sur la lutte contre les insectes en milieu tropical, Chambre de Commerce et d'Industrie, Marseille, France, pp. 127 - 144.
24. Cauquil J. & Vaissayre M., 1994. Nouvelle politique de protection et choix des pesticides. Agriculture et Développement, 3 :13-23.
25. Cavana J.F., 2002. Filière coton en Ivory Coast. Agence AFD d'Abidjan, 24 p.
26. Chabrier C., Mbolidi-Baron H. & Wicker E., 2007. Techniques de lutte alternative. Les cahiers du PRAM, 5: 43 - 49.
27. Cherin P., Voronska E., Fraoucene N. & Jaegerb C., 2012. Toxicité aiguë des pesticides chez l'homme. Médecine & Longévité, 4: 68 - 74.
28. Chevalier A., 1927. Le *Melaleuca Leucadendron*. Sa répartition géographique et ses produits. Journal d'Agriculture Traditionnelle et de Botanique Appliquée, 67 : 175 - 183.
29. Chisowa E. H., Hall D. R. & Farman D., 1998. Volatile Constituents of the Essential Oil of *Cymbopogon citratus* Stapf grown in Zambia. Flavour and Fragrance Journal, 13: 29 - 30.
30. CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), 2006. Le coton, fil des temps, des marches et des cultures. Exposition du salon international de l'agriculture de Paris, 25 février-5 mars 2006, Montpellier, France, 15 p.
31. Cloyd R. & Chiasson H., 2007. Activity of an essential oil derived from *Chenopodium ambrosioides* on Greenhouse Insect Pests. Journal of Economy Entomology, 100: 459 - 466.
32. Conti B., Benelli G., Flamini G., Cioni P. L., Profeti R., Ceccarini L., Macchia M. & Canale A., 2012. Larvicidal and repellent activity of *Hyptis suaveolens* (Lamiaceae) essential oil against the mosquito *Aedes albopictus* Skuse (Diptera: culicidae). Parasitology Research, 110: 2013-2021.
33. Couilloud R. & Giret M., 1980. Multiplication de *Heliothis armigera* (Hubner) (Lepidoptera: Noctuidae) : Amélioration possible grâce à l'adoption d'une technique d'élevage en groupe des chenilles. Coton et Fibres Tropicales, 35: 217 - 224.
34. Deguine J-P. & Vaissayre M., 2000. Proposition pour une gestion durable des populations de puceron, d'aleurodes chez les petits producteurs de coton africain. In: Actes de la Réunion Phytosanitaire Coraf – Réseau Coton, 22-25 Février 2000, Lomé, Togo, pp 209 - 218.
35. De-Oliveira A. C. A. X., Ribeiro-Pinto L. F. & Paumgartten F. J. R., 1997. *In vitro* inhibition of CYP2B1 monooxygenase by b-myrcene and other monoterpenoid compounds. Toxicology Letters, 92: 39 - 46.
36. Desramaux M., 2018. Huiles essentielles en dermocosmétologie. Thèse de Doctorat, Université De Bordeaux, U.F.R. Des Sciences Pharmaceutiques, France, 145 p.
37. Dhara J., Chinna B. N. V., Kranthi S., Kranthi K. R. & Valarmathi R., 2015. Viable mass production method for cotton pink bollworm, *Pectinophora gossypiella* (saunders). The Journal of Basic & Applied Zoology, 73: 9 - 12.
38. Didi G. J. R., Koné P. W. E., Ochou G. E. C., Dekoula S. C., Kouakou M., Bini K. K. N. B., Yao M. D. S., Mamadou D. & Ochou O. G., 2018. Evolution spatio-temporelle des infestations de la mouche blanche *Bemisia tabaci* (Gennadius, 1889) associées à la culture cotonnière en Ivory Coast. Journal of Applied Biosciences, 121: 12202 - 12210.
39. Diomandé L. B., Dougba W. H., Kanko C., Koné B. & Yao-Kouamé A., 2015. Les teneurs en carbone, azote et phosphore du sol sur l'occurrence de *Lippia multiflora* M. (théier de savane) et la composition chimique de son huile essentielle extraite des feuilles au Nord-Ouest de la Ivory Coast. International Journal of Biological and Chemical Sciences, 9 (1): 492 - 503.
40. Doffou N. M., 2013. Impact de la gestion de la résistance aux pyréthrinoïdes sur les populations de *Cryptophlebia leucotreta* Meyrick (Lepidoptera: tortricidae) et de *Pectinophora gossypiella* Saunders (Lepidoptera: gelechiidae) en culture cotonnière en Ivory Coast: de la sensibilité aux mécanismes. Thèse unique, Université Felix Houphouët-Boigny, UFR Biosciences, Ivory Coast, 178 p.
41. Elajjouri M., Satrani B., Ghanmi M., Aafi A., Farah A., Rahouti M., Amarti F. & Aberchane M., 2008. Activité antifongique des huiles essentielles de *Thymus bleicherianus* Pomel et *Thymus capitatus* (L.) Hoffm. & Link contre les champignons de pourriture du bois d'œuvre. Biotechnology Agronomy Society and Environment, 12 (4): 345 - 351.
42. Elidrissi M., Elhourri M., Amechrouq A. & Boughdad A., 2014. Etude de l'activité insecticide de l'huile essentielle de *Dysphania ambrosioïdes* L. (Chenopodiaceae) sur *Sitophilus oryzae* (Coleoptera: Curculionidae) [Study of the insecticidal activity of the essential oil of *Dysphania ambrosioïdes* L. (Chenopodiaceae) on *Sitophilus oryzae* (Coleoptera: Curculionidae)]. Journal of Materials and Environment Science, 5 (4): 989 - 994.
43. EPPO (Organisation européenne et méditerranéenne pour la protection des plantes), 1994. Decision-making scheme for the environmental risk assessment of plant protection products, Chapter 9, Arthropod natural enemies. EPPO Bulletin, 24: 17 - 35.

44. Fall R., Ngom S., Sall D., Sembène M. & Samb A., 2017. Chemical characterization of essential oil from the leaves of *Callistemon viminalis* (D.R.) and *Melaleuca leucadendron* (Linn.). Asian Pacific Journal of Tropical Biomedicine,7 (4): 347 - 351.
45. Farag R. S., Shalaby A. S., El-Baroty G. A., Ibrahim N. A., Ali M. A. & Hassan E. M., 2004. Chemical and biological evaluation of the essential oils of different Melaleuca species. Phytotherapy Research, 18: 30 - 5.
46. FARM (Fondation pour l'agriculture et la ruralité dans le monde), 2005. Le coton : quels enjeux pour l'Afrique ? Fondation pour l'Agriculture et la Ruralité dans le monde, les dossiers de FARM, Paris, 19 p.
47. Ferron P., Deguine J.P. & Mouté J.E., 2006. Evolution de la protection phytosanitaire du cotonnier : un cas d'école. Cahiers Agricultures, 1 (15): 128 - 134.
48. Fleischer G., Andoli V., Coulibaly M. & Randolph T., 1998. Analyse socio-économique de la filière des pesticides en Ivory Coast. Institut des Sciences Economiques, Faculté d'Horticulture, Université de Hanovre, Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH Eschborn, Allemagne, 112 p.
49. Gonzalez C. A., Reina M., Diaz C. E., Fraga B. M. & Santana-Meridas O., 2013. Natural Product-Based Biopesticides for Insect Control. In: Reedijk, J., Ed. Elsevier Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Waltham, MA, Spain, 55 p.
50. González-Trujano M. E., Hernández-Sánchez L. Y., Ocotero V. M., Dorazco-González A., Fefer P. G. & Aguirre-Hernández E., 2017. Pharmacological evaluation of theanxiolytic-like effects of Lippia graveolens and bioactive compounds. Pharmaceutical Biology, 55: 1569 - 1576.
51. Guèye M. T., Seck D., Wathelet J. P. & Lognay G., 2011. Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale: synthèse bibliographique. Biotechnology, Agronomy Society and Environment, 15 (1): 183 - 194.
52. Hafsa J., Smach M. A., Ben Khedher M. R., Charfeddine B., Limem K., Majdoub H. & Rouatbi S., 2016. Physical, antioxidant and antimicrobial properties of chitosan films containing *Eucalyptus globulus* essential oil, LWT. Food Science and Technology, 68: 356 - 364.
53. Hassane S. O. S., Ghanmi M., Satrani B., Farah A., Amarti F., Achmet S. M. & Chaouch A., 2011. Composition chimique et bioactivité des huiles essentielles de deux provenances de *Ocimum canum* de l'île de la Grande Comore. *Phytothérapie*, 9 : 18 - 24.
54. Hernandez L. R., Pajaro C. N., Caballero-Gallardo K., Stashenko E. & Olivero-Verbel J., 2015. Essential oils from plants of the genus *Cymbopogon* as natural. *Journal of Stored Products Research*, 62: 81 - 83.
55. Hussein K., Perret C. & Hitimana L., 2005. Importance économique et sociale du coton en Afrique de l'Ouest : rôle du coton dans le développement, le commerce et les moyens d'existence. OCDE SAH/D 556, Novembre 2005, 71 p.
56. Iwu M. M., 2014. Handbook of African medicinal plant. 2e édit. CRC Press Inc, Boca Raton, Floride, USA, 202 p.
57. Jigam A. A., Akanya H. O., Ogbadoyi E. O., Dauda B. E. N. & Egwim C. E., 2009. *In-vivo* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippia multiflora* mold. *Journal of Medicinal Plants Research*, 3 (3): 148 - 154.
58. Johnson F., Seri-Kouassi B., Aboua L. R. N. & Foua-Bi K., 2006. Utilisation de poudres et d'extraits totaux issus de plantes locales des genres *Ocimum sp*. et *Mentha sp*. comme biopesticides dans la lutte contre *Callosobruchus maculatus* fab. *Agronomie Africaine*, 18 (3) : 221 - 233.
59. Johnson F., Oussou K. R., Kanko C., Tonzibo Z. F., Foua-Bi K. &Tano Y., 2018. Bioefficacite des Huiles Essentielles de Trois Especes Vegetales (*Ocimum Gratissimum*, *Ocimum Canum* et *Hyptis Suaveolens*) de la Famille des Labiees dans la Lutte Contre *Sitophilus Zeamais*. *European Journal of Scientific Research*, 150 (3) : 273 - 284.
60. Jouault S., 2012. La qualité des huiles essentielles et son influence sur leur efficacité et sur leur toxicité. Thèse de Doctorat d'état en Pharmacie, Université de Loraine, Faculté de Pharmacie, France, 137 p.
61. Kaan P., Ömer C. K., Yasemin Y., Salih G., Betül D., Fatih D. & Kemal C. B., 2016. Insecticidal activity of *Salvia veneris* Hedge, essential oil against coleopteran stored product insects and *Spodoptera exigua* (Lepidoptera). *Industrial Crops and Products*, 97: 93 - 100.
62. Kanko C., Sawaliho B., Koné S., Koukoua G. & N'Guessan Y. T., 2004. Etude des propriétés physico-chimiques des huiles essentielles de *Lippia multiflora*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon giganteus*. *Comptes Rendues Chimie*, 7 : 1039 - 1042.
63. Karima T. T., 2015. Extraction et caractérisation des huiles essentielles de dix plantes aromatiques provenant de la région de Kabylie (Nord Algérien). Evaluation de leurs effets sur le bruche du niébé *Callosobruchus maculatus* (Coleoptera : Bruchidae), Thèse de Doctorat en Sciences Biologiques, Université Mouloud Mammeri de Tizi-Ouzou, Faculté des Sciences Biologiques et Sciences Agronomiques, Département de biologie Animale et végétale, Algérie, 206 p.
64. Karunamoorthi K., Ilango K. & Murugan K., 2010. Laboratory evaluation of traditionally used plant-based insect repellent against the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae). *Parasitology Research*, 106 (12): 17 - 23.
65. Koba K., Sanda K., Guyon C., Raynaud C., Chaumont JP. & Nicod L., 2009. *In -vitro* cytotoxic activity of *Cymbopogon citratus* L. and *Cymbopogon nardus* L. essential oils from Togo. *Bangladesh Journal of Pharmacology*, 4: 29 - 34.
66. Kobenan K. C., Tia V. E., Ochou G. E. C., Kouakou M., Bini K. K. N., Dagnogo M., Dick A. E., Ochou O. G., 2018a. Comparaison du potentiel insecticide des huiles essentielles de *Ocimum gratissimum* L. et de *Ocimum canum* Sims sur *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), insecte ravageur du cotonnier en Ivory Coast. Eur. Sci. J., 14 : 236–301.
67. Kobenan K. C., Ochou G. E. C., Kouakou M., Dick A. E., Ochou O. G., 2018b. Essential oils of *Cymbopogon citratus* (DC.) Stapf, *Cymbopogon nardus* L. and Citrus sp: Insecticidal activity on the Pink Bollworm *Pectinophora gossypiella* Saunders. Int. J. Inn. Appl. Stud., 24 :389–397.
68. Kobenan K. C., Ochou G. E. C., Sinan K. I., Kouakou M., Bini K. K. N., Ramazan C., Zengin G., Boka N. R. K., Ochou O. G., 2022. Chemical Composition, Antioxidant Activity, Cholinesterase Inhibitor and in Vitro Insecticidal Potentiality of Essential Oils of *Lippia multiflora* Moldenke and *Eucalyptus globulus* Labill. on the Main Carpophagous Pests of Cotton Plant in Ivory Coast, Chem. Biodiversity, 19, e202100993.
69. Koffi A. M., Tonzibo Z. F., Delort L., Ruiz N., Caldefie-Chézet L. & Chalchat J. C., 2013. Corrélation entre la composition chimique et l'activité antifongique des huiles essentielles à prédominance thymol sur *Candida albicans* et *Aspergillus fumigatus*. *Phytothérapie*, 11 : 134 - 139.
70. Koffi S. Y., 2013. Libéralisation de la filière coton en Ivory Coast quinze ans après : Empreinte spatiale et organisationnelle. *Cinq Continents*, 3 (7) : 5 - 17.

71. Koné P. W. E., Ochou G. E. C., Didi G. J. R., Dekoula S. C., Kouakou M., Bini K. K., Mamadou D. & Ochou O. G., 2017. Evolution spatiale et temporelle des dégâts du jasside *Jacobiella fascialis* Jacobi, 1912 (Cicadellidae) en comparaison avec la distribution de la pluviométrie au cours des vingt dernières années dans les zones de culture cotonnière en Ivory Coast. *International Journal of Biological and Chemical Sciences*, 11 (3): 1190 - 1201.
72. Konstantopoulou L., Vassilopoulou L., Mauragani T. & Scouras Z. G., 1992. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *D. auraria*. *Experientia*, 48 (6): 535 - 619.
73. Kouamé N. M., Kamagate M., Koffi C., Die-Kakou H. M., Yao N. A. R. & Kakou A., 2015. *Cymbopogon citratus* (DC.) Stapf : ethnopharmacologie, phytochimie, activités pharmacologiques et toxicologie. *Phytothérapie*, 14 (6): 384 - 392.
74. Kpoviessi S., Bero J., Agbani P., Gbaguidi F., Kpadonou-Kpoviessi B., Sinsin B., Accrombessi G., Frederich M., Moudachirou M. & Quetin-Leclercq J., 2013. Chemical composition, cytotoxicity and *in-vitro* antitrypanosomal and antiplasmodial activity of the essential oils of four *Cymbopogon* species from Benin. *Journal of Ethnopharmacology*, 151 (1): 652 - 9.
75. Maciel M. V., Morais S. M., Bevilaqua C. M., Silva R. A., Barros R. S., Sousa L. C., Brito E. S. & Souza-Neto M. A., 2010. Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary Parasitology*, 167 (1) : 1 - 7.
76. Marchand P. A., Isambert C. A., Tournant L., Oste S. & Larrieu J-F., 2014. Evaluation des caractéristiques et de l'intérêt agronomique de préparations simples de plantes, pour des productions fruitières, légumières et viticoles économes en intrants. Programme de protection des plantes par les plantes, 14 p.
77. Markus E. & Francis T. 2010. Le Ghana et la Ivory Coast: une inversion des rôles, *International Development Policy, Revue internationale de politique de développement*, 1 : 37 - 54.
78. Martin T., Ochou O. G., Hala N. F., Vassal J. M. & Vaissayre M., 1997. Perte de sensibilité aux pyréthrinoïdes chez *Helicoverpa armigera* (Hübner). *In* : Actes de la réunion phytosanitaire de l'Afrique de l'Ouest et du Centre, 27 - 31 janvier, Réseau Coton - CIRAD - INRAB, Cotonou, Bénin, pp 173 - 179.
79. Merghnia A., Noumia E., Haddeda O., Dridia N., Panward H., Ceylane O., Mastouria M. & Snoussif M., 2018. Assessment of the antibiofilm and antiquorum sensing activities of *Eucalyptus globulus* essential oil and its main component 1,8-cineole against methicillin-resistant *Staphylococcus aureus* strains. *Microbial Pathogenesis*, 118: 74 - 80.
80. N'cho B. S., 1991. Modélisation de l'accès des racines de maïs (*Zea mays*) à l'azote du sol. Mémoire de fin de cycle, IDESSA (Institut des savanes), Bouaké, Ivory Coast, 27 p.
81. Ngamo L. S., Ngassoum M. B., Jirovetz L., Ousman A., Nukenine C. E. & Mukala O. E., 2001. Protection du maïs stocké contre *Sitophilus zeamais* Motsch. Par l'utilisation d'huiles essentielles d'épices en provenance du Cameroun. Meded Rijks univ Gent Fak Landbouwkd. *Toegepaste Biologische Wetenschappen*, 66 (2): 473 - 478.
82. Ngom S., Faye F. D., Diop M., Kornprobst J. M. & Samb A., 2012. Composition chimique et propriétés physico-chimiques des huiles essentielles de *Ocimum basilicum* et de *Hyptis suaveolens* (L.) Poit. récoltés dans la région de Dakar au Sénégal. *Bulletin de la Société Royale des Sciences de Liège*, 81 : 166 - 175.
83. N'goran K. E., 2008. Effets des légumineuses à grain et des plantes de couvertures sur la fertilité des sols et la production de l'igname en zone soudano-guinéenne de Ivory Coast. Thèse de Doctorat, Université de Cocody, UFR Biosciences, Ivory Coast, 183 p.
84. Nguemtchouin M. G., 2012. Formulation d'insecticides en poudre par adsorption des huiles essentielles de *Xylopia aethiopica* et de *Ocimum gratissimum* sur des argiles camerounaises modifiées. Thèse de Doctorat, ENSC de Montpellier, France, 293 p.
85. Ntonga P. A., Baldovini N., Mouray E., Mambu L., Belong P. & Grellier P., 2014. Activity of *Ocimum basilicum*, *Ocimum canum*, and *Cymbopogon citratus* essential oils against *Plasmodium falciparum* and mature-stage larvae of *Anopheles funestus* s.s". *Parasite*, 21 (33): 1 - 8.
86. Ochou G. C. E., 2019. Evolution de la sensibilité aux pyréthrinoïdes chez trois lépidoptères [*Helicoverpa armigera* Hübner (Noctuidae), *Thaumatotibia leucotreta* Meyrick (Tortricidae) et *Pectinophora gossypiella* Saunders (Gelechiidae)] : approches agroécologiques de gestion de la résistance en culture cotonnière en Ivory Coast.Thèse de Doctorat, Université Nangui Abrogouauët-Boigny, Option : Biodiversité et Gestion Durable des Écosystèmes, Spécialité : Entomologie Agricole, Ivory Coast, 222 p.
87. Ouattara G., Koffi G. B. & Yao A. K., 2012. Contribution des images satellitales Landsat 7 ETM+ à la cartographie lithostructurale du Centre-Est de la Ivory Coast (Afrique de l'Ouest). *International Journal of Innovation and Applied Studies*, 1 (1) : 61 - 75.
88. Ouattara N., 2001. Note thématique sur les ressources génétiques forestières. Situation des ressources génétiques forestières de la Ivory Coast (zone de savanes), 43 p.
89. Ouédraogo I., Sawadogo A., Nebie R. CH. & Dakouo D., 2016. Evaluation de la toxicité des huiles essentielles de *Cymbopogon nardus* (L) et *Ocimum gratissimum* (L) contre *Sitophilus zeamais* Motsch et *Rhyzopertha dominica* F, les principaux insectes nuisibles au maïs en stockage au Burkina Faso. *International Journal of Biological and Chemical Sciences*, 10 (2) : 695 - 705.
90. Pascual M. E., Slowing K., Caretero E., Mara K. D. & Villar A., 2001. Lippia: Traditional uses, chemistry and pharmacology. *Journal of Ethnopharmacology*, 76: 201 - 214.
91. Patana R., 1977. Layered Diet for Pink Bollworm Rearing, U.S. Departement of Agriculture, Agricultural Research Service, 10 p.
92. Pavela R., 2008. Insecticidal Properties of Several Essential Oils on the House Fly (*Musca domestica* L.). *Phytotherapy research*, 22: 274 - 278.
93. Popp J., Petö K. & Nagy J., 2013. Pesticide productivity and food security. *Agronomy for Sustainable Agriculture*, 33: 243 - 255.
94. Priestley C. M., Williamson E. M., Wafford K. A. & Sattelle D. B., 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA receptors and a homooligomeric GABA receptor from *Drosophila melanogaster*. *British Journal of Pharmacology*, 140 : 1363 - 1372.
95. Saxena R. C., Jilani G. & Kareem A. A., 1989. Effect of the neem on stored grain insects. In: Focus on phytochemical, Vol I, The neem tree, pp 1-18.
96. Sharma A., Singh H. P., Batish D. R. & Kohli R. K., 2019. Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of *Hyptis Suaveolens*. Ecotoxicology and Environmental Safety, 171: 863 - 870.

97. Sigrist J. C., Martin T. & Renou A., 1994. Effet non intentionnel des pesticides sur l'entomofaune utile des cotonniers. In: Actes de la réunion phytosanitaire de coordination Cultures Annuelles-Afrique Centrale, 26-29 janvier 1994, Maroua, Cameroun, pp 154 - 175.
98. Soro L. C., 2016. Caractérisation biochimique et activités biologiques des feuilles de *Lippia multiflora* Verbenaceae "théier de savane" cultivé en milieux forestier et savanicole de Ivory Coast. Thèse de Doctorat, Nutrition et Sécurité Alimentaire, Université Nangui Abrogoua, Ivory Coast, 204 p.
99. Soro S., N'guessan K. A., Traoré D. & Ouattara N. 2006. Impact des jachères sur la flore adventice Au nord de la Ivory Coast. Agronomie Africaine, 18 (3): 253 - 265.
100. Tchoumbougnang F., Dongmo J. P. M., Sameza M. L., Mbanjo N. E. G., Fotso G. B. T., Amvam Z. P. H. & Menut C., 2009. Activité larvicide sur *Anopheles gambiae* Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnology, Agronomy Society and Environment, 1 (13): 77-84.
101. Thiam A. & Mamadou B. S., 2009. Monitoring des pesticides au niveau des communautés à la base. Rapport Régional Afrique PAN Africa, Dakar, Sénégal, 57 p.
102. Tia E. V., Lozano P., Lozano Y. F., Martin T., Niamké S. & Adima A. A., 2013. Potentialité des huiles essentielles dans la lutte biologique contre la mouche blanche *Bemisia tabaci* Genn. Phytothérapie, 11 (1): 31 - 38.
103. Tiendrebeogo A., Ouedraogo I., Bonzi S. & kassankogno A. I., 2016. Etude de l'activité antifongique d'extraits de *Cymbopogon citratus* (DC.) Stap, *Eclipta alba* L., *Lippia multiflora* M. et *Agave sisalana* P. International Journal of Biological and Chemical Sciences, 11 (3): 1202 - 1211.
104. Traoré S. K., Dembele A., Kone M., Mambo V., Lafrance P., Bekro Y-A. & Houenou P., 2008. Contrôle des pesticides organochlorés dans le lait et produits laitiers: Bioaccumulation et Risques d'exposition. Afrique Sciences, 04 (1): 87 - 98.
105. Tunç I., Berger B. M., Erler F. & Dagli F., 2000. Ovicidal activity of essentiel oils from five plants against two stored-product insects. Journal of stored products reasearch, 36: 161 -168.
106. Vaissayre M., Sement G. & Trijau J. P., 1984. Aspects phytosanitaires de la culture cotonnière en Ivory Coast d'après le réseau d'essais à trois niveaux de protection. Coton Fibre Tropical, 39 (2): 1 - 7.
107. Viana G. S., Do Vale T. G., Silva C. M. & Matos F. J., 2000. Anticonvulsant activity of essential oils and active principles from chemotypes of *Lippia alba* (Mill.) N.E. Brown. Biological and Pharmaceutical Bulletin, 23: 1314 - 1317.
108. Wei L. S. & Wee W., 2013. Chemical composition and antimicrobial activity of *Cymbopogon nardus* citronella essential oil against systemic bacteria of aquatic animals. Iranian Journal of Microbiology, 5 (2): 147 - 152.
109. Wu H. H., Wu K. M., Wang D. Y. & Guo Y. Y., 2006. Flight potential of pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Environmental Entomology, 35: 887 - 893.
110. Yarou, B. B., Silvie, P., Komlan, F. A., Mensah, A., Alabi, T., Verheggen, F., Francis F. (2017). Plantes pesticides et protection des cultures maraîchères en Afrique de l'Ouest (synthèse bibliographique). Biotechnol. Agron. Soc. Environ. 2017 21(4), 288- 304.
111. Yoro G. R., 2000. Les principaux sols de Ivory Coast et leur corrélation avec les groupes de référence de la base mondiale de données des sols (WRB). Quatorzième réunion du sous-comité Ouest et Centre Africain de Corrélation des Sols, 9 - 13 Octobre 2000, Cotonou, Bénin, 19 p

Interactive Effects of Abiotic Factors on Abundance of Sucking Pests on Bt Cotton

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Abstract

Background

Crop productivity primarily gets highly influenced by biotic and abiotic stress. Several biotic fauna influence the growth of which insects are the most limiting factor to obtain the desired yield. On the other hand abiotic factors play an important role for the biotic stress abundance. Cotton (Gossypium hirsutum L.) is an important commercial crop and globally a claimed as white gold which is attacked by various type of insect pests like sucking and bollworms which results in significant reduction in productivity which ravages the crop growth throughout cropping period. Though genetically engineered Bt cotton provides effective management of bollworm complex, sucking pests still pose a great threat in cultivation of Bt cotton. Different abiotic factors influence the seasonal activity and population dynamics of sucking pests in cotton agroecosystem. Hence, the attempt is made here to ascertain the role of weather parameters on incidence of sucking pest complex viz., leaf hopper, aphids and thrips at ARS Dharwad, Karnataka, India during 2020-21 and 2021-22

Results *Among sucking insect pests, the incidence of a leaf hopper, thrips and aphids remained active through the cropping season in varying population density. Thrips (Thrips tabaci L.) population was only observed in early stage of the crop growth and peak activity of thrips notices from 40-55 days onwards and the average population ranged from 31.80 - 41.70 thrips per 3 leaves. The peak incidence of 41.20-47.30 no. of thrips population per three leaves was observed from August 2nd FN- September months which coincides with maximum temperature of 27.80 - 29.19 °C and minimum temperature of 20.37-20.60 °C. The correlation of thrips population with minimum temperature was significant positive (r=0.322*) relationship. Cumulative effect of both maximum and minimum temperature designated that (R^2 = 0.233) 23.30 per cent role on population abundance. Multiple regression analysis revealed that every increase in 1°C temperature will lead to increase in 3.97 thrips per 3 leaves along with decrease in 1°C temperature will also lead to increase in 4.41 thrips per 3 leaves. Leaf hopper attained peak during 2nd FN of September and was maximum of 11.25 average nymphs per 3 leaves in consecutive years (2020-21 and 2021-22). The population of leaf hopper A biguttula showed highly significant positive correlation (r= 0.478**) with maximum temperature (29.50-30.61°C). Effect of maximum temperature by multiple regression analysis showed that 22.80 per cent influence on population (R^2 = 0.228) which indicates that every increase in 1°C temperature will lead to increase in 1.11 nymphs per 3 leaves. Further peak activity of aphids notices from 120-130 days after sowing and with maximum of 37.54 average aphids per 3 leaves in 2nd FN of November in 2020 and 1st FN of December in 2021. The peak average incidence of 37.54 aphids population per three leaves was observed in November and December months of two consecutive years which coincides with maximum (27.37-30.50 °C) and minimum temperature (16.19-17.35 °C). Correlation studies revealed that minimum temperature (r= -0.687**) exhibited negative and highly significant relationship with no. of aphids per 3 leaves. However, aphid population showed positive and highly significant relation with maximum temperature (r=0.378*), but negative and highly significant relationship with minimum temperature (r=-0.687**), maximum relative humidity (-0.607**) and minimum relative humidity (r=-0.669**). Whereas, rainfall exhibited negative relationship with aphid population (r=-0.272). Among all factors, influence of minimum temperature showed that (R^2 = 0.472) 47.20 per cent on the population, which indicates every decrease in 1°C temperature will lead to increase in 6.05 no. of aphids per 3 leaves.*

Conclusion *Considering the role of weather factors which influences population buildup of sucking pests in transgenic Bt Cotton, which found to be helpful in gauging likely buildup of the pest population, there by aiding in forewarning and timely action before infestation in conducive weather.*

Key words: *Correlation, Regression, Leaf hopper, Thrips, aphids, Bt cotton, abundance*

Introduction

Crop productivity primarily gets highly influenced by biotic and abiotic stress. Several biotic fauna influence the growth of which insects are the most limiting factor to obtain the desired yield. On the other hand abiotic factors play an important role for the biotic stress abundance. Survival and thriving at extreme physical conditions require peculiar adaptations and plastic responses. Among abiotic factors, temperature and humidity stand out as the most important ones constraining abundance and distribution of insect. Furthermore, it is well documented that abiotic factors, regulate the ecology of insect communities. Although effects of temperature on survival, development, and reproduction of insects have been exhaustively explored over several decades, there is still a lot of interest on how temperature and other abiotic factors set the limits of distribution and define abundance of insect species. Cotton, (*Gossypium hirsutum* L.) is the important cash crop in India due to its high industrial demand. Despite of huge share in areas the productivity of cotton (290 kg/ha) is still very lower than even the world average productivity. It is anticipated that this low production is mainly attributed due to infestation of pest problem. An array of insect pests has been reported to infest the crop rendering the low yield. About 162 species of insects has been known to occur in cotton at various stages of growth, of which 8 are key pests (Dhawan, 2000).

Cultivation of cotton under diversified agro climatic situations makes the crop to suffer a lot by different kinds of pests and diseases. Large area under rainfed situations and extensive replacement of conventional varieties with superior hybrids made the crop easily vulnerable to insect pests. The major reason for the low productivity in cotton is damage caused by insect pests. In India, as many as 162 species of insect-pests are known to attack cotton from sowing to maturity which cause up to 50-60 per cent loss (Agarwal et al., 1984). Cotton pests can be primarily divided into bollworms and sucking pests. Among sucking pests, aphid, *Aphis gossypii* (Glover), leafhoppers, *Amrasca biguttula biguttula* (Ishida), thrips, *Thrips tabaci* (Lind.) and whitefly, *Bemisia tabaci* (Genn.) are of major importance. These sucking pests occur at all the stages of crop growth and responsible for indirect yield losses. A reduction of 22.85 per cent in seed cotton yield due to sucking pests has been reported by Satpute et al. (1990).

Materials and Methods

A field experiment was conducted at the Agricultural Research Station, Dharwad under unprotected conditions (for sucking pests) to study the seasonal abundance pattern of sucking insect pests in popularly grown Jaadoo BGII cotton genotype and was kept unsprayed throughout the cropping season. The ARS Dharwad is located between15 0 N latitude and 76.46 0 E longitude at an altitude of 678 meters above mean sea level with annual average rainfall 922.7 mm. The plot size was 8.1 x 5.4 m with 10 rows of 10 plants for each genotype under 90 x 60 cm spacing replicated four times. All the recommended agronomical practices were followed to raise the crop successfully as per package of practices prescribed for the region (Anon., 2020).The population of sucking pests were estimated from 15 days after sowing (DAS) on population of adults as well as nymphs of thrips, aphids and nymphs of leafhoppers at weekly intervals on three leaves (top, middle and bottom) in ten plants selected randomly. Later the population was averaged to present as number per three leaves.

Results and Discussion

Seasonal incidence of sucking insect pests

Among sucking insect pests, the Thrips (*Thrips tabaci* L.), Leaf hopper (*A. biguttula*) and Aphids (*Aphis gossypi*) were key pests and remained active through the cropping season. *T. tabaci* population was only observed in early stages of the crop growth.

Thrips (*Thrips tabaci* L.): Similarly peak activity of thrips notices from 40-55 days onwards with average population ranged from 31.80-47.30 thrips per three leaves during both the consecutive year. The peak incidence of 41.20-47.30 no. of thrips population per three leaves was observed from August 2nd FN-September months which coincides with maximum (27.80 - 29.19 ^{o}C) and minimum temperature (20.37-20.60 ^{o}C). A population of *Thrips tabaci* L showed significant positive relationship with minimum temperature (r= 0.322*). However, the correlation with maximum temperature (r= 0.264), maximum relative humidity (r= 0.201) and minimum relative humidity (r=0.185) was positive relationship during all the years. Based on correlation was negative relationship with rainfall (r=-0.047). Cumulative effect of both maximum and minimum temperature designated that 23.30 per cent population (R^2 = 0.233). Multiple regression analysis revealed that every increase in 1^{o}C temperature will lead to increase in 3.97 no. of thrips per 3 leaves along with decrease in 1^{o}C temperature will also lead to increase in 4.41 no. of thrips per 3 leaves (Table 1 and 2).

Leaf hopper (*A. biguttula*): The population build up of sucking pests in relation to the abiotic factors were ascertained through the correlation studies along with multiple regression analysis. The results revealed that the peak activity of leaf hopper starts from 50 days after sowing and the average nymphal population ranged from 6.40 -11.70 per three leaves. The peak incidence of 10.80-11.70 nymphs per 3 leaves was observed from September-October months which coincide with maximum temperature of 29.50-30.61^{o}C and minimum temperature of 19.9-20.96 ^{o}C during two consecutive years. The multiple regression analysis between abundance of *A. biguttula* as a dependent variable and weather parameters revealed 22.80 per cent (R^2= 0.228) influence on population. Among the parameters maximum temperature has great role in activity of leaf hopper that every increase in 1^{o}C temperature will lead to increase in 1.11 leaf hopper per 3 leaves (Table 1 and 2).

Aphids (*Aphis gossypi*): Further peak activity of aphids notices from 120-130 days after sowing and the average population ranged from 30.40-41.50 per three leaves. The peak incidence of 30.40 - 41.50 aphids population per three leaves was observed in November month which coincides with maximum (27.37-30.50 ^{o}C) and minimum temperature (16.19-17.35 ^{o}C) during two consecutive years. However correlation showed that the mean temperature also showed positive correlation with incidence of *Aphis gossypi* and it was significant (r=0.387**). The regression analysis incidence of aphid showed negative and highly significant relationship (r=-0.687**) with minimum temperature and 47.20 per cent influence on aphid population. Which indicates that every decrease in 1^{o}C temperature will lead to increase in 6.05 aphids per 3 leaves (Table 1 and 2).

The understanding of the ecological factors (either biotic or abiotic) affecting the population of the insect pest is a prerequisite for planning the effective and more precise management strategy for a particular pest. The increased incidence of aphid towards tail end of the season irrespective of genotypes might be due to positive correlation of aphids with maximum temperature as disclosed by Mohapatra (2008). Sitaramaraju et al. (2010) who also observed population of *A. biguttula* throughout the cropping season. However, Gosalwad et al. (2009) reported the peak population of *A. biguttula* during 2nd week of October and the end of October at Parbhani (Maharashtra). In Guntur (Andhra Pradesh), Sitaramaraju et al. (2010) and Soujanya et al. (2010) observed the peak abundance of leafhoppers in 2nd week of October to 3rd week of November. Mohapatra (2008) also reported its peak in 2nd week of October in Western Orissa.

The leafhopper incidence was recorded in two peaks *i.e.* during second fortnights of August and November in all Bt genotypes, which recorded peak leafhopper incidence during second fortnight of September (Phulse and Udikeri., 2014). Selvaraj et al. (2011) and Prasad et al. (2008) reported abundance of *A. biguttula* with maximum and minimum temperature ranging from 30.5 to 33 °C and 20 to 24 °C, respectively. According to Selvaraj *et al*. (2011), morning relative humidity from 82 to 90 percent and evening relative humidity from 55 to 66 percent favours the multiplication of *A. biguttula* which corroborates with the present findings. Mean peak population of aphid *Aphis gossypii* (Glover) and jassid *Amrasca biguttula biguttula* (*Ishida*) was recorded at 34 and 37 meteorological week, respectively coinciding with abiotic factors 30–32°C maximum temperature, 21–22°C minimum temperature and more than 70% mean relative humidity in rainfed *Bt* cotton. The population dynamics of pest reaches above ETL (Economical Threshold Level) or to lower and higher vital limit are associated with changing abiotic factors (Pralhad and Shinde, 2021). Seasonal abundance and crucial role of different weather parameters on the population fluctuation of *A. biguttula* and *B. tabaci* in cotton agroecosystem which can be helpful in forecasting and formulating effective management strategies for these insect pests.

Table1: Seasonal Incidence of Sucking pests in transgenic Bt Cotton, 2020-22

Fort Night (FN)	2020-21			2021-22		
	Leaf hopper	Thrips	Aphids	Leaf hopper	Thrips	Aphids
			August			
1st FN	0.00	0.00	0.00	0.00	0.00	0.00
2nd FN	2.20	18.33	2.20	1.60	11.80	1.20
			September			
1st FN	4.00	32.90	3.40	3.80	31.80	4.50
2nd FN	6.70	41.20	8.9	8.10	47.30	6.80
			October			
1st FN	10.80	38.10	16.50	11.70	36.70	14.20
2nd FN	6.70	22.62	20.20	9.40	28.62	20.20
			November			
1st FN	6.20	17.10	30.40	7.10	16.20	24.60
2nd FN	3.90	9.20	38.89	6.00	8.20	31.40
			December			
1st FN	5.90	1.30	36.30	3.80	2.20	36.20
2nd FN	5.70	1.10	13.60	4.60	1.00	14.20

Table 2: Population of sucking pests on Bt Cotton vs. Climatic factors

S. No.	Climatic factors	Correlation Coefficients		
		Leaf hopper	Thrips	Aphids
1.	Maximum Temperature	0.478**	0.264	0.378**
2	Minimum Temperature	-0.170	0.322*	-0.687**
3	Relative Humidity (Max)	-0.253	0.201	-0.607**
4	Relative Humidity (Min)	-0.277	0.185	-0.669**
5	Rainfall (mm)	-0.226	-0.047	-0.272

Table 3: Correlations and Regression analysis with weather factors

Insect pests	MaxT (X_1)	MinT (X_2)	RHmax (X_3)	RHmin (X_4)	Rainfall (X_5)	Regression equation	R^2 (%)
Thrips	0.264	0.322*	0.201	0.185	-0.047	$Y=-181.699+4.41X_2+3.97X_1$	23.30
Leafhopper	0.478**	-0.170	-0.253	-0.277	-0.226	$Y=-26.633+1.11X_1$	22.80
Aphids	0.378**	-0.687**	-0.607**	-0.669**	-0.272	$Y=132.22-6.01\ X_2$	47.20

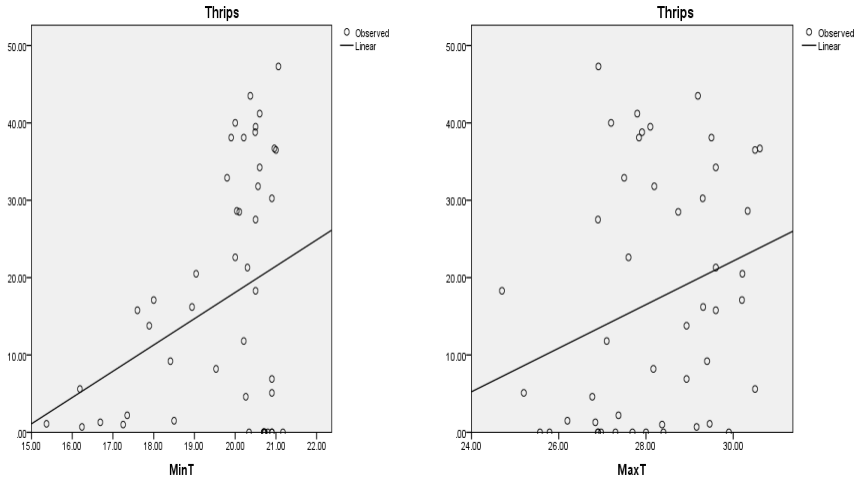

Fig 1: Incidence of thrips in relation to temperature

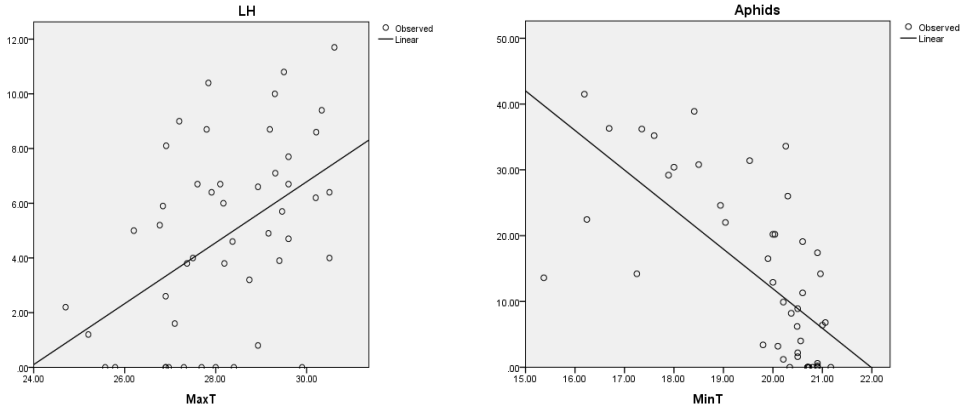

Fig 2: Incidence of Leaf hoppers and aphids in relation to temperature

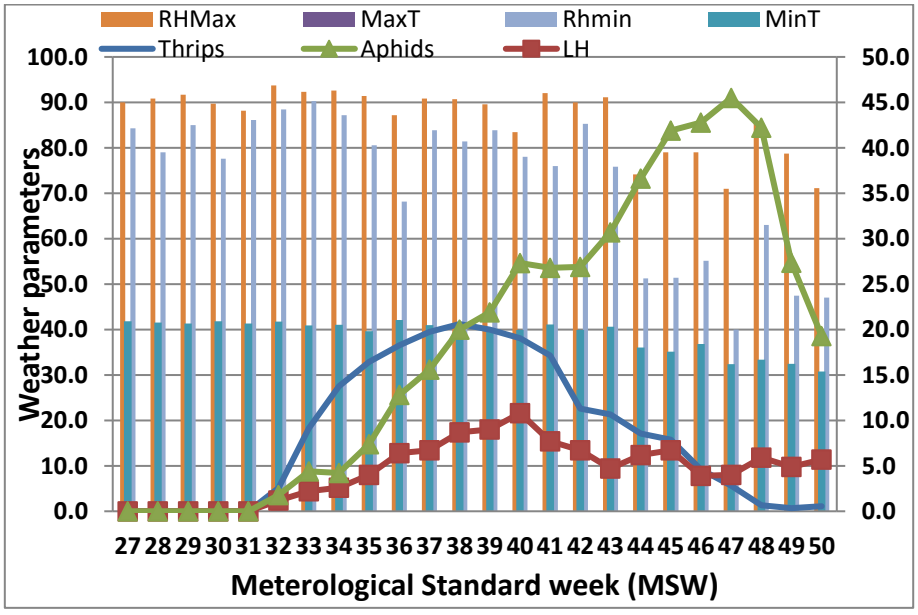

Fig 3: Incidence of sucking pests in Bt cotton in relation to abiotic factors during 2020-21

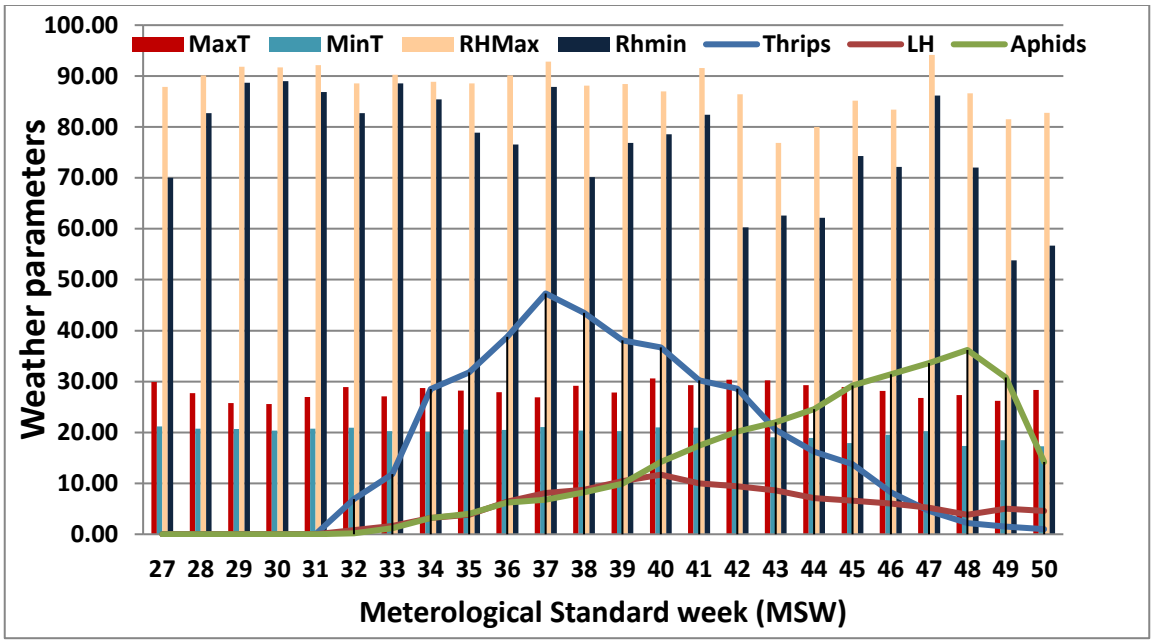

Fig 4: Incidence of sucking pests in Bt cotton in relation to abiotic factors during 2021-22

References:

1. Agarwal, R. A., Gupta, G. P. and Garg, D. O., 1984, Cotton pest management in India. *Res. Publn*., Azadnagar, Delhi, pp. 1-19.
2. Anonymous, 2019, Improved Package of Practices for Higher Yields.UAS, Dharwad.
3. Dhawan AK. Cotton pest scenario in India: Current status of insecticides and future perspectives. Agrolook. 2000;**1**(1):75-85
4. Gosalwad, S. S., Kamble, S. K., Wandnerkar, D. W. and Awaz, B. H. 2009, Population dynamics of major insect pests of cotton and their natural enemies. J. Cotton Res. Dev. 23, 117–125.
5. Mohapatra, L. N., 2008, Population dynamics of sucking pests in *hirsutum* cotton and influence of weather parameters on its incidence in western Orissa. *J. Cotton Res. Dev*., 22 (2): 92-194
6. Phulse V. B. and S. S. Udikeri, 2014, Seasonal incidence of sucking insect pests and predatory arthropods in desi and Bt transgenic cotton. *Karnataka J. Agric. Sci.,* 27 (1): 28-31.
7. Prasad, N. V. V. S. D., Rao, N. H. P. and Mahalakshmi, M. S. (2008): Population dynamics of major sucking pests infesting cotton and their relation to weather parameters. J. Cotton Res. Dev. 22, 85–90.
8. Pralhad J. and Shinde P.B., 2021, Prediction of aphid and jassid in rainfed Bt cotton using regression models based on abiotic factors. Indian Journal of Dryland Agricultural Research and Development. 35(1) : 43-49
9. Satpute, U. S., Patil, V. N., Katole, S. R., Men, V. D. and Thakare, A. V., 1990, Avoidable field losses due to sucking pests and bollworms in cotton. *J. Appl. Zool. Res.*, 1 (2): 67-72.
10. Selvaraj, S., Adiroubane, D. and Ramesh, V. (2011): Population dynamics of important insect pests of bhindi inrelation to weather parameters. Pestology 34, 35–39.
11. Sitaramaraju, S., Parsad, N. V. V. S. D. and Krishnaiah, P. V. (2010): Seasonal incidence of sucking insect pests on Bt cotton in relation to weather parameters. Ann. Pl. Prot. Sci. 18, 49–52.
12. Soujanya, P. L., Prasad, N. V. V. S. D. and Rao, P. A. (2010): Population dynamics of sucking pests and their relation to weather parameters in *Bt*, stacked *Bt* and non *Bt* cotton hybrids. Trends in Biosciences 3, 15–18.
13. Udikeri, S. S., Patil, B. V., Basavanagoud, K., Khadi, B. M., Kulkarni, K. A. and Vamadevaiah, H. M., 2012, Impact of Bt transgenic cotton on population dynamics of aphids and natural enemies. *Indian J. Agri. Sci.,* 82(6): 555-60.

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Management strategy against pink bollworm, *Pectinophora gossypiella* (Saunders) in Bt cotton – What next?

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Abstract

Background: *The health status of cotton production is vital to the Indian economy as it provides >59% of the raw material to the textile industry and contributes to about 4.9% value of agriculture output and >29% of the total textile exports. In 2021-22, cotton production dipped to 315 lakh bales (1 bale=170 kg lint) on account of aberrant rains coupled with crop damage due to pink bollworm and plant diseases. Regular insect resistance monitoring studies indicated that prevalence of Bt cry toxin (BG I and BG II) resistant populations of pink bollworm (PBW) is widespread in the Central and Southern cotton growing zones. In 2021, report on severe crop damage due to pink bollworm came from the north zone states of Punjab and Haryana. The pest has emerged as a serious yield limiting factor in all the cotton growing zones with yield loss estimated at 20-30%. A series of project initiatives/ interventions were launched for monitoring and dissemination of crop-window-based management strategies for the management of cotton pink bollworm. The results are discussed and way forward is suggested.*

Results: *Pest surveys and surveillance is undertaken in all the cotton growing states. In the north Indian cotton growing states, incipient infestation of PBW was first recorded from cotton fields adjoining ginning cum oil extraction mills in Jind, Haryana in 2017-18. In 2019, the incidence of PBW crossed Economic Threshold Level (ETL, >10% green boll damage) at only one out of 2125 field locations. During 2020, this incidence crossed ETL in 69 out of 1622 locations surveyed in the north zone (4.3%). pink bollworm incidence was restricted only to Punjab and Haryana during both these years. However, in 2021-22, field surveys at 763 locations indicated sharp rise in pink bollworm incidence, above ETL in Punjab (25.2%), Haryana and (27.9%). For the first time the incidence was also noticed in Rajasthan to the extent of 11.2%. During 2021 season, random surveys indicated PBW infestation was above ETL in 107 locations (76.4%) out of the 140 locations surveyed. State-wise survey data indicated incidence in green bolls was above ETL at 77.6% locations in Haryana, 90.9% locations in Punjab and 54.5% locations in Rajasthan. Severity of infestation recorded at above ETL locations was in the range of 10-100% green boll damage in Haryana and Punjab and 10-30% in Rajasthan. Similar field surveys using pheromone traps and field sampling indicated widespread incidence of PBW in the Central and southern cotton zones. Insect resistance management (IRM) initiative for dissemination of PBW management strategy*
In 2017-18, outbreak of pink bollworm (PBW) in cotton was reported in the states of Maharashtra, Telangana, Andhra Pradesh, Karnataka, Gujarat and Madhya Pradesh with infestation ranging between 8 to 92% and yield losses ranging between 10-30%. A package of interventions was devised in 2018 and demonstrated at 1050 locations for management of PBW in 21 affected districts across 8 cotton-growing states every year. PBW infestation was 30 to 35% lower in demonstration plots compared to control plots. Results of mass trapping of adult moths of PBW evaluated at 8 to 40 pheromone traps/ ha treatments in field clusters in 6 districts indicated significant reduction in green boll damage due to PBW during October/ November (<130 days crop age).

Conclusion: *Pest monitoring, crop-window and ETL based management interventions as part of IRM and evaluation of mass trapping for PBW on BG II cotton were effective in minimizing crop damage in early pickings. Two mating disruption formulations for management of PBW in cotton have received approval from the Central Insecticide Board Registration Committee (CIBRC) for commercial use. This strategy is perhaps the immediate option for area-wide management of PBW as new gene technologies effective against PBW await development and short season cotton that can escape PBW infestation attains wider adoption. One constraint for adoption of mating disruption technique is cost of application for season long effect (3-4 applications). Need for community action in technology adoption across contiguous field clusters is a serious challenge. Conversely its adoption in individual small holdings may not be as effective. Concerted and integrated effort by all stakeholders in cotton value chain including State Departments of Agriculture, cotton processing industry, textile industry, researchers and farmers is warranted for sustainable and profitable cultivation of Bt-Cotton in India.*

Keywords: *Bt Cotton, pest management, pink bollworm, insect resistance management, pest monitoring*

Background

Cotton (*Gossypium hirsutum* L.) is the most important commercial crop in India cultivated in an area of >12.0 million ha with a production of 5.3 million tonnes of lint (Cotton Corporation of India 2021). Cotton is cultivated in three major agro-ecological zones of the country, *viz.,* north, central and southern and domestic production provides >59% of the raw material to the textile industry.

Cotton cultivation is the prime source of agricultural income for farmers in large tracts of rainfed region in the central and southern zones. Insect pests are the key constraints limiting yield and profitability of small holder farmers. In India, transgenic Bt cotton genotypes were approved for commercial cultivation in 2002. Rapid and widespread adoption of GM cotton led to a spectacular growth in cotton area by about 4 million ha and yield increase from 300 to 560 kg lint/ha in a short time span by 2013. However, the first field level failure in resistance of Bt cotton came in with the survival reports of pink bollworm (PBW) (*Pectinophora gossypiella* Saunders) on BG-II cotton genotypes during 2009 season. By 2015, wide spread incidence of PBW attack on BG-II cotton has been noticed in the Central and southern cotton growing states of Gujarat, Andhra Pradesh, Maharashtra and parts of Karnataka states (Kranthi 2015). In the North Zone, PBW was noticed during the 2018 season from Jind district of Haryana and two other field locations in Punjab; all from fields adjoining to cotton ginning-cum-oil extraction mills (Prasad and Kumar 2022). Naik et al. (2018) recorded resistance ratios of 704-2060 and 1306-9366 folds against *Cry 1Ac* and *Cry 2Ab* in different PBW populations of India. Hence, the reason for outbreaks or control failure is mainly that some alleles confer resistance against the insecticides and *Bt* toxins (Naik et al. 2017). PBW has emerged as a serious yield limiting factor in all the cotton growing zones (Fand et al. 2019).

Pink bollworm immature stages in its life history are difficult to detect in the field. Just hatched neonate larvae of PBW spend hardly few hours on plant/ bolls in the open before entering into fruiting structures, particularly the bolls where it targets to feed on raw seeds. Unlike other insect pests it is not easily amenable for insecticidal control. A holistic approach involving different tactics of insect resistance management has been felt essential to manage PBW successfully. The present study documents the results of project initiatives/ interventions that were launched for monitoring and dissemination of crop-window-based management strategies for the management of PBW infesting *Bt* cotton in the country.

Materials and Methods

Field surveys

Field surveys for assessing PBW infestation on cotton was regularly carried out in the cotton growing zones of India at weekly intervals from mid-August to first week of November. During the 2021 season, random surveys were conducted to assess pink bollworm infestation on BG II cotton hybrids at 94 locations in four districts of Haryana, 24 locations in three districts of Punjab and 22 locations in two districts of Rajasthan between 20 August to 20 October. Field surveys on PBW boll infestation were also conducted from farmers' field locations in central and southern cotton growing states at periodical intervals under the 'Insect Resistance Management (IRM)' initiative for dissemination of PBW management strategies to farmers in 21 districts across cotton growing states (Maharashtra, Telangana, Andhra Pradesh, Karnataka, Gujarat, Madhya Pradesh and Tamil Nadu). Field demonstrations were conducted in a total of 1050 acres of cotton area and mass awareness was created through outreach activities. The package of crop stage window-based interventions comprised of timely sowing, installation of pheromone traps for monitoring, botanical spray with neem formulation, release of egg parasitoid, pesticide sprays based on economic threshold level for PBW based on pheromone catches (8 moths trapped/ night consecutively for 3 days), flower and green boll damage (>10%) and timely termination of crop.

Mass trapping for management of PBW

Field experiments were conducted to evaluate the performance of mass trapping of PBW adults in reducing crop damage in rainfed cotton. Field trials were laid out at three locations (Nagpur, Chandrapur and Amravati districts of Maharashtra, India) during June to December 2021 rainy season crop. Bt cotton variety was planted at Nagpur and hybrids at the other two locations in farmers' fields by dibbling method in the second fortnight of June. Imposed seven mass trapping treatments comprising T1: Control (No traps), T2: 8 traps/acre, T3: 20 traps/acre, T4: 25 traps/acre, T5: 30 traps/acre, T6: 35 traps/acre and T7: 40 traps/acre was evaluated in four replications against pink bollworm. Each replication comprised of one acre with traps installed at 10 m apart from each other at 30 cm above canopy using bamboo stakes/wooden sticks. The observations were recorded

from 70-220 days after sowing at 15 days interval. Number of moth catches per trap and number of infested green bolls from 25 randomly selected bolls per plot was recorded at each observation.

Results

Field surveys

Incidence of PBW in the North zone states

Surveys for pink bollworm infestation on cotton was regularly carried out in the cotton growing regions of north India i.e. Punjab, Haryana and Rajasthan at weekly intervals from mid-August to first week of November (Table 2). In 2019, the incidence of pink bollworm crossed the Economic Threshold Level (ETL >10% green boll damage) at only one out of 2125 field locations. During 2020, this incidence crossed ETL in 69 out of 1622 locations surveyed in the North Zone (4.3%). PBW incidence was restricted only to Punjab and Haryana during both these years. However, in 2021-22, field surveys at 763 locations indicated a sharp rise in pink bollworm incidence, above ETL in Punjab (25.2%) and Haryana (27.9%). For the first time the incidence was also noticed in Rajasthan to the extent of 11.2%. During the 2021 season, random survey was conducted to assess pink bollworm infestation on BG II cotton hybrids at 94 locations in four districts of Haryana, 24 locations in three districts of Punjab and 22 locations in two districts of Rajasthan between 20 August to 20 October (Table 2). The infestation was above ETL in 107 locations (76.4%) out of the 140 locations surveyed. State-wise survey data indicated incidence in green bolls was above ETL at 77.6% locations in Haryana, 90.9% locations in Punjab and 54.5% locations in Rajasthan. The severity of infestation recorded at above ETL locations was in the range of 10-100% green boll damage in Haryana and Punjab and 10-30% in Rajasthan (Table 3).

Table 2. Extent of spread of pink bollworm infestation in recent years in the north zone

States	# survey locations and number above ETL (>10% green boll damage)					
	2019-20		2020-21		2021-22	
	# locations	> ETL	# locations	> ETL	# locations	> ETL
Punjab	1474	1	1082	20	360	91
Haryana	388	0	288	49	136	38
Rajasthan	263	0	252	0	267	30
Total	2125	1	1622	69	763	159

Table 3. Random surveys and severity of green boll damage during 2021-22

States/ District	# Villages surveyed	# locations reporting PBW green boll infestation (%) in the range of				
		0-10%	10-30%	31-50%	51-80%	81-100%
Haryana						
Sirsa	36	10	12	8	5	1
Fatehabad	6	2	4	0	0	0
Jind	20	8	7	4	1	0
Hisar	32	1	23	8	0	0
Total	94	21	46	20	6	1
Punjab						
Muktsar	2	2	0	0	0	0
Bathinda	12	0	0	3	2	7
Mansa	10	0	0	2	5	3
Total	24	2	-	5	7	10
Rajasthan						
Sri Ganganagar	10	6	4	0	0	0
Hanumangarh	12	4	5	3	0	0
Total	22	10	9	3	0	0
Grand total	140	33	55	28	13	11

Incidence of PBW in Central and Southern cotton zones

Green boll infestation of PBW over the season ranged between 40-83% in 2020-21 and 19-90% in 2021-22 in the central zone states; 22-85% in 2020-21 season and 14-68% in 2021-22 season in the south zone states (Table 4). Infestation in demonstration plots could be brought down by 35% in 2020-21 and 33% in 2021-22 in the demonstration plots compared to control plots without the package of interventions.

Table 4. Annual report of pink bollworm infestation range in central and southern cotton growing states

State	Peak infestation (%)across farmers' fields (based on sampling of 20 green bolls per acre at each observation)	
	2020-21	2021-22
Central Zone states		
Maharashtra	83	90
Gujarat	40	19
Madhya Pradesh	68	52
Southern zone states		
Telangana	28	16
Andhra Pradesh	85	49
Karnataka	40	68
Tamil Nadu	22	14

Effect of mass trapping of PBW on green boll damage

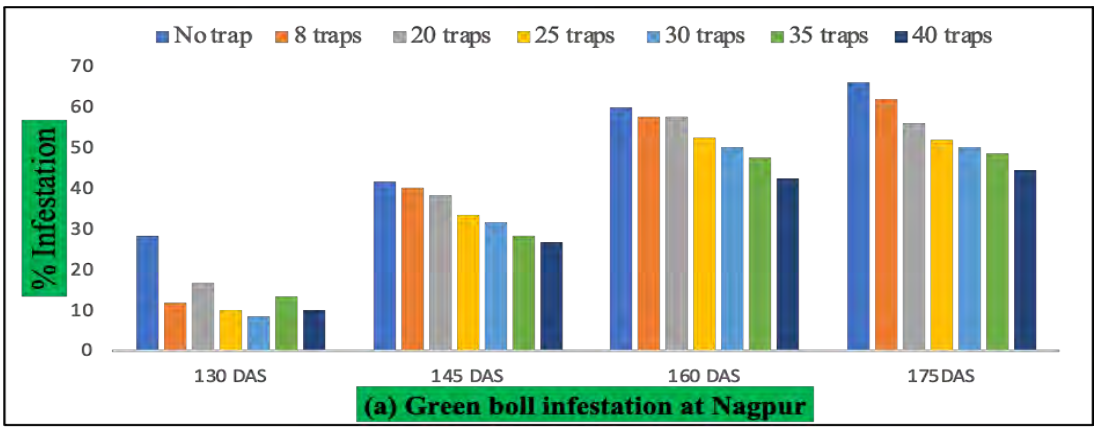
Peak infestation of green boll damage was recorded on 175 days old crop at Nagpur (66%), Chandrapur (42%) and Amravati (30%) in 'no trap' control plots. Green boll infestation was progressively lower at higher trap densities across crop age with few exceptions. At all the three locations, significantly lower (7-20%) green boll damage was recorded compared to no trap control at trap densities between 30-35/ 0.4 ha at 130 days of crop age. In extended crop at 175 days crop age, green boll infestation was lower by 14-22% at Nagpur; 23-26% lower at Chandrapur and 8-12% lower at Amravati At higher trap densities between 25 to 40 traps/0.4 ha compared to 'no trap' control (Fig. 1).

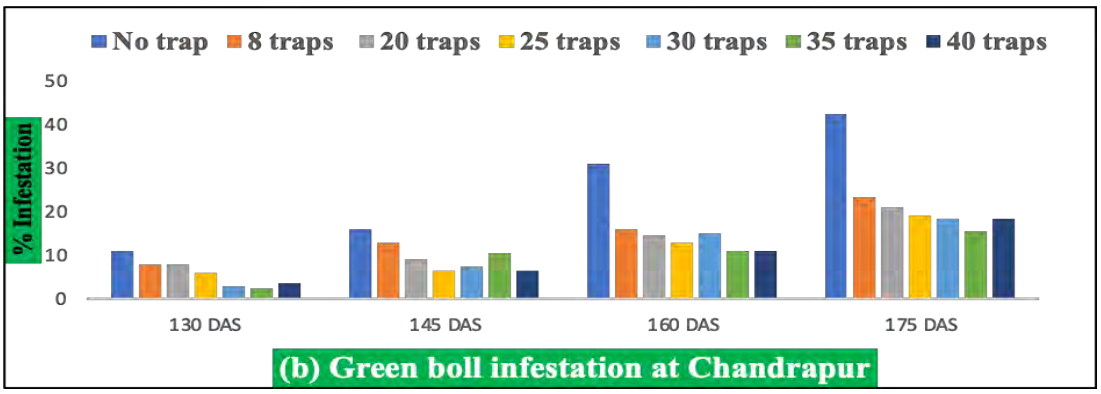
Discussion

The efficacy of Bt transgenic traits in cotton against pink bollworm is not appearing to be sustainable any more in India. First reports of heavy loss due to PBW infestation in Bt cotton in India came during 2013 and 2014 (Kranthi 2015). The reported resistance in PBW to Cry 1Ac toxins (Ojha 2014; Dhrua and Gujar 2011) or *Cry 1Ac+ Cry 2Ab* (Naik et al. 2018) reiterates the use of other management options in addition to GM technology. In this study, field surveys in Bt cotton fields across the cotton growing states indicates serious and growing losses due to PBW in Bt cotton. Currently reliance on chemical control by farmers appears to be less effective against PBW due to its cryptic nature and concealed feeding habit. Integrated pest management (IPM) approaches are more optimal for its management (Henneberry and Naranjo 1998). In the past several efforts have been made to manage PBW using different pheromone-based tools/technologies viz., mating disruption (Lykouressis et al. 2005) and mass trapping (Agenor and Mohamed 1996). In the present study we have selected a pheromone trap and lure model for its high trapping efficacy. Increase in numbers of traps per acre (30 to 40 traps/acre) decreased the green boll damage at various cotton picking stages. Installation of 30 to 40 traps per acre for mass trapping two weeks before first flowering reduced the chance of establishment of first-generation larvae in the flowers. Increasing the number of pheromones traps in the field captures males of the newly emerged moths to reduce the number of adults for mating and thus suppress the population and delay the build-up of the subsequent generation using mass trapping technique. Pink bollworm generally requires multiple generations for population build up in the field. Mass trapping appears to have the potential to reduce this build up to alarming levels. The infestation of the pink bollworm was reduced on an average by 10-15 per cent in fields where mass trapping was employed as compared to the control fields during the experimentation. Effectiveness of mass trapping technique is best achieved by its adoption in larger contiguous clusters of farms.

Conclusions – what next

Pest monitoring, crop-window and ETL based management interventions as part of insect resistance management and evaluation of mass trapping for PBW on BG II cotton were effective in minimizing crop damage. Mass trapping technique attempted in this study is to be compared with two mating disruption formulations for management of PBW in cotton that have received approval from the Central Insecticide Board Registration Committee (CIBRC) for commercial use. One constraint for adoption of mass trapping or mating disruption techniques is cost of application for season long effect. Deployment requirement across contiguous field clusters in view of the smaller land holdings is another challenge. Various options for area-wide management of PBW are to be made available to farmers as new gene technologies effective against PBW await development and short season cotton that can escape PBW infestation attains wider adoption.





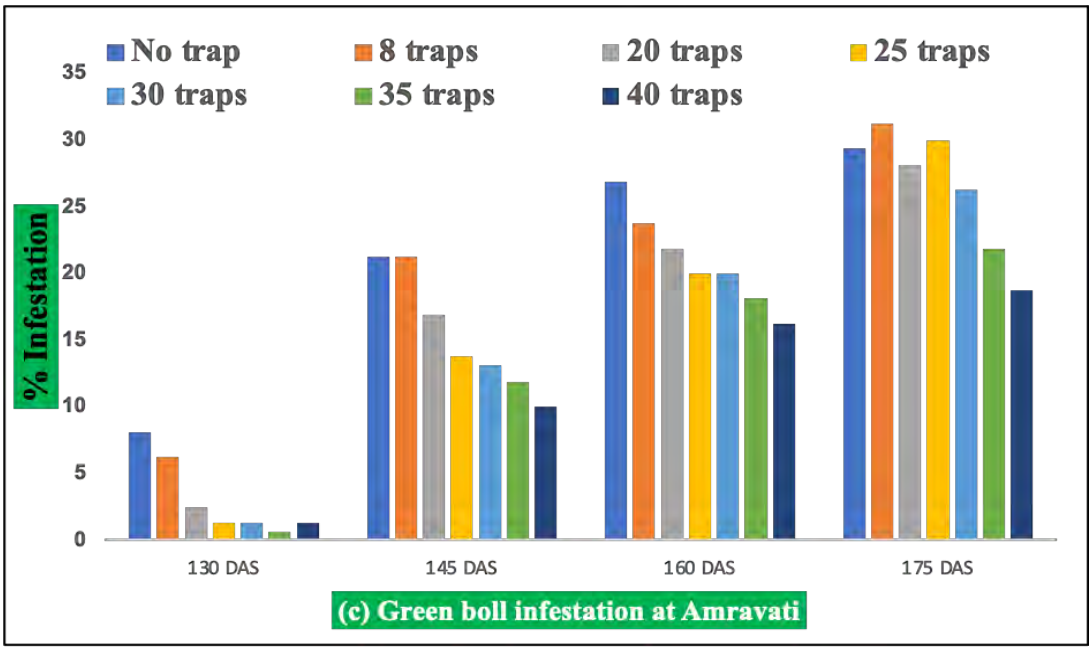


Fig. 1. Green boll infestation due to PBW recorded in mass trapping experiments at different trap densities at three locations (a) Nagpur (b) Chandrapur (c) Amravati in Maharashtra, India

References

1. Agenor M, Mohamed H (1996) Evidence that mass trapping suppresses pink bollworm populations in cotton field. Entomologia Exp. Et Appl. 81(3), 315-323.
2. Anonymous (2019) Pocketbook of agriculture statistics. Govt. of India, Ministry of Agriculture & farmers welfare, Dept. of Agriculture, cooperation & farmers welfare, Directorate of Economics & Statisitics, New Delhi, pp 22-34.
3. Carrière Y, Crickmore N, Tabashnik BE (2015) Optimizing pyramided transgenic Bt crops for sustainable pest management. Nat. Biotechnol 33, 161.
4. Cotton Corporation of India (2021) https://cotcorp.org.in.
5. Dhaliwal GS, Arora R Dhawan AK (2004) Crop losses due to insect pests in Indian agriculture: an update. Indian J. Ecol. 31, 1-7.
6. Dhrua S, Gujar GT (2011) Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manage Sci. 67(2), 898–903.
7. Fand BB, Nagrare V, Gawande S, Nagrale D, Naikwadi B, Deshmukh V, Gokte-Narkhedkar N, Waghmare V (2019) Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) on Bt cotton in Central India: A new threat and concerns for cotton production. Phytoparasitica. 47, 1–13.
8. Henneberry TJ, Naranjo SE (1998) Integrated management approaches for pink bollworm in the southwestern United States. Inte Pest Manage Revie. 3, 31-52.
9. Kranthi KR (2015) Pink bollworm strikes Bt cotton. Cotton Statistics and News. 35, 1-5.
10. Lykouressis D, Perdikis D, Samartzis D, Fantinou A, Toutouzas A (2005) Management of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by mating disruption in cotton fields. Crop Prot. 24(2), 177-183.
11. Naik VC, Kumbhare S, Kranthi S, Satija U, Kranthi KR (2018) Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (Bt) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. Pest Manag. Sci. 74, 2544-2554.
12. Naik VCB, Kranthi S, Kumbhare S, Nagrare VS (2017) A manual on pink bollworm resistance monitoring and management. ICAR-Central Institute for Cotton Research, Post bag, (2).
13. Ojha AA, Sowjanya SK, Sachdev B, Rashmi MA, Ravi KC, Suresh PJ, Mohan KS, Bhatnagar RK (2014) Analysis of resistance to Cry1Ac in field collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera:Gelechiidae). GM Crops & Food. 5(4), 280-286.
14. Patil SB (2003) Studies on the management of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepideptera: Gelechiidae). Doctoral Dissertation. University of Agricultural Sciences, Dharwad, India.
15. Prasad YG (2022) Cotton institute successfully tests pheromone traps for pink bollworm. Lokmat Times. p4.

16. Prasad YG, Kumar R (2022) Pink bollworm in north cotton growing zone of India. Cotton Statistics and News. 3, 1-5.

17. Rameash K (2021) Pheromone technology for check mating pink bollworm. Cotton Statistics and News. 21.

18. Ranjith MT, Prabhuraj A, Srinivasa YB (2010) Survival and reproduction of natural populations of *Helicoverpa armigera* on Bt-cotton hybrids in Raichur, India. Curr. Sci. 99, 1602–1606.

Controlled Release Emission Mating Interruption Technique (CREMIT): A novel and viable approach for area wide management of pink bollworm, *Pectinophora gossypiella* (Saunders) in *Bt* cotton ecosystem

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Abstract

Back ground : *A novel mating disruption technique through the wax-based pheromone formulation CREMIT-PBW for area-wide management of pink bollworm, Pectinophora gossypiella (Saunders) was exploited in Bt cotton ecosystem.*

Results: *The efficacy studied of CREMIT-PBW was carried out over an area of 154 acres (2017-18) and 206 acres (2018-19) in Raichur district of Karnataka, India. Dose optimization study comprising of 500, 750 and 1250 g/acre were evaluated during the first season. In the subsequent season, the optimum dosage of 500g per acre was split and applied at 40, 70, 100 and 130 days after sowing.The outcome of the investigation is quite promising and recorded minimum rosette flower (11.76%) and green boll damage (10.20%). During the second season, the rosette flowers and green boll damage was further reduced to 4.70 % and 4.52 %, respectively. Similarly, locule damage of 8.65% and8.18% was recorded during first season, and second seasons, respectively. Ultimately, the higher yield of 33.59 q/ha was obtained from CREMIT treated plots in contrast to farmers' practice who even after 5-6 rounds of spray have got 22.33 q/ha of yield.CREMIT being a mating disruptant has direct impact on moth trap catches, wherein, the number of pink bollworm male moths caught per trap per week was minimum at higher doses viz., 1250 and 750g per acre. At optimum dose the moth trap catches were 9.29 in the first season and an average of 18.92 male moths caught per trap per week in the second season. On the contrary, the average numbers of male moths caught were highest in conventional practice (240.38/trap/week) and (155.03/trap/week) indicating that incompetence of insecticides to deliver expected level of control in comparison with CREMIT-PBW.*

Conclusion: *Impact of CREMIT-PBW is prominent at all the applied dosages which is clearly depicted in terms of lower bollworm incidence and higher cotton yields. The highest yield of 43.33 q/ha was recorded from plots treated with the highest application rate of CREMIT-PBW i.e., 1,250 g/ha. While, the cotton yields obtained from the fields treated with other two dosages (750 g/ha and 500 g/ha) of CREMIT-PBW, were almost similar, recording 42.50 q/ha and 40.50 q/ha, respectively in the first season. In contrast, the cotton yields obtained from the conventional farmers' practice plots were 24.68 q per ha and 20.00 q per ha from two seasons respectively. This trend clearly depicts the effectiveness of CREMIT-PBW in managing the pest, reflecting in terms of increased yield as compared to the conventional farmer's practice. The gain in crop yield ranged from 15.80 to 18.63 q per ha in the first season and additional gain of 6 to 7 quintals per hectare in the next season which is outstanding result making the mating disruption as breakthrough tool in the field of pest management.*

Background

Cotton (*Gossypium* sp.) is one of the most important commercial fiber crops cultivated in the country and is often referred as 'king of fibers'. In IndiaCotton is cultivated over 12.0 to 13.5 million hectares that account for about 37% of world area under cotton production. Across the country cotton spans over123.50 lakh hectares with production of 340.62 lakh bales (one bale = 170kg) (CCI, 2021).The yield potentiality of the cotton is majorly hindered by the pests and diseases making it unremunerative for the farmers. Among the insect pests, the damage due to sucking pest and bollworm complex comprising of *Helicoverpa armigera* (Hubner), *Spodoptera litura* (Fabricius), *Erias insulana* (Boisduval), *E. vitelli* (Fabricius) and *Pectinophora gossypiella* (Saunders) causes yield loss up to 50-60% (Dhaliwal *et al*., 2004). During 2002, transgenic cotton expressing crystalliferous toxins against lepidopteran pest was introduced and approved for cultivation in India. Soon after the

introduction the area under cultivation of transgenic cotton shoot up from a mere 72,000 acres (2002-03) to 30,00,000 ha (2006-07) (Manickam *et al*., 2008). Among the bollworm complex pink bollworm (PBW) is the pest of major concern due its potentiality of causing significantly higher yield losses in *Bt* cotton ranging from 20-90% (Patil, 2003 and Fand *et al*., 2019).But first case of resistance against PBW was noted from Gujarat in 2008 against Cry 1Ac toxin (Dhurua and Gujar, 2011). Subsequently Naik *et al*. (2018) has reported that PBW has evolved resistance against BG-II during 2014. Hence, the adoption of transgenic events for the management of the PBW has become unreliable.Due to the cryptic feeding behaviour of the pest made it less prone for the insecticidal spray making it difficult for the farmers to manage the pest through chemical means (Singh *et al*., 1988). This has made farmers desperate to go for multiple spraying of the insecticides at much higher doses to bring down the pest population. This has ultimately resulted in resistance against insecticide molecules and residue of persistent insecticides in the ecosystem. Due to this indiscriminate use of pesticides and non-compliance of refugia has led to the major resistance problem. Hence, Significant yield losses and control failure was evident noticed even after taking extensive measure of management.

The major tools for the management of pest like the transgenics and insecticides are ruled out making the situation desperate for the farmers. Present scenario of pink bollworm management has depicted that none of the major tools for the management are long lasting and fool proof. Even though novel chemicals are released into market regularly and simultaneously pest is developing resistance leading to the pesticide treadmill. The present Integrated Pest Management protocols formulated for the pest management still requires refinements for the significant management of pest.This has paved the way for a novel, eco-friendly approach or tool that addresses the above-mentioned problems. One such green and long-lasting tool is the utilization of mating disruption technology. Mating disruption technology is pest specific, safe to natural enemies and humans, and the problem of development of resistance and residue problems are addressed. At current several mating disruption technologies are available in the market like PB-Knot, CREMIT- PBW and Pheromone traps installed at higher number per acre. Deploying this technology over large area thorough the community approach has huge scope in near future for managing the pest. These aspects make the mating disruption an equal contender with other management tools and effects will be much more magnified if adopted over large area. Hence, a large-scale trial with CREMIT-PBW was executed in farmer's fields of Kurdi cross, Chikallaparvi, Hussain nagar camp, Hokrani cross, Matamari, Marchathal and Satyanarayan camp of Manvi and Raichur taluka of Raichur district to determine and confirm the optimum dosage of the formulation. The cost economics was also elucidated against pink bollworm management in cotton ecosystems, in comparison with farmers' practice which is solely an insecticide based.

Materials and Methods

Mating disruption is a proactive way to protect crops by preventing pests from reproducing in the first place. By simulating the natural pheromone emission of female insects, mating disruption technologies cause males to become confused and incapable of locating a female to mate with. Controlled Release Emission Mating Interruption Technology (CREMIT) is a wax-based formulation having sustained-release pheromone which works on the principle of mating disruption. These formulations will result in reduced rates of mating success which will lead tocollapse of insect pest populations, avoiding the application of toxic and potentially ecologically hazardous pesticides. Point sources of pheromone-based CREMIT formulations are applied to the crop. Each CREMIT dollop emits precise amounts of sex pheromone required to shut down mating. Males are diverted away from females due to the powerful allure of the pheromone being emitted by each CREMIT dollop. Unlike females, CREMIT dollops emit high doses of pheromone continuously.

2.1 Field Experiment

Experienced cotton growing farmers were selected and explained about the mating disruption technology, and regarding when and how to use and not use the insecticides for the pink bollworm management in thetreated plots. Precautions were taken to maintain a minimum isolation distance of 2 km between each dosage of CREMIT-PBWduring the first season trail. The popular *Bt* cotton hybrid Jadoo was used by most of the farmers and sown during the last week of June 2017 and 2018.

Mating suppression delivered by CREMIT-PBWwas preliminarily demonstrated in an area of 154 acres with three different dosages *viz*., 500, 750, and 1,250 g per acre. Each of these doses was split and applied four times (total quantity was split in to four application) starting from 35–40, 65–70, 95–100 and 125–130 days after sowing. For the dose optimization study, the farmers field of Kurdi cross, Hokrani cross and Hussain nagar villages of Manvi taluka almost 30 kms from Raichur, were selectedduring *kharif* 2017–18. Subsequently, the effective dosage of 500 g per acre was

demonstrated out in a large area of 216 acres in the farmers field of Kurdi cross, Hokrani cross, Chikallaparvi, Matamari, Marchathal and Satyanarayan camp of Manvi taluka and Raichur taluka of Raichur district, during *kharif* 2018-19.

To compare the effectiveness of mating disruption (CREMIT-PBW), the efficacy of insecticides on PBW was also recorded. In conventional practice,farmers use 4–5 rounds of insecticide chemicals *viz*., profenophos 50% EC, lambda-cyhalothrin 5% EC, chlorantraniliprole 18.5% SC, emamectin benzoate 5% SG, and lambda-cyhalothrin 5% EC + chlorantraniliprole 10% SC.

2.2 Application of SPLAT

In general, the incidence of PBW begins from 65–70 days after sowing, but the application of CREMIT-PBW was made intentionally from 35–40 days. This pre-emptive application was done to ensure that by the time PBW began to appear in the field, the field would already be effectively permeated by the pheromone released by CREMIT-PBW, maximizing the effect of the mating disruption strategy on the pest population. Farmers were instructed in prior not to apply any insecticides against pink bollworm in CREMIT treated blocks. The wax-based formulation is applied at the primary branch axil, below 3 to 4 inches from the terminal portion of the plant for first application and 6 inches from the terminal part

of the plant for subsequent applications.

2.3 Observations recorded

From each section, ten plants were selected randomly to document observations on pink bollworm which includes the rosette flowers, green boll damage and locule damage. The observationswere recorded at weekly intervals starting from 45 days of sowing. Similarly, adult male moths captured in pheromone traps (installed previously at the rate of two per acre) per week were also recorded. Subsequently, observation was recorded at weekly intervals till the complete harvest of the crop.

2.4 Economic analysis of CREMIT-PBW

The standard cost of cotton crop cultivation was same for all the treatments. It was obtained from the recommended agricultural practice of the University of Agricultural Sciences, Raichur, India. The total cost of cultivation was calculated by adding the common cost of cultivation and treatment cost. The gross return per treatment was computed by multiplying the total yield per hectare by the prevailing market price, while net returns for each treatment were realized by subtracting total cost from grass returns. Each treatment's benefit-cost ratio was derived by dividing gross returns from net returns.

2.5 Statistical Analysis

Data generated on impact of PBW on percent rosette flowers, percent green boll damage, and percent locule damage were transformed to arc sin values prior to statistical analysis. Data on number of moth trap catches per trap were converted to square root values ($\sqrt{x+1}$) prior to statistical analysis. All data recorded, including kapas yield, was subjected to statistical analysis following the Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results

3.1 Efficacy of CREMIT-PBW at various dosages

The outcome ofmating disruption technology *i.e*., CREMIT-PBW is promising at all the applied dosages (500, 750, and 1,250 g per acre).Similarly, the effective dosage of 500g per acre applied during second season in four splits has revealedgood results.The overall incidence of pink bollworm in terms of rosette flowers and green boll damage was minimum (11.24 and 9.70%) in 1,250g per acre CREMIT applied treatment, respectively. This did not differ significantly with its lower dosages of 750 g (11.80 and 10.27%) and 500g (11.76 and 10.20%) in the first season and 4.70 % of rosette flowers and 4.52 % green boll damage observed from the application of optimum dosage of 500 g/acre in the second season. In contrast to the farmers practice, all the dosages tested in both seasons differed significantly, wherein rosette flowers and green boll damage recorded was 20.96% and 37.93% in the first season and 13.67 % and 23.97% in the second season respectively. Similarly, per cent locule damage was found to be minimum (7.86%) in 750 g per acre CREMIT applied treatments, followed by 8.05% (1,250 g/acre) and 8.65% (500 g/acre) in first season trail and in the

second season it is 8.18 % (500 g/acre) showing a significant difference with the conventional farmers' practice (34.34%) and (37.63 %) even after four to five rounds of insecticides spray exclusively for pink bollworm management (Fig. 1a). CREMIT being a mating disruptant has impacted on moth trap catches, wherein, the number of pink bollworm male moths caught per trap per week was minimum *viz.,* 7.59, 7.20 and 9.29 in 1250, 750 and 500 g per acre in the first season and an average of 18.92 male moths caught per trap per week in the second season. This has avoided the availability of male for mating with wild females. On the contrary, the average numbers of male moths caught were highest in farmers' practice (240.38/trap/week) and (155.03/trap/week) indicating that the insecticide being used in these fields failed to deliver equivalent control to CREMIT-PBW (Table 1).

3.2 Impact of CREMIT-PBW on Yield of cotton

CREMIT-PBW applied at three dosages from beginning of cropping season significantly reduced the pest population by the mechanism of mating disruption, which in this study resulted in lower bollworm incidence and higher cotton yields. The cotton yield realized from the fields treated with CREMIT-PBW ranged from 40.50 to 43.33 quintals per ha. The highest yield of 43.33 q/ha was recorded in plots treated with the highest application rate of CREMIT-PBW (1,250 g/ha). While, the cotton yields obtained from the fields treated with other two lower dosages (750 g/ha and 500 g/ha) of CREMIT-PBW, were on par with the highest dose as they recorded 42.50 q/ha and 40.50 q/ha, respectively in the first season and higher-level yields of 26.67 quintals per hectare obtained in the second season. In comparison, the cotton yields realized in plots under conventional farmers' practice were 24.68 q per ha and 20.00 q per ha from two seasons respectively. In total, CREMIT-PBW applications led to gain in crop yield ranging from 15.80 to 18.63 q per ha in the first season and additional gain in yield of 6 to 7 quintals per hectare in the next season (Table 1).This study is first of its kind demonstrating against PBW population reduction in *Bt* cotton ecosystem. Hence, the literature available on other crop pests is being utilized for comparison.

3.3 Population of natural enemies in the CREMIT treated plots

In general, the population of Coccinellids and *Chrysoperla* spp. were found throughout the cropping period, but their population were found maximum during the month of November, so in CREMIT applied treatments irrespective of dosages, wherein, the population ranged from 6.20 to 9.80 per plant with *Chrysoperla* and 3.10 to 4.40 per plant in Coccinellids across different dosages and they found on par with each other. On the contrary, the lowest population of natural enemies *viz*., *Chrysoperla* (2.0-3.0 & 1.50 of eggs and grubs/ plant) and Coccinellids (1.0-2.10 & 1.20-2.0 of grubs and adults) was recorded in conventional farmers' practices (Table 2).

3.4 Cost economics of CREMIT-PBW

CREMIT-PBW tested at three dosages (500, 750 and 1,250 g/acre) did not show any significant difference in PBW menace among their dosages in the first season. The lowest dosage of 500 g per acre applied in four splits was found to be sufficient for mating disruption of pink bollworm. Hence, the net returns obtained from CREMIT-PBW treated at 500 g per acre was Rs. 136,000 per ha, with highest benefit: cost ratio (B:C) of 3.04, followed by Rs. 141,500 in 750 g per acre treatment (B:C ratio of 3.0), and Rs. 136650 in 1,250 g per acre treatment (B:C ratio of 2.70) obtained from first season and the net returns of Rs. 66685 per hectare with highest benefit: cost ratio (B:C) of 1.84 observed in second season trail (Table 3 & Fig.1b).

Discussion

The results of present study are in accordance with a study conducted in Florida (USA) reported by Lapointe and Stelinski (2011), where a field trial was conducted in Florida demonstrating effective disruption of more than 90 % of male moth catch in traps baited with pheromone lures characterized by high potency following each of four applications of 250 or 500 g per ha of CREMIT-CLM, containing 0.15 % (*Z*, *Z, E*)-7,11,13-hexadecatrienal. Similarly, the obtained results of present investigations are comparable with the Stelinski *et al.* (2009) who reported a season-long mating disruption trial of citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) employing a newly developed, emulsified wax dispenser of pheromone, CREMIT-CLM. A formulation of CREMIT-CLM containing a 3:1 blend of (*Z*,*Z*,*E*)-7,11,13hexadecatrienal : (*Z*,*Z*)-7,11-hexadecadienal, at a 0.2% loading rate of active ingredient by weight, was deployed twice over the course of the season (24 weeks total) at a rate of 490 g per ha. This treatment achieved season-long disruption of male moth catch in pheromone traps, as well as reduced leaf infestation. The CREMIT formulation evaluated herein appears to be an effective release device for (*Z*,*Z*,*E*)-7,11,13-

hexadecatrienal, given that approximately 100 days of steady release occurred following an initial brief (7 days) burst of higher release.

Findings are also in line with the earlier studies by Ksenia *et al.* (2010), evaluating CREMIT-GM as a mating disruption technology against the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). CREMIT-GM is a sprayable formulation designed for the controlled release of the gypsy moth sex pheromone, disparlure [(2R,3S)-2-decyl-3-(5-methylhexyl) oxirane], to disrupt gypsy moth mating. The study was conducted in 2006, 2007, and 2008 in forested areas in Virginia, USA. Mating success of gypsy moth females was reduced by more than 90%, and male moth catches in pheromone baited traps were reduced by more than 90 %, in plots treated with CREMIT-GM at dosages ranging from 15 to 75 g of active ingredient (a.i) per ha. Dosage response tests conducted in the final year of the studyindicated that CREMIT-GM applied 7.5 g a.i per ha was as effective as against a dosage of 15 g a.i per ha.

Patrick *et al.* (2012) tested the mating disruption formulation CREMIT Grafo+Bona (SG+B) against two pests of apples, *Bonagota salubricola* and *Grapholita molesta* (Lepidoptera: Tortricidae), compared to standard insecticides used for management in the Integrated Apple Production (IAP) system. The formulation was applied at a rate of 1 kg/ha (300point sources per ha) in experimental units 7 ha in size. In the CREMIT-treated experimental units, a significant reduction was observed in the number of *B. salubricola* and *G. molesta* males caught in Delta traps, compared to the experimental unit treated via the IAP system. Damage by *B. salubricola* at harvest ranged from 1.63 to 4.75%, while damage by *G. molesta* was near zero. Mating disruption using SG+B was sufficient to control *B. salubricola* and *G. molesta* to an equivalent degree as that achieved through the use of IAP procedures. Application of CREMIT-Grafo + Bona also reduced the number of insecticide applications made to control the target pests to an extent of 43%.

The results are also comparable with the investigations carried by Bheemanna *et al.* (1998), evaluating the efficacy of Sirene, disruption formulation containing the PBW sex pheromone, as a component in IPM strategy in cotton. This study was conducted from 1995–1997 in farmers' fields of Kasbe camp village Raichur, Karnataka. Sirene was applied on fully opened terminal leaves of cotton plants using a fabricated paintball gun developed specifically for this purpose. Cotton plots treated with Sirene recorded minimum moth catches (3.0 male moths/trap/night) and maximum cotton yield (19.27 q/ha), compared to untreated plots, where more numbers of moths were captured and lower yields were reported.

Conclusion

Mating disruption through wax-based pheromone formulation CREMIT-PBW has fulfilled its purpose by ceasingthe mating process of pink bollworm. Males of PBWare diverted away from females due to the powerful scent of the pheromone being emitted from each source point. The controlled and extended emission of the pheromone scent is the added advantage of CREMIT to lure the males as compared with the normal females. Area wide management of pink bollworm conducted over an area of 350 acres (2017-18 & 2018-19) in Raichur district with CREMIT-PBW a mating disruption tool applied at 500 gm/acre (in 4 splits at 40, 70, 100 & 135 days). Results revealed more than 80-90% control of pink bollworm with maximum yield gain of 33.58 q/ha (B:C ratio of 2.44) compared to conventional farmers practice who realized 22.34 q/ha (B:C ratio of 1.93) even after 4-5 rounds of chemical spray. The higher benefit: cost ration suggests the viability of the tool and even remunerative. The natural enemy population observed during the investigation period depicts no harm to their population. Hence, under present circumstances the best way to curb the menace of notorious pest like pink bollworm is through insect family planning via-a-vis mating disruption technology. The present investigation proposes CREMIT –PBW as a potential tool in area-wide management of pest through mating disruption.

References

1. Bheemanna, M., Sreenivas, A.G. and Patil, B.V. (2000). Efficacy of Sirene – A New PBW Pheromone against pink bollworm, *Pectinophora gossypiella* of cotton. Journal of cotton Research and Development**, 14** (2) 247- 249.
2. Cotton Corporation of India, 2021, https://cotcorp.org.in
3. Dhaliwal, G.S., Arora, R., & Dhawan, A.K. (2004) Crop losses due to insect pests in Indian agriculture: an update. *Indian J. Ecol.*, 31, 1–7.
4. Dhurua, S. and Gujar, G.T., 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders)(Lepidoptera: Gelechiidae), from India. *Pest management science*, *67*(8), pp.898-903.
5. Fand, B. B., Nagrare, V. S., Gawande, S. P., Nagrale, D. T., Naikwadi, B. V., Deshmukh, V., Gokte-Narkhedkar, N& Waghmare, V. N. (2019). Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders)(Lepidoptera:

Gelechidae) on Bt cotton in Central India: a new threat and concerns for cotton production. *Phytoparasitica*, 47, 313–325. https://doi.org/10.1007/s12600-019-00738-x

6. Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research, John Wiley and sons, New York, p. 680.
7. Ksenia, S. O., Kevin, W. T., Andrea, D. H., Patrick, C. T., Donna, S. L. and Anderson, E. R. (2010). Effects of CREMIT-GM sprayable pheromoneformulation on gypsy moth mating success. *Journal of Applied Entomology*, 132: 461–468.
8. Lapointe, S. L. andStelinski. L. L. (2011). An applicator for high viscosity semiochemical products and intentional treatment gaps for mating disruption of *Phyllocnistiscitrella. Entomologia Experimentalis et Applicata*, 90: 267–277.
9. Manickam, S.,GururajanK. N.and Gopalakrishnan, N., 2008. Bt cotton vis-à-vis environmental protection: An Indian Perspective
10. Naik, V. C., Kumbhare, S., Kranthi, S., Satija, U., &Kranthi, K. R. (2018). Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (*Bt*) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. *Pest Manag. Sci.*, 74, 2544-2554.
11. Patil, S. B. (2003). Studies on the management of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepideptera: Gelechiidae). Doctoral dissertation, University of Agricultural Sciences, Dharwad, India.
12. Patrick, L. P., Cristiano, J. A., Marcos, B., Lino, B. M., Lyndsie, S., Agenor, M. (2012). Integrated control of two tortricid (Lepidoptera) pests in apple orchards with sex pheromones and insecticides. *RevistaColombiana de Entomologia,* 38(2): 224-230.
13. Singh, J.P., Lather, B.P.S., Mor, B.R., 1988. Exit behaviour of pink bollworm (Pectinophora gossypiella) larvae. Indian J. Agric. Sci. 58 (3), 236–237.
14. Stelinski, L. L., Lapointe, S. L., Meyer, W. L. (2009). Season long mating disruption of citrus leafminer, *Phyllocnistis citrella* Stainton, with an emulsified wax formulation of pheromone. *Journal of Applied Entomology*, 134: 512–520.

Table 1. Evaluation of CREMIT-PBW against pink bollworm on *Bt* cotton during *Kharif* 2017-18 and 2018-19.

Treatment details	Rosette flowers* (%)		Green boll damage* (%)		Locule damage* (%)		Mean PBW incidence* (%)		% decrease in PBW incidence over farmer's practice		Average number of moths catches/week**		Percentage mating disruption#		Cotton yield (q/ha)	
T_1: CREMIT-PBW@ 1250g/acre	11.24 (19.59)	-	9.70 (18.14)	-	8.05 (16.49)	-	9.66	-	69.29	-	7.59 (2.93)	-	96.84	-	43.33	-
T_2: CREMIT-PBW@ 750 g/acre	11.80 (20.09)	-	10.27 (18.69)	-	7.86 (16.28)	-	9.97	-	65.75	-	7.20 (2.86)	-	97.00	-	42.50	-
T_3: CREMIT-PBW @ 500 g/acre	11.76 (20.05)	4.70 (12.52)	10.20 (18.62)	4.52 (12.27)	8.65 (17.10)	8.18 (16.61)	10.20	5.80	63.57	76.88	9.29 (3.20)	18.92 (4.40)	96.14		40.50	26.67
T_4:Conventional farmer's practice (Control)	20.96 (27.24)	13.67 (21.69)	37.93 (38.01)	23.97 (29.31)	34.34 (35.87)	37.63 (37.83)	31.07	25.09	-	-	240.38 (15.33)	155.03 (12.47)	--		24.68	20.00
S. Em (±)	0.98	0.58	0.86	1.47	0.68	2.14	-	-	-	-	0.15	0.55	-	-	0.99	0.56
CD @ 0.05	3.03	1.81	2.64	4.54	2.09	6.60	-	-	-	-	0.46	1.70	-	-	2.97	1.68
CV (%)	10.13	12.33	8.20	13.52	7.13	11.38	-	-	-	-	5.40	12.72	-	-	10.20	10.23

* Figures in the parentheses are arc sin transformed values

** Figures in the parentheses are square root ($\sqrt{x+1}$) transformed values

Table 2.Effect of CREMIT-PBW on natural enemies in cotton ecosystem during *Kharif* 2017-18 and 2018-19.

Treatments	*Chrysoperla*				Coccinellids			
	Eggs/plant*		Adults/plant*		Grubs/plant*		Adults/plant*	
Treatment Season	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
T_1: CREMIT-PBW @ 1250 g/acre	9.40 (3.22)^{ab}	-	5.60 (2.56)^{b}	-	3.20 (2.04)^{b}	-	3.80 (2.19)^{b}	-
T_2: CREMIT-PBW @ 750 g/acre	8.70 (3.11)^{b}	-	6.30 (2.70)^{b}	-	3.60 (2.14)^{b}	-	4.00 (2.23)^{b}	-
T_3: CREMIT-PBW @ 500 g/acre	9.20 (3.19)^{b}	9.80 (3.20)	5.10 (5.10)^{b}	6.20 (2.58)	3.10 (2.02)^{b}	4.40 (2.21)	4.20 (2.28)^{b}	3.20 (1.92)
T_4: Conventional farmer's practice	3.00 (2.00)^{a}	2.00 (1.58)	1.50 (2.46)^{a}	1.50 (1.41)	1.00 (1.41)^{a}	2.10 (1.61)	2.00 (1.73)^{a}	1.20 (1.30)
S. Em (±)	0.17	0.14	0.11	0.18	0.08	0.15	0.12	0.12
CD @ 0.05	0.51	0.43	0.35	0.54	0.24	0.44	0.37	0.36
CV (%)	12.92	9.56	10.93	10.11	9.17	9.43	12.70	9.67

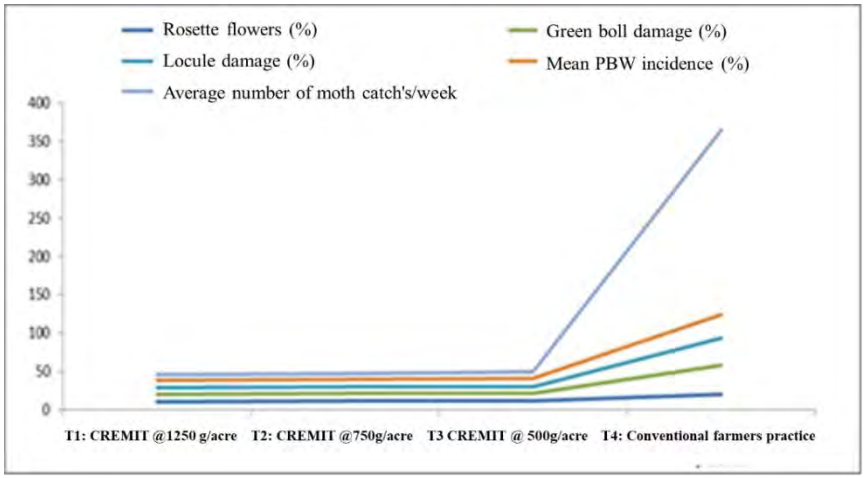
*Average of 50 plants

Table 3. Cost economics of CREMIT-PBW in cotton ecosystem during 2017-18 and 2018-19.

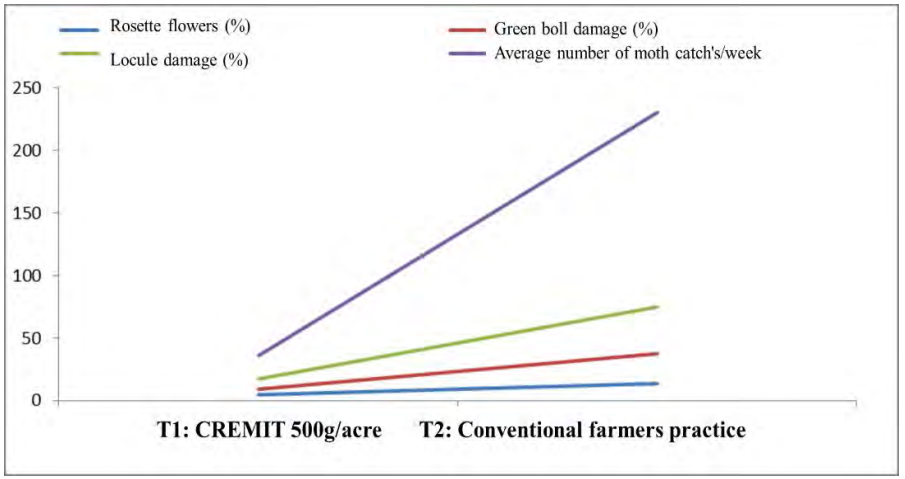
Treatments*	Cotton yield (q/ha)		Cost of cultivation (Rs./ha)		Cost of Treatment (Rs./ha)		Total Cost (Rs./ha)		Gross returns (Rs./ha)		Net Returns (Rs./ha)		B:C ratio	
Treatment season	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
T_1 : CREMIT-PBW @ 1250 g/acre	43.33	-	55,000	-	25,000	-	80,000	-	216650	-	136650	-	2.70	-
T_2 : CREMIT-PBW @ 750 g/acre	42.50	-	55,000	-	16,000	-	71,000	-	212500	-	141500	-	3.00	-
T_3 : CREMIT-PBW @ 500 g/acre	40.50	26.67	55,000	55,000	11,500	25,000	66,500	80,000	202500	146685	136000	66685	3.04	1.84
T_4:Conventional farmer's practice (control)	24.68	20.00	55,000	55,000	-	12500	55,000	67500	123400	110000	68400	42500	2.24	1.62

*applied in 4 splits

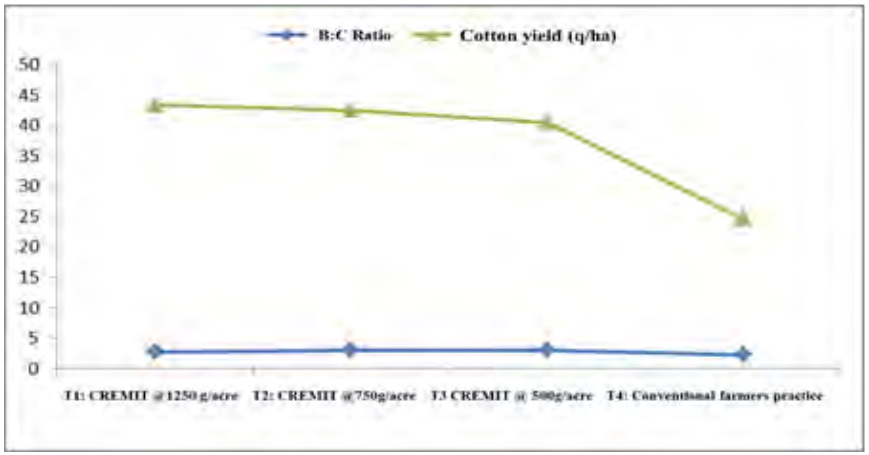
Note: Price of cotton: Rs. 5500/qt.
Cost of CREMIT: Rs. 11500/ha (Rs. 2250/application hence, for 4 applications it is 9000 plus cost of application Rs.2500/ha for four times)
Cost in farmer's practice: Rs. 12500/ha (Rs. 10000 is chemical cost and Rs. 2500 for labour four times application)



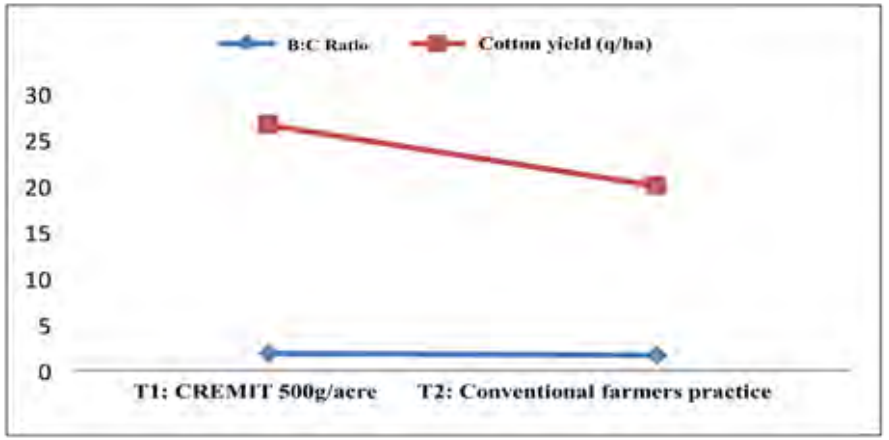
a



b



c



d

Fig.1.Effect of CREMIT-PBW mating disruption tool against pink bollworm, Pectinophora gossypiella on Bt cotton during 2017-18 and 2018-19. (a) and (b): PBW incidence (c) and (d): cost economics

Cotton Farming Typology as A Guide for Actions in Cote d'Ivoire and Beyond

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Abstract

Background: *In West and Central Africa, cotton production is of socio-economic importance by involving millions of people in rural areas and bringing hard currencies to the related country. Such an outcome has resulted, partly, from the backstopping by national, bilateral and multilateral development funding organizations for decades. Information nevertheless lacks on the impacts of the procured aids, related or not to technology transfer, in terms of the socio-economic status of the cotton farms by lack of application of a device to assess and follow-up what these features are.*
The objective of this communication is to draw out a typology of cotton farms in Cote d'Ivoire to compensate for the mentioned lack in view of guiding actions and assessing their impacts.
Results: *Through the data of a study implemented in 2014, a typology was based on the single criterion of cattle possession in relation with the tradition of hoarding. This typology clearly differentiates four types of farms according to their technical and financial performance in cotton growing, the characteristics of farmers and that of their families as well as their well-being through the possession of some durable goods.*
Conclusion: *The proposed typology is adapted, simple for application and flexible for evolution. It could fit all cotton-producing countries of West and Central Africa where the same tradition of accumulating capital in hoarding remains and it would allow country comparison to assess distinct cotton policies.*

Keywords: *Typology criteria, animal-drawn agriculture, hoarding, farm size, farm family, farm differentiation, durable goods, Africa*

Background

In West and Central Africa, cotton production is of socio-economic importance by involving millions of people in rural areas and bringing hard currencies to the related country. A recent study has precisely estimated that a population of 1.3 million is involved in the single country of Cameroon (Fok et al. 2019), which ranks fifth in production in the concerned African region, while the figure of ten millions people is commonly claimed in the mentioned African region.

The mentioned outcome has resulted, partly, from the backstopping by national support and more importantly by bilateral and multilateral development funding organizations for decades, seldom with clear indication on the expected implications of the procured aid, frequently related to technology transfer but not necessarily, on the socio-economic status of the cotton farms. Indeed, France has backstopped cotton production for historical reason, supplemented by European Union, The World Bank or other bilateral aid agency like GIZ of Germany. We have no knowledge of any report from these aid agencies informing on the evolution of the socio-economic status of cotton farms in the concerned countries.

The mentioned shortfalls result from the lack of application of a device to assess what the socio-economic of cotton farms are. There have been, and there still are, typologies based on the implementation of ox-drawn agriculture in countries like Mali (Kleene, Sanogo, and Vierstra, 1989), but the evolution of the farm distribution according to the followed typology is rarely reported and only partially (Samake, Bélières and Bosc, 2007). Such a typology has never been used to guide actions and assess the impacts of the implemented actions.

The lack of an applied typology to guide and access actions is also true at global level, with a single exception. Most research work on farm typologies have been implemented to understand farmers' perceptions or practices at a given time, kind of snapshots. There was generally no ambition of applying

over time typologies carried out through a statistical approach combining multivariate analysis and identification of homogenous groups of farmers or clusters. The single exception is encountered in the USA where a typology was used for example to identify specific actions for small farms (USDA 2002) or assess the variable adoption of conservation or irrigation techniques (Tavernier and Vic 2004). The applied typology is based mainly on the farm size, indicated by the level sales (Hoppe, Perry and Banker, 2000; Newton, 2002) replaced later on by the level of income (Hoppe and MacDonald, 2013). The objective of this communication is to draw out a typology of cotton farms in Cote d'Ivoire to provide a vision of the socio-economic status of cotton farms and that can be used to guide actions, related or not to technology transfer, and assess their impacts. The proposed typology has been identified through a study implemented in 2014. A combination of statistical and expert-based approaches has been followed to identify the factors influencing cotton yield and to select a typology criterion among the influencing factors pertaining to farm structure.

Results

Cotton yield was influenced by various factors (Table 1) which are not all suitable for farm typology. These factors pertained to farmers' characteristics (age and education), a single farm characteristic (possession of cattle), farmers' objectives or capabilities (related to the extension of cotton area, the share of cotton in total income and manure production) and farmers' cultivation practices in growing cotton (fertilizer dosages and number of insecticide sprays).

The expertise on farm functioning and cotton areas allows to observe that only the first three factors corresponded to structural features of farms, as farmers' objectives, capabilities and practices could evolve from one year to another. As the age increments over time and education can be compensated by training, the possession of cattle, a traditional practice of accumulation in hoarding, singled out to be more relevant for farm differentiation.

Table 1: influential factors of cotton yield (extract)

Farmer's characteristics	Coef.	p value
Education below secondary school	-0.377	0.001
Field work of children below 17 year old	-0.103	0.037
Farm characteristics		
Possession de bovins	0.146	0.045
Farmer's objectives or capabilities		
Objective of extending cropped area	-0.171	0.001
No capability for more manure	0.101	0.024
Objective of cotton >40% of income	-0.513	<0,0001
Farmer's cultivation practices of cotton		
Cotton area per adult family worker	0.131	0.027
Intensification en intrants		
Compound fertilizer dosage	0.111	0.061
Urea dosage	0.154	0.001
Number of insecticide sprays	0.262	<0,0001
Statistical test indicators		R2=0,335
		F =4,085; Pr>F : <0,0001

An iteration of farm clustering, by modifying the thresholds of the number of cattle heads possessed, has led to four farm types with the number of cattle heads possessed varying from none to more than 10. Table 2 shows a clear differentiation for various domains of characteristics between farm type D (no cattle at all), type C (less than four cattle heads), type B (4 to 10 cattle heads) and type A (more than 10 cattle heads). Domains of characteristics reported here pertain to farm heads and families, farm features, cotton growing and performance, and finally possession of the considered durable goods. In general terms, better was the accumulation in cattle,

- more wives the farm head had, as well as family field workers from bigger families;
- bigger was the area available, because of higher frequency of renting land in;
- better was the ox-drawn equipment level and more frequent was the use of manure;
- bigger were the total cotton area and cotton area by family worker;
- better was the seedcotton yield as well as the resulting margin;
- better was of the possession of the various durable goods in terms of frequencies and numbers, although not significantly for cellphones whose possession was widespread.

It is worth noting that the number of years of farm settlement had some effect on the farm typed by the level of hoarding. Farms of type A were settled a bit earlier than those of type D were. The period of civil turmoil, which lasted from 2002 to 2011, might have upset the accumulation process over years. This period had surely affected negatively the schooling of farmers' children. The schooling rate of the children of school age was rather low, with little difference between the farm types although slightly better for farms of type D and C; such a difference probably derives from the younger age of the concerned farm heads who had more children reaching the school age when the civil turmoil had settled down.

The differentiation observed between by the number of cattle heads was greatly related to the correlation between this accumulation feature and various farm characteristics as shown in Table 3, related to farm heads, their families, farms or cotton growing. A strong correlation was notably observed with farm production equipment in ox-drawn agriculture.

Discussion

The proposed typology is valuable with regard to its capability to clearly differentiating farms according to various characteristics related to farmers and their families, their cotton growing features and material well-beings. At the period of the study, two farm types lagged behind the other two types while representing 68% of all farms. The proposed typology has value for action as it gives a vision of the socio-economic status of farms according to the farm distribution among the four types. This value results from the correlation between the typology criterion (cattle possession) and the various socio-economic characteristics mentioned above. Hence, actions could be identified through the objective of improving the status of specific socio-economic characteristics of the observed farm distribution mong four types. Similarly, the impact of the actions conducted could be assessed through the alteration of the farm distribution.

The proposed typology is consistent with the few existing and can be claimed to be better. The few typologies that have been defined and mainly in Mali and Burkina Faso, albeit not operational, are based on the equipment for ox-drawn agriculture. Our typology is consistent with farm differentiation based on ox-drawn equipment because this latter criterion is correlated with that of cattle possession. Our typology nevertheless looks better because the quantitative and continuous nature of the criterion used enables to categorize all farms; this is not the case for example with the typology defined for Mali[1]. Our typology is also better because its criterion is an indicator of wealth by tradition. It is established not only to inform about production capability but also many other socio-economic characteristics. The value of the proposed typology lies on the simplicity of the criterion used and on the feasibility of its application. We cannot reject the possibility that farmers might be reluctant to indicate the number of cattle heads they have because of its wealth indication, although this reluctance was not encountered in our study. Nevertheless, the issue can be turned around easily by adopting the following sequence of simple and mainly Yes/No questions: a) do you have cattle? b) Do you have more than 3? c) Do you have more than 10? and d) If you do not mind, how many do you have? Even if the last question is not answered, the type of the related farm can be identified by the first three questions.

However, over time, the application of the proposed typology asks more than getting information to categorize farms into types. The various socio-economic characteristics of interest correlated to the typology criterion of cattle have to be updated. The collation of the related data could be more complex or demanding than the criterion of cattle accumulation itself. A pre-assessment of the difficulty in data collation should help determine which socio-economic characteristics to retain. The value of the proposed typology derives also from its potential for evolution without risk of discontinuity. Indeed, in

[1] For instance, it is impossible to classify farms having two sets of ox-drawn equipment but less than ten heads of cattle.

case of continuing economic development in cotton zones, hoarding will increase with less farms without cattle, if any, and more farms having more cattle. If so, the A type can be split into two sub-types, e.g. A1 for farms with 11-20 cattle heads and A2 with more than 20 cattle heads. Such a process of splitting the upper type can be extended over time.

Finally, the value of the proposed typology goes beyond Cote d'Ivoire and can be of interest in other countries where the same tradition of accumulation in hoarding has prevailed. Of course, the thresholds for the four farm types should vary because cattle in Cote d'Ivoire has particularly suffered from the period of civil turmoil. However, if the same typology were adopted in various countries, comparison would be easier to assess the impacts of actions conducted with financial support.

Table 2: Farm typology and differentiation based on cattle possession

	Farm type *				Total
	D	C	B	A	
Number of farms	146	156	108	29	439
% farms	33,3	35,5	24,6	6,6	100,0
Farmer's age	40.9 a	40.8 a	45.1 b	45.6 b	42,2
Number of farmer's wives	1.2 a	1.5 b	1.8 c	2.1 d	1,5
Number of family field workers	2.7 a	3.8 b	4.9 c	6.2 d	3,9
Primary school age kid at school, %	71,4	52,5	57,8	63,0	60,0
Primary & secondary school age kid at school, %	65,4	45,9	52,6	57,0	54,2
Number of years of farm settlement [1]	13.2 a	14.5 ab	17.1 ab	18.2 b	15,0
Total area available, ha	8.9 a	11.8 b	17.8 c	25.9 d	13,4
Frequency of renting land in, %	14.4 a	13.2 a	23.8 ab	29.4 b	17,4
Area under fallow, ha	1.6 a	1.8 a	3.3 ab	5.9 b	2,4
Level of ox-drawn equipment [2]	0.6 a	2.3 b	2.9 c	3.7 d	1,9
Number of cattle	0.0 a	2.1 b	4.8 c	43.7 d	4.8
Frequency of manure use, % [3]	6.0 a	22.1 b	29.3 bc	41.6 c	19,60
Cotton area in 2013, ha	1.7 a	3.3 b	5.1 c	6.2 d	3,4
Cotton area per family worker, ha	0.6 a	0.8 b	1.2 c	1.5 c	0,9
Seedcotton yiekd, kg/ha	1,079 a	1,076 a	1,269 b	1,410 b	1 146
Margin, 10^3 CFA/ha [4]	188 b	156 a	202 bc	242 c	183
Frequency having motorbikes, %	55.3 a	75.3 b	91.5 c	100.0 c	74,3
Number of motorbikes by those possessing	1.1 a	1.2 b	1.6 c	2.1 d	1,4
Frequency having bicycles, %	78,7	88,3	91,5	91,2	86,0
Number of bicycles by those possessing	1.3 a	1.7 b	2.1 b	2.5 c	1,8
Frequency having radioset, %	33,3	46,8	53,8	64,7	45,3
Frequency having Tvset, %	22,0	20,8	26,4	44,1	24,3
Frequency having cell phones, %	86,7	87,0	95,3	94,1	89,4

* D for no cattle; C for less than 4 cattle heads; B for 4-10 cattle heads ; A for more than 10 cattle heads

Distinct letters refer to statistical significant mean differences between the farm types

[1] Number of years till 2014

[2] Equipment level is related to the figure as the result of the way it was coded : 0 = 0 to 1 ox without plough; 1 = at least 2 oxen but without plough; 2 = at least one plough but less than two oxen; 3 = at least oxen and plough for a full equipement set; 4 = oxen and ploughs for at least two full equipment sets.

[3] Manure use was coded by 1 and 0 otherwise. The mean value was hence the frequency of manure use

[4] Value of production minus inputs provided by cotton companies on credit

Conclusions

A combination of statistical and expert-based approach has singled out the criterion of cattle possession for farm typology that fits with the tradition of hoarding. In Cote d'Ivoire, farm types according to this criterion are pretty well differentiated in cotton performance and in socio-economic terms. Farm typology is hence a guide for actions to evolve from an observed distribution of farm types, as well as a tool to assess the impacts of conducted actions through the alteration of farm distribution. As hoarding keeps on prevailing in all cotton producing countries of West and Central Africa, typology based on cattle

possession is of value beyond Cote d'Ivoire and its adoption could help country comparison of various cotton policies.

Table 3: Correlations of cattle possession with various farm characteristics

Farmer's characteristics	Coef.	y p value
Education below secondary school	-0377	0.001
Field work of children below 17 year old	-0,103	0.037
Farm characteristics		
Possession de bovins	0,146	0,045
Farmer's objectives or capabilities		
Objective of extending cropped area	-0,171	0,001
No capability for more manure	0,101	0,024
O Objective of cotton >40% of income	-0,513	<0,00010
Farmer's cultivation practices of cotton		
Cotton area per adult family worker Intensification en intrants	0,131	0,027
Compound fertilizer dosage	0,111	0,061
Urea dosage	0,154	0,001
Number of insecticide sprays	0362	<0,0001
Statistical test indicators	R2=0335	
Statistical test indicators	F=4.085: Pr>F: <0.0001	

Methods

The work has been based on a survey conducted in 2014 in the framework of an expertise study (Fok et al. 2016). The survey covered the thirteen administrative regions of Cote d'Ivoire where cotton production was marketed by five cotton companies and varying a lot geographically. A representative sample of 1108 farms was extracted from the whole list of cotton producers recorded for the previous year of 2013 by the sectoral coordination body "Intercoton". The survey was implemented to address the characteristics of farm heads (age, education, number of wives…), their families (through a census of family members in terms of sex, age, and education), their farms (size, equipment), their cotton growing practices (area, fertilizer, pest control…) and possession of durable goods (motorbikes, bicycles, cellphones, radio-set, TV-set).

The specific and reliable data on cotton production and inputs (fertilizers and pesticides) provided on credit basis were asked to cotton companies in charge of delivering inputs and marketing seed cotton. Data were eventually obtained for a sub-sample of 439 cotton farms that is indeed cope with in this study. These data were used to compute yield, cost and income.

In view of identifying a typology criterion, a first stage of statistical approach was conducted and differing from the common analysis of multiple factors like Principal Component Analysis (e.g. (Schwarz, McRae-williams, and Park 2010). We conducted instead a logistic regression to isolate factors explaining the seed cotton yield in farms because farm productivity used to be a matter of concern of cotton companies. Independent factors to explain the seed cotton yield were retained from the domains of characteristics related to farm heads, farmers' families, farms and cotton practices. Among the influential factors identified, those that were associated to farm structure were considered as potential for typology criterion. Among these ones, the typology criterion was selected by calling upon the expertise on farm functioning in cotton areas.

Once a typology criterion was identified, namely the cattle possession on farms, an iteration of typologies was implemented by varying the number of types and the thresholds of cattle possession for the various types. The number of types, varying from 3 to 5, was based on our expert knowledge of the cotton areas and the functioning of the involved cotton companies. The quality of each tested typology was assessed according to the statistical differentiation of farms (by Newman-Keuls test) with regard to characteristics related to farm heads, their families, their farms, their cotton production practices and performance and possession of durable goods.

Acknowledgement

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References

1. Fok, Michel et al. 2019. Analyse de La Chaîne de de Valeur Du Coton Au Cameroun. Rapport Pour l'Union Européenne, DG-DEVCO. Value Chain Analysis for Development Project (VCA4D CTR 2016/375-804).
2. Fok, Michel, Siaka Koné, Francis Akindes, and Marceline Soro. 2016. Mission d'observation Des Exploitations Cotonnières En Côte d'Ivoire. Abidjan (Côte d'Ivoire).
3. Hoppe, Robert A., and James M. MacDonald. 2013. "Updating the ERS Farm Typology." SSRN Electronic Journal (110).
4. Hoppe, Robert A, Janet E Perry, and David Banker. 2000. Agriculture Information Bulletin ERS Farm Typology for a Diverse Agricultural Sector. http://ageconsearch.umn.edu/bitstream/33657/1/ai000759.pdf.
5. Kleene, Paul, Bakary Sanogo, and Gerben Vierstra. 1989. A Partir de Fonsébougou... Présentation, Objectifs et Méthodologie Du "Volet Fonsébougou" (1977-1987). Bamako (Mali): IER.
6. Newton, Doris. 2002. "Typology of America's Small Farms." In Proceedings of the Third National Small Farm Conference, Washington D.C. (USA): USDA, 9–10.
7. Samake Amadou, Bélières Jean-François, Bosc Pierre-Marie, Sanogo Ousmane. 2007. Les Implications Structurelles de La Libéralisation Sur l'agriculture et Le Développement Rurale. Première Phase: Synthèse Nationale.
8. Schwarz, Imogen, Pam McRae-williams, and Danielle Park. 2010. "Identifying and Utilising a Farmer Typology for Targeted Practice Change Programs : A Case Study of Changing Water Supply in the Wimmera Mallee." Extension Farming Systems Journal 5(1): 33–42.
9. Tavernier, Edmund M., and Tolomeo Vic. 2004. "Farm Typology and Sustainable Agriculture: Does Size Matter? Edmund." Journal of Sustainable Agriculture 24(2): 33–46.
10. USDA. 2002. "Proceedings of the Third National Small Farm Conference." In eds. Denis Ebodaghe and Edmund Gomez. Las Cruces, NM (USA): New Mexico State University's Department of agricultural communications, 201.

Cotton Smallholders' Innovation in Agribusiness Organization and Management to Increase Productivity, Case of Taiyiba in Sudan Gezira Scheme

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Abstract

***Background**: A case study was conducted in Taiyiba Block in Sudan Gezira Scheme; one of the World's main irrigation projects and was known for extra-long staple cotton. Taiyiba represented an embryonic phase in the development of the Gezira Scheme since initiation of the Gezira Scheme in the first quarter of the last century. The Scheme infrastructure was deteriorating and sold by the Government by the onset of the 3rd millennium. There was an arbitrary decision by the smallholders to return to farming and especially cotton. The research described the plight of the small farmers after the Government had suddenly pulled out of the Scheme financing; their innovation in agribusiness organization and management in order to increase cotton productivity and maximize profits.*
***Results:** Social research methods were used to tap data from various sources, including key informants, semi-structured interviews, and other library and archival sources. A simple random sample of 30 farmers was selected. Data was analyzed using SPSS software; to compare means, one-sample T test was applied. The Block Group was able to locally innovate many small business projects, including feeder calves, poultry, and seed propagation and experimented with different organizational forms, e.g. partnership with national and international financial entities. It was found that there was no significant difference between the cotton production mean of the sample test (0.24 t/ha) and the Scheme's Seed Farm (0.29 t/ha), under the best crop management for the GMO variety under concern, Chinese 1. The holding size was also investigated and showed 0.84 ha and 1.68 ha as minimum and mode, respectively of this variable, an indication of land fractionation trend.*
***Conclusions:** The Taiyiba Group's evolutionary experience of development was based on self-initiative. The existence of production organizational and natural hazards and risks was still evident and need to be minimized. So as to achieve higher variety potential and organizational change, what the Government had promised to input, Research and Extension support remains to be seen. Recommendation made to improve agribusiness organization and management tools in the Taiyiba/Gezira Scheme area should be considered.*

Keywords: *: Gezira Scheme; Taiyiba Block Group; cotton smallholders; agribusiness management; organizational innovation; GMO cotton; cotton yield; farm size*

Background

Sudan Gezira Scheme (cultivable irrigated area: about 0.9m ha) was once described as the biggest long staple cotton farm worldwide, under single management and backbone of Sudan economy since the 1st quarter of the last century. Before the close of the last century the Government sold or privatized the Scheme infrastructure including the ginning factories, workshops, stores and the Gezira Light railroads (GLR) etc. Further, the Government stopped from financing farm operations and the small scale farmers had almost abandoned farming. In the 1990s cotton production had fallen to the lowest level in the history of Sudanese cotton. (ICAC, 2019): Table 1, below shows production and areas in Sudan under GMO cotton during the last five years. It shows Sudan increasingly coming back to cotton production.

Table 1: GMO Cotton Areas and Production in Sudan (2016-2020)

Season	2015/16	2016/17	2017/18	2018/19	2019/20*
Area (thousand ha)	74	122	190	200	190
Production (thousand tons of lint)	97	170	263	260	260*

*Estimate of the season; Source: (ICAC, 2019):

During the last decade farmers have resorted to organizational innovations, contract farming with local and foreign dealers and there was a comeback to cotton again especially GMO cotton.

Chinese 1(GMO) Stand: Season 2019/2020

Taiyiba is central to this evolutionary process since it was a success story as an experimental farm for the Gezira Scheme in 1911(Motwakil, 2000). It is hoped that Sudan will revive cotton farming and history be made again in Taiyiba.
Sudan agriculture for a long time had been characterized by low productivity as compared to home research and international standards. This was especially true for field crops including cotton. This situation was further exacerbated by low quality of lint. It was due to honeydew stained bolls as a result of infection by thrips, other cotton pests and bad labour picking practices. The cost of production was also very high. In consequence, Sudan lost its place in the international market as long staple cotton exporter.

Cotton is often described as a social development crop involving in its production and processing different labour categories: vis. Skilled, casual, seasonal, gender etc. When cotton area and production had decreased in the Gezira, quality of life was declining in the countryside area of the Gezira Scheme and a large number of the population fled the area to live in the capital Khartoum. Farm animals also suffered as cotton fields were good pasture areas immediately after cotton picking seasons had ended. It is against this background that Taiyiba initiatives and agribusiness management innovation is described.

The general objective of this research is to document in Taiyiba success stories, agribusiness initiatives and organizational innovation so that these might be replicated in the Gezira Scheme area and elsewhere in Sudan.

The specific objectives are:

- Compare Taiyiba to Gezira Scheme Seed Farm GMO cotton yield.
- Show Sudan cotton areas and yields as background to Taiyiba.
- Recommend successes and point out agribusiness management lacking in tools.

Results

Farmers' local innovations

Taiyiba farmers were known for their innovativeness and as early adopters of improved seed varieties, low production- and- storage cost of onion and mechanization of carrots and groundnuts. They also had used laser leveling and some farmers' organizations had been active for signing partnerships with the Arab Organization for Development and Agricultural Investment and with Bank of Khartoum. But the most important initiative had been Wadballal Company (Taiyiba Society, 2017).

Wabballal Company Office Feeder Calves Fences.

Seed Propagation by Taiyiba

Following are the initiatives that have been completed:

Improved Seeds Propagation: In season 2018/2019 some 420ha were sown with Chinese1 (GMO) from foundation seed. The production was very high of which 100 tons were produced as seeds after they had been physically and chemically treated by the Seed Production Unit of the Sudan Cotton Company. These seeds also had been certified by the Federal Ministry of Agriculture Biosafety Corporation and the Seeds Division of the Ministry. This season, 2019/2020 eighty four hectares have been approved by the Federal Ministry's Seeds Unit after they had paid an inspection field visit (Key informant).

Closely linked to the above small project is Wadballal Commercial Ginning Factory. In season 2018/2019 Taiyiba Society agreed with the Erada Company (part of Khartoum Bank) to finance their members for cotton. For the season 2019/2020 they also signed a contract with a Gin Factory that had been built near the production point, to pay each basic society or individual farmer in cash an amount of SDG6000/kantar of seed cotton (equivalent of less than $60 per kantar) (1kantar=315lb). The advantages of the Wadballal Factory as said by the Manager (Key Informant): 1) Availability of a cotton market in the Area 2) Cuts of transportation costs to members especially in view of the scarcity of petrol in Sudan 3) The target area extends beyond the irrigated area to the rain fed cotton in Gedarif and Damazin. 4) Absorption of unemployed labour in seasonal or otherwise permanent jobs.

Baled cotton for export

Seed cotton

III. The newly established Taiyiba Centre for Technology Transfer and Training offers opportunities and activities for farmers such as capacity building sessions etc. that had never been seen before. There are still other projects that are envisioned by the Society, for example a Marketing Board for groundnuts and also to get into partnerships with other societies within the irrigated area for the developments of sunflower, groundnuts, wheat and other field crops (Key Informant).

Achievements by the sampled farmers

Table 2 shows the size of the sample (30 farmers), their output mean (about 0.24 tons per hectare), the standard deviation and the standard error of the mean (see also appendix for actual figures). This figure (mean) is low compared to last season average (0.4 t/ha). This had been due to late sowing and water logging by continuous rainfalls (Block Supervisor). However, it was reported (Bakheit, 2016) that Bt. cotton had been infected by mildews (possibly: Oxycarenus hylinipennis) in season 2015/2016 in the Gezira Scheme Area. This also reflects on standards of technology package application and crop management.

Table 2: Yield of the sample of farmers

	N	Mean	Std. Deviation	Std. Error Mean
Farmyld	30	.2417	.12485	.02279

The item (sig. 2-tailed) in table3 shows the figure (0.043) which is greater than α/2=0.025). This indicates statistically no significant difference between the two means; which illustrates Taiyiba compared well with best variety conditions in this season 2019/2020. Low cotton productivity in the Seed Farm had been also due to continuous rainfalls (Seed Farm Manager). However, these figures were taken for one season because of lack of records at all levels: Farmer, Society, Block Office and Headquarters.

Table 3: Comparative yield performance of the sample of farmers

	Test Value = .29					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Farmyld	-2.120-	29	.043	-.04833-	-.0950-	-.0017-

Table 3 shows the sample mean compared to the Gezira Seed Farm's mean (Chinese1under the best crop management and complete technological package application):

H_0: sample mean= Seed Farm mean

H_1: sample mean≠ Seed Farm mean

Discussion

Production Risks and Uncertainties

The Taiyiba Group faces all types of agricultural risks and uncertainties: pests, diseases, shortages of irrigation water (surface irrigation) and water flooding by rains or bad field irrigation control (Key informant). The recent outbreaks of pests and diseases on GMO cotton such as mentioned above might be due to the now increasing trend of mixed cropping (lupin, groundnuts, okra, wheat, chickpea etc.) with cotton and the distortion of the famous Gezira Scheme 5-course rotation (cotton used to be the main crop in the rotation). The standard of technology is very low and the farmers are still using sticks, hoes and hand picking.

The problem of lack of financing was mentioned earlier and there are times when finance becomes very critical: at land preparation and harvesting time. The latter facts are understandable in view of the political and economic situation of Sudan today: shortages of gasoline, cash etc. and in light of the demanding nature of the crop for production inputs. The Taiyiba Group had to locally innovate to overcome all these hazardous production conditions. As said 'need is the mother of creativity'

Land fractionation

The mean, mode and median of farm size are shown as 1.65 ha, 1.68 ha and 1.68 ha, respectively (Figure 1, appendix 2). In the Gezira Scheme old standard rotation the mean and mode of tenancy size used to be more than 4 ha of cotton per tenant. The minimum of the sample (0.84) also indicates this smallness of farms. This has now led to land fractionation. It might be due to Islamic laws of inheritance and relaxation of Gezira Scheme's strict Rules and Regulations (R&R). The former crop rotation allowed for aerial spraying of chemicals and other farm operations on cotton on a larger scale. The field unit

used to be: (1 Number = 37.8 ha). But, no research is being done to address these socio-economic trends in the Gezira Scheme. Details of this issue are beyond the scope of this study.

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Chickpeas Mixed with Cotton

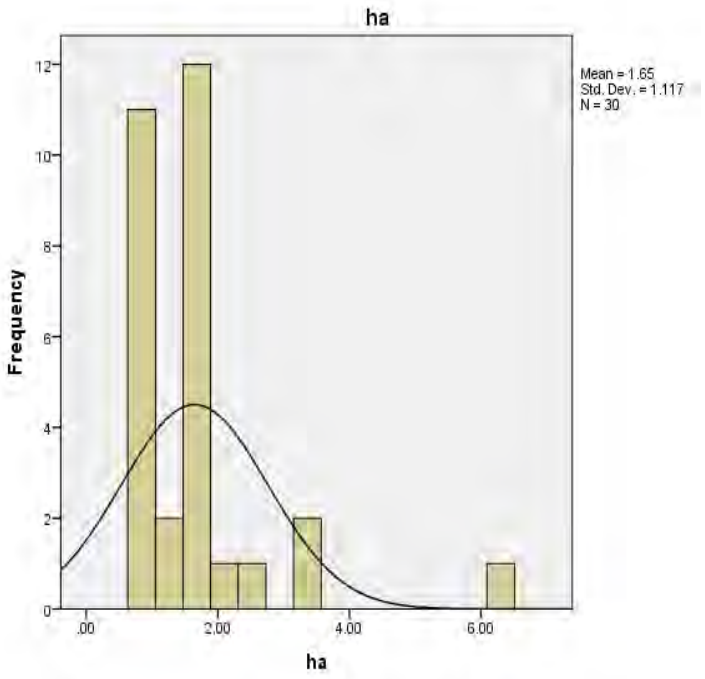

Figure 1: *Sample distribution of farm sizes*

Conclusion

The Government does need to give support to such local initiatives technologically and organizationally so that they can be expanded in the whole Gezira and other areas of Sudan. The Gezira latest Rule, 2005 and amended 2014 stipulated that extension and research would be the responsibility of the new Management. The Government has not lived to its promise. The following are recommendations by the study:

1. Develop farmers' organizations into full-fledged cooperatives.
2. The leaders of the cooperatives should be selected according to leadership criteria by democratic means, not by political orientation as before.
3. Strengthen links with research sources in proximity of the research area such as The Agricultural Research Corporation (ARC) and the University of Gezira for validation and authentication of local innovation.
4. Increase the coverage of extension to village level rather than block level.
5. Train farmers, farmers' leaders and supervisors on bookkeeping, farm records, agricultural accounting and agricultural entrepreneurship.
6. The value of cotton seeds are not included in the proceeds of cotton as had been the case with former Gezira Scheme Management, so this situation need to be corrected.
7. Consolidate small scattered farmers into complete numbers and streamline the previous Gezira course rotation so as to make use of scale economies.
8. Diversify income sources capitalizing on rural tourism: flat land scape, natural scenery and local culture.

Methods

The research area, Taiyiba: a locational advantage

Taiyiba is located at the centre of the Gezira Scheme Area in the Central region of Sudan. It is about 14km from Barakat, the Headquarters of the Gezira Scheme. It is nearby Wad Madani, the capital of the Central State and where many financial and agricultural support institution are found e.g., the Agricultural Research Corporation (ARC), the University of Gezira, the Federal Ministry of Irrigation and Water Resources, Masaad Centre for Testing Agricultural Machinery and many other private and Government businesses, banks etc. It is also in proximity of the main highway that connects almost all parts of Sudan with the Capital. Many feeder roads have recently been built, mainly by self-help to link the Area with the major road. Taiyiba Society is composed of 13 villages; some of these enjoy ethnic homogeneity e.g. Wadballal of the Shigiya tribe. This was advantageous in group cohesion and helped them complete together many small projects, e.g. feeder calves' project poultry, etc. The total cultivated area in Taiyiba is 9660ha.

Sample and Sample frame

The total number of farmers is 1300, of whom there are 117 cotton growers who comprised the sample frame of this study. The sample size of 30 farmers was chosen by simple random sampling and using the Table of Random Numbers (The Rand Corporation, 1955).

Data Sources

A large part of information for the study was in the qualitative form. This was taken from primary sources, from field visits and key informants, for example the Gins Manager and the Irrigation Responsible of the Society on 7th and 8th of February, 2020, respectively. A lot of information and images were exchanged by mobile phone calls and by WhatsUp, thanks for the new media channels! The latter included sources like Seed Farm Manager in the Gezira, Research people and the Taiyiba Block Supervisor. The quantitative data was from secondary sources, mainly from the Supervisor Block Office, and from some World Records. One major handicap to conducting research was lack of keeping proper production records and bookkeeping systems at all levels, at field, block and headquarters.

Data Analyses:

Data was analyzed using the computer software: Statistical Package for Social Sciences (SPSS). The following variables were obtained for area and yield: Averages, minima, maxima and standard deviations. One-sample T-test for significance of results

Acknowledgement

I thank Taiyiba people for their generosity and hospitality. I especially mention my friend Abdelrahim and his family and their neighbour Shopaly in El azaza Village. Also thanks go to Wadballal Society Head in Charge, Mr. Hamdoun. The role of Yousif Babikir, the Taiyiba Block Supervisor cannot go unmentioned.

References

1. Bakheit N. I (2016), Poster Session: WCRC-6, Goiania, Brazil: Nageeb Ibrahim Bakheit, May 2-6, 2016: Vision for Reforming Gezira Scheme by Strengthening Cotton Smallholders Field-Level Organization and Decision Making: unpublished poster presentation: PPP (14 slides).
2. Hassan Motwakil (2000), Development of Agriculture in the Sudan. 1st ed. Khartoum: Dar hyle Printing. Sudan
3. Sudan Cotton Company (2019), Sudan Country Statement: the 78th Plenary Meeting of the ICAC.
4. Taiyiba Society (Undated): Taiyiba Pilot Project: Taiyiba 2021.
5. The Rand Corporation (1955), A Million Random Digits with 100,000 Normal Deviates. Glencoe, III: The Free Press.

Appendix 1: Sample areas and production (season 2019/2020)

Serial	Area (ha)	Production (lint in tons)	Yield (t/ha)
1	1.68	0.42	0.25
2	2.52	0.53	0.21
3	1.68	0.36	0.21
4	1.26	0.26	0.21
5	1.68	0.35	0.21
6	0.84	0.23	0.27
7	3.36	1.21	0.36
8	0.84	0.17	0.20
9	1.68	0.35	0.21
10	0.84	0.11	0.13
11	0.84	0.29	0.35
12	3.36	0.65	0.19
13	1.68	0.33	0.20
14	1.68	0.30	0.18
15	1.68	0.33	0.20
16	0.84	0.17	0.20
17	1.68	1.36	0.81
18	0.84	0.18	0.21
19	0.84	0.20	0.24
20	1.68	0.30	0.18
21	0.84	0.23	0.27
22	2.1	0.30	0.14
23	6.3	2.27	0.36
24	0.84	0.14	0.17
25	1.05	0.38	0.36
26	1.68	0.21	0.13
27	0.84	0.20	0.24
28	0.84	0.14	0.17
29	1.68	0.30	0.18
30	1.68	0.35	0.21

Appendix 2: Statistics of hectares and yields

		ha	**Yield**
N	Valid	30	30
	Missing	0	0
Mean		1.6450	.2417
Median		1.6800	.2100
Mode		1.68	.21
Std. Deviation		1.11679	.12485
Skewness		2.804	3.492
Std. Error of Skewness		.427	.427
Range		5.46	.68
Minimum		.84	.13
Maximum		6.30	.81

Inefficiency using Stochastic Frontier Analysis (SFA) in Mali

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Abstract

Background: *Agriculture in least developing countries is considered as an engine of economic growth, food security and nutrition and poverty reduction in rural areas. As one of Malian's economy crops, cotton cropping plays an important role in generating national income and creating rural employment for the thousands of smallholder farmers. Cotton cropping has had a positive effect on agricultural production systems and living conditions of smallholder farmers. Investing in cotton farming is one of the main strategies that the government is using to increase agricultural productivity and reduce extreme poverty in the country. Few technical efficiency studies have been conducted on cotton production in Mali. This study aims to contribute to understanding of the factors that influence technical efficiency among cotton producers and determinants of inefficiency and fill this knowledge gap by conducting a Stochastic Frontier Analysis. Cross sectional survey data collected from 126 cotton producers and 126 plots of cotton in three selected villages was used in this study.*
Results: *The technical efficiency (TE) of the sampled cotton producers ranged from 0.29 to 0.90 with a mean of 0.84. Hence, the results show that the average output of cotton could be increased by 16 percent under the present technology. The variable quantity of NPK/ha was significant and positively affected technical efficiency. The variables of quantity of insecticide applied and family size positively affected technical inefficiency while age and frequency of weeding negatively influenced technical inefficiency.*
Conclusion: *This study revealed that the NPK/ha was the most important variable influencing cotton farmer's technical efficiency (TE). On the other hand, the socio-economics and practical variables determine farmer's technical inefficiency. The findings suggest that the policy makers should invest on agricultural inputs, provide better extension services and farmers training program for the efficient use of resources.*

Keywords: *Technical efficiency, Stochastic Frontier Analysis (SFA), Cotton, Mali*

Background

Many least developing countries rely on agriculture to support the livelihood of their populations. Moreover, the development of agricultural policies is targeting increasing agricultural productivity, reducing poverty, and increasing food security in rural areas. Mali is one of low-income and food shortage country, located in the Sahel region of West Africa. Agricultural sector account for more than 35 percent of Gross Domestic Product (GDP) and constitutes 80 percent of primary source of livelihood of population (FAO, 2017). The sector is characterized by cotton cropping as cash crop and cereals mainly (maize, millet sorghum and rice) as staple foods. Cotton accounts for 15 percent of GDP and 30 to 45 percent of agricultural export earnings. Thus, cotton is an important and strategic commodity for Malian economy as a source of rural employment. In addition, cotton feeds over four million people directly. Cotton is cultivated by two hundred thousand farming families hence 674704 ha under cotton in 2017-2018 (CMDT,2018). Mali is one of largest producer of cotton in West and central Africa (Bank, 2018). In 2017-2018, the country has produced 725000 tons with an average 1011 kgha-1 and 674704 planting area (CMDT, 2018). The cotton production is carried out in Southern Mali which covers 11 percent of national territory, stand for 27 percent of Malian population.). A part from its contribution to food security and monetary to cotton producers, many activities related to cotton value chain are provided such as transport, ginning, inputs even micro finance among others generate rural employment and contribute to improving livelihood. Cotton still remains to be the number one among cultivated crops in Mali due to its contribution to the livelihood of small scale farmers and at the country level. Despite, the allocated arable land under cotton and improved agricultural technologies available, there has been steady decline in cotton yield over the past decade. Therefore, that increase in land area under cotton and technologies have not widely adopted to increase cotton production. Thus, there

is limited study based on cotton producer's efficiency and inefficiency in Southern Mali. This study intended to address this knowledge gap the technical efficiency among cotton producers in Southern Mali.

In the literature, Technical Efficiency (TE) can be defined as a producer's ability to maximize outputs given a set of inputs and technology (Battese, 1992; Coelli et al., 2005). It refers to upward or downward shift to the production function. Thus, the frontier model is divided into two types; parametric and non-parametric. The parametric model is used in this study. It corresponds widely in developing countries cases, where the data are likely to be influenced by the measurement errors and others effects such as weather, diseases among others. It has two components deterministic and stochastic. The deterministic component assumes that any deviation from the frontier is due inefficiency while the stochastic approach allows the statistic noise. Previous studies have applied stochastic frontier (SF) to measure the efficiency for various agricultural products such as maize, rice, cotton wheat (Moraine, Grimaldi, Murgue, Duru, & Therond, 2016; Theriault & Serra, 2013). Therefore, in this study the SF analysis is used to estimate the efficiency of cotton production in Southern Mali. In addition, few and limited technical efficiency analysis studies have been conducted in Southern Mali. This study measured the technical efficiency of cotton farmers and determined the factors influencing technical inefficiency respect of socio-economics and farmer's practices on cotton. This study aims to fill the gap in the literature and inform policy maker's decision with regard to improving the given set of cotton production inputs.

Results

Statistics descriptive

Table 1 provides descriptive statistics of household characteristics. The average ages of family head in Beguene, Nafegue and Ziguena were 55; 57 and 58 years respectively. In terms of cultivated area of cotton, the averages were 3.09; 4.74 and 9.21 ha respectively and statistically significant among villages. In addition, cotton occupied 28 %; 42 % and 51% in the crop rotation for Beguene, Nafegue et Ziguena respectively. As for cereals the average cultivated areas were 5.71; 6.68 and 7.15 ha in Nafegue, Ziguena and Beguene respectively. Family size were 23; 24 and 25 people respectively. The farming equipment's owned were on average 5 and 6 for Nafegue, Ziguena, and Beguene respectively. The tropical livestock unit own was on average 16, 25 and 10.06 for Beguene, Nafegue and Ziguena respectively and statistically significant at 5%.

Table 1: Descriptive statistics of continuous variables of household characteristics

	Nafegue		Ziguena		Beguene			
	Mean	SD	Mean	SD	Mean	SD	F_stat	P-value
Age head family	57	13	58	15	55	16	0.65	0.53
Cotton(ha)	4.74	2.16	9.21	7.90	3.09	2.23	17.99	0.000***
Cereals(ha)	5.71	2.16	6.68	3.60	7.15	5.09	1.56	0.21
Family size	24	14.84	25	17.63	23	24.19	0.07	0.93
Equipment	5	2	6	3	6	3	0.69	0.50
Livestock owned	15.76	20.49	24.65	31.42	10.06	11.68	4.50	0.013**

;*. respectively significance at 5% and 1%

Institutional variables

Descriptive statistics for institutional variables are presented in

Comparison **of cotton yield per ha and per village**

The comparison of output yield per hectare and main inputs used such as NPK and Urea per hectare for cotton production among tree villages are presented in Figure. 1. The yields per hectare were 1046 kg/ha; 1138 kg/ha and 1146 kg/ha for Beguene, Nafegue and Ziguena respectively. Ziguena and Nafegue zones recorded high yielding per hectare as compared to Beguene. This is explained by over cultivation of the land under cotton, thereby putting more pressure on natural resources which results in low fertility of soil. With respect to, NPK used per hectare, 126 kg/ha was used in (Nafegue), 140 kg/ha in (Beguena) and 152 kg/ha in (Ziguena). These quantities of NPK applied in the first two villages were below the normal quantity recommended by the research institute and extension services. The normal quantity allocate per hectare for NPK cotton is 150 kg/ha delivered by the company in charge of cotton cropping through the extension services. The difference among villages in terms of kg/ha applied can be attributed to farming practices. Some farmers divert an amount of quantity to other crops such as sorghum or millet which are not included in the inputs subsidy. Similarly, quantity of urea

applied per hectare was 50 kg/ha in Nafegue, 53 kg/ha in Ziguena and 56 kg/ha in Beguene respectively. These quantities were almost in line with quantity of urea recommended by the research and extension services which is 50 kg/ha.

Table 2. The acquisition of formal education of head of family was statistically significant at 5% across villages. The education was important in Beguene Ziguena and Nafegue 68.18%; 51% and 45% respectively. The extension services were available and provided training and information to farmers based on good practices on cotton cropping techniques. A majority of the respondents had access to agricultural credit and were statistically significant at 10%, about 65%; 77% and 93% respectively. However, about 43% 59% and 83% of the respondents indicated that they had poor infrastructure (road) and had difficulty in accessing their farms. These were statistically significant at 1%.

Comparison of cotton yield per ha and per village

The comparison of output yield per hectare and main inputs used such as NPK and Urea per hectare for cotton production among tree villages are presented in Figure. 1. The yields per hectare were 1046 kg/ha; 1138 kg/ha and 1146 kg/ha for Beguene, Nafegue and Ziguena respectively. Ziguena and Nafegue zones recorded high yielding per hectare as compared to Beguene. This is explained by over cultivation of the land under cotton, thereby putting more pressure on natural resources which results in low fertility of soil. With respect to, NPK used per hectare, 126 kg/ha was used in (Nafegue), 140 kg/ha in (Beguena) and 152 kg/ha in (Ziguena). These quantities of NPK applied in the first two villages were below the normal quantity recommended by the research institute and extension services. The normal quantity allocate per hectare for NPK cotton is 150 kg/ha delivered by the company in charge of cotton cropping through the extension services. The difference among villages in terms of kg/ha applied can be attributed to farming practices. Some farmers divert an amount of quantity to other crops such as sorghum or millet which are not included in the inputs subsidy. Similarly, quantity of urea applied per hectare was 50 kg/ha in Nafegue, 53 kg/ha in Ziguena and 56 kg/ha in Beguene respectively. These quantities were almost in line with quantity of urea recommended by the research and extension services which is 50 kg/ha.

Table 2: Descriptive statistics of categorical variables

Variables		Nafegue	Beguene	Ziguena	Chi2	p-value
Education attendance	No	54.76	31.82	49.21	8.67	0.01**
	Yes	45.24	68.18	50.79		
Extension services	No	11.90	18.18	2.50	9.51	0.01**
	Yes	88.10	81.82	97.50		
Access to credit	No	7.14	22.73	35.00	5.26	0.07*
	Yes	92.86	77.27	65.00		
Poor infrastructure	No	83.33	59.09	42.50	14.17	0.00***
	Yes	16.67	40.91	57.50		

*;**;***. respectively significance at 10%; 5% and 1%

Comparison of cotton yield per ha and per village

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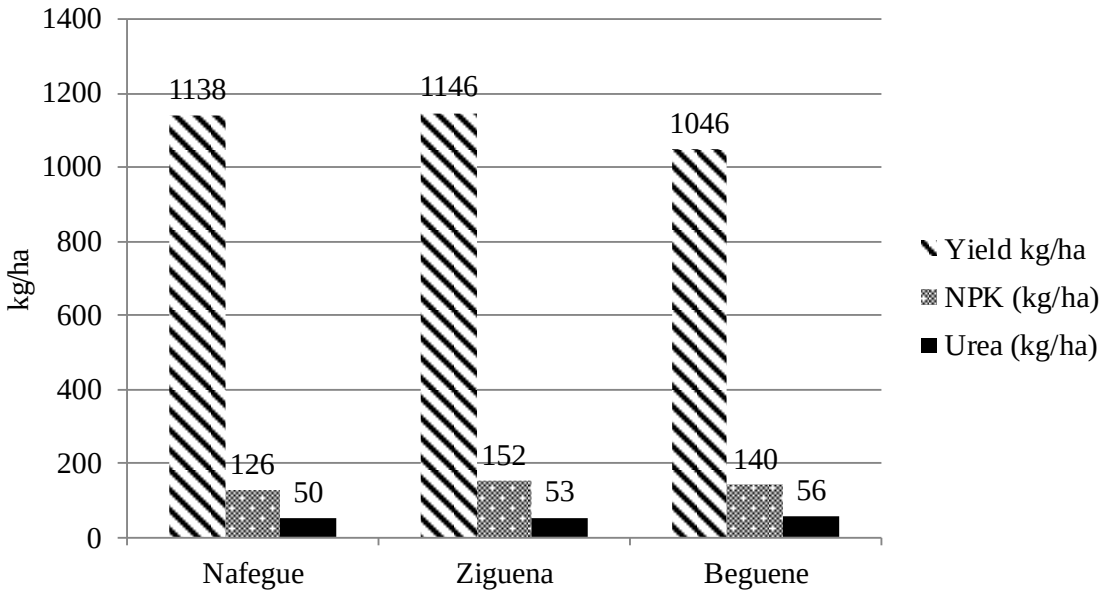

Figure. 1: *Cotton yield per ha and NPK and Urea per ha and per village*

Econometric results

Table 3 presents the maximum likelihood estimates (MLE) of the parameters of Cobb Douglas stochastic frontier production function. The total input coefficient used in the production was 0.197. It also shows the inefficiency effects and idiosyncratic effects in the model. The results revealed that quantity of NPK per hectare positively influenced cotton at production 1 % significance level. That is, an increase in the use of NPK input per hectare would lead to increase cotton output. The coefficient of NPK applied was 0.36 which showed that cotton output was elastic to changes in the application of NPK. A 1 % increases in NPK use will lead to a 36.2 % increase of cotton output. This implies that NPK is one of the most important factors of cotton production and had a strong influence on yield per hectare.

Idiosyncratic effects

Table 3 shows the specific characteristic of smallholder farmers cotton producers. The explanatory variables such as frequencies of insecticide application and weeding are the greatest challenges in contributing to the best cotton output. The coefficient of insecticide applied per hectare had a positive and significant relationship with cotton output at 5% significance levels. In cotton cropping, the frequency of applying insecticide is at least 6 times starting from the point of a distinguished first flower until harvesting. This implies that at least cotton farmer applied insecticides at the normal frequency recommended by research and agricultural extension services. Additionally, the positive coefficient for frequency of insecticide applied influenced cotton output per hectare. Hence applying the optimum frequency of insecticide at the appropriate time is expected to increase cotton output per hectare due to the reduction of insect pests. The variable frequency of weeding was negatively and significantly associated with output of cotton at 1% level. This was unexpected, since a higher frequency of weeding (twice) would be expected to have a positive effect on cotton output. This would be attributed to the availability of labour at the time where the agricultural work was at its peak. However, decreasing effect of higher frequency of weeding could also be explained by the number of crop enterprises that farmers are engaged in cotton cropping area.

Factors influencing technical inefficiency

The parameter estimates of the inefficiency effects of stochastic production frontier model are shown in the last section of Table 3, on socio - economics and institutional factors. The parameters include age of family head, education, family size, land and oxen ownership, agricultural tools as well as institutional factors such as access to agricultural credit and access to agricultural extension services. The results show that, age of family head and family size were significant on cotton cropping system. The coefficient of age was negative and significantly associated with cotton output at 5% levels. This implies that an increase in age of family head led to an increase in technical efficiency. In other words, young farmers were more technically inefficient than the older ones. In addition, this could be explained by the importance of cotton in the family economy in Southern Mali that young farmers ignore it. The coefficient of family size was positive and significant at 5% levels of probability. This implies that a larger family

sizes were more technical inefficient than smaller family sizes. This could be explained by larger family sizes having pressure on available resources hence tend to be poor as compared to small sized families and struggling to survive. This finding is inconsistent with Abdul-rahaman (2016) who reported that age increased technical efficiency of cotton farmers in Northern region of Ghana.

Table 3: Maximum likelihood estimates of Cobb Douglas Stochastic Frontier Analysis (SFA)

Ln Yield/ha cotton	Coef.	Std. Err.	P>z
Production function			
Ln plot size(ha)	-0.082	0.062	0.184
Ln NPK/ha	0.362***	0.092	0.000
Ln Urea/ha	-0.118	0.143	0.408
Ln Labour	0.023	0.045	0.610
Ln Qty insect	0.012	0038	0.758
Constant	5.899	0.658	0.000
Idiosyncratic effects			
Frequency insecticide applied	0.403**	0.193	0.037
Frequency of weeding	-2.559***	0.727	0.000
Constant	-0.853	0.960	0.374
Inefficiency effects			
Age of family head	-0.035**	0.015	0.018
Family size	0.030**	0.015	0.040
Land ownership	0.273	0.616	0.658
Education	0.077	0.378	0.839
Extension services	0.249	0.728	0.732
Access to credit	-0.288	0.517	0.577
Oxen ownership	-0.151	0.138	0.274
Agricultural tools	-0.125	0.146	0.393
Number of plot	126		
Wald chi2 (5)	16.15		
Prob>chi2	0.006		
Log likelihood	-35.389		

Note: **; ***. Significant at 5% and 1%

Distribution of technical efficiency (TE) scores

The mean technical efficiency (TE) was 84 % and ranged between 29% and 0.92 % as shown in Table 4. This implies that cotton producers of Southern Mali are more technically efficient which could be explained by the level of TE scores. The mean level of technical efficiency was 84%, which indicates that on average, cotton output falls 16 % in the short run of the maximum possible level. Therefore, in the short run it is possible to increase cotton production by an average of 16% by adopting the available technologies. The results mean that cotton producers operated at above half of the production frontier. On the other hand, farmers with the best agricultural practices are very close to the production frontier while, the worst producers had a TE of 29%. The distribution of technical efficiency scores across 126 cotton producers showed that 11.11% of farmers had technical efficiency scores that were less than 60%. Further, 32.54% level farmers had technical efficiency scores between 60 to 89% and the majority of farmers 56.35% of farmers had the most technical efficient score above 90%.

Table 4: Distribution of technical efficiency scores

TE score	Frequency	Percentage %	Cumulative %age
0.29-0.39	3	2.38	2.38
0.40-0.49	2	1.59	3.97
0.50-0.59	9	7.14	11.11
0.60-0.69	10	7.94	19.05
0.70-0.79	14	11.11	30.16
0.80-0.89	17	13.49	43.65
>0.90	71	56.35	100.00
Number Observation	126		
Mean	0.84		
SD	0.16		
Min	0.29		
Max	0.92		

Discussions

Farm and institutional characteristics

Smallholder farmers in cotton production of West Africa allocate more arable land under cotton to increase income earn from cotton and assure food security. Therefore, the allocate land size to cotton obtained in this study is in line with the findings from the study conducted by Theriault and Serra (2013) in three West African countries. Many studies reported that the education and institutional factors are among the main asset of agricultural development and highly associated with agricultural productivity (Teklewold et al., 2015; Sibhatu & Qaim, 2017). In addition, better educated farmers and access to institutional factors are more likely to affect agricultural output and food security.

Stochastic frontier analysis (SFA)

In relation to stochastic frontier of production function results, the inputs used to produce cotton mainly NPK, urea, the pesticides, labour among others should influence positively the cotton output. Therefore, the NPK applied per hectare was statistically significant and associated with cotton yield Table 3. These findings are consistent with previous studies reported that there was a positive effect of quantity of NPK used per hectare on cotton and cereals (Theriault & Serra, 2013; Karimov, 2014; Mango et al., 2015). In terms of household head age, the old farmers are more engaging in cotton cropping in order to meet the family expenditure. This is consistent with finding reported by (Bozoglu & Ceyhan, 2007; Okoye et al., 2016). The finding is attributed that older cotton farmers possess an importance about cotton effect on household livelihood in Southern Mali. In addition, cotton constitutes a strategic crop in Mali and specially its contribution in rural development. As for the distribution of technical efficiency scores, the findings is in line with (Hameed et al., 2014). In other words, the findings reported by Ali & Kpakpabia (2019) which is 48.33% in Togo is not in line with this study finding.

Conclusion

This study used the stochastic frontier analysis (SFA) to estimate the technical efficiency and socio economics determinants of small scale cotton producers in Sothern Mali. The results showed that the quantity of NPK applied per hectare was the most important that influence the level of cotton producer's technical efficiency. Thus, increasing the quantity of NPK applied under cotton can significantly contribute to increase cotton yield per hectare. Small scale cotton producers had a mean technical efficiency of 0.84%. This implies that there is a potential to increase cotton output by about 26% with available technologies. Age of household head, the frequency of weeding are the main factors that significantly enhanced the technical efficiency of small scale farmers. The findings suggest that policies implications should be based on dissemination of agricultural technologies practices in order to increase the level of cotton producer's technical efficiency and inputs subsidy. This can be achieved by regular training on improved agricultural technologies practices through extension services in charge of cotton production.

Methods

Study area

This study was carried out in Southern Mali. The selection of the study area was based on the importance on the potential of arable land allocated for cotton cropping and diversified agriculture compared to rest of country. In addition, the Southern Mali receives numerous intervention from public and private sector such as the research centers, NGos among others. The access and use of agricultural technologies such as improved agricultural practices applying inorganic and organic fertilizer, pesticides, crop residues management, compost among others. Agro-ecological condition is favorable for supporting diverse agricultural production system. Three villages were chosen based on their accessibility and level of cotton cropping in the production system.

Beguene in Old basin of cotton production located in Bla (province). It is characterized by the high pressure on the natural resources and cropping systems is based cotton-maize. The rainfall is between 600-800 mm per year.

Ziguene (Sikasso province) in the immediate zone, it corresponds to the isohyet 1000 -1100 mm per year. Climate risk and pressure on natural resources are low compared to the old basin (Beguene). There is an important development of agricultural diversification (potato, sweet potato.

Nafegue (Kadiolo province) located in sub humid zone, and characterized by the low population density and maize production is the first in the cropping system. It corresponds to the isohyet more than 1200mm per year with weak pressure on natural resources.

The population of interest constituted all small scale cotton producers and the sample unit was the household. Therefore, 126 cotton producers have been randomly selected in three villages from 2017 planting season. The primary cross sectional data was collected using a semi structured questionnaire.

Stochastic Frontier Analysis (SFA) model

Efficiency of a farm refers to its performance in the utilization of available resources (Battese & Coelli, 1995; Coelli et al., 2005). Therefore, the SFA and Data Envelopment Analysis (DEA) are the two main methods to measure a farm efficiency. According to Aigner, Lovell, & Schmidt (1977), the stochastic production frontier analysis with two error terms can be modelled as:

$Y_i = f(X_i\beta)Exp(V_i - U_J)$ where Y_i is the production of the i^{th} farm X_i I is a vector of functions of inputs quantities applied by the i^{th} farm, β is a vector of unknown parameters to be estimated, V_i are random variables to be independently and identically distributed $(N(0\delta^2))$. The error terms $V_i \; and \; \mathrm{U_J}$ capture respectively the random shocks and the effects of technical inefficiency. And the technical efficiency of the i^{th} farm denoted by TE is given by: $TE = \frac{Y_i}{Y^*_i} = \frac{\exp(X_i\beta + V_i - U_i)}{\exp(X_i\beta + V_i)} = \exp(-U_i)$. The empirical model of the stochastic production function for the small scale farmers is specified as:

$\ln Y = \beta_0 + \beta_1 \ln area + \beta_2 \ln NP + \beta_3 \ln urea + \beta_4 \ln pesticides + \beta_5 \ln labour + V_i - U_i$ where:

Ln =logarithm base e;

β_0 = constant or intercept;

$\beta_1 \; to \; \beta_5$ = parameters to be estimated;

Y= yield per hectare

V_i = Stochastic errors

U_i = Technical inefficiency

References

1. Abdul-rahaman, A. (2016). Stochastic frontier analysis (SFA) of technical efficiency , insights from smallholder cotton farmers in the Northern Region of Ghana. Global Journal of Agricultural Economics, Extension and Rural Development, 4(1), 361–367.
2. Aigner, D., Lovell, C. A. K., & Schmidt, P. (1977). Formultion and estimation of stochastic frontier production function models. Journal of Econometrics, 6, 21–37.
3. Ali, E., & KPAKPABIA, K. T. K. (2019). Determinants of technical efficiency of cotton farmers in Togo. Review of Agricultural and Applied Economics, 2(1991), 88–95. https://doi.org/10.15414/raae.2019.22.02.88-95
4. Bank, W. (2018). Growth and Diversification, Volume I . (Vol. I). Washington DC 20433.
5. Battese, G E, & Coelli, T. J. (1995). A Model for Technical Inefficiency Effects in a Stochastic Frontier Production Function for Panel Data. Empirical Economics, 20, 325–332.
6. Battese, George E. (1992). Frontier production functions and technical efficiency : a survey of empirical applications in agricultural economics. Agricultural Economics, 7, 185–208.
7. Bozoglu, M., & Ceyhan, V. (2007). Measuring the technical efficiency and exploring the inefficiency determinants of vegetable farms in Samsun province , Turkey. Agricultural Systems, 94, 649–656. https://doi.org/10.1016/j.agsy.2007.01.007
8. Coelli, T. J., Rao, P. D. S., O'Donnell, C. J., & Battese, G. E. (2005). An introduction to efficiency and productivity analysis. Australia.
9. FAO. (2017). Nations, Food and Agriculture Organisaztion of the Unted (FAO) Mali Country fact sheet on Food and Agriculture Policy trends (Vol. 3).
10. Hameed, A., Padda, H., & Salam, A. (2014). Estimation of Productivity and Efficiency of Cotton Farmers : A Case Study of District Dera Ghazi Khan. Business & Economic Review, 6(2), 63–82.
11. Karimov, A. A. (2014). Factors affecting efficiency of cotton producers in rural Khorezm , Uzbekistan : Re-examining the role of knowledge indicators in technical efficiency improvement. Agricultural and Food Economics, 1–16.
12. Kassie, M., Teklewold, H., & Jaleta, M. (2015). Understanding the adoption of a portfolio of sustainable intensification practices in eastern and southern Africa. Land Use Policy, 42, 400–411.
13. Mango, N., Makate, C., Hanyani-mlambo, B., Siziba, S., & Lundy, M. (2015). A stochastic frontier analysis of technical efficiency in smallholder maize production in Zimbabwe : The post-fast-track land reform outlook. Cogent Economics & Finance, 6(1), 1–14. https://doi.org/10.1080/23322039.2015.1117189
14. Moraine, M., Grimaldi, J., Murgue, C., Duru, M., & Therond, O. (2016). Co-design and assessment of cropping systems for developing crop-livestock integration at the territory level. AGSY, 147, 87–97. https://doi.org/10.1016/j.agsy.2016.06.002
15. Okoye, B. C., Abass, A., Bachwenkizi, B., Asumugha, G., Alenkhe, B., Ranaivoson, R., … Ralimanana, I. (2016). Effect of transaction costs on market participation among smallholder cassava farmers in Central. Cogent Economics & Finance, 33(1), 1–20. https://doi.org/10.1080/23322039.2016.1143597

16. Sibhatu, K. T., & Qaim, M. (2017). Rural food security , subsistence agriculture , and seasonality. PLOS One, 12, 1–15. https://doi.org/10.1371/journal.pone.0186406
17. Theriault, V., & Serra, R. (2013). Institutional environment and technical efficiency : A Stochastic frontier analysis of cotton producers in West Africa. Journal of Agricultural Economics, 1–23. https://doi.org/10.1111/1477-9552.12049

Integrated Extension Management Module for Bridging up the Yield and Knowledge Gaps among Indian Cotton Growers

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Abstract

Background: India has the laurels of holding top places in acreage and production of cotton at world level for many times. But, it always has a concern about yield of the crop. The country's cotton research system produces many technologies and extension system devises many mechanisms to disseminate those technologies but there is always a gap between potential yield of the technologies claimed by the technology inventors and the actual yield realized by the farmers in the fields. Studies conducted to find out the reasons behind this gap revealed that knowledge gap was the major reason in addition to the other researchable and non researchable constraints. In order to bridge up the yield gap in cotton, at first, the knowledge gap has to be bridged up with novel extension interventions.

Results: Analysis on the impact of popular knowledge delivery program in India named Front Line Demonstration (FLD) on cotton yield using secondary data revealed that there is significant gap between the yields of FLD and farmers' own practices. The assessment of the program on knowledge delivery revealed the existence of knowledge gap even among the FLD farmers and the major cause cited was lack of continuous information flow in FLD extension model. To overcome this, a new extension module was conceptualized with all possible extension innovations along with yield gap closing options. The concept module was validated, piloted, modified and an empirical module was developed.

Conclusion: Study results suggest a new extension mechanism titled "Integrated Extension Management Module" for bridging up the yield and knowledge gaps among Indian cotton growers which has the potential to be replicated in other similar situations.

Keywords: Cotton; Transfer of Technology; yield gap; knowledge gap; Integrated Extension Management module; Front Line Demonstration

Background

Since time immemorial cotton has been the towering commercial crop in India. In ancient days, cotton played an important role in the history of the British and independent India, and continues to be an important commodity for many decades. India has the laurels of owning top places in acreage and production of cotton at world level by cultivating 12-13 million hectares and producing 35 to 37 million bales (Indian bale 170kg lint) every year. It has the unique distinction of being the only country in the world to cultivate all four cultivable Gossypium species. Even though only one per cent of India's cotton production is organic, it is the world's largest producer, producing 56 per cent of the world's organic cotton (Business Line, 2018). Apart from providing 60 per cent of the fiber used in textile industries, the crop is also a source for 11.5 lakh tonnes of oil, 90 lakh tonnes of animal feed, about 200 lakh tonnes of cotton stalk that is used for fuel and value addition as particle boards.

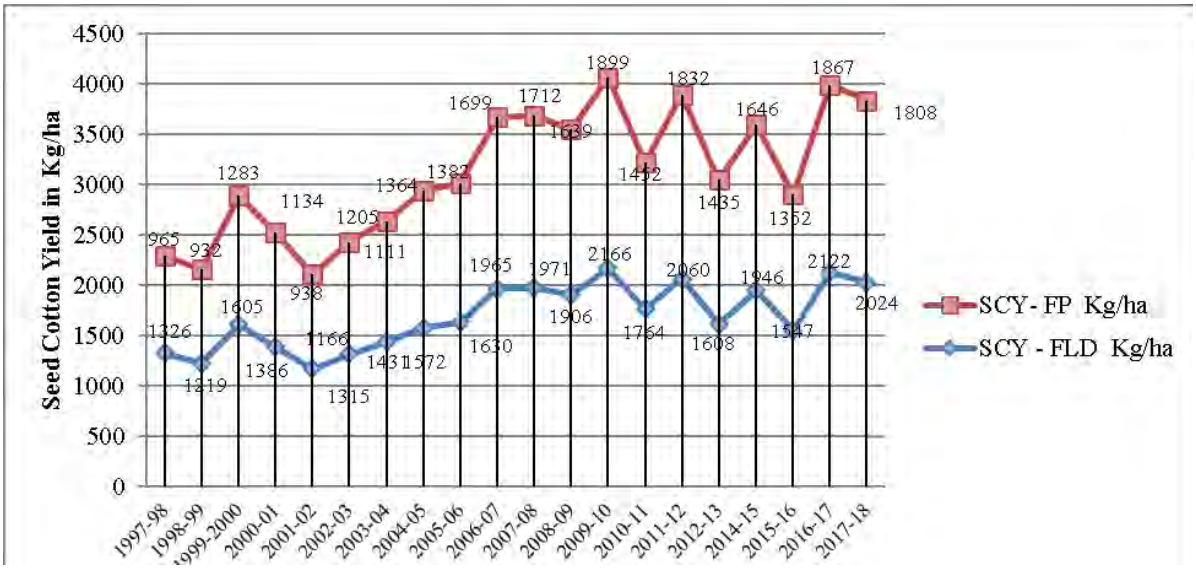
In midst of all these glories, Indian cotton sector always has a concern as regards its average national productivity. The country's average cotton lint yield has been ranged from 475 to 567 kg/ha for the past few years (Rani, 2019). To overcome this constraint, the cotton research system in the country both in public and private sectors, release many high yielding varieties / hybrids, yield enhancing agro technologies and management strategies for damages caused by the insects, diseases and deficiencies. Similarly, the country's cotton extension system devises many Transfer of Technology (TOT) programs to disseminate these technologies to the end users. But there is always a gap between the potential yield of the technologies claimed by the technology inventors and the actual yield realized by the farmers in the fields. To study the reasons behind this gap, many attempts have been made by the cotton scientists and the studies revealed that the knowledge gap was the major reason in addition to the other researchable and non researchable factors. So, to bridge up the yield gap in cotton, at first, the knowledge gap has to be bridged up. To bridge up the knowledge gap, novel extension

innovations need to be developed to speed up the reachability of cotton technological information to the unreached in appropriate forms. “Integrated Extension Management Module for Cotton knowledge transfer” is such an approach developed for bridging up the knowledge gap among Indian cotton growers and thereby the yield gap in Indian cotton, of which this paper is detailing about.

Results

Assessment of Popular Knowledge Delivery Program “Front Line Demonstration” in Bridging up the Yield Gap in Cotton in India

The field demonstration conducted under the close supervision of scientists of the National Agriculture Research System in India including the Scientists from Indian Council of Agricultural Research, State Agricultural Universities, Krishi Vigyan Kendra (Farmers Science Centre) is called Front Line Demonstration. The intentions of FLD are demonstrating the usefulness of the latest improved crop production and protection technologies to the farmers as well as extension workers with a view to reduce the time gap between technology generation and its adoption. It also enables the scientists to obtain direct feedback from cotton farmers and suitably reorient their research programs, develop appropriate technology packages and to create effective linkage among scientists, extension personnel and farmers. In cotton, for the first time in 1995-96, the program was conducted extensively covering all the cotton growing states with 812 demonstrations. Since then, the ICAR-CICR through its All India Coordinated Research Program (AICRP) on Cotton has been conducting FLD in cotton throughout the country. Until 2017, a total of 19500 demonstrations have been conducted in eleven cotton growing states of India with a budget outlay of 102.8 million Indian rupees (13.73 million USD) by sixteen participating centers. The average seed cotton yield (kg/ha) obtained in FLDs conducted nationwide from 1997-98 to 2017-18 were given in figure 1.



Source: FLD Annual Reports from 1997-98 to 2017-18

Fig.1. Average yield obtained in FLDs conducted nationwide from 1997-98 to 2017-18 in India

The average seed cotton yield obtained in the demonstrations conducted by ICAR – AICRP centers throughout the country from 1997-98 to 2017-18 on cotton production technology and Integrated Crop Management ranged from 1166 kg/ha to 2166 kg/ha. The average seed cotton yield obtained in FLD over twenty years was 1686 kg/ha. Similarly, the average seed cotton yield obtained in the farmers’ own practices without the advice of Scientists were also collected for the said years. It ranged from 932 kg/ha to 1899 kg/ha. The average seed cotton yield obtained in farmers’ practices in the past twenty years was 1432 kg/ha. The average yield increase because of FLD program over farmers’ own practice was 254 kg/ha (Figure 2). It reveals that, there is a possibility of increasing average seed cotton yield to a tune of 18.00 % by proper TOT intervention viz., FLD.

The causes for gaps between yields at FLD and farmers’ own practices were observed and they were inefficient pest management, water scarcity, labor scarcity, lack of information on yield enhancing

technologies and lack of extension support in farmers' own practice. At FLD also few concerns were documented. They were pest attack (but could manage partially with the help of scientists' advice), labor scarcity, water scarcity and few visits of Scientists could not meet out the information and continuous extension need of the farmers.

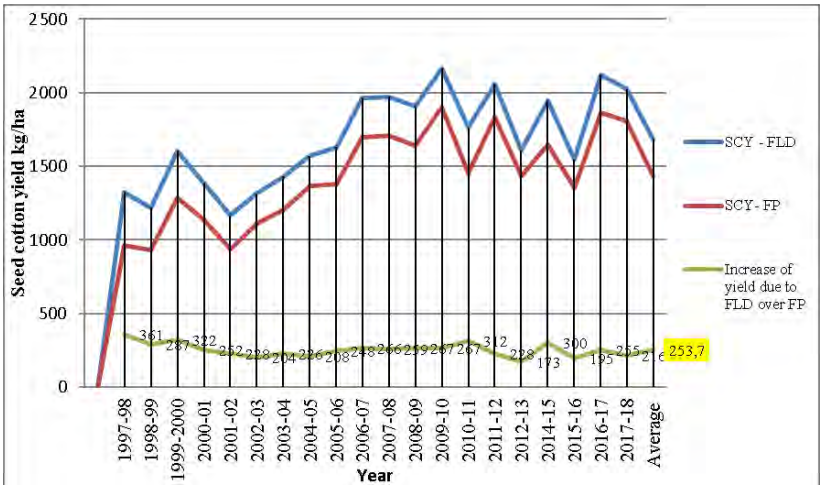


Fig.2. *Average yield increase obtained in FLDs over Farmers' own practice from 1997-98 to 2017-18*

Assessment of Popular Knowledge Delivery Program "Front Line Demonstration" in Bridging up the Knowledge Gap among Cotton Growers in India

To assess the effectiveness of FLD in delivering the knowledge among cotton growers, an ex post facto research designed study was conducted among the 94 FLD beneficiaries of University of Agricultural Sciences, Dharwad in Dharwad and Belgaum districts of Karnataka, in Southern cotton growing zone of India. The results (Table 1) revealed that the average area under FLD was 0.25 to 1.0 ha. The average seed cotton yield obtained in FLD was ranged from 1269 kg/ha to 2950 kg/ha. Similarly the average seed cotton yield obtained in farmers' own practices ranged from 1108 to 2600 kg/ha. The yield gap between the attainable yield (FLD) and actual yield (Farmers' practices) ranged from 160 to 350 kg/ha. The net profit in FLD ranged from INR 32714 (425 USD using conversion factor 1 INR = 0.013USD) to INR 70200 (913 USD) and in farmers' practice ranged from INR 26230 (341 USD) to INR 43200 (562 USD). The additional benefit due to adoption of technologies disseminated through FLD ranged from INR 6484 (84 USD) to INR 27,000/ (351 USD). The technologies learnt by the farmers due to FLD were potential ruling Bt cotton Hybrids, balanced nutrition, split application of N & K, foliar nutrition, management of reddening, intercropping, correct sowing time, spacing, ETL of various pests, need based chemical sprays for pests, proper storage of cotton, management of Pink Boll worm and Sucking pests.

Table 1 Assessment of Front Line Demonstration in terms of yield and technologies learnt according to the experience of the respondents (n=94)

Number of Beneficiaries	94 (beneficiaries of the years 2016-17 and 2017-18)
Component of FLD	Integrated Crop Management in cotton
Range of area under FLD / farmer	0.25 ha to 1.0 ha
Range of seed cotton yield obtained in FLD in the study area	1269 kg/ha to 2950 kg/ha
Range of seed cotton yield obtained in farmers' own practices in the study area	1108 kg/ha to 2600 kg/ha
Range of yield difference between FLD and farmers' own practice in the study area	160 kg to 350 kg/ha
New Technologies learnt through FLD (Farmers' perception)	Potential ruling Bt cotton Hybrids, balanced nutrition, split application of N & K, foliar nutrition, management of reddening, intercropping, correct sowing time, spacing, ETL of various pests, need based chemical sprays for pests, proper storage of cotton, management of Pink Boll worm and Sucking pests.

The data collected on the FLD farmers' perception on assessment of FLD as a cotton extension mechanism (Table 2) revealed that majority of the farmers (> 80.00 per cent) perceived the FLD

program as an efficient, compatible, observable, trial able and a program with multiple advantage as well as risks.

As regards cultivation behavior, few desirable changes were observed in adoption of new varieties/ hybrids, management strategies for weeds, diseases, pests, physiological disorders and in harvesting & post harvesting practices by the FLD farmers. But the knowledge test conducted among them revealed that more than half of them could not tell the correct answers as regards the how to do and principle knowledge behind the technologies viz., cotton varieties and hybrids, management strategies for weeds, nutrients, diseases, pests, physiological disorders and in harvesting & post harvesting practices. This explains the existence of knowledge gap (Table 3) even among the FLD farmers who could adopt few technologies after knowing through FLDs.

Table 2 Distribution of respondents based on their perception about FLD as a knowledge delivery cotton extension program (n=94)

Sl. No.	Attributes	Agree	Undecided	Disagree
1	Efficiency	80 (85.10)	12 (12.77)	2 (2.13)
2	Feasibility	56 (59.17)	3 (3.19)	35 (37.24)
3	Immediacy of returns	78 (82.93)	10 (10.64)	6 (6.38)
4	Compatibility	89 (94.68)	2 (2.13)	3 (3.19)
5	Observability	84 (89.36)	5 (5.32)	5 (5.32)
6	Profitability	78 (82.98)	10 (10.64)	6 (6.38)
7	Perceived risk	85 (90.43)	6 (6.38)	3 (3.19)
8	Availability	56 (59.57)	20 (21.28)	18 (19.15)
9	Continuity	50 (53.19)	4 (4.26)	40 (42.55)
10	Complexity	57 (60.64)	7(7.45)	30 (31.91_
11	Trialability	84 (89.36)	2 (2.13)	8 (8.51)
12	Multiple advantage	90 (95.74)	2 (2.13)	2 (2.13)

Table 3 Distribution of respondents based on their Scores in Knowledge test about questions related to cultivation practices of Cotton (n=94)

Sl. No	Particulars (questions related to)	Number of Respondents according to their answers	
		Correct	Incorrect
1	Land preparation	80 (85.11)	14 (14.89)
2	Cotton Varieties and Hybrids	45 (47.87)	49 (52.13)
3	Seed and sowing, spacing	65 (69.15)	29 (30.85)
4	Weed Management	35 (37.23)	59 (62.77)
5	Irrigation Management	69 (73.40)	25 (26.60)
6	Nutrient Management	42 (44.68)	52 (55.32)
7	Pest Management	23 (24.47)	71 (75.53)
8	Disease Management	29 (30.85)	65 (69.15)
9	Management of Physiological disorders	23 (24.47)	71 (75.53)
10	Harvesting and Post harvest practices	24 (25.53)	70 (74.46)

The constraint analysis revealed many researchable and non researchable constraints in cotton cultivation. But taking into consideration of the constraint and suggestion expressed as regards Extension Methodology, majority of the (> 95.00 %) of the FLD farmers expressed that continuous information flow is lacking in FLD extension model. So, they suggested for an extension approach which could supply the information continuously during the entire season and suggested few extension components to be included in the new approach.

Conceptual Integrated Extension Management Module for Bridging the Yield and Knowledge Gaps in Cotton

Based on the suggestions offered by the respondents and ideas given by the cotton growers in South India through focus group discussions, an extension module like the popular Integrated Pest Management module in cotton was conceptualized. This conceptual extension module was planned to have all possible extension innovations to supply information from cotton sowing to harvesting with the

name “Integrated Extension Management Module for bridging up the yield and knowledge gaps in Cotton”. The module was conceptualized to have both yield gap closing technological options and knowledge gap closing extension options for each crop stage. It also conceptualized to have both conventional and contemporary extension innovations. To identify the yield gap closing technological components and knowledge gap closing extension components, an opinion survey was conducted among the cotton Scientists in both biological and social fields. Also, the secondary data available in the literature also used to select the components. With the identified components, the following conceptual module was developed.

Components of Conceptual Integrated Extension Management Module for bridging the yield and knowledge gaps in cotton

Crop Stage	Yield Gap Closing Technology Option	Knowledge Gap closing extension innovation
Pre Sowing	High yielding Bt cotton varieties and hybrids preferably compact varieties	Preseason training On Farm Varietal Demonstration Result Demonstration on newly released varieties / hybrids Decision Support System
Sowing	High Density Planting System	Method demonstration Short Video films
Growth period	Best management practices specific to the biophysical conditions focusing on management of deficiencies, insect pests and diseases including drip irrigation, foliar application, poly mulching, precision farming and other yield enhancing technologies in ethical and responsible way.	A ready reckoner (booklet) with entire package of practices for ready reference during cultivation Field visit by experts Mobile phone based voice SMS Web based crop advisory services Radio or Television programs on specific topics Using Whatsapp group for clarifying the suddenly occurring pests/diseases/ disorders, deficiencies etc., Mobile App Field Day
Harvesting	Clean harvesting practices Machine picking	Method Demonstrations Short video films
Post harvesting	Proper Storage Clean transportation Proper Marketing Value addition of by products	Short video films Market information through web and mobile Training program

Empirical Integrated Extension Management Module for bridging the yield and knowledge gaps in cotton

The conceptual module was validated again with the cotton Scientists in both biological and social fields and piloted among few cotton growers. Based on the assessment results, few modifications were done in the conceptual module based on the availability of the components and the empirical one was developed. Most of the modifications were done due to unavailability of proven technologies.

Discussions

This study was planned to assess the yield gap in Indian cotton sector since it is common to consider yield as an indicator of production competitiveness for a country or an indicator of its profitability for the producers (Fok, 1998). The yield gap analyzed here was the gap between the attainable yield obtianed in FLDs and the actual farm yield obtained in faremer’s own practices. This can be explained in view of the definition given by Jha et al., (2011) for yield gap in his study as the difference between the maximum-attainable yield and the farm-level yield. The results of the yield increase due to FLD program over farmers’ own practice reveals that, there is a possibility of increasing average seed cotton yield to a tune of 18.00 % by proper TOT intervention viz., FLD.

Equal to yield, knowledge is regarded as a capital and an economic resource. Knowledge includes information, understanding, insights and other information that has been processed by individuals through learning and thought. Knowledge is the basic of any development action. Sharing of Knowledge is critical to support the three dimension of sustainable development in society viz., social, economic and environmental. Studies state that knowledge is one of the forms of wealth and is often differentially distributed throughout a social system. There is always a gap existing between the growers about the knowledge of novel yield enhancing technologies. Cotton growers are not exempted community for having such knowledge gap between the technical knowledge available in the

experimental stations and with them. The present study revealed that the FLD program could partially satisfy the knowledge requirement of the cotton growers but could not succeed fully. It was considered as the most important cause for yield gap in Indian cotton sector. This is confirmed by the results of Rani et al., (2018) that knowledge gap due to lack of timely technical information tops with more than 90 per cent of cotton growers' responses.

Figure 3 *Empirical Integrated Extension Management Module with Yield and Knowledge Gaps Closing Options for Cotton*

To have yield be improved, there is either to extend the existing yield potential or to decrease the gap to existing potential yield (Fok, A. C. M., 1998). To extend the existing yield potential we need to extend the technologies. Technologies make a difference. Cotton is one of the crops tremendously influenced by the technological breakthroughs. Technologies with genetic modification, inter specific and intra specific hybrids/varieties, novel pesticides, management of diseases, insects and nematode pests, weeds, nutrients, soil, water and climatic aberrations and mechanization can contribute significant increase in the yield (Kranthi, 2015). Hence, a module with yield gap closing technological options and knowledge gap closing extension options for each crop stage was developed in this study.

Way Forward

The empirical "Integrated Extension Management Module" has to be field tested for bridging the yield and knowledge gaps in cotton. Similar to this, localized "Integrated Extension Management" modules suitable to different climatic conditions, crops and growers can be developed.

Conclusion

The study was conducted to develop an extension innovation to bridge up the yield and knowledge gaps in cotton in India. The results presented here clearly support that there is a significant gap between yield obtained at FLDs and farmers' own practices. The results also revealed that the knowledge gap present among the FLD beneficiaries and call for developing a new extension approach that supply continuous information to bridge the knowledge gap. To ensure the continuous knowledge support to the cotton growers during the crop season, an "Integrated Extension Management Module" with yield gap closing technological options and knowledge gap closing extension options is conceptualized in this study. The newly developed module can be envisioned as an extension option for bridging the yield and knowledge gaps in cotton in India and can be replicated for further related countries.

Methods

To examine the impact of popular knowledge delivery program in India called "Front Line Demonstration (FLD)" in bridging up the yield gap in cotton secondary data on average yield obtained in FLD and farmers' own practices were analyzed for twenty years since the inception of the program (1997-98 to 20017-18). To find out the knowledge gap in cotton, the impact of various earlier implemented cotton extension or Transfer of Technology programs on yield and knowledge level cotton farmers was studied. Even though, there are many cotton extension programs have been implemented since pre and post independence era in India, Front Line Demonstrations in cotton is the program which has a long history and has been conducted from 1995-96 season. To study the impact created by FLD, among the 16

FLD implementing centres which have been consecutively conducting the FLD since 1995-96, University of Agricultural Sciences, Dharwad was selected purposively since it had conducted more number of FLD in recent past from 2014-15 to 2018-19 under the new sponsoring scheme by Government of India called National Food Security Mission on Cotton (NFSM) – Commercial Crops in southern cotton growing zone of India. Among the FLD implemented districts by UAS, Dharwad in the state Karnataka, two districts namely Dharwad and Belgaum where majority of the FLD conducted were selected as study area. A total of 94 FLD beneficiaries as one third of total FLD beneficiaries over five years have been selected as respondents for conducting the impact survey. Primary data were collected through seven Focus Group Discussions, fourteen case study methods and personal interviews using semi structured interview schedules, analyzed and documented. Based on the constraints revealed and suggestions offered by the respondents, a concept extension module called "Integrated Extension Management to close the yield and knowledge gap in cotton" was developed with yield gap closing options from seed to seed cotton and extension innovations for each stage to offer continuous information flow to close the knowledge gap. The technologies and extension components were identified and validated through opinion survey among the subject matter specialists both in cotton production and extension. Based on the suggestions an empirical module was developed.

Acknowledgement

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References

1. Aggarwal PK, Hebbar KB, Venugopalan MV, Rani S, Bala A, Biswal A and Wani SP. Quantification of Yield Gaps in Rain-fed Rice, Wheat, Cotton and Mustard in India. Global Theme on Agro ecosystems Report no. 43. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 2008;36 pages
2. Business Line, Weaving Success through Organic cotton. June 25, 2018
3. Evans LT, Fischer RA Yield potential: its definition, measurement and significance. Crop. Sci. 1999; (39):1544-1551.
4. Fok ACM, Cotton yield stagnation: addressing a common effect of various causes, in World Cotton Research Conference 2 Athens, (Gillham, F. M., Ed.). Sept. 5-11, 1998; (1): 38-45
5. Hall, AJ, Feoli C, Ingaramo J, Balzarini M. Gaps between farmer and attainable yields across rainfed sunflower growing regions of Argentina. Field Crops Res. 2013; (143): 119-129.
6. Jha GK, Burman, RR, Dubey, SK, Singh, G. Yield Gap Analysis of Major Oilseeds in India. 2011;6 (2): 209–216.
7. Kranthi, KR. Technologies are breaking down – What next? Cotton Statistics and News. 2015; (9):1-2.
8. Usha Rani J, Prakash, AH. Front Line Demonstration in Cotton – As a good Transfer of Technology Practice Advocated for Africa from the Experiences of India - Perspectives on Cotton Research and Ideas for Africa - Proceedings & Recommendations of the XIV Meeting of the Southern & Eastern Africa Cotton Forum (SEACF) Ed. Keshav R. Kranthi, Lawrence Malinga & Washington Mubvekeri. The ICAC Recorder. September 2018; 34(3):4-12.
9. Usha Rani S, Extension strategies to reduce the yield and knowledge gaps in cotton, Compendium of Lead and Invited papers – National Symposium on cotton production technologies in the next decade : problems and perspectives Ed. Chauhan MS, Saini RK, Manmohan, Asish J. 2019;177-184

Issues and challenges in the Cotton supply chain and way forward: An Indian perspective

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Abstract

Background: *Cotton is an important agricultural crop, responsible for employment and income generation across the world and in India too. The supply chain of cotton is very complex and is interspersed with various issues. In order to list out the possible challenges and suggest a way forward, there is a need to study the supply chain of cotton in India. The purpose of this paper is to discuss the supply chain of cotton sector in India and explain the issues which are affecting it.*

Results: *The supply chain of cotton in India mainly consists of step-by-step activities, starting from production, harvesting of seed cotton, ginning to separate lint and seed, spinning the lint into yarn, using yarn to produce fabric, using the fabric to manufacture garment, which then goes to retailers and finally to the consumers. The supply chain is fraught with various challenges, the most common of which include: Water intensive crop; Unavailability and costly labour in peak season; Inappropriate and excessive use of pesticides and fertilisers; Biodiversity depletion; Adverse impact on human heath due to pesticide exposure; Quality issues due to high contamination & trash content; Lack of appropriate market information to the farmers & their poor negotiating power; Insufficient and well-equipped warehousing facilities; Forced labour and child labour; Price volatility and market uncertainty; Stagnant productivity; Disastrous effects of inefficient waste disposal of textile mills; Stiff competition from artificial fibres; Susceptible to climate changes- flood, drought, heat, storm; Low incomes realised by the smallholder farmers.*

Conclusion: *After discussing the issues and challenges hindering the growth of Indian cotton sector, the paper tried to enlist the corresponding mitigation and adaptation strategies to overcome and deal with the identified issues. The Government needs to pay attention towards: Improved Warehousing facilities; Increased Research and development funding for biotic and abiotic stress resistant/tolerant cotton varieties; Stricter Labour laws to curb forced and child labour and ensure no gender discrimination; Setting up competitive MSP rates for cotton; adequate insurance coverage and timely payment to farmers for any crop losses; Access to protective equipments to labourers handling pesticides. The extension scientists need to conduct Trainings, Awareness Campaigns, Educational Programmes on IPM, IDM, INM, IWM, Organic Cotton, Improved crop varieties and Good Agricultural Practices; Provide timely Weather & Extension advisories, accurate and timely Market Information to the farmers; Mobilise cotton farmers into groups/unions to increase their bargaining power; Analyse the Crop Information Database and forge suitable marketing linkages to bridge the demand-supply gap; Continuous monitoring & Impact assessment study of pesticide exposure on the farmers/laborers. The importance of growing and sourcing sustainable cotton for the textile mills and retail giants has been highlighted in the paper.*

Keywords: *Sustainability, Pesticide, Forced labour, Child labour, Cotton Supply chain, Challenges, Price Volatility, Market Uncertainty, Environmental impact, Strategies*

Background

Cotton accounts for 25 per cent of the total global fibre production and is an important cash crop also in India. It is privileged to enjoy the most favoured fibre status in the Indian textile industry because of the fact that it is the major raw material contributor i.e. around 59% (Ministry of Textiles, 2018). It plays a pivotal role in sustaining the livelihood of an approximate 5.8 million cotton farmers and 40-50 million people engaged in related activities such as cotton processing and trade (Ministry of Textiles, 2017). India stands first in terms of global cotton area and production and is the second largest cotton exporting country in the world. It is cultivated in about 341.37 lakh hectares across the world and in around 124.44 lakh hectares in the country. Thus, India accounts for around 36% of the global cotton area, which is the largest in the world (Ministry of Textiles, 2019). India recorded an annual production of 348.88 lakh bales of 170 kg each in 2017-18 (DAC&FW, 2019). India is the only country in the world where all the four cultivated species- *Gossypium arboreum* and *herbaceum* (Asian cotton), *G. barbadense* (Egyptian cotton) and *G. hirsutum* (American Upland cotton) are grown on commercial scale, which is a great

advantage and can be tapped profitably. India is also a leading consumer of cotton. The average annual consumption of cotton is more than 300 lakh bales (Ministry of Textiles, 2018). The importance of cotton globally is celebrated in form of World Cotton Day, which was observed for the first time in 7th Oct, 2019 at Geneva by World Trade Organisation (WTO, 2019). This year, it will be celebrated in the World Cotton Research Conference-7 in Egypt on the same day. The details of production and consumption of cotton during the last 10 years is given in Table 1.

Table 1 Production and consumption of cotton in last 10 years (In lakh bales of 170 kg each)

Year	Production	Consumption
2009-10	305	259.00
2010-11	339	259.61
2011-12	367	375.28
2012-13	370	283.16
2013-14	398	299.55
2014-15	386	309.44
2015-16	332	315.28
2016-17	345	310.41
2017-18	370	319.06
2018-19 (P)	337	311.50

Source: Cotton Advisory Board (CAB) P-Provisional as estimated by CAB on 18.6.2019

The textile industry, which consumes cotton principally, contributes about 14% to industrial production, 4% to the GDP and 17% to the Country's export earnings. It is the second largest provider of employment (directly to about 35 million people) after agriculture (Ministry of Textiles, 2018). Thus, the growth and all-round development of this industry has a direct bearing on the improvement of the economy of the nation. The cotton crop is grown majorly in three diverse agro-ecological zones, Northern zone (Punjab, Haryana and Rajasthan), Central zone (Madhya Pradesh, Gujarat, Maharashtra & Orissa) and Southern zone (Telangana, Andhra Pradesh, Karnataka and Tamil Nadu). Cotton is also grown in small patches in non-traditional states like Uttar Pradesh, West Bengal and Tripura. The highest production is from the states of Maharashtra, Gujarat and Telangana. The details of state-wise area, production and yield is given in Table 2. Growth and development of cotton industry can lead to the overall development of the Indian economy. This sector affects significant portions of the population directly and indirectly, and thus it is evident that the sector occupies a strategic position in the development of poverty reduction policies and programmes. Given these facts, it becomes imperative to study the supply chain of cotton, in order to identify the various issues arising at various stages of the supply chain and thereby find suitable ways and means to tackle these issues. A clear picture of the supply chain relationships has tremendous potential to make difference in the quality, sustainability, delivery and cost of the final products, as well as the lives of all the stakeholders involved.

Table 2 State-wise area, production and yield

Year	Area (In Lakh hectare)		Production (In Lakh bales)		Yield (In Kgs/hectare)	
State	2017-18	2018-19(P)	2017-18	2018-19(P)	2017-18	2018-19(P)
Punjab	2.91	2.68	11.76	11.50	687.01	729.48
Haryana	6.65	7.08	21.48	23	549.11	552.26
Rajasthan	5.84	6.29	23.26	25.00	677.09	675.68
Gujarat	26.24	26.59	103.84	87	672.74	556.22
Maharashtra	43.51	42.54	83.35	77	325.66	307.71
Madhya Pradesh	6.03	6.14	22.14	24	624.18	664.5
Telangana	18.97	18.27	54.44	47	487.87	437.33
Andhra Pradesh	6.46	6.21	21.26	15	559.47	410.63
Karnataka	5.47	6.88	17.32	15	538.28	370.64
Tamil Nadu	1.83	1.31	5.5	6	510.93	778.63
Odisha	1.45	1.58	3.65	4.5	427.93	484.18
Others	0.50	0.50	2	2	680	680
All India	125.86	126.07	370	337	499.76	454.43

Source: Cotton Advisory Board (CAB) P-Provisional as estimated by CAB on 18.6.2019

Results

Supply Chain of Cotton

Supply chain is a system of actors, activities, information, resources, functions, processes, and organizational ties involved in supplying a product or service to a consumer. Supply chain management encompasses the planning and management of all activities involved in sourcing and procurement,

transformation of natural resources, raw materials, and components into a finished product that is delivered to the end customer, and all logistics. Importantly, it also includes coordination and collaboration with channel partners, which can be suppliers, intermediaries, third party service providers, and customers (Irina et al. 2015). Supply chain is an integration of key functions of marketing channels, logistics, purchasing, and operations within and across multiple firms (Frankel et al. 2008; Hult, Closs, and Frayer 2014).

The Indian cotton supply chain, which begins with fibre production, post procurement of raw cotton, culminates in textile and garment products. It is very complex in structure with a profusion of numerous small-scale, decentralised and fragmented units along with some large-sized integrated enterprises (composite mills). The small-scale units are largely unorganised and labour-intensive unlike the organised and capital-intensive large-scale enterprises. The supply chain of cotton is comprised of various steps of production, harvesting at field level, ginning & pressing, classification and storage, spinning, and marketing the fabrics, which undergo further treatments like weaving, knitting, dyeing, finishing, etc and later used for garment manufacturing. The cotton supply chain has been diagrammatically presented below in Fig 1.

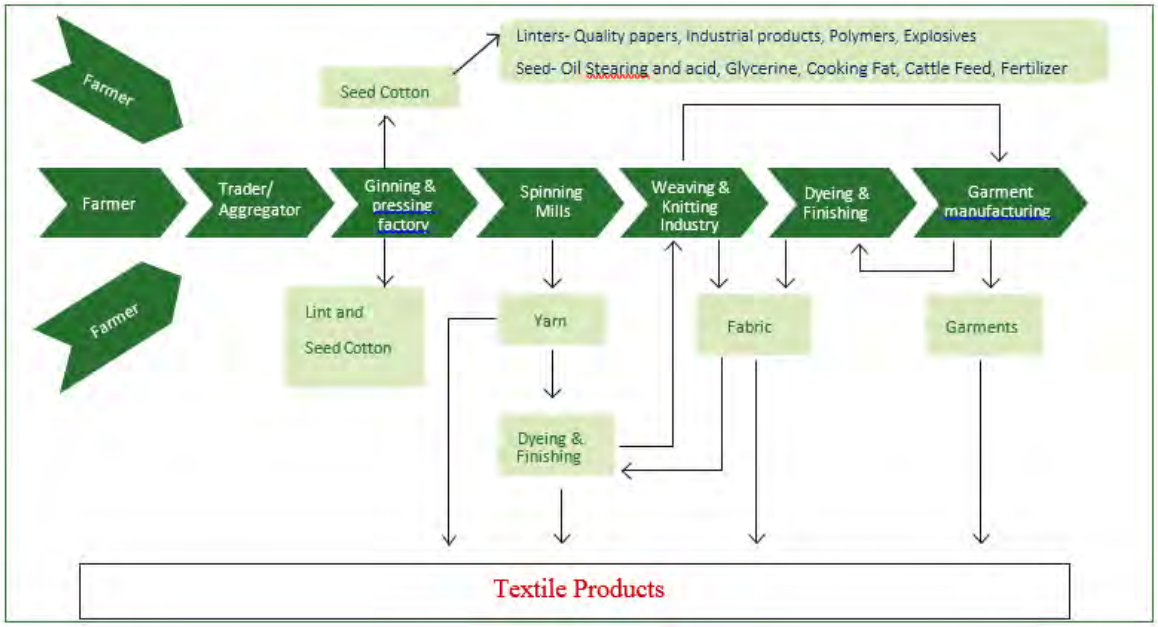


Figure 1: *Cotton supply chain in India*

The cotton supply chain can be broken down into the following steps:

Production and Harvesting

The cotton farming in India is mainly done through regular farming or contract farming. Contract farming of cotton crop has an important place in India. Many agencies like Cotton Corporation of India and private mill consumers have entered into contract farming in a big way. The parties involved in contract farming are producers (farmer groups), co-ordinating agencies (textile mills/ ginning factories), input suppliers, technology providers and facilitators (State Departments of Agriculture), credit and insurance agencies.

A co-ordinating agency enters into a contractual agreement with the producer to cultivate a particular variety of cotton required by it and promises to purchase the entire crop harvested by the farmer(s) at pre-agreed price with premium for quality. The farmer group in turn agrees to sell the cotton exclusively to the co-ordinating agency. The farmer group is then instructed by a team of experts engaged by the co-ordinating agency on how to cultivate cotton with the technical advice on crop management including IPM practices. The coordinating agency also ensures timely supply of quality inputs including seeds, fertilisers, plant protection chemicals, manures, bio-pesticides, etc. to the farmers at affordable prices. It also helps the farmers to secure institutionalised credit and insurance at low premium rates. Contract farming results in a win-win situation for all the stakeholders as farmers are assured of market and remunerative prices, the industry gets supply of quality raw material and risks are minimised for financial institutions, banks and insurance companies. Technology Mission on Cotton in India has offered huge support to farmers under its various contract farming schemes.

The cotton can either be picked by hand or by machines. In India, farmers still rely heavily on hand picking unlike Australia, Israel and U.S.A where it is completely machine-picked (Hazarika, 2012). Over 90 per cent is machine picked in Greece, Mexico and Spain. Almost 75 per cent of total production is machine- picked in Brazil (DAC&FW, 2017). Normally, farmers go for two to five pickings of cotton till the final stage of crop harvesting. It is observed that 85 per cent of the seed cotton (kapas) is picked during the initial three pickings and the subsequent pickings may not be economical.

Cotton picking is not only tedious but ten times costlier than irrigation and twice of weeding operation. The cost of picking accounts for 30 to 35 per cent of the total cost of cultivation. During the peak season of cotton picking, the labour availability becomes even more scarce. Due to unavailability of labourers when required, cotton picking gets delayed causing yield loss which may be up to 15 per cent and also affecting the overall quality of lint. After picking, it should be sun-dried for 3-4 days with proper care and should be stored in a clean and dry place.

Trader/Aggregator buys seed cotton from several farmers and combines into bulk.

The Agriculture Produce Marketing Committee (APMC) is the primary market infrastructure in the country through which cotton is marketed. APMC regulates market practices such as weighing, process of sale, method of grading, payment process, and also provides storage, boarding and lodging facilities for buyers, sellers, etc. Cotton in India is primarily marketed through three marketing agencies:

Private Sector (traders, ginners, etc.)

Public Sector (Cotton Corporation of India, Maharashtra State Co-operative Cotton Growers Marketing Federation)

Co-operative Sector

Only about 20% of the cotton transaction is done in the Public and the Co-operative Sector, while the remaining cotton is handled by the Private Sector. In private setup, farmers sell cotton directly to ginners, primarily in the form of kapas (raw cotton or seed cotton). Aggregators collect raw cotton from farm gate of 10-15 farmers and sell the consolidated produce to ginners in a radius of 100-150 km. The produce is bought by stockists, ready dealers, ginners, brokers or commission agents, etc.

Cotton Corporation of India (CCI), a Government Organisation, makes purchases to the extent necessary, as part of its price support operation and not for maintaining buffer stock of cotton. To check sharp fluctuations in cotton prices, CCI can undertake necessary export / import operations.

Co-operative societies established in cotton sector not only undertake the marketing of cotton but ginning and pressing too. The co-operatives provide the members with credit, agricultural inputs, and sell the produce to the mills. The credit advanced to the grower members is recovered from the sale proceeds after marketing the cotton.

Ginning factories undertake drying and cleaning activities of the seed cotton for separation of lint (ginned fibre) and seed.

The fibre or lint is later pressed and compressed into dense bales, which is used in making cotton cloth. Linters – the short fuzz on the seed – provide cellulose for making plastics, explosives and other products. Linters also are incorporated into high quality paper products, padding mattresses, polymers, furniture and automobile cushions. The cottonseed is crushed in order to separate its three products – oil, meal and hulls. Cottonseed oil is used primarily as a cooking oil and salad dressing. The meal and hulls are used either separately or in combination as livestock, poultry and fish feed or as fertilizers.

After ginning, cotton is generally shipped to a warehouse/gin yard and sampled to establish the fibre characteristics and quality.

This is called Cotton classification, or classing, the process of describing the quality of cotton in terms of grade, staple length and percentage of contamination. Graded cotton can then remain in storage for extended periods.

The fibre is then supplied to spinning units who spin the lint into thread (yarn).

The fabric mills knit or weave the yarn into fabric and undertake dyeing activities.

Trader buys the fabric and sells to garment manufacturers, who further may have subcontractors to dye, launder or embellish their products.

Manufacturers create the products/textiles for retailers. Products appear on shelves at the retail store, which is ultimately bought and used by the consumers.

To put the supply chain of cotton simply, flow of cotton lint is follows:

Grower → Ginner → Yarn Spinner → Fabric Mill → Garment Manufacturer → Retailer → Consumer

The supply chain of cotton is not linear as it seems from above, but is much more complicated. Large volumes of raw cotton are aggregated from many farmers through numerous local intermediaries before reaching to local mills or to major merchants. Many agents, intermediaries, controllers and supervisors of shipments are involved. Big merchants (traders) buy cotton from different parts of the world and sell it through global markets. A ginning mill receives produce from multiple traders. Spinners use a mixture of cotton that ranges in origin and quality (and cost) to produce yarn. Fabric mills take a similar approach to produce the final fabric. Garment manufactures may have varied sources and quality of fabric. Retailers may source the same product from a variety of garment manufacturers. In addition to value chain actors, there are financial, reputational and other influences, like input providers, traders, Government, NGOs, etc. that affect the cotton supply chain. When we put this in a global perspective, we can visualise that the cotton supply chain is really a very complex web of players worldwide.

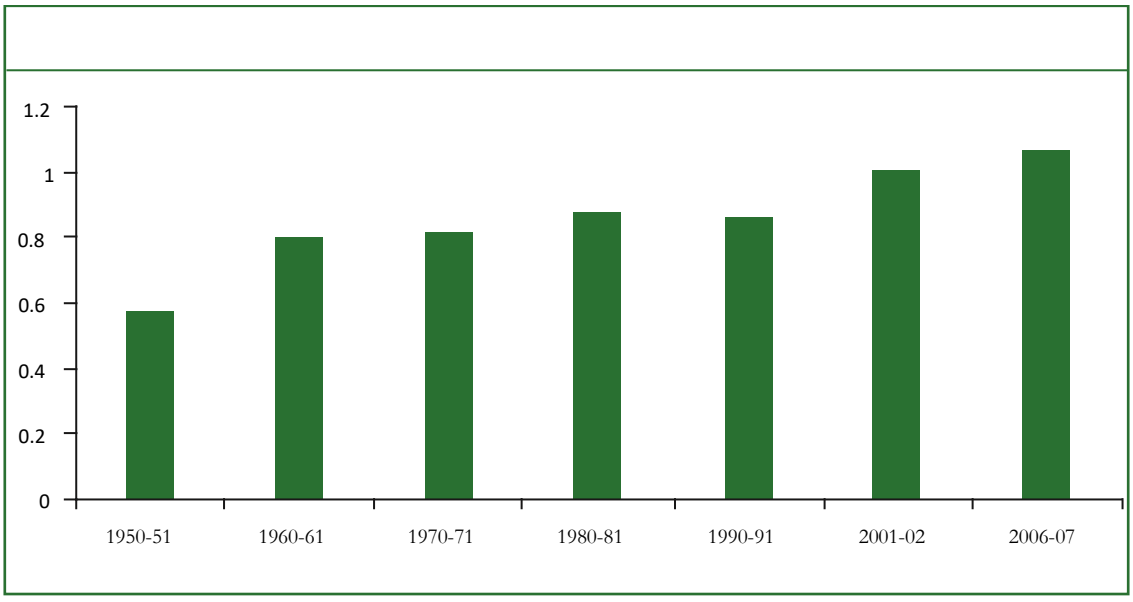
Challenges in the Cotton Supply Chain

Thirsty crop

Cotton is a particularly demanding and energy-intensive crop. The sheer quantity of resources it consumes is a serious problem. Cotton is a thirsty plant. The World Wildlife Fund (WWF) estimates it takes 20,000 litres of water to produce 1 kg of cotton; equivalent to a single t-shirt and pair of jeans. Almost 62 per cent of the area under cotton in India is rainfed, mainly in the southern and central states (Ministry of Textiles, GoI, 2019), with erratic and poorly distributed rains during the cropping season. Satiating the needs of cotton crop can lead to compromising of water security severely in such areas.

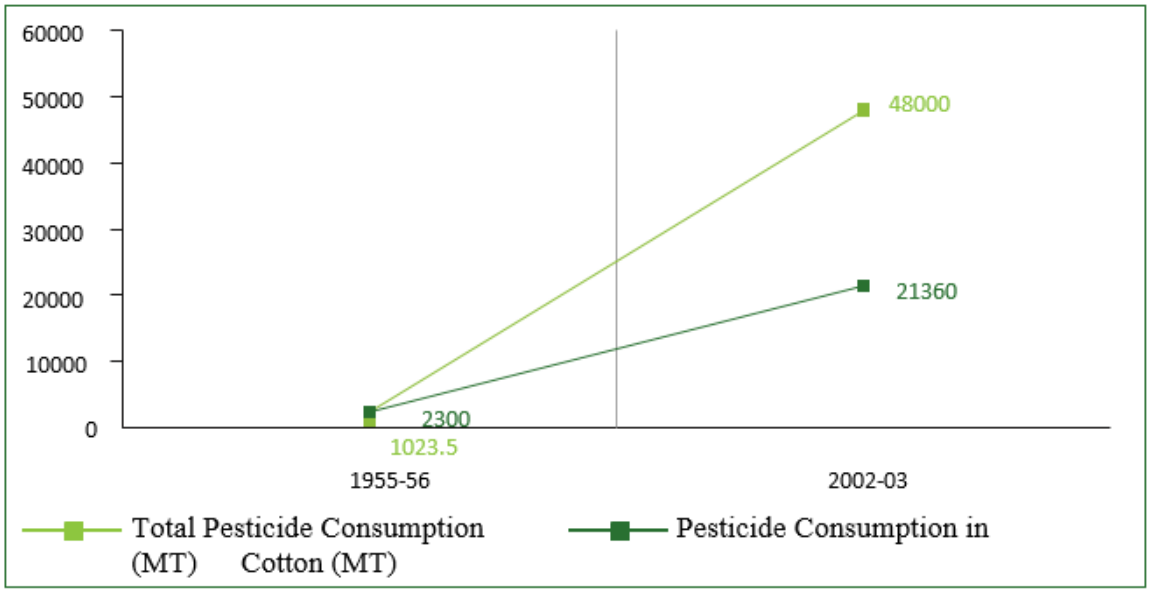
Use of fertilisers and pesticides

The crop is subjected to severe attack of pests and diseases. Thus, cotton cultivation uses a lot of fertilisers (Figure 2) and pesticides (Figure 3), which negatively impacts the health of soil, water, biodiversity and the health of farmers.



Source: WWF, 2012

Figure 2: Fertilizer consumption over years, million metric tons



Source: PK Gupta, 2004

Figure 3: Cotton and total consumptions of pesticides in India, metric tonnes

Cotton crop which makes up 2.4% of the world's crop land, accounts for a staggering 24% and 11% of global sales of insecticide and pesticides, respectively (WWF, 2020). In India, cotton accounts for 54% of all pesticides used annually – despite occupying just 5% of land under crops (EJF, 2007). The proportion of global insecticide sales for cotton was 18.4% in 2003 and 14.1% in 2009 (cited in FAO report, 2015). The farm workers work in cotton fields with limited safety precautions to protect them from hazardous pesticides (Pesticide Action Network, 2011). Endosulfan is a commonly used pesticide for cotton, which can cause a range of health problems for humans by attacking the central nervous system (EPA, 2012). Cotton production can adversely affect the environment by releasing these chemicals in water (Allwood et. al., 2006; WWF Global, 2003). In a 5-month observation period, 97 cotton farmers of Andhra Pradesh experienced 323 separate incidents of ill health. Of these, 39% were associated with mild poisoning, 38% with moderate poisoning, and 6% with severe poisoning (Mancini et al. 2005).

Stagnant productivity

Despite the increase in production, cotton crop has been experiencing a plateau in productivity for quite some time, which needs to be broken. During 2018-19, India's Productivity is estimated at 454.43 kg/ha. In terms of productivity, India ranks poorly compared to USA (955 kg/ha) & China (1764 kg/ha) (Ministry of textiles, 2019). The yields of cotton in India are much below the world average and less than half of USA's cotton yield (Figure 4).

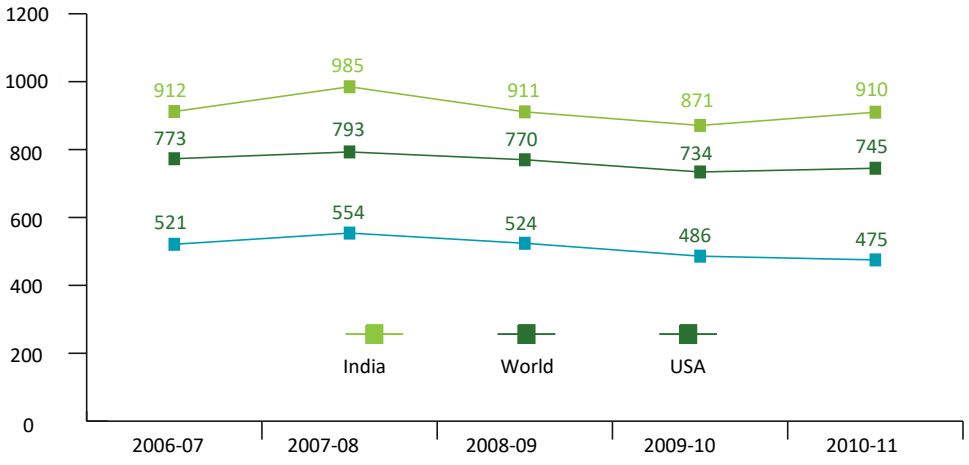


Figure 4: Comparison of cotton productivity (Source: Cotton Advisory Board, 2012)

There is potential for increasing the productivity through continuous improvement in land use efficiency and following of the good agricultural practices. The productivity of cotton in India for the last 10 years is given in Table 3.

Table 3 Productivity of cotton for the last 10 years

Year	Acreage (In lakh hectare)	Productivity (In Kg /hectare)
2009-10	103.10	502.91
2010-11	112.35	512.95
2011-12	121.78	512.32
2012-13	119.78	525.13
2013-14	119.60	565.72
2014-15	130.83	493.77
2015-16	122.92	459.16
2016-17	108.26	541.75
2017-18	125.86	499.76
2018-19 (P)	126.07	454.43

Source: Cotton Advisory Board (CAB) P-Provisional as estimated by CAB on 18.6.2019

Contamination

Though the cotton produced in India possess very good properties in terms of fibre characteristics, yet it suffers from high level of contamination and trash content. There are inconsistencies in the strength, length, micronaire, colour and reflectance of cotton as different varieties of cotton fibre with different physical properties are mixed together. Admixture also makes the grading and testing of cotton very difficult. The high incidence of trash and contamination in cotton affects both quantity and quality, as it reduces yarn realization on the one hand and causes high level of yarn imperfections on the other.

Contamination with non-cotton fibres is a major problem for mills as it affects their processing and dyeing abilities. In many states, the warehousing facilities are inadequate or are poorly managed. Required shade space or godown to keep the cotton is not available in most of the ginning units. Some farmers keep the produce in the open, day and night. It is also one of the greatest reasons for production of more contaminated cotton.=

Climate change

The quantity and quality of cotton is increasingly affected by the impacts of climate change, as cotton growing regions experience more frequent floods, droughts and extreme heat and storms. This presents difficulties across the entire supply chain of cotton. If rain comes during the harvesting season, the cotton becomes wet in the vicinity of ginning and pressing areas and in market yard, where it is kept for selling or ginning purpose. The percentage area insured under all insurance schemes have risen from 12.10 % (15.32 lakh ha out of the gross cropped area of 126.60 lakh ha) in 2014-15 to 20.55 % (26.02 lakh ha out of the gross cropped area of 126.60 lakh ha) in 2017-18 (DAC&FW, 2018).

Dangerous dyeing process

The dyeing process for cotton is highly chemically intensive. Irresponsible use and disposal of dyes or chemicals used in the fabric dyeing or garment manufacturing process can have devastating environmental consequences. Chemicals that have not been properly treated before disposal can lead to serious pollution problems. For example, the East River in Xintang, China, whose factories produce 300 million denim articles per year, has turned blue due to wastewater from dyeing being dumped directly into the river (Cable News Network CNN, 2010).

Price volatility and Market uncertainty

The cotton price changes every day and due to the inadequate networking/communications, sometimes farmers are quoted the lowest price by the traders as they do not have much knowledge on current price. The price of cotton can be volatile, due to a range of factors such as national regulation, stockpiling, and government subsidies for farmers. This, combined with other factors, creates an uncertain market for farmers. Restrictions on exports and frequent changes in the policy have hurt cotton trade and resulted in the country being side-lined in the international cotton market. The financialization of cotton is a lesser-known issue significantly impacting the stability of cotton markets. Where these markets were once used to manage risk, they are now used in times of low returns in conventional stock markets and investments as a source of profit. This results in significant fluctuations in price and therefore instability in the cotton price, despite having no real connection to physical supply and demand.

Forced labour and Child Labour

Cotton growing is known as less mechanised farming in India, so needs cheap labour. Forced labour is still rife in cotton. Labour issues in the cotton supply chain have grown beyond that of poor working conditions and overworked, underpaid and underaged employees.

Child labour is a particular issue because much of the supply chain requires low-skilled labour and some tasks are even better suited to children than adults. In cotton picking, employers prefer to hire children for their small fingers, which do not damage the crop. Children work at all stages of the supply chain: from sowing, weeding, harvesting, yarn spinning, right to the different phases of putting garments together in factories. They are subjected to long working hours, exposed to pesticides and they are often paid below the minimum wage (SOMO report, 2014).

One of the biggest challenges in tackling child labour in the fashion supply chain is the complex supply chain for each garment. Even when brands have strict guidelines in place for suppliers, work often gets sub-contracted to other factories that the buyer may not even know about. Various accreditation schemes exist (Table 4), such as the Fairtrade Label Organisation, the Global Organic Textile Standard and the Ethical Trading Initiative, but all of them struggle with the lack of transparency in the textile and garment supply chain (UNICEF, 2015).

Stiff competition

The traditionally produced hand spun and hand-woven textiles are facing stiff competition from the fast changes in fashion trends in this dynamic era and there is fear of the death of the ancient indigenous textile forms and handlooms. Cotton fibre is increasingly facing competition from artificial fibres, notably polyester. This is attributed mainly due to rising price volatility in cotton trade and variations in fibre characteristics, because of genetic, environmental, harvesting and ginning factors.

Table 4: Global initiatives towards sustainability in cotton production

Initiatives	Standards	Highlights
Better Cotton Initiative (BCI)	Environmental and Decent Work Conditions	Formation of learning groups, self-assessment, creation of chain of custody in supply chain, no premium for the produce
Fair Trade (FT)	Labour, Development, and Environmental	Product certification system, social premium for doing social/ community development activity
Organic Cotton	Environmental	Product certification system, premium for the quality produce

Question of sustainability

In retail chain, present day requirement is the concept of sustainable cotton use. Sustainability is a rising concern for consumers, especially those in emerging markets such as China, India, and Mexico, highlighting the need for brands to address the environmental impact of their products. Producing more ethical products requires full knowledge of the supply chain to ensure that environmental and social practices are respected by all. In an era where consumers are more mindful of their purchases, supply chain transparency is of utmost importance. The volume of sustainable cotton fibre has increased significantly over the years, which can be clearly visible from the Table 5.

Table 5 Volume of sustainable cotton fibre (in 1000 metric tonnes)

Years	Volume of sustainable cotton fibre (in 1000 metric tonnes)
2008	163
2009	232
2010	332
2011	432
2012	933
2013	1052
2014	2173
2015	3000

Source- WWF, 2016

Discussion

A study by Aditya Shrinivas in Vidharbha region of Maharashtra, reported that cotton prices were very volatile. Analyzing data from 2002-12, he found, on average, a farmer selling 50 quintals of raw cotton

would earn an additional 2,300 rupees if domestic prices increased by 250 rupees per quintal. However, if prices dropped by the same amount, the farmer would lose 3,464 rupees. Lack of pricing information hurts the interests of farmers as they can not bargain with the middlemen who control the market. Other challenges the cotton farmers face are the oligopsony structure of market yards—many sellers and few buyers, and the absence of farmer cooperatives in Vidarbha, which leads to reduced farmers' negotiating power with traders.

India was the only country named by the U.S. Department of Agriculture report in 2016 for having child and forced labour in both cottonseed production as well as cotton growing. A 2014 study by Davuluri Venkateswarlu, Glocal Research, 2014 found that 200,000 children under 14 were working on cottonseed farms, double the number since 2010, particularly in the seed production sector (Thomson Reuters, 2019).

A report by the Centre for Research on Multinational Corporations (SOMO) and the India Committee of the Netherlands (ICN) revealed that recruiters in southern India convince parents in impoverished rural areas to send their daughters to spinning mills with promises of a well-paid job, comfortable accommodation, three nutritious meals a day and opportunities for training and schooling, as well as a lump sum payment at the end of three years. Their field research shows that “in reality, they are working under appalling conditions that amount to modern day slavery and the worst forms of child labour”. It was found that 60 per cent of workers at the mills in India were under 18 when they started working there. In garment factories, children perform diverse and often arduous tasks such as dyeing, sewing buttons, cutting and trimming threads, folding, moving and packing garments. In small workshops and home sites, children are put to work on intricate tasks such as embroidering, sequinning and smocking (making pleats).

Eighty-six per cent of consumers across the world and 81 per cent in India express concern for sustainability. Consumer concern for sustainability affects shopping behaviour as 63 per cent of consumers globally – including 81 per cent of consumers in Indian markets – put time and effort into finding sustainable clothing. Consumers think of sustainability in terms of preserving – or at least not damaging – the environment. (Cotton Incorporated Report, 2018).

Sustainable cotton systems typically share common benefits, including: reduced use of hazardous chemicals on farms, or safer handling and more efficient use where chemicals are still used; less water use for irrigating cotton crops, which can benefit other local water users and natural habitats, particularly in water-stressed regions; reducing poverty for cotton farmers and workers on cotton farms through higher yields (cottonupguide.org).

There has been an increase in organic cotton growth, which clearly expresses a wider sustainable textile strategy by major retailers and manufacturers (Ferrigno & Lizarraga et al., 2009). Organic cotton production has been continuously increasing. As per the data reported in the Organic Exchange Farm and Fiber Report (2009), organic cotton is grown in 22 countries globally with the top ten producing nations led by India, followed by Turkey, Syria, Tanzania, China, United States, Uganda, Peru, Egypt and Burkina Faso.

Adidas, Ikea, and H&M have been recognised for efforts promoting sustainability in the cotton sector. The three retail giants have topped the 2020 Sustainable Cotton Ranking amongst the 77 global companies, compiled by Pesticide Action Network UK and WWF. The ranking was based on their policy (on pesticides, water, biodiversity, human rights, and recycling), traceability (sourcing targets, transparency of the cotton supplier) and uptake or percentage of sustainable cotton. The major British retailer 'Marks and Spencer' has pledged to increase their amount of Fair-trade and organic clothing and support fabrics that can be recycled more easily (BBC News, 2009).

The organic farming project by Green hotelier has helped to restore soil fertility in an area where the soil and water table were depleted, benefitting 16,000 farmers in six districts of Maharashtra and Odisha. The company driving the project buys a majority of the farmers' produce at a fair premium, then transports it from farm to market. This approach benefits the farmers directly, as logistical problems and intermediaries that cut into their profit are eliminated. The company sells the cotton to brands and textile companies in Vietnam, Germany and Canada, as well as the domestic market. A focus on farmer training and education has also helped to promote the use of superior varieties of cotton. These measures, combined with seeds grown in-house and natural pesticides, have helped cut input costs for farmers by up to 30 per cent. A percentage of profits are also used for social welfare activities, from supporting women to set up micro-enterprises, to encouraging environmental improvements which in turn can support additional crops and income. (greenhotelier.org)

WWF-India's Sustainable Cotton Project is working towards developing improved sustainable cotton production systems, in which farmers, by adopting Better management practices (BMPs), are equipped to produce quality cotton by using environment friendly organic fertilisers produced from locally available resources. The project started in 2007 with a few farmers in Warangal and now has presence in different agro-climatic cotton-growing regions of the country—Andhra Pradesh, Maharashtra, and Punjab. By using BMPs, these farmers managed to reduce their water application by 30–51 per cent pesticide application by 38–80 per cent, chemical and fertiliser use by 32–53 per cent and GHG emissions by 57 per cent on an average, as compared to the farmers who were engaged in conventional farming and did not use BMPs that year. The cotton produced under WWF-India's BMPs is procured by the local suppliers of the global brands and retailers, such as IKEA and Marks and Spencer. Thus, a supply chain is ensured, focusing on production and uptake of sustainable raw materials. (WWF, 2010)

The suggested strategies on part of various fronts- Government machinery, Social Scientists and the Retail Giants to deal with the issues listed out in this paper can be summed up in the Table 6.

To sum up,

- Accelerate contract farming and make appropriate arrangements with farmers and Sanctity of contracts to be fully observed.
- Hedging facilities for farmers through cotton futures, enabling them to choose appropriate varieties of cotton for sowing and better price realization
- Farmers to have ready access to modern production technology, pure and good quality seeds, essential inputs and credit through networking and link up with concerned agencies including research and development organisations

Table 6 Strategies and Way Forward

Government Steps

Improved and adequate warehousing facilities
Research for pest and disease resistant, climate resilient cotton varieties
Dedicated Fund allocation for Cotton machinery
Stricter Labour laws to curb forced and child labour
Ensure no gender discrimination
Set up competitive MSP rates for cotton
Access to protective equipments

Social Scientists

Accurate and timely Market Information

Timely Weather Advisories Trainings, Awareness Campaigns, Educational Programmes on IPM, INM, IWM, Organic Cotton, Improved crop varieties and Good Agricultural Practices Mobilise cotton farmers into groups/unions to increase their bargaining power Analyse the Crop Information Database and forge suitable marketing linkages Continuous monitoring & Impact assessment of pesticide exposure

Retail Giants

Maintain supply chain transparency
Follow Reuse and Recycling of the produce
Folow the labour laws ethically & stringently

- Awareness campaign regarding the Minimum Support Price (MSP) rate through advertisements in newspapers, radio and TV, distribution of pamphlets to individual farmers and displaying banners in APMCs in order to avoid distress sale of cotton by farmers and also create awareness of the quality parameters in local languages
- Awareness campaigns amongst farmers may be conducted encouraging them to adopt best pre and post-harvest practices to minimise contamination and keep the moisture content upto 8 per cent, which will fetch the best price in market.
- Processing of bales in renovated, modernised factories accredited by the Technology Mission on Cotton
- Quality evaluation only through instrument testing unlike by visual inspection in earlier times
- Continued thrust on sustained improvement in quality through modernisation of the Ginning and Pressing factories and market yards
- Farmers should be made aware of the Government schemes for cotton so as to avail benefits from the same. Under National Food Security Mission (NFSM), Cotton Development Programme is being implemented in 15 major cotton growing states with an aim to enhance production and productivity. The major activities implemented under the scheme are transfer of latest technology to cotton growers through Front Line Demonstration (FLD) on Integrated Crop Management (ICM), Intercropping & Desi/Extra Long Staple Cotton and trials on High Density Planting System.
- Extension workers can play a major role in dissemination of knowledge through training programmes among the cotton farmers regarding the various crop management practices like Integrated Pest Management (IPM), Integrated Disease Management (IDM), Integrated Nutrient Management (INM), Integrated Water Management (IWM). Following such practices will lessen the burden of inputs, reduce cost of cultivation, reduce negative impact on environment while increasing productivity.
- The farmers should be taught to utilise the organic inputs (like tank silt application, crop residues, vermicompost, farm yard manure, etc.) available at the farm level in conjunction with inorganic fertilisers, which have to be applied only after the soil testing and nutrient profiling of the soil.
- There should be frequent farmer-scientist meetings to discuss the various crop activities and impart them knowledge on the mix of application of various cultural, mechanical and judicious chemical practices; timely sowing, Use of good quality genetically pure cotton seeds, use of pest and disease resistant varieties/hybrids, monitoring of insects, insecticides spray schedule, etc. to control the commonly emerging pest and diseases in cotton. Farmers should also be informed about the classification of the commonly available pesticides as ‘Extremely Hazardous’ or ‘Highly Hazardous’ as per the World Health Organisation (WHO) standards.
- Efforts should be made through extensive education programmes to raise awareness of the problems arising due to excessive use of fertilisers and pesticides and instead, promote organic cotton production. Research in non-chemical pest management, and research in seed varieties adapted to organic agriculture should be encouraged and promoted. Social scientists should make efforts to raise awareness of organic cotton production techniques and its benefits. Switching to organic cotton would directly benefit both the local and global environment, it will have lighter environmental impact.
- The scientists may conduct an urgent assessment of the impact of cotton pesticides on the health of the cotton farming communities and should regularly monitor the occurrence of pathological exposures to pesticides.
- Social scientists may spread awareness and knowledge among the farmers about the various climate resilient cotton varieties. Government should also invest more funds towards research and development of high yielding varieties, biotic and abiotic stress tolerant/resistant varieties for increased productivity and reduced crop losses.
- The Government should ensure that all agricultural workers involved in cotton production have adequate access to protective equipments, and receive training in the responsible use of hazardous cotton pesticides.
- Government should ensure that the cotton farmers should be adequately covered under the insurance schemes, so that they are protected from the unforeseen setbacks such as pest and disease invasion, crop failure and natural disasters.
- Social scientists can also play a major role in sending timely advisories to the farmers regarding the market information by disseminating the Minimum Support Price (MSP) rates to cotton farmers. There should be regular and parallel dissemination of advisories through electronic, print & mobile platforms to all the stakeholders.

- Awareness campaigns can be organised time and again to educate the farmers on the importance to ensure quality of the produce. Emphasis should be given to clean cotton picking practices that reduce the likelihood of contamination. Separate ergonomically designed aprons should be provided to the women labourers for reducing the contamination of hair and threads from polypropylene bags.
- Rate of the cotton is decided by the quality of the produce. Well-designed and adequate space of warehousing facilities should be available in the state which can be used before and after ginning to reduce chances of contamination. The trash and contamination level can be reduced significantly by improving work practices and by modernization of existing ginning and pressing factories and market yards.
- Indian policymakers can shape a better functioning market by eliminating the information asymmetries and levelling the information playing field. They need to invest in an effective system so that market information gets to the farmer. Because this information, if well managed by farmers, farmer cooperatives and producer groups, may empower them to be in a better position to negotiate with the traders.
- The real time data and scientific assessment through satellite-based cotton crop assessment system of ISRO can prove beneficial to arrive at realistic crop conditions and assessment of cotton availability in the country. The next step is to forge marketing linkages or buy- back arrangements from the surplus areas to the demand areas, and thus try to bridge the gap between demand and the supply.
- Mechanical picking machines can be introduced to minimise the drudgery involved in hand-picking. Mechanical picking will also enhance the production of cleaner grade of seed cotton. Further, mechanical cotton-picking system will also be helpful in achieving timeliness of operation, for the next crop.
- The state should train officials from various government departments, including labour and education, to identify and stop any form of forced labour and gender discrimination in cotton fields, with an aim to promote social dialogue and empower workers in keeping with the fundamental principles and rights of the workplace.
- Labour injustices and sustainability issues can often be traced several levels down the supply chain, where many brands lack visibility. Supply chain transparency is of utmost importance. Brands need to discover their supply chains from source to store, identify uncertified suppliers and ensure following of strict environmental and labour guidelines.
- Big fashion retail outlets should implement the eco-friendly practices in its supply chain to reduce the use of energy, water, chemicals and other materials in the apparel industry. This includes sourcing 100% sustainable or recycled cotton.
- Green marketing: Moving toward “circular” manufacturing, which emphasizes recycling clothes and reusing resources.
- Since the cotton industry is mostly an unorganized sector, the workers are not adequately represented by the trade unions. So, its suggested to form trade unions which will provide opportunity to represent the unorganized sector workers.
- Changes to traditional supply chain metrics include giving equal weightage to sustainability and social measures, like increased income levels through Fairtrade minimum price and premium, gender equality and poverty alleviation.
- In order to enhance the productivity and quality of Indian cotton and making it competitive globally, we need to work towards: (i) bringing down the cost of cultivation and enhancing its productivity and quality, (ii) rendering our cotton globally attractive, (iii) keeping Indian cotton free of trash content.

Conclusions

Cotton being called as White Gold, is an important cash crop, which provides fibre, fuel and food (such as cotton seed oil). It is the basic raw material for Textile Industry. The Indian Textile Industry has an overwhelming presence in the economic life of the country and plays a pivotal role through its contribution to industrial output, employment generation, and the export earnings of the country. India’s cotton sector is a sunrise sector and presents huge opportunities for the agribusiness, small-scale industries and thereby, the economic and social development of the rural areas through a well-established, sustainable cotton supply chain. However, there are various factors affecting the supply chain as discussed above which pose serious challenges for this sector in particular and to the Indian textile industry in general. Cotton cultivation can have negative environmental and social impacts. These range from indiscriminate chemical usage to child and bonded labour. These require immediate

attention and need to be curbed, so as to provide impetus to supply chain of cotton sector. The solution to this lies in sustainable cotton. Sustainable cotton is grown in a way that can maintain levels of production with minimal environmental impact, can support viable producer livelihoods and communities, and can do so in the face of long-term ecological constraints and socio-economic pressures. It has therefore enormous potential to lift millions of people out of poverty by providing a more stable income and improved working conditions, which leads to the development of Indian economy as a whole. Reducing the land, water and social impacts of cotton production will considerably play a part in moving the world closer to achieving the UN's Sustainable Development Goals (SDGs).

Methods

Descriptive research has been used for this study. The present study undertakes a thorough review of basic and contemporary literature available on the various facets of cotton sector in India. The supply chain of cotton sector in India has been explained and attempt has been made towards identifying the issues affecting the supply chain of this sector. And effort is made to suggest some suitable strategies to overcome or mitigate such issues in future. The paper is based upon secondary data collected from Textile Committee, Ministry of Textile, Government of India; Dept. of Agriculture, Government of India and from other published sources. Along with this, research report of various government agencies, research papers from peer-reviewed journals, conference proceedings, white papers and presentations from the industry were also collected, which were reviewed for this study.

References

1. Allwood, J. M., Laursen, S. E., & Bocken, N. M. (2006). Well Dressed? Institute for Manufacturing. Cambridge: University of Cambridge.
2. BBC News. (2009, February 20). Bid to buck 'fast fashion' trend. Retrieved August 17, 2012, from BBC News: http://news.bbc.co.uk/2/hi/uk_news/7899327.stm
3. Chang, E. (2010, Apr 28). China's famed Pearl River under denim threat. *CNN News.* Retrieved from http://edition.cnn.com/2010/WORLD/asiapcf/04/26/china.denim.water.pollution/index.html
4. Cotton Advisory Board. (2012). *Cotton balance sheet.* Retrieved from https://cotcorp.org.in/ statistics.aspx#area
5. Cotton Advisory Board. (2019). Retrieved from http://www.caionline.in/articles/cotton-advisory-board-cab-pegs-2018-19-cotton-crop-at-36-1-mln-bales
6. Department of Agriculture, Cooperation & Farmers Welfare. (2017). Statistical Year Book India 2017. Retrieved from http://mospi.nic.in/statistical-year-book-india/2017/177
7. Department of Agriculture, Cooperation & Farmers Welfare. (2018). *Agricultural Statistics at a glance 2018*. Retrieved from http://agricoop.gov.in/sites/default/files/agristatglance2018.pdf
8. Department of Agriculture, Cooperation & Farmers Welfare. (2018). *Annual report: 2018-19*. Retrieved from http://agricoop.nic.in/sites/default/files/AR_2018-19_Final_for_Print.pdf
9. Environmental Justice Foundation. (2007). *The deadly chemicals in cotton*. ISBN No. 1-904523-10-2. Retrieved from https://ejfoundation.org/resources/ downloads/ the_deadly_ chemicals_ in_ cotton.pdf
10. FAO. (2015). *Measuring Sustainability in Cotton Farming Systems: A guidance framework.* ISBN 978-92-5-108614-8. Retrieved from http://www.fao.org/3/a-i4170e.pdf
11. Ferrigno, S., Lizarraga, A., Nagarajan, P., & Tovignan, S. (2009). Organic Cotton Farm and Fiber Report. Retrieved from http://farmhub.textileexchange.org /upload /library/ Farm%20 and%20fiber%20report/Farm_ Fiber%202009%20-%20Executive%20Summary_final.pdf
12. Frankel, Robert, Yemisi A. Bolumole, Reham A. Eltantawy, Antony Paulraj and Gregory T. Gundlach (2008), "The Domain and Scope of SCM's Foundational Disciplines – Insights and Issues to Advance Research," Journal of Business Logistics, 29 (1), 1–30
13. Gupta P K. (2004). *Fundamentals of Toxicology: Essential concepts and applications*. 198(1-3): 83-90. Academic Press. ISBN: 9780128054031
14. Hart, C. (2020, Feb 19). Adidas leads the way in sustainable cotton use. *Supply Chain, Sustainability,* Retrieved from https://www.cips.org/en-SG/supply-management/news/2020/february/adidas-leads-the-way-in-sustainable-cotton-use/
15. Hazarika, M. (2012). *The green cotton fields back home.* Peace by peace cotton – supporting smallholder cotton farmer households in Odisha. Retrieved from https://www.jica.go.jp/india/english/office/about/ngodesk/c8h0vm0000a2nsnz-att/ngodesk_03_01.pdf
16. Hult, G. Tomas M., David Closs and David Frayer (2014), Global Supply Chain Management, New York, NY: McGraw Hill
17. Irina V.,Tomas M., Donald J., Jeannette A. & Pinar K. 2015. The Role of Marketing Channels in Supply Chain Management. *Journal of Retailing*. 91 (4): 586–609. doi:10.1016/j.jretai.2015.03.003.
18. Mancini et al. 2005. Acute pesticide poisoning among female and male cotton growers in India. International Journal of Occupational Environmental Health 11: 221-232 Retrieved from http://www.ijoeh.com/pfds/IJOEH_1103_Mancini.pdf
19. Ministry of Textiles. (2017). *Annual report: 2016-2017.* Retrieved from http://texmin.nic.in/sites/default/files/ar_16_17_ENG.pdf
20. Ministry of Textiles. (2018). *Annual report: 2017-2018.* Retrieved from http://texmin.nic.in/sites/default/files/AnnualReport2017-18%28English%29.pdf
21. Ministry of Textiles. (2019). *Annual report: 2018-2019.* Retrieved from http://texmin.nic.in/sites/default/files/Textiles-AnnualReport2018-19%28English%29.pdf
22. Moulds J. 2015. Child Labour in the fashion supply chain- Where, Why and What can be done. The Guardian. Sponsored by UNICEF, Retrieved from https://labs.theguardian.com/unicef-child-labour/

23. Nagaraj, A. (2019, Aug 5). Indian state to map cotton supply chain to stamp out child labour. *The Thomson Reuters.* Retrieved from https://www.reuters.com/article/us-india-slavery-cotton/indian-state-to-map-cotton-supply-chain-to-stamp-out-child-labor-idUSKCN1UV0YG
24. Organic Exchange. (2009). Organic Exchange 100 Standard - 2009 Version 1.3. Retrieved from Textile Exchange: http://textileexchange.org/sites/default/files/te_pdfs/integrity/oe100_stand.pdf
25. Pesticide Action Network. (2011, May 2). Problems with conventional cotton. Retrieved from http://www.panna.org/resources/cotton
26. Shrinivas A. 2016. Price transmission, asymmetric adjustment and threshold effects in the cotton supply chain: a case study for Vidarbha, India, Agricultural Economics. DOI: 10.1111/agec.12242
27. Siobhan O'Neill, 2015. India and the cotton supply chain, February 27, 2015 at Retrieved from https://www.greenhotelier.org/our-themes/supply-chain/talking-point-india-and-the-cotton-supply-chain/
28. SOMO and ICN, 2014. Flawed Fabrics- The abuse of girls and women workers in the South Indian textile industry. Retrieved from http://www.indianet.nl/pdf/FlawedFabrics.pdf
29. United States Environmental Protection Agency. (2012, April 4). EPA. Retrieved from Endosulfan, fact sheet: http://www.epa.gov/wastes/hazard/wastemin/minimize/factshts/endoalfa.pdf
30. USDA Foreign Agricultural Service, 2016. Production Supply and Distribution, United States Department of Agriculture
31. Why source sustainable cotton? How cotton can contribute to a sustainable future. (n. d.). Retrieved from http://cottonupguide.org/why-source-sustainable-cotton/how-cotton-can-contribute-to-a-sustainable-future/
32. World Wildlife Fund Report. (2020). *Sustainable agriculture: Cotton.* Retrieved from https://www.worldwildlife.org/industries/cotton
33. WTO. (2019). *Agriculture and development: Cotton*. Retrieved from https://www.wto.org/english/tratop_e/agric_e/wcd2019_e.htm
34. WWF Global. (2003). Cotton Farming, cotton: a water wasting crop. Retrieved from WWF Global: http://wwf.panda.org/about_our_earth/about_freshwater/freshwater_problems/thirsty_cro ps/cotton/
35. WWF. (2016). *Sustainable cotton.* Retrieved from https://www.wwfindia.org/about_wwf/making_businesses_sustainable/cotton/
36. WWF-India report 2010 and 2012– Developing a policy framework for promoting better management practices in four cotton states

Smallholders' Differentiated Contribution to the Value of Cotton Chain in Cameroon

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Abstract

Background: *In West and Central Africa, cotton economic and social importance of cotton production used to be claimed, in terms of income distributed to farmers and contribution to GDP, balance of payments and livelihood of rural population in the concerned production areas. Figures put forward are seldom updated. Besides, farmers benefitting from cotton were presented as a whole regardless of their possible differentiation in terms of farm size and endowments.*
This communication focusses on the results of the economic analysis of a study by using the VCA4D method, a method promoted by a specific European program to deal with value chains through their economic, social and environmental dimensions. The study was implemented in 2019 in Cameroon where a single cotton company (Sodecoton) operates in marketing and ginning seedcotton and crushing cottonseeds. Fiber processing, on an industrial or artisanal scale, was taken into account.
Results: *For the campaign of 2017-18, with a seedcotton production of 254,181 tons by 152,612 cotton growers on 182,610 ha, the total added value was FCFA 95.9 billion (€ 146 million) corresponding to 0.6% of GDP. The total generated income was FCFA 48.2 (€ 73.5 million). Out of total added value and income, growers represented 38.4% and 67.8%, respectively. The shares of the cotton company in the two mentioned economic indicators were 39.3% and 17.6%. Its exportation of cotton fiber generated FCFA 106 billion (€ 161.5 million) representing 16.8% of all agricultural exportations, 5% of balance of payments of the country. After deduction of the Value Chain importations, net foreign currency return was FCFA 51 billion (€ 77 million). Through tax payments, the cotton value chain contributed to the national budget for FCFA 9.2 billion (€ 14.0 million), out of which 68.0% came from the cotton company. In terms of employment, around 27,000 people in villages got remuneration from the management and marketing activities related to cotton production while 3,200-3,500 persons got salaries by the cotton company on permanent or temporary basis.*
Cotton growers' contribution was differentiated according to the size of cotton acreage in farm. The four types of farms with cotton acreage of less than one ha, 1-5 ha, 5-10 ha and more than 10 ha represented 70.1%, 24.8%, 4.0% and 1.1% of all farms, respectively. However, their respective shares in farmers' contribution to added value were 29.1%, 35.6%, 23.8% and 11.5%. Small "cotton farms" were the most numerous. The greatest share of children at primary school age were found in these farms where cotton income should account to help materialize the schooling purpose.
Conclusion: *In a country where the very low level of textile industry hampers value addition, smallholders producing cotton account substantially to the total Added value and extracts an even higher share of the total income distributed. Even in an essentially manual agriculture, clear farm differentiation can be observed through the size of cotton acreage in farms. Relatively big cotton farms (over 5 ha of cotton) represent about 5% of all farms but contribute to more than 35% of added value. The concern for economic performance bears the risk of excessive attention to big farms at the expense of smaller ones which are far much numerous and for which the preservation if not improvement of cotton income matters in a context of lack of alternative cash income.*
Referring to the results highlighted, our work shows that the VCA4D method, at least for the dimension of economic analysis, is valuable and manageable. Probably like any other method, the accuracy of the estimation of economic indicators depends on how comprehensively players involved in the chain are taken into account and the extent to which real accounting data are supplied.
The study conducted also demonstrates that the VCA4D method is of particular relevance in cotton producing countries in West and Central Africa. The administered feature of their cotton sectors makes the method easier to apply by ensuring the needed collaboration from players directly involved in producing and processing cotton. When applied on a more or less permanent basis, the method provides an evolution of economic indicators which helps to properly administer the cotton sector.

Keywords: *Value chain analysis, added value, income, economic analysis, farm differentiation*

Background

In West and Central Africa, cotton production is important. Despite a production by family smallholders, this part of Africa accounts for 3-4% of world production, but up to 12% of world exportations in some years because local textile industry is little developed.

The economic and social importance of cotton production used to be claimed by West and Central African countries, in terms of contribution to GDP, balance of payments and livelihood of rural population in the concerned production areas. This kind of claim was at the basis of the well-known initiative1 introduced in September 2003 by the C4 group (Benin, Burkina Faso, Chad and Mali) at the Ministerial meeting of WTO in Cancun against the market distorting policies of subsidy implemented by few countries (WTO, 2003). After about 17 years, what is called the "Cotton Dossier" at WTO is not yet fixed despite the set-up of a specific sub-committee at the Organization which makes all items publicly available about the ins and outs of the case2.

Figures were put forward to sustain the economic and social importance, but the sources were not indicated and they have been not updated since then. It was claimed that cotton contributed to 5-10% of GDP, around 30% of total export earnings and over 60% of earnings from agricultural exports in each of the four countries (WTO, 2003). In demographic terms, it was stated that around 10 million people depended directly on cotton production, to which several millions depended indirectly. The extent to which farmers benefitted from cotton production was not elaborated, not mentioning how farmers could benefit differently according to differentiated characteristics of their farms.

This communication provides a detailed and updated vision of the economic impact of cotton production in one Central African country, taking into consideration the reality of farm differentiation which implies that the benefit to farms could be unequal. It is based on a study implemented in Cameroon by using the VCA4D method, a method promoted by a specific eponym European program to deal with value chains through their economic, social and environmental dimensions. It focusses on the results of the economic analysis conducted in 2019 to estimate various economic indicators of the cotton sector where a single cotton company (Sodecoton or SDCC) operates in marketing and ginning seedcotton and crushing cottonseeds. Fiber processing, on an industrial or artisanal scale, was taken into account in the study.

Results

Value chain activities and representation

In 2019, the cotton value chain in Cameroon was the most concentrated in West and Central Africa, the following description remains applicable at the time of writing this communication. A single company, SDCC, was in charge of supplying services and inputs to cotton growers, paying growers' seedcotton, ginning seedcotton, crushing cotton seeds, manufacturing animal feeds at crushing mills and selling all products obtained. Commercialization of growers' seedcotton was implemented by village Cotton Growers Groups (CGG) properly trained by SDCC and the technical staff of CNPC-C, the federation of CGG.

A full cooperation of SDCC and CNPC-C enabled to collect a great part of the needed data to follow the value chain at its various stages of processing, as illustrated in Figure 1, complemented by data supplied by CICAM, the single textile company still operational. The study conducted integrated the activities of village artisans involved in manufacturing ceremonial garment (locally called "Dimol") from seedcotton to final product. These activities were conducted free of any capital and any input (except seedcotton produced in villages) as spinning was manual and weaving machines were homemade from pieces of wood and straws.

The cotton value chain was excessively export oriented with regard to fiber, accounting for 98.8% of total production. Products resulting from the crushing of cotton seeds were essentially, almost exclusively consumed locally with distinct success depending on products. The offer of oil was below local demand, so there was not any trouble in selling. It was another story with seed meals and animal feed: unsold production was a threat to the profitability of the whole cotton company.

[1] Document available at https://www.wto.org/english/tratop_e/agric_e/negs_bkgrnd20_cotton_e.htm
[2] https://www.wto.org/english/news_e/archive_e/cott_arc_e.htm

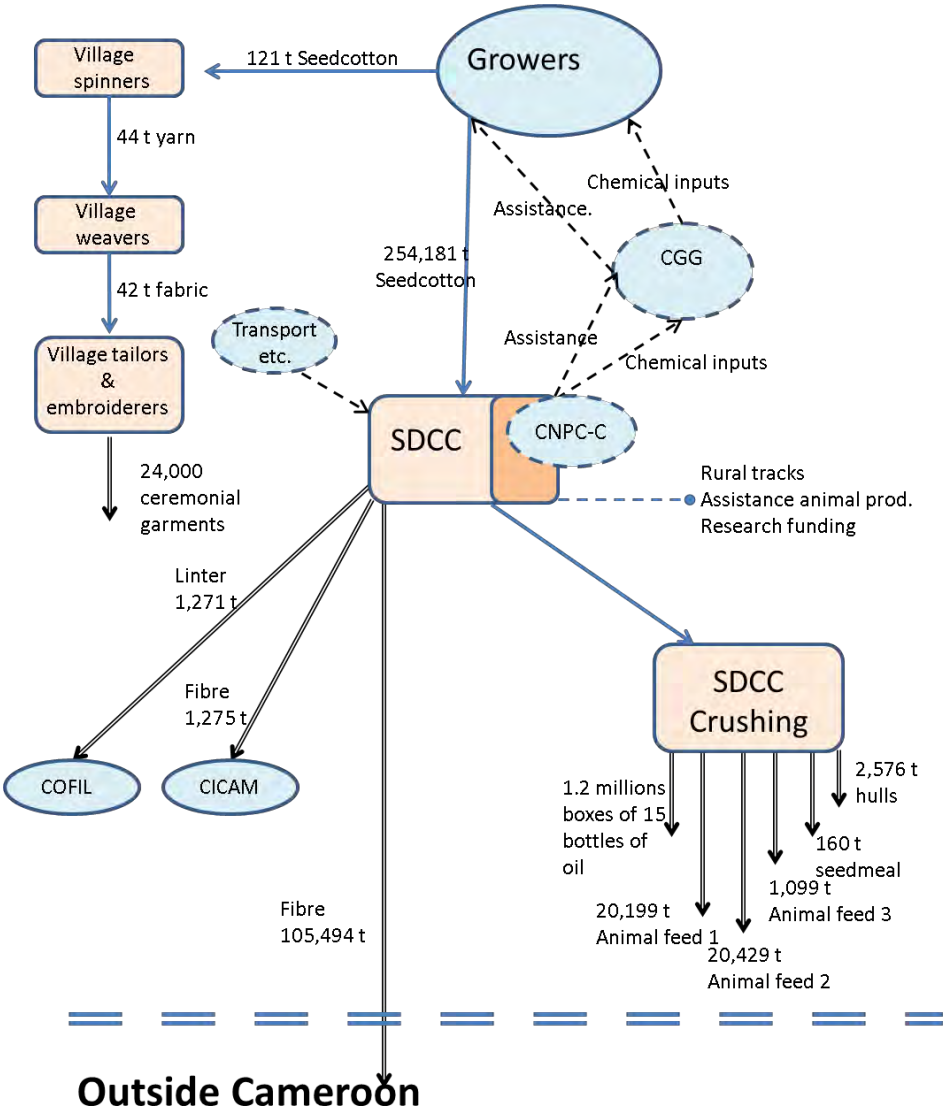

Figure.1. *Graph of the assessed cotton value chain*

Cotton chain value and contribution to national economy

For the production campaign of 2017-18, with a seedcotton production of 254,181 tons by 152,612 cotton growers on 182,610 ha and leading to a 106,769 tons of fiber, the added value created by the cotton value chain (Table 1) was estimated at FCFA 95,9 billion (or € 145.6 million at one euro for 656 FCFA). The added value created by each kg of seedcotton was FCFA 377. This added value corresponded to 0.6% of Cameroon's GDP.

The exportation of cotton fiber generated FCFA 106 billion (€ 161.5 million) representing 16.8% of the value of all agricultural exportations and 5% of balance of payments of the country. After deduction of the Value Chain importations, net foreign currency return was FCFA 51 billion (€ 77 million).

The cotton company and farmers, as main direct players in the value chain, contributed at almost equal level to the whole added value, at about 39.0% each. Indirect players, not consisted only of companies providing goods and services, brought 19.0% of total added value; growers' groups and their national representation accounted for a total 7.3% of total added value through their activities in marketing

seedcotton and supplying chemical inputs. The whole value chain was profitable with a total net income of FCFA 48,2 billion (or € 73.5 million). It was profitable to all players involved, except for the industrial textile company. Growers' share in total net income was 67.8%, much higher than their contribution to added value creation. Reversely, that of the cotton company was only 17.6%.

The value chain was also profitable to the government through the tax payments of FCFA 9.2 billion (€ 14.0 million). The cotton company paid the biggest share of 68.0%. Growers did not have to pay tax directly, but indirectly through the chemical inputs and production equipment supplied by the federation of growers' groups, at a substantial share of 17.1%.

Table 1 Profit and loss accounts in cotton value chain (in millions FCFA)

	Direct players					Indirect players				Whole
	Farmers	SDCC	Artisans	CICAM	Total	CGG*	FCGG**	Providers	Total	Total
Turnover (TO)	64,607	222,034	5,040	12,005	303,686	4,242	23,394	32,031	59,667	363,353
Added Value (AV)										
Amount	36,878	37,696	2,830	275	77,679	3,260	3,609	11,380	18,249	95,928
% TO	57.1	17.0	56.2	2.3		76.9	15.4	35.5	30.6	
% total AV	38.4	39.3	3.0	0.3	81.0	3.4	3.8	11.9	19.0	
Salaries										
Amount	2,280	12,582	0	2,630	17,492	1,723	425	2,324	4,472	21,964
Occasional staff	2,280	2,502			4,782	1,092		465	1,556	6,339
Permanent staff		10,080		2,630	12,710			1,859	1,859	14,569
Other		0			0	632		0	632	632
% AV	6.2	33.4		957.3	22.5	52.9	11.8	20.4	24.5	22.9
Financial expenses & insurances										
Amount	0	5,248	0	346	5,594	18	925	1,134	2,077	7,671
% AV	0.0	13.9		125.8	7.2	0.5	25.6	10.0		8.0
Taxes										
Amount	0	5,967	0	308	6,275	0	1,582	1,370	2,952	9,227
% AV	0.0	15.8		112.1	8.1	0.0	43.8	12.0	16.2	9.6
% Total taxes	0.0	64.7	0.0	3.3	68.0	0.0	17.1	14.9	32.0	
Gross income	34,598	13,899	2,830	-3,009	48,318	1,519	772	6,552	8,843	57,162
Amortization	1,868	5,430	0	971	8,270	200	65	388	652	8,922
Net income	32,730	8,469	2,830	-3,980	40,048	1,320	707	6,164	8,191	48,239
% total net income	67.8	17.6	5.9		83.0	2.7	1.5	12.8	17.0	

* CGG = Cotton Growers Groups; **FCGG = Federation of CGG named CNPC-C

Beyond the above financial terms, the cotton value chain implied more than 51,000 employment opportunities especially in villages (Table 2), although not mainly on permanent basis. Permanent employments, for a figure of 3,200 – 3,500, were provided mainly by formal economic organizations such as the cotton company and the textile industry. Growers' groups in villages accounted for the biggest figure of 27,000 employments to manage field advice, input and equipment supply and seedcotton marketing, although for a duration of eight months per year. The total of non-permanent employment, in various forms, was more than four times that of permanent ones.

Growers' differentiated contribution to the chain value

Cotton growers did not represent a perfectly homogeneous group. According to the criterion of the size of cotton area that the cotton company tended to prioritize in its extension activities, four types could be distinguished with cotton area ranging from less than one ha, one to five, five to ten and more than ten ha. These four types were quantitatively very unbalanced: the type of lowest cotton area accounted for about 70.0% of farms, while the two types of bigger area represented 4.0 and 1.1%, respectively. In other terms, most growers grew less than one hectare of cotton, and about 95% of growers had less than 5 ha of cotton (Table 3).

Table 2 Employment linked to cotton value chain

	Growers & groups		SDCC	CICAM	Artisans	Goods & Service providers	Total
	Growers	CGG					
Number employments							
Permanent	---	6,308	1,900	1000	---	n.a.	9,208
Other	n.a.	17,036	1,500	n.a.	24,000	n.a.	42,536
Salaries distributed, 10^6 FCFA	4,003		12,582	2,630		2,324	21,539
in number of months SMIG* salary	111,202		349,505	73,051		64,543	598,301
in number of years at SMIG*salary for one person	9,267		29,125	6,088		5,379	49,858

Note: Monthly Minimum guaranteed salary, at the rate of CFA 36,000

Table 3 Farm characteristics according to four types depending on the size of cotton area

	Total	Type 1*	Type 2*	Type 3*	Type 4*
Cotton area, ha	1.20	0.63	1.75	5.14	10.71
Number of growers	152,612	106,981	37,848	6,104	1,679
% growers		70.1	24.8	4.0	1.1
Family size	10.4	7.4	10.0	15.2	22.5
Number of wives	1.7	1.2	1.6	2.5	3.9
Population involved	1,301,440	791,769	379,121	92,697	37,853
% population involved		60.8	29.1	7.1	2.9
Population of children	911,874	542,186	273,222	68,468	27,997
% children <= 12 year old		57.2	31.4	8.2	3.1
Number oxen	1.5	0.7	1.5	2.8	3.9
Cattle heads, oxen excluded	2.2	0.6	2.0	4.3	11.7
Total cultivated area, ha	6.0	2.8	5.2	11.6	24.3

* Farm typed according to four cotton area sizes: <1ha, 1-5 ha, 5-10 ha and > 10 ha

The size of cotton area was illustrative of the size and wealth status of farms. Bigger cotton areas were found in farms of larger size (total cultivated area), of bigger families where the heads (men) had on average more wives and of better equipment for ex-drawn agriculture (as illustrated by the number of oxen). The number of cattle heads, indicator of wealth status in a context of traditional hoarding in cattle, was also higher. Cotton production in field involved a total population of 1.3 million people, out of which 61.0% were living in small cotton farms. When considering the population of children below 12 year-old which is the primary school age, small cotton farms accounted for 57.2%. Farm differentiation also led to differentiation of growers' contribution to the cotton value chain (Table 4). Bigger cotton growers had a contribution higher than their quantitative representativeness as a joint effect of larger cotton area and higher yield. Consequently, the two types of bigger cotton farms accounted for 23.8% and 11.5% of all growers' added value, respectively, or a total of 35.3% while representing only 5% of all growers. In terms of net income, the same discrepancies were somewhat found.

Table 4 Value contribution of four types of farms based on the size of cotton area

	Unit	Total	Type 1	Type 2	Type 3	Type 4
Seedcotton yield	kg/ha	1,392	1,341	1,315	1,618	1,470
Production/grower	kg	1,666	842	2,297	8,315	15,742
Total seedcotton produuction	ton	254,181	90,054	86,939	50,760	26,427
% production			35.4	34.2	20.0	10.4
Production value	10^6 CFA	64,607	23,153	22,026	12,781	6,647
Production cost	10^6 CFA	27,729	12,420	8,898	3,995	2,416
Cost of chemical inputs	10^6 CFA	24,829	11,471	8,072	3,474	1,812
% chemical cost		89.5	92.4	90.7	87.0	75.0
Added Value (AV)	10^6 CFA	36,878	10,733	13,128	8,785	4,231
% production value		57.1	46.4	59.6	68.7	63.7
% Growers AV			29.1	35.6	23.8	11.5
Salaries	10^6 CFA	2,280	1,666	488	111	14
FE&I	10^6 CFA	0	0	0	0	0
Taxes	10^6 CFA	0	0	0	0	0
Gross income	10^6 CFA	34,598	9,067	12,640	8,674	4,216
Amortization	10^6 CFA	1,868	1,107	588	132	41
Net income	10^6 CFA	32,730	7,960	12,052	8,542	4,176
% AV		88.8	74.2	91.8	97.2	98.7
% Growers net income			24.3	36.8	26.1	12.8

Discussions

Our study gives an estimation of the value creation for a specific campaign in a cotton chain with little industrial textile production. Cotton fiber is almost exclusively exported, there is very limited value addition from the local industrial processing of cotton fiber. This is unfortunately a situation common to all cotton producing countries in West and Central Africa. Hence, the features found in Cameroon for the value creation and distribution among players should also somehow apply to other countries of this region.

Value creation and distribution have been estimated through a method whose results depends on how comprehensively and precisely the accounts of production and of profit and loss could be established; in other words, the application of the VCA4D method could hardly be perfect. A value chain involves direct players that can be easily identified and approached, but also much more indirect players who provides goods and services to the direct players. It is much more difficult to identify all indirect players and furthermore approach them to obtain the needed economic data. In our study, almost all direct players were approached and the needed data obtained. For the main direct players (namely SDCC and CICAM), calculations were made through real accounting data and not estimated ones. However, it was impossible to identify all indirect players, not mentioning approaching them and get access to their accounts. Except for CGG and their federation, only three groups of indirect players were isolated (transporters, providers of goods, providers of other services) and their accounts were estimated from the data of the transportation which was internalized within SDCC but which represented only partially all transportation costs.

The application of the method nevertheless is not excessively difficult, it can be appropriated by the cotton sector itself to proceed calculations over a period to assess the evolution of the economic indicators. The main advantage of the method is not to get a snapshot but the possibility to provide a dynamic vision of the value creation and distribution within a sector when the method is applied over time. As long as the same system of players are considered and the same approximations are followed, the advantage of dynamic vision remains.

In a country where industrial textile industry was little developed in the 2017-18 campaign, economic indicators found were totally different from those claimed in the "Cotton initiative" introduced by four countries in 2003 (for example contribution of 15% to GDP instead of 0.6% found in our study). The related document did not mention any sources for the figures put forward. As the document was built with the assistance of some NGO, it is likely that the figures of economic indicators were exaggerated for communication purpose.

Our results are more consistent, albeit more informative, with those estimated in former studies about the cotton value chains in Mali and Togo by people without purpose of protest (Benhamou, Macrae, & Raymond, 1992; Raymond, Schulman, & Schwartz, 1983), at least as far as cotton production at growers' level is concerned. The former studies did not encompass textile industry (although it was also of tiny size as well in the two related countries). They did not isolate the contribution of cotton growers's groups which indeed existed, nor the federation of these groups which was not yet set-up. The former studies weere implemented in a period of unfavorable world market price which jeopardized the survival of the cotton sectors and which called upon budgetary intervention of the related government, in the opposite of the contribution to national budget that was found in our study.

The indicators of the contribution of the cotton value chain to the economy of Cameroon are rather but not fully consistent with the figures obtained in more recent studies in Cameroon. The contribution of the cotton chain to GDP was claimed at 5% in 2016 (Geocoton, 2016) or at 1% for the same campaign (Texier et al., 2017). Added value by each kg of seedcotton is estimated at FCFA 377 in our study, quite consistent with the figure of FCFA 341 found by Texier et al. (2017). The exportation of almost all cotton fiber produced represented 16.8% of all agricultural exportations, consistent with the figure of 15% in 2016 (Geocoton, 2016).

In our study, the contribution of cotton production in field to total added value is substantial (about 40%) but it comes out that value addition results more from downstream of field production. It would be furthermore so if textile industry is more developed than what it is.

With regard to the profitability of cotton production to smallholders, and furthermore to the equity of income distribution, farmers benefitted of a rather favorable situation as they got 67.8% of total net income, at a rate much higher than their contribution in added value. This (favorable) discrepancy is consistent with two features of cotton production in field: it is essentially if not exclusively a family

production with little recourse to paid labour and it is a very lowly capital intensive production with old ox-drawn tools, so the added value was almost totally transmitted to net income because of very low levels of salaries, financial expenses and amortization.

Although cotton production is profitable to smallholders, it is worth noting that it could be more profitable were the cost related to their landlocked isolation shared. At growers' level, input cost are high, accounting for almost 90% of all production inputs and services (Table 3), because they pay input at real cost without any support from the government.

Smallholders' contribution to the cotton value chain is differentiated because of the differentiation among farmers in the size of cotton areas. Although farmers grow on average 1.2 ha of cotton each, a large majority of 70% grow less than one ha (precisely 0.6 ha), while a minority (4.1%) grow more than 5 ha (precisely 5.1 ha) and a further tiny proportion of farmers (1%) grow more than 10 ha (precisely 10.7 ha). Consequently, about 35% of added value came from a minority of about 5% of farmers who have bigger farms, bigger families and more assets, and achieve a relatively higher productivity in field. The large majority of cotton growers (95%) contributed to 65% of farmers' share in the cotton chain added value, where cotton income should matter for paying the schooling fees of about 90% of children at primary school age (Table 3).

The observation above elaborated has policy implications depending on political orientation. The enhancement or better support to bigger cotton growers may sound desirable from the perspective of improving the cotton sector competitiveness. However, if such enhancement implies that the cotton opportunity will become less accessible to smaller cotton growers, the social implications might be dramatic in areas where economic opportunities lack and where insecurity linked to Boko Haram is already sustained by the recruitment into djihadist ranks of youngsters lacking education and who felt abandoned (Seignobos, 2014).

Conclusion

In the specific campaign of 2017-18 and in a country where the very low level of textile industry hampers value addition, smallholders producing cotton account substantially, at about 40%, to the total Added value. Their share in distributed income is higher because of a family and essentially manual production implying little capital.

Even in an essentially manual agriculture, clear farm differentiation can be observed through the size of cotton acreage in farms. Relatively bigger cotton farms, about 5% of all farmers, contribute for 35% of added value brought by all as a result of better productivity on larger cotton areas. Bigger farms could deserve more attention for a matter of competitiveness of the value chain, but smaller farms should not be left alone as cotton production procures income to the majority of population concerned.

Our work shows that the VCA4D method, at least for the dimension of economic analysis, is valuable and manageable. Probably like any other method, only approximation of the real value of a commodity chain can be computerized. The accuracy of the estimation depends on how comprehensively players involved in the chain are taken into account and the extent to which real accounting data are supplied.

The study conducted also demonstrates that the VCA4D method is of particular relevance in cotton producing countries in West and Central Africa, giving rationale for political decision of applying the method over time. Indeed, the administered feature of their cotton sectors makes the method easier and more desirable to apply. On one hand, the administered feature ensures better collaboration from players directly involved in producing and processing cotton. On the other hand, the application of the method on a more or less permanent basis provides a vision on the evolution of economic indicators, hence helping to administer properly the cotton sector.

Methods

This communication was based on a study of the cotton value chain in Cameroon (Fok, Gian, Meier, Balarabé, & Calaque, 2019), implemented by following the VCA4D method, or Value Chain Analysis for Development, as presented by Dabat, Orlandoni, & Fabre (2018). The method is being promoted by a program at the European Union to appraise agricultural commodity value chains in their economic, social and environmental dimensions. With regard to the economic dimension, the method presented by Chervel et al. (1997) was applied in the 1980s in Africa on cotton sector in Mali , in Togo (Raymond et al., 1983) or peanut sector in Senegal (Amselle, Baris, & Papazian, 1982).

This communication is focused on highlighting the results of the economic analysis for which the method started by identifying the system of players involved, beginning by those directly involved in producing

seedcotton and in processing it at various stages. Indirect players were those involved in providing goods or services in the activities of the direct players.

In Cameroon, the cotton value chain was very concentrated in 2019, and still is by the time of writing this communication. A single company, Sodecoton or SDCC, was in charge of supplying services and inputs to cotton growers, paying growers' seedcotton, ginning seedcotton, crushing cotton seeds, manufacturing animal feeds at crushing mills and selling all products obtained. Commercialization of growers' seedcotton was implemented by village Cotton Growers Groups (CGG) properly trained by SDCC and the technical staff of CNPC-C, the federation of CGG. There was only one company operational in industrial textile production (essentially yarn and fabric for loincloth) while the production of traditional ceremonial garments (locally called "Dimol") by artisans remains active in about one hundred villages. This garment manufacture started from manually spinning seedcotton (produced by artisans themselves), then weaving and tailoring and embroidering. Traditional weaving machines were made by artisans from pieces of locally available wood and straws. Through their tasks of marketing seedcotton in villages and supplying inputs or ox-drawn tool to growers, CGG and CNPC-C were indirect players, along firms providing goods and services (including transportation) needed at the ginning and crushing stages.

For each direct player, production account were established through formula 1 and 2, respectively:

Formula 1 $AV = PV - PI - PS$

Where AV is Added Value, PV is Production value, PI is production inputs and PS is production services

Formula 2 $AV = S+FEI+T+A+NI$

In formula 2, added value was split into salaries (S), Financial expenses and insurances (FEI), Taxes (T), amortization (A) and Net income (NI)

Providers of inputs and services had to be identified, as indirect players, for which the estimation of AV had to be computerized according to formulas 1 and 2. Theoretically, the same operation should be iterated again and again, but in practice experience shows that the gain in precision was minimal beyond one iteration.
In the study conducted, the access to the accounting of SDCC, CNPC-C and CICAM allowed to implement calculation of their profit and loss account on real data. That of CGG was based on the data obtained on a sample of twenty of them. The iteration of the calculation for indirect players providing goods and services at various stages of product processing by SDCC was extrapolated through the data of part of the transportation which was internalized within the company.
For village artisans manufacturing traditional ceremonial garment, interviews conducted in one of the biggest villages involved helped to obtain the needed information. For growers, we got access to a detailed survey that SDCC conducted in 2017 along 956 farmers. The processing of the available data allow to estimate growers' production account and to categorize them according to the criterion of cotton area size, the one that SDCC has been considering to adapt its assistance in villages.

References

1. Amselle, J. L., Baris, P. P., & Papazian, V. (1982). Evaluation de la filière arachide au Mali. Ministère de la Coopération, Paris.
2. Benhamou, J. F., Macrae, J., & Raymond, G. (1992). Evaluation macro-économique de la filière coton et des effets du Contrat Plan sur la période 1989/90-1993/94 : Rapport de synthèse (p. 59). p. 59. Bamako (Mali): CMDT.
3. Chervel, M., Fabre, P., Kane, R., & Saldarriaga, G. (1997). Manuel d'évaluation des projets d'investissement par la méthode des effets. Orbiter.
4. Dabat, M.-H., Orlandoni, O., & Fabre, P. (2018). Bridging research and policy: evidence based indicators on agricultural value chains to inform decision-makers on inclusiveness and sustainability. 166th EAAE Seminar Sustainability in the Agri-Food Sector. August 30-31, 2018, National University of Ireland, Galway, Ireland.
5. Fok, M., Gian, N., Meier, M., Balarabé, O., & Calaque, R. (2019). Analyse de la chaîne de coton au Cameroun. Rapport pour l'Union Européenne, DG-DEVCO. Value Chain Analysis for Development Project (VCA4D CTR 2016/375-804).
6. Geocoton. (2016). Plan de redressement de la Sodecoton et élaboration des mécanismes de sa mise en oeuvre. Paris.
7. Raymond, C., Schulman, J. P., & Schwartz, A. (1983). Evaluation de l'activité coton au Togo. Ministère de la Coopération, Paris.
8. Seignobos, C. (2014). Boko Haram : innovations guerrières depuis les monts Mandara Cosaquerie motorisée et islamisation forcée. Afrique Contemporaine, (252), 149–169.
9. Texier, H., Liboum, M., Hotina, L., Granel, A., Ghouzi, C., & Prunaret, H. (2017). Etudes portant sur la contribution du secteur cotonnier dans l'émergence socio-économique et la création d'emploi dans la zone cotonnière au Cameroun. C2G Conseil, Paris.

10. WTO. (2003). Poverty reduction: sectoral initiative in favour of cotton. Joint proposal by Benin, Burkina Faso, Chad and Mali. Accessible at https://www.wto.org/english/news_e/news04_e/sub_committee_19nov04_e.htm link to WT/MIN(03)/W/2/Add.1 document.

Status and Implications of Seed Purity Seven Years After Bt Cotton Use in Burkina Faso

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Abstract

Background: *Since the commercial release of Bt cotton the issue of seed purity in producers' fields has been little addressed and in an unbalanced way when it was. It is well documented that the loss of purity in conventional seeds has endangered the continuation of organic cotton production. However, studies are rare on the purity of Bt-cotton seeds despite its implications on the effectiveness and sustainability of their use.*

This communication compensates for the mentioned lack of literature by analyzing data collected in 2015 in Burkina Faso, namely results of ELISA tests on samples of seeds from 646 fields grown with conventional or Bt varieties.

Results: *According to the conservative criteria retained to declare the presence of Bt gene (more than 10% and 90% of controlled seeds for conventional and Bt variety, respectively), seed purity was very questionable for both types of varieties. For the conventional variety, the presence of Bt gene was observed on 63.6 and 59.3% of samples for Cry1Ac and Cry2Ab, respectively. Only 29.3% of samples corresponded to pure conventional seeds while 52.2% were double Bt seeds. Conversely, for the Bt variety, the presence of Bt gene was observed on 59.6 and 53.6% of samples for Cry1Ac and Cry2Ab, respectively. Actually BG2 seeds with both Bt genes were found in 40.4% of samples against 27.2% of samples of actually conventional seeds while the remaining of 32.4% of samples corresponded to single Bt gene seeds.*

Two factors affected the severe lack of seed purity. As regard to conventional seeds, it clearly resulted from a phenomenon of contamination, indicative of a failure in adjusting the seed production scheme to the use of Bt-cotton. With regard to the Bt variety, the lack of purity of the original seeds provided to Burkina Faso accounted and should even be the major factor.

The observed lack of seed purity is a threat to the initiative of organic cotton production, albeit a very minor production mode in the country. It also calls upon the effectiveness and furthermore the sustainability of Bt cotton to control target pests.

Conclusion: *Our results show the extent of purity loss when no especial attention is paid to the preservation of seed purity. Pure conventional seeds could totally vanish while Bt seeds become a combination of seeds of various types encompassing or not the expected Bt genes.*

Any country willing to embark the use of Bt cotton, or to resume this use like Burkina Faso, must previously adjust its seed production scheme and enforce its operation. This is a condition to preserve pure seeds both to enable the launch or the continuation of identity-cotton production and to ensure a sustainable effectiveness of Bt-cotton.

Keywords: *Conventional cotton, organic cotton, GM cotton, seed production scheme, seed control*

Background

Since the commercial release of genetically modified cotton, especially Bt cotton made resistant to some damaging insects, the issue of seed purity has been little addressed and mainly from the perspective of non-GM fields. Studies pointing out the loss of purity of conventional seeds are related to the concern of producers of identity-cotton, namely organic cotton, for not being able to meet the requirement of absence of GM at the imposed threshold (Cederholm 2014). In the USA, the coexistence of GM and non-GM crops is perceived as an illusion because the contamination by GM crops is thought to be impossible to escape (Food Decmocracy Now! 2014), so the continuation of organic cotton production is threatened. In Europe, like in France, coexistence is found to be possible (Vinck 2003), at least on theory as GM crops are little developed in this continent.

However, published studies dealing with the purity of GM, more precisely Bt-cotton seeds, are rare. Many research works have dealt with the expression of Bt genes in varieties, by measuring the concentration of Bt toxins. It was observed for instance variety effect or seed generation effect in India where F1 hybrids are released and where the F2 offprings might have been used by farmers (Singh et al. 2016). The issue of purity of Bt-cotton seeds was only specifically addressed in Pakistan, where discrepancies were found between farmers' declaration on the type of cotton they were growing and the real type checked through lab analysis of plant samples, in a context where only the single Cry1Ac Bt gene was encountered (Spielman et al. 2017). Even in works about the issue of coexistence, the rate of contamination of non-GM seeds by GM traits is addressed, from 0.1% to 5.0%, but the rate of GM purity of GM seeds is seldom mentioned just like if there is no issue about it. GM trait is not considered distinct from any other genetic trait for which the general genetic purity should be met, at a level generally established at 95.0 % to 99.9% depending on the type of seeds in the USA or Europe. In China, the standard of GM purity rate is established at 90% (Seed world 2017), and 90-95% in India (Mohan and Sadananda 2019).

The lack of work on the seed purity of Bt-cotton varieties is regrettable because of the possible implications on the effectiveness and sustainability of Bt-cotton use. In case of insufficient seed purity, seeds used would pertain to in-pack seed mixture with debatable outcome. Sun et al. (2013) have shown that 20 to 50% share of conventional in seeds mixtures of Bt cotton led to poor if not very poor pest resistance effectiveness. Although the presence of conventional seeds in the mixture might procure kind of refuge within plot, it was documented that such type of refuge was less effective (Tabashnik 1994) and dominance of resistance was promoted (Brévault, Tabashnik, and Carrière 2015) while good stewardship is required for the success of its implementation (Mohan and Sadananda 2019). The objective of this communication is to compensate for the mentioned lack, by analyzing data collected in 2015 in Burkina Faso, at the eve of the decision to suspend the use of Bt cotton that had been legally released in the country in 2008 with BG2 varieties, e.g. stacking two Bt genes (Cry1Ac and Cry2Ab). The data corresponded to ELISA tests conducted to assess the presence of Cry1Ac and Cry2Ab genes in samples of seeds from 646 fields representing more or less equally the conventional variety (FK37) and the Bt variety (FK95 BG2, derived from the introgression of the two Bt genes into FK37). The knowledge gained with regard to the level of seed purity and influencing factors should be helpful to other developing countries willing to embark Bt-cotton use or even Burkina Faso in case of decision to resume such use.

Results

Because of the organization of seed distribution in Burkina Faso, fields could be easily referred to the types of seeds supplied to farmers. Seeds were supplied by the cotton company after registering what each farmer wanted to grow, although some arbitration might apply according to seed availability. In general, farmers do not get seeds apart from the unique cotton company in their area, although there might be some arrangement between individuals to get additional seeds from others. Hence, sampling of our study was made based on the records of the extensionists of Sofitex, the main cotton company representing 80% of national production. With reference to the results of ELISA tests implemented on the samples made from fields cultivated with conventional seeds supplied by Sofitex, the presence of Bt genes was clearly revealed in seeds collected from these fields (Table 1). As cotton is mainly an autogamous specie, the results shown were illustrative of the feature and extent of contamination by Bt genes in seeds supplied to farmers. Out of 297 fields cultivated with so-called conventional seeds, 63.6% and 59.3% showed the presence of Cry1AC and Cry2Ab, respectively. In both cases, the level of presence was high (more than 40%, corresponding to positive test observed for 4-5 seeds out of 11). Besides, the frequency of conventional cotton fields where both Bt genes were present was 52.2%, indicating that about half of the sampled fields were indeed Bt-cotton fields.

Table 1: Presence of Bt genes in seeds from fields cultivated with conventional variety

	Presence* of Cry 1 A	Presence* of Cry2Ab	Double presence
Number samples	297	297	297
% samples being Cry +	63.6	59.3	52.2
% presence of CrylAc			
Mean	41.5		43.1
Std. Deviation	23.2		22.5
% presence of Cry2Ab			
Mean	39.3		40.3
Std. Deviation	23.2		23.3

* Presence was claimed when observed on at least one out of 11 seeds of each sample

The status of conventional cotton in the 297 fields (where such a cotton was assumed to be cultivated) was not real for most of them. In Table 2 synthesizing the above results, it is shown that out of the sampled fields grown from supplied conventional seeds, only 29.3% were actually conventional fields, against 52.2% which were BG2 ones (double Bt genes). There were 11.4% and 7.1% of fields that were single-Bt cotton fields, expressing Cry1Ac and Cry2Ab genes, respectively.

Table 2: Contamination status of conventional seeds in samples from conventional cotton fields (% of 297 samples)

Contamination* by CrylAc	Contamination* by Cry2Ab		Total
	No	Yes	
No	293%	7,1%	36,4%
Yes	11,4%	522%	63,6%
Total	40,7%	593%	100,0%

* Contamination was claimed when observed on at least one out of 11 seeds of each sample

Conversely, the Bt status was much less debatable in the 349 fields where Bt-cotton were assumed to be cultivated. With the retained criterion for the Bt status (46% of presence of Bt gene in seeds), around 95% of the related fields met the criterion either for Cry1Ac or Cry2Ab separately (Table 3). Even when both Bt genes were considered jointly, 92.6% of fields reached the threshold set. The level of Bt gene presence was high, in more than 86% of seeds in all cases (or more than 9 out of 11 seeds). With a more stringent criterion of 73% of presence of Bt gene in seeds for the Bt status, we found 65-77% of sampled fields meeting the criterion (results non shown).

However, it would be more difficult to claim that Bt-cotton fields were cultivated with pure Bt seeds (Table 4). The criterion of pure seeds was met for less than 60% of the related fields when the two Bt genes were considered separately (Table 4). It fell to only 40% when the two genes were taken jointly. The level of presence of Bt genes (Cry1Ac or Cry2Ab) in more than 95% of seeds, was consistent with the retained criterion for pure Bt seeds.

Considering the seed purity criterion to define the real status of cotton grown (conventional or Bt), 27.2% of the sampled fields -assumed to have been cultivated with double Bt genes- were in fact of conventional status (Table 5). Only 40.4% of the related fields had actually the status of being cultivated with double Bt gene seeds. The remaining 42.2% (19.2 + 13.2) had the status of Bt-cotton with a single Bt gene.

Discussion

Literature lacks to confront our results about the purity of Bt-cotton seeds. There should be data resulting from control by seed production organizations but they are not disclosed. The reality of lack of seed purity is documented only in a few countries.

In Pakistan, the lack of seed purity for both conventional and Bt cotton has been assessed through a research work confronting farmers' declaration on the type of seeds they used and the biochemical control of plant leaves. It was found that only a single-Bt gene cotton was cultivated (Cry1Ac) and 11% of farmers believed they were cultivating Bt cotton while the Bt gene was not present, and 5% of farmers believed they were cultivating non-Bt cotton when, in fact, the Bt gene was present (Spielman et al 2017). The figures of discrepancies in our study are higher; the fact that a double Bt gene cotton was used should be an explaining factor, although the authenticity of the seeds supplied only by the cotton company should be better.

In China, through the measurement of Bt toxin contents, Pemsl, Waibel, and Gutierrez (2005) have suspected the lack of seed purity in Bt cotton varieties released in a very competitive context. Besides, in the particular case of hybrid varieties destined firstly for cultivation in more southern province of China, the lack of seed purity was also addressed indirectly through the assessment of Bt toxins (Xu et al. 2008). In both cases, the extent of purity imperfection was not estimated.

In Burkina Faso, the control of Bt nature of FK95 BG2 variety is implemented but data were not accessible. The only information available was obtained in an external initiative to check the Bt status of cotton plants in claimed Bt-cotton fields in this country. Out of the tests implemented on 45 samples, 24.4% of samples had no Bt status at all, 17.8% had a single Bt gene status equally distributed between the two Bt genes, and 57.8% had the double Bt gene status (Michel Fok et al. 2016). These figures are quite consistent with those in our present study that is based on a much higher number of samples.

Table 3: Bt status of seeds in samples from fields of Bt cotton

	Presence* of Cry1Ac	Presence* of Cry2Ab	Double presence
Number samples	349	349	349
% samples being Cry +	95.7	94.3	92.6
% presence of Cry1Ac			
Mean	88.1		88.8
Std. Deviation	11.9		11.2
% presence of Cry2Ab			
Mean		86.5	86.6
Std. Deviation		12.2	12.1

* Presence is claimed when observed on no less than 6 seeds out of 11 of each sample

Table 4: Purity status of Bt seeds in samples from Bt-cotton fields

	Purity* for Cry1Ac	Purity* for Cry2Ab	Double presence
Number samples	349	349	349
% samples being Cry +	59.6	53.6	40.4
% presence of Cry1Ac			
Mean	96.0		96.7
Std. Deviation	4.5		4.4
% presence of Cry2Ab			
Mean		95.4	95.9
Std. Deviation		4.6	4.5

* Purity is claimed when the presence of Bt gene was observed on no less than 10 seeds out of 11 of each sample

Table 5: Bt status of seeds in Bt-cotton fields

Cry1Ac status*	Cry2Ab status*		Total
	No	Yes	
No	27,2%	13,2%	40,4%
Yes	19,2%	40,4%	59,6%
Total	46,4%	53,6%	100,0%

*Status refers to the use of pure seeds, i.e. presence on at least 90% of the tested seeds in samples, or on no less than 10 seeds out of 11 of each sample

More literature deal with the phenomenon of contamination of conventional seeds by Bt genes, but with much less, if any, quantitative assessment than in our study. The issue is more documented mainly because the phenomenon has endangered the continuation of organic cotton production, notably in the USA (Hershaw 2013) where it is claimed that no organic cotton producer could be meeting the purity criterion of conventional feature. The contamination status has become so much generalized and unescapable that Endres (2005) advocates a revision of federal and states laws governing seed purity. In India, in almost 30% of cases examined, conventional seeds supplied for refuge purpose contained Bt genes (S. Kranthi et al. 2017) although at non-specified extent. In Burkina Faso, a study mandated

by promoters of organic cotton production pointed out that about 50% of organic cotton producers were provided with seeds containing Bt genes, however with the stringent criterion that Bt presence was declared when found on at least one out of 300 seeds and tests completed with lateral flow strips (Vognan and Bourgou 2014).

Our results clearly show that, at the eve of suspending the use of Bt-cotton, conventional seeds were contaminated at large extent. As we have retained rather conservative threshold of the presence of Bt genes in conventional seeds, the real situation of contamination was indeed worse than indicated in our figures.

The main reason of the observed contamination should be the lack of specific attention to prevent contamination when Bt-cotton was disseminated at large scale. No specific measures were implemented to delineate non-Bt cotton zone where conventional seed production could have taken place. In addition, one may suspect some arrangements between farmers in exchanging seeds, including in seed production area, so that some assumed conventional cotton fields were indeed Bt-cotton ones. The reverse case was also possible as there were farmers unwilling to grow Bt-cotton, particularly at the first years of the shift to this cotton type. Quality control with regard to the Bt traits in seed processing was quantitatively insufficient as it can be observed through the procedures of implementation of ELISA tests (Sofitex n.d.). In addition, these tests were conducted mainly to check the Bt nature and not the level of presence of Bt genes.

Since the suspension of Bt-cotton use in 2016, it is probable that the seed contamination of conventional cotton by Bt genes has not persisted at the quite high level found in our study, but it is hard to claim that Bt genes have totally disappeared from fields. After the suspension decision, seed control was implemented with measures that were more stringent. Burkina Faso also has shifted to using another conventional variety. This variety shift could nevertheless not be total in one campaign. There must remain some level of adventitious and unintentional presence of Bt genes in cotton fields. So, to some extent, cotton producers keep benefitting from some effectiveness of Bt genes to control targeted pests.

At the eve of the suspension of Bt cotton use, the GM nature of the Bt-cotton seeds could be acknowledged although it was not perfect. Our point is based on ELISA test indicating that Bt toxins were detected but not at the levels expected. The inference to the absence of Bt genes might be excessive -because of various factors impacting on the expression of Bt genes (Huang et al. 2014; Iqbal et al. 2013; Rochester 2006; Wan et al. 2005)- but this argument little applies in our study as we observed this expression in falsely-assumed conventional seeds in the same growing conditions.

Our work is the first to quantify the loss of the BG2 status up to a poor level. The BG2 status (based on the double presence of Cry1Ac and Cry2Ab genes) was applicable only to 40% of the seeds supplied. Again, because of the conservative threshold retained for purity with regard to the presence of Bt genes in seeds, the real lack of BG2 status was even worse than indicated by our figures.

Two factors at least are beneath the observed lack of Bt purity in seeds. Because of the insufficient control of the BG2 status at the stage of seed production and processing, the lack of the adjustment of the seed production scheme is to blame. However, as the seeds provided by Monsanto for large scale release were neither stabilized nor homogenous (technically impossible to achieve in two years with at most four cycles), another factor of purity shortfalls dates back to the seeds originally supplied. The original lack of seed purity would have made ulterior quality control more difficult and costly, so this lack -not reported so far in other countries- could be regarded as the principal factor of the poor BG2 status of the Bt-cotton seeds.

The cultivation of Bt-cotton with defaulting purity of its seeds has worrisome implications with regard to the effectiveness and sustainability of the related Bt genes against target pests. A mixture of four cotton types -with regard to the presence of Bt genes (none, only Cry1Ac or Cry2Ab and both Bt genes)- implies disadvantages additional to those indicated in introduction and wipes out the rationale of having opted for stacked genes. The presence of single Bt gene has helped the emergence of resistance to single gene, hence facilitating the emergence of resistance to double Bt genes. It is likely that the process of resistance build-up against the two Bt genes had already started and that resistant alleles could already be encountered, at least at low frequencies. If so, the relaunch of Bt-cotton with the same genes in Burkina Faso would not be ensured of a lasting effectiveness, regardless of the risk of outbreak of secondary pests encountered in most (if not all) countries having adopted Bt-cotton long enough (Zhao, Ho, and Azadi 2011; B. K. Kranthi 2011; M. Fok 2010).

Conclusions

At the eve of suspending its Bt-cotton use, Burkina Faso has not escaped the phenomenon of conventional seeds purity loss and has suffered from a serious lack of seed purity of the disseminated Bt-cotton variety. Such a situation -which probably had prevailed several years before- has resulted from the defaulting adjustment of the seed production and distribution scheme upon the Bt-cotton release and the lack of purity in the original Bt seeds supplied to the country.

The case studied provides the lesson that any country willing to embark using Bt-cotton should ensure the purity of the Bt seeds and must adjust its scheme of seed production and distribution so as to preserve seed purity both for Bt and conventional varieties. This lesson applies also to Burkina Faso in case it resumes the use of Bt-cotton. However, one might fear that the comparative measured effectiveness will not be as strong as expected because conventional seeds are indeed already partially Bt ones. The effectiveness could also last less because the process of building up the pest resistance to the two Bt genes had probably been engaged before the Bt-cotton use was suspended.

Methods

The data were collected in December 2015 in the framework of a study to access the gap in fiber quality between the conventional and Bt cotton varieties being cultivated at this period (Bourgou et al. 2020). The gap observed, at the expense of the Bt-cotton variety, has implied huge financial loss for the cotton companies (Michel Fok 2016) which led to decide in mid-2015 to suspend the Bt-cotton use (Dowd-Uribe and Schnurr 2016). The 2015 season was the last where both conventional and Bt, isogenic cotton varieties were grown in coexistence.

The conventional variety was known as FK37, developed by the national research in Burkina Faso while the transgenic variety was FK95 BG2 obtained via introgression of two Bt genes (Cry1Ac and Cry2Ab) implemented by Monsanto. It is worthwhile to note that FK37 was supplied for introgression in 2004, and seeds of FK95 BG2 BC2 were sent back in 2006 for demonstration of biological effectiveness before its release at large scale in 2008. The supplied seeds were obtained after at most four cycles (by cultivation in both northern and southern hemispheres), quite insufficient to claim for a stable and homogenous genetic material.

For the mentioned study, samples collection was conducted in districts over the whole intervention area of Sofitex, representing about 80% of total production (Figure 1). Three cotton producer groups (CPG) were randomly selected by district and then three producers by CPG. Within CPG, fields of producers growing both types of cotton were sampled preferentially; they were complemented by producers growing one of the two types. In 2015 season, most farmers planted Bt-cotton (62.53% of of the total 520,428 ha), consequently, the share of FK95 BG2 in sampled fields was higher (349 and 297 for FK95 BG2 and FK37, respectively).

In each field, a sample of one kilogram of seedcotton was collected randomly from 135 cotton plants across diagonal lines of the field. Three bolls were picked at the bottom, in the middle and on top of each plant. After ginning, eleven seeds were randomly isolated to undergo ELISA test and check the presence or not of Cry1Ac and Cry2Ab, according to the procedures followed at the seed quality control lab of Sofitex (Sofitex n.d.) and probably set up in line with Monsanto stewardship.

For each field sample, the presence of Bt genes was controlled for each of eleven seeds but the data we highlighted were the percentages of seeds being tested positive (e.g. 9.09% means one seed tested positive out of eleven). For each sample, separated figures were obtained for the presence of Cry1Ac and Cry2Ab.

In our study, we retained to declare that a so-called conventional cotton field was cultivated with seeds contaminated by a Cry gene when the corresponding sample showed an ELISA test result above 10% (or ELISA test was positive for more than one out of eleven seeds). Conversely, we opted to declare a so-called Bt-cotton field was cultivated with pure seeds with regard to a specific Cry gene when the ELISA result of the corresponding sample was above 90% (or test positive for at least 10 out of 11 seeds). In other words, we retained a maximum threshold of 10% of Cry gene presence to approve purity of conventional seeds and a minimum threshold of 90% for that of Bt cotton seeds. These thresholds are much higher than those commonly referred to in the USA or Europe, at 1.0-5.0% and 95.0-99.0%, respectively, and which are quite stringent and hard to achieve in practice. The thresholds we have retained are consistent with a conservative approach to shelter from false-positive or false-

negative errors (Remund et al 2001). Our purpose is to show a status of loss of purity, which would be furthermore real when following a more stringent criterion of seed purity.

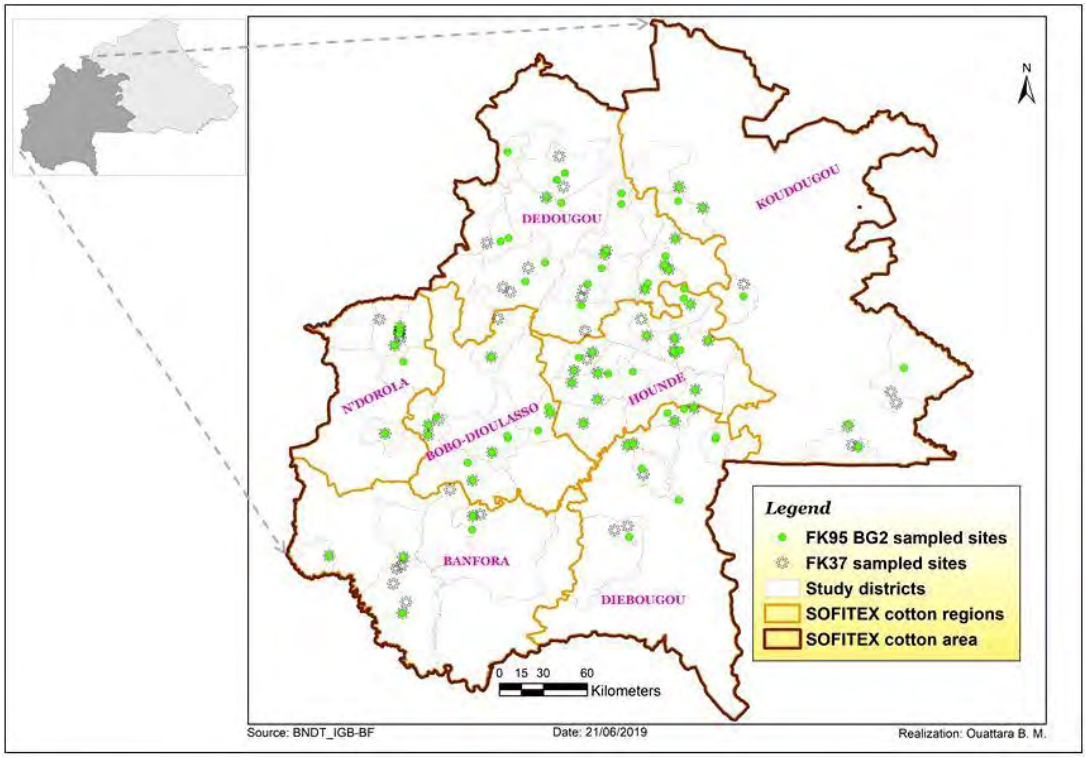

Figure 1: *Sofitex cotton zone, showing study sampled districts and sites*

However, a field might be considered to have a Bt-cotton status although the seeds used were not pure as defined above. This is a situation seldom addressed as it is assumed that GM seeds being supplied are necessarily pure. For a first study taking into account the possibility of growing Bt cotton with seeds which are no pure, we have retained a minimal threshold of 46% of Bt gene presence in seeds (in our case, presence in at least 6 out of 11 seeds) to assume that supplied seeds were of Bt nature.

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References

1. Bourgou, Larbouga et al. 2020. “Impact of Transgenic Conversion on the Characteristics of Burkina Faso Cotton.” *African Journal of Biotechnoly* 19(1): 8–17.
2. Brévault, Thierry, Bruce E. Tabashnik, and Yves Carrière. 2015. “A Seed Mixture Increases Dominance of Resistance to Bt Cotton in Helicoverpa Zea.” *Scientific Reports* 5: 1–7. http://dx.doi.org/10.1038/srep09807.
3. Cederholm, Holli. 2014. *Protecting Organic Seed Integrity. The Organic Farmer’s Handbook to GE Avoidance and Testing*. Organic Seed Grower and Trade Association (OSGATA).
4. Dowd-Uribe, Brian, and Matthew A. Schnurr. 2016. “Briefing: Burkina Faso’S Reversal on Genetically Modified Cotton and the Implications for Africa.” *African Affairs* 4(January 2016): adv063. http://afraf.oxfordjournals.org/lookup/doi/10.1093/afraf/adv063.
5. Endres, A. Bryan. 2005. “Revising Seed Purity Laws to Account for the Adventitious Presence of Genetically Modified Varieties: A First Step towards Coexistence.” *Journal of Law and Policy* 1(479): 131–63. http://dx.doi.org/10.1016/j.mpaic.2009.01.006%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/17533935%0Ahttp://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2014-14372-001&site=ehost-live%5Cnjenifer.ware@unt.edu%0Ahttp://www.bmj.com/cgi/doi/10.1136/bmj.396.
6. Fok, M. 2010. “Gone with the Wind of Transgenic Cotton Use in the United States a Vision of the Discussions and Presentations at the New Orleans (Louisiana, USA) ‘Beltwide Cotton Conferences’ in January 2010 | Autant En Emporte La Culture Du Coton Transgénique Aux États.” *Cahiers Agricultures* 19(4).
7. Fok, Michel. 2016. “Impacts Du Coton-Bt Sur Les Bilans Financiers Des Sociétés Cotonnières et Des Paysans Au Burkina Faso.” *Cahiers Agricultures* 25(3): 1–13.
8. Fok, Michel, Claire Neirac, Alain Renou, and Dominique Dessauw. 2016. *Etude de Faisabilité Du Coton Génétiquement Modifié En Côte d’Ivoire*. Montpellier (France).

9. Food Decmocracy Now! 2014. “Coexistence Equals Contamination : Tell the USDA to Stop Monsanto ’ s GMO Contamination.” https://www.fooddemocracynow.org/blog/2014/mar/3/tell_the_USDA_2_stop_Monsantos_GMO_contamination/ (June 25, 2016).
10. Hershaw, Eva. 2013. “Back to Mobile Seeds of Discontent : A Texas Organic Cotton Farmer Takes On Monsanto.” http://www.texasobserver.org/seeds-of-discontent/ (April 16, 2014).
11. Huang, Jikun et al. 2014. “Effect of Farm Management Practices in the Bt Toxin Production by Bt Cotton: Evidence from Farm Fields in China.” *Transgenic Research* 23(3): 397–406.
12. Iqbal, Arshad et al. 2013. “Comparative Account of Bt Gene Expression in Cotton under Normal and Salt Affected Soil.” *International Journal of Agriculture and Biology* 15(6): 1181–86.
13. Kranthi, By Keshav. 2011. “Part III : 10 Years of Bt in India.” : 2010–11. http://cotton247.com/news/?storyid=2171.
14. Kranthi, S. et al. 2017. “Non- Bt Seeds Provided by Seed Companies in India – Are They Suitable as Refuge for Bt-Cotton ?” *Current Science* 112(10): 25–26.
15. Mohan Komarlingam, S., & Sadananda, A. R. (2019). Success of refuge-in-bag for Bt-cotton hinges on good stewardship. Current Science, 117(5), 739–740. https://doi.org/10.1371/journal.pone.0021863.8.
16. Pemsl, D, H Waibel, and A P Gutierrez. 2005. “Why do some Bt-cotton farmers in China continue to use high levels of pesticides.” *International Journal of Agricultural Sustainability* 3(1): 44–56.
17. Remund KM, Dixon DA, Wright DL, Holden LR. 2001. "Statistical considerations in seed purity testing for transgenic traits." *Seed Sci Res.* (11): 101–19.
18. Rochester, Ian J. 2006. “Effect of Genotype, Edaphic, Environmental Conditions, and Agronomic Practices on Cry1Ac Protein Expression in Transgenic Cotton.” *The Journal of Cotton Science* 10: 252–62.
19. Seed world. 2017. “Variety Certification Standard Committee: Certification Standard of Varieties of Main Crops at National Level (in Chinese).” https://m.sohu.com/a/158948011_266055 (March 23, 2020).
20. Singh, B. P. et al. 2016. “Assessment of Bt Trait Purity in Different Generations of Transgenic Cottons.” *Indian Journal of Experimental Biology* 54(4): 237–44.
21. Sofitex. “Le Processus de CQS de La Semence (Tests Spécifiques).” : 6.
22. Spielman, D. J., Zaidi, F., Zambrano, P., Khan, A. A., Ali, S., Cheema, H. M. N., … Ali, G. M. (2017). What are farmers really planting? Measuring the presence and effectiveness of Bt cotton in Pakistan. PLoS ONE, 12(5), 1–10. https://doi.org/10.1371/journal.pone.0176592
23. Sun, Guo-qing et al. 2013. “Influence of Seed Purity on Insect Resistance and Yield in Transgenic Cotton.” *Current Biotechnology* 5(1): 27–31.
24. Tabashnik, Bruce E. 1994. “Delaying Insect Adaptation to Transgenic Plants: Seed Mixtures and Refugia Reconsidered.” *Proceedings of the Royal Society B* 255: 7–12.
25. Vinck, Evelyne. 2003. “Cultures OGM, conventionnelles et biologiques : la coexistence est possible.” *GNIS*. http://www.gnis.fr/index/action/page/id/100/title/Cultures_OGM_conventionnelles_et_biologiques_la_coexistence_est_possible (August 3, 2003).
26. Vognan, Gaspard, and Larbouga Bourgou. 2014. Rapport d’étude sur la Contamination du coton Biologique par le Cotonnier Génétiquement Modifié (CGM), 63 p.
27. Wan, Peng, Yongjun Zhang, Kongming Wu, and Minsong Huang. 2005. “Seasonal expression profiles of insecticidal protein and control efficacy against Helicoverpa armigera for Bt cotton in the Yangtze River Valley of China.” *Journal of Economic Entomology* 95(1): 195–201.
28. Xu, Naiyin, Michel Fok, Lixin Bai, and Zhiguo Zhou. 2008. “Effectiveness and Chemical Pest Control of Bt-Cotton in the Yangtze River Valley , China.” *Crop Protection* 27: 1269–76.
29. Zhao, Jennifer H, Peter Ho, and Hossein Azadi. 2011. “Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China.” *Environmental monitoring and assessment* 173(1–4): 985–94. http://www.ncbi.nlm.nih.gov/pubmed/20437270 (August 10, 2012).

Women's Wealth Status and Factors on Cotton Farms in West Africa

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Abstract

Background: *Gender mainstreaming in rural development has mainly led to appraise women's performance in agricultural production comparatively to men. Studies are rare in understanding the impact of women's agricultural role on their own economic plight, even in Africa where women often have the opportunity to carry out various economic activities and to produce for their own account in fields. Our study in 2014 compensate for the lack by analyzing the characteristics, economic activities, income and assets of women on cotton-growing farms in relation to the characteristics of their husbands who headed farms. It was conducted in three countries with distinct cotton production evolution: continuous and great increase in Burkina Faso, chaotic in unstable cotton sector in Benin and stagnating in Togo having been put aside for decades by the international community.*

Results: *The status of cotton production in the studied countries affected somewhat the economic fate of heads -men- on cotton farms, as well as that of women's albeit a bit less clearly. Men in Togo lagged behind as compared to the other two countries but men in all countries had their wealth positively influenced by their number of wives. The economic situation of women was generally weak but it was better in Benin, particularly in terms of animal assets. Several factors affected women's wealth, notably that of their husbands which influenced positively.*
Conclusion: *Tradition keeps on, through the status of polygamy, but the observed men-women synergy in wealth accumulation is a sign of positive evolution and that should persist because of a context of increasing economic exchanges in rural areas. The mentioned synergy deserves to be integrated into the approaches dealing with the gender and development issue.*

Keywords: *Women, gender & development, household, polygamy, economic activities, poverty, assets, animal production*

Background

Since the 1970s, gender mainstreaming in rural development has led to a focus on the agricultural role of women (FAO, 1983) who are entrusted with multiple tasks, as in Africa (Blackden & Wodon, 2006). This agricultural role of women is particular in Africa because they often have the opportunity to carry out various economic activities (Boserup, 1970) and to produce for their own account in the fields allocated to them (Lilja, Randolph, & Diallo, 1998).

Studies on women's agricultural role have focused on their performance, which is generally penalized by the lesser access to factors of production (Quisumbing, 1996). In the case of cotton cultivation, Wang & Fok (2017) indeed showed that women in China performed similarly to men when they had equal access to factors of production.

Studies are rare in understanding the impact of women's agricultural role on their own economic plight. In China, despite performing at least as well as men by working more (Wang & Fok, 2017), the impact on women's well-being has been found to be rather negative (Chang et al., 2011; Mu & van de Walle, 2011), owing to the burden of caring for ascendants and children. There are very few studies in West African cotton-growing countries where the local factors of patriarchal management, polygamy and ability to farm for their own influence women's income and time availability differently. A rare and long-standing study in Côte d'Ivoire highlighted the negative impact of women being more involved in working on their husbands' cotton fields at the expense of their own fields (Peltre-Wurtz & Steck, 1991). This view has been recently questioned by Aboudou & Fok (2019) who found that women in Benin enjoying the right to produce cotton for their own had a more favorable economic position without affecting negatively their husbands' production.

This study contributes to reducing the lack of information on the economic plight of women on cotton farms in West Africa. The study conducted in 2014 analyses the characteristics, economic activities, income and assets of women on cotton-growing farms of three West African countries in relation to the characteristics of their husbands who headed farms. The three countries had distinct evolution of their cotton production: Burkina Faso with continuous increase up to becoming the African Champion, Benin with chaotic production related the turmoil in its cotton sector, and Togo with stagnating production for lack of international support for decades.

Results

Cotton farms to which women belonged differed in characteristics. These characteristics were distinct as regard to the farm heads (age, high illiteracy rate, frequency of polygamy and therefore the average number of wives and family size). They differed also with regard to the farm features (total area under cultivation, area under cotton, ratio of cotton area in the all crop area, possession of the material goods taken into account, frequency in cattle possession and asset values). Wholly speaking, farms in Burkina Faso were characterized by a higher rate of polygamy; those in Benin had a higher value of assets in the opposite of those in Togo (Table 1).

Women differed between the three countries in terms of age and frequency of being their husbands' single wife. They differed less in the frequency of access to land than in the area of land they had use right for; women in Benin being relatively privileged. However, the areas actually cultivated were less different, although women in Benin were still at an advantage (Table 2).

Women were engaged in growing various cash crops but also in trading and processing agricultural products, with notable differences between countries. Women's involvement in cotton cultivation for their own account was lowest in Burkina Faso (unlike Benin), in contrast to their involvement in sesame and soybean cultivation. The frequency of women's involvement in trade was quite high (at a lower degree in Benin) but not in the processing of agricultural products.

The economic situation of women varied greatly among the three countries. This was true for monetary income and for the value of assets, mainly composed of animals. Women in Benin, being more frequent to have cattle, were much better off than those in Togo were. Reported to annual basis, the level of annual accumulation was low (average of US$ 36) but highly variable between and within countries (indicated by standard deviation values not shown in Table 2). Women in Benin had a better level, but still only at US$ 48.

Table 1: Characteristics of farms to which women belong

Characteristics	Total	Country			p value
		Togo	Benin	Burkina Faso	
Number of farms	546	178	215	153	
About farm heads and families					
Farm head's age	43,5	43.4 b	41.5 b	46.5 a	0,000
Proportion of being illiterate, %	49,1	59.6 a	46.1 b	41.2 b	0,002
Proportion being polygamous, %	42,9	48,3	31,2	52,9	<0.0001
Number of wive	1,5	1.7 a	1.4 b	1.6 a	<0.0001
Number of family members	8,9	9.6 a	8.8 ab	8.2 b	0,013
Abour farm and cotton cultivation					
Cultivated area, ha	12,3	5,5	17,6	12,8	<0.0001
Cotton area, ha	2,8	0.9 b	3.9 a	3.6 a	<0.0001
Cotton area ratio over all crops, %	40,0	35.3 b	46.3 a	36.5 b	<0.0001
Per ha added value, 10^3 FCFA	186	189 a	213 a	146 b	<0,0001
About farm assets					
Proportion of farms having motorbike, %	65,8	37.6 b	83.3 a	73.9 a	<0.0001
Proportion of farms having bike, %	92,1	89.3 b	89.7 b	98.7 a	0,001
Proportion of farms having mobile phone, %	79,8	74.7 b	70.7 b	98.7 a	<0.0001
Proportion of farms having cattle, %	65,4	57.9 b	60.5 b	81.0 a	<0.0001
Asset value in material goods, 10^3 FCFA	565	256 b	758 a	653 a	<0.0001
Asset value in animals, 10^3 FCFA	1 337	892 b	1,534 a	1,577 a	<0.0001
Total asset value, 10^3 FCFA	1 902	1,148 b	2,292 a	2,230 a	<0.0001

Notes : Statistically significant differences at the probability of 95% are marked with distinct letters.
Exchange rate: Euro = 656 FCFA or US$ = 550 FCFA

Table 2: Women's characteristics

Characteristics	Total	Country			Valeur p
		Togo	Benin	B. Faso	
Number of women	509	86	261	162	
Age	34,1	36.3 a	32.2 b	36.0 a	<0.0001
Available area with use right, ha	3,1	1.5 b	4.8 a	1.3 b	<0.0001
Cultivated area, ha	1,0	0.8 b	1.3 a	0.8 b	<0.0001
Proportion of women, %					
Being single wife of their husband	51,3	45.3 b	57.9 a	43.8 b	0,009
Having access to land	94,7	94.2 ab	91.6 b	100.0 a	0,001
Benefitting husband's help					
in farm equipment	79,2	51.2 c	78.5 b	95.1 a	<0.0001
in field work	77,6	69.8 b	69.7 b	94.4 a	<0.0001
in technical advice	58,3	54.7 b	37.2 c	94.4 a	<0.0001
in financial support	43,6	55.8 b	14.2 c	84.6 a	<0.0001
Having:					
Mobile phone	30,3	4.7 b	39.1 a	29.6 a	<0.0001
Bicycle	39,9	5.8 c	23.4 b	84.6 a	<0.0001
Cattle	6,9	0,0	10,0	5,6	0,005
Sheeps	27,5	15.1 b	35.2 a	21.6 b	0,000
Having income from					
Cotton	17,7	20.2 a	24.3 a	4.9 b	<0.0001
Soybean	23,9	30.9 a	11.7 b	34.4 a	<0.0001
Sesame	18,9	15.4 b	3.2 c	46.6 a	<0.0001
Commerce	32,4	34.6 a	23.5 b	43.6 a	<0.0001
Processing of agricultural products	6,4	7.4 b	0.0 b	14.7 a	<0.0001
Total monetary income, 10^3 FCFA	230	183 b	409 a	145 b	<0.0001
Women's assets, 10^3 FCFA					
Assets in material goods [1]	29	3 b	13 b	69 a	<0.0001
Assets in animals [2]	114	54 b	173 a	51 b	<0.0001
Total assets	143	57 b	186 a	120 ab	0,001
Annual accumulation [3]	20	6 b	26 a	17 ab	0,043

[1] Mobile phone, bike, motorbikes, motorized tricyle, motorized grinder; [4] cattle, sheeps, goats; [3] Mean value calculated over the period from women's age of 20 to her age in year 2014.
Notes : Statistically significant differences at the probability of 95% are marked with distinct letters.
Exchange rate: Euro = 656 FCFA or US$ = 550 FCFA

When all factors that could potentially influence the level of women's wealth (indicated by the estimated assets) were considered, multivariate regressions showed that the respective effects of country and type of wife status were not significant (Table 3, box a). Women's wealth was positively influenced by the equipment of women with cell phones and bicycles as well as their involvement in trading activities. It was also positively influenced by their husbands' assistance in the form of access to their equipment as well as their husbands' wealth indicated by their assets in animals.

With regard to the assets of women's husbands, farm heads, they were positively impacted by their education, the number of wives present on the farm, and the size of the cotton area (Table 3, box b). On the other hand, the effect of the share of cotton area in all crop area was negative, indicating the unfavorable effect of an imbalance between crops. The positive effect of women's animal assets on men's assets was highly significant.

Table 3. Regression analyses to explain women's and men's respective assets

a. Depending variable of women's total assets

Explaining factors	Coef.
Country referred to Togo	
Burkina Faso	-0.101
Benin	0.080
Women's characteristics	
Age	0.021
Having polygamous husband	-0.017
Being wife of first rank	-0.028
Number of cell phones	0.114 **
Number of bikes	0.236 ***
Share of activities in the estimated income	
Cotton growing	0.087
Legumes growing	-0.008
Selling of cereals	0.059
Trade	0.190 ***
Factors related to women's husbands	
Husbands' assets in material goods [1]	0.005
Husbands' assets in animals [2]	0.343 ***
Husbands' help in field work	0.082
Husbands' technical advice	-0.001
Husbands' financial help	-0.042
Use of husbands' cultivation equipment	0.146 **
Children's help in field work	
In sowing	0.021
In weeding	0.009
In harvesting	-0.044
p value of Fisher test	<0.0001
DDL and R^2	484 and 0.263

[1] Cell phones, bikes and motorbikes; [2] Cattle, sheeps and goats.
Notes : *, ** and *** for statistical significance at 95, 99 and 99.9% of probability

b. Depending variable of men's total assets

Explaining factors	Coef.
Country referred to Togo	
Burkina Faso	0.055
Benin	-0.005
Factors of farm heads and families	
Farm head age	-0.001
Farm head having been to school	0.093 *
Farm head having got literacy program	-0.05
Number of wive of farm heads	0.085 *
Number of family members	0.004
Number of children at secondary school	0.058
Women's characteristics	
Women's assets in material goods	-0.014
Women's assets in animals	0.290 ***
Farm characteristics	
Total cultivated area	0.037
Cotton area	0.490 ***
Cotton share in cultivated area	-0.094 *
More than 20 years of experience in cotton growi	0.033
Maize share in cultivated area	0.022
p value of Fisher test	<0.0001
DDL and R^2	527 and 0.422

Notes : *, ** and *** for statistical significance at 95, 99 and 99.9% of probability

Discussion

Our study sought to identify the situation of women on cotton farms in three countries where cotton production has evolved differently. Given the importance of cotton in farm income, one would expect that the economic situation on cotton farms would differ between countries, as would the situation of women on these farms.

The results of our study do not really confirm the expectations in terms of the economic situation of farm heads and their wives. For farm heads, those in Togo are indeed disadvantaged, but not those in Benin. Within a country, polygamy affects positively as farm heads' wealth is linked to the number of wives. For women on farms, the differences observed are under the more subtle influence of various factors as elaborated below.

The majority of women are not or not yet, on polygamous farms. The tradition of polygamy of farm heads is maintained in all three countries, affecting nearly half of the farm heads and which is comparable to the range of 21-51% observed by Boserup (1970) for several West African countries. The rate of polygamy observed could even increase if we consider that the average age of farm heads is rather young, indicating that some farmers have not had the time needed to accumulate enough money to pay the dowry for marriage, while the dowry tends to increase.

The study allowed a first quantification of women's assets, generally very low, indicative of their material poverty resulting from two factors. On the one hand, the husbands of these women themselves have a low accumulation. On the other hand, these women are rather young (being in their early thirties, ten years younger than their husbands are) and have not had the time to start accumulating. The low level of women's assets has nevertheless to be nuanced as our study did not take all durable goods into account (like kitchen utensils) and the cost of children's schooling that women might take charge of was deducted.

The variation observed between the three countries calls into question the impact of the husbands' cotton production on the economic fate of their wives. Women in Burkina Faso are certainly better off than those in Togo are, but they are barely at the same level as women in Benin. Women in Burkina Faso may have less time to devote to their own economic activities because they are too busy with their husbands' cotton fields. On the contrary, in Benin, the turpitudes of the cotton industry have been favorable to women in Benin who have gained more freedom in their economic activities (Aboudou & Fok, 2019).

Women are equal among themselves in terms of the income generated and the assets built up regardless of their status as wives, even though women in the position of sole or first wife may cultivate more land. In reality, the possible effects of the type of wife status can be counteracted by that of age. Women at first rank spouse might have more freedom in their economic activities but their age might imply less strength in doing so. In their economic activities, the access to land does not appear to be that much problematic as frequently claimed such as in Quisumbing (1996), in the opposite of the capabilities of fully exploiting the land made available to them.

The negative effect of women's age on the level of annual accumulation is an original result that may indicate a favorable trend for their economic fate. This negative effect can be interpreted in three ways. One would be that older women have lower incomes compared to younger women, while the average age of women is relatively low. This interpretation is only valid if it is accepted that age rapidly affects rural women in the three countries studied; this assumption is realistic given that women are subjected to very long days and physically demanding tasks. A second way is that older women has higher education cost for their children who might be at university, hence reducing their assets in material goods. Finally, another possible interpretation is that the rural economy has become more active (the frequency of motorcycle and telephone equipment may be an indication of this) and that economic opportunities for women have increased in recent years, with the result that older women had not benefited as much as younger ones. The three explanatory hypotheses are nevertheless not antagonistic.

The study shows a positive impact of the husbands' assets on that of the wives, especially that of animal assets, and reversely. The study does not make it possible to explain the mechanism at work, but plausible hypotheses can be put forward. The increase in farmers' animal hoarding may reduce women's crop production activities in favour of more profitable livestock production. On the one hand, women are more likely to be called upon to intervene in the herds of their husbands, in particular to take care of milking and selling milk or cheese, sales traditionally entrusted to women and the proceeds of which go to them (Meyer & Denis, 1999). On the other hand, women may be more easily "rewarded" by their husbands in the form of donated animals, thus providing them with first capital to engage in livestock production on their own account. This hypothesis is consistent with the idea, put forward by Beneria & Sen (1981) that the process of accumulation on farms interacts with the changing roles of men and women. Wholly speaking, women would make their husbands' business their own ones so that their assets were mutually influenced as it was observed. Some extent of wealth synergy hence emerges between men and women. Such a synergy calls upon the frequent approach in gender & development by dealing separately men and women, an approach that Mccune (2011) has denounced as an underlying Western conceptual framework.

Conclusions

In the three countries studied in West Africa, the economic situation of women is weak, although it varies between countries. This situation is reflected in the estimated assets after deduction of the cost for the education of children. The share of animals in assets explains the differences observed between the countries.Tradition keeps on but with signs of evolution favorable to women. On one hand, polygamy is being maintained. On the other hand, the economic accumulation of men, greater for those who are polygamous, has in return a positive effect on that of wives, particularly through the exploitation of animals. A synergistic effect between women and their husbands is thus revealed, albeit not generalized, under conditions that deserve to be further analyzed and taken into account in addressing gender and development issues.

Methods

The data came from a study conducted in 2014 in the cotton-growing areas of three countries with distinct production evolution. Cotton production has experienced strong and continuous growth in Burkina Faso, a country which has benefited of long support from the international community, unlike

Togo where production rather stagnated. In Benin, production has been chaotic because of the turpitudes of the cotton sector linked to its reform.

Data collection in each country was carried out by focusing on two provinces, 4 villages per province. In each village, the sample of 20-30 cotton farms has resulted from discussions with village officials to represent the various farm sizes.

Farm surveys were implemented with separate interviews of men and women. They covered the characteristics of the farm heads (sex, age, education) and their families (number of wives, family size), durable goods owned (limited to cellular phones, bicycles, motorcycles), structural characteristics of the farm (size, cultivation equipment, livestock), cropping systems, and finally, costs and revenues in cotton production. The questions asked to women were quite similar but limited to what they were (as wives), had (as material goods, including equipment for processing agricultural products) and conducted as economic activities.

As women were illiterate, the income of each woman was estimated by asking whether she could recall and indicate the amount(s) corresponding to five possible sources of income (sale of cotton, soybeans, sesame, processed agricultural products and income from trade). If so, when faced with 10 stones displayed in front of her to represent her total cash income, the woman was asked to indicate the number of stones that could represent the share of each income amount she had mentioned.

The assets, in terms of equipment and animals, were valued at their replacement cost, taking into account market prices in 2015. For men, agricultural equipment was not taken into account because it was generally very old. For women, an average annual accumulation capacity was in addition deducted from the estimated assets, based on the distribution of assets over the number of years between the age of 20 and women's age in 2014. The option of the age of 20 years (women got married before this age) is an approximation but sufficient to highlight the differences between countries.Multivariate regressions were carried out to explain the wealth of farm heads and that of women, respectively, based on the characteristics of the farmers, their families and their farms. The assets accumulated by the spouses were specifically integrated in order to determine the degree of antagonism or synergy in the economic accumulation between spouses on a farm. The size constraint of the article does not allow for the presentation of the anticipated effects of the explanatory variables.

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References

1. Aboudou, F., & Fok, M. (2019). Women ' s empowerment in cotton growing : a case in Northern Benin. 04(01), 13–29. https://doi.org/10.19268/JGAFS.412019.2
2. Beneria, L., & Sen, G. (1981). Accumulation, Reproduction, and Women's Role in Economic Development : Boserup Revisited. Signs, 7(2), 279–298. https://doi.org/10.1086/493882
3. Blackden, M. C., & Wodon, Q. (2006). Gender, Time Use, and Poverty in Sub-Saharan Africa (No. 73). Washington D.C. (USA).
4. Boserup, E. (1970). The economics of polygamy. In Women's role in economic development (pp. 37–50). London: George Allan and Unwin.
5. Chang, H., MacPhail, F., & Dong, X. (2011). The Feminization of Labor and the Time-Use Gender Gap in Rural China. Feminist Economics, 17(4), 93–124.
6. FAO. (1983). The state of food and agriculture. World Review, the situation in Sub-Saharan African. Women in developing agriculture. Roma: FAO.
7. Lilja, N., Randolph, T. F., & Diallo, A. (1998). Estimating gender differences in agricultural productivity: Biases due to omission of gender-influenced variables and endogeneity of regressors. American Agricultural Economics Association Annual Meeting. Salt Lake City, Utah.
8. Mccune, J. (2011). Problematic Aspects of Ester Boserup ' s Woman ' s Role In Economic Development. Retrieved April 20, 2016, from Africa website: http://www.africaresource.com/essays-a-reviews/gender/131-problematic-aspects-of-ester-boserups-womans-role-in-economic-development?showall=&limitstart=
9. Meyer, C., & Denis, J. P. (1999). Elevage de la vache laitière en zone tropicale. Retrieved from https://books.google.fr/books?id=7sEiCwAAQBAJ
10. Mu, R., & van de Walle, D. (2011). Left behind to farm? Women's labor re-allocation in rural China. Labour Economics, 18, S83–S97. https://doi.org/10.1016/j.labeco.2011.01.009
11. Peltre-Wurtz, J., & Steck, B. (1991). Les charrues de la Bagoué. Gestion paysanne d'une opération cotonnière en Côte d'Ivoire. Bondy (France): ORSTOM.
12. Quisumbing, A. R. (1996). Male-Female Differences in Agricultural Productivity :Methodological Issues and Empirical Evidence. World Development, 24(10), 1579–1595.
13. Wang, G., & Fok, M. (2017). Are women less capable in crop managing? Insights from cotton production in northern China. Feminist Economics, 23(4), 117–142. https://doi.org/10.1080/13545701.2016.1241416

Are farmers in Cote d'Ivoire technically conservative in growing cotton?

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Abstract

Background: *Under unfavorable natural and economic conditions, a great stability in the cultivation techniques of cotton contributes to the low productivity level in West and Central Africa. Techniques are available at the world level to help increase field productivity, but their adoption is not ensured without improved farmer-extension-research linkage, notably through experiential actions to consider farmers' views and accompany their appropriation of new and alternative techniques. This communication refers to an experiential action about a transplanting technique to improve plant density, targeting at addressing farmers' perception about their current cultivation practices and alternative cultivation techniques in various domains.*

Results: *Farmers differ little in their perceptions. They seldom contemplate using less chemicals to improve their productivity and profitability. They were rather happy of the plant densities they achieved, despite of being by 25% lower than the recommended density of 83300 plants/ha. Very variable yields were observed for similar plant densities, indicating that the current sowing technique is not optimally implemented.*
The impacts of transplanting varied according to three observed factors, notably the period of cotton sowing. This result illustrates that new techniques are not necessarily to substitute to an existing one but to complement it ~~i~~during evolving and uncertain production conditions. Reactions observed do not show farmers being technically conservative but rather suffering from a lack of information stemming from a technical assistance process not dedicated enough into experiential actions to open farmers' horizon of technical options.

Conclusion: *The results obtained in demonstrating the transplanting technique give more rationale to implement similar experiential actions to make know and debate on alternative techniques for their adjustment and appropriation. At the world level, there is a valuable reservoir of techniques already widely applied. The application of some of these techniques, even the most adapted to Africa at first glance, could not materialize without a plan and means for experiential actions to introduce them.*
Keywords: *Productivity, experiential action, plant density, transplanting, farmers' perception, adoption, Côte d'Ivoire*

Background

Many developing countries lag behind in cotton field productivity, especially in West and Central African despite its significant position in production and furthermore in exportation. Wholly speaking, the six major cotton-producing countries of the mentioned region (Benin, Mali, Côte d'Ivoire, Burkina Faso, Cameroon and Togo) accounts for around 10% of world exportation from a production share of 3-5%. The average seed cotton yield of around 1,000 kg/ha (or 430 kg/ha of cotton lint) is much below the world average (ICAC 2019) and it has been stagnating, if not decreasing, for the last two decades.

Natural and economic factors account substantially to the poor field productivity above mentioned ; they should prevent from excessive optimism about the degree and speed of the impacts on field productivity by introducing alternative techniques that are effective in other countries. Cotton production is totally rain-fed while climate change is manifest both in the erratic feature of rain settlement and amount. In the opposite of Eastern and austral Africa, soils are poor, badly structured and with very low organic matter content. In Côte d'Ivoire, cotton crop soils have very low levels of

total organic matter, total nitrogen, available phosphorus, calcium, potassium and low cation exchange capacity values (Kouadio et al, 2018). Consequently, the effect on yield of fertilizer use is attractive but the cost of fertilizers has been continuously increasing while farmers are financially resource-poor.

The poor field productivity, although hampered by unfavorable natural and economic factors, is nevertheless related to a great stability in the cultivation techniques of cotton. The way cotton is being cultivated is little distinct from what it was four to five decades ago, in terms of installing cotton crop, fertilizing and protecting against various pests. The control of cotton pests is the only area where technical changes have been observed, either through a better use of chemical (Silvie et al. 2001; Renou et al. 2012) or through the implementation of topping (Renou, Téréta, and Togola 2011), but still at limited scale and only in a single country (Mali). Otherwise, the plant density being recommended (83,300 plants/ha, two plants per hole) has not been altered in any sense and growth regulator to control plant height and canopy is seldom used. These two examples of techniques are purposely indicated because of their wide application in many countries and of the enormous change of cropping patterns that they have implied, notably in narrow row planting in USA (Larson, Roberts, and Cooney 2003) that has inspired cotton growers in Argentina and Brazil.

Techniques are available to help increase field productivity in West and Central Africa, but their availability is not sufficient to anticipate that they would be adopted successfully. Many works have pointed out that the lack of application of research outputs in developing countries has resulted from the lack of appropriation by farmers on one hand and the lack of approach of researchers to understand and consider farmers' perception of their production environment on the other hand. Such an observation is at the basis of the advocacy for improved farmer-extension-research linkage by considering three extension models –technology transfer, advisory and facilitation of learning– identified by Van den Ban (1998).

Nowadays, more support is given to approaches based on experiential education like Farmer Field School because knowledge should no longer be regarded as a commodity that only needs to be transferred from researchers to farmers (Angstreich and Zinnah 2007).

This communication is related to an experiential action conducted in Côte d'Ivoire on a transplanting technique destined to improve plant density. The action involved the capture of farmers' perception about their current cultivation practices and alternative cultivation techniques in various domains, in line with the view that the perception of technology-specific characteristics is key for the adoption of new technologies (Adesina and Zinnah 1993). The alternative techniques were expressed through the alterations implied in managing soil and plot, using chemicals, arranging plant density and canopy.

Results

In Côte d'Ivoire, cotton farming was somehow diverse even when production was essentially manual complemented by ox-drawn equipment. For sake of simplicity, cotton farms were distinguished by two types according to their size (Table 1). Big cotton farms demonstrated a cotton area double than that of the smaller ones, thanks to more land available for cropping and bigger families. Their wealth status was much better, with reference to the number of cattle heads: this criterion was relevant in a country where the tradition of economic accumulation through cattle hoarding still prevailed.

Cotton production mattered a lot for farmers who in great majority found it profitable and seldom could afford not growing cotton for several years. Almost all farmers (93.3%), regardless of the size of cotton farming, wanted to increase the income deriving from cotton production but only 69.6% expected to achieve this through the increase of field productivity (meaning that about 30% relied on extending cotton area).

The two types of farmers did not differ neither about how to achieve a better field productivity of cotton. Most farmers expected reaching higher yield of cotton and did not contemplate using chemicals less than the current levels which were rather low (200-250 kg/ha of fertilizers composed of 150-200 kg of compound fertilizers and 50 kg of urea; 5-6 sprays of insecticide). They expected higher productivity through paying less for external services including labor hiring. They were in majority willing to harvest a crop additional to cotton, especially the heads of smaller cotton farms. Farmers seldom indicated referring to means distinct from those suggested during the survey.

Table 1: Farmers' characteristics and opinions on cotton growing

Characteristics of farmers/farms and farmers' opinions on cotton cropping	Total	Farm size		p value
		Small	Big	
Number of farms	188	118	71	
Farmers' age	42.2	41.4	43.7	0.090
Number of wives	1.3	1.3	1.2	0.627
Number of family members at field work	5.6	5.1	6.5	0.008
Number of cattle heads	8.6	0.6	21.4	<0.0001
Total area available for crops, ha	20.0	18.1	23.2	0.000
Cotton area, ha	3.4	2.4	5.0	<0.0001
Seedcotton yeld, kg/ha	1,199	1,154	1,262	0.169
Number of farmers expressing their mind about cotton cropping	209	135	74	
Shares of farmers, %				
saying cotton is profitable	92.8	91.9	94.6	0.631
saying capable of skipping cotton for a while	15.3	14.8	16.2	0.946
Wishing to increase cotton income	92.8	93.3	91.9	0.918
Wishing to increase cotton plot productivity	69.6	74.0	61.8	0.119
by harvesting more cotton on the plot	82.8	84.4	79.7	0.514
by using less fertilizers	15.8	14.8	17.6	0.751
by using less herbicides	15.3	15.6	14.9	1.000
by using less insecticides	9.1	9.6	8.1	0.907
by using less labor	40.2	40.7	39.2	0.943
by paying less for services	43.5	39.3	51.4	0.123
by harvesting another crop on the plot	62.7	68.1	52.7	0.041
by other means	12.0	11.9	12.2	1.000

Table 2: Farmers' approvals of suggested alternative techniques

	Cotton farms			p Value
	All	Small	Big	
Plot and soil management				
Manage plots of smaller size	40.7	40.0	41.9	0.905
Use of manure	52.2	51.1	54.1	0.793
Conduct alternative action to improve the soil organic status	53.1	51.9	55.4	0.728
Conduct other soil management action	42.1	42.2	41.9	1.000
Use of chemicals				
Use more fertilizers	46.9	47.4	45.9	0.954
Modify the modalities in using fertilizers	20.1	18.5	23.0	0.565
Use other types of fertilizers	37.3	37.0	37.8	1.000
Use more insecticides	29.7	29.6	29.7	1.000
Stop using insecticides	1.9	0.7	4.1	0.347
Use other products in addition to insecticides	43.1	42.2	44.6	0.853
Modify the modalities in using insecticides	21.1	14.8	32.4	0.008
Use other techniques not related to insecticides	37.8	36.3	40.5	0.650
Management of plant density on plot				
Modify the modalities in sowing cotton	36.8	33.3	43.2	0.208
Increase the number of plants per unit area	27.8	25.2	32.4	0.347
Decrease the number of plants per unit area	5.7	4.4	8.1	0.471
Management of plot canopy and harvest				
Increase the plant size	9.1	8.9	9.5	1.000
Decrease the plant size	23.0	21.5	25.7	0.611
Implement plant topping	20.6	16.3	28.4	0.072
Use of alternative plant shape	8.6	6.7	12.2	0.308
Use of varieties with grouped harvest	36.8	36.3	37.8	0.944
Implement technique to group harvest	36.4	34.1	40.5	0.440
Crop settlement				
Associate cotton with food crops	7.2	4.4	12.2	0.112
Associate cotton with cash crop	2.4	3.0	1.4	0.775
Intercrop at the early stage of cotton plants	3.8	3.7	4.1	1.000
Intercrop at the late stage of cotton plants	9.6	12.6	4.1	0.041
Implement technique adapted to late settlement of rains	70.3	75.6	60.8	0.045
Implement technique adapted to early settlement of rains	60.8	66.7	50.0	0.028

When asked whether they approved a series of suggested alternative techniques or ways in various domains (plot and soil management, use of chemicals…etc.) and which were already implemented in other cotton countries, approval rarely dominated just as total disapproval (Table 2). Dominating approval was observed for techniques to adapt to erratic settlement of rains and farmers were in slight majority for actions to improve the soil organic status although the use of manure has been promoted for a long time.

Farmers were in minority, and sometimes at tiny proportions, to approve other ideas related to the management of plant density and plot canopy, through either specific cultivation technique or adapted plant shape. The approach of intercropping cotton gained little interest.

With regard to the use of chemicals, farmers were rather open to complement chemical use by cultivation techniques but rejected the idea of stopping using insecticides. For fertilizers, they had no clear position about using more, using new types or altering the modalities of use.

With regard to the specific domain of cotton crop settlement at sowing, a bit more than half of all farmers claimed to be happy with the plant density they achieved (Table 3). Similar proportion however wished to achieve a higher density but without really questioning the planting arrangement, in terms of plant hole distance or the number of seeds or plants per hole. Despite being supplied with 30 kg of fuzzed seeds per hectare, farmers did not feel they were using too many seeds while they did not always implement plant thinning and not at the optimal period. Only one third of farmers claimed that the current sowing mode was detrimental to yield while two thirds claimed in the same time that they would accept to evolve from the current mode.

The issue of plant density achieved by farmers was showcased by the tests implemented to assess the impacts of the transplanting technique (Table 4), although the implementation was not optimal

(Table 6). Plant density achieved by farmers according to their current sowing mode was about 60,000 plants/ha, below the recommended density of 83,300 plants/ha. Plant densities achieved by farmers varied, but less than seedcotton yield, indicating that similar density levels could lead to very distinct yields.

Table 3: Farmers' opinions on their sowing practices

Farmers' opinions on sowing practices of cotton	Total	Farm size		p value
		Small	Big	
Number of farmers concerned	59	32	27	
Distribution of farmers, %				
being happy with plant density achieved	52.5	56.3	48.1	0.719
wishing a higher plant density	54.2	40.6	70.4	0.033
considering using too many seeds per unit area	10.2	6.3	14.8	0.523
considering not enough seeds per hole	18.6	18.8	18.5	1.000
conidering insufficient plant holes per row	1.7	3.1	0.0	0.428
not implementing, not always, plant thinning	20.3	18.8	22.2	0.524
claiming to always thin plant holes on time	66.1	59.4	74.1	0.351
indicating right optimal thinning period*	84.7	81.3	88.9	0.645
considering the current sowing detrimental to yield	33.9	28.1	40.7	0.456
accepting the evolve from the current sowing mode	66.1	68.8	63.0	1.000

*before 15 days after emergence

The impact of compensating plant densities by transplanting to increase yield led to variable effect but less than expected because the density compensation achieved was limited and did not allow getting closer to the recommended density. The impact of transplanting was also lessen by the delay of its implementation which should not have existed were the tests implemented properly. The signs of the coefficients in the regressions conducted to explain the observed gains of transplanting, either partial or total, over farmers' current mode showed that the impact of transplanting was higher when implemented with little delay and on tests sown lately, although statistical significance was not always observed because of the insufficient number of tests (Table 5).

Although the implementation of the program to demonstrate the transplanting technique was not optimal, and by far, valuable information was revealed on farmers' perception about the introduction of a new technique. Indeed, less than half of the tests was implemented according to instructions to compare farmers' sowing mode and transplanting at partial or total level (Table 6). Farmers nevertheless claimed that the technique could be useful although they were particularly aware of the associated constraint of labour requirement to prepare and maintain plantlets in nurseries. They saw the advantages of seed saving, of potential yield gain and better adaptation to climate change making erratic the settlement of rains.

Table 4: Densities and yields on tests demonstrating the transplanting technique

	Extent of transplanting delay [2]	Number of tests	Plant density of FM [4]	Seedcotton yield of FM [4]	Gain from Partial Transplanting [5]		Gain from Total Transplanting [6]	
					Density	Yield	Density	Yield
Tests of good yield level [1]	<1 week	8	64,797	3,909	531	92	-1,219	356
	1-2 weeks	8	48,781	1,542	3,688	50	8,406	177
	>2 weeks	2	54,000	1,625	9,875	-313	-7,125	-188
	Unknown [3]	2	36,347	1,854	-1,375	21	-1,306	-375
	Total	20	54,466	2,529	2,538	28	2,032	157
Test of poor yield level [1]	<1 week	4	66,594	775	2,521	23	615	51
	1-2 weeks	7	63,024	835	3,625	9	5,829	49
	>2 weeks	2	77,250	1,063	1,375	63	250	0
	Unknown [3]	13	66,311	852	2,939	21	3,366	42
	Total							
All tests	<1 week	12	65,396	2,865	1,194	69	-608	255
	1-2 weeks	15	55,428	1,212	3,658	31	7,204	117
	>2 weeks	4	65,625	1,344	5,625	-125	-3,438	-94
	Unknown [3]	2	36,347	1,854	-1,375	21	-1,306	-375
	Total	33	59,132	1,868	2,696	25	2,558	112

[1] The threshold for seedcotton was set at 1,200 kg/ha corresponding to the average yield of Cote d'Ivoire over 2014-2018; [2] Delay of transplanting with regard to the sowing of the plot of farmer's mode of crop installation; [3] Cases of missing dates about sowing or transplanting; [4] Farmer's mode of installing cotton crop by sowing; [5] Lack of emergence was compensated by transplanting; 6 Plot totally installed by transplanting plantlets obtained from sowing seeds on nutrblocks and kept in nursery;

Table 5: Explaining the variable impact of transplanting

Explaining variables	Gain of partial transplanting over FM[1]				Gain of total transplanting over FM[1]			
	in plant density		in yield		in plant density		in yield	
	Coef.	p Value	Coef.	p Value	Coef.	p Value	Coef.	p Value
Setting period of FM[2]	-0.331	0.066	-0.163	0.371	-0.225	0.094	-0.057	0.667
Achieved plant density of FM	-0.077	0.672	-0.121	0.519	**-0.362**	**0.008**	-0.094	0.475
Transplanting delay[3]	0.032	0.860	-0.160	0.391	-0.220	0.103	**-0.483**	**0.001**
Number of tests	31		31		47		47	
F Fisher	1.393		0.691		4.813		4.886	
p Value of F	0.264		0.565		0.005		0.005	

[1] FM = Farmers' current mode of sowing cotton

[2] Five setting periods of two weeks were considered from end of may

[3] Delay in days between transplanting and the sowing date of the FM plot, although transplanting was recommended to be implemented the same day

Discussion

In our study, farmers differ little in their perceptions and they are attached to cotton growing in spite of rather low yield achieved; this is consistent with what was observed in a former study on more than one thousand farmers (Fok et al. 2016). This observation gives more rationale to improve its productivity and profitability, and explore technical ways suitable and acceptable to farmers.

Clearly, farmers seldom contemplate using less chemicals to improve their productivity and profitability although they are relatively pricey. The amounts currently used are indeed relatively small as compared to other cotton producing countries like China (Fok 2011) or Brazil (Mendez del Vilar, Alvez, and Keita 2006). Consequently, cotton responses to chemicals remain high and negative effects of excessive use are not yet perceived. Our observation warns against the temptation of advocating the reduction of chemicals regardless of the levels of their use.

Table 6: Farmers' perception of constraints and advantages of transplanting

	Total	Cotco 1*	Cotco 2*	p Value
Number of implemented tests	74	43.0	31.0	
Proportion of tests, %				
implemented till harvest	82.4	93.1	67.7	0.015
implemented till harvest with 3 installation modes**	44.6	60.5	22.6	0.001
Proportion of farmers, %				
declaring transplanting technique useful	83.6	95.3	66.7	0.002
indicating their will to adopt transplanting	76.7	86.0	63.3	0.053
stating prefering partial transplanting	75.8	78.9	71.4	0.683
Proportion of farmers seeing little constraint for, %				
getting the right materials for plantlet substrate	47.3	39.5	58.1	0.174
manufacturing the substrate	43.2	37.2	51.6	0.317
manufacturing nutriblock	40.5	23.3	64.5	0.000
watering nutriblocks in nurseries	43.2	41.9	45.2	0.964
committing the needed labour	16.7	9.8	25.8	0.128
Proportion of farmers indicating advantages of transplanting, %				
in saving seeds	80.8	83.3	77.4	0.742
in saving labour	16.7	2.4	35.5	0.001
in gaining yield	61.6	64.3	58.1	0.767
in adapting to climate change	56.9	63.4	48.4	0.298

* Mainly two cotton companies committed with implementing tests at farmers' level but they are designated by their real names; ** Tests were to be implemented with three treatments: two lines sown according to farmers' mode current mode, two lines of partial transplanting compensating the lack of emergence after sowing according to farmers' mode, two lines of total transplanting with previously prepared seedlings.

In the area of cotton crop installation, densities achieved are by 25% below what is recommended despite using a rather high seed dosage, but farmers are rather happy with the plant densities they obtain. Because the recommendation of plant density dates back to research done several decades ago, while soil fertility and climatic conditions have evolved, it would be relevant to update the research on cotton plant density and arrangement.

Plant density is a factor to yield but the way it is achieved impacts differently on productivity. The demonstration tests –conducted to inform about the transplanting technique– shows that yields could differ with similar plant densities obtained by farmers according to their current way of sowing at high seed dosage and not systematically thinning on time. When thinning is not implemented –this often happens when sowing has been late– four to six plants at the same hole contribute to increase plant density but not too much to yield because of the intra-hole plant competition. This observation means that the sowing technique being adopted currently is not optimal and needs to be complemented.

A first attempt to make know about the transplanting technique –as an alternative way to install cotton crop to compensate or substitute to sowing– provides valuable information in spite of results hampered by non-optimal implementation of the tests. With regard to the three characteristics conducive to innovation adoption –compatibility to needs, relative advantage and complexity–revealed by Tornatzky and Klein (1982), transplanting is not perfect. The need for the technique might not be felt by farmers who are not paying much attention to the issue of plant density. The technique is not that much complex but the labour requirement reduces its relative advantage.
The non-optimal implementation of the tests has implied that the observed effects on yield were lower than expected, but it comes clear that the effects are better when the current mode (by sowing at high seed dosage) cannot be installed early enough in season. This result illustrates the fact that new techniques are not necessarily to be contemplated in view of totally substituting to an existing one but rather to complementing it, notably when conditions for optimal implementation of an existing technique are not met.The observation above also warns against promising or letting to expect miraculous techniques that would work for all, under any conditions. The challenge is to help farmers adapting to fluctuating production conditions through the supply of more knowledge about possible techniques, in a learning process (Angstreich and Zinnah 2007), so that farmers could combine them to fit to their specific producing conditions.
While farmers' perceptions on possible techniques suggested to them (Table 2) might show them technically conservative, this view has to be nuanced. A majority of farmers is seldom found to approve the alternative techniques suggested to them, but farmers' mind openness can be observed by the rare total rejection of these techniques. The reaction of those who were involved in the

demonstration tests of transplanting (Table 6) indicate that farmers are fully capable of identifying the advantages and constraints associated to this new technique, confirming the view of Adesina and Zinnah (1993). In fact, farmers of our study are not technically conservative but lack information on alternative techniques to combine them into their own baskets of techniques.

The current technical status of cotton farmers reflects the way cotton sectors had been reformed in West and Central Africa and more specifically in Cote d'Ivoire. In this latter country, the reform of the cotton sector since 1998 has been mainly administrative (more elaboration on this point would lead us too much out of topic) and has not been conducive to promote technical knowledge and alternatives. Despite training sessions prior to the test implementation, the substantial rate of tests not properly set up indicates that the implementation to show new techniques was not frequent and not on top of the extensionists' priorities. Demonstration of technical novelties was rare because inside cotton companies operating in Côte d'Ivoire, there is also a strong belief that new techniques should only be shown to farmers when they are totally finalized, implicitly to fit to all farmers under any conditions. This belief, corresponding to the technology transfer model of extension (Van den Ban, 1998), is associated to the idea that when a promising technique is obtained at research level, it suffices one or two years of promotion to have it adopted at large scale in the field. This belief overlooks the other two models for technology progress, notably that of facilitation for learning by farmers.

The challenge of improving substantially the productivity and profitability of cotton growing implies the challenge of evolving from the current technical assistance based on top-down relationship towards more joint experiential actions on alternative techniques. In such an approach, alternative techniques are demonstrated not in view of adoption as such, but a material for technical interaction between extensionists and farmers. Through such an interaction, a new technique, if actually valuable, can be adjusted and the conditions of its application are clarified.

Conclusions

We have a balanced view on how farmers are technically conservative in growing cotton. On one hand, they appear to be conservative when confronted to ideas of alternative techniques. On the other hand, when confronted with the implementation of a specific alternative technique, farmers show their capabilities to catch the associated advantages and constraints. So, farmers' apparent conservative behavior stems from a lack of information related to the current process of technical assistance that should invest more to help farmers know about alternative techniques. The results obtained in demonstrating the transplanting technique –albeit not optimally implemented– give more rationale to implement similar experiential actions to make know and debate on alternative techniques for their adjustment and appropriation. At the world level, there is a valuable reservoir of techniques already widely applied. The application of some of these techniques, even the most adapted to Africa at first glance, could not materialize without a plan and means for experiential actions.

Methods

The study is based on the data collected in the framework of a project funded to demonstrate the transplanting technique destined to compensate the lack of density after seed emergence. The technique is inspired from what China has been applying since the late 1980s and described in detail by Fok and Xu (2007). The projects involved two cotton companies (COIC and Ivoire Coton) operating in Northern Cote d'Ivoire to set up 160 demonstration tests in 2019 after due training of extensionists and concerned farmers to prepare plantlets and manage nurseries. Unfortunately, only half of the program has been implemented mainly because of the late settlement of rains that pushed farmers to focus on their own production plots at the expense of the tests.

Each test was composed of three treatments of two lines of 25 meters without replications:

- FM = farmers' current sowing mode at high seed dosage of 4-6 seeds per hole (0,80 m of inter-rows, 0.30 m of inter-holes in rows, two plants per hole after recommended thinning for a targeted plant density of 83,300 plants/ha, compensation of empty holes by resowing);
- PT = partial transplanting which is FM whose empty holes by lack of emergence are compensated by transplanting;
- TT = total transplanting or crop installed by transplanting instead of sowing.

It was instructed to implement transplanting of 14 day-old plantlets at the date of sowing of the FM lines (meaning that nurseries of plants had to be prepared in advance), but unfortunately transplanting was generally delayed, particularly because the lack of rains did not give sufficient soil moisture to secure the survival of plantlets once transplanted.

Collaborating farmers were requested to give their opinions about their current practices in installing their cotton crop as well as about the constraints and possible advantages of the transplanting technique. In addition to the descriptive analysis of farmers' opinions on transplanting, regressions were conducted to explain the gains that transplanting might bring to increase plant densities or yield. Three factors of potential influence were considered: the period of sowing, the level of plant density achieved on FM and the delay at which transplanting was actually implemented with regard to the sowing of FM lines. A bigger set of farmers (not only those conducting the demonstration tests) was asked about their farm characteristics and whether they would approve or not a series of alternative ideas of growing cotton. The series of questions is summarized below (Table 7) with indication of the corresponding techniques adopted elsewhere.

Table 7: Domains of questions and corresponding cultivation techniques

Areas addressed in questions asked*	Corresponding technique
Use of manure to improve soil	Manuring has been promoted for decades in West Africa
Other action to improve soil organic matter status	e.g. green manure
Soil improvement action not on organic matter content	e.g. control of soil erosion or water run-off
Use more fertilizers	Fertilizing is much higher out of Africa
Alter ways or timing of fertilizing	Various ways existing
Use of alternative types of fertilizers	Alternative types in terms of composition or nutrient release
Use more insecticides	More and more types of insecticides are used elsewhere
Not using at all inseciticdes	Some production modes are prohibiting insecticide use
Use of alternative products than insecticides to control pests	e.g. biocides
Alter ways or timing of insecticide sprays	e.g. sprays based on scouting
Use of techniques not based on insecticides to control pests	e.g. use of trap plants
Alter the sowing mode to achieve the targeted plant density	e.g. precision sowing and use of quality seeds
Increase the number of plants at harvest	High plant density like in ultra narrow rows
Decrease the number of plants at harvest	Plant density adapted to hybrid varieties
Increase the size of cotton plants	Plants are quite tall if not too tall already in Cote d'Ivoire
Decrease the size of cotton plants	Size is much smaller in countries with mechanical harvest
Top cotton plants at a given period	Common practice in China, and under promotion in Mali
Use cotton plants of alternative shapes	e.g. varieties with monopodial stems
Use cotton varieties with grouped boll maturing	Varieties with monopodial stems
Implement technique to group boll maturing	Program of growth regulation
Intercrop cotton with food crops	e.g. various intercropping patterns in China
Intercrop cotton with other cash crops	e.g. various intercropping patterns in China
Intercrop at early stage of cotton cycle	e.g. various intercropping patterns in China
Intercrop at late stage of cotton cycle	e.g. various intercropping patterns in China
Use of technique adapted to late rain settlement	e.g. adjusing cotton cycle length
Use of technique adapted to early rain settlement	e.g. adjusing cotton cycle length

* Farmers were asked if they could approve the suggested ideas

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List of references

1. Adesina, Akinwumi A. and Moses M. Zinnah. 1993. “Technology Characteristics, Farmers’ Perceptions and Adoption Decisions: A Tobit Model Application in Sierra Leone.” *Agricultural Economics* 9(4):297–311.
2. Angstreich, Michael and Moses Zinnah. 2007. “A Meeting of the Minds: Farmer, Extensionist, and Researcher.” *Journal of International Agricultural and Extension Education* 14(3).
3. Van den Ban, Anne Willem. 1998. “Supporting Farmers, Decision Making Processes by Agricultural Extension.” *Journal of Extension Systems* 14:55–67.
4. Fok, M. 2011. “Variety Market Development: A Bt Cotton Cropping Factor and Constraint in China.” *AgBioForum* 14(2).

5. Fok, Michel, Siaka Koné, Francis Akindes, and Marceline Soro. 2016. *Mission d'observation Des Exploitations Cotonnières En Côte d'Ivoire*. Abidjan (Côte d'Ivoire).
6. Fok, Michel and Naiyin Xu. 2007. "Technology integration and seed market organization: The case of GM Cotton diffusion in Jiangsu Province (China)." *Life Sciences International Journal* 1(1):59–72.
7. Kouadio, EN, Koffi, EK, Julien, KB, Messoum, GF, Brou, K., & N'guessan, DB (2018). Diagnostic de l'Etat de Fertilité des Sols Sous Culture Cotonnière Dans les Principaux Bassins de Production de Côte d'Ivoire. *Revue scientifique européenne, ESJ* , *14* (33), 221. https://doi.org/10.19044/esj.2018.v14n33p22
8. ICAC. 2019. *Cotton Data Book*. Washington D.C. (USA): ICAC.
9. Larson, J. A., R. K. Roberts, and M. B. Cooney. 2003. "Yield and net revenue response to plant population for UNR cotton." in *PROCEEDINGS OF THE 2003 BELTWIDE COTTON CONFERENCES. Jan. 6-10, Nashville (TN)*, edited by D. A. Richter. National Cotton Council (Memphis).
10. Mendez del Vilar, Patricio, Lucillo R. A. Alvez, and Manda Sadio Keita. 2006. "Facteurs de performance et de compétitivité des exploitations cotonnières au Brésil, aux Etats-Unis et au Mali." *Cahiers Agricultures* 15(3):23–34.
11. Renou, Alain, Idrissa Téréta, and Mamoutou Togola. 2011. "Manual topping decreases bollworm infestations in cotton cultivation in Mali." *Crop Protection* 30:1370–75.
12. Renou, Alain, Mamoutou Togola, Idrissa Téréta, and Thierry Brévault. 2012. "First Steps T,owards 'Green' Cotton in Mali." *Outlooks on Pest Management* 23(4):173–76.
13. Silvie, P., J. P. Deguine, S. Nibouche, B. Michel, and M. Vaissayre. 2001. "Potential of Threshold-Based Interventions for Cotton Pest Control by Small Farmers in West Africa." *Crop Protection* 20(4):297–301.
14. Tornatzky, Louis G. and Katherine J. Klein. 1982. "Innovation Characteristics and Innovation Adoption-Implementation : A Meta-Analysis of Findings." *IEEE TRANSACTIONS ON ENGINEERING MANAGEMENT* 29(1):28–44.

Robotic Cotton Harvest Design Criteria and Concepts

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Abstract

Several companies have developed prototype autonomous weed control systems and there has been on-going research to develop robotic harvest systems for fruit and vegetable crops. Cotton harvest is in a unique position to benefit from this progress as frequent harvest would limit risk from yield and quality loss due to rainfall and extreme weather events. There are many design criteria that must be considered in developing an automated systems for cotton harvest, including the number of passes to be made through the field per season, the mass of seed cotton to be transported, and number of machines needed to harvest a given area. This paper examines how these design parameters vary for a base scenario of a single row system traveling at 5 km hr^{-1}, with an end of season yield of 1500 kg fiber per ha and an average field length of 0.5 km for 800 ha of cotton. For this base scenario, if harvest was all conducted in a single event as is currently done with most mechanized systems, the system would need 8 single row machines each harvesting 95 bolls per second and have a storage capacity of 2 cubic meters in order to cross the field one time over a period of 28 days. Increasing the number of harvest events per year reduces both the bolls removed per second and storage capacity requirements but increases the number of machines needed. For example, increasing to 5 harvest events per season over 60 days for the otherwise same base scenario would increase the number of machines needed to 17, but reduce the bolls harvest rate to 19 bolls per second and storage capacity to 0.4 m^3 per machine. Based on the rate of bolls that must be harvest per second in all of the scenarios considered, it likely the first commercially viable for system for cotton harvest will require a number of individual "arms" with 1 to 2 degrees of freedom. Additional design criteria will be very dependent on economic considerations.

Keywords: Robotics, automation, harvest

Background

Over three decades ago Sistler (1987) provided a review of robotic applications and future possibilities for robotic applications in agriculture. That vision is becoming reality, as for example the dairy industry has rapidly adopted automation for milking cows, and Salfer et al. (2019) estimate over 35,000 robotics milking systems are currently in use globally. For row crops, weed control with the rise of herbicide resistant weeds and lack of new herbicide modes of action is a significant concern and robotic systems are one of the proposed solutions (Westwood et al., 2018). Most of the major agricultural machinery companies have announced autonomous machinery plans, have prototype machines and/or have filed patents on autonomous robotic systems for agriculture (e.g., Murray et al., 2018). The concept of an autonomous platform with several interchangeable implements is emerging as a preferred concept for agricultural robots (Feldschwarm ® technologies: http://www.feldschwarm.de; Dot Power Platform: https://seedotrun.com/).

Barnes et al. (2019) discussed the potential advantages autonomous cotton harvest could bring by conducting multiple harvests after first open boll such that would improve fiber quality and reduce risk of yield loss due to extreme weather events. They also observed that cotton is an ideal crop for robotic harvest as when the boll is mature, it is white surrounded by a dark background making identification easier and it is difficult to damage in removing from the plant relative to fruits and vegetables. Multiple harvesting events will also allow for a greater uniformity of cotton fiber quality characteristics and fiber grades from each harvest event (Kothari et al., 2015). This fiber uniformity should provide additional market opportunities and premiums for farmers.

In 2019, Cotton Incorporated sponsored projects at seven U.S. universities to explore robotic harvest. Texas A&M, the University of Georgia (UGA), and Clemson University evaluated robotic platforms for cotton harvest. Clemson and UGA also evaluated robotic weed control along with collaboration from North Carolina State University. Scientists at the University of North Texas examined the possibility of genetically modifying the plant architecture to simplify robotic harvest. Economists at Kansas State University have started an economic model to compare robotic harvest system configurations to the current one-pass harvest system. To have data on the potential economic impact of frequent harvest for the model, field studies where cotton were hand-harvested twice weekly was conducted by Texas A&M (two locations), University of Georgia, and University of Tennessee. For more details see Barnes et al. (2020). Fue et al. (2019) provide details on the boll detection and removal system being developed at the University of Georgia that has autonomously harvest individual cotton bolls.

In addition to work on robotic cotton harvesting in the U.S., the use of machine vision to identify cotton bolls is underway in India (Rao, 2013) and China (Wang et al., 2008). A mechanical gripper for removal of cotton from the boll has been designed in India by Limbasiya et al. (2015). An Indian company has also developed a prototype robotic harvester that uses a vision system to identify a cotton boll and then remove it using a combination of mechanically rotating spikes and a vacuum system (http://www.grobomac.com/). The objective of this paper is to develop design criteria for cotton robotic harvest systems and discuss a possible prototype based on those criterea.

Results

To examine the impact of different system parameters for robotic harvesters, the base scenario summarized in Table 1 was used.

Using the values in Table 1, Figure 1 illustrates the variation in the number of harvest events during the season on the bolls that would have to be harvested per second and volume of material that would need to be stored to complete a pass the length of the field. The demands on the system decrease rapidly in transitioning from a single harvest pass per season to 5 harvest events. Bolls to be harvested per second decreases from nearly 100 to less than 20 and the storage volume from 2 to 0.4 m^3 in going from 1 to 5 harvest events.

Table 1. Base values used in scenario Evaluations

Parameter	Value
Row Spacing (m)	1
Speed (km hr^{-1})	5
Fiber Yield (kg ha^{-1})	1500
Lint Fraction	0.4
Row Length (km)	0.5
Rows per Pass	1
Fiber per Boll (g)	2.2
Total area harvested (ha)	800
Harvest per Year	1

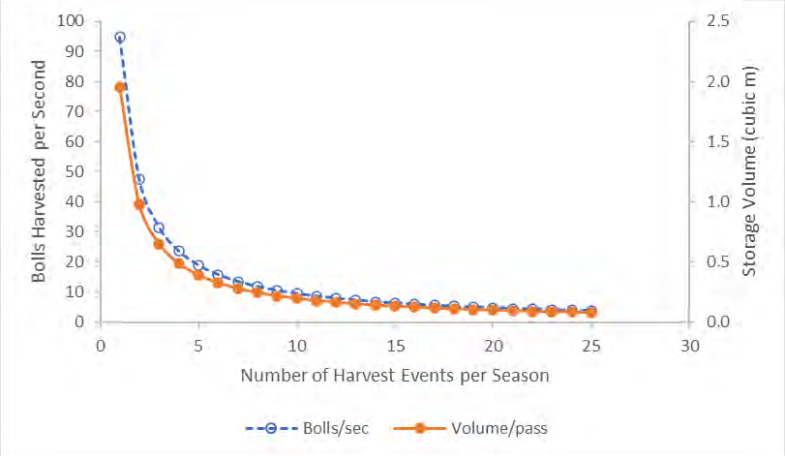

Figure 1. Variation in bolls harvested per second and storage volume need to complete one pass across the field as the number of harvest events per season increase.

The impact of boll size on the number of bolls harvested per second is shown in figure 2 using the parameters from table 1 except assuming 5 harvest events per season. The range of bolls size on the x-axis is approximately the range observed in U.S. variety trials (RBTN, 2017).

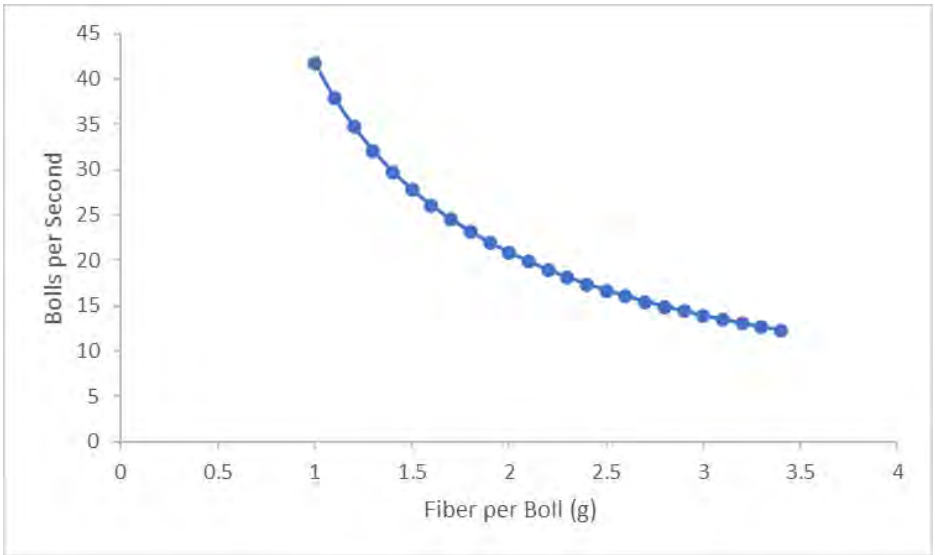

Figure 2. Relationship between bolls harvest per second and mass per boll for five harvest events per season and fixed yield of 1500 kg ha-1.

The impact of machine speed through the field and number of harvest per season on the bolls to be harvested per second for a 800 ha field with 1500 kg ha^{-1} total yield is shown in Figure 3. Increasing speed results in an increase in bolls harvested per second, while increasing harvest events decreases the bolls per second. The boll harvest rate increases linearly with travel speed while there is an inverse power function relationship between harvest events and bolls per second.

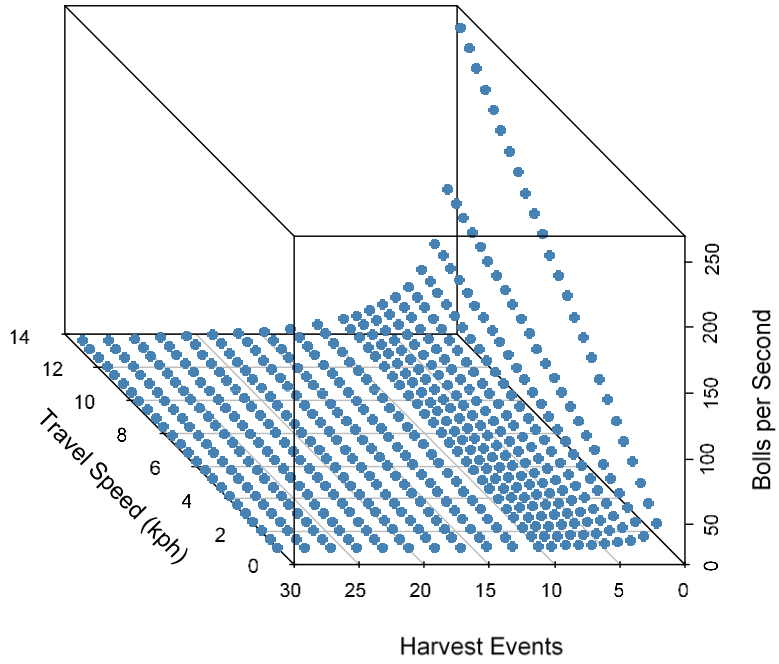

Figure 3. Impact of travel speed and harvest events on the bolls harvested per second to harvest 800 ha of cotton. Note that harvest events are portrayed in descending order to better display the data.

The reduced risk of damage to open bolls can be decreased with more frequent harvest; however, as the number harvest events increase, so will the number of machines needed as shown in Figure 4. From an objective of protecting cotton yield and quality, daily harvest would be the ideal; however, increasing the number of machines to complete that frequent of a harvest is not likely to be justified. For example, harvesting every other day (30 harvest events) results in 102 machines needed with all

else set to the base scenario of Table 1. Note in this analysis, the minimum number of machines was set to a level where the entire area could be harvested in 28 days, and once that level was reached, varied as a function of harvest events spread across a 60 day harvest window (see details in the methods section). For the range of values considered in this study, the adjustment was only needed when the number of harvest events were three or less.

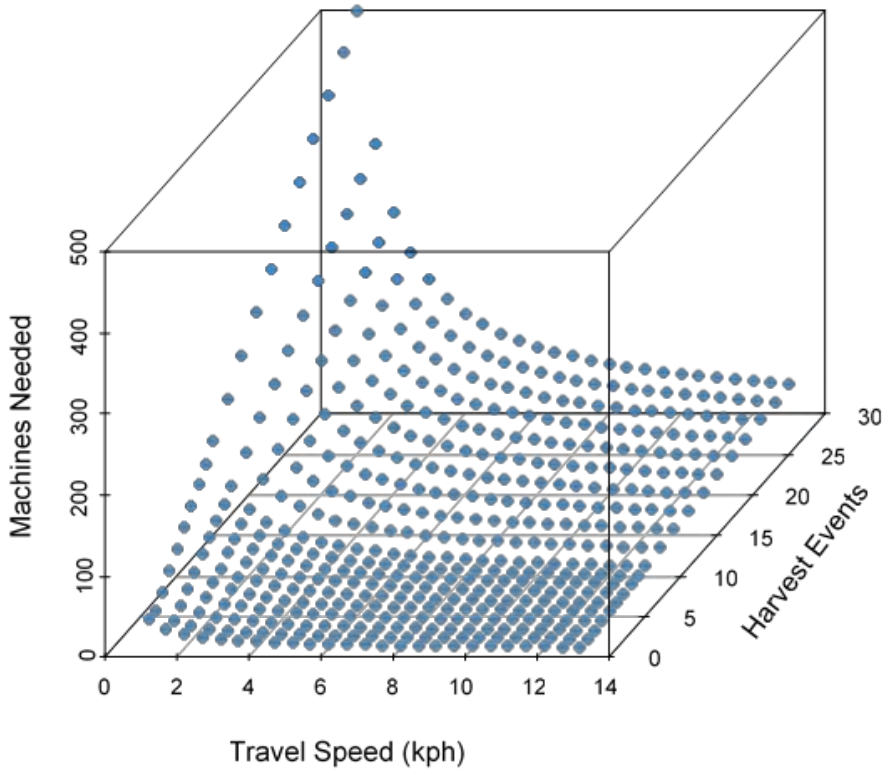

Figure 4. Impact the number of harvest events per season and machine travel speed have on the number of machines needed to harvest 800 ha.

Discussion

There are several variables that could change design requirements for robotic cotton systems, especially for systems where the focus is on removal of individual bolls using a machine vision system. The number of harvest events per year will be a key variable. Increasing the number of harvest events will decrease the demands on the system to identify and remove cotton bolls; however, that will also increase the number of machines needed to harvest a given area. It is also clear that larger boll varieties would be of benefit for selective robotic cotton systems. Areas of the world where hand harvest is utilized maintain larger boll sizes and so those systems will be better adapted to robotic systems.

Based on Figures 1 and 3, at least 5 harvest events per season will be needed to reduce the demands on the detection and removal system and also significantly reduce the storage capacity needed per machine. From Figure 4, 5 harvest events would require 17 single row machines to harvest 800 ha over a 60 day period. The estimated number of machines is optimistic as the assumptions used in this analysis does not account for time for turning, unloading and transporting machines between fields- is not accounted for. One conceptual design that could potentially meet the previously discussed criteria is pictured in Figure 5.

The black arrows represent a retractable array of “spears” that could be extended when a cotton boll is detected at that height on the plant. By having several independent spears on each side of the harvester the ability to obtain a high total boll removal rate (great than 10 per second) is more feasible as opposed to a single articulated arm. The end of each spear could contain a device like a modern cotton spindle. The red arrow represents an arm that has been extended to harvest a cotton boll (side view). This only requires one degree of freedom of control when machine speed is accounted for and should allow a high boll removal rate. Looking from the top view, the array of arms are set up to harvest plants on both sides of the machine and are at an oblique angle to the row to allow them to be longer than if

perpendicular to the machine (minimizes machine width to fit between the rows). A system with a forward-looking detection system allows more processing time before spindle needs to be triggered and also allows multiple view angles of the plant so bolls behind leaves or stems can be detected. The doffing action occurs when spear is retracted into housing – the boll is dislodged and falls to bottom of unit (boll catcher). The boll catcher could then mechanically conveyed seed cotton to a small trailer behind the unit. The trailer would then empty to a "boll buggy" robot at the end of the row that could then deposit the seed cotton into a stationary round module builder at the end of the field.

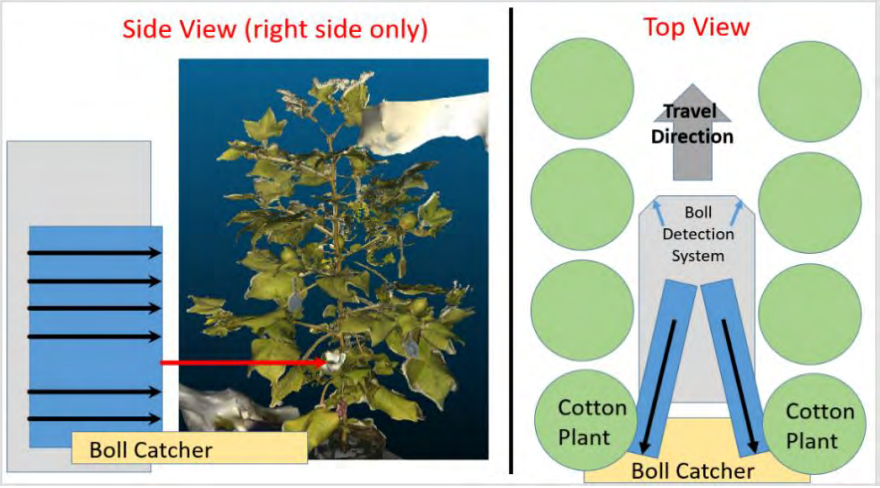

Figure 5. Conceptual robotic cotton harvest system (cotton plant representation shown in side view from the USDA-ARS, Maricopa, AZ).

The previous description assumes a small independent harvester similar to the Clemson ClearPath unit (Figure 6). Alternatively, the system in Figure 5 could be treated as a row unit and several units mounted on a high clearance platform such as used by the UGA Red Rover platform (Figure 7). With a high clearance platform, the system should still be capable of multi-harvest without significant plant damage.

Another possible scenario is one where there is non-selective harvest of a limited part of the plant (for example, bottom 5 fruiting nodes in the first harvest cycle) at a high rate of speed (no individual boll detection, but some automate control of the height to correspond to open bolls on the plant). A slower "gleaner" robot then follows using a machine vision system to collect any bolls that were not captured. It is likely many additional approaches will be envisioned in the future.

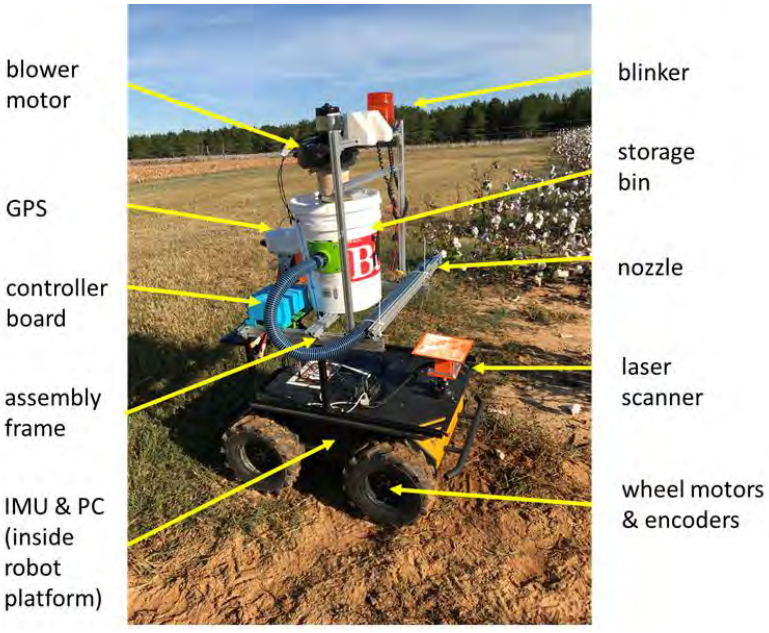

Figure 6. Clemson robotic system based on a ClearPath Husky platform (Burce et al., 2019).

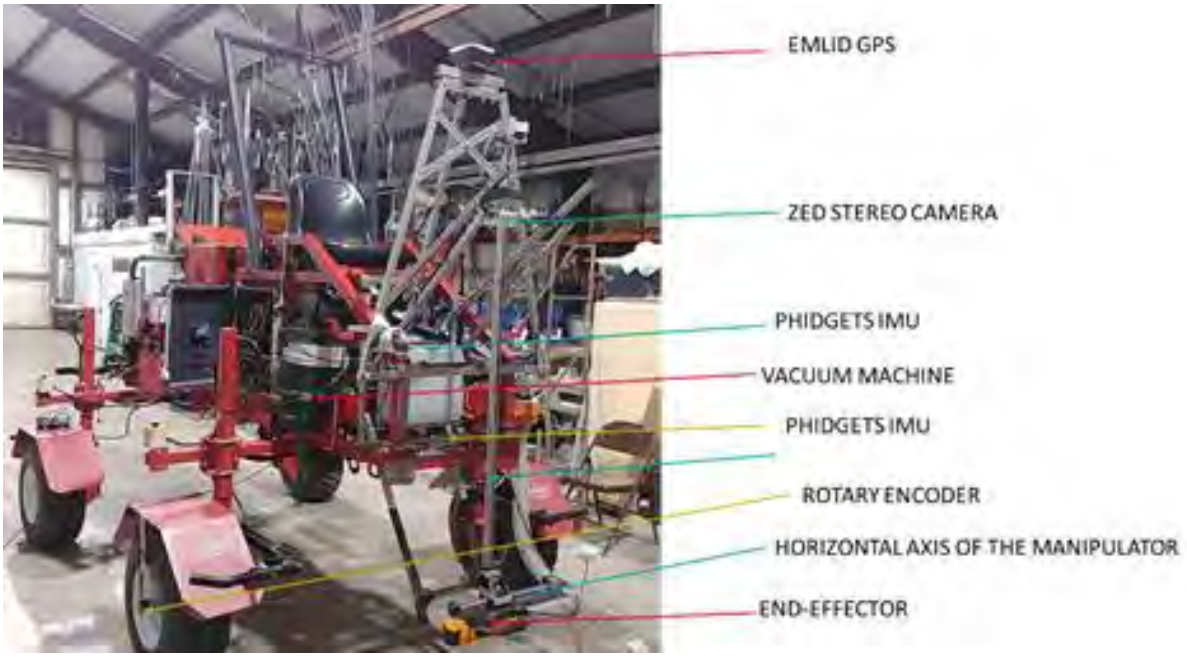

Figure 7. Red Rover" platform used at the University of Georgia for evaluation of autonomous cotton boll removal (Fue et al., 2020).

For lower yielding environments, slower boll removal rates could be possible; however, it is difficult to envision a scenario where the rate of boll removal is less than a boll per second. For example, with a total yield of 500 kg/ha, five harvest events in the season, and reducing the machine speed to 2 kph, a removal rate of 2 bolls per second would still be required.

This analysis is only a starting point in identifying the design criteria for a robotic cotton harvest system. An economic model to carefully balance the number of harvesters versus value gained from high frequently harvest is in development by economists at Kansas State University. The economic model is also needed help identify the costs per harvest unit that would be competitive with current harvest system costs whether that be machine-based or hand harvest. Lost in efficiencies due to materials handling need to be considered in future analysis as do possible interruptions to harvest events due to rainfall.

Conclusions

There are many variables that must be considered in designing an autonomous system for cotton harvest. Frequent harvest after mature bolls have formed has promise to preserve yields and quality as well as reduce the demands on a system to detect and remove individual cotton bolls; however, this has to be balanced with the costs of increasing the number of machines needed for harvest. Based on the calculations for boll removal rates it is unlikely a robotic arm with several degrees of freedom will be able to remove bolls at the required rate of at least 2 bolls per second. A system that selectively identifies and remove bolls will likely require several individual "spears" to obtain the boll removal rate required. In the future, Cotton Incorporated will continue to support autonomous weed control and harvest applications for cotton and have all results made available in an open source format when possible.

Methods

Several design criteria need to be considered for a cotton harvest system. One item of importance is the rate of boll removal, especially if the goal is individual boll identification and removal using a computer vision system. The rate of boll removal per harvest event can be estimated from equation 1:

$$NB = FY / (MB/1000) / HE \qquad \text{eq. 1}$$

Where,

NB = number of bolls per ha per harvest event,
FY = fiber yield for the entire season (kg fiber ha^{-1}),
MB = mass of fiber from a single boll (g),
1000 is used to convert from g to kg,
HE = number of harvest events during the season.

A significant assumption of equation 1 is that the number of mature bolls is uniformly distributed during the season.

Other important design criteria will be around machine speed and capacity. To determine the area a single machine can cover per hour, equation 2 is used:

$$APH = Rows * RS * V /10 \quad \text{eq. 2}$$

Where,

APH = area harvested per hour (ha hr^{-1}),
Rows = crop rows per harvest pass,
RS = row spacing (m),
V = travel velocity (km hr^{-1}), and
10 is used to convert from m^2 to ha.

The number of bolls that are harvest per second per harvest event (BPS) then becomes:

$$BPS = NB * APH / 3600 \quad \text{eq. 3}$$

Where,

BPS = bolls harvester per second per harvest event, and
3600 is used to convert from hours to seconds.

Another parameter to consider is the minimum storage capacity needed to make one pass across the field assuming there will be a collection system for the seed cotton when exiting the field. Equation 4 is the field area per pass through the field:

$$RA = Rows * RS * RL / 10 \quad \text{eq. 4}$$

Where,

RA= Area harvested in one pass across field (ha), and
RL = row length (km), and
10 is used to convert from m*km to ha.
The mass of seed cotton harvested in one pass across the field can then be determined as:

$$MA = RA * FY / LF / HE \quad \text{eq. 5}$$

Where,

MA = mass of seed cotton harvested in one field pass per harvest event (kg), and
LF = fraction of lint (mass of fiber divided by mass of fiber and seed),
The volume of that cotton to be stored is then calculated as:

$$VA = MA / r \quad \text{eq. 6}$$

Where,

VA = volume of cotton per pass per harvest event (m^3);
r = density of seed cotton (kg m^{-3}).

To develop a base set of values for evaluation, characteristics of a typical U.S. cotton farm were used. Yan and Roy (2016) report most U.S. cotton fields have a mean row length of approximately 0.5 km. Hardin and Searcy (2008) for seed cotton loaded into trailers of 96 kg m^{-3}. Data from the Regional Breeders Testing Network (RBTN, 2017) shows that an average cotton boll contains 2.2 grams of fiber and ranges from 1.3 to 3.6 g per boll for data in the 2017 tests. While global average yield from 2015 to 2019 (FAS, 2020) has been 760 kg ha^{-1}, projections in this study assumes continued yield increases and examines a more challenging scenario (from a robotics standpoint) of 1500 kg ha^{-1}.

Another key parameter in harvest system evaluation is the number of machines needed to timely harvest a given area when multiple harvest events occur in a season. The harvest rate needed when multiple harvest events occur is calculated as:

$$ER = A / (AD / HE) \quad \text{eq. 7}$$

Where,

ER = effective harvest rate required (ha per d)
A = the total area to be harvest (ha),
AD = the total number of days cotton is to be actively harvested (e.g., cumulative time from approximately first open boll to date when all bolls are mature and harvest is to be complete), d).

The time available to harvest that area is a function of when the first bolls mature and how long until the last boll of interest is harvested. Using data from USDA (2018), the date when 50% of acres in state had open bolls and were harvest was determined based on 5-year average conditions ending with the 2017 crop season. From that data, the average time between open boll and harvest was 47 days. This window may be somewhat expanded in larger farming operations where planting dates will be spread out; however, this will be minimal as cotton is typically planted at a much faster rate than it is harvested (Griffin and Barnes, 2017). To account for the fact there are cases in mechanical harvest where immature bolls are not harvested to reduce risk to mature bolls, the value for AD in equation 7 was assumed to be 60 days.

Combining the results from equations 7 and 1 and assuming an average harvest time of 8 hours per day, the number of machines needed (M) is calculated as:

M = CINT(ER / (APH * 8)) eq. 8

Note, CINT is a function that rounds the resulting calculation up to the next highest integer.

The total area of cotton per farming operation will vary across the globe, and in this analysis the area used for evaluation is 800 ha that corresponds to the approximate area harvested by a modern six row cotton harvester in 220 hours in the U.S. mid-south (Wanjura et al., 2015). Chen et al. (1992) determine the value of 220 hour based on prevalent weather conditions in Mississippi and the costs from lost value due to delayed harvest. The total time for harvest will be highly influenced by climatic conditions, where arid regions can tolerate longer harvest windows than humid regions. When evaluating the number of harvest events, the minimum number of machines needed was set to provide the harvest capacity would accomplish a single harvest event in 220 hours (27.5 days for 8 hr assumption). This was done to prevent a scenario when harvest events were less than 3 that the extended harvest window would result in a situation where the predicted capacity would be less than needed for a once over harvest.

To evaluate the impact of machine speed and harvest events on the number of machines required per 800 ha as well as the impact on bolls per second, machine speeds of 0.5 to 13 m s^{-1} were considered while varying the number of harvest events from 1 to 30 (up to every other day for a six day harvest window). To help visualize the interaction of speed and harvest events impact on the number of machines needed and the bolls harvest per second, three-dimensional scatter plots were generated using the “scatterplot3d” function in R version 4.0.0. (R Core Team, 2020).

The current calculations assume no time is lost for unloading of seed cotton or in turning at the end of the row. Those are additional parameters that need to be considered but are ignored in this analysis. Data from current harvest systems indicate that the machines are harvesting between 75 to 90 percent of the time (Wanjura et al., 2015). Additionally, the analysis does not consider the fact there could be times in the season when rainfall prevents a harvest event. Therefore, these results should be treated as a best-case scenario.

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Many people working on the Cotton Incorporated robotic initiative have inspired some of the concepts discussed in this manuscript. Lead investigators on this initiative include Dr. Joe Maha, Clemson University; Drs. Glen Rains and John Snider, University of Georgia; Dr. Alex Thomasson, Mr. James Griffin and Dr. Emi Kimura, Texas A&M; Drs. Terry Griffin, Greg Ibendahl, and Ajay Sharda, Kansas State University; Dr. Tyson Raper, University of Tennessee; Dr. Gaylon Morgan, Dr. Kater Hake and Mr. Jon Devine, Cotton Incorporated.

References

1. Barnes, E., K. Hake, J. Devine, T. Griffin, G. Ibendahl, G. Rains, K. Fue, J. Maja, M. Bruce, J. Thomasson, J. Griffin, E. Kimura, G. Morgan, M. Burce, B. Ayre, and M. Pelletier. 2019. Initial possibilities for robotic cotton harvest. Beltwide Cotton Conferences, New Orleans, LA, January 8-10, 2019. Pp. 133-143. National Cotton Council, Memphis, TN.
2. Burce, M., J.M. Maja, E. Barnes. 2019. Adaption of Mobile Robot Platform for Cotton Harvesting. Beltwide Cotton Conferences, New Orleans, LA, January 8-10, 2019. National Cotton Council, Memphis, TN.
3. Chen, L.H., T. Ahmad, and M.H. Willcutt. 1992. Simulation model for cotton harvesting machinery. Trans. ASAE 35(3):1071–1077. doi:10.13031/2013.28702
4. FAS, 2020. Production, supply and distribution. United States Department of Agriculture, Foreign Agricultural Service. https://apps.fas.usda.gov/psdonline/app/index.html#/app/advQuery (accessed 14 April 2020).
5. Fue, K., W.M. Porter, E.B. Barnes, G.C. Rains. 2020. Field Testing of the Autonomous Cotton Harvesting Rover in Undefoliated Cotton. Beltwide Cotton Conferences, Austin, TX, January 8-10, 2020. National Cotton Council, Memphis TN.
6. Griffin, T.W. and Barnes, E. 2017. Available Time to Plant and Harvest Cotton across the Cotton Belt. Journal of Cotton Science. 21(1):8-17.

7. Hardin, R.G., and S.W. Searcy. 2008. Viscoelastic properties of seed cotton. Transactions of the ASABE 51(3): 803-810.
8. Kothari, N., J. Dever, S. Hague, and E. Hequet. 2015. Evaluating intraplant cotton fiber variability. Crop Science 55:564-570.
9. Limbasiya, Y.G., and J.P. Maheta. 2015. Mechanism design of cotton picking gripper. International Journal of Advance Research in Engineering, Science & Technology (IJAREST), ISSN(O):2393-9877, ISSN(P): 2394-2444 2(5):8pp.
10. Murray, C.L., and M.E. Barker. 2018. Autonomous robotic agricultural machine and system thereof. United States Patent No. 9,891,629 B2. Feb. 13, 2018.
11. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/
12. Rao, USN. 2013. Design of automatic cotton picking robot with machine vision using image processing algorithms. 2013 International Conference on Control, Automation, Robotics and Embedded Systems (CARE), 16-18 December, Indian Institute of Information Technology, Design and Manufacturing, Jabalpur India. Institute of Electrical and Electronics Engineers, Inc.
13. RBTN. 2017. Regional Breeders Testing Network (RBTN). https://rbtn.cottoninc.com/files/ - 2017 Results.
14. Salfer J., M. Endres, W. Lazarus, K. Minegishi, B. Berning. 2019. Dairy Robotic Milking Systems – What are the Economics? DAIReXNET. https://dairy-cattle.extension.org/2019/08/dairy-robotic-milking-systems-what-are-the-economics/
15. Sistler, F.E. 1987. Robotics and Intelligent Machines in Agriculture. IEEE Journal of Robotics and Automation RA-3(1):3-6.
16. USDA, 2018. National Crop Progress Reports accessed through the USDA, National Agricultural Statistics service Quick Stats site https://quickstats.nass.usda.gov/. Data for 5-year average conditions ending with the 2017 cotton crop year retrieved on May 1, 2018.
17. Wang, M., J. Wei. J. Yuan, and K. Xu. 2008. A Research for Intelligent Cotton Picking Robot Based on Machine Vision. Proceedings of the 2008 IEEE International Conference on Information and Automation June 20 -23, 2008, Zhangjiajie, China.
18. Wanjura, J.D., E.M. Barnes, M.S. Kelley, and R.K. Boman. 2015. Harvesting (book chapter) in Cotton, 2nd Edition, D.D. Fang and R.G. Percy, Editors. Agronomy Monograph 57.
19. Westwood JH, Charudattan R, Duke SO, Fennimore SA, Marrone P, Slaughter DC, Swanton C, Zollinger R. 2018. Weed Management in 2050: Perspectives on the Future of Weed Science. Weed Sci. 10.1017/wsc.2017.78
20. Yan, L., and D.P. Roy. 2016. Conterminous United States crop field size quantification from multi-temporal Landsat data. Remote Sensing of Environment 172 (2016) 67–86.

Energy Use Efficiency for Cotton Production in the Mechanized Rainfed Areas Eastern Sudan

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Abstract

Agricultural production in Sudan, especially the mechanized rainfed schemes, is lacking behind in energy use efficiency analyses. The objectives of this study were to analyze energy input-output and to identify the energy use patterns for cotton production in the mechanized rainfed schemes eastern Sudan. The data was collected through structured questionnaire from 54 farmers. The results showed that 19% of the surveyed farmers grew cotton crop in an area of 874 ha on the average. The results revealed that the total energy input used to produce cotton was 2234.62 MJ ha-1 and the total energy output was 7802.10 MJ ha-1, indicated that cotton production was efficient in energy consumption. The results showed that energy use efficiency was more than three. The results indicated that the average net energy, the energy productivity, and the specific energy was 5567.48 MJ ha-1, 0.3 kg MJ-1, and 3.38 MJ kg -1, respectively. Herbicide energy input was the highest among energy input items. The results showed that the direct energy was greater than the indirect energy. In addition, the non-renewable energy was greater than the renewable energy. In conclusion, more investigations are needed on balanced utilization of energy sources for cotton production in the mechanized rainfed schemes eastern Sudan.

Keywords: *Energy ratio; Direct and indirect energy; Renewable and non-renewable energy; Rain-fed cotton; Sudan*

Introduction

Cotton “Gossypium” is one the major textile fiber crop worldwide. Cotton is a strategic crop in the Sudan; it is one of the most important crop produced science centuries ago. Historically, it had been grown in eastern Sudan since 1867. In Sudan, it grown under various soil types, utilizing different methods of irrigation and using several methods of production managements. The famous areas for it is cultivation are silt soil in Tokar eastern Sudan, clay soils in Gezira scheme and heavy clay soils in Nubian mountain, Blue Nile and Gedarif (Faki, 2006). Cotton had played a major role in Sudan’s economy as a cash crop in both irrigated and rainfed sectors. It is a multi-sectoral source of employment, which generates income to satisfy family needs and to provide services. The intensive labor demand in cotton farming and cotton- based industries provides employment, reduces poverty, improves quality of life and encourages settlement in rural areas. This stimulated the stability, development and security of the society. Cotton gained its importance because of its higher economic value; it supports the economy and social life of the local community besides that it is a source of hard currency.

Cotton crop in Gedarif State, is produced under rainfed conditions in areas where annual rainfall exceeded 800 mm. Figure 1 illustrates cotton cropped area (000 ha) and productivity (ton/ha) in Gedarif rainfed areas during the last twenty years (2000/2001 to 2019/2020). The cropped area and productivity fluctuated from year to year. The maximum and minimum-cropped area by cotton during this period was 63445 and 840 ha, respectively; and the productivity was ranged between 0.058 to 1.016 ton/ha.It evident that there is growing (increasing) trend in the cropped area and productivity of cotton crop,especially in the recent years.

Energy is an important issue in crop production. Crop production is an energy consumer and at the same time is energy producer. The production activities that consume energy are hand labor, fuel, machinery, agrochemicals and seed. Yield is one of the main energy producer in crop production. Energy requirements in crop production can be divided into direct and indirect energy, either of these divisions could also be categorized as renewable or non-renewable. The direct energy includes fuel and hand labors (Uzunoz et al., 2008; Canakci et al., 2005; Yilmaz et al., 2005; Zahedi et al., 2014). The indirect energy includes seeds, agrochemical and machinery (Ozkan et al. 2004). The Renewable

energy includes hand labors and seeds while non-renewable energy consists of fuel, agrochemicals and machinery (Uzunoz et al., 2008). The share of these energies in crop production differs according to crops grown, production management practices and sector (Erdal et al., 2007). The energy ratios in crop production are closely related to the used production techniques, quantity of inputs used by producers and the obtained crops yield. Therefore, there is a range of energy input and output relationships for the same crop depending on the region.

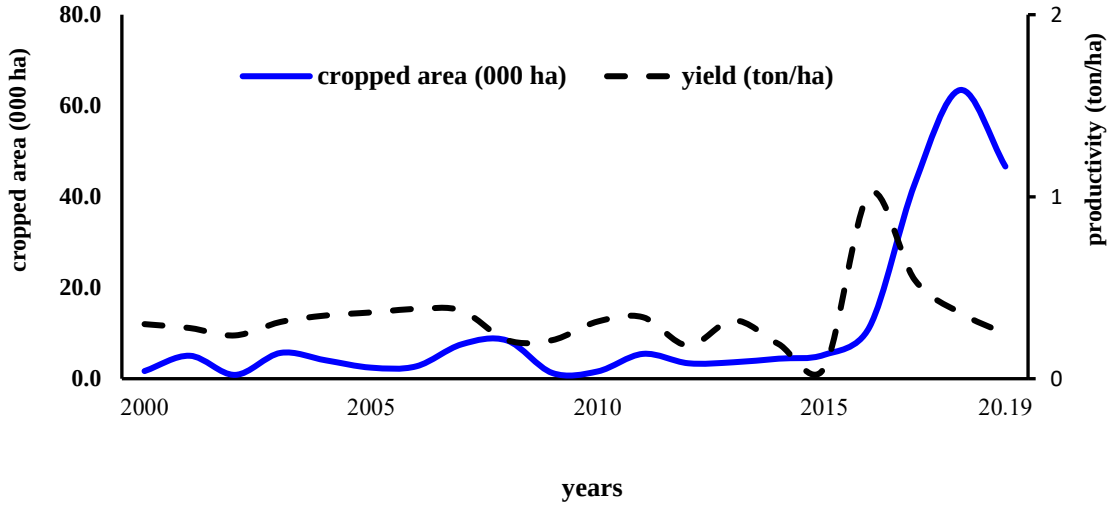

Fig.1. Cotton cropped area (000 ha) and productivity (ton/ha) in Gedarif rainfed schemes during the years from 2000 to 2019

The energy input-output analysis usually made to evaluate the efficiency of the production system. Several other indicators are often used in energy analysis, which include; total energy input, total energy output and energy use efficiency. In addition to that, energy productivity, specific energy and net energy are also common indicators. Numerous studies on the subject of energy use, energy input–output analysis and their relationships have been conducted on crop production elsewhere (Chandra et al., 2001; Canakci et al., 2005; Yilmaz et al., 2005; Safa et al., 2010; Zahedi et al., 2014; Kheiry and Dahab, 2016). However, cotton has been paid relatively a little attention. Singh (2002) found that cotton consumed maximum energy among the wheat, mustard, maize and cluster bean in Haryana, India

The use of energy in crop production augmented in response to the growing demand for food production to meet the rapid growth of population. On the other hand, excessive use of energy causes problems threatening public health and environment (Rafiee et al., 2010). However, the excessive and unconscious use of input in the production causes increasingly negative effects to both the environment and farmers. Therefore, understanding the use of energy in agricultural production will promote sustainable crop and decrease environmental harms. In addition, efficient use of the energy resources is vital for improving the productivity and competitiveness (Hatirli et al., 2005).

Despite the much research work that has been carried out to improve cotton production in Sudan, studies considering energy use are limited. The objectives of this study were to analyze energy input-output and to identify the energy use patterns for cotton production in the mechanized rainfed agricultural schemes eastern Sudan.

Materials and methods

The large-scale rainfed agricultural schemes eastern Sudan are mainly located in Gedarif State (Fig. 2). Gedarif State lies between latitudes 12.67º and 15.75º N and longitudes 33.57 º and 37.0º E, covering 71000 km2. The State encompasses three-climate zones arid zone in the North to dry monsoon zone in the South (Adam, 2008). The total area suitable for cultivation is about 3.4 million hectares; the soil is heavy clay (Vertisols). Effective rainfall occurs during June - July and extends to

September-October and accordingly there is a single growing season a year. Cotton is one of the crops grown in the area. The crops production in the study area has been practiced by private farmers.

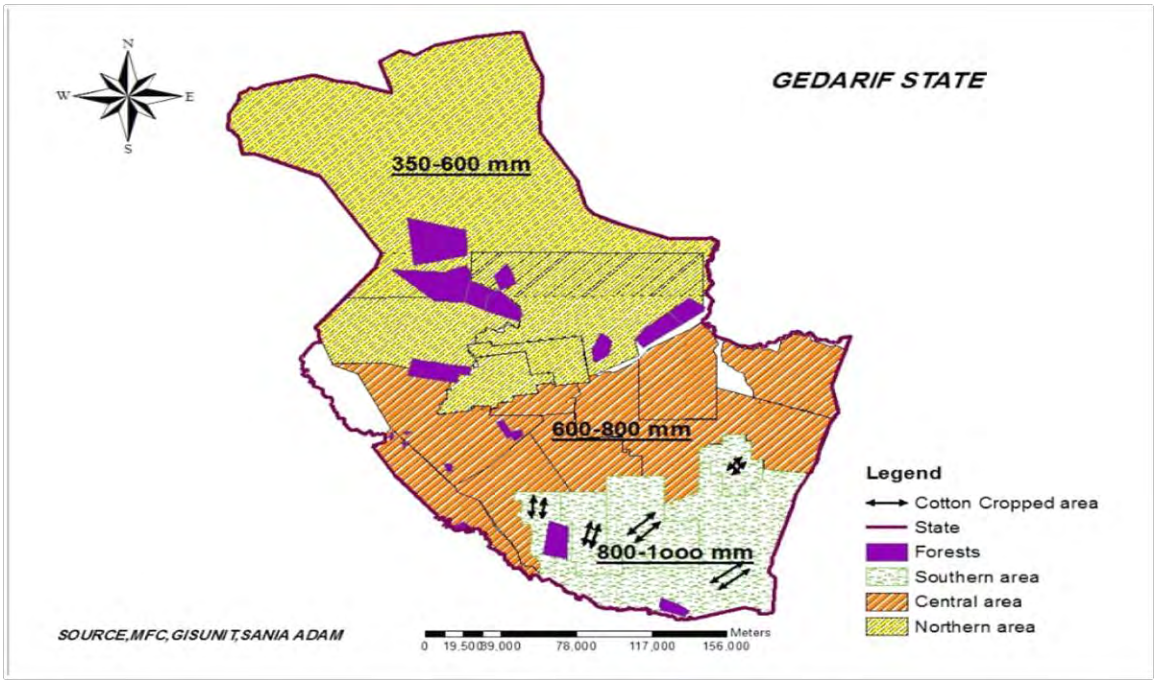

Fig.2. Gedarif State map displays cotton cropped areas and annual rainfall amount (mm)

The farmers in the mechanized rainfed schemes have used wide level disk harrow with attached seeder box for seedbed preparation and sowing the cotton crop in broadcasting pattern by the same implement. Tractors of 56 to 60 kW have been used for powering the necessary implements. The sowing date starts during the second week of June and extends up to late-July. The average seed rate of 12.7 kg/ha is sown and no fertilizer is applied. Manual weeding is a common practice; however, the farmers recently have adopted chemical herbicide to control weed. The crop is ready for harvest in about 100 to 120 days from emergence. Harvesting normally carried out manually in the late of November and extends until end of March.

The required data was collected through structured questionnaire from 54 farmers. The respondent farmers were randomly selected and interviewed. The number of the respondent farmers considered sufficient for the purposes of this study, as the farmers are using similar implements, adopting similar operations and farming system besides that they face similar constrains. The questionnaire considered details about the inputs used and operations for cotton production from seedbed preparation to harvest. In addition, the questionnaire focused on hand labor, area cultivated, diesel fuel and tractor power as well as cotton seed yield. Human labor, machinery, diesel fuel, agrochemicals, seed rate and output values of cotton crop were considered as energy analysis components.

The energy equivalent of inputs and outputs of cotton production are shown in Table 1. All these values attained from those studies that have addressed energy analysis in agricultural production.

Table 1. Energy content for inputs and outputs of cotton crop production

Item	Unit	Energy content (MJ/unit)	Source
Labor	h	1.96	Yilmaz, *et al.* (2005); Zahedi, *et al.* (2014); Gokdogani and Sevim (2016)
Machinery	h	62.7	Yilmaz, *et al.* (2005); Zahedi, *et al.* (2014);
Fuel	l	47.8	Kheiry and Dahab (2016); Kızılaslan (2009)
Herbicides	kg	454	McLaughlin *et al.,* (2000)
Cotton Seeds	kg	11.8	Yilmaz, *et al.* (2005); Zahedi, *et al.* (2014)
Cotton Yield	kg	11.8	Yilmaz, *et al.* (2005); Zahedi, *et al.* (2014)

The collected data was prepared in an excel worksheet and thereafter, the intended indicators were calculated. The amounts of each input used by farmers was calculated per hectare and multiplied by

its energy equivalent (Kizilaslan, 2009). The calculation of energy indices; energy ratio (energy use efficiency), energy productivity, specific energy (energy intensity) and net energy were calculated as suggested by Rafiee et al. (2010),Ghorbani et al., (2011) and Zahedi et al. (2014) as follows:

Energy efficiency = Total energy output (MJ/ha) /Total energy input (MJ/ha)……(1)
Energy productivity = Grain yield (kg/ha)/Total energy input (MJ/ha)…… (2)
Specific energy = Total energy input (MJ/ha)/Grain yield (kg/ha)…… (3)
Net energy = Energy output (MJ/ha) – Energy input (MJ/ha)…….. (4)

Moreover, direct and indirect energies inputs as well renewable and non-renewable energies inputs were computed according to the procedure described by Uzunoz et al. (2008).

Results and discussion

The number of the surveyed farmers and their holding size were depicted in Table 2. A total number of 54 farmers practiced crop production in the large-scale rainfed schemes (120042 ha) eastern Sudan were interviewed. Of whom 10 farmers (19%) grew cotton crop. The total area owned by the surveyed cotton growers was 58403 hectares. Cotton crop was grown in an area of 8739 hectares, which represented 7% of the total area owned by the surveyed farmers and 15% of the total area owned by surveyed cotton farmers. The average farm size grown by cotton crop was 874 ha. Other crops such as millet, sesame, sunflower and cotton were grown in the remaining of the area.

Table 2. Number of the surveyed farmers and the holding size of cotton farms in the mechanized rainfed schemes eastern Sudan

Items	Value
Total number of surveyed farmers	54
Total area owned by the surveyed farmers, ha	120042
Number of the surveyed cotton growers	10
Percentage of cotton growers from total surveyed farmers, %	19
Total area owned by surveyed cotton growers, ha	58403
Area grown by cotton crop, ha	8739
Average cotton farm size for surveyed cotton growers, ha	874
% of cotton cropped area from total area owned by the surveyed farmers	7
% of cotton cropped area from area owned by surveyed cotton farmers	15

The quantities of input and output for cotton production and their energy equivalents were shown in Table 3. The results showed that about 332.843 man-hour per hectare were used in cotton production. Its respective energy equivalent was 652.36 MJ ha-1. The used hours per hectare by machinery were about 1.52 hrs, and its respective energy equivalent was 95.40 MJ ha-1. The results also showed that the total fuel consumption in cotton production was 13.72 l ha-1 which equivalent to 656MJ ha-1. On the other hand, the quantities of seeds and herbicide that used to produce cotton were 12.7 kg ha-1 and 1.5 kg a. i. ha-1, respectively. The energy equivalent for cotton seeds and herbicide was 149.86 and 681.0 MJ ha-1, respectively. The total energy input for cotton production in the studied mechanized rainfed schemes was 2234.62 MJ ha-1 (Table 3). The obtained total energy input was much lower than that reported by other authors elsewhere. Zahedi et al., (2014) found that the total input energy used in cotton production in the Esfahan province of Iran was 52507.8 MJ ha-1. Yilmaz et al (2005) and Dagistan et al., (2009) in Turkey found that energy consumed in cotton production was 49730 MJha-1 and 19 558 MJha-1, respectively. The results showed that the total energy output for cotton production in the mechanized rainfed schemes was 7802.1 MJ ha-1 (Table 3).

Table 3. Amount of inputs, outputs and their energy equivalent for cotton production in the mechanized rainfed schemes eastern Sudan

Inputs	Quantity per hectare	Total energy equivalent (MJ ha^{-1})
Labor (h)	332.84	652.36
Machinery (h)	1.52	95.40
Diesel fuel (l)	13.72	656.01
Herbicides (kg)	1.50	681.00
Seeds (kg)	12.70	149.86
Total energy input (MJ ha^{-1})		2234.62
Outputs		
Cottonseed yield (kg)	661.20	7802.10

The obtained energy output is far below to that reported by some researchers. Yilmaz et al., (2005), Dagistan et al., (2009) and Zahedi et al., (2014) found that energy output from cotton production was

36729.9, 46156.88 and 32308.4MJ ha-1, respectively. These variations in the total energy input and output for cotton production between this study and other studies may be due to the variation in the amount of the used inputs, cultivars and management practices. It is first time to establish such information about energy consumed in cotton production in the mechanized rainfed schemes eastern Sudan; therefore it is hard to judge on the values on energy input and output. It is thought that the furnished information will help researchers and engineers as well as farmers to understand the relationship between energy input and production techniques; and this will help in allocating inputs to optimize and sustain the productivity.

Energy input-output relationships were presented in Table 4. The results showed that the energy ratio (energy use efficiency) was 3.49%. This result indicates that cotton production in the mechanized rainfed schemes was efficient in term of energy consumption. As Safa et al., (2010) mentioned that if the energy input-output ratio is higher than one, the system is earning energy, whereas if it is less than one, the system is losing energy. However, Zahedi et al., (2014) found that the energy ratio for cotton production in Esfahan province of Iran was 0.7%. The results also showed that the energy productivity was 0.3 kg MJ-1 (Table 4). This means that one Mega Joule energy input was used to produce 300 grams of cotton on the average. The specific energy for cotton production was 3.38 MJ kg-1.This means that every 3.38 MJ energy was used to produce one kilogram of cotton. The results revealed that the net energy was 5567.48MJ ha-1 (Table 4). This indicated that production of cotton gained energy, however, the obtained net energy was much lower than in the developed countries. As an example, Dagistan et al (2009) found the net energy was 26663 MJ ha-1.

On the other hand, Table 4 shows the total energy input in form of direct, indirect, renewable and nonrenewable energies used in production of cotton. The direct energy input was 1308.37 MJ ha-1.The indirect input energy was 926.25MJ ha-1. Furthermore, the renewable energy for cotton production in the mechanized rainfed schemes was 802.22 MJ ha-1 and the non-renewable energy was1432.40 MJ ha-1.

Table 4. Some energy indices in cotton production in the mechanized rainfed schemes eastern Sudan

Inputs	Unit	Quantity
Energy ratio	-	*3.49*
Energy productivity	*kg MJ-1*	*0.30*
Specific energy	*MJ kg -1*	*3.38*
Net energy	*MJ ha -1*	*5567.48*
Direct energy	*MJ ha -1*	*1308.37*
Indirect energy	*MJ ha -1*	*926.26*
Renewable energy	*MJ ha -1*	*802.22*
Non-renewable energy	*MJ ha -1*	*1432.40*
Total energy input	*MJ ha -1*	*2234.62*

Figure 3 illustrates the share of energy input sources for cotton production. Out of the total energy input, the share of herbicide energy was found to be the highest (30.46%). The highest share of herbicide energy implies that cotton production in the mechanized rainfed schemes depend heavily on herbicides for controlling weeds. However, the share of the consumed energy from the total input energy in fuel was 29.35%. Several authors had found that fuel energy was the highest energy consumed for crop production (Canakci et al., 2005; Umar and Ibrahim, 2012). The share of energy excreted by hand labor from the total energy input was 29.18%., whereas machinery and seeds represented lower energy sources 4.28% and 6.73%, respectively. Efforts should be focused on herbicides, fuel and hand labor to balance energy use for cotton production in the study area.

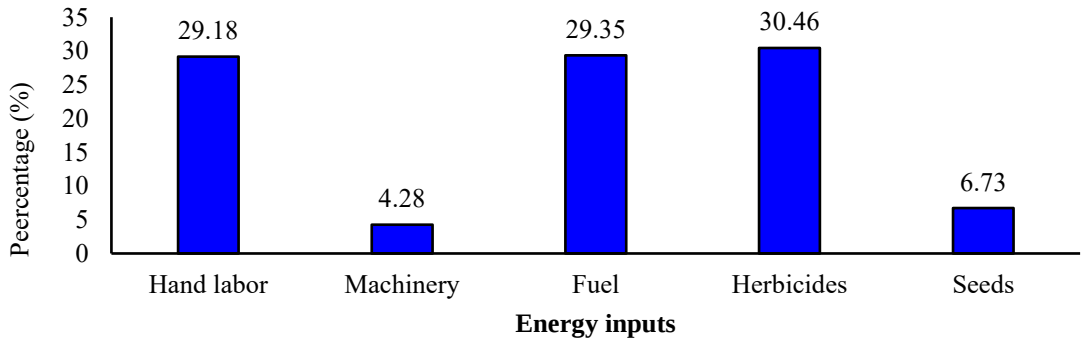

Fig. 3. The shares of energy inputs for cotton production

The direct energy consumed in cotton production represented 58.5% and the indirect energy (machinery, herbicides and seeds) represented 41.5% (Fig. 4). The results indicated that the direct energy in form of hand labor and fuel for cotton production was to some extent more than indirect. Zahedi et al., (2014) reported similar result and found that direct energy was 68.3% of the total input energy. In contrast, Yilmaz et al., (2005) and Dagistan et al., (2009) found that indicated energy was higher than direct energy which was 57.5% and 71.13% for the former and later authors, respectively. On the other hand, the results showed that the non-renewable energy was greater than the renewable energy that used for cotton production. Therefore, researchers need to investigate more on balanced utilization of energy sources for cotton production in the mechanized schemes in rainfed areas eastern Sudan. This could be achieved by examining different management practices.

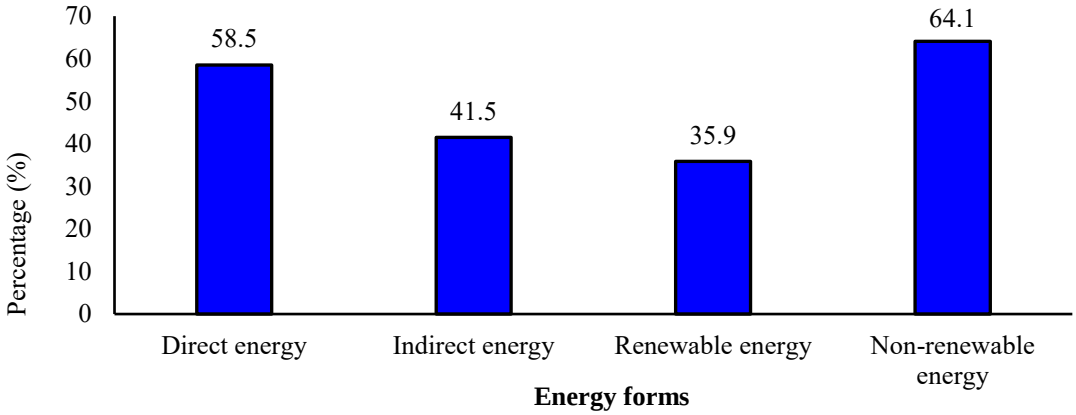

Fig. 4. Distribution of energy forms in cotton production

Conclusion

- The average energy input consumed to produce cotton in the mechanized rainfed schemes was 2234.62 MJ ha-1 and the total energy output was 7802.10 MJ ha-1.
- Cotton production was efficient in energy consumption as the energy use efficiency was higher than three. The average net energy, the energy productivity and the specific energy was 5567.48 MJ ha-1, 0.30kg MJ-1 and 3.38 MJ kg -1, respectively. Herbicide energy input was the highest among the energy input items.
- The direct energy was greater than the indirect energy. Likewise, the non-renewable energy was greater than the renewable energy. Investigations on balanced utilization of energy sources for cotton production in the mechanized rainfed schemes eastern Sudan are necessary.

References

1. Adam, H. S. (2008). Agro climatology, Crop Water Requirement and Water Management; Gezira
2. Canakci, M., Topakci, M., Akinci, I., and Ozmerzi, A. (2005). Energy use pattern of some field crops and vegetable production: Case study for Antalya Region, Turkey. Energy Conversion and Management, 46, 655–666.
3. Chandra H, De Dipanker, Singh, R. S. (2001). Spatial variation in energy use pattern for paddy cultivation in India. Proc. Of National Workshop on Energy and Environment Management for Sustainable Development of Agriculture and Agro Industrial Sector, July 8–9, 2001, 48–51.
4. Dagistan, E., Akcaoz, H., Demirtas, B., and Yilmaz, Y. (2009). Energy usage and benefit-cost analysis of cotton production in Turkey. African Journal of Agricultural Research, 4 (7): 599-604, Available online at: https://www.academicjournals.org/AJAR.
5. Erdal, G., Kemal, G., Esengun, K., Erdal, H. and Gunduz, O. (2007). Energy use and economical analysis of sugar beet production in Tokat Province Turkey. Energy 32:35-41.
6. Faki, Hamid H. (2006). Sustainable Cotton Production Forum. Sudan's Report ICAC, Washington, April 4-13.
7. Ghorbani, R., Mondani, F., Amirmoradi, S., Feizi, H., Khorramdel, S., Teimouri, M., Sanjani, S., Anvarkhah, S., and Aghel, H. (2011), A case study of energy use and economical analysis of irrigated and dryland wheat production systems. Applied Energy, 88, 283-288.
8. Gokdogani, O., and Sevim, B. (2016). Determination of Energy Balance of Wheat Production in Turkey: A Case Study of Eskil District of Aksaray Province, Journal of Tekirdag Agricultural Faculty, 13(04):36-43.
9. Hatirli, S. A., Ozkan, B., and Fert, C. (2005). An econometric analysis of energy input–output in Turkish agriculture. Renew. Sustain. Energy Rev. 9:608–623.
10. Kennedy S. (2000). Energy use in American agriculture. Sustainable Energy Term Paper 2000. 5/1/2000.

11. Kheiry, A. N. O. and Dahab, M. H. (2016). Energy input-output analysis for production of selected crops in the central clay Vertisols of Gezira agricultural scheme (Sudan). International Journal of Science and Research (IJSR) (5)3: 1215-1220.
12. Kızılaslan, H. (2009). Input-output energy analysis of cherries production in Tokat province of Turkey. Applied Energy, 86:1354-1358
13. McLaughlin, N. B., Hiba, A., Wall, GJ. and King, OJ. (2000). Comparison of energy inputs for inorganic fertilizer and manure based corn production. Canadian Agricultural Engineering 42(1): 9 -17.
14. Ozkan, B., Akcaoz, H., and Fert, C. (2004). Energy input-output analysis in Turkish agriculture. Renew. Energy 29:39–51.
15. Rafiee, S.; Mousavi Avval, S. H. and Mohammadi, A. (2010). Modeling and sensitivity analysis of energy inputs for apple production in Iran. Energy 35: 3301-3306.
16. Safa, M. Mohtasebi, S. S., and Lar, M. B. (2010). Energy use in wheat production (A case study for Saveh, Iran), World Journal of Agricultural Sciences 6 (1): 98-104.
17. Singh J. M. (2002). On farm energy use pattern in different cropping systems in Haryana, India. Germany: Int. Inst of Management, University of Flensburg, Sustainable Energy Systems and Management, Master of Science Thesis, Germany, 2002.
18. Umar, H. S. and Ibrahim, H. Y. (2012). Energy use and gross margin analysis for sesame production in organic and inorganic fertilizer user farms in Nigeria. African Crop Science Journal, (20)1:39 – 45.
19. Uzunoz, M., Akcay, Y., and Esenguni, K. (2008). Energy input-output analysis of sunflower seed (Helianthus annuus L.) oil in Turkey. Energy Sources, Part B, 3:215–223.
20. Yilmaz, I., Akcaoz, H. and Ozkan, B. (2005). An analysis of energy use and input costs for cotton production in Turkey, Renewable Energy 30: 145–155.
21. Zahedi, M., Eshghizadeh, H. R., and Mondani, F. (2014). Energy use efficiency and economical analysis in cotton production system in an arid region: A case study for Isfahan Province, Iran, International Journal of Energy Economics and Policy, (4)1:43-52.

What lessons from the motorization program of the National Union of Cotton Producers of Burkina Faso for a sustainable development of motorization in cotton areas?

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Abstract

This article analyzes the effects of the introduction of the tractor on the management and performance of family farms benefiting in 2014 from the "motorization" program of the National Union of Cotton Producers in the Hauts Bassins region of Burkina Faso. Tractors replaced animal traction and labor for soil preparations. In FAE, there is an increase in cultivated area and labor productivity for soil preparation, lower yields and incomes. The fragmentation and remoteness of some plots lead to additional time and operating costs for tractors. Overall breakdowns and repairs on equipment are numerous especially for lack of training and skills of tractor drivers and farmers. As a result, many farmers are failing to reach their early planting goal because of failures that can immobilize tractors for several days. The result is an increase in tractor operating costs and difficulties for farmers to meet their financial commitments. This study shows that it is not enough to distribute / sell tractors to make the engine viable and sustainable. To this end, there is a need to: i) promote sustainable soil and land management techniques in FAE, ii) build capacity of actors on the maintenance and use of motorization, iii) conduct studies thorough prerequisites before the introduction of any agricultural motorization program, iv) to reflect on other forms of use of tractors and on the mechanization of other agricultural operations, v) to solicit the State to support sustainable development motorization through incentives, support to the private sector for the development of after-sales services, training and support-advice, and reflections on land management.

Keywords: *motorization, performance, maintenance, skills, management, cotton, Burkina Faso.*

Introduction

The continued growth of cotton in Burkina Faso from 2,772 tons (t) in 1960, to more than 749,000 tons in 2016/2017 (ICAC, 2019), places it among the top cotton-producing countries in Africa (Sba-Ecosys-Cedres, 2011). Cotton activity contributes on average to 65% of household incomes in cotton areas (Dembele, 2012), and reduces poverty 4 times faster in cotton production areas (WAEMU, 2010). Cotton cultivation has a knock-on effect on cereal production (Schwartz 2007): the back effect of cotton fertilizers contributes to increasing cereal yields by 20 to 30%.

The increases in cotton and cereal production are mainly the result of the extension of areas, as yields have stagnated over the last fifteen years. Animal traction is used by more than 70% of family farms (FAEs) in cotton areas for soil work (ploughing, tooth work), crop maintenance (weeding, butting), sowing cotton and maize. Motorization affects about 1% of EAFs despite the programs of recent decades by the Burkinabe Society of Fibers and Textiles (SOFITEX) (Bouyer TE tractors in the 70s and 80s (Tersiguel, 1995), Hindustan tractors, Agrimex). The "motorization" operations of the State follow one another. In 2007, 700 tractors were set up (Team 9 program with India). In 2017, the State gave agricultural producers subsidized equipment on credit: 500 tractors, 100 tillers and 750 motor pumps, all equipped. This last gesture contributes to the concretization of Axis 3 of the National Economic and Social Development Plan (PNDES). With these programs, the ambition of the Burkinabe government, like those of several sub-Saharan African countries since the hunger riots in 2008, is to accelerate the process of modernization of agriculture, including agricultural mechanization, with a focus on

motorization (Side and Havard, 2015).

For its part, the National Union of Cooperative Societies of Cotton Producers of Burkina Faso (UNPCB-CA-SCOOP), as part of the modernization of cotton FAEs, implemented in 2014, an acquisition operation of 300 tractors with their accessories composed of ploughs, disc sprayers and trailers. This operation was supported by the Burkinabe authorities through a reduction in customs duties and value added tax (VAT) of 568,151,373 FCFA (19.2% of the amount of tractors and equipment supplied by Datong Entreprise Group (DTE) based in Bobo Dioulasso).

The objectives of this operation, in line with the UNPCB's Five-Year Strategic Plan (2014-2018) and State policy, are: improving the living conditions of producers, contributing to considerably reduce the rural exodus and making a significant and sustainable contribution to agricultural production. In the cotton zone of Burkina Faso, the introduction of tractors has resulted in an increase in the area sown without leading to an increase in yields of the main crops (Tersiguel, 1995; Sanou et al., 2019). Large mechanized farms can more easily carry out direct sowing, one of the elements of conservation agriculture (Lankoande, 2013). The increase in the level of mechanization translates into an improvement in the ability to manage fertility (Ouedraogo, 2012). In addition, farmers and tractor operators face difficulties in the use and maintenance of tractors: numerous breakdowns, unavailability of spare parts, lack of qualified technicians and mechanics (Sanou et al., 2019). This leads to the following question: what assessment can be drawn from the UNPCB's motorization operation after 4 years of operation (2014-2017)? what are its effects on the performance of beneficiary FAEs? How and under what conditions do the beneficiary producers use and maintain the tractors and equipment? This article aims to evaluate the effects of 4 years of implementation of the UNPCB motorization program on the beneficiary FAEs (structure, operation, performance, living conditions) and to draw lessons for the development of sustainable agricultural mechanization in cotton areas.

After this introduction, the methodological approach is described, the results are presented and discussed, and the conclusion is accompanied by recommendations.

Material and method

To assess the effects of the introduction of the tractor with tillage and transport equipment on the beneficiary FAEs, a comparative analysis was carried out. It covers the data, results and performance of FAEs, the year before the acquisition of the tractor (2013-2014 crop year), and the years following the introduction of the tractor (2014-2015 to 2017-2018 seasons). Data from different years are collected in a single survey of a sample of beneficiaries, based on their memory. What is sustainable agricultural mechanization and mechanization (FAO website: http://www.fao.org/sustainable-agricultural-mechanization/fr/008): "Mechanization includes all levels of agricultural and processing technologies, from the simplest hand tools to motorized and more sophisticated equipment. Mechanization facilitates and reduces the arduousness of work, compensates for the lack of staff, improves productivity and the timing of agricultural operations, allows better use of resources, facilitates access to the market and helps mitigate climatic hazards. Sustainable mechanization takes into account technological, economic, social, environmental and cultural aspects by contributing to the sustainable development of the agri-food sector. It supports the sustainable development of food supply chains through improved agricultural practices that increase production and enhance food security."

Study sites

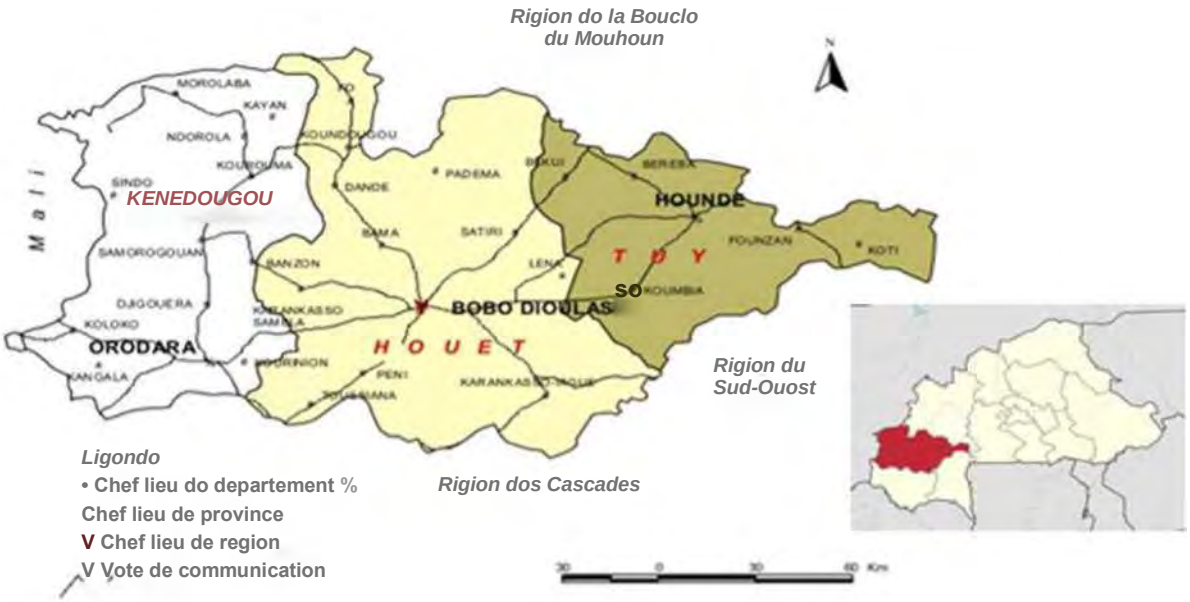
The study was carried out in the Hauts-Bassins region of western Burkina Faso (Map 1) (MAH, 2011). The distribution of annual rainfall (900 to 1200 mm), favorable to the cultivation of cereals, legumes, cotton, etc., distinguishes a wet season (6 to 7 months from May to October/November) and a dry season (5 to 6 months from November/December to April) (MAH, 2011). This region was chosen because it has 129 of the 300 beneficiaries of the UNPCB "motorization" programme.

The soils of the cotton zone are not very evolved, ferruginous and ferralitic (good agricultural value), hydromorphic to pseudo-gley (chemically rich), and gravel (low agricultural value). They have a low content of organic matter and exchangeable bases, which makes them fragile and easily degradable by continuous tillage carried out in poor conditions (inappropriate and misused agricultural equipment).

Choice of agricultural holdings in the sample

The producers were classified according to the power of the tractors: 40, 50, 60 and 80 hp (Table I). Then 60 producers were chosen in a reasoned way to have all the combinations of tractors and

equipment chains (plough, sprayer, seeder, weeder and trailer): i) those acquired by one or two producers were all retained; (ii) for the others, combinations were made to have 50 % of the producers in the sample with the disc plough and 50 % with the sprayer.



Source: Regional Directorate for the Economy and Development of the Hauts Bassins, 2003 Map 1: Administrative map of the Hauts Bassins region

Table I: Sample of agricultural holdings surveyed

Tractor	Equipment chains	Mother	Retained	% of mother
	40 CH+PUL	46	13	30
	40 CH+CHAR	23	11	48
40 CH	40 CH+CHAR+Rem5T2R	1	1	100
(28)	40 CH+PUL+Sem+Sarcl	1	1	100
	40 CH+CHAR+Sem	1	1	100
	40 CH+PUL+Rem5T4R	1	1	100
	50 CH+PUL	19	9	48
	50 CH+CHAR	17	9	50
50 CH	50 CH+PUL+Rem5T4R	2	2	100
(23)	50 CH+CHAR+Rem5T4R	1	1	100
	50 CH+CHAR+Sem	1	1	100
	50 CH+CHAR+Rem5T2R	1	1	100
60 CH	60 CH+PUL	8	4	50
(8)	60 CH+CHAR	6	4	70
80 CH (1)	80 CH+CHAR+Rem10T+Sarcl+Sem	1	1	100
Total		129	60	

Legend: CH=horses; CHAR=plough; PUL=sprayer; REM=trailer; T= tonne; R=wheel; Sarcl=weeder; **Source:** UNPCB

Data collection

In 2018, an exploratory survey of agents of the Departmental Unions of Cotton Producers (UDPC) identified the number and types of tractors and villages in the sample. In June-July 2018, producers were surveyed using a three-part questionnaire:

- EAF structure data: i) identification of the producer (age, number of workers, etc.); (ii) area and type of crops in 2013/2014, 2014/2015 and 2015/2016, (iii) equipment and infrastructure; (iv) numbers and types of livestock;
- constraints encountered in the use of the equipment: date, breakdown lists, maintenance and repair costs, availability of qualified mechanics;
- adoption of sustainable land management practices: identification and description of the techniques used, characterization of soil and land management practices, level of adoption of practices,
- use and place of motorization in the EAF: i) perception by producers of the effects on the soil of mechanization (motorization), work within the EAF and agricultural production; (ii) the reasons for the use of different types of tools and soil preparation depending on the plot and crop.
- maintenance and repair of the tractor: types, quantities and costs of the parts purchased, maintenance expense, list, location and parts of the various suppliers, cost of the service of the mechanics,

Data processing and analysis

Qualitative data were analyzed manually. Quantitative statistics were entered in Access, and descriptive statistics (average, minimum, maximum, ratios) were performed in Excel. The changes that occurred with the introduction of the tractor in the beneficiaries' FAEs, on the structure, operation, and technical and economic performance were highlighted by comparative analyses between the year before the acquisition of the tractor, and the following years when the tractors were used.

The average net margin is the average gross income minus the total expenses including input, labour, investment and operating expenses. It makes it possible to highlight the evolution and profitability of the activity.

Agricultural input charges are obtained from the valuation of the unit cost of inputs and the overall quantity used during the crop year. The workforce represents the remuneration paid to the tractor operator and those inherent in the services in the work of soil preparation, crop maintenance and harvesting ....

Tractor operating expenses include routine maintenance (expenses remain roughly constant each year if the amounts of work are the same between years), repairs (expenses increase with the aging of tractors).

The cumulative repair cost of a tractor is the cost of all the repairs that a machine has required from the date of its acquisition until the date considered. For the cost/benefit analysis and calculation of the technical and economic performance of the tractor and its equipment, the use of tractors with their various equipment was done using a grid (Havard, 2002). The hourly costs of using the tractors are taken into account, the other costs (per ha, per kg, etc.) are deducted. This method distinguishes between fixed costs (interest on invested capital, insurance, shelter, taxes), variable costs under certain conditions (depreciation, repairs) (Table II), and essentially variable costs (fuel, lubricant, driving, maintenance). Estimated repair costs are calculated using the following formula for one hour of work: Material price * Repair coefficient / Number of hours of use over the lifetime. Thus, for a tractor of 6 million CFA francs with a service life of 8,000 hours, with a repair coefficient of 100% (Table II), the average estimated repair cost for one hour of work is 750 CFA francs / h of use.

Results

Some characteristics of motorized farms

Of the 60 producers in the study, 58 are indigenous and two are migrants. The majority of UNPCB FAEs have only the UNPCB tractor with its equipment; the few others had either other tractors or other equipment: ploughs with ploughs, gins, rice threshers (Table III).

Table II. Expected service life and repair coefficients according to the types of agricultural equipment.

Material	Possible service life		— Repair coefficient as % of new price
	Years	Hours	
Tillers	8	3 000	60
Wheeled tractor	10	8 000	100
Tracked tractor	15	10 000	80
Tillage equipment	10	2 500	120
Sowing and spreading equipment	10	1 000	100
Thresher	10	5 000	100
Harvesting and self-propelled	8	2 000	60
Trailers	10	5 000	20
Shellers and mills	10	2 000	50

Legend: The figures are given as an indication because they are subject to variations according to the contexts of use.

Source: Havard, 2002

Table III : Rate of equipment of agricultural holdings with motorized equipment

Materials	Equipment Proportion of FAEs equipped (%)	Proportion of FAEs purchase from own funds (%)	Average number per EAF
Tractor	100	5	1,02
Disc plough	57	7	0,62
Sprayer	82	32	0,88
Trailer	93	83	1,05
Seeder	8,3	3,3	0,08
Weeder	3,3	0	0,03
Plough plough	8	8	0,08
Egreneuse	62	62	0,68
Rice thresher	1,6	1,6	0,016

Legend: The figures are given as an indication because they are subject to variations according to the contexts of use.
Source: Havard, 2002

On all farms, the average population is 24.5 people with 54% active, 52% women, and 53% women among the active. The average population increases with the power of tractors: 18 people for the 40 HP to 38 people for the 80 HP. The % of the working population, women and women among the working population vary little around the averages whatever the type of tractor. The majority of those who are not active are children in school. During the holidays, they can keep the herd, drive draught oxen during agricultural work, participate in the application of fertilizers and organic manure. All FAEs use daily labour, some use seasonal workers, but few have permanent employees.

The motorized farms in the sample mainly produce cotton and maize (nearly 85% of crop rotation), and to a lesser extent sesame, and other speculations such as cowpea, groundnuts, millet, sorghum... (Table IV).

Table IV: Evolution of the average rotation of a motorized holding of the sample between 2013 and 2017

Year	Cotton	Maize	Sesame	Over crops	Fallow	Total (ha)
2013	50,0%	34,2%	2,6%	10,5%	2,6%	38
2014	53,2%	31,9%	0,0%	12,8%	2,1%	47
2015	54,0%	32,0%	0,0%	12,0%	2,0%	50
2016	41,7%	43,8%	2,1%	8,3%	4,2%	48
2017	51,0%	31,4%	0,0%	13,7%	3,9%	51

Source: Survey

Almost all the respondents claim to alternate the production of cotton with that of maize and incidentally sorghum.

The 2016/2017 crop year recorded a decrease in areas due to the late installation of the season. Almost all producers use their tractor as a service for tillage (on average per EAF 17 ha for ploughing and 25 ha for spraying at 25,000 FCFA/ha), ginning maize (on average per EAF, 513 bags ginned at 500 FCFA/ginned bag), transport of inputs, construction materials and organic manure (on average 5,000 to 10,000 FCFA/trip depending on distances).

Motorized farms are also developing services with the tractor.

Farms combine tractor work with animal traction

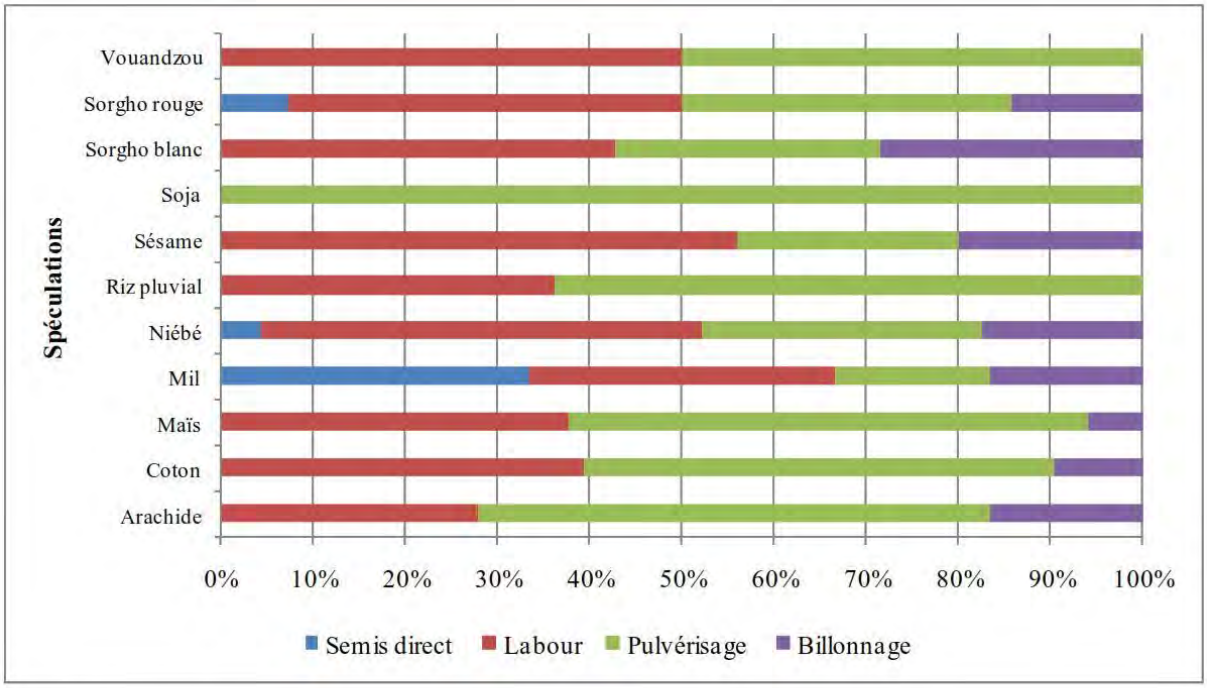
All producers in the study retained their animal traction equipment. They practice cleaning, desoiling if necessary, and fires on their plots before tillage: ploughing (animal traction and tractor), tractor spraying and some producers the ridge (animal traction and tractor) (Figure 2). Manual tillage and direct sowing without tillage are rare in FAEs.

Animal traction is used on 6 %, 9 %, 34 %, 73 % and 80 % of the average area of the EAF respectively for ploughing, ridge, sowing, weeding and butting because: i) some plots are not well sealed, small, difficult to access, nor prepared for the use of seeders, weeders and motorized multirow bumpers, (ii) sowing with animal traction may be carried out simultaneously with motorized tillage. The latter is carried out with a sprayer on 51% of the areas cultivated on average by EAF, especially on groundnuts, cotton, maize, rice, soybeans and cowpeas. Motorized ploughing is carried out on 31% of the EAF area, mainly on millet, sesame, red sorghum, vouandzou and white sorghum. Motorized sowing is carried out on 2% of the average area of the EAF on cotton and maize.

Manual maintenance, especially weeding, is practiced by all producers on 90% of the cultivated areas, because after the use of weeders, buttors and herbicides, manual weeding is often necessary. On line crops (cotton, maize, millet and sorghum), weeding and butting between the lines is followed by manual weeding on the lines between the plants. Herbicides were used on 65% of the average EAF area: 96% of the area under cotton, 91% from sesame, 82% from rice, and 67% from maize. On cotton and maize, plots treated with selective herbicides are rooted only if the state of bgrassing is critical. Mechanical butting will be carried out after the second application of fertilizer,

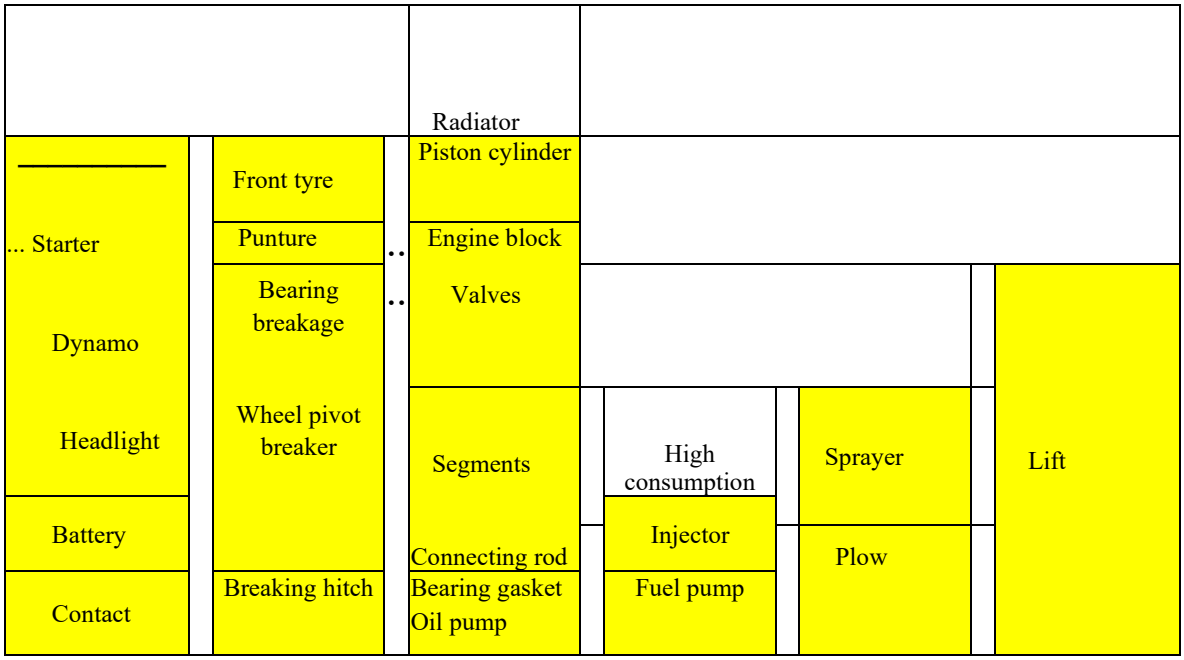
The many failures affecting the engine are due to poor maintenance: the diesel filters are not changed in time, in other cases, the original filters are replaced by adaptable filters, etc., the drain periods are not respected, poor quality oil is used, the radiator is not cleaned often or there is a lack of water causing the engine to overheat, etc.

Breaks of front bearing, wheel pivot, puncture ... are due to inappropriate driving, excessive working speed, dead wood stumps in plots, degraded roads or rural tracks.



Source: Survey

*Fig 2.*Distribution of Types of Tillage by Crop in 2015-2016



Source: survey.

Fig 3.Distribution of the type and average number of breakdowns per tractor over 4 years

Table V: Summary of annual hourly volumes and repair costs in FCFA per tractor and its accessories by type of power

Tractor power	Year	2015	2016	2017	2018	Total
40 CH	Real	407 647	321 059	307 647	381 176	1417 529
	W(h/an)	155	128	124	129	536
50 CH	Real	345 385	279 038	239 654	394 231	*0* 258 308
	W(h/an)	161	131	130	140	562
60 CH	Real	677 625	805 000	691 250	706 250	*2* 880 125
	W(h/an)	284	190	187	180	841
80 CH	Real	60 000	80 000	30 000	200 000	370 000
	W(h/an)	225	229	210	284	948

Legend: Forecast; W. Working time

Source: Survey

Disappointing economic results

Average net margins per ha decreased between 2014/15 and 2017/18, years with the tractor. Net margins per hectare decreased by 22.12% without services, by 22.50% with services (Table VI). The EAFs have therefore recorded a decrease in their earnings since the acquisition of the tractor.

Between 2013/2014, the year before the tractor, and 2014 to 2018, years with the tractor, the average gross income per farm increased significantly (about 50%) in the first year with the tractor, then gradually fell back in 2018 to practically the same income as the year before the tractor. In the years with the tractor, the operating costs doubled compared to the year before the tractor. As a result, the net margin per farm increased in the first year with the tractor, and then gradually decreased to half of the net margin of the year before the tractor in 2018.

Diversified but poorly adopted sustainable land management practices Motorized producers all produce organic manure: manure pits, barns, garbage cans, etc. The introduction of the tractor did not change the usual practices of manure production. Manure is supplied primarily on cotton (7.5 tonnes per EAF, i.e. less than 500 kg/ha), and maize (2.75 tonnes per EAF, i.e. less than 150 kg/ha). These doses are far below the recommendations of the Institute of the Environment and Agricultural Research (INERA) which vary from 3 to 4 tons of organic manure per hectare.ns of 150 kg/ha).

Motorization has also not changed water and soil conservation/soil defense and restoration (CES/DRS) practices, but it has facilitated tasks and motivated some producers. 21% of the FAEs surveyed practice CES/DRS techniques, 2% of EAFs plant live hedges. All these practices of sustainable land management, little adopted, are essential to be part of the dynamics of sustainable mechanization.

Effects of motorization perceived by producers

For producers, motorization makes it possible to extend the areas of pure crops, which is confirmed by the survey (Table III). Between 2013/2014 and 2017/2018, the area under cultivation per holding increased from 38 ha to 51 ha (+34%) with the largest increases in areas for cotton (+37%), maize (+23%).

Table VI: Evolution of the generation of income account in motorized FCFA/EA from 2014/15 to 2017/18

Sample de 35 EAF	Front tractor	With tracteur			
	2013/14	2014/15	2015/16	2016/17	2017/18
Average gross income without benefit (1)	10 862 699	15 737 338	14 384 384	12 800 399	11 350 772
Input costs (2)	3 591740	4471006	4585353	4 398 736	4 425 689
Tractor annuity and accessories (3)	0	2 000 940	1 553 080	1 531 000	2 011 080
Operating expenditure (4)	0	1 062000	1 047 966	1 075 137	817 365
Labour costs(5)	0	356 428	350 600	305 080	275 700
Total expenses (6)=(2)+(3)+(4)+(5)	3 591 740	7 569 594	7 536 999	7 309 953	7 529 834
Total expenses (FCFA/ha)	86757	161 055	150 740	152 291	147 644
Net margin without services (7)=(1)-(6)	7 270 959	8 167 744	6 847 385	5 490 446	3 820 938
Net margin per hectare without benefit income	178 042	173 782	136 948	114 384	74 920
Income from benefits (8): Labour, transportation	0	201 428	83 023	70 571	41 899
Average gross income with benefits (9)=(1) +(8)	10 862 699	15 938 766	14 467 407	12 870 970	11 392 671
Net margin with services(10)=(9)-(6)	7 270 959	8 369 172	6 930 408	5 561 017	3 862 837
Evolution of the net margin between years		+1 098213	-1 438764	—1 369391	—1 698 180
Average net margin/hectare with benefit income	178 042	178 067	138 608	115 855	75 742

Source: Survey

On the other hand, for other crops the areas have increased overall, but with variations between years. Rice and sorghum areas decreased in 2014, and cowpea and sesame in 2015. The average area per family worker increased from 2.9 ha/active in 2013/2014 before the tractor, to 3.9 ha in 2017/2018 with the tractor.

Compared to animal traction, motorization makes it possible to carry out soil preparations faster, to sow in conditions more favorable to germination and emergence of crops and to reduce late sowing. In addition, 80% of producers attribute the same effects related to animal traction compared to manual work. For 73% of producers, motorized tillage improves yields, while 23% do not see a difference.

For producers, tractor tillage is deeper than that carried out with animal traction and hand tools, the soil is better loosened and more from the second year, weeds are better buried, clods are larger, but signs of erosion are accentuated with the sprayer and disc plough.

Producers prefer the plough sprayer for time saving (it works faster) and a lower negative impact on the soil (it works less deeply and turns the earth less). After the passage of the sprayer, the soil is looser, less mottled and better leveled on the surface; it does not require recovery before sowing. Plough tillage is deeper, turns over the fertile layer of soil, buries weeds and promotes the creation of paths for erosion. A recovery before sowing (never carried out by farmers) is necessary to break the clods and level the soil surface.

For producers, motorization has not changed the organization of work within the EAFs. But the expansion of the areas cultivated with the tractor has increased the need for family and salaried labour for crop maintenance, fertilizer and pesticide application, harvesting and transport.

Discussion

The results of this study are comparable to those of previous studies (Tersiguel, 1995; Ouedraogo, 2012), showing that the way in which the engine is used in FAEs has not changed in recent decades, and very few FAEs own the tractor. Tractors and their equipment are underutilized (200 to 300 hours/year), as they only carry out soil preparation, a little corn ginning and transport of crops and manure. Other agricultural work (sowing, weeding, butting, harvesting, etc.) is carried out using human and animal energies. This underutilization found in previous studies (Tersiguel, 1995; Sims et al., 2018) questions the profitability of tractor use on motorized farms. The average annual use of a medium-sized four-wheel tractor must be at least 700 to 800 hours to make it profitable (Sims et al., 2018). Under current conditions of use, agricultural monetary income remains relatively stationary or decreasing when motorization comes into play, following a sharp increase in fixed costs (Raymond, 1991).

FAEs face unavailability of spare parts needed for maintenance and repairs. 30% of FAEs are not satisfied with suppliers and go to other horizons including Mali. Some parts such as connecting rods, ball joints, bearings, etc. are often out of stock at the tractor supplier (DTE) and at the merchants. In THE FAEs, tractor operators do the maintenance of the tractor. However, they only received three days of training on driving and maintaining the tractor. With a low level of education, they are unable to note and meet drain dates, resulting in serious engine failures, as shown by high repair costs.

The EAFs are all part of a logic of extension of the areas with the acquisition of the tractor, but without always respecting the good practices of use of the equipment especially for the preparation of the soils. Land degradation is exacerbated by the un skilledness of tractor drivers, and the misuse and choice of tillage equipment (Seone, 1999).

This UNPCB motorization programme, like those of the State, uses imported equipment, for the use of which the workforce is not well trained, and the after-sales service (spare parts, maintenance and repair) is not adequately provided. To promote the emergence of sustainable mechanization, training programs for qualified human resources in the design and production of certain spare parts on site, and the establishment of units capable of manufacturing them are essential.

In general, the loss of vegetation cover is more accelerated among motorized FAEs, because of the operations of de-soiling, reduction of the number of feet of useful trees (Shea, Nere, Tamarind ...) to promote the mobility of the tractor and its equipment. Hedge and tree plantations are essential to curb rain and wind erosion in plots and regulate biological processes (pollination, biological regulation of pests). According to Moureaux et al., (1969) and Piraux et al., (1997) the degradation of the structural state of the soil is more accentuated in mechanized cultivation than in the traditional system integrating long-term fallow preceded by a cultivation of 2 to 4 years. Soil degradation inevitably leads to lower yields (FAO, 2007), and thus production and income.

Conclusion

This study shows that the introduction of the tractor promotes the extension of areas, and early sowing in EFAs, but does not change their practices. It also highlights a slight decrease in cotton and maize yields to be confirmed over a longer period, and a significant drop in farm incomes linked to a very sharp increase in their expenses. In fifty years, the terms of use of the engine in the EAF have remained almost the same, only the tractors are different (Chinese and Indian brands, and formerly European) and a little more powerful. The availability of spare parts, qualified mechanics, and competent tractor drivers, remain strong constraints to the use of the tractor and its equipment. The tractor is little used for short periods, mainly for tillage and transport, in combination with animal traction, but also for services. Motorized FAEs want to keep animal traction because in addition to the work provided, it valorizes crop residues, produces organic matter to fertilize the fields and is a form of standing savings.

The results of this study show that the conditions for the development of sustainable motorization are not met in this UNPCB programme. It is urgent to put in place accompanying measures for the development of sustainable motorization in cotton areas, by mobilizing the various actors, and by soliciting the support of the State. These are:

- promote sustainable land management techniques in FAEs: integration of agriculture and livestock, soil conservation and soil defence and restoration techniques (CES/DRS),

agroforestry;

- strengthen the capacities of stakeholders (farmer, tractor operator, mechanics, craftsmen, etc.) on good practices in the use, maintenance and repair of tractors and its equipment to avoid soil degradation, and high maintenance and repair costs;
- carry out feasibility studies before the implementation of any agricultural motorization program,
- reflect on other forms of tractor use to enable small producers to use them, such as collective management by several producers, service centres, etc. and on the mechanisation of other agricultural operations to increase the annual use of tractors;

References

1. Dembele, 2012. Possible alternative to traditional cotton production in Africa from a sustainable development perspective? the case of the organic and fair trade production system in Mali, Original dissertation presented with a view to obtaining the degree of Doctor in Agronomic Sciences and Biological Engineering, University of Cork - Gembloux Agro Bio Tech
2. FAO, 2007. How to address the challenges faced in the supply of inputs for agricultural mechanization and the processing of agricultural products. Deliberation of a workshop FAO, World Congress of Rural Engineering, 5-6 September, Bonn, Germany. FAO, Technical Report on Rural and Food Engineering No. 5.
3. Havard M., 2002. Motorization: technical choice of material and cost of equipment. CD-ROM. In: Memento de l'agronome, Montpellier, CIRAD, GRET, Ministere des Affaires Etrangeres.
4. ICAC. 2019. World cotton statistics, December 2019, www.icac.org (limited access).
5. Lankoande, 2013. Analysis of the influence of agricultural mechanization on the development possibilities of conservation agriculture in the Boucle du Mouhoun Region. Thesis of engineering degree in agricultural extension, Institute of Rural Development, Bobo Dioulasso, Burkina Faso.
6. MAH, 2011. National survey on household access to family sanitation facilities (2010); Hauts- Bassins regional monograph. Ministry of Agriculture and Hydraulics, General Directorate of Wastewater Sanitation and Excreta, Burkina Faso, 60 p + annexes.
7. Moureaux C.I., Fauck R. and Thomann C. H., 1969, Bilan de l'evolution des sols de Sefa (Casamance, Senegal) apres 15 annees de culture continue. Agron. Too.,3: 263-301pp.
8. Ouedraogo I., 2012. Importance of agricultural mechanization and its impact on cotton yields and certain soil chemical characteristics in the Central (FASO COTON) and West (SOFITEX) cotton agrosystems of Burkina Faso. Agronomist Engineer's Thesis, UPB/IDR16, Burkina Faso. 67 p
9. Piraux M., Bock L., Buldgen A., Lefort., 1997. Influence of climatic, topographical and intensification conditions on the physical and chemical fertility of ferruginous soils in the Sahelo- Sudanian region. Drought; 8(4): 247-256pp.
10. Raymond, 1991. The technical data necessary for economic calculation in agriculture. Rural economy. N°18, 1953. pp. 17-24;
11. Sanou F., Havard M., Coulibaly K., Nana S., 2019. Effects of the introduction of the tractor on agricultural practices in the cotton zone of Burkina Faso. In: Soumare and Havard (under the direction of). Actes du collque les zones cotonnieres africaines, dynamique et durabilite, 21 au 23 novembre 2017, Bamako, Mali.
12. Sba-Ecosys-Cedres, 2011. Economic analysis of the cotton sector. Links between poverty and the environment. Final report. Ministry of Environment and Living Environment (MECV), Poverty Environment Initiative (IPE) project, Ouagadougou, Burkina Faso.
13. Schwartz, 2007. Brief history of cotton cultivation in Burkina Faso. ORSTOM. Bondy, France, 28 p.
14. Seone H., 1999. Contributions to the microeconomic analysis of motorized farms in the western cotton zone of Burkina Faso. Dissertation. Agronomy - IDR /UPB, 84p + annexes
15. Side C.S., 2013. Strategy for the mechanization of family farming in sub-Saharan Africa, Dissertation de fin d'etudes. Specialized Master's Degree in Innovations and Policies for Sustainable Food (IPAD), SupAgro, Montpellier, France.
16. Side C.S., Havard M., 2015. Sustainably develop mechanization to improve the productivity of family farming in Sub-Saharan Africa. Int. J. Adv. Stud. Res. Africa. 6(1&2) : 34-43. Available from: http://www.ijasra.org/
17. Sims B., Hilmi M., Kienzle J., 2016. Agricultural mechanization: A key input for sub-saharan Africa smallholders. FAO, Integrated Crop Management, vol. 23. http://www.fao.org/documents/card/en/c/51868c45-4ed7-4733-94ae-751b2e5589f7/.
18. Tersiguel P., 1995. The tractor's bet: The modernization of cotton agriculture in Burkina Faso. Editions de 1'Orstom, Paris, 1995.
19. UEMOA, 2010. WAEMU Cotton, West African White Gold conquering the World Market Sba-Ecosys-Cedres, 2011.

Economic analysis of the cotton sector. Links between poverty and the environment. Final report. Ministry of Environment and Living Environment (MECV), Poverty Environment Initiative (IPE) project, Ouagadougou, Burkina Faso.

20. Schwartz, 2007. Brief history of cotton cultivation in Burkina Faso. ORSTOM. Bondy, France, 28 p.

Development of Reference Materials for Calibration of Micro-Ginning Machines to Monitor the Performance of Industrial Ginning Plants

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Abstract

It is utmost important to regularly monitor the performance of industrial ginning plants in order to avoid any quality loss arising due to improper settings or wear and tear of any machinery. For this purpose, mostly, fibre quality results obtained from the ginning plants are compared with that of the micro-gins. However, there is lack of any reference materials for calibration of micro-gins. Hence, the end users are unsure whether fibre quality results obtained from micro-gins are reliable. Hence, there is need to develop standard reference materials (seed cottons) for calibration of micro-ginning machines on lines of reference materials for calibration of fibre testing equipment such as HVI. Reference material (seed-cotton) with known fiber characteristics may serve as tool for verifying the performance of the micro gins. In this work, attempts have been made to develop reference material for calibration of the micro gins employed in ginneries for monitoring the performance of industrial plants.

Keywords: *Ginning, Material standard, calibration, HVI, Mali*

Background

In sub-Saharan countries, it is normal practice to compare the fibre quality results of the micro-gins to that of industrial plants for its diagnostic. This micro-gin has neither seed-cotton cleaner nor lint-cleaner (Boykin et al., 2010). Assuming that the micro-gin is properly maintained and set, one may consider that it is constantly preserving fiber quality at its best during the ginning of any seed-cotton (ICAC, 2001). Once the micro-gins are calibrated properly, it may be considered as reference tool for verifying the performance of the industrial ginning plants.

However, the only existing method to check if the micro-gin could remain a reference is to periodically check that no part has been damaged during the last ginning experiments. But some damages, such as saw or grid wearing, build up slowly along time and may not be detected by maintenance technicians. This may induce drifting performances of the micro-gin along time. Therefore, the reference micro-gin slowly would become unreliable and would no longer stay a proper reference to make a valid comparison with the industrial ginning plants performances, resulting into improper diagnostic of the ginning plants.

We propose to develop the specific and homogenized Seed-Cotton as Reference Materials (SCRef) that serve the purpose of calibration materials for checking the micro-gin performance on the long run. This requires the selection of seed-cotton in large quantities, its homogenization, and its storage for several ginning crops (Anthony & Mayfield, 1994). During the first micro-ginning experiments of SCRef, several fiber samples would periodically be taken and characterized for their fiber properties: the mean values and the level of homogeneity of each SCRef would be established based on these first fiber characterization results (Anthony, 1982).

Then, processing one SCRef sample in each successive micro-gin experiments on other industrial ginning mills samples and measuring all fiber characteristics from the corresponding fiber samples would allow the tracing of the micro-gin performance itself along time in comparison to SCRef established values. In these experiments, the periodical check of SCRef data based on any fiber characterization result would allow the detection of any result outside the confidence intervals of the SCRef established values and would then indicate a drift or a setting problem of the micro-gin itself. This assumption would be then extended to the other experiment seed-cotton samples, among which the ones from the industrial ginning mills in order to produce a better diagnostic.

Therefore, we propose that any well homogenized seed-cotton material, available in large quantities, could be considered as SCRef. However, caution is to be taken in its selection by choosing a uniform cotton field and/or by applying a gentle homogenization process, even though it is known that any manipulation leads to a degradation(Falconer, Bash, Parker, & Parker, 2000). Therefore, the seed-cotton homogenization process should stop as soon as the beneficial improvement in homogeneity of the seed-cotton is lower than the observed degradation of the fibers (ITC, 2008).

The purpose of this research is to check if it is possible to establish reference values for large quantities of homogeneous SCRef materials based on fiber characterization results obtained by the testing of any fiber sample taken during the micro-ginning of any subset mass of these SCRef.

Results and Discussion

Results on HVICC testing controls at the laboratory level: Characterization results obtained for the LS and SW controls showed that there was no drift and no deviation from their established reference values for any of the measured characteristics. In addition, no critical nor significant difference was highlighted between the two replicates of measurements. Therefore, laboratory results being reliable and stable for these HVICC controls, we considered it possible to establish reference values for SCRef1 and SCRef2 and check their stability along time as given hereafter.

Comparison between all measurement results obtained on ginning sets H1 and H2 ginned one year apart: Table 1 and Table 2 represent the mean and standard deviation values of the measurements of ginning sets H1 and H2, respectively. The fibre quality results given in Table 1 and Table 2 represent 60 characterizations (3 bags x 10 samples/bag x 2 characterization replicates/sample) and 80 characterizations, (with 4 bags instead of 3) for H1 and H2, respectively.

Table 1: Characterization results on subsets H1 and H2 of SCRef1 ginned three months apart.

Material	Criteria/unit	IM index	UHML mm	UI %	Str cN/tex	Rd %	+b
H1	Mean	4.4	28.6	81.9	28.5	74.5	10.5
	Standard deviation	0.08	0.93	1.73	1.60	1.19	0.34
H2	Mean	4.5	28.6	81.7	28.4	75.0	11.0
	Standard deviation	0.09	0.72	1.19	1.39	1.01	0.29

Table 2: Characterization results on subsets H1 and H2 of SCRef2 ginned three months apart.

Material	Criteria/unit	IM index	UHML mm	UI %	Str cN/tex	Rd %	+b
H1	Mean	4.0	28.5	81.7	28.9	75.9	11.9
	Standard deviation	0.09	0.84	1.40	1.16	0.91	0.47
H2	Mean	4.1	28.6	81.7	29.5	75.2	12.5
	Standard deviation	0.09	0.74	1.10	1.28	1.04	0.40

On both cottons all characteristics but the color and brightness remain virtually unchanged after the micro-gin has been used extensively during all the campaign.

Detailed evaluation of UHML and UI measurement results obtained on ginning sets H1 and H2 Comparison between ginning sets" Figure 1 and Figure 2 indicate that no difference was observed between ginning sets (H1, H2) for both SCRef1 and SCRef2 for UHML and UI.

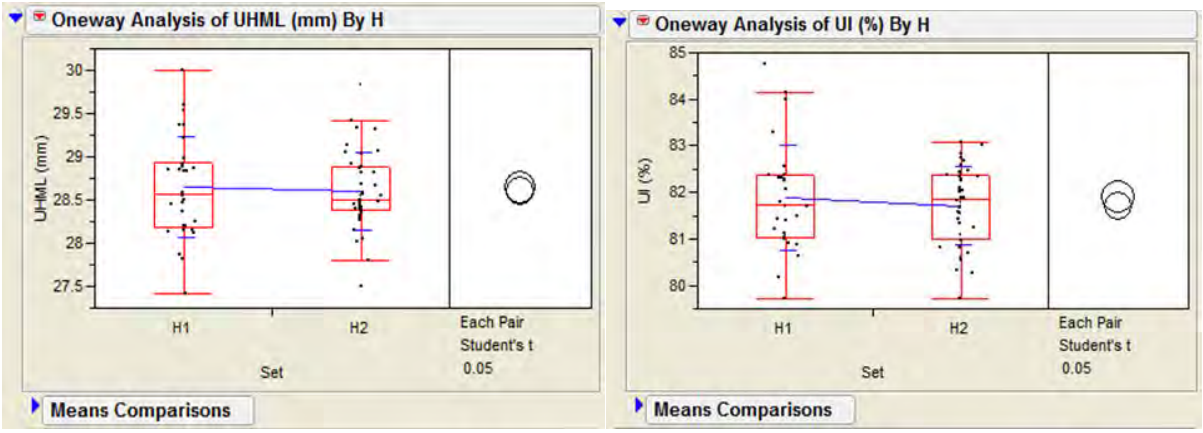


Figure 1: Distributions of UHML and UI of cotton SCRef1 in the two ginning tests H1 and H2

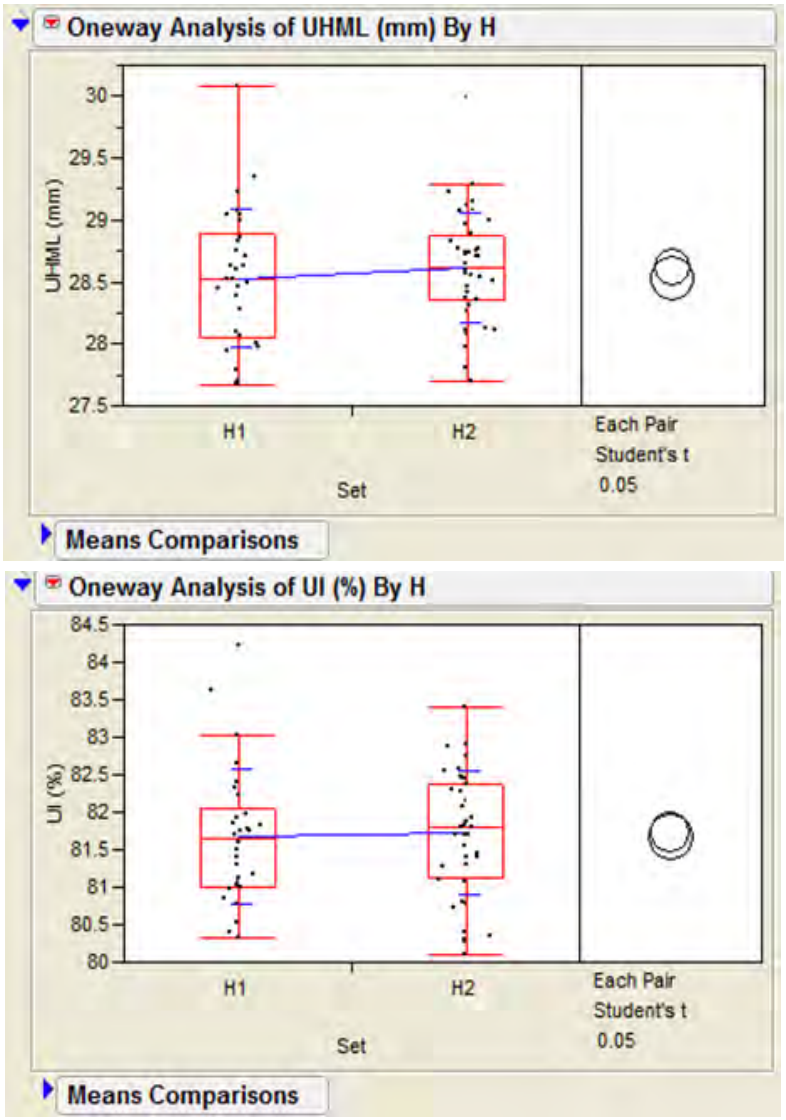
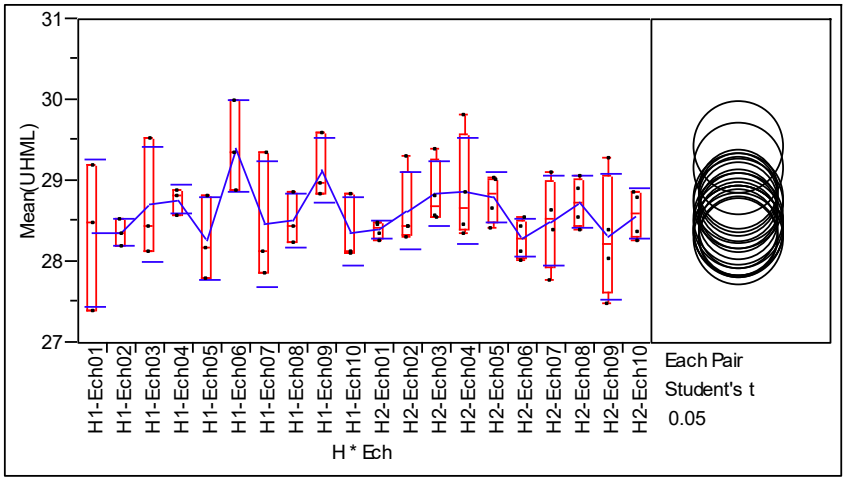


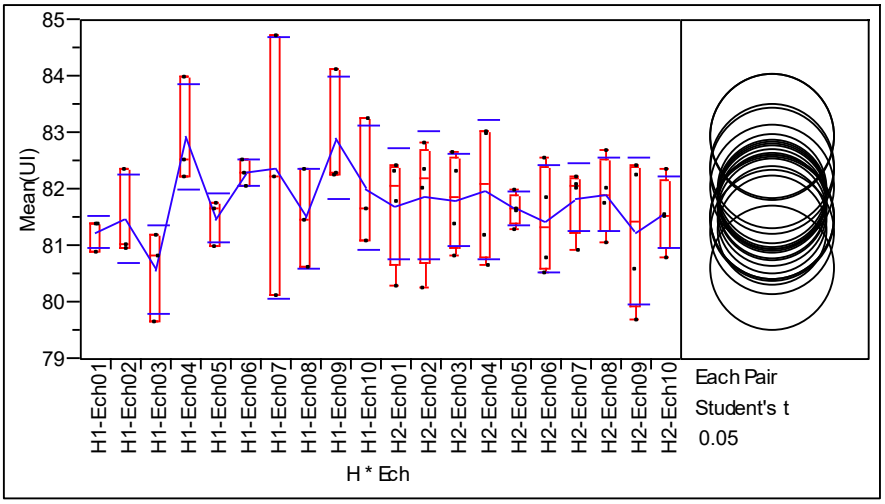
Figure 2: Distributions of UHML and UI of cotton SCRef2 in the two ginning tests H1 and H2

Note: Each point corresponds to the fiber testing mean result for one sample of one SCRef bag

Comparison between samples within each ginning set

For checking the stability of results along ginning duration, Figure 3 and Figure 4 display quartiles, standard deviations and mean comparisons using the combination of set (H1 or H2) and sample name (from 01 to 10) for each SCRef respectively and for UHML and UI.





Notes: results in the charts are ranked in the order of sample production at the micro-gin from left to right (each point corresponds to the fiber testing mean result for one SCRef bag; the blue broken line joints the means, the blue horizontal segments are one standard deviation from the mean, and the red boxes ends are quartiles)

Figure 3 : Comparison of UHML and UI for samples for all set by sample name combinations (SCRef1)

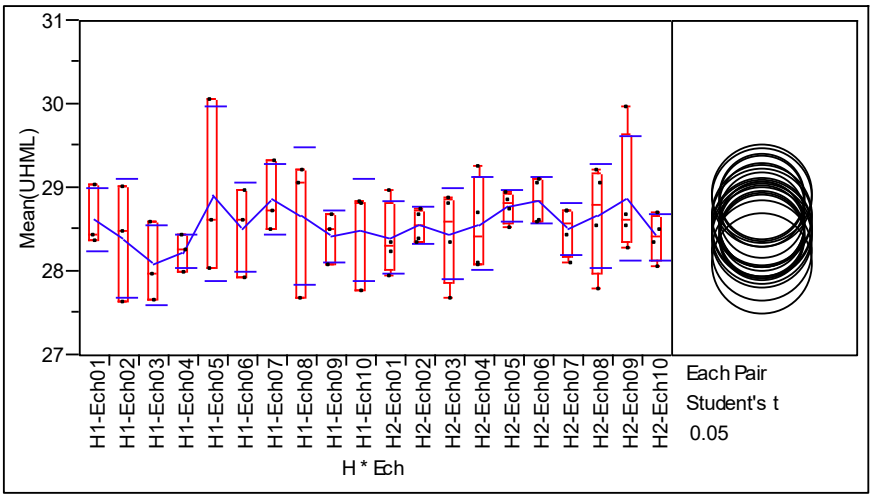
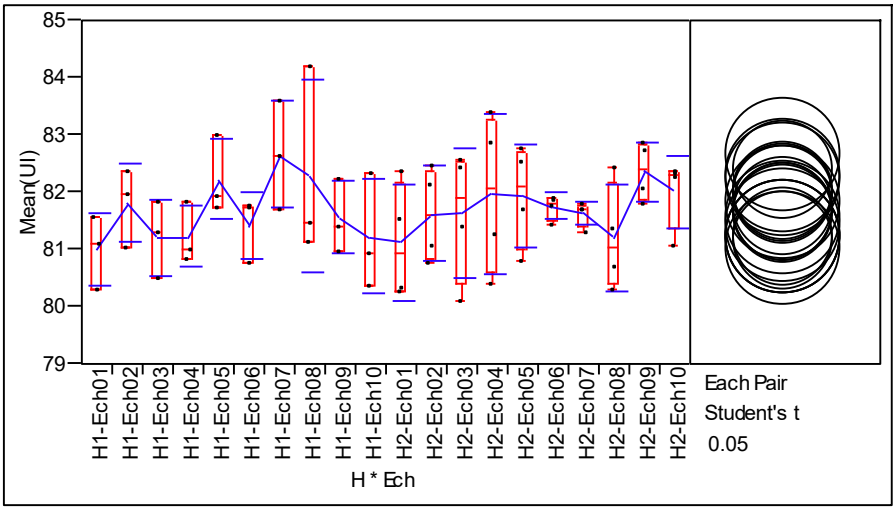


Figure 4 : Comparison of UHML and UI for set by sample name combinations(SCRef 2)

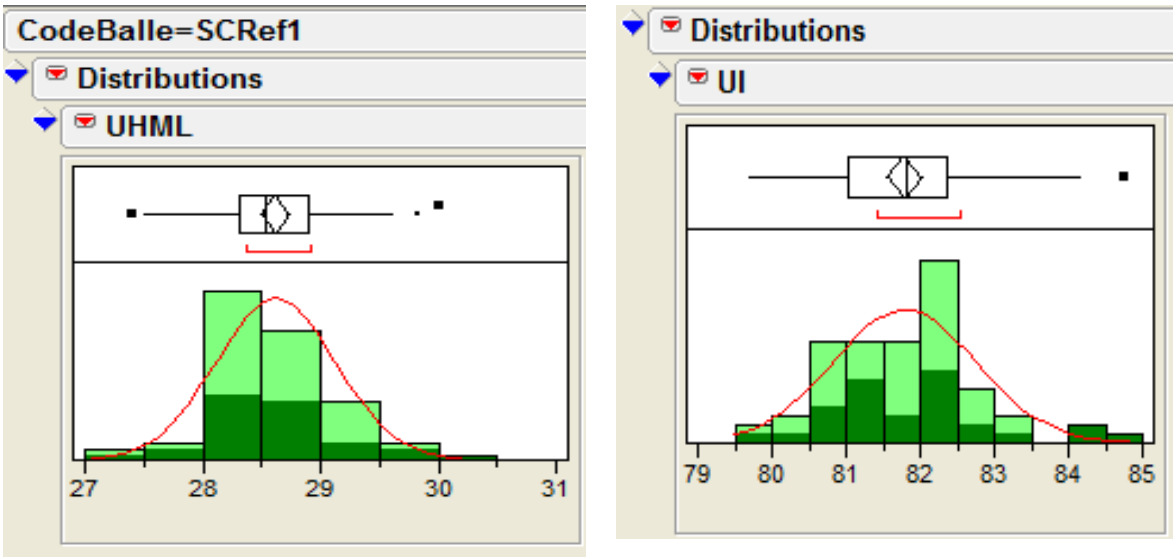


Notes: results in the charts are ranked in the order of sample production at the micro-gin from left to right (each point corresponds to the fiber testing mean result for one SCRef bag; the blue broken line joints the means, the blue horizontal segments are one standard deviation from the mean, and the red boxes ends are quartiles)

These figures show that some variation may appear at some moments. These variations were attributed to a potential variability in the SCRef corresponding fiber sample characteristics, and not to any ginning or machine effect. We observe that there was no trend in the results for the most sensitive fibers characteristics to bad ginning practices appearing over a period of one month of intense ginning for our (un-shown here) experiments. This assures that no fatigue or bad setting appeared at any time of our experiments.

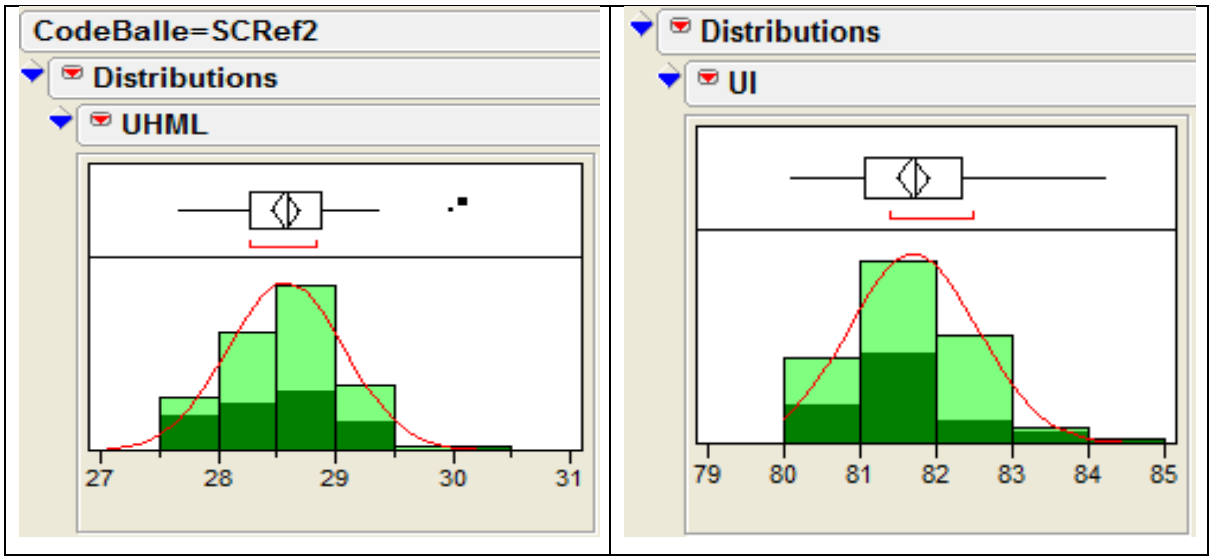
Another way to compare the distributions of observed results on both sets of ginning is using stacked histograms in Figure 5 for UHML and Figure 6 for UI. The dark shaded color represents the distribution of results obtained during the first ginning set H1, the brighter color during the second set of ginning H2. It can be seen that data was consistent between sets of ginning and that accumulating results ease the process of matching the Normal-Gaussian distribution; this gives more precision to reference data to be considered before alerting on any ginning problem in future experiments.

Figure 5: Distributions of UHML (mm) and UI (%) of SCRef1



Notes: ; darker color represents the data from first ginning set H1, the brighter color represents the data from second ginning set H2) in the overall distribution

Figure 6: Distributions of UHML (mm) and UI (%) of SCRef2



Notes: ; darker color represents the data from first ginning set H1, the brighter color represents the data from second ginning set H2) in the overall distribution

Reference data for our future experiments

Based on fibre quality results obtained in this work as indicated in Table 3, it may be considered that the homogenized seed cotton material may serve as reference material for calibration of the micro-gins.

Table 3: Results from SITC fiber characterization for SCRef 1 and SCRef2, based on all available results, and serving as reference data for future experiments.

Material	Criteria/unit	IM index	UHML mm	UI %	Str cN/tex	Rd %	+b
SCRef1	Mean	4.52	28.62	81.75	28.40	74.86	10.87
	Standard deviation	0.09	0.78	1.36	1.45	1.08	0.37
SCRef2	Mean	4.04	28.59	81.71	29.38	75.38	12.37
	Standard deviation	0.10	0.77	1.19	1.27	1.06	0.52

Standard deviations observed between samples in our experiment (Table 3) were at the same level as the usual standard deviations observed in periodic CSITC international round trials for SITC (Table 4, ICAC-CSITC, n.d.). This is all the more remarkable since more variation factors contribute to standard deviations under our conditions than under those prevailing for the preparation and tests of the CSITC standards (Table 5). Therefore, the conclusion is that the preparation method of SCRef in large quantity seemed feasible for potentially checking the micro-ginning practices along time.

Table 4: Limits used in CSITC international round-trial evaluations.

	IM Index	UHML mm	Uniformity %	Str cN/tex	Rd %	+b
Limits	0.2 unit	0.030 inch or 0.76 mm	2%	2 g/tex	1.5 units	0.5 unit

Table 5: Variance components contributing to the observed standard deviations for Seed-cotton reference (SCRef) and CSITC limits.

Sr. No.	Component	CSITC
1	Seed-cotton heterogeneity	-
2	Seed-cotton sampling effect	-
3	Ginning practices	-
4	Ginning conditions	-
5	Fiber sampling effect	Fiber sampling effect
6	Testing practices	Testing practices
7	Testing conditions	Testing conditions

Discussion and Conclusion

A properly set and maintained micro-gin commonly used as a reference device for checking performances of industrial ginning mills, especially for fiber quality preservation. However, unless checking the mechanical parts of the micro-gin, there is no other way to assure that it is preserving fiber quality at its best, so that it could really serve as a reference. Therefore, we made the assumption that periodically ginning samples of some homogeneous seed-cotton reference materials (SCRef) available in large quantities would serve as long-time control of the micro-gin itself.

In this study it has been proved that SCRef, well homogenized with limited variability level and available in large quantities, whose corresponding fiber characteristics were established, may serve the purpose of monitoring the performance of the micro-gin along time in order to detect any malfunction or any drift. These SCRef can serve as periodic control of the micro-ginning experiments based on SITC fiber characterization results and with the same precision. Therefore, another way of controlling the micro-gin is proposed in addition to checking its parts.

It has been shown that it was possible to create seed-cotton reference materials with known fiber characteristics. However, many factors may potentially affect these established reference data (duration of storage, moisture content, variation in moisture content throughout the stored mass, temperature of the seed cotton, weather factors) during storage time. These may induce differential evolution along time in established cotton fiber characteristics depending on the chosen material. This evolution was not significantly detected in this experiment. However, it has to be verified and periodically taken care of potential ageing of the materials, unless a new H1 type experiment should be performed at each ginning campaign.

Material and Methods

Large masses of seed-cotton were selected for making two seed-cotton reference materials (SCRef1 and SCRef2). For each SCRef, around 1 700 kg seed-cotton was sampled from a specific region in the country (Mali). Each SCRef was produced by a farm having homogeneous field growing conditions, hand picking, and a single cotton variety planted leading to specific fiber characteristics.

Each SCRef was homogenized several times by hand from the farm to its storage place at least at each time the seed-cotton was transferred from one place to the next (at the field, to the trailer, to the primary storage, to the truck). Then, bags of around 20 kg of seed-cotton was filled with many handfuls of materials taken from all over the overall available mass of seed-cotton. In these conditions, 86 bags were prepared once for each SCRef (Figure 7).

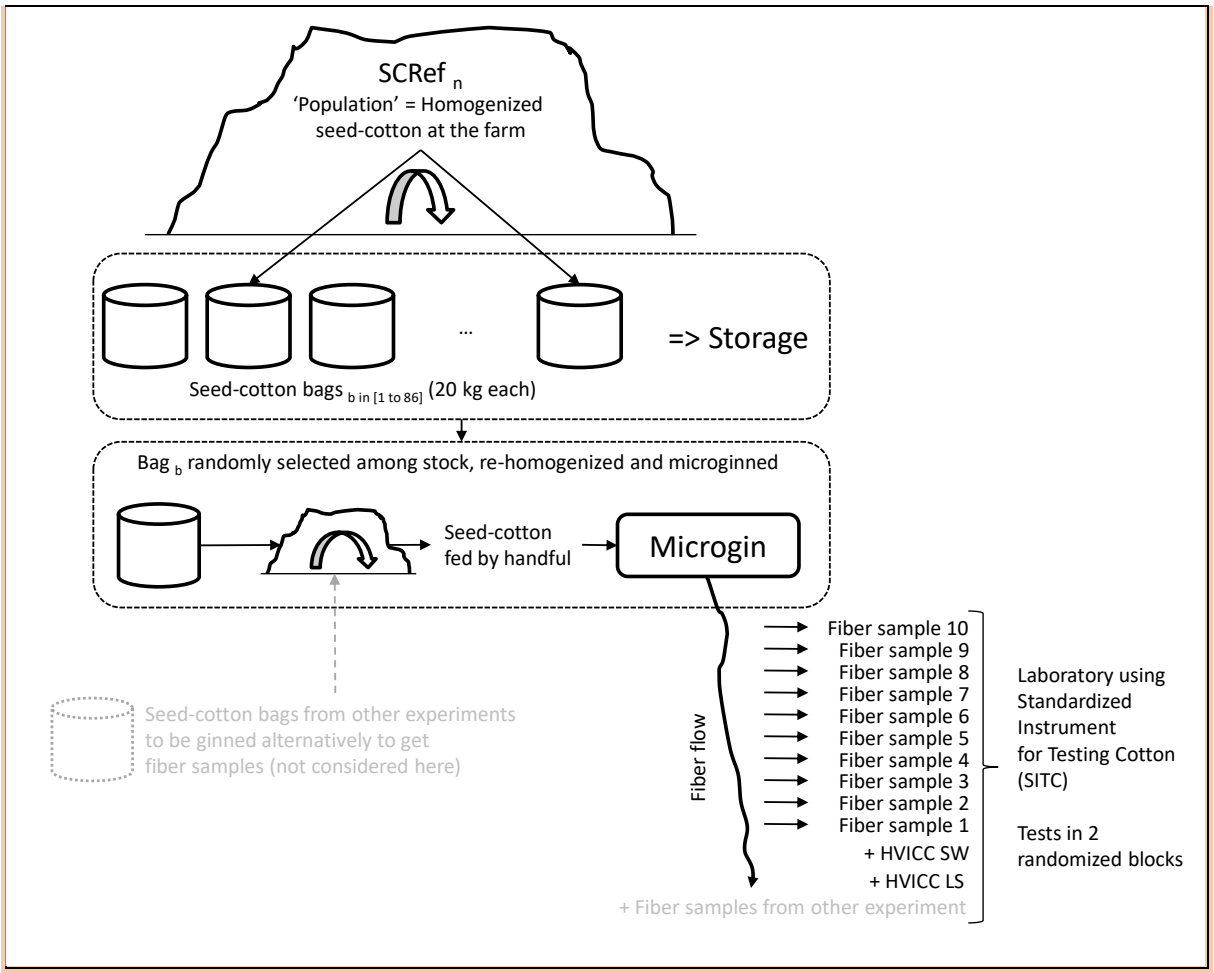


Figure 7: Seed-cotton reference material (SCRef) and fiber flow in this experiment.

SCRef bags were then introduced in-between seed-cotton samples from a bigger experiment (not discussed here and in light grey in Figure 7 in the series for controlling the overall stability of the micro-ginning process along time. Each processed SCRef bag was randomly selected within the 86 prepared bags from each SCRef. Each randomly selected SCRef bag was further homogenized before feeding the micro-gin (Continental 20 saws gin stand) by successive handfuls taken all over from the 20 kg SCRef mass until its exhaustion.

This experiment was performed in two sets of ginning: the first set, named H1, was performed on 6 bags, that is three bags of SCRef1 and three bags of SCRef2, for value setting. The second setH2 was each performed using 7 or 8 bags for each SCRef. This second set was performed after some delay during which many seed-cotton samples from other experiments (not considered here) were processed, leading to potential change in settings or some wearing or degradations of mechanical parts.

While ginning each single selected bag of SCRef, ten cotton fiber samples were periodically taken from the fiber flow and numbered1 to 10 for fiber quality characterization in a laboratory in accordance with the CSITC Guidelines (Drieling, Gourlot, & Knowlton, 2012). Moisture content of seed cotton and lint

were recorded during ginning. The recorded moisture content of both seed cotton and lint did not vary to a point that could affect fiber properties.

Similarly, all samples of the SCRef were characterized under ambient conditions using Uster technologies HVI® 1000/700, which was calibrated as standard operating protocol.

Measured parameters considered in this study are: Upper Half Mean Length (UHML, mm), Uniformity Index (UI, %), Micronaire (IM, index), Strength (Str, cN/tex), Reflectance (Rd, %) and yellowness (+b, no unit). Two replicates of testing were performed on each sample of the whole experiment in a randomized complete block design. Each replicate consisted of one measurement of micronaire and two measurements of UHML, UI, Str, Rd and +b per sample (Aboé, Gourlot, Gozé, Huble, & Sinoimeri, 2012; Lukonge, Aboé, Gozé, Sinoimeri, & Gourlot, 2013).

Statistical analysis was performed using JMP® Graphics from SAS Institute (SAS Institute Inc., n.d.). Tests were performed using a significance level of 0.05.

Acknowledgements and Funding

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References

1. Aboé, M., Gourlot, J.-P., Gozé, E., Huble, P., & Sinoimeri, A. (2012). New findings on within bale repeatability of measurements obtained with standardized instruments for testing cotton (SITC) in Gossypium Hirsutum Fiber produced in West and Central Africa. Textile Research Journal, 82(14), 1469–1484. https://doi.org/10.1177/0040517511424532
2. Anthony, W. S. (1982). Response of Fiber Properties to Gin Machinery and Moisture, During Ginning as Measured by HVI. Cotton Gin and Oil Mill Press, 1982-11–27(24), 16–20.
3. Anthony, W. S., & Mayfield, W. D. (1994). Handbook for cotton ginners.
4. Boykin, J. C., Whitelock, D. P., Armijo, C. B., Buser, M. D., Holt, G. A., Valco, T. D., … Watson, M. D. (2010). Predicting Fiber Quality After Commercial Ginning Based on Fiber Obtained with Laboratory-Scale Gin Stands. The Journal of Cotton Science.
5. Drieling, A., Gourlot, J.-P., & Knowlton, J. (2012). Guideline for Standardized Instrument Testing of Cotton. (A. Drieling, J.-P. Gourlot, & J. Knowlton, Eds.) (Version 1.). ICAC Task Force on Commercial Standardization of Instrument Testing of Cotton and ITMF International Committee on Cotton Testing Methods. Retrieved from http://csitc.org/index.php?lien1=/instrument_testing/public_documents_it
6. Falconer, L. L., Bash, E., Parker, J. L., & Parker, R. D. (2000). Cotton quantity, quality and value: a comparison of hand and mechanical harvest results from a large-scale South Texas test. In Cotton Beltwide Conferences (Vol. 1, pp. 390–392). https://doi.org/10.1017/CBO9781107415324.004
7. ICAC-CSITC. (n.d.). CSITC Global round-trials website. Retrieved from https://csitc.org/index.php?lien1=/rounds_tests/round_trial_results
8. ICAC. (2001). Report of an expert panel on ginning methods. ICAC Working Group on Ginning, (September).
9. ITC. (2008). Quality and Marketing of Cotton Lint in Africa, in Cotton Exporter's Guide.
10. Lukonge, E. E., Aboé, M., Gozé, E., Sinoimeri, A., & Gourlot, J.-P. (2013). Application of a published methodology for evaluating within-bale repeatability of measurements obtained with standardized instruments for testing cotton: Gossypium hirsutum in Eastern and Southern Africa. Textile Research Journal, 84(2), 187–199. https://doi.org/10.1177/0040517513487787
11. SAS Institute Inc. (n.d.). JMP®, Version 5. Cary, NC.

Comparative Performance of Spiked and Saw Type Pre-Cleaners for Trash Removal in Seed Cotton

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Abstract

A study was conducted to assess the comparative performance of spiked cylinder, saw type pre-cleaners and their combinations on trash removal efficiency in seed cotton prior to ginning. Four different treatments namely spiked cylinder, saw type, saw type followed by spiked cylinder and spiked cylinder followed by saw type pre-cleaner were studied to find out the most appropriate pre-cleaning system for Indian ginneries. The trash removal efficiency and effect of different cleaning treatments on various fibre attributes were compared. Seed cotton of three cotton cultivars was used for this experiment. The highest trash removal efficiency was observed in case of spiked cylinder followed by saw type pre-cleaner combination for all three cotton varieties. The test results revealed that spiked cylinder pre-cleaner was least effective. Saw type pre-cleaner was found to be more effective in removing trash content than spiked cylinder pre-cleaner. No significant difference in fibre attributes was observed for all pre-cleaning combinations and cotton cultivars.

Keywords: *Spiked cylinder pre-cleaner, saw type cleaner, trash removal efficiency, fibre quality attributes*

Background

India produces about 27% of world's cotton from about 11 million ha area. Indian cotton contains almost negligible amount of trashes at the time harvesting primarily due to 100% hand picking and well opening of bolls on maturity. However, improper storage, handling, cleaning and processing practices result into significant amount of trashes and contaminants in Indian bales leading to fetching of lower prices in the international market. On contrary, countries like USA and Australia managed to produce cotton bales free from trashes and contaminates from mechanically harvested cotton, though it contains a large amount of trashes at the time of harvesting. These countries employ a series of spiked cylinder and saw-type pre-cleaners that removes most of the leaves, vegetative matters and other foreign matters prior to ginning (Patil and Shah, 1999). Further, any foreign matter present in the lint after ginning is removed in series of lint cleaners. Whereas, in India, it is normal practice to employ one number of spiked cylinder pre-cleaner and lint cleaner in each ginnery for removal of foreign matters. The number and type of pre-cleaners used in Indian ginneries are inadequate to remove all foreign matters.

The spiked cylinder pre-cleaner is mainly useful for removal of fine foreign matters like sand, dust, immature locks, trash particles, etc. (Mayfield et al., 1983) whereas saw type pre-cleaner is considered better for removal of coarse and heavy foreign matter like stems, burrs, hulls, immature locks, stones etc. Spiked cylinder type pre-cleaners employed in Indian ginneries have hardly 20-25% trash removal efficiency (P.G. Patil et al., 2006). There is a misconception in India that saw type pre-cleaners deteriorate the fibre attributes while cleaning. However, the saw type pre-cleaners are considered as essential machinery in developed countries for seed cotton cleaning. The saw type pre-cleaners are considered to be more effective than cylinder pre-cleaners as they comb the seed cotton with the help of saw band tooth during cleaning operation unlike cylinder cleaners where in only centrifugal action is used for removal of foreign matters.

The objective of this study was to evaluate the comparative performance of spiked and saw type pre-cleaners and their combinations for trash removal. The trash removal efficiency and different fibre attributes of control were compared with four sets of pre-cleaning operations viz., spiked cylinder, saw type, saw type followed by spiked cylinder and spiked cylinder followed by saw type pre-cleaners.

Results

The initial trash content present in case of cotton cultivar A was 4.1% (Table 1). The pre cleaning operations with SC cleaner and ST cleaner brought down trash contents to 3.7 % and 3.4%,

respectively, which is reduction of trash content in A cotton cultivar by 9.8 and 17.1 percent, respectively. The combination of SC cleaner followed by ST cleaner resulted in reducing the trash content in cotton to 3.2 percent thereby reducing the trash content by 21.9 percent. The combination of ST cleaner followed by SC cleaner resulted in trash content of 3.3 percent thereby reducing the trash in lint by 19.5 percent.

Table. 1 The effect of different pre-cleaning combinations on trash removal efficiency for three cotton cultivars

S. No.	Cultivars	Treatments	Visible Trash (%)	Std. Dev. (%)	Invisible Loss (%)	Std. Dev. (%)	Total Trash (%)	Trash Removal Efficiency (%)
1		SC	1.3	0.10	2.5	0.20	3.7	9.8
2		ST	1.3	0.15	2.1	0.26	3.4	17.1
3		SC followed by ST	1.4	0.20	1.8	0.18	3.2	21.9
4	A	ST followed by SC	1.6	0.26	1.7	0.16	3.3	19.5
5		Control	1.8	0.15	2.3	0.23	4.1	-
6		SC	1.4	0.16	1.8	0.18	3.4	12.8
7		ST	1.2	0.11	0.9	0.08	3.3	15.4
8		SC followed by ST	1.3	0.18	2.3	0.20	3.1	21.5
9	B	ST followed by SC	1.3	0.09	2.5	0.23	3.2	17.9
10		Control	1.8	0.20	2.1	0.24	3.9	-
11		SC	1.7	0.17	0.6	0.06	3.0	11.8
12		ST	1.9	0.21	1.2	0.12	2.9	14.7
13	C	SC followed by ST	1.7	0.19	0.8	0.07	2.5	26.5
14		ST followed by SC	1.5	0.23	0.7	0.09	2.6	20.5
15		Control	2.1	0.27	1.3	0.10	3.4	-

Table. 2 The effect of different pre-cleaning combinations on fibre attributes for three cotton cultivars

S. No.	Cultivars	Treatments	HVI fibre properties						
			UHML (mm)	UI (%)	MIC (µg/inch)	Strength (g/tex)	SFI (%)	Rd (%)	+b (%)
1		SC	29.1	83	4.3	29.5	7.3	75.9	8.2
2		ST	29.5	82	4.2	29.7	7.4	75.4	8.1
3		SC followed by ST	29.8	84	4.3	29.6	7.5	76.6	8.1
4	A	ST followed by SC	29.5	83	4.3	29.5	7.3	76.4	8.2
5		Control	29.6	83	4.2	29.6	7.1	73.3	9.3
6		SC	29.1	82	4.1	29.8	7.7	75.9	8.3
7		ST	29.3	82	4.0	29.3	7.1	75.6	8.3
8		SC followed by ST	29.0	83	4.1	29.2	7.8	76.3	8.9
9	B	ST followed by SC	29.4	81	4.0	29.6	7.4	77.8	8.9
10		Control	29.0	82	4.0	29.5	7.5	74.1	9.4
11		SC	26.3	81	4.9	25.5	13.2	78.0	9.3
12		ST	26.2	80	4.8	25.7	13.6	76.4	9.4
13		SC followed by ST	26.3	81	4.9	24.6	13.2	79.6	9.9
14	C	ST followed by SC	25.9	82	4.8	25.5	13.8	78.7	9.8
15		Control	25.8	81	4.9	24.6	13.1	77.0	10.1

The initial trash content present in case of B cotton cultivar was 3.9 percent (Table 1). The pre cleaning operations with SC cleaner and ST cleaner brought down trash contents to 3.4 % and 3.3%, respectively thereby reducing the trash content in B cotton cultivar by 12.8 and 15.4 percent, respectively. The combination of SC cleaner followed by ST cleaner resulted in reducing the trash content in cotton to 3.1

percent thereby reducing the trash content by 20.5 percent. The combination of ST cleaner followed by SC cleaner resulted in trash content of 3.2 percent thereby reducing the trash in lint by 17.9 percent.

The initial trash content present in case of C cotton cultivar was 3.4 percent (Table 1). The pre cleaning operations with SC cleaner and ST cleaner brought down trash contents to 3.0 % and 2.9%, respectively thereby reducing the trash content in C cotton cultivar by 11.8 and 14.7 percent, respectively. The combination of SC cleaner followed by ST cleaner resulted in reducing the trash content in cotton to 2.5 percent thereby reducing the trash content by 26.5 percent. The combination of ST cleaner followed by SC cleaner resulted in trash content of 2.6 percent thereby reducing the trash in lint by 20.5 percent.

It may be observed from Table 2 that all averaged fibre parameters including UHML, UI, MIC, Tenacity and SFI values varied within tolerance limits of the instrument for all cleaning treatments. However, the averaged reflectance percentage (Rd%) and yellowness percentage (+b) values grades were improved for all cleaning treatments as compared to the control.

Discussion

The highest percentage of trash removal was observed in SC cleaner followed by ST cleaner combination followed by combination of ST cleaner followed by SC cleaner, ST cleaner and SC cleaner in case of all the three cotton varieties tested. It is mainly due to reason that the lumps or clods of seed cotton, which was fed into spiked cylinder cleaner were loosened and the thickness of cotton layers were reduced. This well-opened and thin layered cotton was fed to saw type pre-cleaner, which removed trashes more effectively by means of saw tooth and brushing action. The test results revealed that ST cleaner followed by SC cylinder cleaner combination was less effective than SC cleaner followed by ST cleaner for cleaning of all three cotton varieties tested. When seed cotton is fed to ST pre-cleaner prior to proper opening in SC, saw cylinders receive cotton in the form of lumps or clods. The saw cylinders are formed by fitting specially designed saw bands on its periphery. The saw bands have teeth for gently combing the thin layered of hardly 2 to 3 mm thickness. If the lump thickness is more than teeth depth (2 to 3 mm), the clods cotton was not broken, hence, cleaning occured only on the outer surface of clods of cotton and trash content present in the inner surface of lumps remained as such in the seed cotton even cleaning.

The test result further revealed that ST cleaner was more effective in removing trash content than SC cleaner for all the three varieties of cotton tested. It was due to reason that ST cleaner removes trash content with combing and brushing action, which leads to removal of trash content not only from cotton outer surface but also from interior surface of cotton. In contrast, the SC cleaner removes the trash content from cotton with scrubbing action over grid bars, which leads to removal of trash content mostly from outer surface of cotton.

The fibre properties evaluation using HVI showed that there is no significant difference in UHML, UI%, MIC, Tenacity and SFI% in all treatments as the readings were observed to be within the limits for margin of instruments error (Table 2). Therefore, it may be inferred from this study that SC cleaner and ST cleaner do not injure the fibre during its cleaning operations. However, there were slight improvements noticed in the reflectance and yellowness grades in all cleaning treatments. It was due to reason that some trash content (non- lint material) was removed in each cleaning treatment, which leads to improvement in the values of reflectance grades and yellowness grades. Past studies (Thompson, 1990; Anthony, 1994; Patil P.G. et al., 2006) have also reported the same findings.

Conclusion

The combination of spiked cylinder cleaner followed by saw band pre-cleaner was found to be the best with highest trash removal efficiency of 26.5%. The combination of saw band pre-cleaner followed by spiked cylinder cleaner was the second best with trash removal efficiency of 21.9%.

The saw type and spiked cylinder pre-cleaners were less effective with trash removal efficiencies of 17.1 and 12.9%, respectively. The saw band pre-cleaner was more effective in removing trashes than spiked cylinder pre-cleaner for all the three varieties of cotton tested. The fibre properties evaluation showed that there is no significant difference for all three cotton cultivars tested as the readings were observed to be within the limits for margin of instruments error. There were slight improvements noticed in the reflectance and yellowness grades in all cleaning treatments.

Materials and Methods

In this work, a (six cylinder) spiked cylinder (Fig. 1) and a two stage saw type pre-cleaners (Fig. 2) were utilized for all cleaning treatments. The rotating speed and diameter of the spiked cylinders were 250

rpm and 76 mm, respectively. The power requirement, capacity and width of this pre-cleaner were 5.0 hp, 2.5-3.0 tonnes/h and 1400 mm, respectively. There were 17 spikes per row per cylinder fitted in total four rows over each spiked cylinder. The diameter and height of spikes were 12.5 and 140 mm, respectively. A 0.5 geared motor was used to drive feed rolls of the spiked cylinder pre-cleaner. The grid bar comprised of 17 numbers of rods of 5.4 mm diameter each fitted at an angular spacing of 5^0.

The power requirement and capacity and width of saw type pre-cleaner were 5 HP, 2.0 tonnes/h, 1650 mm respectively. The diameter and operating speed of the saw cylinders were 760 mm and 140 rpm, respectively whereas the diameter and operating speed of reclaimer cylinder were 300 mm and 35 rpm, respectively. The operating speeds of the kicker conveyor and doffing brush were 35 and 300 rpm, respectively.

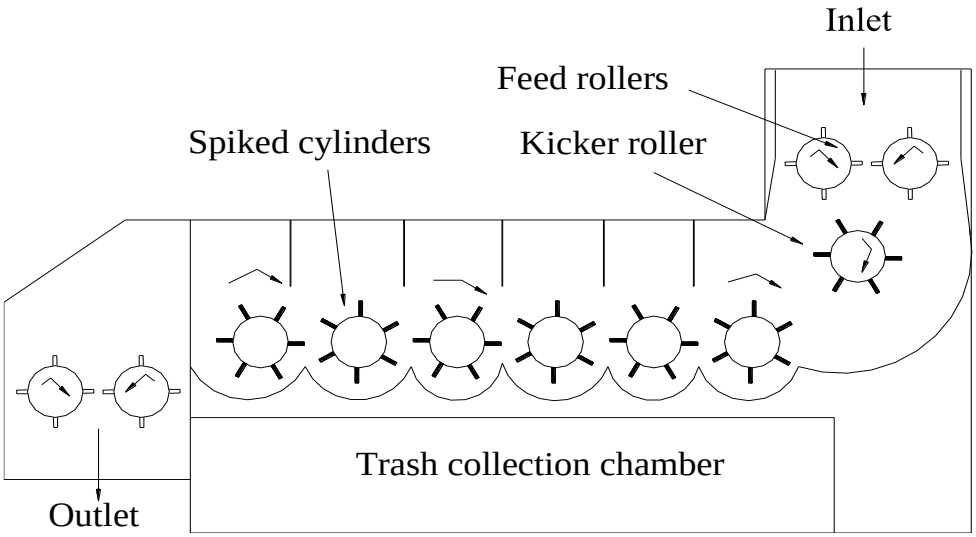

Fig. 1: Spiked cylinder type horizontal pre-cleaner

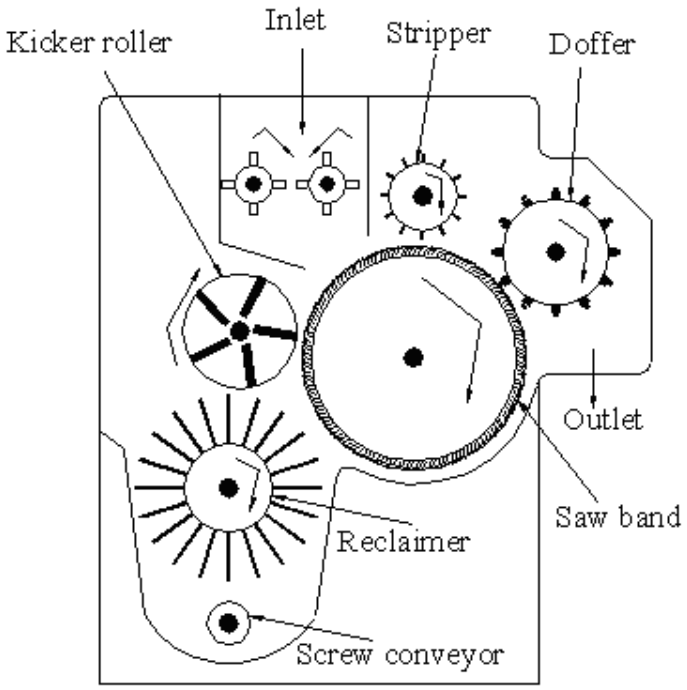

Fig. 2 Internal view of a single stage saw band pre-cleaner

Three different cotton cultivars named as A, B and C were used to study the comparative performance of different combination of pre-cleaners. Seed cotton was maintained at 8% initial moisture content prior to pre-cleaning. There were 12 treatments (four cleaning treatments by three cultivars) replicated three

times each for total 36 lots. One twenty-five kg cotton was processed in each replication. The cleaning treatments included spiked cylinder (SC), saw type (ST), saw type followed by spiked cylinder and spiked cylinder followed by saw type pre-cleaners. The cleaned and the control seed cotton were ginned using a Double Roller (DR) gin. The trash content and fibre properties of the ginned cotton were determined using MAG-SITRA trash analyzer and Uster High Volume Instrument (HVI-900), respectively.

References

1. Anthony WS. 1994. The effect of gin machinery on measurement of high-volume instrument color and trash of cotton. Trans. ASAE 37(1):373–380.
2. Baker RV; Anthony WS; Sutton RM. 1994. Cotton Ginners Handbook. ARS, Agricultural Handbook No. 503. U.S. Govt. Print Office, Washington, DC. 69-90.
3. ITMF. 2007. Cotton Contamination Surveys 1999-2001-2003-2005. International Textile Manufacturers Federation, Am Schanzengraben 29, CH-8002 Zürich.
4. Mayfield W; Baker RV; Childers R E. 1983. Ginning cotton to preserve quality. US Department of Agriculture, Agricultural Handbook. 503.
5. Patil NB; Shah SR. 1999. Ginning and processing with special reference to problem of cotton contamination. Hand book of cotton in India. ISCI , 292-302.
6. Patil PG; Arude VG; Anap GR. 2006. Design and development of cylinder type cotton pre-cleaner. Agricultural Mechanization in Asia, Africa and Latin America (AMA), 37(3), 46-51.
7. Santhanam V; Sundaram V.. 1999 Harvest and Post–harvest measures to reduce contamination in cotton. Hand book of cotton in India. ISCI, 283-289.
8. Shukla SK; Manojkumar TS. 2004. Performance evaluation of spiked cylinder and saw band cylinder pre-cleaners using trashy seed cotton. Journal of the Indian Society for Cotton Improvement (ISCI), 29(3), 170-174.
9. Thompson JA. 1990. Effects of non lint material on HVI colour measurements. ASAE paper No. 90-1031. St. Joseph, Mich.:ASAE.

Comparative Study of Cotton Fibre and Yarn Quality Ginned on Both Reciprocating and New Rotary Roller Gin-Stand

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Abstract

Egyptian Government has a policy objective to restore Egypt's position as the world's leading or among the world's leading producers of fine has demonstrated that an ambitious program of the development of fine cotton output is a realistic objective. The Cotton and textile industries Holding Company "HC" has an important part to play in achieving the policy objective. First, it is the Holding Company's responsibility to provide the sector with ginning capacities that separate lint from the annual seed cotton output to the highest quality standards. Second, the HC has a role also to play in contributing with other responsible government agencies in the policy programs to promote cotton agriculture and the enabling mechanisms to assist and incentivize farmers to grow more cotton. The current research paper was carried out at New Fayyoum Gin-plant "Rotary knife, Bajaj Continental" and Old Fayyoum Gin-plant "Single roller Gin Stand in both Giza 95 cotton varieties grown in Upper Egypt and Giza 94 cotton variety grown in Delta Egypt. Fibre quality, seed coat fragments, contaminations, and the number of neps were compared with both ginning types.

Therefore, it is important to conclude that the rotary gin-stand reduces the trash content, impurities, and short fibres in cotton lint and consequently, the waste percentage in blowroom . High productivity, which resulted in maintenance of the fibre quality despite an increase in the number of neps. Hence, it is preferable to redraw the policy of ginning in Egypt to develop the ginneries sector with rotary gins equipped with seed-cotton and lint cleaners.

Keywords: Ginning technology, reciprocate gin, Rotary gin, fibre, and yarn quality.

Background

Almost all Egyptian cotton is extra fine cotton classified as Long-Staple or Extra Long Staple (ELS). The government intends to implement policies that will restore Egypt's position among the world's leading producers of LS and ELS. The country's installed ginning capacity to process the cotton crop that is aged and technically obsolete does not permit the processing of raw cotton to high quality and efficiency standards. In parallel with agricultural policies to promote regeneration of cotton production, a pre-condition of restoring Egypt's leadership position in fine cotton will be therefore the installation of new ginning and processing plants to replace the current inadequate facilities. The Holding Company's ginning subsidiaries are involved also in cotton exporting and domestic cotton trading activities. However, this ginning technical diagnostic concentrates on the physical processing of the cotton crop. It addresses the status of the Holding Company's ginning facilities against criteria for quality and efficient processing and indicates the configuration of facilities and the ginning technologies and capacities required to fulfill the objective of restoring Egypt's position as a leading fine cotton producer.

There are 25 ginneries in the three subsidiaries. There has been no capital replacement program for 40 years and the ginning park is antiquated between 40- and 140-year-old. All the ginneries are using the basic same model roller gins that are technically obsolete and with yield and production rates a fraction of modern standards, high energy consumption, and critically important low ginning performance. The ginned lint contains contaminants, residual trash, and the ginning process damages the lint quality. The ginneries have no mechanical handling of cotton to the gins, no pre-cleaning or post-ginning cleaning, there is no moisture measurement or control and the ginneries do not have laboratories for fibre testing. Human contact with seed cotton and lint due to manual handling increase the risk of contamination.

Most of the ginneries work one or two shifts per day and starting and stopping a gin requires the use of more electric power consumption. McCarthy roller gin gained worldwide acceptance during the late 18th century and early 19th century and continued to be in extensive use in many countries among them Egypt. Its ginning capacity, however, remained relatively low, about 30Kg of lint per hour. The rotary knife-gins have virtually eliminated the use of McCarthy gins in the USA. In Egypt, The Cotton and Textile Industries Holding Company installed

new four ginning mills equipped with Bajaj Continental machine and complete fibre laboratory fitted with HVI instrument. Due to the importance of cotton ginning in Egypt, the Cotton and Textile Industries Holding Company, in its effort to continuously improve the Egyptian cotton competitiveness in world markets, will install a new three rotary gin plants to cover all areas of cotton production, as a start point for the development of the textile sector in Egypt. Further work is necessary to better define the quality effect of ginning type “conventional reciprocating and rotary-knife gin-stand” on lint out-turn, fibre properties of two Egyptian cotton varieties. Sharma (2008) stated that the sole purpose of all ginning technology developments is to obtain optimum fibre parameters at the lowest cost.

The main objectives of the development in cotton ginning technologies are (to obtain the maximum length of fibre on seed without breakage, to preserve inherent qualities of fibre, to obtain undamaged clean seed, to obtain lint-free of trash and contaminants, and with the lowest cost per unit of ginning. Therefore, cotton ginning technology should be such which is adjustable for different varieties of cotton and compatible with various practices, such as machine picking, manual picking, etc. Moreover, volumes of cotton available at different places vary; hence the technologies should have capabilities to provide optimum output at the lowest cost for different needs of higher or lower volumes available. Van der Sluijs (2015) compared the impact of saw and roller ginning on Long Staple Upland cotton variety.

There was a significant difference between the two ginning methods in some of the average fibre results, with the roller ginned fibre longer and more uniform with fewer short fibre and fibrous neps, as well as stronger with higher elongation with slight but significantly smaller seed coat, nep, and total trash size. By the roller gin increasing the lint out- turn except for bundle strength and increasing the micronaire values. Delhom et al.(2017), studied new Upland cultivars processed by both saw and roller ginning. Four diverse Upland cultivars were processed by saw ginning and high-speed roller ginning and analyzed by ginning method. Results overall showed that the roller gin, when compared to the saw/ gin, produced staple length longer, higher length uniformity, and less short fibre, and contained 25% fewer neps. The overall objective of the research reported here was intended to determine the practical benefits that Rotary roller ginning Egyptian cotton delivers to a textile mill. Specific objectives were: (1) to compare the differences in fibre quality due to Rotary “high-speed roller ginning or Macarthy reciprocating ginning using two cultivars of Egyptian cotton; (2) to highlight the textile processing differences of Rotary and conventional roller-ginned cotton for both carded and compact ring-spun yarn production.

Results and Discussion

Lint out-turn and gin-stand capacity and lint grade

The two methods of ginning were exerted a significant difference at 0.05 probability levels on the lint out-turn. The reciprocating ginned lint of Giza 94 showed a significant increase in lint out¬turn of 2.32% more than rotary ginned lint (38.73% and 36.41% respectively). The reciprocating ginned lint of Giza 95 showed a significant increase in lint out-turn of 2.25% more than rotary ginned lint (39.38% and 37.13% respectively). The relatively higher value of lint out-turn for reciprocating ginned cotton compared with rotary ginned could be explained by the fact that roller ginned lint has extra materials composed primarily of foreign matter, non-lint content, aborted seed motes, and short fibres.

The decrease in lint out-turn due to cleaning machinery in the rotary gin plant system could be probably, ascribed to the removal of stones, trashes, unopened immature seed cotton locks during seed-cotton cleaning, in addition to the removal of a proportion of immature fibres during lint cleaning. Regarding gin-stand capacity, both ginning methods significantly affected the gin-stand capacity at a 0.05 probability level. The capacity of the McCarthy roller gin is almost 31 Kg/h, while the capacity of the rotary knife is 400 kg/h.

The composite Giza 95 and Giza 94 seed-cotton, regardless of ginning treatment, had a similar grade namely “Good”, but in the case of the lint cotton ginned on reciprocating knife gin-stand, the grade was Good +3/16 for each variety. While, in the case of lint ginned on rotary gin¬stand, lint cotton was Good+7/16 for each of varieties, according to Egyptian cotton classification. The seed-cotton and lint cleaners improve the lint grade due to the extraction of a lot of trashes and impurities. Regarding machines’ sequence of rotary gin-stand, the seed¬cotton and lint cleaners were the most important machines in ginnery in relationship to maintaining and improving fibre quality and accordingly, lint grade. This finding is agreed with El-Sayed et. Al. 2008 and Gordon et.al. 2011, who reported that processing lint through lint cleaner decreases the amount of trash grade and improved the color and grade index.

It could be fairly stated that in seed-cotton and lint cleaning in rotary knife ginning plant in Fayyoum, the removal of contamination, impurities, is usually accompanied by shortening of the length distribution. In practice, the trash content of lint cotton is a major concern for, mainly, spinning mills. Therefore, it is important to conclude that the rotary gin-stand reduce the Contamination, trash content, impurities, and short fibres, high productivity, which maintains the fibre and yarn quality despite reducing the ginning out-turn, meaning that it is important to redraw the policy of ginning in Egypt to development the ginneries.

Raw fibre properties and lint grade

The HVI Results were summarized in Table 1. Quality attributes measured by the HVI were superior in fibre length parameters for the rotary ginning than for the roller ginning treatment. Generally, the effects of ginning treatment on fibre length, uniformity index, and short fibre index measurements were statistically insignificant at a 0.05% level of probability. Although insignificant between both ginning types, the rotary ginning recorded a high level of quality in all fibre properties compared with single roller reciprocating ginning. For Giza 94, the Upper half mean length recorded 33.40 and 33.51 mm, uniformity index averaged 85.69% and 86.77%, short fibre Index averaged 6.7 and 6.3%, trash content averaged 23 and 47, trash area averaged 0.36% and 0.59%, nep content averaged 165 and 101 on the Rotary gin and reciprocating knife gin, respectively.

Fibre strength, elongation, Micronaire, and maturity ratio were insignificantly affected regarding ginning treatment. For Giza 95, the Upper half mean length recorded 30.69 and 28.7 mm, uniformity index averaged 85.1% and 83.5%, short fibre Index averaged 6.6 and 8.3%, Micronaire reading averaged 4.45 and 4.29 trash content averaged 31 and 59, trash area averaged 0.24% and 0.59%, nep content averaged 79 and 93 on the Rotary gin and reciprocating knife gin respectively. Fibre strength and elongation were insignificant regardless of ginning treatment, the rotary gin recorded 37.6 g/tex while the McCarthy roller gin recorded 35.34g/tex.

The presence of trash and its subsequent removal during the ginning process affects color characters due to getting rid of a considerable proportion of foreign matter mixed with fibres that led to decreased non-lint content and improving the grade and the fibre brightness. While foreign matter remaining mixed with the seed cotton, as in the case of reciprocating ginned lint, resulted in decreasing the fibre brightness. Rotary ginned cotton receive more aggressive cleaning through the seed cotton cleaner and lint cleaner, which reduces nonlint content and can improve color appearance through combing and aligning of the fibres but also can cause entanglements of fibres, known as neps, to form more readily than in roller ginning and its associated lint cleaning (Tables 1 and 2).

The Rotary-ginned cotton had an average of 25 additional neps per gram than the Reciprocate roller-ginned cotton (Table 2). Fibrous neps can cause appearance issues in yarns and fabrics and must be reduced substantially during the carding process or the mill risks quality problems in downstream processing. It is advantageous for a textile mill to begin processing with cotton that contains fewer neps as it reduces the need for the mill to remove material during processing.

Yarn Quality

Spinning mills always focus on realization (output versus input) and, therefore, many spinning mills install elaborate systems to capture and accurately record waste figures from the various processes. There was a significant difference in the total waste (blowroom to carding) extracted from the two ginning methods, with the total trash extracted from the Rotary ginned fibre almost 3% less than that extracted from the reciprocate roller ginned fibre.

The reciprocate roller ginned cotton's increased waste was extracted during the blowroom (3.6%). For average Giza 94 yarn results, spinning end breakage varied from 26 to 18 end/1000spindle/h for Ne 40 in both reciprocate and Rotary gin, respectively. yarn strength ranged from 24.33 to 25.86, (C.V%) ranged from 11.45% to 12.11%, in terms of imperfections; thin places ranged from 15 to 12 per 1000 meters, thick places ranged from 43 to 35 per 1000 meters and neps ranged from 132 to 70 per 1000 meters, while the yarn hairiness index recorded 4.4 for Ne 40 in both reciprocate and Rotary gin, respectively.

For average Giza 95 yarn results, spinning end breakage varied from 35 to 23 end/1000spindle/h for Ne 40 in both reciprocate and Rotary gin, respectively. yarn strength ranged from 17.65 to 19.14, (C.V%) ranged from 14.32% to 13.23%, in terms of imperfections; thin places ranged from 32 to 28 per 1000 meters, thick places ranged from 55 to 48 per 1000 meters and neps ranged from 100 to 65 per 1000 meters, while the yarn hairiness index recorded 4.4 for Ne 40 in both reciprocate and Rotary gin, respectively. The average yarn results for the two ginning methods are listed in Tables 3 and 4 and illustrated in figures1 and 2.

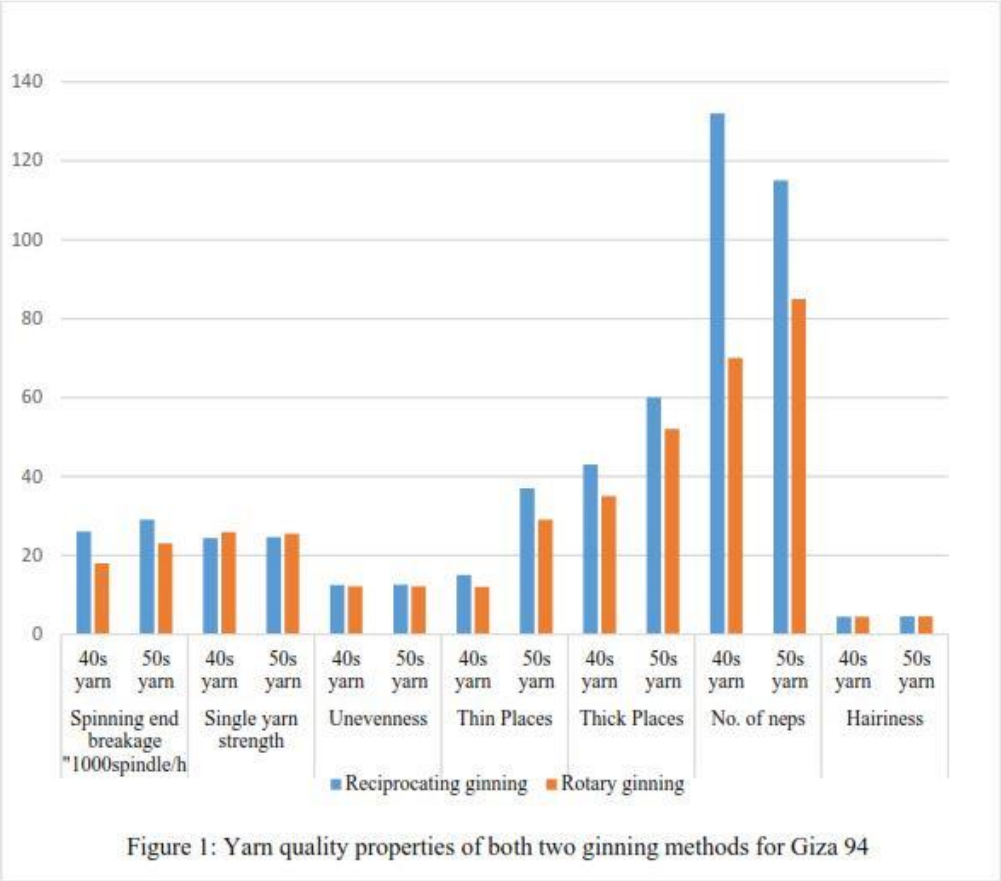

Figure 1: Yarn quality properties of both two ginning methods for Giza 94

Fig 1: Yarn quality properties of both two ginning methods for Giza 94

Table 3. Yarn quality properties of both two ginning methods for Giza 94

	Count	Reciprocating ginning	Rotary ginning	L.S.D at 0.05%
Blow-room and card waste		7.80 %	4.20 %	1.08
Spinning end breakage "1000 spindle/h"	40s yarn	26	18	N.S
	50s yarn	29	23	N.S
Single yarn strength cN/Tex	40s yarn	24.33	25.86	N.S
	50s yarn	24.57	25.44	N.S
Unevenness (%)	40s yarn	12.45	12.11	N.S
	50s yarn	12.53	12.12	N.S
Thin Places (-50%)	40s yarn	15	12	N.S
	50s yarn	37	29	N.S
Thick Places (+50%)	40s yarn	43	35	N.S
	50s yarn	60	52	N.S
No. of neps	40s yarn	132	70	N.S
	50s yarn	115	85	N.S
Hairiness	40s yarn	4.4	4.4	N.S
	50s yarn	4.5	4.5	N.S

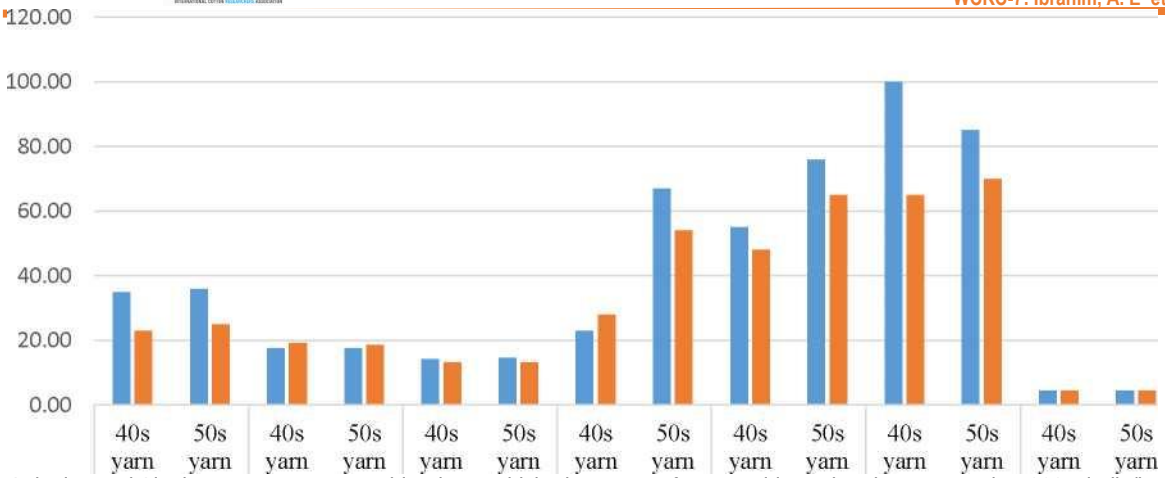

Fig 2: Yarn quality Properties of both two ginning methods for Giza 95

Table 4. Yarn quality properties of both two ginning methods for Giza 95

	Count	Reciprocating ginning	Rotary ginning	L.S.D at 0.05%
Blow-room and card waste		8.50%	4.80%	0.51
Spinning end breakage "1000 spindle/h"	40s yarn	35.00	23.00	N.S
	50s yarn	36.00	25.00	N.S
Single yarn strength cN/Tex	40s yarn	17.65	19.14	N.S
	50s yarn	17.50	18.63	N.S
Unevenness (%)	40s yarn	14.32	13.23	N.S
	50s yarn	14.55	13.32	N.S
Thin Places (-50%)	40s yarn	23.00	28.00	N.S
	50s yarn	67.00	54.00	N.S
Thick Places (+50%)	40s yarn	55.00	48.00	N.S
	50s yarn	76.00	65.00	N.S
No. of neps	40s yarn	100.00	65.00	N.S
	50s yarn	85.00	70.00	N.S
Hairiness	40s yarn	4.40	4.40	N.S
	50s yarn	4.50	4.50	N.S

Conclusions

Undoubtedly, the overall quality improvements of Rotary roller-ginned Egyptian cotton over reciprocating cotton are consistent. High production Rotary roller ginning of Egyptian cotton consistently reserves the longer and more uniform length fibres for the same cotton. The reciprocate roller-ginned cotton is processed through the gin with more than two percentage points higher turnout and higher in trash area, trash content, and foreign fibre matter. This was simply a matter of increased nonlint content, as the higher turnout was not preserved through blowroom operations in the spinning mill with an average of 3.6 percentage points more loss for reciprocate ginned cotton compared to Roller ginned cotton. Carded yarn production for medium count yarns was more efficient, with fewer thin, thick, unevenness and ends down using Rotary-ginned cotton.

Material and Methods

Two experiments were conducted at the Fayyoum gin-plant, Misr for Cotton Ginning, Cotton and Textile Industries Holding Company, to evaluate the effect of conventional reciprocating and rotary-knife gin-stand of Bajaj Continental make on lint out-turn, fibre quality of Giza 95 grown in Upper-Egypt and Giza 94 transferred from Delta Egypt to Upper Egypt. 2000 Tons of homogenous bulk of seed cotton for each of Giza 95 and Giza 94 cotton varieties of 2018/2019 crop were used in this study.

The seed-cotton grade was classified as Good + %. The ginning machinery sequence was typical of that found in Fayyoum ginnery. 1000 Tons of each Giza 95 and Giza 94 seed cotton were ginned in the process sequence in the rotary gin i.e., rock trap, Big J, inclined cleaner, extractorfeeder, roller gin stand, Pima lint cleaner, and the other 1000 tons was ginned in the ordinary reciprocating-knife roller gin-stand, figures 3 and 4 and table 5. The time required for ginning each ton and the weight of ginned lint was recorded. The lint out-turn was estimated as the percentage of ginned lint about the seed cotton weight.

The principal raw cotton fibre properties of the ginned lint were measured on the High-Volume Instrument (HVI) and Advance Fibre Information System (AFIS). The lint grades were determined by qualified lint classers. All fibre properties were carried out under standard atmospheric conditions of (65 % ± 2) relative humidity and (21.0°C ±1) temperature degree.

Table 5. Ginning treatment, production speed, and outturn

Variety	Seed Cotton Grade	Gin type	Outturn	Production/machine/h	Lint Grade
Giza 95	Good	Rotary	37.13	400 Kg/h	Good +7/16
Giza 95	Good	Reciprocate	39.37	30 Kg/h	Good +3/16
Giza 94	Good	Rotary	36.41	400 Kg/h	Good +7/16
Giza 94	Good	Reciprocate	38.73	30 Kg/h	Good +3/16

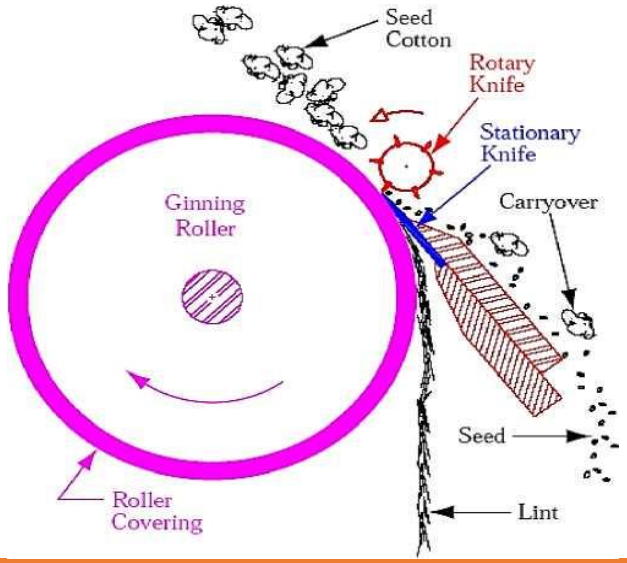

Figure 3: Principle of Rotary Knife Gin

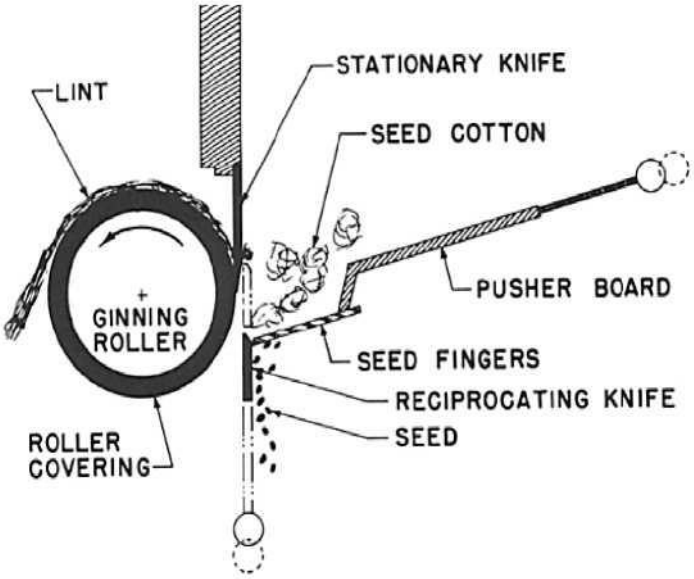

Figure 4: Principle of McCarthy Roller Gin (Ref. Carlos et al. 2017)

Table 1: Average HVI test Results for Rotary and conventional ginning mill

	UHM (mm)	UI (%)	SFI (%)	Strength (g/Text)	Elongation (%)	Micronaire	Mat. Ratio	Rd	+b	Tr Cnt	TrAr	Nep count
Average new Rotary Gin Giza 94	33.40	85.69	6.70	40.10	6.36	4.00	0.86	77.70	9.00	23.00	0.36	125
Average Variety in old Gin Giza 94	33.51	86.77	7.30	40.55	6.20	4.00	0.87	76.20	9.00	59.00	0.59	101
Average new Rotary Gin Giza 95	30.69	85.10	6.60	37.60	6.80	4.45	0.86	69.70	11.20	31.00	0.24	79.00
Average Variety in old Gin Giza 95	28.70	83.53	8.30	35.34	6.20	4.29	0.88	68.00	11.80	59.00	0.59	93.00
Observed Significance Level for Mean Difference												
LSD at 0.05%	3.686	2.150	1.241	3.842	0.451	0.355	0.015	7.580	2.328	29.831	0.277	30.653

NS: Non-Significant at 0.05% level of probability

Table 2: Average AFIS test Results for Rotary and conventional ginning mill

	SFC(w) %<12.7 nun	**UQL(w) (mm)**	**L(n) (mm)**	**Fineness (mtex)**	**Maturity Ratio**	**IFC (%)**	**Total Nep Cnt (Cnt/g)**	**SCNep Count (Cnt/g)**	**Trash Count (Cnt/g)**	**VFM (%)**
Average new Rotary Gin Giza 94	4.9	35.4	23.7	143	0.94	5.4	160	5	20	0.52
Average Variety in old Gin Giza 94	5.1	34.8	23.3	147	0.91	4.7	176	7	59	1.66
Average new Rotary Gin Giza 95	5.2	31.4	21.2	155	0.95	5.3	165	5	18	0.55
Average Variety in old Gin Giza 95	5.7	31.3	21.0	160	0.92	4.5	210	8	66	1.76
Observed Significance Level for Mean Difference										
LSD at 0.05%	0.54	3.46	2.22	12.21	0.03	0.70	35.80	2.38	40.24	1.08

NS: Non-Significant at 0.05% level of probability

Spinning Processing. The ginned lint cotton was processed and opened, carded, and drew on a Truetzschler line, while roving, spinning was processed on a Marzoli line as shown in Fig. 5 to produce carded ring spun yarns, Ne 40 and Ne 50. Yarn evenness and imperfections (thin and thick places and neps), as well as hairiness, was measured with a Uster Tester 3. While single yarn strength was measured with a Statimat ME.

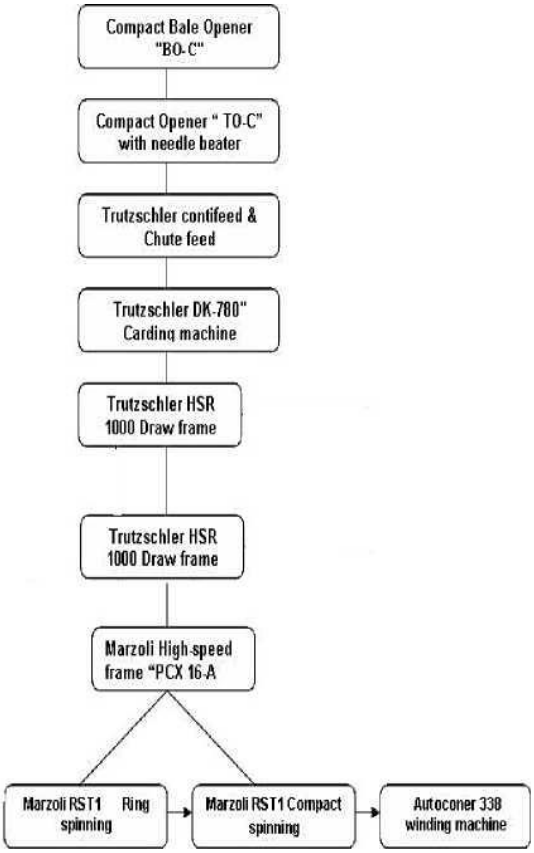

Figure 5. Outline yarn mechanical process

References

1. Abdel-Salam, M.E. 1999. The Egyptian Cotton. Pp 487. El-Kalema Press, ISBN 16105/99. EGYPT
2. A.S.T.M., standards (1982). Designations: 168496. American Society for Testing and Materials, 556Philadelphia, Pa., U.S.A.
3. Carlos B. Armijo, Derek P. Whitelock, Joe W. Thomas, Sidney E. Hughs, and Marvis N. Gillum. 2017. Engineering and ginning roller gin. The Journal of Cotton Science 21:199-209 (2017). http://journal.cotton.org, © The Cotton Foundation 2017.
4. Delhom, C.D., C.B. Armijo and S.E. Hughs (2017). High-quality yarn, produced via high-speed roller ginning of Upland cotton.Textile Technology. The J. of Cotton Sci., 21: 81-93.
5. El-Sayed M.A. M. M. A. Naguib and H. H. El- Feky. 2008. Comparative study of cotton quality ginned on both reciprocating and rotary knife gin-stands in Sids gin-plant. Egypt. J. Agric. Res., Vol. 86 (3) 1027: 1037, 2008.
6. Gordon, K. M. Bagshaw, F. A. Horne. 2011. The Effect of Lint Cleaner Elements, Settings, and Fibre Moisture Content on Fibre Quality. American Society of Agricultural and Biological Engineers, St. Joseph, Michigan www.asabe.org. 54 (6):2267-2278. (doi:10.13031/2013.40640) @2011.
7. Sharma, M.K. (2008). New Developments in cotton ginning. Fourth breakout session 67th Plenary Meeting of the ICAC in Ouagadougou, Burkina Faso., Page 1-11.
8. Vander Sluijs, M.H. (2015). Impact of the ginning method on fibre quality and textile processing performance of Long Staple Upland cotton. Tex. Res. J. 85(15): 1579-1589.
9. Werner International Management Consultants. 2016. Survey report; Assessment of the ginning operation, Presented to Cotton and Textile Holding Company.

Effect of Single Locking Cotton Feeders on Ginning Efficiency of Double Roller Gin for Short Staple Cotton

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Abstract

***Background**: Low ginning efficiency of double roller (DR) gins provides an economic barrier to their widespread adoption. Appropriate feeding mechanism to DR gin plays a significant role in enhancing ginning efficiency. Conventional autofeeder encounters problems of uneven supply and feeding in lumps of cotton to DR gin thereby resulting in poor ginning efficiency. To address this issue three cotton feeding mechanisms viz. spike cylinder feeder (M1), saw band cylinder feeder (M2) and combined feeder (M3) was developed based on principle of single locking of cotton bolls with an aim to unlock cotton bolls and to maintain constant and optimum feeding rate of individual locules to DR gin. Response Surface Methodology was used to optimise developed feeders and the performance of DR gin with these single locking feeders was compared to conventional autofeeder (Control) for ginning short staple cotton.*

***Results:** Degree of unlocking of cotton bolls found to be improve by 17, 14 and 17% respectively for M1, M2 and M3 respectively as compared to Control. Ginning output increased by around 62, 36 and 48% and specific energy decreased by around 27, 18 and 22 % for M1, M2 and M3 respectively as compared to Control. Highest ginning output of 41.23 kg/h and lowest specific energy consumption of 0.0685 kWh/kg was found for M1. Spike cylinder feeder showed higher colour grade improvement as compared to other two mechanisms and other fibre quality parameters tested by HVI and AFIS remain unaffected. Post-hoc analysis with Tukey's test revealed that among three feeder, spike cylinder feeder (M1) was best in terms of improvement in ginning performance of DR gin.*

***Conclusions:** Spike cylinder type single locking cotton feeder is a better option as compared to conventional autofeeder to enhance ginning efficiency of double roller gin for ginning short staple cotton and to make ginning business more remunerative.*

Keywords: *Ginning, Short staple cotton, Single locking feeder, Double roller gin*

Background

Cotton is an important commercial crop and India has emerged as the largest producer of cotton in the world with a total production touching 6.15 million tons in 2017-18 (ICAC, 2018). Double roller (DR) gins are more prevalent in Indian ginneries than saw gins. DR gins has prominent share in ginning industries across the world. About 95% of the cotton produced in India is subjected to ginning by using DR gins. As compared to saw gin and rotary knife roller gin, the ginning capacity of DR gins is very low. Low production capacities of DR gin is the barrier for its widespread adoption across the world except in India and some African counties (Sharma, 2014).

Indian researchers have made efforts to improve DR gins to increase capacity and ginning efficiency. Improved models of DR gins were developed by increasing roller length from 1065 to 1525 mm with an increase in output from 40 to 90 kg lint/h. Roller and beater drives were seperated and driven independent of each other to develoep 'Variable Speed' DR gin (Jadhav et al., 2007). To increase the gin productivity and to eliminate the chromium contamination a 'Self grooving rubber roller' was developed as a substitute to chrome composite leather roller used in DR gin (Arude et al., 2017). The ginning efficiency is governed by lint output, lint quality and energy conssumption. Type of cotton, moisture content, ginning roller speed, beater speed, setting and adjustments and feeding mechanism to DR gin influences the ginning output to a great extent (Patil et al., 2007).

The way cotton is fed at the knife edges of DR gin plays an important role in ginning process. Manual feeding never offer uniform feeding which results in loss of efficiency up to 20% (Sharma, 2014). In Indian ginneries, autofeeder is commonly used as feeding mechanism to DR gin. Uneven, inconsistent, and feeding in lumps of cotton through conventional autofeeder to DR gin results in low ginning

efficiency. For maximizing ginning efficiency, the feeding rate of seed cotton should be constant and should be limited to such an extent that, it ensures smooth and trouble free ginning (Antony, 1994).

Appropriate feeding mechanism to DR gin plays a significant role in enhancing ginning efficiency. Single locking of seed cotton ensures controlled feed rate and increases the production capacity with the increased bale value (Baker et al., 1994). Feeders in saw and rotary knife roller gins are based on the principal of single locking of cotton bolls which results in higher ginning capacities. Since there was a need for a suitable feeder to enhance ginning efficiency of DR gins, the feeder based on the principal of single locking of cotton bolls was developed and evaluated. The purpsoe behind developing these feeding mechanism was to replace conventional autofeeder used in ginning industry in order to make ginning business more remunerative.

Cotton ginner's experienced difficulties in ginning short staple cotton with the present DR ginning system. Further it was observed that, DR gins are not effective in ginning short staple cotton as compared to medium, long and extra-long staple cottons. Ginning of short staple cotton with present DR gin is uneconomical due to low ginning output and poor energy efficiency. It was expected that, if DR gins are employed with feeder based on single locking principal, it would result in improvement in ginning output and efficiency. Therefore in the present work attempts have been made to develop single locking feeding mechanisms and evaluate their performances while ginning short staple cotton on double roller gin.

Results and Discussion

Results of face centered CCD experimental design

Ten experimental runs with two independent variables at three levels were conducted for each mechanism. The experimental face centered CCD design and actual results of dependent variables i.e. ginning output (Y1), specific energy for ginning (Y2) and decrease in Bulk density (Y3) are presented in Table 1. Ginning output was found to vary from 27.5-42, 26.0-36.2 and 26.3-38.6 kg/h, and specific energy was found between 0.0695-0.0916, 0.0744-0.0904 and 0.0725-0.0905 kWh/kg and decrease in Bulk density was found in the range of 11.97-23.34, 9.66-18.14 and 11.75-22.91 % for mechanism M1, M2 and M3 respectively.

Table 1. Experimental face centered CCD design and actual results of dependent variables for different feeding mechanisms

Standard run	M1					M2					M3				
	Actual variables		Dependent variables			Actual variables		Dependent variables			Actual variables		Dependent variables		
	X_1	X_2	Y_1	Y_2	Y_3	X_1	X_2	Y_1	Y_2	Y_3	X_1	X_2	Y_1	Y_2	Y_3
1	9	200	27.5	0.0916	15.89	9	150	26.0	0.0904	12.01	9	200	26.3	0.0905	15.63
2	9	300	31.0	0.0803	19.83	9	200	28.0	0.0854	16.03	9	300	29.4	0.0830	19.31
3	9	400	29.7	0.0865	23.34	9	250	27.2	0.0871	18.14	9	400	28.4	0.0870	22.91
4	12	200	34.0	0.0762	13.42	12	150	31.5	0.0794	10.75	12	200	32.6	0.0779	13.17
5	12	300	42.0	0.0695	17.18	12	200	36.2	0.0744	13.84	12	300	38.5	0.0731	16.95
6	12	300	41.8	0.0694	17.28	12	200	36.0	0.0746	14.04	12	300	38.6	0.0725	17.29
7	12	400	37.6	0.0747	20.56	12	250	33.5	0.0770	15.89	12	400	35.1	0.0761	20.31
8	15	200	31.5	0.0781	11.97	15	150	30.4	0.0813	9.66	15	200	30.9	0.0799	11.75
9	15	300	36.0	0.072	14.22	15	200	33.6	0.0768	12.72	15	300	34.6	0.0749	13.87
10	15	400	33.0	0.0764	17.30	15	250	32.0	0.0791	14.20	15	400	32.6	0.0779	16.98

The result revealed that moisture content and spike cylinder speed played a vital role in unlocking cotton bolls as evidenced from the decrease in Bulk density. Single locking of cotton bolls in M1 was achieved as the spike tips were spaced closer to the feed rollers than the thickness of a lock of cotton and spiked cylinder travelled at a greater linear speed than feed rollers, thus striking the bolls of cotton momentarily held between the feed rollers. Single locking of cotton bolls was increased with increase in spike cylinder speed and decreased with increase in cotton moisture content.

It was observed that moisture content and saw band cylinder speed played a key role in unlocking the cotton bolls as seen from the decrease in Bulk density. Single locking of cotton bolls in M2 was achieved by saw band cylinder which does the function of opening and unlocking the cotton bolls. Single locking of cotton bolls was increased with increase in saw cylinder speed and decreased with increase in cotton moisture content. It can be seen that saw band cylinder speed had positive effect whereas the moisture content has the negative effect on single locking of cotton bolls. The lowest ginning output was observed at 5% moisture content. This is due to the fact that as moisture content of cotton becomes less, fibre becomes more brittle and as relative humidity lowers static electricity begin to build which leads to fibres wrapping on rollers or failing to doff properly resulting in negative impact on ginning efficiency. Conversely, if relative humidity is allowed to increase too high there are potential problems with the fibres drafting and doffing improperly as well as the propensity for blockages during ginning operation.

Establishment of regression models

The analysis of variance (ANOVA) and lack-of-fit (LOF) tests were generated for three responses from experimental data. Table 2 depicts model summery for three feeding mechanism.

Large F-values for ginning output (Y_1), specific energy (Y_2) and decrease in Bulk density (Y_3) and p-values <0.001 indicate that developed regression models are extremely significant at 5% level of significance for all three mechanisms. The lack of fit was not significant as evidenced from the p-values for all three responses and mechanisms. Quadratic model was fitted for prediction of responses Y_1 and Y_2 whereas linear model was fitted for prediction of response Y_3. Values of R^2 exceeding 0.95 indicated that the fitted models are in excellent agreement with the experimental values.

The optimum solution for each response and mechanism i.e. critical values of moisture and spike cylinder speed were generated through SAS analysis. Table 3 illustrates the critical values of moisture content and speed for maximizing ginning output and minimizing specific energy.

Multiple regression analysis: Optimization of responses and desirability

Table 2. Model summary for different feeding mechanisms for short staple cotton.

Response variable	RSM model	df	SS	MS	MSE	F	p-value	R^2	R^2 Adj	p-value
Mechanism 1 (M1)										
Output (kg/h)	Quadratic	5	210.23	42.05	1.3112	24.46	<0.0043*	0.968	0.928	0.0686
Specific Energy (KWh/kg)	Quadratic	5	0.0004	0.0002	0.0012	115.71	<0.0004*	0.987	0.971	0.0372
Decrease in Bulk density (kg/m^3)	Linear	5	107.66	21.53	0.4125	220.37	<0.0001*	0.996	0.992	0.1437
Mechanism 1 (M2)										
Output (kg/h)	Quadratic	5	111.04	22.21	0.7288	41.81	0.0015*	0.981	0.975	0.1233
Specific Energy (KWh/kg)	Quadratic	5	0.0003	0.0001	0.0002	1751.81	<0.0001*	0.999	0.998	0.5010
Decrease in Bulk density (kg/m^3)	Linear	5	59.08	11.82	0.1398	604.52	<0.0001*	0.998	0.997	0.6154
Mechanism 3 (M3)										
Output (kg/h)	Quadratic	5	148.19	29.64	0.8987	36.69	0.0019*	0.978	0.951	0.0501
Specific Energy (KWh/kg)	Quadratic	5	0.0003	0.0001	0.0007	143.11	0.0001*	0.994	0.987	0.3999
Decrease in Bulk density (kg/m^3)	Linear	5	104.25	20.85	0.39	133.40	0.0002*	0.994	0.986	0.3811

Notes: *Significant at 5% level of significance

Table 3. Critical values of moisture and cylinder speed for maximizing output and minimizing specific for different mechanism.

Parameters	M1	M2	M3
Ginning Output			
Moisture content (%)	12.45	12.86	12.66
Speed (RPM)	313	208	315
Output at critical values (kg/h)	41.27	36.03	38.24
Specific energy			
Moisture content (%)	13.08	12.99	13.05
Speed (RPM)	313	209	312
Specific energy at critical values (kWh/kg)	0.0681	0.0738	0.0717
Specific energy at critical values (kWh/kg)	0.0681	0.0738	0.0717

The aim of the study was to find out the optimal parameters i.e. cylinder speed and cotton moisture content to enhance ginning efficiency on one hand and to reduce specific energy on the other hand without affecting fibre quality. The different critical values of moisture and cylinder speed were given by

the fitted models for ginning output and specific energy. Therefore single optimum solution was obtained separately by multiple response analysis.

Desirability technique determines optimum settings of input factors that achieve optimum performance levels for one or more responses. The optimum settings may be maxima, minima, target settings or a combination of these. The JMP software allows multiple optimization using models derived from designed experiments. JMP's approach to optimization for multiple response is based upon the concept of desirability (Philip et al., 2005).

Optimization process was conducted, by assigning the constraints 'in the range' for independent variables for maximizing output and minimizing specific energy. In case of response variable decrease in Bulk density; neither maximum nor minimum could be obtained (saddle point). Since the interest is to achieve maximum output, minimum specific energy with desired unlocking of the bolls, the multiple response optimization was done keeping the target values for the response decrease in Bulk density in the range of 17.9±0.5%, 14.0±0.3% and 17.0±0.62% for M1, M2 and M3 respectively to get maximum ginning output and minimum specific energy.

The single optimum solution was obtained by multiple response analysis for mechanism M1 (Fig.1), mechanism M2 (Fig. 2) and Mechanism 3 (Fig. 3). For mechanism M1, ginning output of 41.23 ± 2.1172 kg/h and specific energy of 0.0686 ± 0.001953 kWh/kg was observed at optimum levels of moisture content of 12.5% and speed of 324 RPM with desirability of 0.9047. For mechanism M2, ginning output of 36.03 ± 1.161 kg/h and specific energy of 0.0737 ± 0.00028 kWh/kg was observed at optimum levels of moisture content of 12.9% and speed of 209 RPM with desirability of 0.919386. For mechanism M3, ginning output of 38.10208 ± 1.39998 kg/h and specific energy of 0.071869 ± 0.001029 kWh/kg at optimum levels of moisture content of 13.0 % and speed of 330 RPM with desirability of 0.914954. Ginning output was higher and specific energy was lower for M1 as compared to M3 at optimum solutions obtained by using multiple response analysis and desirability function.

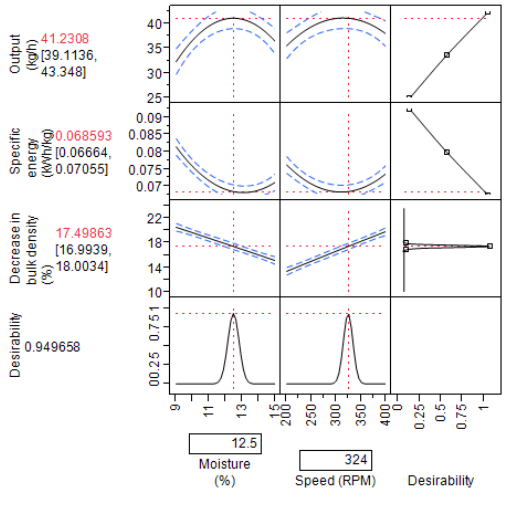

Fig. 1. Desirability and levels of moisture and speed by multiple response optimisation for M1

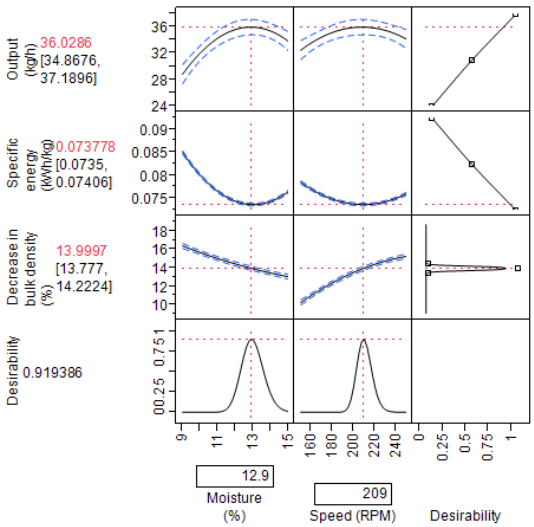

Fig. 2. Desirability and levels of moisture and speed by multiple response optimisation for M2

Effect of different feeding mechanisms on colour grade of cotton

The colour grades were assigned to each sample following USDA HVI colour chart for American upland cotton. The first two digits in the colour grade indicates colour grade and last digit after hyphen indicates leaf grade. Colour grades of cotton were improved with application of different feeding mechanisms as compared to application of conventional autofeeder. Mechanism M1 showed higher colour grade improvement as compared to other two mechanisms. Higher cylinder speeds resulted in better colour grades as compared to lower speed because of better unlocking of cotton and removal of fine dust. Comparatively low colour grade was observed at higher moisture content than lower moisture content for all cottons and all mechanisms.

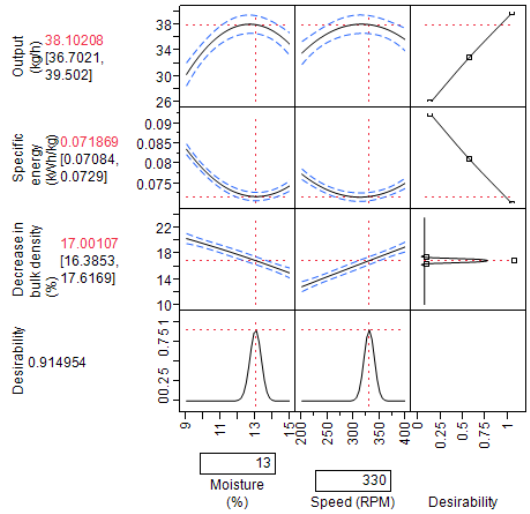

Fig. 3. Desirability and levels of moisture and speed by multiple response optimisation for M3

For mechanism M1, colour grade improved from middling light spotted (32-2) to middling light spotted (32-1) at 12% moisture content and 300 RPM cylinder speed. For mechanism M2, colour grade improved from middling light spotted (32-2) to middling light spotted (32-1) at moisture content and speed of 12% and 300 RPM respectively. For M3, cotton colour grade improved from middling light spotted (33-2) to middling light spotted (32-1) at 12% moisture content and saw band cylinder speed of 200 RPM (Table 4).

Table 4. Effect of single locking on colour grade of short staple cotton

Independent variables		Colour grade		Independent variables		Colour grade		Independent variables		Colour grade	
X_1	X_2	M1	Control	X_1	X_2	M2	Control	X_1	X_2	M3	Control
9	200	32-2	32-2	9	150	32-2	32-2	9	200	32-2	32-2
9	300	32-1		9	200	32-1		9	300	32-1	
9	400	22-2		9	250	22-2		9	400	22-2	
12	200	32-2	32-2	12	150	32-2	33-2	12	200	32-2	32-2
12	300	32-1		12	200	32-1		12	300	32-1	
12	300	32-1		12	200	32-1		12	300	32-1	
12	400	22-2		12	250	32-1		12	400	22-2	
15	200	33-2	32-2	15	150	33-2	43-1	15	200	33-2	32-2
15	300	32-2		15	200	32-2		15	300	32-2	
15	400	22-2		15	250	32-1		15	400	22-2	

Enhancement in ginning efficiency of double roller gin

The main focus of investigation was to enhance ginning efficiency of DR gin by using developed single locking feeding mechanism. All three mechanisms attempted were found to significantly enhance ginning efficiency of DR gin to that of conventional autofeeder. Enhancement in ginning efficiency was worked out in terms of percentage increase in ginning output, degree of unlocking in terms of percentage decrease in Bulk density and reduction in specific energy. Multiple regression analysis with desirability had provided the optimum solution for ginning performance parameters for each mechanism. The response values for output, specific energy and decrease in Bulk density obtained by multiple regression analysis for M1, M2 and M3 were compared to the mean values of respective response of control. Table 5 represents enhancement in ginning efficiency of DR with different feeding mechanisms. Ginning output increased by around 62, 36 and 48% and specific energy decreased by around 27, 18 and 22 % for M1, M2 and M3 respectively as compared to conventional autofeeder. The highest ginning output of 41.23 kg/h and lowest specific energy consumption of 0.0685 kWh/kg was found for M1 i.e. spike cylinder type feeder.

Table 5. Enhancement in ginning performance of DR gin with different feeding mechanisms

Parameter	M1	Control	Change (%)	M2	Control	Change (%)	M3	Control	Change (%)
Ginning output (kg/h)	41.23	25.40	62.32	36.02	26.33	36.80	38.10	26.0	48.53
Decrease in Bulk density (%)	17.00	0	17.00	14.0	0	14.0	17.0	0	17.00
Specific energy (kWh/kg)	0.0685	0.095	27.89	0.0737	0.0909	18.92	0.0718	0.0921	22.04

Results revealed increase in output of DR gin for short staple cotton with all three mechanisms. It is pertinent to mention that, it was difficult to gin short staple cotton with double roller gin and conventional autofeeder. But single locking feeding mechanisms significantly increased ginning output of DR gin while ginning short staple cotton. It was mainly due to unlocking of cotton bolls which created more opportunity for more fibres to come in contact with ginning roller and get ginned faster which otherwise not possible with the conventional mechanism.

Post-Hoc analysis for comparative assessment of different feeding mechanisms

In order to explore the specific differences between the different feeding mechanisms, the post-hoc test was carried out. For the post-hoc analysis, mean values of ten replications of ginning performance parameters and fibre quality parameters obtained using different feeding mechanism were taken. The comparisons of means of the ginning performance parameters and fibre quality parameters obtained using three different feeding mechanism and control (conventional autofeeder) was done using Tukey's test or Tukey's HSD.

The effect of different feeding mechanism to improve the ginning efficiency for short staple cotton was analyzed using the t-test. The results showed that the feeding mechanisms (M1 M2 and M3) improved ginning efficiency in terms of ginning output, decrease in bulk density, specific energy and trash content compared to control. The different feeding mechanism did not affect quality parameters of cotton as measured by HVI and AFIS. The analysis also revealed that use of feeding mechanisms has significantly improved colour grade of the cotton being processed as reflected from the degree of reflectance. Overall results showed improvement in ginning efficiency with use of feeding mechanisms developed based on the concept of single locking of cotton bolls.

Table 6. Post-hoc analysis of different feeding mechanisms on ginning efficiency

Parameters	M1	M2	M3	Control
Ginning performance parameters				
Output (kg/h)	33.6^{a}	30.9^{a}	32.0^{a}	25.9^{b}
Bulk density (kg/m^3)	49.24^{a}	50.73^{a}	49.09^{a}	59.00^{b}
Decrease in bulk density (%)	17.08^{a}	13.69^{a}	16.76^{a}	0.00^{b}
Specific Energy (kWh/kg)	0.078^{a}	0.081^{a}	0.080^{a}	0.093^{b}
Trash content (%)	2.21^{a}	2.64^{b}	2.24^{a}	2.67^{b}
HVI fibre quality parameters				
UHML (mm)	18.9^{a}	19.0^{a}	19.0^{a}	19.0^{a}
UI (%)	74.1^{a}	74.3^{a}	74.3^{a}	73.7^{a}
MIC (µg/inch)	7.2^{a}	7.2^{a}	7.1^{a}	7.2^{a}
Tenacity (g/tex)	21.2^{b}	21.3^{a}	21.4a	21.4^{a}
SFI (%)	60.9^{a}	62.6^{a}	63.0^{a}	60.3^{a}
Rd (%)	73.2^{a}	72.8^{a}	73.0^{a}	70.9^{b}
Plus b	10.3^{a}	10.3^{a}	10.4^{a}	10.4^{a}
AFIS fibre quality parameters				
UQL (w) (mm)	19.3^{a}	19.3^{a}	19.4^{a}	19.4^{a}
Fibre neps (c/g)	85.9^{a}	86.0^{a}	88.5^{a}	86.3^{a}
Seed coat neps (c/g)	34.4^{b}	34.5^{b}	34.3^{b}	28.2^{a}
Total trash (c/g)	338^{a}	433^{ab}	347^{a}	502^{b}

Table 6 depicted the post-hoc analysis of different feeding mechanisms for short staple cottons. The post-hoc analysis done to differentiate the significant difference among the different mechanism being

studied. Post-hoc analysis showed that Mechanisms M1, M2, M3 are significantly different from Control in respect of ginning performance indicators (ginning output, bulk density, specific energy). Post-hoc analysis revealed no significant difference in M1, M2 and M3 and control in respect of fibre quality parameters as measured by HVI except for Rd values. Single locking feeding system has improved the colour grade of the cotton compared to conventional feeding mechanism. It was also observed that mechanism (M2) created more seed coat neps which is not desirable. Mechanism M2 did not aid in reducing the trash content in cotton. Further the improvement in ginning efficiency was comparatively less in case of M2 than M1 and M3. The study revealed that mechanism M3 performed comparatively better than M2 in terms of improvement in ginning efficiency. The comparative analysis revealed that among the three mechanisms (M1, M2 and M3) studied, spike cylinder type single locking cotton feeder (M1) was the best mechanism followed by M3 and M2 in terms of improvement in ginning efficiency of double roller gin. Overall results indicated that spike cylinder cotton feeder (M1) based on the principle of single locking of seed cotton ensured uniform feeding with improved ginning efficiency as compared to conventional autofeeder for ginning of short staple cotton.

Conclusions

Three mechanisms viz. spike cylinder single locking cotton feeding mechanism (M1), saw band cylinder single locking cotton feeding mechanism (M2) and combination of spike cylinder single locking cotton feeding mechanism with conventional autofeeder (M3) were successfully designed and developed based on the concept of single locking of cotton bolls. It is concluded that among three mechanism spike cylinder single locking cotton feeder (M1) is a better option to improve ginning performance of double roller gin for short staple cotton as compared to conventional autofeeder. Ginning output increased by around 62, 36 and 48% and specific energy decreased by 27, 18 and 22 % for spike cylinder feeder, saw band feeder and combined feeder respectively as compared to conventional autofeeder. Spike cylinder feeder showed higher colour grade improvement. Other fibre quality parameters tested by HVI and AFIS remain unaffected.

Materials and Methods

Development of single locking feeding mechanisms

Three different feeding mechanisms spike cylinder single locking feeder (M1), saw band cylinder single locking feeder (M2) and combined feeding mechanism (M3) comprising of spike cylinder feeder and conventional autofeeder were desinged and developed based on the concept of single locking of cotton bolls; with an aim (a) to unlock cotton bolls, (b) to maintain uniform feeding rate of individual locules and (c) to ensure feeding locules near to the ginning point across the knife edges of DR gin. Spike cylinder single locking cotton feeding mechanism (M1) (Fig.4) was developed with a pair of feed rollers and spiked cylinders, grid bar housed in a feeder hopper and chute for cotton distribution on either side of the beater of DR gin. Saw band cylinder single locking feeding mechanism (M2) (Fig.5) was developed with a pair of saw band cylinders with hopper, reservoir box, doffing brushes and feeding aprons.

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Fig. 4. *Spike cylinder feeder (M1)*

Fig. 5*. Saw band cylinder feeder (M2)*

Fig. 6. Combined feeder (M3)

Fig. 7. Conventional autofeeder (Control)

The conventioanl autofeeder (Control) (Fig. 7) consist of two endless belts, hopper, spike patti, rollers for mounting belt, UCT bearings and drive mechanism. In hopper of autofeeder, two counter rotating endless belts with metal strips are fixed at an angle to the vertical. The strips move through seed cotton in the hopper and cotton gets entangled to the spikes and carried over and fed it along the knife edges of beater

Experimental method for performance evaluation

Performance of three feeding mechanisms was evaluated by mounting each mechanism on commercial DR gin with roller length of 1360 mm. Experimental setup of mechanism M1 with DR gin is depicted in Fig. 8. Similarly other feeding mechanisms and conventional autofeeder were mounted on same DR gin for evaluation of their performance and optimization. Performance was measured in terms of degree of unlocking of cotton bolls, ginning output, specific energy consumption and fibre quality. Short staple cotton with 2.5% span length of about 22 mm was ginned. The cylinder speed was varied with the help of a variable frequency drive. The clamp on power meter (CW240; Yokogawa, Japan) was used for measurement of energy consumption. Fogging machine was used to apply moisture in mist form on cotton to the desired extent. Moisture content of cotton was measured with portable C - 2000 moisture meter from Delmhorst. Degree of single locking of cotton bolls was determined by measuring change in Bulk density of seed cotton before and after passing through the feeder. The effect of the developed feeders on cotton quality was assessed by measuring the fibre quality parameters on High Volume Instrument (HVI) and Advanced Fibre Information System (AFIS). The trash analyser was used to measure the trash content and thereby the cleaning efficiency of the developed prototype.

Fig. 8. Pictorial view of mechanism M1 with DR gin

Optimization by response surface methodology (RSM)

Response surface methodology (RSM) was used to optimize performance of developed feeders. Face-Centered Central Composite Design (CCD) was used to optimise machine and crop parameters and to generate experimental matrix and model equations by using SAS JMP software (Praveen et al., 2009). Face-Centered CCD consisted of two independent factors with three levels each i.e. cotton moisture content (X_1) and cylinder speed (X_2) of developed feeder. The ginning efficiency parameters viz. ginning output (Y_1) and specific energy consumption (Y_2) of DR gin and degree of unlocking of cotton bolls in terms of decrease in Bulk density of cotton (Y_3) were the dependent variables. The developed feeders were optimised with these variable to maximize Y_1 and to minimize Y_2. Experiments were carried out for each mechanism as per design matrix that consisted of 10 runs for short staple cotton. The analysis of variance (ANOVA) and lack-of-fit (LOF) tests were generated for the responses from the experimental data and used to analyze models for significance. Single optimum solution was obtained by multiple response analysis and using desirability function (Philip et al., 2005).

Comparative analysis of different feeding mechanisms

The purpose of comparative analysis was to identify which among the three mechanisms performed better in comparison to conventional mechanism. The developed mechanisms M1, M2 and M3 were compared to that of conventional autofeeder (Control) on the basis of ginning performance. Improvement in ginning performance was indicated by increase in ginning output, decrease in Bulk density, reduction in specific energy, reduction in trash content, improvement in colour grade of cotton and impact on fibre quality. The effect of different mechanism on colour grade of cotton was studied. Post - hoc analysis using Tukey's test was done to compare different mechanisms.

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References

1. Antony W S. Overview of ginning process, in: Anthony W S, Mayfield, W D. (Eds.), Cotton Ginners Handbook. United States Department of Agriculture, (USDA), USA. 1994; 43-46.
2. Arude V G, Manojkumar T S, Shukla S K. Self-grooving rubber roller - A Substitute to chrome composite leather roller in double roller cotton ginning machine. Agricultural Mechanization in Asia, Africa and Latin America (AMA). 2017; 48 (3): 94.
3. Baker R V, Antony W S, Sutton R M. Seed cotton cleaning and extracting, in: Anthony W S, Mayfield W D, (Eds.), Cotton Ginners Handbook. United States Department of Agriculture, (USDA) USA. 1994; 69-71.
4. ICAC. International Cotton Advisory Committee (ICAC), Cotton this month (July 2018). https://www.icac.org/cotton_info/publications/updates/2018/English/ectm7_18.pdf, (accessed on 28.07.18).
5. Jadhav S B, Paralikar K M. Roller and beater speed combinations for higher lint outturn in double roller gin. IJFTR. 2007; 32: 377-384.
6. Patil P G, Padole P M, Agrawal J F. Effect of roller speed and moisture content of cotton on ginning rate, lint quality and electric energy consumption in double roller gins. Text. Res. J. 2007; (77): 635-644.
7. Philip J Ramsey, Mia L Stephens, Marie G. Multiple optimization using the JMP statistical software. Kodak Research Conference, May 9, 2005. North Haven Group, http://www.northhavengroup.com.
8. Praveen S, Lakhvinder S, Neeraj D. Response surface methodological approach for the decolorization of simulated dye effluent using Aspergillus Fumigatus Fresenius. J. Hazard Mater. 2009; 1081–1086.
9. Sharma M K. Advances in cotton ginning technology in India during 2010-2013. Proceedings, National seminar on advances in cotton ginning and testing technology, Indian Fibre Society (IFS) and CIRCOT, Mumbai, India. 2014; 1-11.

Fire Retardancy of Cellulosic Materials by using Waste Plant Bio-macromolecules

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Abstract

The field of flame retardancy especially in cellulosic materials like cotton is facing a lot of challenges, due to the concerns of eco-friendliness, sustainability etc. At present the quantity of the chemicals used to impart flame retardancy is high, these are toxic, expensive and have detrimental effect on the strength and hand value of the material at required add-on percentage. Therefore, there is a need to find an easy to apply, cheap eco-friendly fire retardant chemical which can be effective even at low add-on% and has no detrimental effect on the material. Scientists and researchers from different fields all over the world are trying to find a solution to this problem. In this regard, recently plant based bio-molecules have been tried for imparting fire resistant properties of cellulosic material. This paper, examines the effect of various plant based bio-molecules on the fire retardant property of cellulosic textiles and the mechanism behind the thermal stability of the treated textile materials.

Keywords: *Fire retardant, plant bio-macromolecule, Textile, Cotton, Thermo-gravimetry, Char*

Background

Cellulosic textiles like cotton catch flame readily and it is quite difficult to extinguish the same, resulting in serious health risk and damage to the textile products. Different chemicals have been used from sixteenth century to impart flame retardant property to cellulosic textiles. The most simple and common non- durable chemicals available in the market are inorganic salts like aluminium trihydrate, calcium carbonate, borax and boric acid mixture, diammonium phosphate, urea, sodium metasilicate etc,. [Horrocks et al 2011]. Most of these work by heat sink, heat barrier and condensed phase mechanism. Antimony in combination with halogen though popular in this field, was not very successful due to the negative environmental impact of the halogen (especially bromine, chlorine) based compounds [Horrocks et al 2011]. These chemicals have been mostly applied by back coating mechanism for maintaining the physical properties and hand value of the treated textile material. Phosphorous based flame retardants along with a nitrogenous compound is the most effective treatment reported so far due to their synergistic effect and condensed phase mechanism (char formation and change in pyrolysis path) of fire retardancy. Therefore, for the last fifty years, flame retardants based on phosphorous, nitrogen and halogen compounds like Tetrakis phosphonium salt and N-alkyl phosphopropionamide derivatives have dominated the market as commercial flame retardant products [Horrocks et al 2011]. However, when such formulations are applied on cotton fabric, the tear and tensile strengths of the fabric get reduced, fabric colour changes and it becomes hard and stiff as these are applied in acidic condition. Besides, the treatment is toxic, hazardous, expensive and also, time consuming due to involvement of high quantities of chemical and high temperature curing process. In addition, due to the use of formaldehyde containing resins in the process, toxic formaldehyde is released during the process and also from the treated fabric during its use. On account of this problem, there has been a thrust to develop more cost effective, environmentally friendly and sustainable fire retardant chemicals which when applied to cotton fabrics can maintain its quality to a great extent. In this direction, attempts have been made to reduce the quantity of formaldehyde released from fire retardant fabric by using low formaldehyde alternatives. However, development of an easily applicable, effective and environmentally friendly, cheap fire retardant for cellulosic material is still a major concern. Further, due to recent awareness about human health and hygiene, cellulosic materials finished with natural products are getting attention of the researchers [Alongi et al 2013, Alongi et al 2012, Basak et al 2015a, Bosco et al 2013]. As some plants are known to contain polyphenolic tannin, various oxides, salts and minerals, they may be suitably utilized to impart flame retardant properties to paper and textile material [Basak et al 2015b, Basak et al 2016a, Basak et al 2016b]. However, very few studies are reported on the application of plant extracts for flame retardant finishing of any textile and/or polymeric material.

In the present paper, an attempt has been made to present the studies connected with the exploration of the different plant based bio-products for making fire retardant cellulosic textiles and the mechanism of their action.

Results and discussion

Flame retardancy by plant based bio products

Banana pseudo-stem sap (BPS) is obtained as a by-product when fibres are extracted from the outer sheaths of the pseudo-stem of banana plant (Musa sp.). It looks like colourless clean water immediately after extraction. However, with the passage of time, it slowly turns into natural light khaki colour due to the oxidation of phenolic rings present in it [Basak et al 2015b]. Apart from the other end uses, very recently BPS has also been used to impart flame retardancy to the cellulosic, ligno-cellulosic, protein textiles and paper substrates [Basak et al 2015a, Basak et al 2015b, Basak et al 2016b]. On the other hand, extract of pomegranate rind (PRE), a waste product from the pomegranate, also has been explored for making fire retardant cellulosic cotton fabric [Basak et al 2018, Basak et al 2019].

Fire retardancy of a material can be tested by measuring the Limiting Oxygen Index (LOI) which is defined as the minimum quantity of oxygen in the oxygen/nitrogen mixture that is required to support its combustion. Textiles having LOI value more than or equal to 26 are generally considered as fire retardant. The LOI values of the control, mordanted and the BPS, PRE treated cotton and paper samples are given in Table 1.

Table 1: Flammability parameters of the control and the plant bio-products treated

Flammability parameters	Control cotton	Mordanted cotton	BPS and PRE-treated material	
			BPS treated cotton fabric	PRE treated cotton
Add on (%)	nil	1	20	30
LOI	18	18	28	32
		Vertical flammability		
Occurring of flashing over the surface	Yes	Yes	No	No
After flame(s)	60	60	5	nil
Afterglow after extinguishing of flame (s)	25	25	600	140
Char length (mm)	nil/ nil	nil	nil	5cm
State of the fabric in contact of flame	Completely Burnt with flame	Completely Burnt with flame	Burnt initially with flame followed by afterglow	Specific char length obtained

Source: cotton [Basak et al 2016a, Basak et al 2015a, Basak et al 2018]

The results from Table 1 indicate that BPS and PRE both act as fire retardant material for cellulosic products. LOI of the cotton fabric has improved after treatment with BPS. Further, burning rate of the treated cotton fabric has been reduced. Like cotton fabric, BPS treatment is also beneficial on the jute fabric [Basak et al 2015b]. However, most of the cellulosic materials treated with plant extracts, showed afterglow and smoke. This dangerous afterglow can be arrested by the addition of small amount of boric acid into the BPS formulation [Basak et al 2016b]. It is seen that PRE also acts as fire retardant on the cotton material and PRE treated cotton fabric showed high LOI value and specific char length after vertical burning test.

Thermogravimetry and char morphology

Figure 1 shows the vertical burning behaviour of the control and the BPS treated cotton fabric. It is reported that the control cotton fabric was burnt within 1 min whereas BPS treated fabric showed resistance against flame. Only afterglow was present in the BPS treated fabric and it propagated very slowly. Anybody using this material can easily stop the afterglow as temperature generate during burning with afterglow is low. Fig 2 shows the thermogravimetry (TG) curves of the control textile materials in N_2 atmosphere at a heating rate of 10ºC/min. The TG curve of the control cellulosic textile material (cotton) shown in Fig 2 (C) evidenced three stages of progression. Initial stage at temperature below 300°C, the small loss in mass occurred mainly due to removal of bound and unbound absorbed moisture from the cellulose polymer. Above 360ºC temperature, both dehydration and char formation occurred. Similar trend of loss in mass, i.e., degradation was also observed in the only mordanted sample. These cellulosic samples lose approximately 98% of their mass below 500°C. However, the main thermal decomposition occurred in the temperature range of 300-360ºC, where mass of the

sample sharply decreased at around 340°C. This happened mainly due to the pyrolysis of cellulose [Alongi et al 2014b, Alongi et al 2012, Alongi et al 2014a]. On the contrary, BPS treated cellulosic material, depicted in Fig 2 (D), showed slower rate of pyrolysis and more amount of char mass retention at higher temperature. Indeed, different polyphenolic tannins, metallic oxides, salts, phenolic groups present in the BPS together helped to earlier pyrolysis instead of the depolymerisation of cellulose. As a result, 1st derivative peaks of the treated fabric samples shifted towards the lower temperature. Char morphology of the treated cellulosic textile (Fig. 1B) showed multicellular structure with closed cell pockets through which flow of the flammable gases has been restricted whereas char of control cotton fabric (Fig 1A) showed net like fragile structure. Like BPS, thermo-gravimetry curves of the PRE treated cotton samples also showed reduction in pyrolysis temperature from 350°C to around 265-280°C and enhanced the dehydration phenomena of the underlying cellulosic substrate [Basak et al 2019].

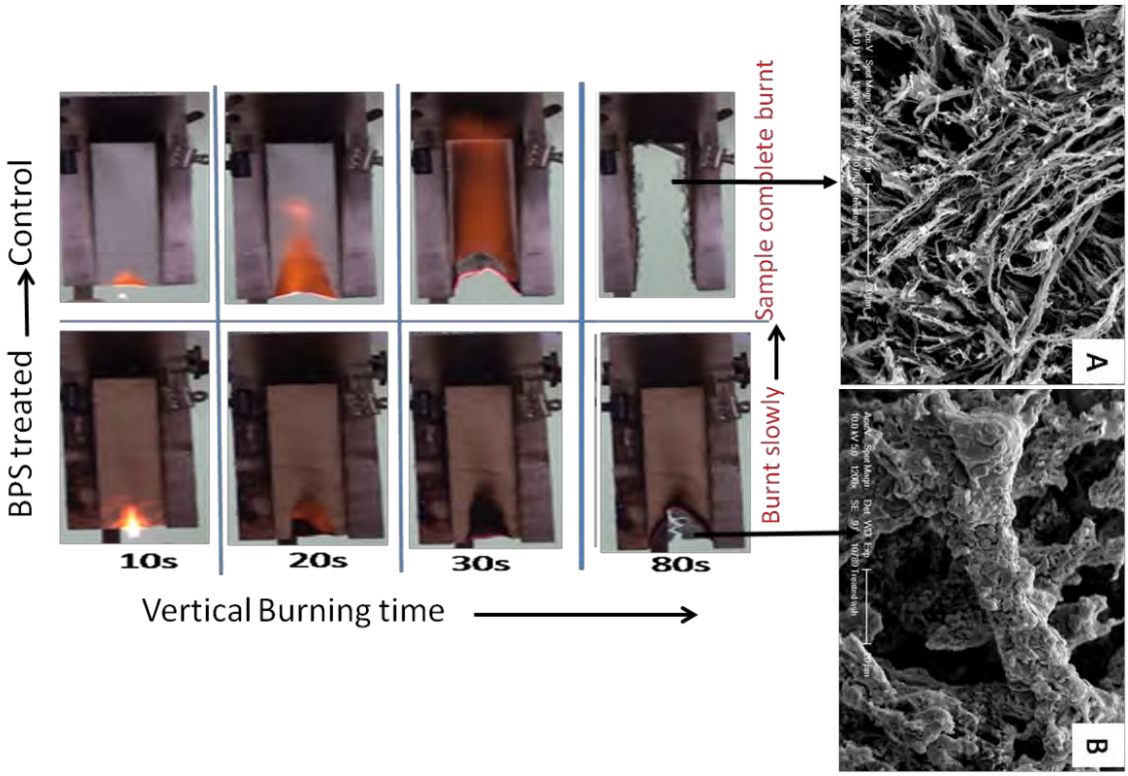

Fig 1: Pictorial depiction of vertical burning behaviour and char morphology of the control and BPS treated fabric

Source: [Basak et al 2016a, Basak et al 2015b]

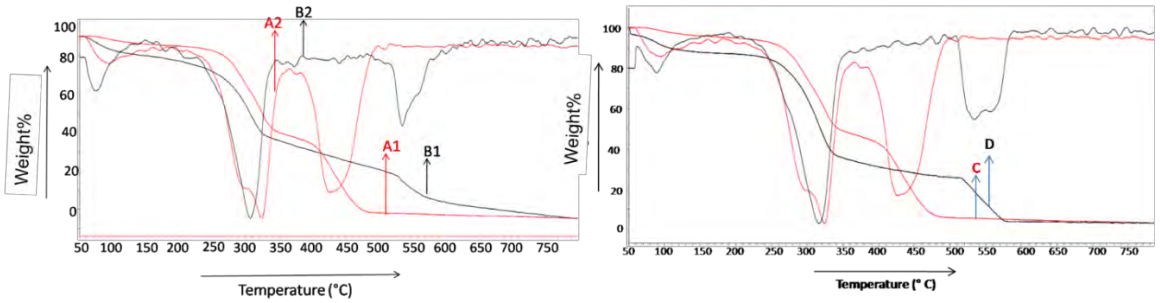

Fig 2: Control jute (A1), BPS treated jute (B1) and control cotton (C), BPS treated cotton fabric (D)

Source: [Basak et al 2016a]

Mechanism behind the fire retardancy

Flame retardancy with BPS biomolecule

The high thermal stability of the BPS treated cellulosic substrates has been attributed to the presence of polyphenolic tannin based compound, various inorganic metals and their salts (Ca^{+}, Mg^{+} , K^{+}, Si^{+}, KCl^{-}, Cl^{-}) which was evident from the energy dispersive X-ray (EDX), Secondary ion mass spectroscopy

(ToF-SIMS) and X-ray fluorescence (XRF) analysis [Basak et al 2015b]. The secondary ion mass spectroscopic analysis **of the BPS showed the presence of the following major ions/molecules at different mass units, such as H^- , C^- , CH^-, N^- , O^- , OH^-, F^-, Cl^-, PO_2^- , PO_3^-, KCl^- , Cl_2^- etc. It also showed the presence of various metal ions, such as Mg^+, K^+, Fe^+ etc. (Basak et al 2015a & 2015b).**

Flame retardancy with PRE biomolecule

Active components present in the pomegranate rind extract are responsible for the flame retardancy. Researchers have reported that branched chain polyphenolic compounds, tannin based components like gallic acid, ellagi tannin, natural colouring matter like anthocyanin, coumarin, nitrogen containing alkaloids, metallic salts and oxides present in the PRE get deposited on the fabric surface which lowered the pyrolysis temperature [Basak et al 2018, Basak et al 2019]. As a result, propensity of the pyrolysis of the treated fabric is more prominent and more char mass was produced. Also, chances of the liberation of the flammable gases (like levoglucosan, pyroglucosan etc.,) are reduced (as proved by GCMS analysis of the volatile species). Both these phenomena establish the condensed phase flame retardant action of the PRE treated cotton fabric.

Conclusion

The concept of using plant based bio-products for imparting fire retardancy to textile materials is novel and relatively a new thought. However, most of the finishes are not durable to home laundering. Further, some finishes produced a lot of smoke due to afterglow after extinguishment of flame. It is also not favourable due to the presence of a lot of carbon monoxide in the smoke. Further research should focus on reducing the add-on%, improving the wash durability and restricting the afterglow duration.

References

1. Alongi J., Blasio A.D., Cuttica F., Carosio F., & Malucelli G. (2014a) - Bulk or surface treatments of ethylene vinyl acetate copolymers with DNA: Investigation on the flame retardant properties - European Polym. J., 51: 112-119.
2. Alongi J., Carletto R.A., Bosco F., Carosio F., Blasio A.D., Cuttica F., Antonucci V., Giordano M. & Malucelli G. (2013) - Caseins and hydrophobins as novel green flame retardant for cotton fabrics- Polym. Degrad. Stabil., 99: 111-117.
3. Alongi J., Colleoni C., Rosace G., Malucelli G. (2012) - Thermal and fire stability of the cotton fabrics coated with hybrid phosphorous doped silica films - J. Therm. Anal. Calorim., 110: 1207-1216.
4. Alongi J., Cuttica F., Blasio A.D, Carosio F., Malucelli G. (2014b) - Intumescent features of nucleic acids and proteins - Thermochim. Acta., 591: 31-39
5. Basak S, Ali SW (2018). Fire resistant behavior of cellulosic textile functionalized with wastage plant bio-molecules: A comparative scientific report. Int J Bio Macromol., 114: 169-180.
6. Basak S, Ali SW (2019). Wastage pomegranate rind extracts (PRE): a one-step green solution for bioactive and naturally dyed cotton substrate with special emphasis on its fire protection efficacy, Cellu., 26: 3601-3623.
7. Basak S. and Ali S.W (2016a) - Sustainable fire retardancy of textiles using biomacromolecules - Polym. Degrad. Stabil., 133: 47-64.
8. Basak, S., Patil, P.G., Shaikh, A.J. and Samanta K.K (2016b) - Green coconut shell extract and boric acid: new formulation for making thermally stable cellulosic polymer J. Chem. Technol. Biotechnol., 91: 2871-2881.
9. Basak, S., Samanta, K.K., Chattopadhyay, S.K. and Narkar, R. (2015b) - Self- extinguishable ligno-cellulosic fabric made by banana pseudostem sap – Curr. Sci., 108: 372-383.
10. Basak, S., Samanta, K.K., Saxena, S., Chattopadhyay, S.K., Narkar, R. and Mahangade R. (2015a) - Fire retardant cellulosic textile using bannana pseudostem sap – Pol. J. Chem. Technol., 17: 123-133.
11. Bosco F, Carletto R.A, Alongi J, Marmo L, Blassio AD & Malucelli G. (2013) - Thermal stability and flame resistance of cotton fabrics treated with whey protein, Carbohydr. Polym., 94: 372-377.
12. Horrocks, A.R. (2011) - Flame retardant challenges for textiles and fibres – Polym. Degrad. Stabil., 96: 377-390.

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Quality Assessment of New Egyptian Cotton Varieties

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Abstract

This study carried out in Cotton research Institute, to assess the quality of Giza 94, Giza 95 and Giza 96. The new promising Extra-long and Long Staple Egyptian cotton varieties. The goal of new variety introduction is to replace a current variety with one that shows significant improvement in particular areas, notably yield, fiber quality, resistance to relevant diseases or pests. From the breeding perspective, new varieties should always be "available". From traders and ginners point of view, new varieties should be higher quality and lint percentage than the existing varieties. From textile and commercial perspective, availability will depend on the probable profit potential of new varieties. Giza 94, Giza 95 and Giza 96 produced to meet these requirements.
Giza 95 presented in Upper Egypt, Giza 94 presented in Delta Egypt, both of them belonging to Long-Staple category. While Giza 96 presented in North Delta as Extra-long staple variety. Long-Staple Egyptian cottons; the commercially grown Giza 90, and the newly introduced Giza 95 were spun into 40s and 50s counts. The commercially grown Giza 86, and the newly introduced Giza 94 were spun into 80s and 100s counts. The commercially grown Giza 92, and the newly introduced Giza 96 were spun into 100s and 120s counts. All the yarns were processed on Compact spinning system. Giza 95 found to be generally better to Giza 90 in single yarn strength, yarn unevenness, yarn neppiness and yarn hairiness. While Giza 94 with same fiber and yarn quality "in some characters" in compared with Giza 86. Giza 96 remarkable similar quality of Giza 92..

Keywords: *Egypt, Cotton, Gossypium barbadense, fiber quality, varieties*

Background

Egyptian cotton fibre quality must improve to remain competitive with other Extra Long and Long Staple producers due to increased demands for lightweight casual garments which require longer, stronger, and finer fibres. Improved cotton yields and fibre quality have continued to be realized through science-based plant breeding, particularly in Egypt and production systems with suitable climate and appropriate management inputs to maximize those improvements. The most significant challenge for cotton breeders has been to combine high yield with improved fiber quality, due to negative associations between yield and quality attributes in G. barbadense, El-Sayed, and Sanad, (2007).

Successful cotton improvement strategy must face both the quantity and year-to-year stability of fibre production to meet producer needs, while the enhancement of the magnitude and uniformity of certain fiber quality traits is needed for the technologically evolving yarn and textile industries. CRI is introduce new three cotton varieties in the last five years. As the new three varieties are clearly distinguished into three quality categories, they could be reviewed accordingly, ARC-CRI (2020).

Giza 96 (2015), Extra Long Staple variety. A cross between [(Giza 84 (Giza70 x Giza 51b)] X Stain PS62. In comparison with improved Giza 92 has a substantially higher fiber length (1.5 mm) and coarser fibers, but it has a slightly lower fiber strength (2 GPT). Giza 92 is highest cotton fibre strength between world cotton cultivars. Released to commercial production in 2015, it rose quickly to 909 hectares in 2015 comprising about 24 % of the ELS cultivated area, and by 2018 it reached its peak, when it covered 3820 hectares, i.e.51% of the ELS area. By year 2020, the total are of cotton production is reduced by almost 29%. In 2020 Giza 96 covered 1300 hectares and 31% of the total Extra Long staple cultivated area, "CATGO (2020) Figure 1.

Giza 94 (2016), Long Staple Delta cotton variety. A cross between Strain 10229 and Giza 86. It proved to be one of the most successful Egyptian cotton varieties. Giza 94 is of white lint and of the same fiber length as Giza 86 and high ginning outturn "GOT is 39.36%", consequently surpassed Giza 86 in cultivated are. Launched into commercial production in 2016, its acreage grew rapidly to 64538 hectares in 2020, about 64% of total cotton cultivated areas. Its acreage reached a peak in 2019 of 82532 hectares 58% of the total cotton cultivated area, (Figure 2).

Giza 95 (2015), Upper Egypt Long Staple varieties. A cross between [(Giza 83 (Giza75 x Strain 5844)] X Giza 80. Released for commercial production in 2015, when it was grown in about 1916 hectares, its

acreage was increased in the following years to 11800 thousand hectares, (Figure 3). However, its yield potential is the higher than Giza 90, its main merit is its earliness

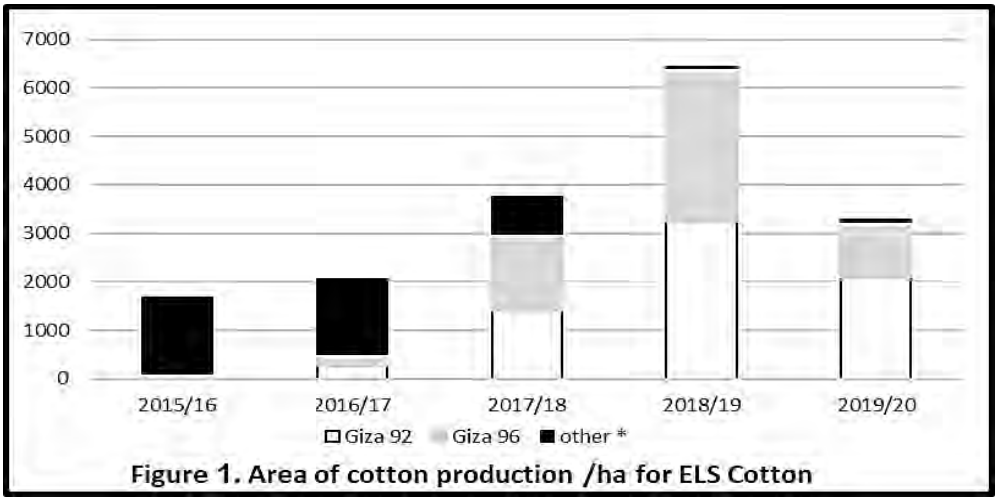

Figure 1. Area of cotton production /ha for ELS Cotton

Other; Extra Long Extra Fine, i.e., Giza 45, Giza 87 and Giza 93.

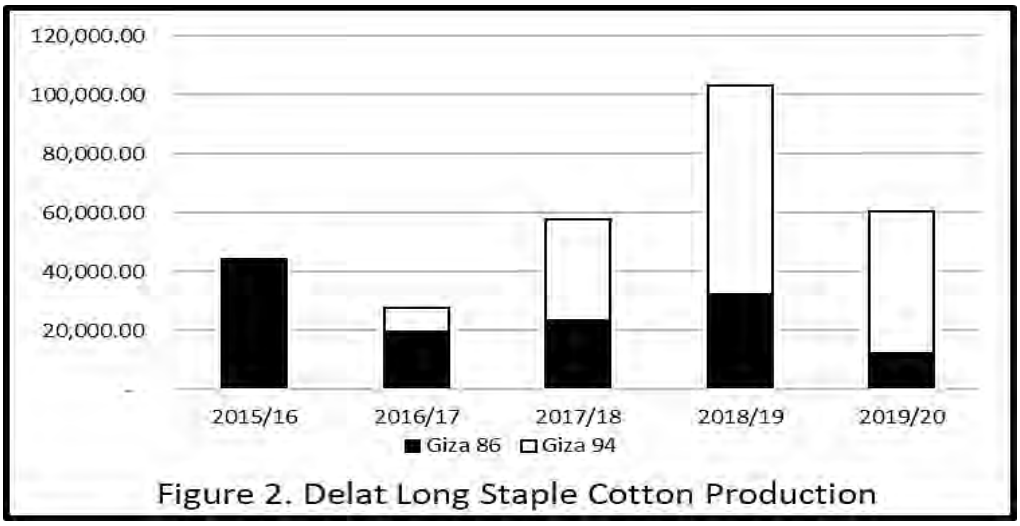

Figure 2. Delat Long Staple Cotton Production

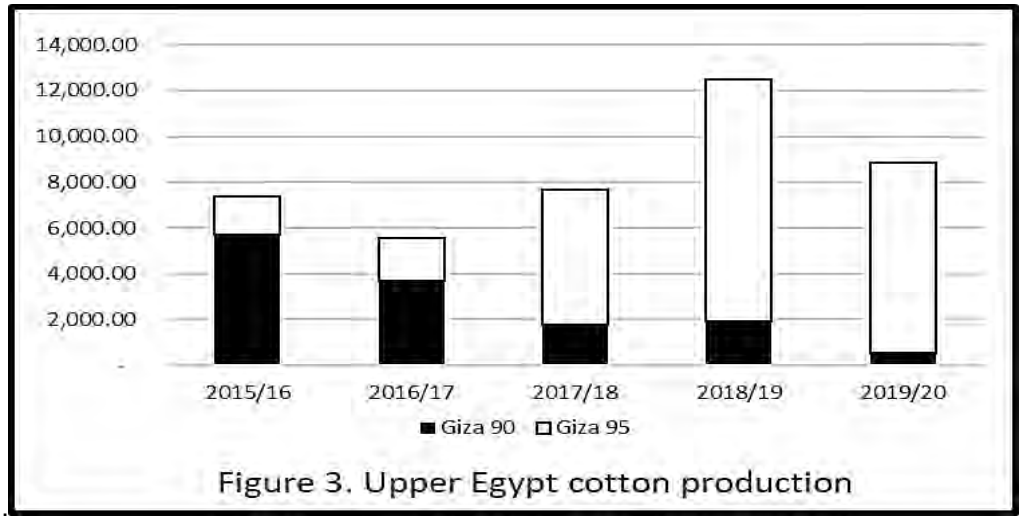

Figure 3. Upper Egypt cotton production

CRI is assessing the quality of the new varieties in their fibre and yarn properties to give the industry guidance or identifying its highest priority fibre quality needs.

In this study, the quality properties of the new commercial varieties Giza 96, Giza 94 and Giza 95 were compared with those of the established commercial varieties, Giza 92, Giza 86 and Giza 90 cotton varieties from the standpoint of quality level.

Results and Discussion

Upper Egypt Long Staple Cotton category

Fiber and yarn properties

Much breeding effort directed towards enhancing cotton fiber length to promote ring-spinning performance. Breeding for fiber quality has focused on increasing the length and strength, typically measured by High Volume Instrument. Egyptian cotton breeders have been very successful for increasing the Upper Half Mean Length and length uniformity and reached the maximum fiber length limit in the LS cotton category, i.e., 30.1 mm for Giza 95. Table 2 demonstrates the relation between fiber length and fiber strength in commercial and new cotton varieties.

Yarn quality

From the data presented in Table 3, it is possible to summarize the differences between the two varieties in the following points:

Regarding to compact ring spinning, ring spinning is the only system that offers a real challenge to Open End spinning for coarser and medium yarn count up to 40 Ne and it offers successful processing of cotton at significantly higher quality than Open End spinning.

Regarding to ring spinning system, single yarn strength of Giza 95 is similarly higher than that for Giza 90. Giza 95 yarns are generally more even than those of Giza 90. As a result, the coefficient of variation of single yarn tenacity is generally lower for Giza 95 than it is for Giza 90, or in other words, Giza 95 yarns are of more regular strength, a property that undoubtedly, lead to higher weaving efficiency when using Giza 90 yarns. The results show that, with increasing twist, yarn tensile properties increased in an approximately linear over the range of twist multiplier considered.

Raw material represents the largest item among operating costs for Egyptian spinning mills. Assuming, raw material represents, on average, 65 % of spinners' estimated operating costs, the minimum yarn count should be spun from Upper Egypt cottons is 50's. Below this count, the spinning mill will achieve lose. Generally, production of coarse or fine yarn counts from specific cotton depends on the demand and costumer desire.

Table 3. Compact Yarn properties, of 40 Ne and 50 Ne spun from Giza 95 and Giza 90.

	Giza 95	Giza 90	Giza 95	Giza 90	L.S.D. at
	40 Ne		50 Ne		0.05 Level
Count	39.6	39.4	50.7	50.9	
C.V. count	0.8	0.8	3.4	1.2	2.45
Tpi	26.0	25.8	29.6	29.4	4.06
C.V. Tpi	3.2	4.5	1.9	4.0	2.22
TM	4.2	4.1	4.2	4.1	0.08
Strength	22.4	22.5	22.9	24.0	1.46
C.V. Strength	6.6	7.9	7.4	9.8	1.05
Elon.	6.3	6.0	5.9	5.5	0.65
CVm	11.0	11.7	13.3	13.0	2.11
Hairiness	2.8	2.7	2.9	3.0	0.25
Thin places	1	1	2	4	2.77
Thik places	6	12	18	25	15.95
Neps	17	23	22	28	8.84

Delta Long Staple Cotton category

Giza 86, the commercial variety is of staple length about 33 mm. While, Giza 94 is of staple length to the upper limit defining the Long-Staple cottons (Table 2), or in other words the lower limit defining the Extra-Long Staple cottons, thus they are really a bridge between these two groups, their staple length

is about 33 mm. The fiber tensile properties, Micronaire reading and colour attribute are of similar trend of both varieties; since Giza 86 is one of Giza 94 parents.

Generally, as fiber length, fineness and strength are the most important factors in determining the spinning limit, and as the two cottons Giza 86 and Giza 94 are equal fiber length and strength, thus both commercial varieties are expected to be more suitable for finer counts.

Table 4 presents the tenacity and yarn imperfections of yarns in the function of linear densities of the yarns formed. With the decrease in linear density of the yarn, the tenacity of the yarn slightly increase.

Table 4. Compact yarn properties of 80 Ne and 100 Ne spun from Giza 86 and Giza 94.

	Giza 94	Giza 86	Giza94	Giza 86	L.S.D. at
	80s		100s		0.05 level
Count	80.5	80.9	101.0	99.0	
C.V. count	2.0	2.4	2.2	1.3	0.94
Tpi	37.5	37.9	43.0	42.0	5.52
C.V. Tpi	2.4	2.9	2.6	2.0	0.74
TM	4.2	4.3	4.3	4.2	0.09
Strength	25.3	26.7	26.7	26.6	1.31
C.V. Strength	8.6	9.6	8.6	8.5	1.02
Elon.	5.3	5.2	5.2	4.4	0.82
CVm	11.2	12.1	12.0	12.5	1.07
Hairiness	2.1	2.2	2.2	2.1	0.11
Thin places	8	4	12	11	7.04
Thik places	23	33	19	25	11.54
Neps	18	23	23	22	4.67

For the Long Staple cotton variety Giza 86 and Giza 94, single yarn strength of the compact yarn with a nominal linear density of 80 Ne compact spun yarn are 25.5 g/tex and 26.7G/tex.

The cost of Delta Long-Staple raw cotton purchased represents between 40% and 45% of the total selling costs of 100% cotton combed yarn count 80 Ne and 100 Ne, respectively. However, the main competitiveness of the Egyptian Delta Long-Staple cotton is only in combed yarn fine counts “more than 70’s” to achieve revenue with 5% Uster quality level, (Uster Statistics 2018).

Extra Long Staple Cotton category

Because of the importance of staple length, or in fact the length properties, in assessing the quality of the Extra-Long Staple cottons, it is useful to compare in some details the length properties of these two varieties, i.e. Giza 92 and Giza 96. Table 2 shows the fiber properties of the two ELS cotton varieties. The UHM of Giza 96 is around 35.1 mm. while, Giza 92 is shorter and still can’t reach the length of ELS category.

Table 5. Compact Yarn properties, of 100Ne and 120 Ne spun from Giza 96 and Giza 92.

	Giza 96	Giza 92	Giza 96	Giza92	L.S.D. at
	100s		120s		0.05 level
Count	98.4	101.1	123.2	122.0	
C.V. count	2.4	3.6	2.3	1.7	1.56
Tpi	42.7	38.2	48.8	48.4	9.94
C.V. Tpi	1.7	2.6	1.0	0.4	1.86
TM	4.2	3.8	4.4	4.4	0.58
Strength	27.3	27.5	27.7	27.8	0.29
C.V. Strength	4.8	6.4	7.8	8.6	3.27
Elon.	5.9	4.4	4.6	4.6	1.36
CVm	11.5	12.3	11.5	11.4	0.82
Hairiness	1.9	1.8	1.9	1.9	0.10
Thin places	12	15	14	21	7.59
Thik places	22	24	23	27	4.23
Neps	22	23	26	26	4.04

Giza 92 is of much higher fiber strength which compensated for its shortness and resulted in its having a higher level of yarn strength. In addition to the priorities, yarn manufacturers have asked for higher fiber strength. Enhancement of fiber strength through introgression from Giza 92 has been successful through Long-term advanced breeding efforts.

Table 5 shows yarn properties of the two cottons grown in north Delta. It is apparent that the mean value of tensile strength of Giza 92 is somewhat higher than that for the Giza 96. Single yarn strength of Giza 92 is substantially higher than Giza 96. This increase in strength is attributed partially to its higher fiber strength and fiber length uniformity. But the most effective factor is, undoubtedly, its substantially higher fiber strength. However, the Giza 92 could be regarded as comparable to Giza 96 and to be grown commercially so as to suffice the requirements of as exporting and local mills as possible.

The yarns spun on the compact spinning system are characterized by higher tenacity, higher elongation at break, smaller mass irregularity measured at short segments, and lower hairiness in comparison with yarns spun on the conventional ring spinning frame. As the Extra Long-Staple are used extensively in combed and fine spinning it is apparent that for combed 120s yarns; both Giza 96 and Giza 92 are of equal strength, However, it should be kept in mind that spinning these cottons at such very high counts (120s) is almost common in India.

Materials and Methods

The present study carried out in Egyyarn spinning Company, to assess the quality of new commercial varieties Giza 96, Giza 94 and Giza 95 in compared with those of the established commercial varieties, Giza 92, Giza 86 and Giza 90 cotton varieties. Two tons of each cotton variety taken and possessed into spinning line to produce winding cones. Fiber yarn properties were determined at the Egyyarn Spinning Laboratories, Egypt. All the Egyptian cotton varieties under study used to produce Compact combed yarns as shown in Table 6.

Fiber characteristics: The cotton fiber properties such as, fiber length, fiber uniformity, fiber strength, fiber elongation, fiber micronaire, fiber maturity, fiber reflectance fiber yellowness were evaluated as per standard, ASTM Committee, 1997a as shown in Table 7.

Yarn preparation: After evaluation of the physical characteristics of the raw material, each cotton lots opened and processed in spinning line. The counts for the yarn samples collected according to Table 1. The yarn samples thus, prepared were tested according to the standard methods as recommended by ASTM Committee (1997b).

Yarn Strength and elongation: yarn Strength and elongation were measured with Statimat ME-Textechno-Germany, (10 bobbins per sample and ten breaks per bobbin).

Yarn imperfections: This involved measuring the mass variation, yarn hairiness, thin and thick places and number of neps, per 1000 meters of the yarn using USTER 4. This was determined by measuring the capacity occurring as the yarn pass through the condenser and record in terms of total number of neps, thick places and thin places. 10 bobbins per sample and 1000m per bobbin of yarn on the USTER 4 equipment in accordance with the procedures of ASTM standards (ASTM Committee, 1997b as specified by American Society for Testing and Materials (ASTM) Standard D1425/D1425M.16.

Yarn count: Yarn count determined by the Lea Count Method according to ASTM standard (ASTM Committee, 1997c) on Uster Autosorter.

Yarn twist: The yarn twist was measured using the opposite twist method on the digital twist tester. In this way, the random error of mean value was less than 2%.

Table 6. Processing outline.

Cotton category	Upper Egypt Long Staple cotton	Delta Long staple	Extra Long Staple
Commercial variety	Giza 90	Giza 86	Giza 92
New variety	Giza 95	Giza 94	Giza 96
Spinning process	Blowroom, Carding, Drawing, Combing, Drawing, Roving, Compact Spinning and winding		
Yarn count (Ne)	40 Ne and 50 Ne	80 Ne and 100 Ne	100 Ne and 120 Ne
Twist multiplier		4.2	

Table 7. Fibre quality properties of Egyptian cotton varieties under study.

Cotton Varieties	Fiber length Parameters		Fibre Tenacity		Micronaire	Maturity Ratio	Value of color attributes	
	UHM (mm)	Uniformity %	Strength (G/Tex)	Elongation %			Brightness Rd %	Yellowness (+b)
Giza 92	33.7	86.5	46.1	6.3	3.7	0.93	77.7	7.9
Giza 96	35.2	86.4	44.8	6.4	4.0	0.93	75.7	8.3
Giza 86	33.3	86.7	44.0	7.3	4.3	0.95	75.9	7.9
Giza 94	34.1	86.5	43.7	7.4	4.3	0.94	72.3	7.6
Giza 90	29.3	85.3	36.0	7.2	4.3	0.92	62.8	11.3
Giza 95	30.1	85.7	36.9	7.1	4.2	0.93	67.4	11.8

References

1. Agricultural Research Center-Cotton Research Institute. 2020. Cotton cultivation and production bulletin-Arabic Version. www.caae-eg.com
2. ASTM Committee, 1997a. ASTM D4605-86 test method for measurement of cotton fibers by High Volume Instruments (HVI). ASTM Standard on Textile and Materials, American Society for Test and Mat., Philadelphia, USA.
3. ASTM Committee, 1997b. Standard method of test for evenness, imperfections and hairiness of yarn. ASTM Designation: D-1425-96, ASTM Standard on Textile and Materials, American Society for Test and Mat., Philadelphia, USA.
4. ASTM Committee, 1997c. ASTM D1907-07 standard test method for linear density of yarn (yarn number) by the skein method. ASTM Standard on Textile and Materials, American Society for Test and Mat., Philadelphia, USA.
5. Cotton Arbitration and Testing General organization. 2020. Weekly report No. 32. Arabic version. http://www.egyptcotton-catgo.org
6. El-Sayed, M. A. M. And Suzan H. Sanad. 2007. General investigation of fiber and yarn quality of Egyptian commercial and promising crosses cottons. Egyptian Journal of Agric. Res. Vol. 85, No. 5. 1829:1856, 2007.
7. Uster Statistics. 2018. Fiber and yarn quality.

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Valuable and Exploitable Bioactivities of Gossypol: A Multifunctional Compound from Cotton

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Abstract

Gossypol (C30H30O8), derived from cotton plant, is a polyphenolic secondary metabolite obtained through a radical coupling reaction of sesquiterpene dimer. It exists in two enantiomeric forms (+) gossypol and (-) gossypol with varying level of biological activities. The structure of gossypol consists of two aldehyde groups and six hydroxyl groups and contributes towards reactivity of the compound. Gossypol participates in various reaction viz. oxidation, ozonoloysis, methylation and Schiff base formation and results in the formation of various derivatives of the gossypol. The researchers have worked extensively to exploit the potential of gossypol and its derivatives in medical field. Gossypol has multifunctional biological activities including anti-cancerous, anti-oxidant, anti-fertility, anti-parasitic anti-microbial and anti-viral. The present article highlights the various bioactivity of gossypol which can be exploited in the field of pharmaceuticals, medicine and health sector.

Keywords: *Gossypol; bioactivities; cottonseed; medical application*

Background

The gossypol content in cottonseed varies from 0.002-6.64 % depending upon cotton variety, the region and the climatic condition under which the crop is grown (Gadelha et al. 2014). Gossypol is found in resin glands having size 50 to 400 µm. These glands are present in the plant petals, leaves, root-bark, bolls and seeds (Gardner et al. 1976). Gossypol is found prominently in the cottonseed kernels in a concentration range of 0.4-1.7% (Wang et al. 2009). It is formed in the cotton plant by dimerization of hemi gossypol molecules and it is also classified as dimeric-sesquiterpenoid (Stipanovic et al. 1986). Gossypol binds to amino acids, which lowers the nutritional value of the protein in the cottonseed meal. Hence, extracting the gossypol can improve the nutritional value of the cottonseed meal and at the same time gossypol can also be utilized as valuable source in the health and medical field. Before evaluating the bioactivity of any compound, the process of extraction is considered as an important step. Researchers have employed different techniques for extraction of gossypol from cottonseed meal viz. gland floatation technique (Boatner et al. 1949), heat treatment and pressure cooking (Gribbins et al. 1951), liquid cyclone process (Smith, 1971), super critical CO_2 extraction (Bhattacharjee, 2007), ultrasound-assisted extraction (Jia et al. 2009), membrane separation (Kuk et al. 1989) and adsorption (Kuk and Tetlow, 2005); but solvent extraction method (Dechary et al. 1952; Liu et al. 1981; Kuk et al. 2005; Pelitire et al. 2014; Singh et al. 2015; Li et al. 2016) is the adopted commercial technique. An acidified mixture of acetone, ethanol and water (70:20:10) solvent system at pH 4.5 was optimized in our laboratory for the extraction of the gossypol from the cottonseed meal and achieved a decent recovery of crude gossypol (87.3 mg/g on dry weight basis) (unpublished). Though gossypol accounts for a very less concentration in cottonseed kernels, the total production is more than 40000 tons annually in US alone (Wang et al. 2009). Apart from acting as toxic compound, it can play a role of highly functional molecule if the bioactivities exhibited by gossypol is exploited. These bioactivities comprises of anti-fertility activity, anti-oxidant properties, anti-cancer activity, anti-viral activity, anti-microbial activity, cholesterol lowering activity and anti-parasitic activity (Wang et al. 2009). These biological activities impart specific function to the gossypol and can give a potential application in various fields. The use of gossypol as insecticide, anti-feedent, toxicant and detoxicant are few discovered applications of the gossypol. The spectrum of various bioactivities of gossypol are displayed in the Fig. 1.

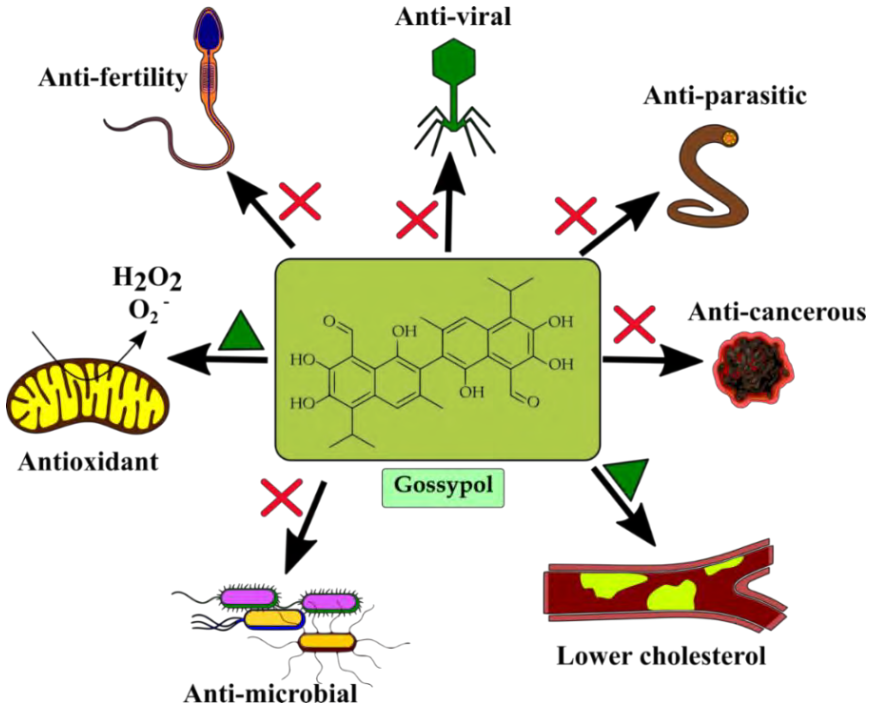

Fig. 1. Bioactivities of gossypol

Recently researchers are working in search of potential bio-activities of the gossypol and its applications.

Results and discussion

Bioactivities of gossypol

The reactivity of two phenolic aldehyde groups and six hydroxyl groups (Fig. 2) contributes towards its biological activities and these bioactivities are follows:

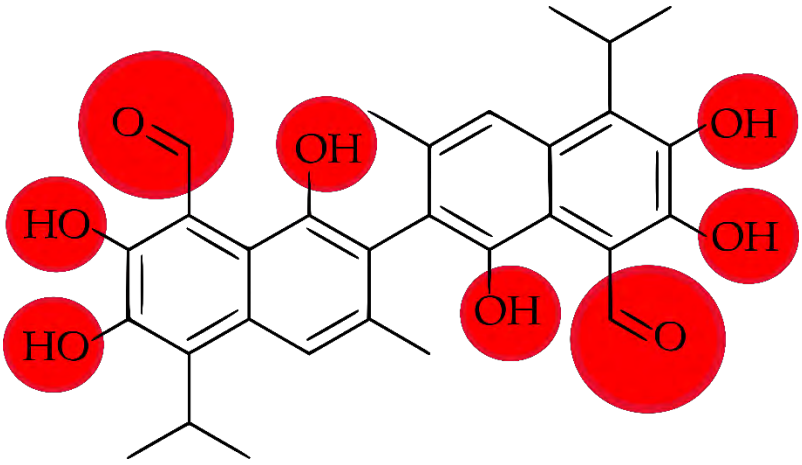

Fig. 2. Structure of gossypol showing two phenolic aldehydic groups and six hydroxyl groups

Anti-cancerous activity

About 4.6 million deaths are reported from various types of cancer every year. To combat the cancer, the use of plants or natural products derived from plants have been determined as beneficial. The hunt for natural anti-cancer agents among the plant products began in early 1950s and led to the discovery of vinca alkaloids (vinblastine, vincristine and podophyllotoxins) (Gorden et al. 2005). Gossypol is known to inhibit the development of carcinomas including pancreatic, liver, lung, skin and colon (Wang

et al. 2011). The anti-cancerous activity of gossypol was first identified by Tuszynski in 1984 where the gossypol induced cell death was found to be maximal in melanoma and colon carcinoma cells. Phenolic group of chiral gossypol and their analogues play a major role in anticancer activity, while the aldehyde group is not linked to the anticancer activity of gossypol (Zhang et al. 2009). Gossypolone has less therapeutic potential than the gossypol in antiproliferative study of breast cancer (Gilbert et al. 1995). Liang et al. 1995 reported anticancer activity enhances with new generation of gossypol.

Anti-fertility activity of gossypol

Gossypol can be considered as versatile anti-spermatogenesis agent (Ciereszko and Dabrowski, 2000). It is a very effective contraceptive agent with reversible mode of action and causes anti-fertility effects in males of mammalian species as well as humans with several dangerous side effects (Hoffer et al. 1988). Gossypol formic acid, gossypol acetic acid and gossypol are most commonly used forms of gossypol for clinical trials (Qian and Wang, 1984). The anti-fertility effects of gossypol appear to be due to its action on both testis as well as epididymis (Srivastava et al. 1989). Gossypol affects the sperm fertility, biochemistry of sperm and disturbs the morphology of testies and epididymis (Hoffer et al. 1988). Gossypol also reduces nuclear expression of androgen receptors in sertoli, myoid and leydig cell and DNA damage by decreasing oxidase activity at cellular level in sertoli cells.

Antioxidant activity of gossypol

Several diseases such as cardiovascular diseases, cancer, neural disorders, arteriosclerosis, skin irritations, and inflammations occur due to accumulation of high level of free radicals in tissue that produces reactive oxygen species. Free radicals are oxidizing biomolecule and make lipid bilayer susceptible to oxidative damage. Generation of peroxy and copper radicals causes lipid peroxidation in cells. Hence, cell produce a defence mechanism to reduce free radicals by making these available to oxidative enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, α- tocopherol and other protein transports, iron, copper, water and lipid soluble anti-oxidants. Oxidative enzymes convert hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) (El-Sharaky et al. 2010; Silakari, 2018; Araujo et al. 2019). Laughton et al. (1989) and Velasquez-Pereira et al. (1998) reported pro-oxidant and anti-oxidant activity of gossypol, their effects on free radical generation and lipid peroxidation is dose and time dependant process. Rhee et al. (2001) reported there is a no correlation between toxicity of both free and bound gossypol on anti-oxidant activity of gossypol after observing highly effective anti-oxidants in cooked meat. Gossypol derivative, Megosin in conjugation with PVP called as rometin, was studied for stability, anti-radical and anti-oxidant activity. Rometin prevents lipid peroxidation accumulation and formed lysophospholipids in mitochondrial membrane to reduce oxidative stress *in vivo* in rat liver. Its stability and anti-oxidant activity also increase *in vitro* and *in vivo* (Ionov et al. 2012). El-Sharakyet al. (2009) reported two strong oxidative damage inhibitors, gossypol acetic acid and gossypol iron complex for lipid peroxidation inhibition in male rats.

Anti-microbial activity of gossypol

Gossypol has been studied extensively for their anti-microbial properties. It has been reported to have anti-microbial nature against various strains bacteria and fungus. In case of bacteria, gossypol attacks cell wall of bacteria and initiates lytic reaction. It has been found that gram negative bacteria are more resistant to gossypol than gram positive bacteria (Keshmiri-Neghab and Goliaei, 2014). Researchers reported racemic mixture of gossypol inhibited growth (3 µg ml^{-1}) of *Edwardsiella ictaluri* which is causal agent enteric septicaemia of catfish (Yildirim-Aksoy et al. 2004). Gossypol may help in enhanced production of catfish by eliminating the deaths caused by enteric septicaemia.

Anti-viral activity

Gossypol exhibited anti-viral activity against a number of viruses; however, most of the reports showed its anti-viral abilities in enveloped viruses such as human immunodeficiency virus (HIV) (Yang et al. 2018), herpes simplex virus type 2 (Radloff et al. 1986), H5N1 (Yang et al. 2013), parainfluenza virus (Dorsett et al. 1975) and the influenza virus (Vichkanova et al. 1970). Gossypol and its derivatives viz. gossylic nitrile-1, 1′-diacetate, gossyliciminolactone, gossylic lactone inhibit the HIV replication at concentrations as low as 0.3 mM (Royer et al. 1991). Lin et al. (1989 and 1993) also have synthesized gossypol analogs exhibiting potential anti-viral activity against HIV-1 and observed that the (-) gossypol derivative (IC50=5.2 µM) was more potent in most biological evaluations than the (+) gossypol

derivative (IC50=50.7µM) against HIV. The inhibitory effect of gossypol on HIV-1 has been displayed in Fig. 4.

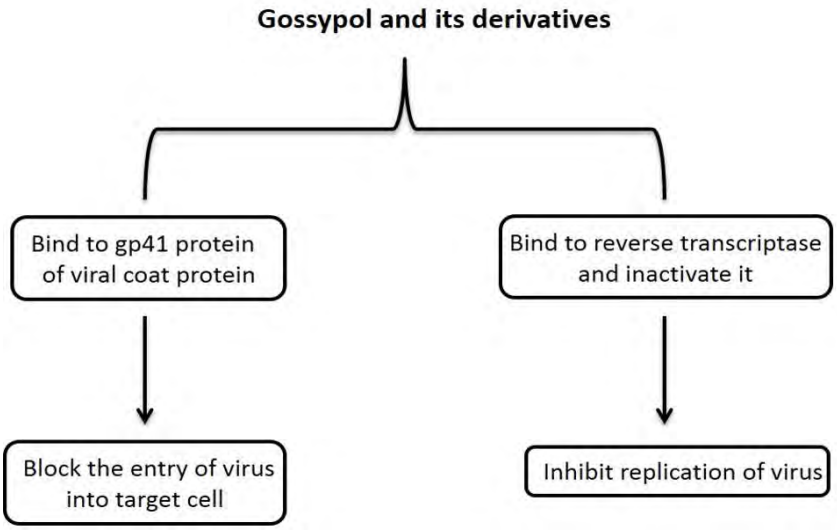

Fig 4. Mechanism of anti-viral action of gossypol and its derivatives in case HIV-1

Anti-parasitic activity of gossypol

Parasites are a heterogeneous group of unicellular and multicellular microorganism which can reside inside and outside of their host for its growth and nutrition. Parasites are categorized into three broad classes: protozoa, helminths and ectoparasites. Parasites got into their host through various means such as contaminated water, food, soil, blood, and even through sexual contact and vectors (Garcia et al. 2018). Numerous parasitic diseases in animals and humans become a sobering cause for millions of death worldwide (Cummings and Turco, 2009). Gossypol from the cotton plant and the tropical tree *Thespesia populnea* has anti-parasitic activity (Keshmiri-Neghab and Goliaei, 2014). Montamat et al. (1982) first time reported the *in vitro* anti-parasitic activity of gossypol, where he reported that 0.01 µm concentration of gossypol hampered the growth rate of *Trypanosoma cruzi a* causal agent of Chagas' disease. Later many reports have supported the anti-parasitic activity of gossypol (González-Garza et al. 1993).

Cholesterol-lowering activity of gossypol

Cholesterol is a fat-soluble compound and functions as a building block for cell membranes and hormones like estrogen and testosterone. High cholesterol directly correlates with higher levels of low-density lipoprotein in the blood, which is linked to serious health issues such as atherosclerosis and coronary heart attack (Wadhera, et al. 2016). A number of studies were conducted in the past which confirms the cholesterol-lowering activity of gossypol, and attribute the effect either by reduction in intestinal absorption of dietary cholesterol or by reducing the hepatic synthesis of low-density lipoprotein (Akingbemi et al. 1995). Achedume et al. (1994) conducted a study in male albino rats divided into two groups one with normal protein diet (25% crude protein), and another with protein-energy deficient diet (6% crude protein). Thestudy reported cholesterol-lowering activity of gossypol in both the group but decrease was more pronounced on normal fed group, with 75% reduction compared to 48% in energy-deficient group with respect to control (Achedume et al. 1994).

Conclusion

Gossypol is a polyphenolic aldehydic compound, and it has been studied for its versatile biological activities. Gossypol and gossypol derivatives play important role in many biological activities based on direct chemical reactions, inhibition of enzymes, and regulation of signal transduction pathways. Gossypol is a naturally available antioxidant which inhibits formation of reactive oxygen species and inhibits lipid peroxidation. Gossypol and gossypol derivatives may have therapeutic potential for different types of cancerous cells and enhances anticancer activity. Gossypol acts as a contraceptive agent and inhibits spermatogenesis in male and it also inhibits steroidogenesis in female **in vivo**. Bacteria, fungi and yeast are sensitive to gossypol due to their antimicrobial activity. Gossypol also shows anti-parasitic activity which are used *in vitro* to solve problem in many diseases. Gossypol also

helps to maintain cholesterol level. However, due to its toxicity, the application of gossypol is sometimes limited.

Acknowledgement

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References

1. Achedume AC, Nwoha PU, Adepoju AY, Abu KA, et al. Biochemical studies of the effect of gossypol consumption on cholesterol and residual glucose in fed and protein-energy deficient rats. Contraception.1994;50(4):391-395.
2. Akingbemi BT, Ogwuegbu SO, Onwuka SK, Oke BO, Aire TA, et al. The effects of protein malnutrition and experimental infection with Trypanosoma brucei on gossypol treatment in the rat: haematological and serum biochemical changes J Comp Pathol. 1995;112(4):361-371.
3. Araujo R, Oliveira A, Sousa F, Dourado L, Guimarães S, Silva W, Biagiotti D, Bayão G, and Sousa K, et al. Effects of cottonseed oil and ferrous sulfate on the performance and expression of antioxidant enzymes in broilers, Poultry Sci. 2019.
4. Bhattacharjee P. Supercritical carbon dioxide extraction of cottonseed oil. J Food Eng. 2007;79:892–898.
5. Boatner CH, Hall CM, Merifield AL, et al. Inventors; Heavy gravity liquid separation of cottonseed. U S Patent 2, 1949;482, 141.
6. Ciereszko A, Dabrowski K. In vitro effect of gossypol acetate on yellow perch (Percaflavescens) spermatozoa. AquatToxicol. 2000; 49:181-187.
7. Cummings R, Turco S. Parasitic Infections. In: Varki A, Cummings RD, Esko JD, et al. editors. Essentials of Glycobiology. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
8. Dechary JM, Kupperman RP, Thurber FH, Altschul AM, Laboratory RR, et al. Removal of gossypol from cottonseed by solvent extraction procedures, J Am Oil Chem Soc. 1952;29:339–341.
9. Dorsett PH, Kerstine EE, Powers LJ, et al. Antiviral activity of gossypol and apogossypol. J Pharm Sci.1975;64(6):1073-5.
10. El-Sharaky A, Newairy A, Elguindy N, Elwafa A, et al. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats, **Food** Chem Toxicol. 2010; 48:3354-3361.
11. El-Sharaky A, Wahby M, El-Dein MB, Fawzy R, El-Shahawy I, et al. Mutual anti-oxidative effect of gossypol acetic acid and gossypol–iron complex on hepatic lipid peroxidation in male rats. **Food** Chem Toxicol. 2009; 47:2735-2741.
12. Gadelha ICN, Fonseca NBS, Oloris SCS, Melo MM, Soto-Blanco B, et al. Gossypol toxicity from cottonseed products. Sci World J. 2014.
13. Garcia LS, Arrowood M, Kokoskin E, Paltridge GP, Pillai DR, Procop GW, Visvesvara G, et al. Laboratory diagnosis of parasites from the gastrointestinal tract.Clin Microbiol Rev. 2018;31(1):00025-17.
14. Gardner HK, Hron RJ, Vix HLE, et al. Removal of pigment glands (gossypol) from cottonseed. Cereal Chem. 1976 ;53:549–560.
15. Gilbert NE, O'Reilly JE, Chang CJG, Lin YC, Brueggemeier RW, et al. Antiproliferative activity of gossypol and gossypolone on human breast cancer cells. Life Sci. 1995;57(1):61–67.
16. González-Garza MT, Matlin SA, Mata-Cárdenas BD, Said-Fernández S, et al. Differential effects of the (+) -and (–) -gossypol enantiomers upon Entamoeba histolytica axenic cultures. J Pharm Pharmacol. 1993;45(2):144-145.
17. Gorden KB, Gorski KS, Gibson SJ, Kedl RM, Kieper WC, Qiu X, Tomai MA, Alkan SS, Vasilakos JP, et al. Synthetic TLR agonistsreveal functional differences between human TLR7 and TLR8. J Immunol. 2005; 174:1259–1268.
18. Gribbins GH. The reduction of free gossypol in cottonseed by pressure cooking. J Am Oil Chem Soc. 1951; 28:41–45.
19. Hoffer AP, Agarwal A, Meltzer P, Naqvi R, Matlin SA, et al. Antifertility, spermicidal and ultrastructural effects of gossypol and derivatives administered orally and by intratesticular injections, Contraception 1988;37:301-331.
20. Ionov M, Gordiyenko NV, Zukowska I, Tokhtaeva E, Mareninova OA, Baram N, Ziyaev K, Rezhepov K, Zamaraeva M, Stability and antioxidant activity of gossypol derivative immobilized on N-polyvinylpyrrolidone. Int J Biol Macromol. 2012 ;51:908-914.
21. Jia G, Zhan Y, Wu D, Meng Y, Xu L, et al. An improved ultrasound assisted extraction process of gossypol acetic acid from cottonseed soapstock. AIChE J. 2009;55(3):797–806.
22. Keshmiri-Neghab H, Goliaei B. Therapeutic potential of gossypol: an overview. Pharm Biol. 2014;52(1):124-128.
23. Kuk MS, Hron RJ, Abraham G, et al. Reverse osmosis membrane characteristics for partitioning triglyceride-solvent mixtures. J Am Oil Chem Soc. 1989;66(9):1374–1380.
24. Kuk MS, Tetlow R, Dowd MK, et al. Cottonseed extraction with mixtures of acetone and hexane. J Am Oil Chem Soc. 2005;82(8):609–612.
25. Kuk MS, Tetlow R, et al. Gossypol removal by adsorption from cottonseed miscella. J Am Oil Chem Soc. 2005;82(12):905–909.
26. Laughton MJ, Halliwell B, Evans PJ, Robin J, Hoult S, et al. Antioxidant and pro-oxidant actions of the plant phenolicsquercetin, gossypol and myricetin: effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. Biochem Pharmacol. 1989; 38:2859-2865.
27. Li Z, Smith KH, Stevens GW, et al. The use of environmentally sustainable bio-derived solvents in solvent extraction applications-A review. Chin J Chem Eng. 2016; 24:215–220.
28. Liang XS, Rogers AJ, Webber CL, et al. Developing gossypol derivatives with enhanced antitumor activity. Invest New Drugs 1995; 13:181–186.
29. Lin TS, Schinazi R, Griffith BP, August EM, Eriksson BFH, Zheng D-K, Huang L and Prusoff WI-I. Selective inhibition of human immunodeliciency virus type 1 replication by the (-) but not the (+) enantiomer of gossypol. Antimicrob Agents Chemolher. 1989; 33:2149-2151.
30. Lin TS, Schinazi RF, Zhu J, Birks E, Carbone R, Yikang S, Prusoff WH, et al. Anti-HIV-1 activity and cellular pharmacology of various analogs of gossypol. Biochem Pharmacol. 1993;46(2):251–255.

31. Liu FK, Jou SY, Jung LY, et al. A new method of detoxification of cottonseed by means of mixed solvent extraction. J Am Oil Chem Soc.1981;58(2): A93–A96.
32. Montamat EE, Burgos C, Gerez de Burgos NM, Rovai LE, Blanco A, et al. Inhibitor action of gossypol on enzymes and growth of Trypanosoma cruzi, Science. 1982; 218:288-289.
33. Pelitire SM, Dowd MK, Cheng HN, et al. Acidic solvent extraction of gossypol from cottonseed meal, Anim Feed Sci Technol. 2014;195:120–128.
34. Qian S, Wang Z. Gossypol: a potential antifertility agent for males. Annu Rev Pharmacol. 1984; 24:329-360.
35. Radloff RJ, Deck LM, Royer RE, Vander Jagt DL, et al. Antiviral activities of gossypol and its derivatives against herpes simplex virus type II. Pharmacol Res Commun. 1986 ;18(11):1063-73.
36. Rhee K, Ziprin Y, Calhoun M, et al. Antioxidative effects of cottonseed meals as evaluated in cooked meat. Meat Sci. 2001; 58:117-123.
37. Royer RE, Mills RG, Deck LM, Mertz GJ, Vander Jagt DL, et al. Inhibition of human immunodeficiency virus type I replication by derivatives of gossypol. Pharmacol Res. 1991;24(4):407-12.
38. Silakari O. Key heterocycle cores for designing multitargeting molecules. 2018 Elsevier.
39. Singh S, Sharma SK, Kansal SK, et al. Extraction of Gossypol from Cottonseed. Rev Adv Sci Eng. 2015;4:301–318.
40. Smith AK. Practical considerations in commercial utilization of oilseeds. J Am Oil Chem Soc. 1971; 48:38–42.
41. Srivastava A, Gupta G, Setty B, et al. Studies on mechanism (s) of antifertility action of gossypol in rat and hamster, Contraception. 1989; 39:337-355.
42. Stipanovic R, Stoessl A, Stothers JB, Altman DW, Bell AA, Heinstein P, et al. The stereochemistry of the biosynthetic precursor of gossypol. J Chem Soc Chem Commun. 1986; 2:100–102.
43. Tuszynski GP, Cossu G. Differential cytotoxic effect of gossypol on human melanoma, colon carcinoma, and other tissue culture cell lines. Cancer Res. 1984; 44:768 – 71.
44. Velasquez-Pereira J, Prichard D, McDowell L, Chenoweth P, Risco C, Staples C, Martin F, Calhoun M, Rojas L, Williams S, et al. Long-term effects of gossypol and vitamin E in the diets of dairy bulls. J Dairy Sci. 1998;81:2475-2484.
45. Vichkanova SA, Oĭfa AI, Goriunova LV, et al. Antiviral properties of gossypol in experimental influenza pneumonia. Antibiotiki. 1970; 15(12):1071-3.
46. Wadhera RK, Steen DL, Khan I, Giugliano RP, Foody JM, et al. A review of low-density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality. J Clin Lipidol. 2016;10(3):472-489.
47. Wang X, Duan X, Yang G, Zhang X, Deng L, Zheng H, et al. Honokiol crosses BBB and BCSFB and inhibits brain tumor growth in rat 9L intracerebralgliosarcoma model and human U251 xenograftglioma model. PLoS One. 2011; 6(4):e18490.
48. Wang X, Howell CP, Chen F, Yin J, Jiang Y, et al. Chapter 6 Gossypol-A Polyphenolic Compound from Cotton Plant. Adv Food Nutr Res. 2009;215–263.
49. Yang J, Chen G, Li LL, et al. Synthesis and anti-H5N1 activity of chiral gossypol derivatives and its analogs implicated by a viral entry blocking mechanism. Bioorg Med Chem Lett. 2013;23(9):2619–2623.
50. Yang J, Li LL, Li JR, Yang JX, Zhang F, Chen G, Yu R, Ouyang WJ, Wu SW, et al. Synthesis and biological evaluation of water-soluble derivatives of chiral gossypol as HIV fusion inhibitors targeting gp41. Bioorg Med Chem Lett. 2018;28(1):49-52.
51. Yildrim-Aksoy M, Lim C, Dowd MK, Wan PJ, Klesiu PH, Shoemaker C, et al. In vitro inhibitory effect of gossypol from gossypol-acetic acid and (+) - and (-)- isomers of gossypol on the growth of Edwardsiella ictaluri. J Appl Microbiol. 2004;9:87-92.
52. Zhang L, Jiang H, Cao X, Zhao H, Wang F, Cui Y, Jiang B, et al. Chiral gossypol derivatives: Evaluation of their anticancer activity and molecular modelling. Eur. J. Med. Chem. 2009;44(10):3961–3972.

Value and Cotton Supply Chain Analysis for Cotton and Textile Industries in Egypt

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Abstract

In Egypt, the objective to develop the textile sector is an important pillar of the country's Vision 2025 strategy, which is why the sector is promoted by a comprehensive set of policies and institutions. The overall goal is to improve the vertical integration of the sector, modernise the industry, increase exports, and create employment.
The home textile global value chain (GVC) represents a classic example of a buyer-driven value chain that is characterised by decentralised, globally dispersed production networks, coordinated by leading businesses that control the activities that add value to products, such as design and branding, but often outsource all or most of the manufacturing process to a global network of suppliers. Exporting to the EU or the US is highly demanding and-depending on the specific value chain - margins are often very low. Integration with the EU and US value chains can nonetheless have important learning effects and support manufacturers in exporting to markets with higher margins. Egypt has, in addition, not only market opportunities in key consumption markets, but also in Middle east and North Africa regional markets with high growth potential and in the relatively large local market.
This article review highlights that the Egyptian cotton textile sector has many strengths and the potential to increase exports as a regional and global supplier of textile products. The major competitive advantages of the sector include:

- *local availability of high-quality ELS cotton, which is a key input for many high-value textile products;*
- *Almost vertically integrated value chain with a large textile and apparel sector;*
- *Well-developed infrastructure;*
- *Flexible production lines accommodating large as well as small orders;*

Keywords: *Cotton , Value chain, Egypt*

Background

The Egyptian Textile and Apparel sector has been a key sector in the country's industrial development strategies in the last decades (Loewe 2013; ETDS 2015). In recent years, the 6,742 enterprises in the Textile industries sector employed more than half a million people, contributed roughly with 3% to 4% of Egypt's GDP6 (MOTI 2019). 7 The sector accounts for approximately 20% to 30% of employment in Egypt's industrial sector, which in turns accounts for 26% of total employment in the country (ibid.). In 2018, Textile and Apparel exports, excluding raw materials, amounted to almost US$3 billion, representing roughly 12% of total exports (UN Comtrade 2019). The T&A sector is thus one of the most important industrial sectors in terms of value added, employment, and exports (MOTI 2019).

A large share of the high-quality cotton, however, continues to be exported without further processing (UN Comtrade 2019). This is a major problem given the potential value addition of further processing compared to the export of raw cotton is estimated to range from 50% (fine count yarns) to 470% (fine shirting) (MOTI 2019). In the case of home textiles, the value added is estimated to be roughly 106% in case of terry towels and 135% in case of bed sheets (ibid.). Increasing value addition of Egyptian cotton should thus be a key concern for policymakers promoting the Egyptian T&A sector.

Results and discussion

The Egyptian textile value chain (Figure 1) includes: (i) cotton production and ginning, producing long and Extra-long staple Egyptian cotton; (ii) a large textile sector producing yarn and woven or knitted fabrics that are used as inputs by apparel manufacturers, domestic textile companies (which tend to be vertically integrated) and carpet manufacturers. The manufactured products are either consumed locally or exported. Cotton, yarn, and fabrics are also exported to varying degrees. Imported inputs play an important role for the sector, and include different varieties of cotton or cotton yarn and fabrics that are not locally available, such as short staple cotton.

Egyptian Cotton Production

The Egyptian cotton cultivation started in 1818 and over a long period of time it has played a major role in the Egyptian economy, development of the local textile industry development, and is a crucial element in the future of the textile industry in Egypt, (Figure 3).

The Egyptian cotton has long and Extra Long distinguished fibre properties, which made Egyptian cotton in the last 120 years a worldwide renowned natural fibre to manufacturers of textiles, and a world recognized brand to consumers worldwide.

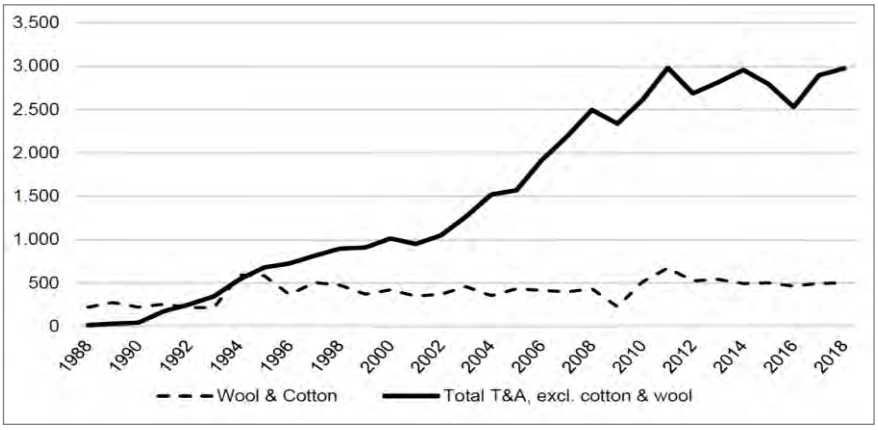

Figure 1: Egyptian Textile and Apparel export development 1988–2018, in US$ million Source: UN Comtrade 2019 (WITS)

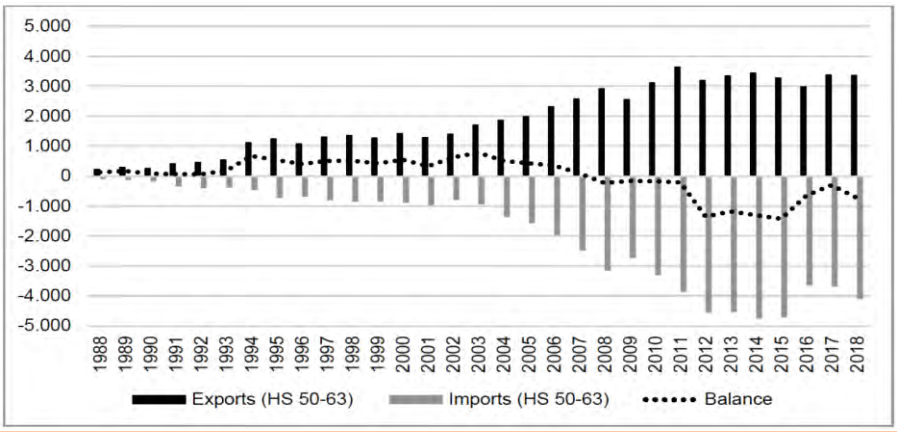

Figure 2: Global Egyptian Textile and Apparel trade balance 1988–2018, in US$ million

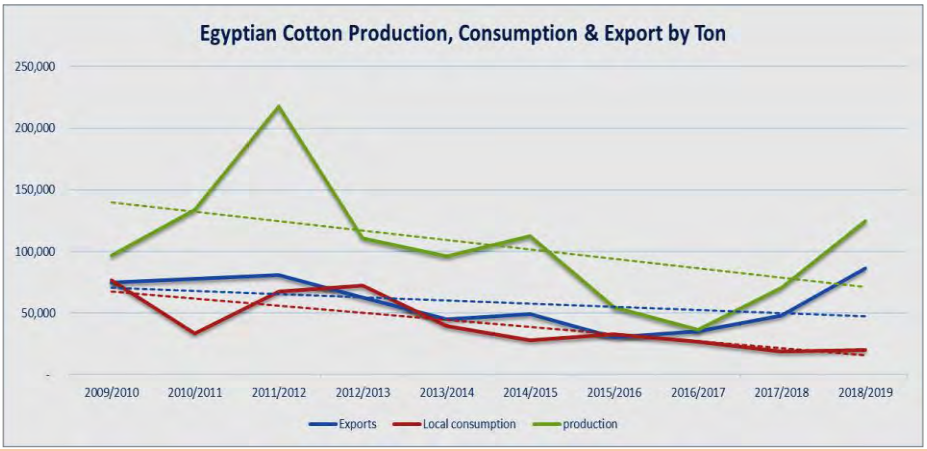

Figure 3: Egyptian cotton production, Consumption and Export

Two key factors have added to the declining production of conventionally grown cotton in recent years. First, growing labour costs have limited collection rounds or harvesting. Second, harvested fibres have been contaminated by fibres from cotton strings used in transportation processes. Egypt's share in the global long and extra-long staple cotton production dropped to only 13% in 2017/18, from 40% in 2004/05, due to these factors, plus the increasing competition in long and extra-long staple production, particularly from American Pima cotton from the US, Xinjiang cotton from China, and India. False labelling and counterfeiting is also a problem for the Egyptian Cotton brand, which the Cotton Egypt Association oversees. The government has recently intensified efforts to increase areas under cultivation.

Until recently, the cotton marketing system used to be regulated by an indicative price system. Indicative prices were based on weight, while quality aspects were not taken into consideration. Traders, ginners and other buyers purchasing cotton at the indicative prices experienced losses, if quality of the cotton purchased turned out to be inferior. Under the new market price system that will be introduced in 2020, public collection rings collect cotton from farmers on a mandatory basis, then a public tender process sells the cotton. A 70% share of the agreed-upon selling price go directly to farmers. Then, after ginning, the remaining 30% of the price is paid to farmers after a quality evaluation. The new system aims at rewarding quality with a premium and driving small traders that exploit farmers out of the market.

Organic Cotton Production in Egypt

The first organic cotton project was started in the year 1990 in Egypt. There were an estimated 182,876 farmers growing certified organic cotton in 2017/18, spread across 19 countries. Organic cotton was planted on a total of 356,131 hectares of certified land. An interesting trend seen in India is that farmers significantly increased the proportion of organic certified land used to grow cotton (as opposed to other organic crops) from 45 to 70 percent in 2017/18.

Organic cotton is not genetically modified, as the use of genetic engineering is prohibited in organic agriculture. Organic cotton is grown without pesticides and insecticides, furthermore organic cotton seeds are not genetically modified. Organic cotton cultivation involves methods and materials that have a low impact on the environment and reduces the use of toxic and persistent pesticides and fertilizers. Organic cotton farming replenish and maintain soil fertility. It builds up biologically diverse agriculture.

The production of organic cotton only plays a minor role in Egypt, with 219 ha organically certified land and 19 organic farmers concentrated particularly in the Nile Delta region (OCMR 2019: 35). During the 2017/18 season, only 287 tonnes of organic cotton fibre were produced, representing 0.2% of global organic cotton output. Compared to 2017, organic cotton production has dropped by 34%. Currently, 1,043 ha of land are in transition to organic cotton cultivation, (Helga et al. 2020). This means expanding the land used for organic cotton cultivation by a factor of five.

A government programme managed by Cotton Research Institute also started to cultivate organic cotton seeds in 2019, making them available starting in 2020. Under the programme set up by a new ministerial decree, contract farmers and private companies grow the seeds on government land. However, an overall government strategy defining targets for organic cotton cultivation is still missing. Local processing of organic cotton is limited and typically does not extend beyond the weaving or knitting stage. Only a few companies manufacture finished organic garments.

Textile Industries

Egypt has a large domestic textile production industry, which however lacks vertical integration and is highly import dependent. Approximately a third (35%) of Egypt's textile production is from 25 state-owned enterprises, which collectively employ 65 thousand people (USDA 2018). The government is currently modernising and partially privatising these state-owned firms. An additional 2,500–3,500 private companies are responsible for the rest of production, but only an estimated 150 to 250 of all textile companies engage in exporting (Figure 4).

The industry has been in decline for 30 years, with many businesses, particularly the state- owned ones using outdated equipment. Investments in spinning, weaving, knitting and dyeing has been very limited, which is why the sub-sector is characterised by various bottlenecks and lack of vertical integration (Figure 5). The major upstream bottlenecks in the following areas are:

(i) man-made fibre;
(ii) cotton yarns;

(iii) (Woven fabrics, in particular narrow woven fabrics, denim from non-Egyptian cotton, and light-weight cotton fabrics used as input in the local apparel sector);
(iv) man-made fibre knitted fabrics and very fine Egyptian cotton fabrics, such as those used for fine underwear and polo shirts;
(v) quality and supply of dying, finishing and printing for woven and knitted fabrics (MOTI 2019).
These bottlenecks contribute to the textile sector's high dependency on imports that emerged particularly after the deregulation in the 2000s and the growing export orientation. Most domestic textile production uses imported short and medium-staple cotton varieties, which are often imported at higher costs compared to spinners due to non-tariff barriers and smaller orders. Common Egyptian products using short staple are denim items and T-shirts (USDA 2018). The textile sector also depends on imports of man- made fibres due to the limited local production. ELS cotton is usually used for home textiles and high-end clothing.

In 2016/2017 local spinning mills were the main consumers of Egyptian cotton, accounting for about half of all use (USDA 2018). Demand from local spinners for cotton increased as they have been receiving higher returns on exports since the devaluation of the Egyptian pound and the growing demand for Egyptian cotton. Recent increases in domestic cotton production are expected to lower prices and create more domestic demand from the textile industry. The top-five global buyers of Egypt's high-content cotton yarns (85% cotton or over) are Italy (36%), Turkey (20%), Portugal (6%), Germany (6%) and France (5%), while almost all of Egypt's low cotton-content yarns (below 85% cotton) go to Turkey (96%) (UN Comtrade 2019).

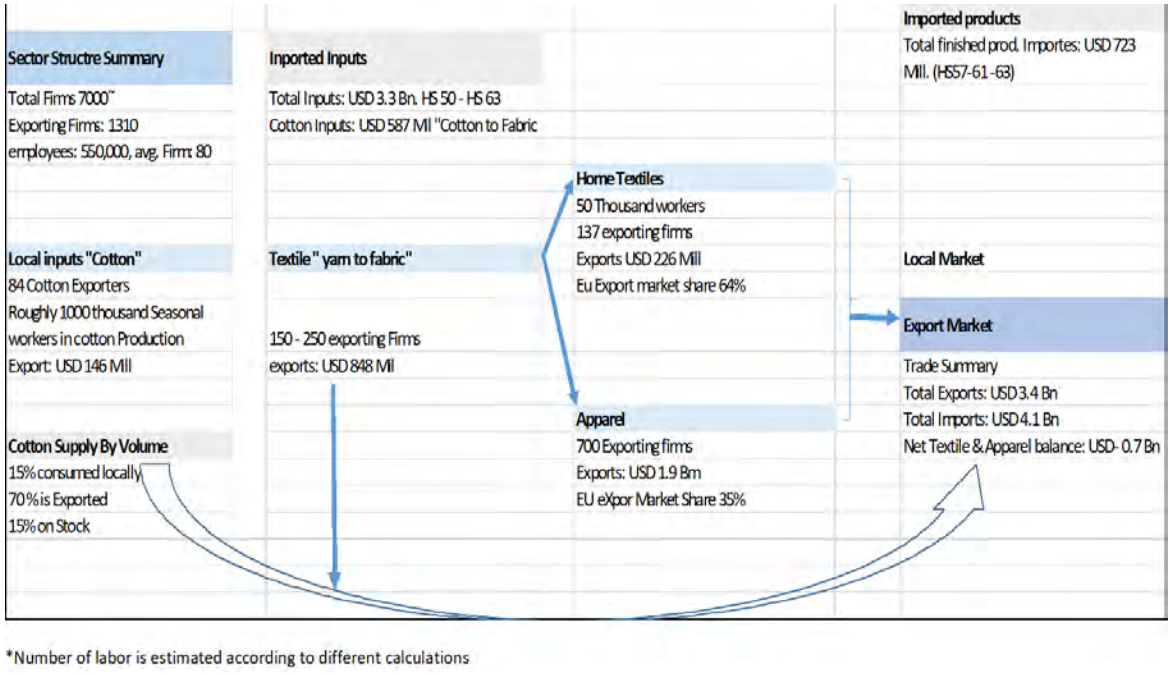

Figure 4 Overview of Egyptian textile Supply chain

Home Textiles

The Egyptian home textile sector is an important sub-sector of the textile and Apparel sector. The sub-sector includes different kinds of companies, including large state-owned enterprises as well as small to large privately owned firms-mostly Egyptian-producing home textiles for the local and export markets. The majority of companies in the textile sector are small and medium-sized enterprises (SMEs), but large companies dominate the sector in terms of production volume and export value. An estimated 137 home textile companies currently export (Figure 4). Micro firms or household manufacturers are sometimes involved in labour-intensive parts of the production and linked to SMEs that have outsourced this processing step. Household manufacturing plays a particular important role in the carpet segment of the sector. Overall, the home textile sub-sector employs an estimated 48 thousand workers.

The home textiles sector, in particular bed linen and terry towel producers, are major local buyers of Egyptian cotton, depending on their vertical integration: cotton lint, yarn, or fabrics. Products that use a high share of Egyptian cotton tend to be exported due to their high prices. Despite Egypt's competitive

advantage in terms of the local availability of Egyptian cotton, the sector to some extent lacks vertical integration, given the lack of local production of cotton yarn and wide-width woven fabrics. Many home textile producers import cheaper cotton inputs for specific components, such as embroidering, or the whole product to supply the local or low-mid segment export markets.

In 2018, Egyptian exports of home textiles (HS 63) and carpets (HS 57) amounted to US$227 million and US$325 million, respectively (Figure 5), representing roughly 2% of total exports (UN Comtrade 2019). The key exported products include various linen and terry towels (HS 6302, representing 75% of total home textile exports), curtains (HS 6303, 12%) and various furnishing articles (HS 6304, 9%) (UN Comtrade 2019).

Export growth of both categories is on a negative trend since 2010. The situation is particularly challenging in the case of carpets. Home textiles, in contrast to apparel, are mostly exported to the EU (64%), in particular Germany (14%), Italy (12%), France (12%), United Kingdom (9%) and the Netherlands (7%), followed by the US (22%) and Saudi Arabia (4%).

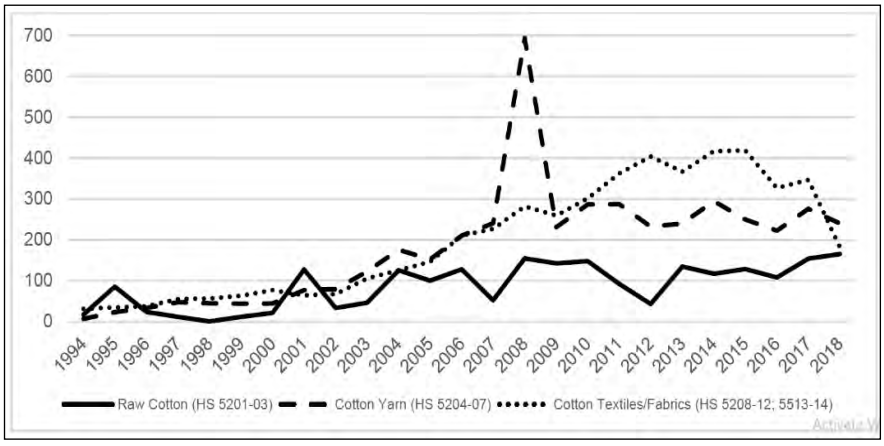

Figure 5: Egyptian cotton product exports 1994–2018, in US$ million

Carpets, on the other hand, are mostly exported to the US (43%), followed by the EU (30%). The largest EU importers of Egyptian carpets are Germany (6% of total Egyptian carpet exports), the UK (5%) and Italy (4%). As has been indicated in the previous sections, both categories lost market share in the EU in recent years, but EU market development has been particularly challenging for exports of Egyptian carpets. The high share of the EU and the US market for Egyptian home textile exports is strongly connected to Egypt's preferential market access to the respective markets, (Figure 6).

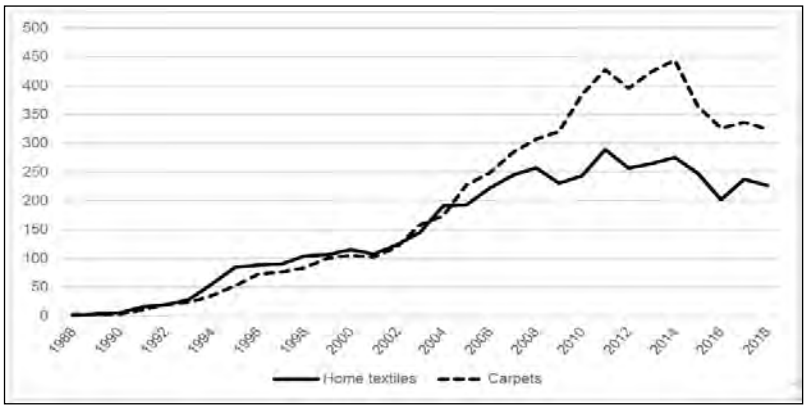

Figure 6: Egypt's home textiles and carpets exports 1988–2018, in US$ million

Table 1 showed summary of comparative and competitive position. Table 2 presents a more detailed analysis of Egyptian home textile and carpets exports to the EU. The value of home textile exports to the EU was on a decreasing trend in the last decade, but exports are currently recovering. The most important export products to the EU include terry towels and linen for various purposes (bed, kitchen, bathroom, etc.) made of cotton. In general, Egypt is highly price-competitive in the key home textile products that are currently exported to the EU, even though there are some differences between products (Figure 7).

Table 1: *Egypt's comparative and competitive position*

Overview	
Key competitors	• Turkey, given its integration into similar regional EU value chains and market segments as well as large market share in the EU, but also other global suppliers, such as China, India, Pakistan, etc.
USP	• Egyptian cotton is a key USP for home textile products, however, its exploitation is limited due to limited local processing, processing and re-exporting by other supplier countries, such as India, false labelling. • Flexible production: Egyptian companies can produce large as well as small orders, which combined with the country's geographical proximity to Europe and efficient transportation infrastructure give them a competitive edge in the EU market.
Reputation with global buyers	• EU buyers have indicated having a partially negative reputation of Egypt as a sourcing location, due to challenging communication, delayed deliveries, etc. The price-quality ratios of the products, however, were considered to be comparatively high. Buyers not currently sourcing from Egypt had a more negative view than those that do.
Comparative Position	
Location	• Egypt is located close to the EU, allowing for an integration into regional EU value chains. The infrastructure and logistics are well-developed and facilitate this integration (Airport Cairo and Port Said are nearby): most production is shipped by sea and takes maximum 8 days to the EU. Air transport is only used for samples or very small orders.
Natural Resources	• Egypt produces large quantities of ELS cotton, however, only few apparel products use ELS as an input. • A large share of Egyptian cotton is exported raw in cotton bales or as semi-finished products. The sector lacks vertical integration. • Production of short-staple varieties is insignificant.
Labour	• Egypt has abundant available labour, however, given the low wages, Egyptians prefer to work in other sectors, forcing the apparel sector to deal with limited availability of skilled workers and high turnover. • Wages in Egypt are low (see Annex: Figure 19), in particular when compared to Egypt's key competitor, Turkey. However, some Asian suppliers have even lower wages, which is a threat to the prevailing business model targeting mass export markets, particularly in the US and EU. Real wages do however erode with high inflation, which will eventually increase wage pressures. The current low-wage low-productivity business model of many companies might thus become untenable in the medium term. • Egypt has abundant available labour, however, given the low wages, Egyptians prefer to work in other sectors, forcing the apparel sector to deal with limited availability of skilled workers and high turnover.
Technology	• Technological capacities and capabilities are in general comparable to competitors in the low to mid-market segments. • The level of technology varies widely among companies, between the private sector and state-owned enterprises, as well as among SMEs.
Infrastructure	• Egyptian infrastructure and logistics are well developed, facilitating integration with regional EU value chains. Transportation logistics are well developed (see above: Location).
Business-enabling environment	• Governmental policies and institutions play a crucial role in the Egyptian T&A sector. Key in Egypt's Vision 2030 strategy, T&A is also supported by the sector-specific Vision 2025 strategy. Given the large number of organisations involved, the institutional policy setup is rather comprehensive and targets various bottlenecks in the sector. In general, however, public support institutions (i) lack the requisite financial means and policy space, (ii) suffer from gaps in knowledge and expertise, and (iii) overlap in activities, complicating the distribution of competences and tasks. • Private supporting services do exist, for instance in certification, but appear underdeveloped in a number of activities, including design services. Companies thus source these services internationally.
Competitive Position	
Companies (SMEs)	• Many SMEs have export experience, but face a variety of bottlenecks in: (i) international communication standards; (ii) export marketing; (iii) financial management (pricing and costing); (iv) knowledge about EU legal and buyer requirements, such as certification, technology (e.g. ICT and integrated systems), communication standards, CSR related issues, etc.; (v) design capabilities; (vi) technological standards (e.g. outdated machinery), and (vii) product quality.
Demand conditions	• Egyptian companies' exports have been decreasing for a variety of internal and external reasons, such as the domestic political environment and the strong competition from abroad. The EU is the most important export market (64% of total home textile exports), followed by the US (22%). Egyptian firms lost market share in the EU over the last decade. • The large internal market, which also offers slightly higher margins, is key for non-exporting companies, but also important for most exporting firms. Though expanding, given the high population growth, the internal market suffered from the uncertainties related to Egypt's political situation in recent years.
Supporting industries	• Transport and logistics aside, supporting industries are in general not well developed in Egypt. Thus, inputs for textile and apparel production have to be imported to a large extent. This relates to agrochemicals for cotton cultivation, such as fertilizers and pesticides, chemicals for textile production, such as dyes, textile and apparel machinery, as well as accessories, such as zippers, buttons, etc.. • Finance is a major bottleneck, in particular for SMEs, but there are also bottlenecks in the textile segment, which limit local sourcing possibilities for (i) man-made fibre; (ii) cotton yarns; (iii) woven fabrics, in particular narrow-width woven fabrics, denim from non-Egyptian cotton and light-weight cotton fabrics used as input in the local apparel sector; (v) knitted fabrics, meaning man-made fibre knitted fabrics and very fine Egyptian cotton fabrics, such as those used for fine underwear or polo shirts; (v) quality supply of dying, finishing and printing for woven and knitted fabrics. • Skilled labour availability is a challenge, in particular with regard to design.

Table 2: Egyptian home textile exports to the EU, in US$ million

	2000	2005	2010	2015	2016	2017	2018	% change 2015–2018
Total Egyptian Home Textile exports	115.33	192.77	243.66	247.61	202.11	237.62	226.61	-8
Exports to the EU	84.02	126.65	156.18	145.80	126.15	141.19	144.40	-1
% of total HT exports	*73*	*66*	*64*	*59*	*62*	*59*	*64*	
Top Home Textile exports to the EU by product								
Bed linen, table linen, toilet linen and kitchen linen of all types of textile materials (excl. floor cloths, polishing cloths, dishcloths and dusters) (HS 6302):	69.74	110.32	135.29	127.01	112.94	114.98	120.90	-5
Toilet linen and kitchen linen, of terry toweling or similar terry fabrics of cotton	24.29	43.40	46.03	33.51	32.89	35.53	32.42	-3
Bed linen of cotton (excl. printed, knitted or crocheted) (HS630231)	17.04	28.70	37.58	36.51	30.07	26.41	28.29	-23
Toilet linen and kitchen linen of cotton (HS630291)	14.11	13.30	20.90	19.36	19.67	17.51	22.57	17
Table linen of cotton (excl. knitted or crocheted)	10.49	12.67	10.35	12.52	10.96	12.04	11.46	-8
Printed bed linen of cotton (excl. knitted or crocheted) (HS630221)	2.37	5.11	4.51	4.77	4.02	6.83	10.58	+122
Bed linen, knitted or crocheted (HS630210)	0.04	1.66	0.47	0.35	1.42	3.58	2.71	+674
Various curtains, almost exclusively of synthetic fibre	2.31	0.73	4.01	8.99	5.28	12.01	11.44	+27
Sacks and bags used for the packing of goods (HS6305)	1.82	7.54	6.54	2.50	2.11	2.42	2.92	+17
Textiles; made up articles (not elsewhere specified.	0.39	0.18	1.63	0.58	0.30	0.67	0.78	+34
Total Egyptian carpet exports	104.82	227.53	384.96	363.76	326.64	334.99	313.67	-14
Exports to the EU	31.13	75.38	142.50	110.58	88.51	95.17	94.36	-15
% of total carpet exports	*30*	*33*	*37*	*30*	*27*	*28*	*30*	
Top carpet exports to the EU by product								
Carpets, not tufted or flocked, e.g. kilim	14.67	27.35	77.68	59.93	44.39	44.68	50.08	-16
Carpets, tufted needle punched	9.75	35.23	45.70	41.22	33.41	40.51	39.02	-5

Source: UN Comtrade 2019 (WITS)

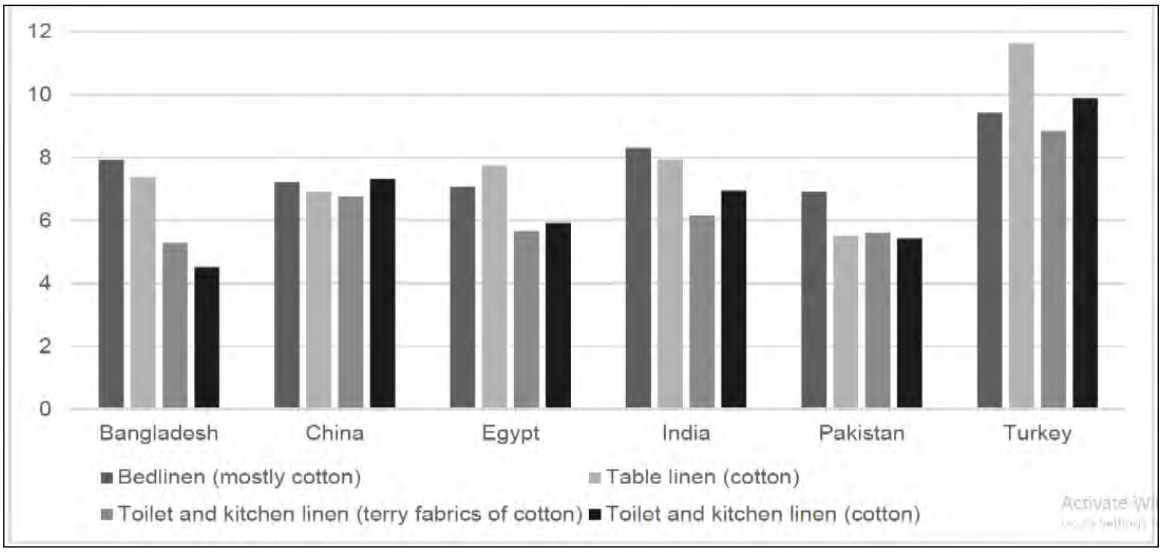

Figure 7: Supplier's unit value of key Egyptian HT export products to EU 2018, in US$/kg)

The strong decline of non-printed, knitted or crocheted bed linen exports in recent years was largely absorbed by the growth of other bed linen product exports printed, knitted or crocheted. The decrease in exports to the EU has been particularly strong for carpets. The key exported carpet products include handwoven, such as kilim, but also a variety of other carpets. A major obstacle for increasing traditional handwoven carpet exports is that they lack an USP, since design is usually inspired by generic 'Persian' or similar styles.

Integrated Manufacturing

Egypt has the only fully vertically integrated textiles industry in the Mediterranean and Middle East region, with the entire production process-from the cultivation of cotton to the production of yarns, fabrics, and ready-made garments-carried out domestically. This is an advantageous situation only

equaled by major textile & clothing producing countries like China, India, Pakistan, Turkey and Brazil. Egypt's ambition should be to operate at the same level.

It is clear from the installed capacity data as shown above that Egypt has not built up its primary textile industry to the same levels of capacity as direct competitors like Turkey, India, Pakistan, ... Low labour cost countries like Bangladesh and Viet Nam, originally focusing on low labour costs for garment production are now moving into completing the textile chain, Bangladesh mainly in knitting, Viet Nam probably focusing more in weaving.

For Egypt, integrated linkages between the upstream cotton suppliers and downstream textile sellers are critical determinants for the sector's long term competitiveness. And this is where the Egyptian textile industry is lacking capacity and quality: the primary textile industry is mainly in the public sector with obsolete equipment and very little integration into the value chain. The future textile policy of Egypt should aim to

- On the one hand moving up the quality ladder in garments, vertically integrating the garment production value chain with the domestic usage of Egyptian cotton, improve design and quality, and, Defend leadership in low-end garmenting by establishing strong brands at both country and supplier levels.
- Restructuring the domestic textile industry by upgrading and gradually privatizing spinning and weaving mills and integrating the domestic supply of high-quality cotton should be the priority for restructuring.

Government policies

The textile industries sector has been at the core of Egypt's industrial development strategies in the last decades. Since the 1990s, the government has increased efforts to promote private- led development. The current sector-specific strategy is part of Egypt's Vision 2030, which emphasises the government commitment to improve the competitiveness of the knowledge-based economic sectors led by the private sector. The vision for the textiles sector aims at both meeting domestic demand and increasing exports to turn Egypt into a key global Textile industries supplier.

Table 3: Textile strategy vision 2025 (preliminary version)

Objective 1: Increase productivity and quality of Egyptian cotton	**Objective 5: Egypt textile value chain integration and export growth**
a. Egyptian cotton yield to reach 9–10 kintar per feddan	a. Increase Egypt textile value chain annual exports to US$12 billion
b. Contamination-free and higher-quality fibre properties	b. Imports not to exceed US$6 billion
c. Grow production to fulfil domestic and export demand	c. Establishing showrooms and distribution hubs in the EU and US markets
d. Establish high yield dessert mechanical plantation of Egyptian cotton varieties	d. Stimulate export demand for Egyptian cotton products
e. Create a sustainable, traceable, and transparent Egyptian cotton blockchain	
Objective 2: Develop management and labour skills, quality and productivity	**Objective 6: Development of innovation and research and development**
a. Upgrading all textile higher and technical education, and training curricula	a. Creation of an Arabic textile knowledge hub
b. Educate and train sustainable highly skilled, and productive human resources (around 1 million people)	b. Encourage start-up innovation projects
c. Increase productivity and efficiency of textile manufacturers	c. Create Egyptian cotton cluster retail stores
d. Enable the creation of a social and environment compliance industry	d. Development of Egypt textile industry 4.0
Objective 3: Enhance Egypt textile value chain growth and industrial development	**Objective 7: Develop institutional support, monitoring, and policy reform (enabling environment)**
a. Create a verified and accurate mapping of the textile sector	a. Implementing the Supreme Council recommendations
b. Commitment of US$6 billion investments in downstream and upstream sub-sectors	b. Establish a National Fibre Policy
c. Development of 20 million m^2 of textile industrial parks ('textile cities') and attraction of FDI	c. Increase textile industry registered Authorized Economic Operator (AEO) – Custom Clearance
Objective 4: Strengthen SMEs	d. Governmental institutions capacity building to support textile strategy implementation
a. Increase textile value chain formal SMEs	
b. Create SME and micro cluster networks through digital platforms	
c. Develop 500 world class competitive SMEs throughout Egypt textile value chain	

Egypt's textile sector strategy, Vision 2025, which has yet to be approved, consists of seven policy objectives (Table 3). Major cornerstones of the strategy include: value-chain integration and export growth (Objective 5), the promotion of human capital, the provision of industrial land and infrastructure, improvements in logistics to decrease lead times, the facilitation of raw materials and production inputs, reforming government policy, and the restructuring and modernisation of state-owned companies. Expansion of theindustrial base by developing and enhancing the local industry's competitiveness, as well as the establishment of 'textile cities' are also key pillars of the strategy (Table 3). The government has also increased efforts to try to slow and reverse the cotton sector's decline.

The overall goal regarding Objective 5 is to become the leading exporter in the Middle East and North Africa region, focusing on supplying medium and high-value products to the world's largest retailers and manufacturers with reliable and agile delivery through a fully integrated value chain (ETDS 2015). The government identified supplying primary textiles and fabrics to LMICs as the main market opportunities, in addition to a niche market strategy to supply finished products to the US and Europe. The textile industry development strategy seeks to tackle a major obstacle of the Egyptian textile industry sector: the limited vertical integration of various segments of the value chain. The plan is to improve vertical integration by attracting foreign direct investment into primary textiles and fibre production, including with a special fund to incentivise new investments. The strategy also aims to increase exports of intermediary products, especially products that use Egyptian cotton as an input, but the overall goal is to increase the exports of finished products. The strategic goal is to increase exports to US$12 billion by 2025, which means a very ambitious quadrupling of the current export value*: US$3.1 billion in 2018,* (UN Comtrade 2019).

The key product opportunities we have identified from government sources, documents, and other government-related actors interviewed during field research include (both genders where not specified):

(i) Knitted garments: T-shirts, underwear, shirts (especially polo shirts), men suits and women dresses, sportswear;
(ii) Woven garments: denim products and trousers, men shirts;
(iii) Children's wear: knitted and woven articles, in particular high-end branded babywear using Egyptian cotton;
(iv) Home textiles: bed linen, terry towels, carpets.

Governmental institutions

Table 4 presents a summary of the key institutions framework of the Egyptian cotton and Textile Industries sector.

Table 4: Mapping of the Egyptian governmental institutions

		Raw Material	Spinning	Weaving	knitting	Dyeing Woven	Dyeing knitted	Yarn Dyeing	woven apparel	knitted apparel	towels	bed linen	carpets	Technical
Ministry of Trade & Industry	Alcotexa													
	Interior Cotton Trade Committee													
	CATGO													
	TCF "Textile Consolidation Fund"													
	Technology Centers													
	Fashion Design Centre													
	IMC "Industrial Modernisation Centre"													
	ITC													
	IDA													
	Industrial Control													
	EDA "Export Development Authority"													
	Import/Export Authority (GOEIC)													
	PVTD "Productivity and Vocational Training Department"													
	EOS (Egyptian Organization for Standardization and Quality)													
	MSMEDA "Micro, Small and Medium Enterprise Development Authority"													
	Export bank													
	Egypt Expo convention													
	Export guarantee													
	National Quality Institute													
	Intellectual Property Unit													
	Egyptian Accreditation Council													
	Federation of Egyptian Industries – Ready-Made Garments Chamber & Textile Industries Chamber													
	QIZ Unit													
	Competition Authority													
	Social Fund for Dev.													
	Cotton Egypt Association													
	Export Dev. Fund													
Ministry of Agriculture	Cotton Research Institute													
	Cotton Council													
	Cooperatives farmers													
	Agriculture Import Dept.													
Ministry of Finance	Custom Authority													
	Sales Tax Authority													
	Corporate Tax Authority													
Ministry of Public Business Sector	Holding Textile Company													
	Holding Insurance													
Ministry of Investment	GAFI													
	Public/Private Free Zone													
	Stock Market / Financial Regulation													
	Donor Development Programs													
Ministry of Manpower	Labor Law/Safety Employement													
	Labor Unions													
	Foreign Expats Approval													
Minister of Higher and Tech Edu.	Textile Universities													
	Intermediate textile college													
Ministry of social Insurance	Labor Insurance													
Ministry of Interior														
Ministry of Internal trade														
Ministry of Petroleum														
Ministry of Electricity and Power														

Utility and Energy Prices

Tables 5, 6 and 7 presented the utility and energy prices in Egypt based on April 2020

Table 5: Drinking and sanitation tariff (USD/M3)

Drinking water and sanitation tariff for FY 2019/2020 (US/m3)							
							Sanitation
		Non Domistic					Percentage of water tariff
	Service	Commercial	Industrial	Touristic	Other		
	0.21	**0.23**	**0.28**	**0.29**	**0.57**		**98%**
	Water tariff in Border governorates (Sinai / Red Sea / Matrouh)						**Sanitation**
		Unified Water Tariff					**Percentage of water tariff**
		0.008					**50%**

Table 6: Prices of petroleum product

Prices of petroleum products for FY 2019/2020					
Commercial LPG	**Gasoline 95**	**Gasoline 92**	**Gasoline 80**	**Kerosene**	**Automotive Natural Gas**
4.14 US/Cylinder	0.54	0.47	0.4	0.35	0.22US/m3
	Fuel Oil				
Fixed prices for food Industries, electricity and Sement					
	248.4 US/Ton				

Table 7: Prices of Electricity

Purpose of use / voltage	Low voltage (380 V)	High voltage (66, 33 KV)	Ultra High voltage (132, 220 KV)	How to apply
Cost (USD/ KW-month)		**0.03**	**0.025**	The wattage is applied on the basis of maximum load per subscriber every three months
Off -Peak (USD/Kw		**0.07**	**0.067**	The usage time tariff is applied according to the smart meters program and the peak period is 4 hours, which is determined by the Ministry of Electricity and Energy
Peak (USD/ kwh)		**0.064**	**0.061**	
Average price of energy (USD/ kwh)	0.079	0.097	0.092	If not available, the power price is applied

Cotton and Textile Sector Donor Project in Egypt

UNIDO – The Egyptian Cotton Project

UNIDO engages with the Egyptian T&A sector through a project known as The Egyptian Cotton Project (2017–2019), funded with US$1.5 million from the Italian Agency for Development Cooperation. The project's full name is 'From cotton seeds to clothing: Enhancing the sustainability, inclusiveness and value addition of the cotton value chain in Egypt'. The project leverages the Cotton for Life initiative41 that aims to promote fully transparent, eco-friendly and socially responsible cotton value chains.42 The Egyptian Cotton Project43 aims to improve the economic, social, and environmental performance of the chain through the promotion of organic and non-contaminated cotton, local value addition (local content and processing), and local linkages. The project also aims to improve the quality, innovation, and scientific knowledge in the area of cotton. In February 2019, UNIDO also launched a Better Cotton Initiative (BCI) pilot project under the framework of the Egyptian Cotton Project. The UNIDO project on the circularity of Egypt's textile value chain is also currently in process of security clearance at MTI. The project tries to tackle the waste produced in the textile industry by using it more efficiently, including with better valorisation. The pilot project will focus on denim scrubs, and introduce mechanical recycling to produce high-quality denim fabrics.

BCI – Better Cotton Initiative

BCI is a global initiative promoting sustainable cotton production through the Better Cotton Standard

System: a holistic approach covering environmental, social and economic aspects of sustainability.45 Training for Egyptian cotton farmers through this programme started in 2019. The project is implemented by UNIDO in collaboration with the MTI, MALR, and local and international private sector stakeholders. In the pilot phase of the project, approximately 5,000 smallholder cotton farmers will be involved. Whether a direct BCI Programme in Egypt shall be implemented, will be decided upon evaluating the pilot phase.

ITC – GTEX Project

The Global Textile and Clothing (GTEX) programme (2018–2021) of the International Trade Centre (ITC) aims to increase export competitiveness in five selected countries: Egypt, Morocco, Tunisia, Kyrgyzstan and Tajikistan. GTEX is funded by the Swiss State Secretariat for Economic Affairs (SECO; CHF 9.8 million) and the ITC (CHF 0.6 million). In-kind country contributions amount to CHF 1 million. The programme envisages to increase employment and income along the value chain. The project goal includes two key outcomes: First, to improve the business environment and the performance of trade and investment supporting institutions in the sector. Second, to improve the competitiveness of 35 SMEs. In Egypt, the programme started in the fourth quarter of 2019. Its budget amounts to roughly CHF 2 million. The institutional partners on the Egyptian side include the RMG Export Council, the Textile Export Council, the Egyptian Exporters' Association (ExpoLink), TrainTex, and the Fashion Design Centre.

USAID – SEED Project

USAID's Strengthening Entrepreneurship and Enterprise Development (SEED) Project is a five-year programme that will end in late 2019, early 2020. The budget is US$22.9 million. The project focuses on strengthening the development of micro, small and medium enterprises (MSMEs) and entrepreneurship through: i) stimulating entrepreneurship and innovation; ii) enhancing formalisation of private enterprises; iii) improving financial and non-financial services to MSMEs; iv) integrating MSMEs and entrepreneurs in progressive value chains; and v) addressing enabling environment policy reform initiatives. Special attention is given to youth and women. SEED provides technical assistance, capacity building, and training to business development services and financial service providers. According to the project website, SEED has so far created 3,328 jobs, helped 8,712 SMEs to access financial services, and more than 200 MSMEs to integrate into value chains.

ILO – Better Work Programme

The Better Work Programme is a collaboration between the ILO and the International Finance Corporation (IFC), aiming to: (i) improve the working conditions in the garment industry; (ii) to ensure the compliance with workers' labour rights; and (iii) to boost the competitiveness of the apparel sector. The programme includes direct cooperation with factories towards complying with ILO core labour standards. Currently, the programme is active in 1,600 factories in seven countries.49 Between July 2017 and December 2018 a pilot of Better Work's services benefiting 30 factories was implemented in the Egyptian garment sector and related industries. However, the Better Work pilot was not extended. The ILO states that an important consideration in this decision was Egypt's lack of conformity in law and practice with international labour standards, as determined by the ILO's supervisory bodies.

Another ILO project in Egypt is the ACCEL Africa project (Accelerating action for the elimination of child labour in supply chains in Africa'). The four-year programme (November 2018–November 2022; €23.5 million) is being implemented in six African countries and focuses on various supply chains.51 In Egypt, cotton growing is identified as a high-risk activity, in which reportedly millions of children are involved each year.52 However, to date, no robust statistical evidence is available, a deficiency the project will address.

EBRD – European Bank for Reconstruction and Development

Egypt has been a member of the EBRD since 1991 and qualified as a recipient country in 2015. In Egypt, the EBRD focuses on four objectives: i) supporting the competitiveness of Egypt's private sector; ii) improving the quality and sustainability of Egypt's public utilities through private sector participation; iii) supporting Egypt's Green Economy Transition; and iv) strengthen governance. The EBRD focuses on : (i) access to national or international consultants; (ii) training for domestic consultants; and (iii) connecting SMEs to domestic banks for finance.

References

1. Loewe, M. (2013): Industrial Policy in Egypt 2004-2011. In: DIE, Discussion Paper 13/2013. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2294507 (11.11.2019).
2. ETDS (2015): Egypt' Textiles Development Strategy "Vision 2025". National Council on Textiles, Ministry of Trade and Industry, presentation by Shady Amin, December 2015, http://texmedclusters.eu/En/home_46_28 (11.11.2019).
3. MOTI (2019): Data and documents provided by MOTI and related institutions during field research. Egypt, 2019.
4. UN Comtrade (2019): UN Comtrade Database. New York, NY: United Nations Statistics Division.
5. OMCR (2019): Organic Cotton Market Report 2018. In: Textile Exchange, Market Report, 2019.
6. Helga W., Schlatter S., and J. Trávníček. 2020. The World of Organic Agriculture Statistics and Emerging Trends 2020. http://www.organic-world.net/yearbook/yearbook-2020.html
7. USDA (2018): 2018 Cotton and Products Annual: Egyptian Cotton on the Rise, EG18011. Washington D.C., USDA.
8. https://www.petroleum.gov.eg/ar-eg/media-center/news/news-pages/Pages/Mop_10042020_4.aspx

Evaluation Reactive Groups of Reactive Dyes on Dyeing Egyptian cotton Fabrics

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Abstract

Background *Cold reactive dyes were studied for their dye fixation and color strength on cotton fabric. Three Reactive dyes namely: procion Mx, Levafix E, and Drimarine with the reactive functional groups, (Di-chlorotriazine), (Di-chloroquinoxaline), and (Di-fluoro chloropyrimidine) respectively were applied on to extralong stable Egyptian cotton fabric of Giza 94 to explore the role of their functional groups on color strength and fastness properties. Exhaustion-fixation method with different reaction times and temperatures revealed that reactive dyes with different functional groups have different reactivity and affinity to the color strength and fastness properties for the cotton fabric.*

Results: *The results obtained revealed that the reactive dyes exhibited high color strength, and fastness properties at optimum conditions of temperature, and reaction time. Among dyes under investigation, the results obtained showed that Procion Mx dye having the reactive group structure Di-chlorotriazine offer higher reactivity at the optimum condition 30ºC at 60 mins. for exhaustion and 30ºC at 5 min. for fixation followed by Levafix E dye having the reactive group structure Di-chloroquinoxaline and finally Drimarine dye having the reactive group structure Di-fluoro chloropyrimidine).*

Conclusion: *The highest dye fixation (%) was about 85% for all reactive dyes used. The fastness properties of cotton fabric for all reactive dyes used were good to excellent at the optimum dyeing process.*

Keywords: *Cotton fabric, Reactive dyes, functional groups, color components, fastness properties*

Introduction

Cotton is the most established and the most significant of the material strands. It has been utilized in the East and Middle East for millennia. Cotton is the most generally utilized of the material strands; it has a blend of properties-toughness, minimal expense, simple wash capacity and solace. This one-of-a-kind blend of properties has made cotton a norm for extraordinary masses of the world's kin who live in warm and subtropical environments, (El-Badry et al. 2012, Bledzki et al. (1997). Dyes which are equipped for responding synthetically with a substrate to form a covalent color substrate linkage, is known as reactive dyes, Daria et al. (2020). Many scientists have been concentrated on the coloring of cotton fabrics with reactive dyes. Among the various dyes, reactive dyes were commercially most popular for the last few decades due to their acceptable price, good color value and reasonably good fastness properties, Alam et al. (2008). The reactive dyes comprise the most ordinarily involved class of colors for coloring cellulosic materials, due to their great all-round properties, for example, water solvency, simplicity of use, assortment of utilization techniques, accessibility of various shades, splendor of variety conceals, great to incredible wash and light quickness and moderate cost, David M and loan Vo (2007). Reactive dyes might be arranged in different ways based on responsive gathering, based on reactivity, and on the premise dyeing temperature. It is vital to upgrade the coloring conditions to improve the color obsession and variety strength of the cotton textile, Ali et al. (2009). Dyeing of cotton fabric with reactive dyes depend upon various parameters such as electrolyte, alkali, liquor ratio, pH of the dye bath and temperature. The exhaustion of a reactive dye depends upon the amount of electrolyte and reactivity of a dye, Ahsanul (2014). The dyeing with reactive dyes is performed in the presence of an alkali medium

of NaCl, Na_2CO_3, NaOH, or Na_2SO_4. The pH value was very important factor since the ionization of hydroxyl group (OH-) in cellulose fibers is accelerated with an increase in PH. The functional group (nature and number) attached to the structure of reactive dye molecule has distinct influence on dyeing behavior. Reactive dye structures consist of two parts: a conjugated chromosphere and reactive groups, Suwanich and Chutima, (2006). The most common reactive groups are mono-chlorotriazine, dichlorotriazine, and vinyl sulfone. Vinyl sulfone is a sulfuric acid ester of β-hydroxyethyl sulfone and reacts with cellulose at a moderate temperature of 60°C, Burkinshaw P, (2011). Molecular shape and size from the main color components of the reactive dyes, had significant effects on the color strength and fixation properties, Soleimani-Gorgani and J. Taylor, (2006). The dyeing properties depend on the co-planarity of the structures; hence, the positions of different groups greatly affect the formation of a suitable molecular geometry, Xie et al. (2014). The positions of the reactive and soluble groups are very important because the dye will undergo substitution reactions and those positions should remain unhindered, Siddiqua et al. (2017). Dyes containing two or more type of reactive group showed higher fixation efficiency versus dyes containing only one type of reactive, Hunger K. (2017). It is important to optimize the dyeing conditions to enhance the dye fixation and color strength, Umbreen et al.)2008). The low fixation of dyes causes environmental issue since dyes lost in dyeing, and about 15% dyes are lost during dyeing process and have adverse effect on the of environmental safety, Iqbal, (2016**).** The hydrolysis in dyeing bath attributes to the lower dye-fixation and is one of the main drawbacks of continuous dyeing method, Broadbent, (2001). The discharge of dyes in wastewater should be reduced to maximum level to ensure sustainable environmental development, Qureshi et al. (2015). which demands the optimization of process variable along with suitable dyes section. In reactive dyeing, the dyeing process can be broadly divided into two phases, namely exhaustion and fixation. The process is lengthy, because much time is spent on the controlled heating of dye bath and portion wise addition of salt and alkali in order to avoid unlevel dyeing and maximizing the exhaustion and fixation, Iftikhar et al. (2001). The dyeing method, dye type, dye concentration, temperature, reaction time, medium pH and salt amount used during dyeing affect the dye fixation. Moreover, dye structure (number of functional group and their position) is also important in controlling the dye fixation, (Sugimoto T, 1994., Blackburn, 2004). The relationship between temperature and reactivity is that higher temperatures require lower alkalinity; to optimize on hydrolysis. They can be broadly grouped under ‘High’ ‘Medium’ and ‘Low’ categories requiring 40ºC. 60ºC and 80ºC respectively, levels of pH 12.5 for High (cold dyeing), 11.5 for Medium (Warm) and 10 – 11.0 for Low (Hot Dyeing) for the reaction to proceed more favorably towards the substrate. The effect of dyeing temperature has been studied on the color strength and color fastness properties of single jersey cotton knitted fabrics dyed with Novacron Red S-B reactive dye (1%) using conventional exhaust dyeing method, Debasree et al. (2017). The effect of dye concentration, electrolyte concentration, dyeing time and dyeing temperature on dyeing performance of cotton fabric dyed with reactive dyes, viz. Reactive Red 6B and Reactive Yellow RL, has been studied, Md. Shamsul et al. (2008).

In the present investigation, three reactive dyes having different functional group (number and positions) were selected and employed on cotton fabric. The dyeing was performed by exhaustion-fixation method. Various process variables were optimized to enhance to color strength and dyeing fastness properties.

Materials and Methods

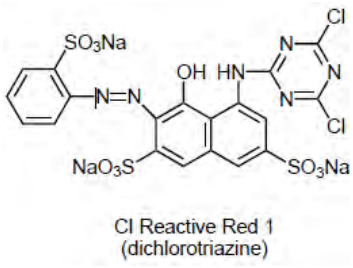
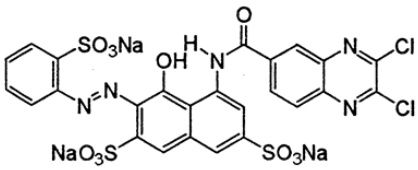
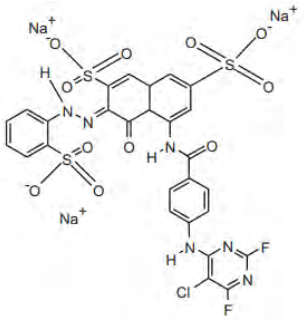
Materials

Scoured woven plain Egyptian cotton fabrics made from Giza 94 was purchased from Misr-El-Mehala Company for Spinning and Textile, Egypt. The fabrics had the following specification: yarn count: 38 x 40tex; weight: 175 g/m^2. Specimens of size of 25 cm x 25 cm were used.

Chemicals

All chemicals used were of analytical grade using doubly distilled water (18.5 MΏ.cm-1). NaOH was analytical grade (Koch-Light Co.), Sodium chloride (LR grade), the wetting agent was the commercially Ttiton X100 supplied by Merck. The reactive dyes used in this investigation were classified as cold reactive dyes were given in Table 1.

Table 1. Structural formula of the reactive dyes

Dye No.	Reactive functional group	Dye Structures
RD1	Dichlorotriazine	 CI Reactive Red 1 (dichlorotriazine)
RD2	Dichloroquinoxaline	 Reactive Red 41
RD3	Difluorochloropyrimidine	 Reactive red 124

Methods

Dyeing bath conditions

Optimization of Dyeing Conditions

The optimum conditions of dyeing with the mentioned reactive dyes were represented in the Fig. 1.

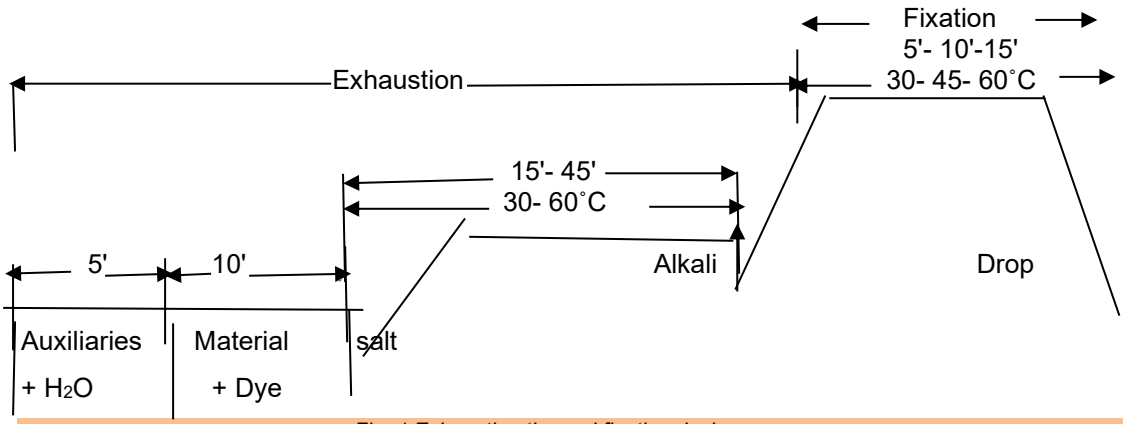


Fig. 1 Exhaustion thermal fixation dyeing curve

The exhaustion thermal-fixation dyeing time and temperature was varied accordingly. The exhaustion dye bath was prepared and the samples were dyed for varying 30, 45, and 60˚C at 30 mins. maintaining the pH at 6-7. At the optimum exhaustion temperature, the dye bath was prepared and the fabrics were dyed for varying exhaustion times 45 and 60 mins maintaining the pH at 6-7. A, the thermal fixation process was carried out containing NaOH electrolyte 10/ l, at 30˚C maintaining the pH at 12.5 and the fixation time were 30, 45, and 60 mins. At the optimum thermal fixation time the fixation process was carried out containing NaOH electrolyte 10/l, maintaining the pH at 12.5 and the fixation temperatures were 45, and 60 ˚C. After dyeing operation is completed the dye samples were put in a bath containing 1% stock solution of acetic acid to neutralizing the fabric. This operation is performed at 60ºC for 10 minutes. The material is then treated with a 1g/L soap solution, which removes the unfixed dye from fabric surface, and makes the surface clean. The material is then treated with a hot water bath and the material is treated with a cold-water bath, as represented in Fig.2. Finally, the material is dried in open air and finally in oven.

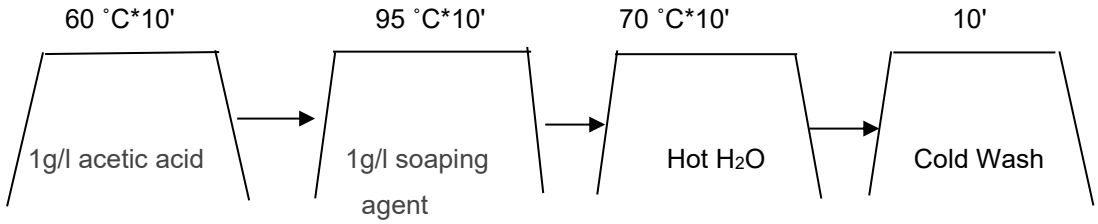


Fig. 2. Washing off of the dyed samples

Evaluation the Properties of the Treated Fabrics

Color strength measurements

The color strength (K/S) of the treated samples using the untreated samples as blank was determined using Perkin Elmer Spectrophotometer, Model Lambda 35 equipped with integrated sphere with applying the Kubelka-Munk equation:

$$K/S = [(1-R)\ 2/\ 2R]$$

Where; R is the reflectance, K is the absorption coefficient and S is the scattering coefficient.

Dye fixation (%)

The dye percentage fixation ratio was calculated considering K/S values before and after washing of the dyed samples according to the following equation

$$\text{Fixation \%} = \frac{(K/S)_1}{(K/S)_1 - (K/S)_2} * 100$$

where K/S_1 and K/S_2 were the color strength before and after washing

Fastness Properties

Washing fastness (WF)

Washing fastness of the untreated samples was done according to ISO 105- C01:1998(E). Two single fiber adjacent fabrics complying with the relevant sections of F01 to F08 of ISO 105-F: 1989. One adjacent fabric of cotton and the second of wool.

Perspiration fastness (PF)

Fastness to synthetic perspiration was measured according to ISO-E04: 1994. (c)

Light fastness (LF)

The dyed cotton fabric was measured according to ISO 105-B01:1994 Textiles- -Tests for color fastness, Part B01: Color fastness to light: Daylight

Rubbing fastness (RF)

The dyed cotton fabric was measured according to ISO 105-X12:2016(en) Textiles- Tests for color fastness, Part X12: Color fastness to rubbing

Results and Discussions

Cotton texture contains a hydroxyl bunch (- OH) in the cellulose chain. At the point when the texture is exposed to coloring with responsive colors of various classes by arrangement process, it shows great dyeability. The responsive colors are fit for consolidating with hydroxyl gatherings of cellulose through covalent bond arrangements which differs from one color to another, contingent on the reactivity. The connection of color particles to the cellulosic tie is viewed as through covalent holding as no color atom strips out from the colored example as demonstrated in the accompanying proposed response:

Cell-OH + X-Dye Cell-O-Dye + HX

Effect of exhaustion temperature on K/S

It has been noted that as the functional groups changed, the temperature sensitivity of dyes also changed, which revealed that functional groups in dyes structure are sensitive to temperature and they may respond differentially under variable temperature values. Fig. 4 exhibits the effect of dyeing temperature on the color strength of cotton fabrics dyed with the three above mentioned dyes. The results obtained revealed that there was variation in the color strength with respect to the reactive functional group of the dye used. The results obtained revealed that K/S value for RD1 showed the maximum at 30ºC, whereas RD2, and. RD3 showed high color strength value at 45ºC. These results may be due to that chlorine imparts medium reactivity, whereas the reactivity of fluorine is the least and its rate hydrolysis is also less. K/S values declined up to 60ºC which mean that this temperature has less impact at the values of dye uptake for fabrics. At 60°C the dye uptake results getting down which may be due to diffusion of dye from the core of the fiber and desorption of already absorbed dye may occur since hydrolytic degradation of dye may occur in aqueous media, Kamel et. al. (2007). Hence, the optimum value of temperature used may be 40°C, which helps in saving water, salt and alkali, Naebe, (2010). In general, RD1 has the highest K/S value followed by RD2, and RD3 respectively.

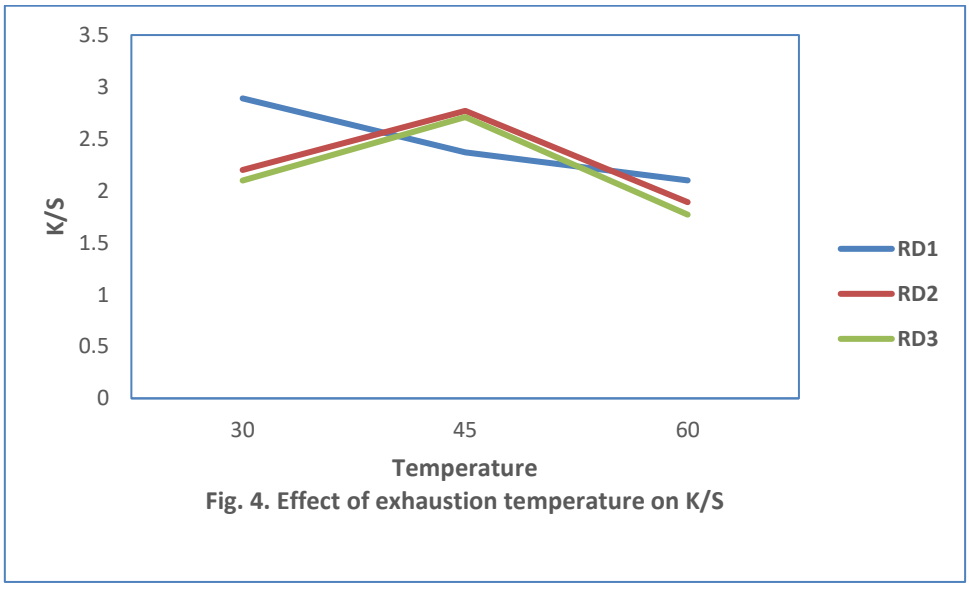


Fig. 4. Effect of exhaustion temperature on K/S

Effect of exhaustion time on K/S

In dyeing process, surface adsorption, diffusion and dye-fabric fixation of dye is involved, Kamel et. al. (2007). This dyeing process reached at an equilibrium stage for specific reaction time and at that specific time, the sorption and desorption equilibrium are reached. However, prolonged dyeing time at higher temperature would facilitate dye desorption more efficiently, Collins, (2001). The hydrolysis of dye is one of main factors, which desorbs the dye molecule at unfavorable condition. The dyeing behavior of dyes under investigation, as a function of time is shown Fig. 5. The results revealed that 60 minutes dyeing time yielded higher color strength

with smooth shade for all the reactive dyes. RD1 showed maximum color strength, while RD3 color strength was least. However, all dyes (RD1 to RD3) furnished color excellent color strength and these findings are in line with reported results of hetero-functional reactive dyes that dyeing time is effective for reactive dyes.

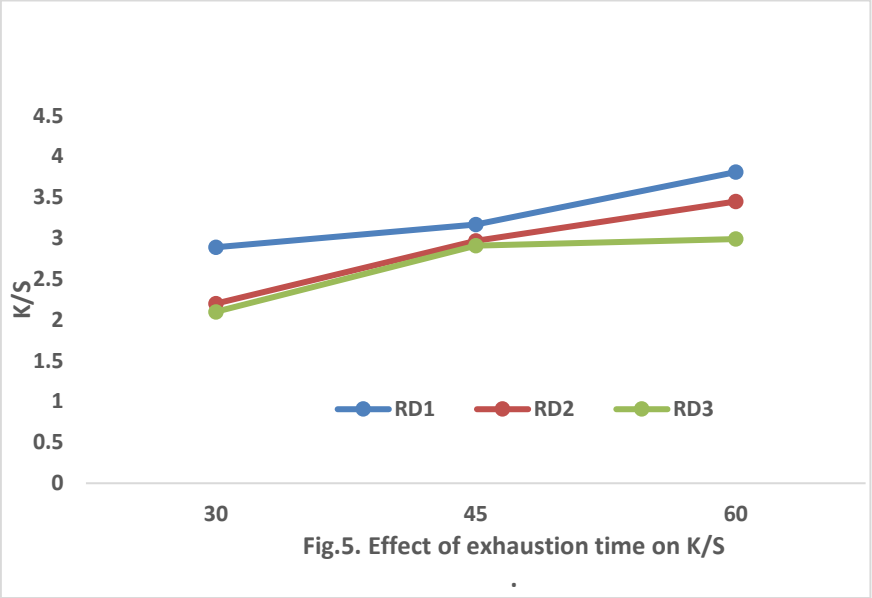


Fig.5. Effect of exhaustion time on K/S

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Effect of time on dye fixation (%)

To compare the dye fixation (%) of the three reactive dyes, the time is taken in x-axis, and the dye fixation% of is plotted in y-axis (Fig. 6). The graph is clearly different from exhaustion graph. Fixation (%) of dye means the reaction of reactive group of dye with terminal –OH group of fiber and thus forming strong covalent bond with the fiber. This is an important phase, which is controlled by maintaining proper pH by adding alkali. The alkali used for this purpose depends on brand of dye and dyeing temperature. In this investigation NaOH is used as alkali depending upon reactivity of dye to create proper pH 12.5 in dye bath and do as the dye-fixing agent. It has been noted that the dye fixation% value for RD1 can be achieved in very short duration (5min), followed by RD2 and then RD3. These results might be due to that RD1 possess such high reactivity that they readily hydrolyze which let it reacts with water. These results were in accordance with the results that the reactive dye fixation can be achieved in very short duration at optimize conditions of temperature and pH, Schmidt, (2003).

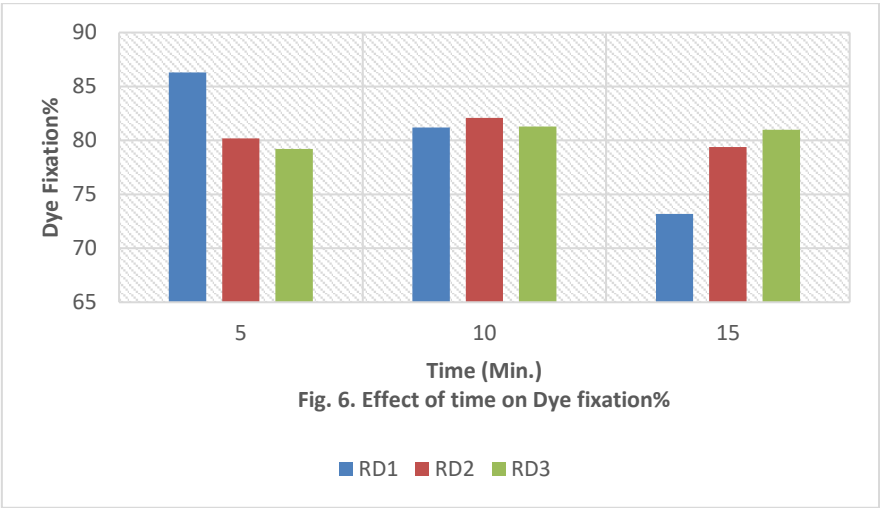

Fig. 6. Effect of time on Dye fixation%

Effect of temperature on dye fixation (%)

The influence of the dye-fixing temperature on dyeing was investigated and the results are shown in Fig. 7. The dye fixation% values all first increased and then decreased, and the optimum dye fixation% was achieved at 45°C for RD1 and 60°C for RD2 and RD3 respectively. The dye fixation% of RD1 possess such high reactivity that they readily hydrolyze at high temperature; the extent of fixation of these dyes is thus lower. The low dye fixation% at lower temperature was due to aggregation of dye molecule and preclusion of dyes to fix on fiber, Umme et al. (2017). Increasing the dye-fixing temperature can improve the diffusion activation energy and dyeing rate of reactive dyes. Therefore, an appropriate increase in the dye-fixing temperature will improve the K/S. However, the K/S values of the dyed fabric decreased when the dye-fixing temperature exceeded 60°C. Increasing the dye-fixing temperature not only increases the reaction rate of reactive dyes with samples but also increases the hydrolysis rate of the reactive dyes. The increase in the reactive-dye hydrolysis rate might be greater than the increase in the reactive-dye dyeing rate upon increasing the dye-fixing temperature.

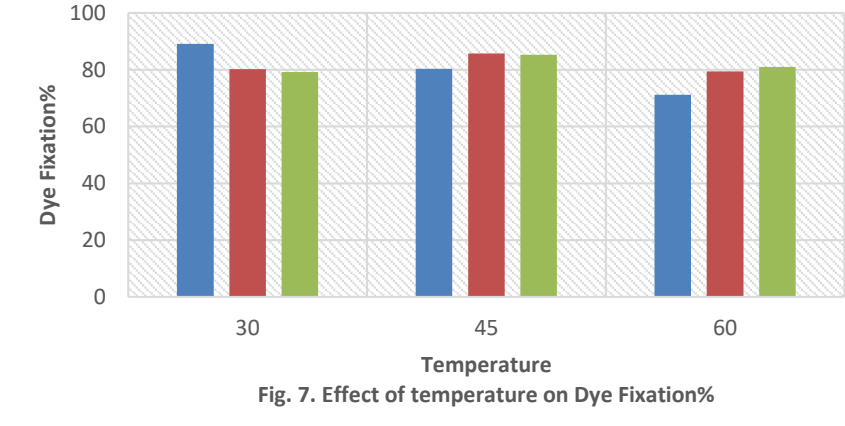

Fig. 7. Effect of temperature on Dye Fixation%

Fastness properties

Fastness to Washing (WF)

At the optimum conditions of exhaustion-fixation time and temperatures, the color fastness of dyed cotton fabric has been and the results are given in the Table 2. The wash fastness of cotton fabric with all the dyes is found to be good. However, it is better for RD1, and RD2 than that RD3. The wash fastness depends upon the physical and chemical properties of the fabric, the class of the dyes, their forces of interaction and their interaction with soap solution. The good washing fastness of the reactive dyes may be due to the chemical fixation of the dye molecules, David M Lewis, (2014).

Fastness to Exposure in Light (LF)

Table 2 showed that the color fastness of cotton fabric to exposure under light decreased and all the dyes is found to be fair. The fastness of a dyed fabric depends upon the dye-fiber interaction and the intensity of light. An intensive oxidation of the fiber occurs due to the capacity of the dye molecule, excited by the light. This oxidation reaction rapidly occurs at the earlier time of light exposure and hence the color of the dyed fiber abruptly changes. Light fastness is the resistance of dyed fiber to fade upon exposure to light. Light fastness of all dyes was fair 3. The explanation of these results may be due to that the strong covalent bond between the dye and cotton fabric seems to have difficult the transfer of energy from the excited dye molecule to the fiber decreasing the stability of the reactive dyes under light exposure, Deepali R et al. (2006).

Color fastness to rubbing (RF)

Color fastness to rubbing is also considering a crucial parameter. Rubbing fastness evaluates the ratio of color which may transfer from the surface of a colored fabric to an uncolored bleached test cloth during the systematic rubbing practice. All dyes furnished good rubbing fastness. The RD1 to RD3 rubbing fastness values were 4/5 for dry treatment, whereas RD1

and RD2 showed the values in the range of 4 and 3/4 for RD3. Results of the rubbing fastness revealed good penetration and fixation of the dyes

Fastness to Perspiration (PF)

Perspiration is slightly acidic. Hydrocellulose is produced by the action of dilute acids due to the cleavage of chains by hydrolysis. Hydrolysis lowers the tensile strength of the fabric and breakdown the formation of covalent bonds between the fabric and the Reactive dyes. It is observed from the results that the color fastness of dyed cotton fabric to perspiration is nearly good. The cotton fabric dyed with RD1 and RD2 exhibits better results as compared to RD3 due to its higher affinity to cotton fabric.

Table 2. Fastness properties of the reactive dyes

Properties / Samples	WF	LF	RF		PF	
			dry	wet	acid	alkaline
RD1	4	3	4/5	4	4	4
RD2	4	3	4/5	4	4	4
RD3	3	3	4/5	3/4	3/4	3

Where WF Fastness to Washing, LF Fastness to Exposure in Light, RF fastness to rubbing, and PF Fastness to Perspiration

Conclusion

Cold reactive dyes were studied for their dye fixation and color strength on cotton fabric. As the functional groups changed, the temperature sensitivity of dyes also changed, which revealed that functional groups in dyes structure are sensitive to temperature and they may respond differentially under variable temperature values. In the present investigation, three reactive dyes having different functional group (number and positions) were selected and employed on cotton fabric. The dyeing was performed by exhaustion-fixation method. Various process variables were optimized to enhance to color strength and dyeing fastness properties. The results obtained revealed that the reactive dyes exhibited high color strength, and fastness properties at optimum conditions of temperature, and reaction time. Among dyes under investigation, the results obtained showed that Procion Mx dye having the reactive group structure Di-chlorotriazine offer higher reactivity followed by Levafix E dye having the reactive group structure Di-chloroquinoxaline and finally Drimarine dye having the reactive group structure Di-fluoro chloropyrimidine.

References

1. Ahsanul H. A. N. Effect of Dyeing Parameters on Dyeing of Cotton Fabrics with Fluoro Chloro Pyrimidene Reactive Dyes, IJRET: International Journal of Research in Engineering and Technology, pp. 125-128, Vol. 03, Issue. 04, Apr-2014.
2. Alam, M.S., Khan, G.M.A., Razzaque, S.M.A., Hossain, M.J., Haque, M.M., & Zebsyn, S. Dyeing of Cotton Fabrics with Reactive Dyes and their Physico-Chemical Properties. Indian Journal of Fiber and Textile Research, 33:58-65, 2008.
3. Ali, S., T. Hussain, R. Nawaz. Optimization of alkaline extraction of natural dye from henna leaves and its dyeing on cotton by exhaust method, J. Clean. Prod. 17, 61–66, 2009.
4. Blackburn, R.S. Natural polysaccharides and their interactions with dye molecules: applications in effluent treatment, Environ. Sci. Technol. 38, 4905-4909, 2004.
5. Blackburn, R.S., Burkinshaw, S.M., & Collins, G.W. The Application of Cationic Fixing Agents to Cotton Dyed with Direct Dyes under Different pH Conditions. Coloration Technology, 114:317-320, 1998.
6. Bledzki A K & Gassan J. Handbook of Engineering Polymeric Materials, edited by N P Charamwasinoff (Marcel Dekker Inc., New Jersey), 787,1997.
7. Broadbent, A.D. Basic principles of textile coloration, Soc. Dyer. Color. West Yorkshire, 2001.
8. Burkinshaw S. and M. Paraskevas, Dyes Pigm, 88, 212, 2011.
9. Collins, G.W. S.M. Burkinshaw, R. Gordon, Dyeing of textiles, US Patent 6200354 (2001).
10. Daria Smigiel-Kami nska, Jolanta W Gubała, Piotr Stepnowski and Jolanta Kumirska, The Identification of Cotton Fibers Dyed with Reactive Dyes for Forensic Purposes, Molecules 2020, 25, 5435.
11. David M Lewis. The chemistry of reactive dyes and their application processes, Review of Progress in Coloration and Related Topics 130(6), (2014).
12. David M Lwis, loan Vo. Dyeing cotton with reactive dyes under neutral conditions Coloration Technology 123(5):306 – 311, 2007.

13. Debasree P, Subrata C. D, Tarikul I, Md. Abu Bakar Siddiquee, Md. Abdullah Al Mamun, Effect of Temperature on Dyeing Cotton Knitted Fabrics with Reactive Dyes, Journal of Scientific and Engineering Research, 4(12):388-393, 2017.
14. Deepali Rastogi, Kushal Sen, Mohan G. Photofading of Reactive Dyes on Silk and Cotton: Effect of Dye-fiber Interactions, Review of Progress in Coloration and Related Topics 117(4):193 – 198, 2006.
15. El-Badry, Kh., Salah M Saleh and Bahlool Shereen O. Effect of Mercerization Techniques on Cotton Towels Properties, Journal of Applied Sciences Research, 9(3): 2386-2393, 2013
16. Hunger, K. Industrial dyes: chemistry, properties, applications, John Wiley & Sons (2007), DOI: 10.1002/3527602011.
17. Iftikhar, M., Jamil, N.A., & Shahbaz, B. Rubbing, Ironing and DryCleaning Fastness of Reactive Dyed Cotton Knitted Fabric as Influenced by Salt, Alkali and Dye. International Journal of Agriculture & Biology, 3(1):109-112, 2001.
18. Iqbal, M. Vicia F. bioassay for environmental toxicity monitoring: a review, Chemosphere. 144 (2016) 785-802.
19. Kamel, M.M. R.M. El-Shishtawy, B. Youssef, H. Mashaly. Ultrasonic assisted dyeing. IV. Dyeing of cationised cotton with lac natural dye, Dye. Pigment, 73 279-284,2007.
20. Md. Shamsul, Alam a, G M Arifuzzaman Khan, S M Abdur Razzaque, M Jamil Hossain & M Minhaz-ul-Haque, Dyeing of cotton fabrics with reactive dyes and their physico-chemical properties, Indian Journal of Fibre & Textile Research, Vol. 33, pp. 58-65 March 2008.
21. Naebe M, P.G. Cookson, J.A. Rippon, X.G. Wang. Effects of leveling agent on the uptake of reactive dyes by untreated and plasma-treated wool, Text. Res. J, 80, 611–622,2010.
22. Qureshi, K. M. Ahmad, I. Bhatti, M. Iqbal, A. Khan, Cytotoxicity reduction of wastewater treated by advanced oxidation process, Chem. Int. 1, 53-59, 2015.
23. Schmidt, A. E. Bach, E. Schollmeyer. The dyeing of natural fibers with reactive disperse dyes in supercritical carbon dioxide, Dye. Pigment. 56, 27-35.57, 58, 2003.
24. Siddiqua, U. H. S. Ali, M. Iqbal, and T. Hussain, Relationship between structure and dyeing properties of reactive dyes for cotton dyeing, J. Mol. Liq., 241, 839 (2017).
25. Soleimani-Gorgani A. and J. Taylor. Dyeing of nylon with reactive dyes. Part 1. The effect of changes in dye structure on the dyeing of nylon with reactive dyes Dyes Pigm., 68, 109 (2006).
26. Sugimoto, T. Neutral-fixing reactive dyes for cotton, J. Soc. Dyer. Color. 108, 497-500, 1992.
27. Suwanich T. and P. Chutima, IOP Conf. Series: Materials Science and Engineering, 215, 012206 (2017).
28. Umbreen, S. S. Ali, T. Hussain, R. Nawaz. Dyeing properties of natural dyes extracted from turmeric and their comparison with reactive dyeing, Res. J. Textile Apparel. 12, 1-11, 2008.
29. Umme H. S, Shaukat A, Munawar I, Tanveer Hu. Relationship between structures and dyeing properties of reactive dyes for cotton dyeing, Journal of Molecular Liquids Volume 241, , Pages 839-844, September 2017.
30. Xie K., A. Gao, M. Li, and X. Wang. Printing properties of the red reactive dyes with different number sulfonate groups on cotton fabric, Carbohydr. Polym., 101, 666 (2014).

Dyeing performance of super-giza 97 egyptian cotton yarns

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ABSTRACT

Background and Rationale: *This investigation was carried out to evaluate the dyeability of the new Egyptian cotton variety and its effect on yarn mechanical properties during 2020 season. The relationship between various yarn properties and natural and synthetic dye types was investigated. The newest commercial Egyptian cotton variety from the Delta area, G. barbadense namely Super-Giza 97 as long staple (LS) was used.*

Methods: *All tests for this new cultivar to determine the physical, mechanical and chemical fiber properties were done at the Cotton Technology Research Laboratories, Cotton Research Institute, Agricultural Research Center (ARC). Fibers were processed to yarns using ring spinning system, and dyed using different dyestuffs, which are natural dye extracted from outer onion skin, reactive dye named Drimarin K-4BL and basic dye (Methyl violet 2B). Yarn Mechanical Properties as yarn strength and yarn elongation was determined before and after dyeing. Evaluation of color measurements and color strength were measured. Furthermore, the changes in surface morphology of cotton yarns after dyeing were identified by Fourier Transform Infrared Spectroscopy (FTIR) analysis.*

Results and Conclusions: *The results revealed that all characteristics of cotton yarns such as color measurements, color strength, color coordinates, tensile strength and elongation differed according to the dyestuff used. An important advantage of using the natural dye extracted from onion skin is the economical and environmental impact by using an agricultural waste for dying. Also, our study helps in understanding the relation between a new Egyptian cotton yarn and different kinds of dyestuff*

Keywords*: Egyptian cotton variety, natural dye, reactive dye, basic dye*

INTRODUCTION

All Egyptian cotton varieties belong to *Gossypium barbadense* species, which plays a great role in economic and social status in the life of Egyptians. Egyptian cotton is one of the most important crops of Egyptian agriculture. Cotton fiber quality is mainly influenced by genotype of the cultivars but agronomic practices and environmental conditions are the secondary factors influencing fiber quality. So, high quality cotton fiber will result in high quality yarn, and high quality final products (Tesema & Hussein, 2015).

Development of this new variety began as promising cross between two cotton genotypes belonging to *Gossypium barbadense L.,* genotype {Giza89xR101)xGiza86} as the female parent characterized by high fiber strength and early maturity with Giza94 as a male parent, which is superior by seed cotton yield and lint percentage in the growing season 2007 at Giza Experimental Station, Cotton Research Institute, Agricultural Research Center, Giza, Egypt during the routine work of Egyptian cotton breeding program followed by the pedigree method of selection (Abdelmoghny *et al.,* 2018).

Now, Super-Giza 97 proudly obtained by Egyptian researchers of the Agricultural Ministry, and commercially approved in 2020, The variety Super-Giza 97 has higher seed cotton yield and higher lint yield than other commercial long staple varieties.

The Egyptian long staple cotton variety (Giza97) had highly significant effects on all cotton fiber properties such as strength, elongation, whiteness and yellowness degrees, as well as spinning constant index, which, significantly differed due to genetic varietal effects (Nassar *et al*., 2019). It is necessary to carefully analyze the structure and the surface properties of cotton fibers in order to enhance the performance of cotton based materials and yarns (El Messirya & Abd-Ellatif, 2013). Egyptian cotton fibers are composed mostly of α-cellulose, the rest is non-cellulosics substances that are located on the outer layers and inside the lumen of the cotton fiber. The chemical composition of cotton fibers varies according to their varieties and growth conditions (Wakelyn *et al*., 2006).

The importance of the relationship between fiber properties and yarn structure has increased due to the need of yarns with best possible quality at optimal cost. The spinning system affects the yarn properties as the tenacity of ring yarns expresses greater value than rotor spun yarns and the elongation of ring yarns has a significantly lower value than that of rotor yarns (Iqbal , 2018).

The skin of onion is not edible and considered as a vegetable waste. Outer onion skin contains a coloring pigment known as “Pelargonidin” which is approximately 2.25% of the weight of the onion (Nurunnesa *et al*., 2018; Chandravanshi & Upadhyay, 2013).

Reactive dyes are among the most popular dyes for dyeing cotton and cellulosic fibers because of their variable color shades and good color fastness properties due to the chemical bonding between the reactive dye and the cellulose (Sharma & Sayed, 2016; Lewis, 2007; Ristić & Ristić, 2012; Bahlool & Saleh, 2020). Reactive dyestuffs can include more than one reactive group and may have more than one type of such groups (Collins, *et al*., 1998). In previous study we used a new technique for evaluating the performance of dyeing cotton fabrics using infrared (IR) heating technique compared to conventional exhaust dyeing method by dyeing Egyptian cotton fabrics made of two Egyptian varieties Giza 90 and Giza 95 using different concentrations of reactive dye using IR laboratory dyeing machine in which the infrared is the source of heating (Bahlool, 2019). Hence, in this study we investigate another reactive dye called Drimarene-K, which is one of the reactive dyestuff suitable for dyeing cotton, linen, rayon and all cellulosic fibers. A unique property of Drimarene-K dye is its stability in water solution (Benkhaya *et al*., 2020; Jamil *et al*., 2019).

Basic dyes are called cationic dyes because the chromophore in basic dye molecules contains a positive charge. Basic dyes are powerful coloring agents. They are applied to wool, silk, cotton and modified acrylic fibers. Usually acetic acid is added to the dye bath to help the take up of the dye onto the fiber because the solubility of these dyes is very good in water, in the presence of glacial acetic acid (https://www.textileblog.com/basic-or-cationic-dye).

FTIR spectroscopy has been one of the useful tools for research development needed for cotton industry**.** Zhongqi and Yongliang, 2021 reviewed the use of FTIR spectroscopy in investigation of cotton fiber and cotton seed components that are impacted by various genetic, cropping, post-harvest processing, and treatments. This was in order to provide a new vision of using FTIR research for the chemistry and quality-evolving mechanisms of cotton and cotton products.

The aim of this study was to investigate the properties of the new Egyptian cotton variety Super-Giza 97 as well as investigate the dyeability of ring spun cotton yarns from this variety using synthetic and natural dyes. We further aimed to study the relation between the cotton

and the dyestuff used by FTIR Analysis. And compare the dyeing performance of the Egyptian cotton variety towards reactive, basic and natural dyestuffs.

MATERIAL AND METHODS

MATERIALS:

Cotton Yarn:

Cotton samples representing Super-Giza 97 Egyptian cotton variety are prepared in order to perform different fiber tests determining their physical properties.

Lint cotton samples were pre conditioned for 24 hours, under the standard conditions of (65 ± 5 %) relative humidity and (20 ± 2 C°) temperature before testing using cotton testing instruments:

All tests of the fibers of this new cultivar to determine the physical, mechanical and chemical fiber properties were performed at the Cotton Technology Research Laboratories, Cotton Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Published in **Cotton research institute annual report season 2020** and summarized in table 1.

Table (1): Egyptian Cotton Fibers' physical, mechanical and chemical properties

Egyptian Cotton Variety	Color			Micronaire	Fiber Length (UHM) mm	Fiber Mechanical Properties		Image Analyzer ; Fiber Perimeter (µ)	Chemical Analysis			
	Color	Bright. (RD%)	Yellow.(+b)			Str.(g/tex)	Elong. (%)		sugar (%)	Wax (%)	Moist. (%)	Ash (%)
Super-Giza 97	White	74.3	9.1	4.4	33.7	46	7.5	47	0.22	1.02	6.16	0.99

Super-Giza 97 commercial Egyptian cotton varieties representing the long staple category season 2020, were processed to yarns Ne 60 using ring spinning system, at the Cotton Technology Research Laboratories, Cotton Research Institute, Agricultural Research Center, Giza, Egypt

CHEMICALS AND AUXILIARIES:

All chemicals used were of analytical grade using doubly distilled water. Glauber salt ($Na_2SO_4 \cdot 10H_2O$) sodium hydroxide (NaOH), sodium chloride (NaCl), sodium carbonate (Na_2CO_3), acetic acid (CH_3COOH), triton x-100 as wetting agent, and detergent.

DYE STUFFS:

Three different dyestuffs have been used and their structure illustrated in **Figures 1, 2, 3 below**

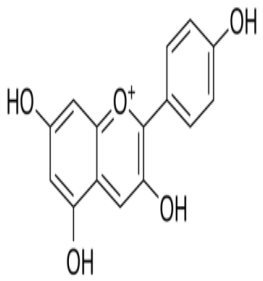


Figure 1. Structural Formula of Pelargonidin" Natural dye extracted from Onion skin (Chandravanshi &Upadhyay, 2013)

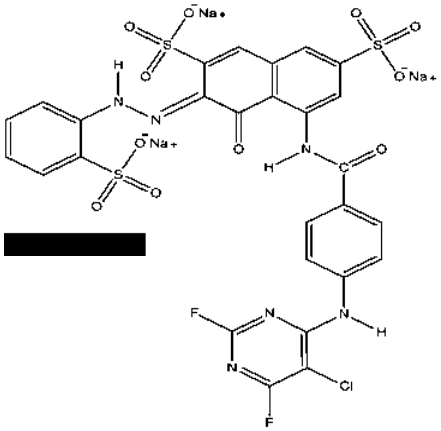


Figure 2. Structural Formula of Reactive dye (Drimarin K-4BL) Fluorochloropyrimidine (Jamil et al., 2018)

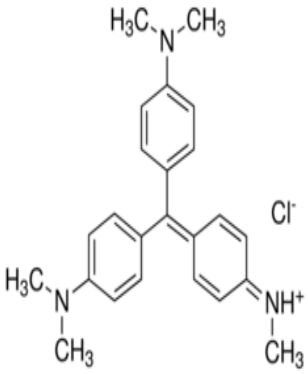


Figure 3. Structural Formula of Methyl Basic Violet Dye

METHODS

(I) Scouring

In order to improve the adhesion of dye to the cotton yarns, an alkaline pre-treatment using sodium hydroxide at concentrations 4.0 % owf with a liquor ratio 1:50 at boiling for 90 minutes. Then yarns were washed with hot water and cold water and air dried at room temperature

(II) Natural dyes extraction from onion skin

About 100g of onion skin that contains the pigment components were boiled in one liter of distilled water and concentrated to 500ml. The extracted liquor was used as the foundation of the dye.

(III) Dyeing procedure

Egyptian cotton yarns Ne 60. were scoured and dyed using three different dyestuffs basic, reactive and natural dye in exhaust dyeing method at the temperatures 50°C , 60°C and 90°C, respectively.

Yarn samples were dyed with 3% owf Drimarene Red, 140 g/L glauber salt (electrolyte) and 12 g/L soda ash (Alkali), Wetting agent 1.0 g/L 1:30 liquor ratio at 60°C.

Cotton yarn was dyed with 3% basic dye (Basic Violet 2), Acetic acid Acetic acid as required to control pH 4.5, Wetting agent 1g/l, M.L 1:30, pH 4.5, Temperature 50c. Salt (20 g/L) used as exhausting agent (Atiqur Rahman., 2020).

Natural dye carried out at a temperature of 90°C with onion skin natural dye extracted using liquor ratio of 1:30.

After dyeing, subsequently rinsed with water hot and cold then, soaping of the dyed yarns were performed in a bath containing 1 g/l non-ionic detergent 10% at 60°C for 15 minutes to remove the unfixed dyes present on the yarn surface, and dried at room temperature. The dyeing procedures are as shown in Figure 4

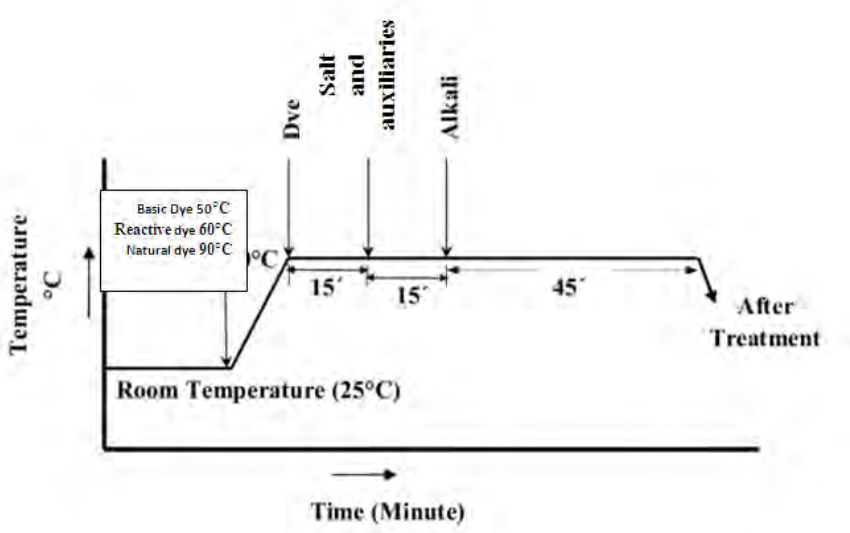


Figure 4. Dyeing procedures of cotton yarns using basic, reactive and natural dyestuffs

- **EVALUATION TESTS**

All cotton samples were conditioned for 48hrs at 65% (±5%) relative humidity and 20°C (±2°C) according to ISO: 6359-1971 standard method.

(I) Yarn Mechanical Properties:

Yarn samples were tested using Statimat ME Tester to determine the yarn strength and yarn elongation before and after dyeing.

(II) Evaluation of Color Strength

Evaluation of Color Strength: The dyed cotton yarns, which were processed in this study, were characterized by color measurements where the reflection spectra wavelength ranges 400-700nm by using a visible spectrophotometer method CIE-Lab 1976/D65. Color measuring instrument (Optimatch spectrophotometer Datacolor international Spectraflash SF450-UK) determines the K/S value of a given yarn through Kubelka-Munk equation as follows (Broadbent, 2001).

The quantitative value for the color strength was obtained by measuring the percent value of reflectance (% R) at the same wavelength and then converted to K/S value with K/S table assistance by Kubelka-Munk (equation 1).

$$\frac{K}{S} = \frac{(1-R)^2}{2R}$$ ---------- **Equation 1**

Reflectance value is the reflection of light amount reflected by objects that contain color.

K is the absorption coefficient (absorbed light); S is the spread light coefficient; R is the percent value of the reflectance (λmax). The color coordinates of the CIE-Lab L* a* b* system and their position in the color space show the color difference values.

Fourier Transform Infrared Spectroscopy (FTIR)

Cotton yarn samples have been crushed and examined using Fourier Transform Infrared Spectroscopy (FTIR 6300) instrument from Jasco Inc., Japan, and the measurements were carried out using an attenuated total reflectance (ATR) technique. All the spectra recorded in the range 4000–400 cm−1 were averaged over multiple scans at a resolution of 4 cm−1. All the recorded data was processed with Spectra Manager II software from Jasco Inc., Japan.

RESULTS AND DISCUSSION

Mechanical properties of cotton yarns before and after dyeing were detailed in Table 2 and Figure 5. We noticed that dyeing has a slight positive effect on the tensile strength of yarns and almost no effect on the elongation percentage

The tensile strength of yarn increases after scouring from 25.5 to 26.8 CN/Tex this increase in strength after scouring possibly is due to fiber swelling and removal of un-cellulosic components, such as wax during wet processing and alkali treatment, which was in agreement with Shrikant *et al*., 2005.

Table 2: Mechanical Properties of cotton Yarns before and after dyeing

Egyptian Cotton Variety G97	Yarn Mechanical Properties	
Treatment	Strength (CN/tex)	Elongation (%)
Raw Cotton Yarn	25.5	5.3
Scoured/ undyed	26.78	5.4
Dyed with Natural dye	26.25	5.3
Dyed with Reactive dye	27.66	5.3
Dyed with Basic dye	26.9	5.4

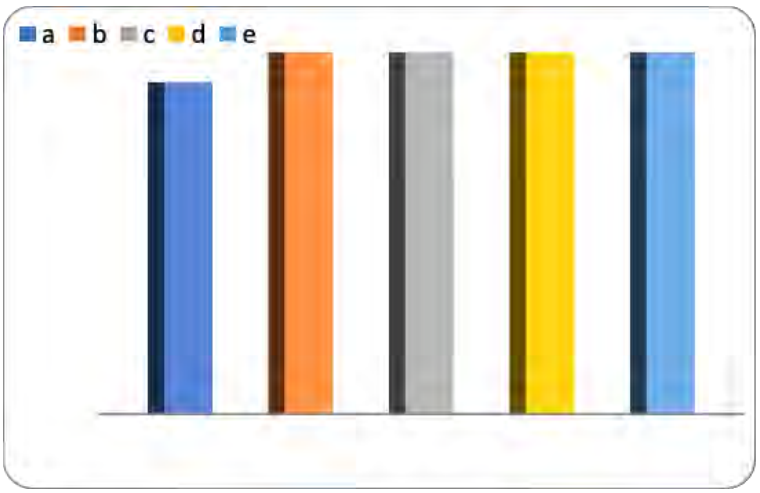
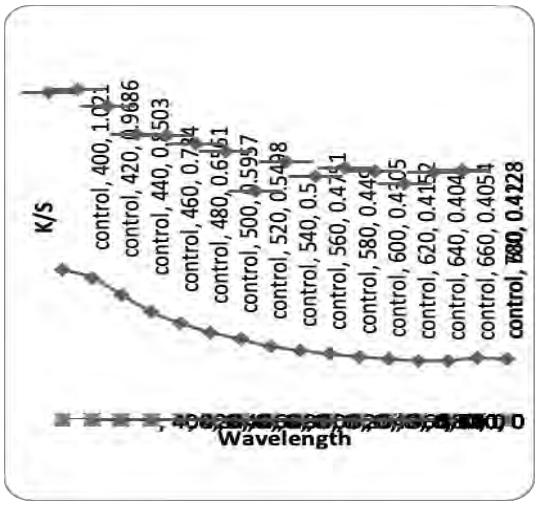


Figure 5. Tensile strength (CN/Tex) of cotton yarns: (a) Raw cotton yarns; (b) scoured cotton yarns; (c) dyed with natural dye; (d) dyed with reactive dyes ; (e) dyed with basic dyes

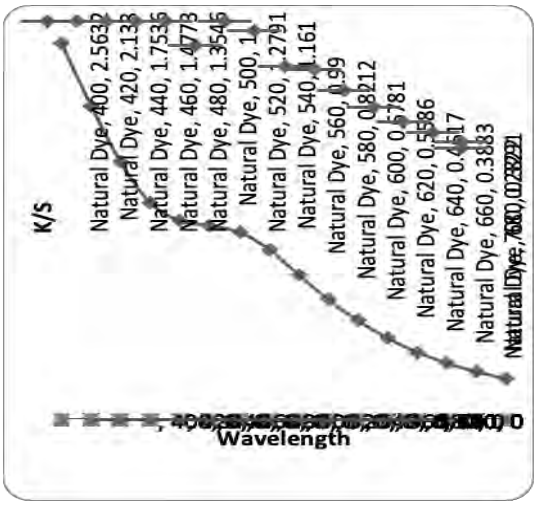
Figure 6 showed the color strength K/S spectra in the wavelength range 400-700nm of cotton yarns by using a visible spectrophotometer, where the color depth of the dyed yarns was analyzed by measuring the K/S values of samples. The higher the value of K/S, the more the amount of dye absorbed into the cotton yarn.

The Outer onion skin contains a coloring pigment called “Pelargonidin” (3, 5, 7, 4 tetrahydroxyantocy anidol) and is used to dye cellulosic fibers (Chandravanshi & Upadhyay 2013).

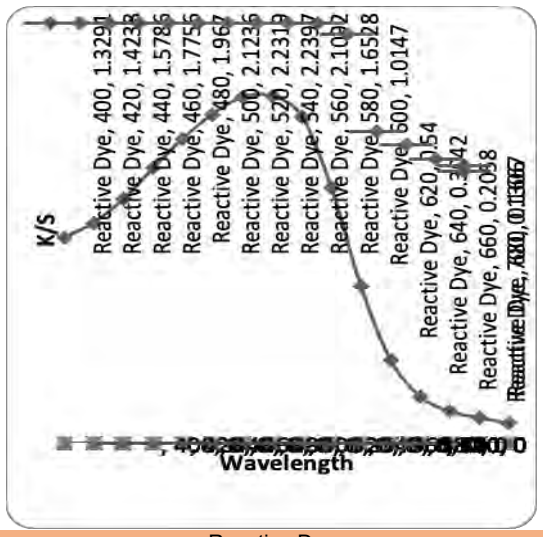
The increase in the reactivity of this kind of basic dyes was made by the substitution of chlorines by fluorine. The bond formed with the textile fiber has been more stable in an acidic medium. The ideal temperature for a good fixation of this type of dye is between 40 °C and 50°C (Benkhaya *et al*., 2020; Jamil *et al*., 2019; Atiqur Rahman & Foisal, 2016). Bi-functional reactive dyes are known for their better exhaustion and fixation properties as they have higher probability to be attracted to the fiber due to double reactive groups.



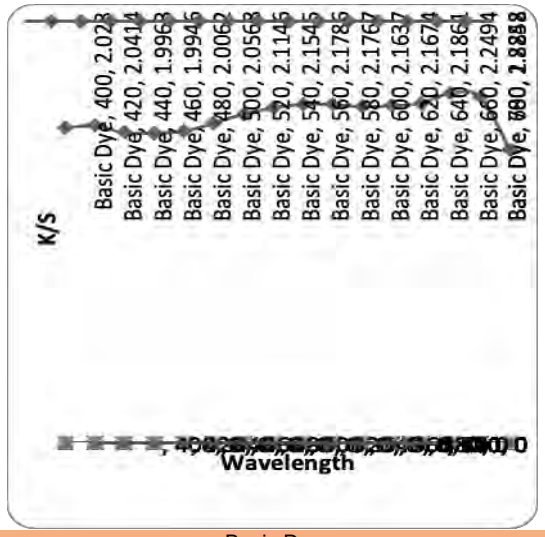
Control



Natural Dye



Reactive Dye



Basic Dye

Figure 6. Color strength k/s spectra wavelength range 400-700nm of cotton yarns:(a) scoured cotton yarns(control); (b) dyed with natural dye; (c) dyed with reactive dyes ; (d) dyed with basic dyes

The K/S values measured at each wavelength, from λ = 400 to λ = 700 nm as Illustrated in figure 6. The color strength was measured at maximum intensity wavelength λmax = 400nm, 400nm, 540nm, 655nm for Natural dye, Reactive dye and Basic dye respectively. The

variation of K/S values is highest at λ = 655 nm and is equal to 2.25 when dyeing with basic dye and at λ = 640 nm and is equal to 2.39 when dyeing with reactive dye, λ = 400 nm and is equal to 2.56 when dyeing with natural dye and at λ = 400 nm and is equal to 1.02 for un dyed yarns

Table 3 Color strength k/s values of undyed and dyed cotton yarns

Dye	K/S	Color Obtained	Color Name
Undyed	1.02		White
Natural dye	2.56		Light Brown
Reactive Dye	2.39		Red
Basic Dye	2.25		Dark violet

Table 4 showed that CIELAB color spaces lightness, values were different as colorant compound is different also by using different light: daylight, artificial light and fluorescent light. The color coordinate values L* for color brightness (brightness, 100 = white, 0 = black), redness (+a*), greenness (-a*), yellowness (+b*), and blueness (-b *)

Table 4. The color coordinate values L*, a* and b* of dyed cotton yarns

Dye		L*	a*	b*
Natural dye	D	68.76	09.12	16.73
	A	70.79	10.95	15.50
	F	68.55	08.78	15.55
Reactive dye	D	52.21	22.09	2.04
	A	55.04	24.49	7.62
	F	52.12	15.99	1.38
Basic dye	D	47.46	-0.06	-1.61
	A	47.33	-0.28	-1.83
	F	47.34	0.10	-1.84

D; daylight, A; artificial light, F; fluorescent light

FTIR spectroscopic analysis

FTIR spectroscopic analysis is used to determine characteristic peaks of functional groups (4,000:400 cm^{-1}) region measured by transmission mode.

From Figure 7, the peak at 3754 cm is attributed to H- bonded and broader band between 3400 and 3300 cm^{-1} is attributed to OH- stretching vibration forming hydrogen bonds between the cellulose molecule located at 3300 and 3334 cm-1cm-1 attributed to intermolecular and intra-molecular hydrogen bonds

The peaks observed at 2917 and 2920cm-1is attributed to –CH2 asymmetric vibrations. We can observe broad peak at 3000–2800 cm^{-1} region for C– H stretching.

Although cellulose has –CH2– groups in their structure, the peaks corresponding to the symmetric and asymmetric stretching modes have never been separated as sharp peaks (Chunga *et al.,* 2004).

Other common bands appearing in different intensities in the spectra showed a peaks 1630 and 1633 cm-1 which is the characteristics of –CH2–symmetrical bending, the spectra

showed a peaks 1420 and 1430 cm-1 which is the characteristics of CH2–scissoring. Peaks at 1372 cm are attributed to CH- bonding deformation stretch. Then we observed peak attributed to C–OH stretching at 1200 cm

1160 and 1170cm^{-1} which is the characteristics of symmetric bridge –C-O-C- , peak attributed to C–O stretching at 1000 cm and 1157 cm^{-1}. Also, Peaks at 1160 and 1170cm^{-1} which are the characteristics of symmetric bridge –C-O-C, Peaks at 850 cm and 900cm^{-1} are attributed to β glucosidic linkage. The C-O stretch corresponds to the peak at 1,162 cm^{-1}, whereas the peaks at 600 and 700 cm-1 represent the symmetric bending of the benzene ring.

The characteristic absorption peaks of cellulose backbone at 3347, 2900, 1160, and 1030 cm^{-1} were assigned to the stretching vibrations of O–H, C–H, C–C, and C–O bonds, respectively, in all samples, which indicated that the cellulose backbone chains of cotton did not change after modification by dyeing. However, the spectrum of dyed yarn showed mild changes in intensity of the peaks.

The results of our study are supported by other researches such as Chunga *et al*., 2004 and Kumar *et al*., 2011who investigate the ATR-FTIR spectral features of cotton plant parts and their functional group assignments. Adapted from Shrikant *et al*., 2005 and Chunga *et al*, 2004 who compared the FTIR spectra of the raw and scoured cotton fabrics.

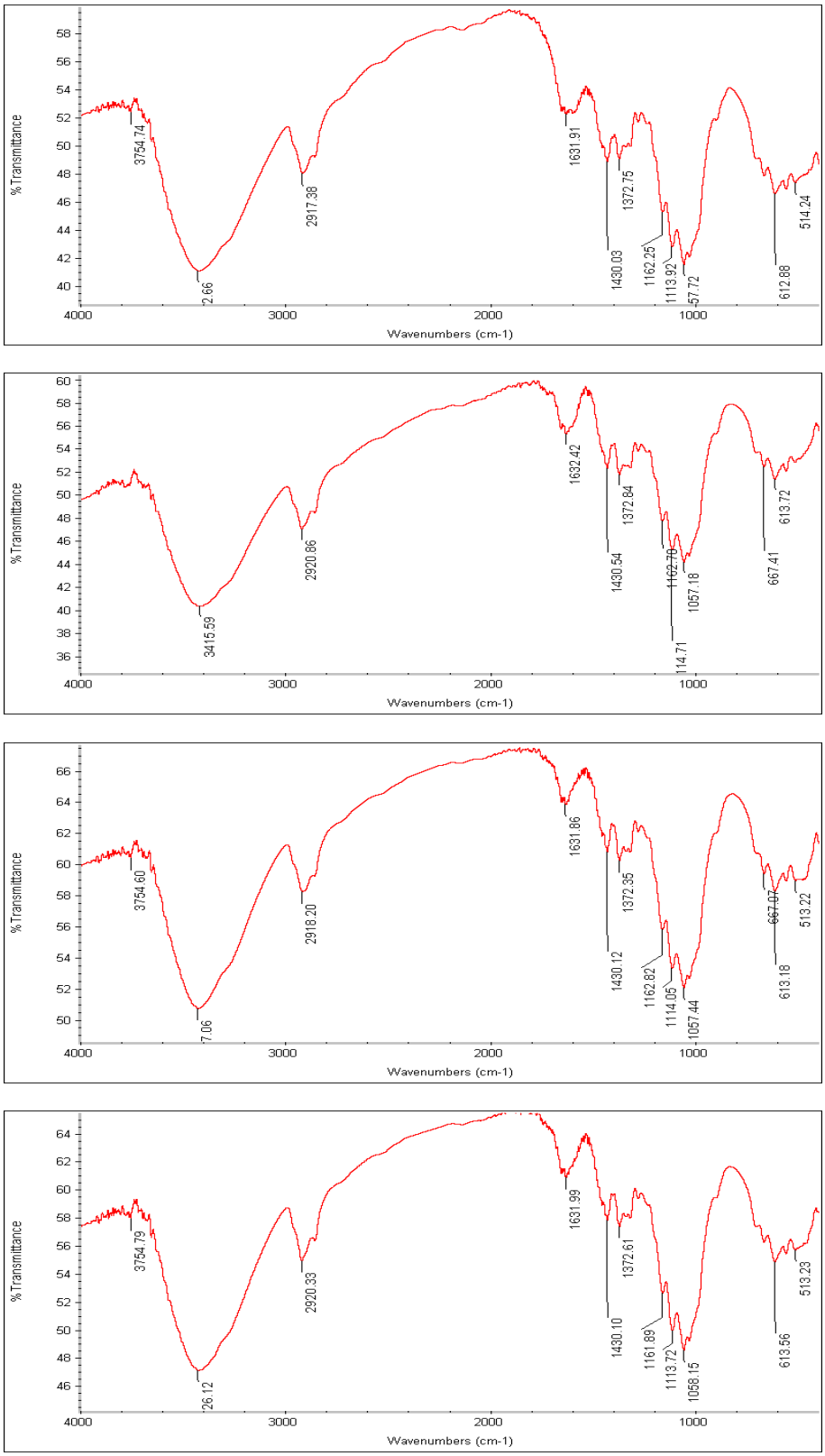


Figure 7. FTIR spectrogram of :(a) scoured cotton yarns(control); (b) dyed with natural dye; (c) dyed with reactive dyes ; (d) dyed with basic dyes

CONCLUSION

The dyeing performance of Egyptian cotton yarn made of Super-Giza 97 variety using reactive, basic and natural dyes has been investigated.

Due to eco-friendliness of natural dyes and the awareness among people regarding the environmental and health hazards associated with the use of synthetic dyes, needing for materials dyed with natural dye is increasing. In the present study the onion outer skin was taken as dye material for the dyeing of cotton yarns as onion is an available and cheap agriculture waste, it will be convenient to produce naturally dyed cotton yarns and eco-friendly fashionable cotton cloths.

Yarns dyed with synthetic dyes, whether reactive or basic showed higher depth of shade than the natural dyed yarns. However, the eco-friendly property of natural dyes especially when extracted from waste materials and applied to a new Egyptian cotton variety is desirable due to the economic and environmental qualities of such treatment.

REFERENCES

1. Abdelmoghny A. M., Orabi M. H. M. and Max Mariz S., A New Egyptian Long Staple Cotton Cross, J. Plant Production, Mansoura Univ., Vol. 9 (12): 1197 - 1202, 2018
2. Atiqur Rahman S., The Preferable Technique of Cotton Yarn Dyeing with Basic Dye, Southeast University Journal of Textile Engineering, Vol. 1, No. 1, January 2021
3. Atiqur Rahman S. and Foisal A. B. M., Dyeing of Cotton Yarn with Basic Dye in Conventional Method and Pretreated with Cationic Polyacrylamide ,SEU Journal of Science and Engineering, Vol. 10, No. 2, December 2016, pp75-80
4. Bahlool S. O. and Saleh S. M., Application of Natural Amino Acid Extracted from Soybean Seed Waste on Egyptian Cotton, Egypt.J.Chem. Vol. 63, No.8. pp.2853-2862 (2020)
5. Benkhaya S. , M'rabet S. , and El Harfi A. , Classifications, properties, recent synthesis and applications of azo dyes, Journal Heliyon, v.6(1); 2020 Jan, PMC7002841
6. Broadbent A. D., “Basic Principles of Textile Coloration,” Society of Dyers and Colourists, West Yorkshire, 2001.
7. Chandravanshi S. and Upadhyay S. K., Interaction of Natural Dye (Allium cepa) with Iconic Surfactant, Journal of Chemistry, Volume 2013. https://doi.org/10.1155/2013/685679
8. Collins G. W., Burkinshaw S. M. and Gordon R., 1998, Dyeing of textiles, https://patents.google.com/patent/WO1998037270A1/en.
9. Chunga C., Leeb M., Choe E. K., Characterization of cotton fabric scouring by FT-IR ATR spectroscopy, Carbohydrate Polymers 58 (2004) 417–420
10. El-Messirya M. & Abd-Ellatif S. A. M., Characterization of Egyptian cotton fibres , Indian Journal of Fibre & Textile Research ,Vol. 38, March 2013, pp. 109-113
11. Iqbal, S. F. (2018) . Influence of yarn structure produced in different spinning systems on the properties of yarn. Int. J. Applied Res., 4(4) : 172-176.
12. Jamil S., Ahmad H., Rauf Khan S., Ramzan M., Janjua S.A., First Synthetic Study of Cube-Like Cobalt Hydroxystannate Nanoparticles as Photocatalyst for Drimarene Red K-4BL Degradation and Fuel Additive, Journal of Cluster Science (2018) 29:685–696.
13. Kumar C. Ganesh, Mongolla P., Basha A., Joseph J., Sarma V.U.M., and Kamal A., Decolorization and Biotransformation of Triphenylmethane Dye, Methyl Violet, by Aspergillus sp. Isolated from Ladakh/ India, J. Microbiol. Biotechnol. (2011), 21(3), 267–273
14. Lewis DM., Dyeing cotton with reactive dyes under neutral conditions, Coloration Technology, 123: 306-311, 2007.
15. Nassar M.A.A., Ibrahim I.A.E., Negm M.A.M., El Bagoury M.I. and Amer R. S. M., Effect of some Egyptian Cotton Varieties and Grades on Fibers and Yarn Mechanical Properties, J. Adv. Agric. Res. (Fac. Agric. Saba Basha)
, Vol. 24 (3), 2019, pp 446-463
16. Nurunnesa Md. Hossain A. , Rahman M., Extraction of Natural Dye Collected from Outer Skin of Onion and its Application on Silk Fabric, Global Journal of Researches in Engineering, Volume 28 Issue 3 Version 1 , 2018 , pp. 1-6
17. Ristić N., Ristić I.,Cationic Modification of Cotton Fabrics and Reactive Dyeing Characteristics, J .Eng. Fabr., Fib., 7,pp. 113-121. 2012.
18. Sharma R.t, Sayed U.,Surface Modification of Cellulosic Fabric, Int. Journal of Advances in Chemical Eng.and Biological Sciences (IJACEBS), Vol. 3, Issue 1, pp. 90-96, 2016.
19. Shrikant V., Pragna K., Someshwar B. and Ashutosh M., Effect of scouring and dyeing on physical properties of the fabric, Journal of the Textile Association - Nov.-Dec. 2005, pp.173-175.
20. Tesema G.B. and K. Hussein (2015). Comparison of different quantification methods to define fiber quality of Ethiopian, Indian & Egyptian cottons .Int. J. of Fiber and Textile Res. , 5 (2) : 9-15.
21. Textileblog, What is Basic or Cationic Dye: Properties and Manufacturing of Basic Dyes, 9, 2021 , https://www.textileblog.com/basic-or-cationic-dye

22. Wakelyn P. J, Bertoniere N., Alfred Dexter French, Devron Thibodeaux , Marie-Alice Rousselle , Barbara Triplett, Wilton Goynes , J Vincent Edwards, Lawrance Hunter, David McAlister & Gary Gamble Lewin, Cotton Fibre Chemistry and Technology (CRC Press, Taylor & Francis Group), 2006.
23. Zhongqi H. and Yongliang Liu, Fourier Transform Infrared Spectroscopic Analysis in Applied Cotton Fiber and Cottonseed Research: A Review, Journal Of Cotton Science, Volume 25, Issue 2, 2021,pp. 167-183.

Rotobar (Rotary Knife Roller Gin), A Boon to Long & Extra-long Staple Cotton

(A paper presented by Dr. M. K. Sharma of Bajaj Steel Industries Ltd. & Dr. Mohamed A. Negm' at WCRC-7 held at Cairo on October 4-7, 2022,)

Abstract:

In the recent past long and extra-long cotton volumes have declined sharply being uncompetitive. The efforts are needed to restore its volumes of production and consumption. If proper ginning technology is used, then the proper processing will help this superior cotton generate premium. Various studies have shown that Rotobar Ginning (Rotary Knife Roller Gin) is found most beneficial ginning technology for long and extra-long cotton. The major producer of long and extra-long cotton such as USA, China Egypt have understood the benefits of this technology and new ultra-modern ginning plants based on Rotobar Ginning technology have been established in Egypt and now China is under process to set up similar ginning mill. This trend is likely to continue which may result in regaining the glory of long and extra-long fine and superfine cotton in near future.

Introduction:

Ginning is the mechanical process for separating cotton in to its constituents namely lint (cotton fibre) and cottonseed. The seed cotton coming from the field has to be subjected to various treatments in the ginning factories depending upon its inherent characteristics such as trash contents, moisture contents, length of the fibre, and variety of the seed i.e. fuzzy or black, method of seed cotton transportation, storage practices, handling practices inside the ginning factories and finally subjected to ginning process for separation of cotton fibre and seeds. After separation cotton fibre (lint) is packed into fully pressed bales (FP Bales) on baling presses and cotton seed is packed / transported to oil mills or for cattle feed. When discussing the various types of ginning the first question that normally comes to mind is, why do we need all these different ginning systems? Why one ginning system can't be utilized for all varieties of cotton? The answer is dictated by the end product and the type of characteristics of cotton needed. There are four ginning technologies being used in the world at present and it is important to select anyone of them for ginning of particular variety of seed cotton for optimal results. The type of ginning technologies are being discussed below:

Type of Ginning:

There are 4 ginning technologies used in the world. Though each Ginning technology can gin most of the cotton but due to the end use and desired quality and quantity parameters of the cotton fibre the selection of most suitable Ginning technology from the following four ginning technologies available in the world have to be selected. Before and after ginning the moisture contents and trash contents have to be dealt with, which requires proper cleaning, dryers or humidifiers and related conveying systems. The Ginning, cleaning and moisture control are different parameters and needs to be handled as such.

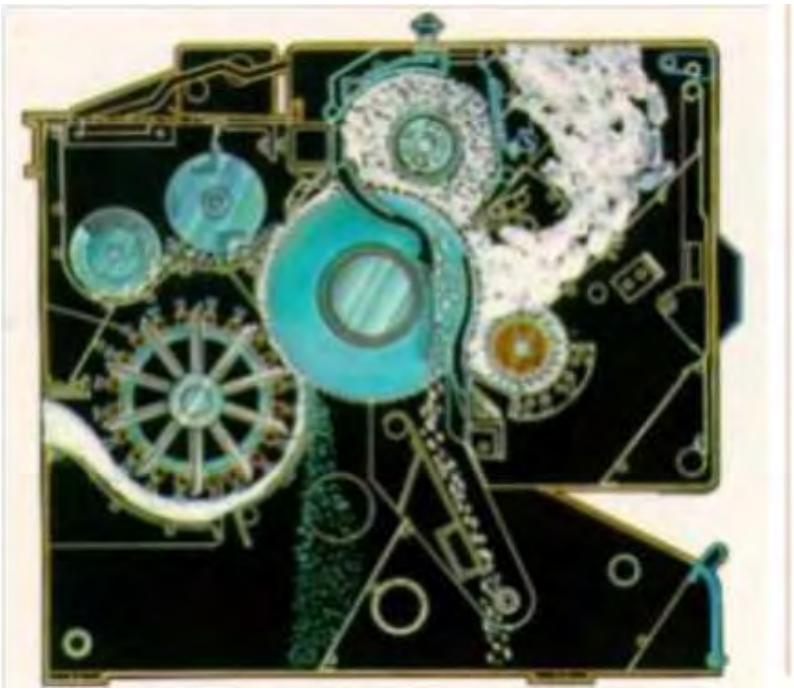
Saw Ginning:

Two type of saw ginning are being used in the world; 1) Brush Doffing Type (High Capacity) and (2) Air Blast Type (Low Capacity). Saw ginning is most suitable for upland fuzzy cotton which adheres strongly to the seed and requires higher force to detach from the seed and the length of the same is up to 29 mm. The saw ginning is having about 55% share in the world cotton ginning. The productivity per unit of electrical consumption is higher in case of brush type saw gins as compared to air blast type saw gins. In the earlier days the space between the two saws was on higher side while in the present the saws have come closer to the optimum extent and highest ginning capacities are being utilized.

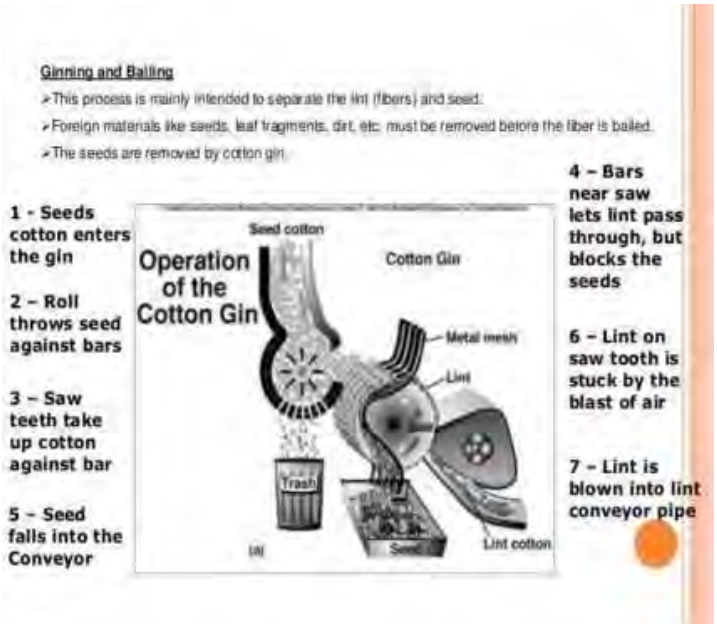
The saw ginning technology is most suitable for high strength, high trash, high moisture, high Micronaire, high maturity cotton varieties having length up to 29 mm, therefore this technology

should be selected for ginning of suitable varieties only. The saw ginning is also used widely where the requirement is for grades by the customer and countries facing problems with bacteria like Honeydew etc.

Pic 1: Saw Gin Machine (Brush Doffing) Plant View



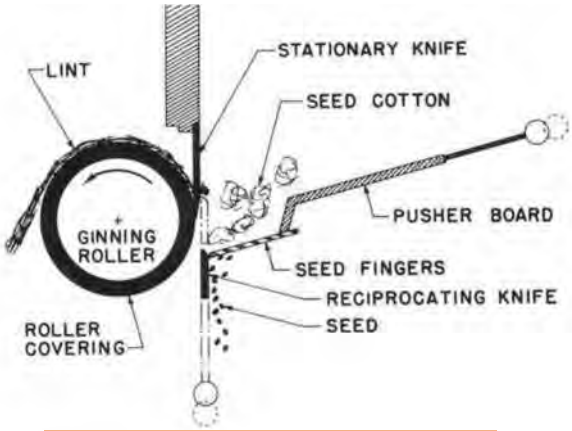
Pic 2: Working Principle of Brush Type Saw Gin



Pic 3: Working Principle of Air Blast Type Saw Gin

Single Roller McCarthy Ginning:

Single Roller Ginning technology is one of the earliest roller gin in which the separation of fibres



Pic 4: Working Principle of Single Roller Gin

Pic 5: A typical Single Roller Gin

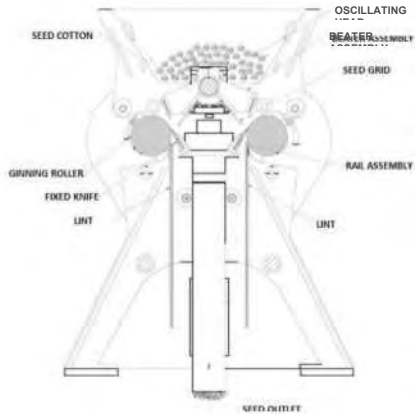
and seed occurs by gentle pulling of fibres by using a roller. This technology has long been preferred method for ginning extra-long staple, fine fibred sea island cotton, Egyptian cotton and Pima cottons (Bennett 1956) while it is possible to gin all types of cotton on single roller gin but it is more suitable for long and extralong cotton varieties and retains maximum natural fibre parameters of the cotton during the ginning, however major disadvantage of McCarthy single roller gin is its lower ginning capacity (about 25 kgs to 40 kgs. lint per hour) The Single Roller ginning is having less than 5% share in the world cotton ginning.

The Single Roller ginning technology can handle higher trash and also gin all kind of cotton whether fuzzy seeded or sleek / black seeded, however the production per unit of electricity consumption and per sq. meter area of space is costly as compared to other technologies, hence the cost of ginning and maintenance is higher as compared to other ginning technologies.

Double Roller Ginning:

This ginning technology is more suitable for clean cotton having above 29 mm length either for fuzzy long staple varieties or sea-island black / naked seeded varieties and preserves fibre parameters near to maximum. This technology is extensively used in India, Myanmar and Eastern Africa and having about 35% share in the world cotton ginning.

Pic 7: Double Roller Gin with Auto Feeder



Pic 8: Working principle of Double Roller Gin

Phoenix Rotobar Roller Gin Designed for Ginning Long Staple "Slick Seed" Cotton and Varieties of Upland "Fuzzy Seed" Cotton.

Pic 9: A view of Double Roller Gin Plant

Double Roller Ginning technology is most suitable for long and extra-long cotton varieties with medium strength (micronaire in the range of 2.2 to 4.2) whether fuzzy seeded or sleek / black seeded having trash in size below 6 mm so that the same may pass through grid. Since the seed coming out of Double Roller gin is having lower fuzz remaining on seed, the seed can be crushed for oil milling without Delinting and oil to seed ratio is better. The neps generation is lower in this ginning technology

Rotobar Rotary Knife Roller Ginning: This technology is most suitable for sea-island or long and extra-long staple cotton where the fibre does not strictly adhere to the seed and the lint can be pulled off, leaving the seed naked i.e. no fuzz on it due to which it comes out very easily and production rate of lint per hour is higher i.e. 200 kgs. to 800 kgs. per hour, when this Rotobar technology is used for fuzzy seeded cotton, the production rate comes down drastically i.e. almost half for same power when used for black seeded cotton, moreover % of seed cut and un-ginned cotton getting mixed with ginned cotton seed is faced due to which this technology is not being widely preferred for fuzzy seeded cotton. For black seeded long and extra-long cotton varieties rotobar is most preferred ginning technology as it gives highest length, retains the luster and fineness.

Ginning by Roller Gins

All the three type of roller gins i.e. (a) Single Roller (McCarthy), (b) Double Roller (Close Type), (c) Rotary Knife Roller Gin (Rotobar) are suitable for ginning long and extra-long varieties and experiments have shown that all the roller ginning technologies can gin upland medium staple varieties with adjustments in settings of operational parameters. These roller gins can be used for handpicked seed cotton as well as machine picked seed cotton after cleaning and moisture control. The Pre-cleaning and Post-cleaning machinery requirements are totally different in both the cases. By using advance pre-cleaning and post-cleaning machinery setup, the machine picked cotton can also be beneficially ginned on roller gins, however capacity per hour and fibre parameters will differ for different varieties.

The roller ginning of cotton to separate fibres from the seed is one of the most ancient processes. In recent times, the importance of roller ginning is increasing rapidly as evidenced by the fact that only about 15% of the world cotton was ginned on roller gin in the year 1990 and now by the year 2022 the quantity of world cotton ginned on roller gins has surpassed 35%. There have been significant change in the cotton varieties as well as various developments in ginning machinery for roller ginning, which is attracting more and more ginners to roller ginning.

Ginning of Long and Extra-long Cotton on Rotobar:

The long and extra-long cotton varieties are produced in following countries:

World LS Output (tonnes)

	2019/20	2020/21	2021/22	2021/22 vs. 2020/21	2020/21 vs. 2019/20
United States	149,000	119,000	80,000	-33%	-20%
Egypt	67,600	58,000	80,000	38%	-14%
India	90,000	85,000	85,000	Unch	-6%
China	60,000	65,000	40,000	-38%	8%
Turkmenistan	21,000	15,000	20,000	33%	-29%
Uzbekistan	5,000	1,000	1,000	Unch	-80%
Tajikistan	1,000	-	-		
Israel	7,800	5,000	4,900	-2%	-36%
Sudan	1,000	1,000	1,000	Unch	Unch
Peru	5,000	4,000	4,000	Unch	-20%
Spain	4,000	3,500	2,500	-29%	-13%
Total	**411,400**	**356,500**	**318,400**	-11%	-13%

Source: Cotton Outlook

Pic 10: Rotobar Gin & working principle

The long and extra-long cotton are most preferred for fine and superfine fabrics and have higher strength. The prices of long and extra-long cotton are significantly higher as compared to upland and other varieties of cotton. In the past the production of long and extra-long varieties were quite higher in Egypt (on an average 1500000 bales/year), American Pima, China and India and other countries, however during the last decade production of long and extra-long staple varieties has declined sharply.

The long and extra-long staple cotton yield per hectare is lower as compared to upland cotton and other varieties of cotton. Further it has very high requirements of temperature, sunshine, heat and rainfall, therefore only selected areas which have suitable climate such as California and West Texas in USA, Awati County, Shaya, Bachu, Korla and Southern Xinjiang (mainly Aksu region) in China etc. To revive the production of long and extra-long cotton countries have to increase plantation area, provide adequate water, and create proper processing facilities (i.e. improvement in harvesting method, cleaning, moisture control, and ginning etc.) which are outdated in many countries. It is also necessary that the research facilities should focus on yield and quality of long and extra-long cotton, which have not made progress in recent years.

For the best ginning parameters of long and extra-long cotton medium speed rotobar gins are found most suitable as this technology is providing longer and clean fibre. The luster and fineness of the long and extra-long cotton is also maintained in rotobar.

Egypt has upgraded their processing to great extent in last 3 years and established four new modern ginning factories with latest Phoenix Rotobar machinery from Bajaj Steel Industries Ltd. India, and have plan for adding further three in coming year, which will improve the fibre parameters in the country and will help for regaining the glory of providing the high quality long and extra-long cotton to the world, however there are some contradictions where the trash reduction is viewed as lowering of ginning outturn. The myth of having more outturn on compromising the quality of the cotton in terms of trash requires detail study with spinners. There is need to add some more up-gradation equipment to achieve uniform feeding, removal of foreign materials for which CDS system with Heavy Particles Removing System is suggested.

Pic. 11: CDS with Heavy Particles Removing System

Apart from uniform feeding , cleaning , the long and extra-long cotton is best ginned when the moisture is around 6 to 8 % hence it is necessary that the dryer should be used for seed cotton if the moisture contents are higher and Humidification System is to be used after ginning when the cotton is pressed in to the bales, it is recommended that the moisture contents should be increased to about 8% by induction of humid-air in the conveying systems, which reduces the pressing force as well as provides desired moisture for better spinning.

With the moisture control, uniform feeding, trained workforce and proper maintenance, rotobar ginning technology will provide best cotton fibre parameters which will be sold on premium and will be commercially competitive as compared to other cotton and competing crops.

Apart from USA, Egypt now China has also realized the advantages of superior technology Phoenix Rotobar Ginning Technology for long and extra-long cotton being provided by Bajaj Steel Industries

Limited India hence now installing modern ginning mill based on Phoenix Rotobar. All these measures are going to help the restoration of glory and volume of long and extra-long cotton and trained may extend to other countries also.

Pic. 11 Mistral Advanced Humidification System

Conclusion:

Various studies have proved that Rotobar Ginning (Rotary Knife Roller Gin) provides best fibre parameters at lowest electrical power consumption retaining luster and fineness of the black seeded long and extra-long cotton which will help in regaining the glory and restoring the volumes of long and extra-long cotton in near future, which will be a boon to this segment.

Recycling textile waste to develop Cellulosic Superabsorbent Polymer (C-SAP) and analyzing its impact in cotton cultivation

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Abstract

There is an increasing global awareness on the need for circularity in the textile industry. The unique Green Machine developed by the Hong Kong Research Institute of Textiles and Apparel Limited (HKRITA) has adopted the world's first hydrothermal separation and decoloring technology. Through hydrothermal treatment, satisfactory separation of polyester and cellulose powder from polyester cotton blends, which is the most common textile blend used in the industry, can be achieved. Heat, water, pressure, and biodegradable green chemicals are employed to separate polyester fibers and turn cotton into cellulosic powder. While polyester fibers are decoloured and the recycled fibers can be re-spun into new yarn and further made into fabrics, the cellulose powder can be processed by dissolution, crosslinking, washing, drying and milling, so as to become Cellulosic Superabsorbent Polymer (C-SAP). C-SAP is known to absorb and retain more than 20 times the volume of liquid relative to its own mass.

The ability of moisture retention opens up scope for utilization of C-SAP in agriculture. Conservation of moisture and mitigating moisture stress during crop growth especially in rainfed conditions and improving productivity under drought conditions assume great importance in agriculture. Even economized use of available limited irrigation water potential by supplementing the root zone with super absorbent polymers becomes very important in boosting low cotton productivity of cotton even in limited irrigation potential zones. The available Superabsorbent Polymers (SAP) are traditionally made from petrochemicals. The synthetic superabsorbent polymers have very high water absorbency (WA=300g/g) and water retention (WR) but they fail in comparison with natural super absorbent polymers because of exceedingly low degradation in soil and harmful side effects in soil and adversely affect plant growth and contribute to soil pollution.

*The results of the initial phase of research experiments conducted at University of Agricultural Sciences Dharwad connected to the use of C-SAP in soil for the possibility of enhancing soil moisture retention, its plausible impact on productivity, fiber quality, and soil property traits are presented here. Main objectives of the experiment were to standardize application of C-SAP by using different levels of C-SAP application rates under rainfed and controlled irrigated conditions (0.5 IW/CPE). The influence of C-SAP was studied on soil related traits like moisture, retention in cotton fields, and micronutrient status. The impact of beneficial changes in soil were assessed on productivity traits and fiber quality. During the study, varying doses of C-SAP were applied in the root zone ranging from 5g/plant to 30 g/plant. To test its role even in economizing irrigation water these doses were tried under limited irrigated situations (**0.5 IW/CPE)**. The comparison of these treatments was done with control treatments such as completely rainfed situation without application of C-SAP, just limited drip irrigation (**0.5 IW/CPE**) and higher level of controlled irrigation (**0.8 IW/CPE**) supplemented with application of recommended dose of fertilizers. The impact was studied on soil moisture (at 80 DAS, 100 DAS and 120 DAS) plant type, productivity and fiber quality traits. The results indicate that soil moisture is enhanced at different stages of crop growth (up to 30%) and this is capable of increasing seed cotton yield by 15 to 20% under rainfed situation followed by limited irrigated situation (**0.5 IW/CPE)**. Fiber properties like fiber length, fiber strength, micronaire values displayed a slight improvement due to the application of C-SAP.. Further, application of C-SAP does not seem to adversely affect different soil properties studied and there is no difference in terms of micronutrient content. C-SAP application does not have any harmful side effects on soil EC. The content of Potassium, Phosphorus, and Sulphur in soil were not affected adversely confirming the safety of using C-SAP in soil.*

Introduction

Demand for circularity within the fashion industry has been growing rapidly in the last few years. The world is looking for practical and scalable solutions to recycle textile waste along the fashion value chain and make them into quality textile material that can be reused in textiles and apparel application. Major fashion brands pledged to use only sustainable and recycled material in 2030. The major surge in demand for recycled polyester currently relies heavily on plastic bottles, which the fashion industry has to compete with the bottle and packaging industries for material. By recovering polyester from blended textile waste, it would provide an alternative and circular source of recycled polyester within the industry. Blended material and mixed color are two pain points in textile recycling. The unique Green Machine has adopted the world's first hydrothermal separation and decoloring technology developed by HKRITA. Through hydrothermal treatment, satisfactory separation of polyester from polyester-cotton blend, which is the most common textile blend used in the industry, can be achieved. "The green machine" a hydrothermal separation technology developed by HKRITA separates cotton and polyester efficiently. Heat, water, pressure, and biodegradable green chemicals are employed to separate polyester fibers and turn cotton into cellulose powder. Polyester fibers are decoloured and the recycled fibers can be re-spun into new yarn and further made into fabrics. While the separated polyester fibers are ready for re-spinning into new products, the cotton decomposed into cellulose powders which can be converted into regenerated cellulose fibers and a durable water repellent finishing reagent. The cellulose powders can also be transformed into Superabsorbent Polymer (SAP) for use in the agriculture sector. SAP can be derived from a number of chemical processes. Here, cellulose powder is dissolved and a crosslinking reaction turns the dissolved solution into a jelly form material. The material is further washed, dried and milled. The Cellulosic Superabsorbent Polymer (C-SAP) derived in this way has impressive hygroscopic properties. It can absorb and retain 20-30 times the volume of liquid relative to its own mass; it is therefore an ideal agricultural water retention agent keeping soil moist. When it rains, the C-SAP in the soil absorbs and retains the rainwater. When drought or simply dry conditions prevails, the water is released to the soil around the roots of cotton. It wets the cotton roots and reduces water stress experienced by the cotton plant whenever the soil is dried up. At the end, C-SAP biodegrades and leaves no trace in the soil.

Many of the synthetic SAP products available in the market are found to have many harmful side effects on soil and plants [(Han *et al* (2010),Yang *et al*.(2014) Chen *et al* (2016) and Chang (2021)]. In the light of this, it is necessary to examine the potential of other types of SAPs. Compared to traditional superabsorbent polymer (SAP) made from petrochemicals, C-SAP has the benefit of being biodegradable in soil and thus can be used in agriculture. No traces are observed after the completion of the crop cycles and thus it is eco-friendly. Since it is high in cellulose content, it can provide several benefits to the root zone of a cotton plant. The initial research on possible influence of C-SAP on soil moisture retention was carried out by Shahi Exports during 2020-21 and a favorable impact on moisture conservation in the cotton root zone was noticed,which also impacted plant growth and ultimately seed cotton yield. Based on these encouraging results, detailed research was planned at University of Agricultural Sciences Dharwad (India).

Material and Methods:

The experiment was laid out in randomized complete block design with three replications and plot size of each treatment was 6.3m X 7.2 m, The spacing followed was 60-120-60 x60 cm paired row planting and sowing was done on 2-7-2021 in medium deep black soil block

Treatment details

Rainfed+	T1	T2	T3	T4	T5	T6	T7	T8
	No C-SAP (Control)	RDF+5gm1 C-SAP	RDF+10gm C-SAP	RDF+15gm C-SAP	RDF+20gm C-SAP	RDF+25gm C-SAP	RDF+30gm C-SAP	RDF+35gm C-SAP
Drip+	T9	T10	T11	T12	T13	T14	T15	T16
	RDF +0.5 IW /CPE +5gm C-SAP	RDF +0.5 IW /CPE +10gm C-SAP	RDF +0.5 IW/CPE +15gm C-SAP	RDF +0.5 IW/CPE +20gm C-SAP	RDF +0.5 IW/CPE +25gm C-SAP	RDF +0.5 IW/CPE +30gm C-SAP	RDF +0.5 IW/CPE + No C-SAP	RDF +0.8 IW/ CPE + No C-SAP

Weather of the season: The rainfall of June and July with the number of rainy days was normal. Hence the sowing was done in time. Though average rainfall from August to October was significantly reduced. Due to reduced rainfall the average growth was reduced during that period. It affected the late formed squares and bolls. However, overall total rainfall received during the crop growth period was more than the average rainfall of 30 years. Rainfall data is presented in Table -4.

Results and Discussion

1. Effect of C-SAP on soil moisture: The data on soil moisture observations recorded at various stages of crop is presented in Table 1. Observations recorded at various stages of crop growth indicated that soil moisture in rainfed treatments and drip treatments are significantly different at 80DAS. In the rainfed treatments all are on par (from T_2 to T_8). Among the drip treatments T_9 (Drip+5gm C-SAP) recorded higher soil moisture (24.4%) as compared to other treatments. However, it was on par with all drip treatments except T_{15} (control no-C-SAP) and T_{16}. Soil moisture observed at 100DAS for drip treatments indicated that T_{12} (Drip+20g C-SAP) recorded significantly higher soil moisture (32.4%) as compared to any other treatments. However, it was on par with all other C-SAP treatments except T_{15} and T_{16}. It shows that at 100DAS drip treatments with any of the dosages of C-SAP had improved soil moisture as compared to treatments without C-SAP application. However, in rainfed treatments (from T_1 to T_8) all were on par in their soil moisture levels. Observations at 120DAS also indicated significantly higher soil moisture in all drip treatments as compared to rainfed treatments. Among drip treatments T_{12} (Drip+20g C-SAP) recorded numerically higher soil moisture (35.2%) as compared to other treatments. However, it was on par with all drip treatments (From T_9 to T_{16}). As compared to this in rainfed treatments, T_6 recorded numerically higher soil moisture (20.2%) as compared to any other rainfed treatments. However, they were also on par (T_1 to T_8) with each other.

2. Effect of C-SAP on growth parameters: Data on plant height, no of monopodials per plant and number of sympodialis per plant are presented in Table 2. Plant height and no of sympodialis per plant were significantly affected due to different treatments. Among the drip treatments plant height was significantly increased with T_{10} (Drip+10g C-SAP) (110.5cm) as compared to any other treatments. However, it was on par with T_9 to T_{16} treatments. No of sympodialis per plant was significantly increased (18.5/plant/) with T_{11} (Drip+15g C-SAP) as compared to any other treatments. However, it was on par with all treatments from T_9 to T_{16}. Whereas number of monopodials per plant were unaffected by different treatments. Both plant height and sympodial numbers in rainfed treatments were on par with each other. Monopodial numbers were unaffected by different treatments.

3. Effect of C-SAP on yield and yield components: Data on number of bolls per plant, number of bolls per sq m, seed cotton yield per plant and single boll weight are presented in Table 2 and 3. Number of bolls per plant and number of bolls per sq. m were not significantly affected by different treatments. Numerically higher no of bolls per plant (29.9) and no of bolls per sq m (55.3) were recorded in T_{12} (Drip+10g C-SAP) as compared to any other treatments. Lowest was recorded in control (T_1) (26.1/plant). Seed cotton yield per plant was significantly affected by different treatments. Drip+10gms C-SAP (T_{10}) recorded significantly higher yield per plant (115.0g) as compared to any other treatments. However, it was on par with all except control and (T_{15}) drip+ no of C-SAP. Whereas single boll weight was unaffected by different treatments.

Seed cotton yield per hectare was significantly affected by different C-SAP treatments. Among the treatments Drip+ 10g C-SAP (T_{10}) recorded significantly higher SCY (2347 kg/ha) as compared to any other treatments. However, it was on par with T_4, T_5, T_9, T_{11}, T_{12}, T_{13}, T_{14} and T_{16} treatments. Similar to the trend of seed cotton yield (SCY) per plant, control recorded significantly lower yield (1710 kg/ha) as compared to C-SAP applied plots**. It was also observed that C-SAP applied plots were showing better yield levels irrespective of dosage levels**. The result also reveals that the average yield levels were higher in drip as compared to rainfed treatments.

Further, it shows that among rainfed treatments Rainfed+ 20g C-SAP (T_5) recorded the highest yield (2277 kg/ha) as compared to other treatments and controls. However, it was on par with T_2 and T_4 treatments. From the rainfed treatments, it can be concluded that C-SAP which is an absorbent molecule by improving soil moisture had improved seed cotton yield though not consistent with various dosages applied.

Table 1: Soil moisture observations as affected by different C-SAP treatments

Treatments	80 DAS	100 DAS	120 DAS
T1 – Control	14.7	15.5	17.7
T2 – RDF+5gm C-SAP	17.6	16.6	19.7
T3 – RDF+10gm C-SAP	17.4	16.5	19.7
T4 – RDF+15gm C-SAP	17.3	17.3	19.1
T5 – RDF+20gm C-SAP	16.7	16.8	19.7
T6 – RDF+25gm C-SAP	17.2	16.3	20.2
T7 – RDF+30gm C-SAP	17.0	16.0	18.3
T8 - RDF+35gm C-SAP	18.3	16.3	19.9
Mean for rainfed (R)	17.0	16.4	19.3
T9 – Drip+0.5 IW/CPE+5gm C-SAP	24.4	29.0	34.4
T10 – Drip+0.5 IW/CPE+10gm C-SAP	22.8	29.0	34.3
T11 – Drip+0.5 IW/CPE+15gm C-SAP	22.2	29.9	34.9
T12 – Drip+0.5 IW/CPE+20gm C-SAP	22.9	32.4	35.2
T13 – Drip+0.5 IW/CPE+25gm C-SAP	23.6	30.6	34.4
T14 – Drip+0.5 IW/CPE+30gm C-SAP	23.9	29.3	33.8
T15 – Drip+0.5 IW/CPE + Control, No C-SAP	20.6	26.4	33.0
T16 – Drip+0.8 IW/CPE + Control, No C-SAP	21.8	27.5	34.1
Mean for drip (D)	22.8	29.2	34.3
For comparison of RxD SE(m)	1.0	0.6	0.5
C.D at 5%	NS	3.8	3.2
For comparison of treatments (from T1 to T16)			
SE(m)	1.3	1.6	1.3
C.D at 5%	3.7	4.7	3.8

Table 2: Growth & yield components as affected by different C-SAP treatments

Treatments	Plant height(cm)	No of monopodials/ Plant	No of Sympodialis/ Plant	No of bolls/ plant	No of bolls/ sq m
T1 – Control	89.0	1.40	15.5	26.1	48.4
T2 – RDF+5gm C-SAP	95.6	1.93	15.4	28.3	52.3
T3 – RDF+10gm C-SAP	97.0	1.67	15.6	27.8	51.5
T4 – RDF+15gm C-SAP	96.2	1.73	16.1	27.3	50.6
T5 – RDF+20gm C-SAP	94.7	1.53	14.9	28.2	52.2
T6 – RDF+25gm C-SAP	93.7	1.53	15.3	26.5	49.2
T7 – RDF+30gm C-SAP	94.5	1.67	15.8	27.0	50.0
T8 - RDF+35gm C-SAP	93.0	1.60	15.7	27.6	51.1
Mean for rainfed (R)	94.2	1.63	15.5	27.4	50.7
T9 – Drip+0.5 IW/CPE+5gm C-SAP	107.3	1.60	18.0	27.1	50.3
T10 – Drip+0.5 IW/CPE+10gm C-SAP	110.5	1.40	18.2	29.9	55.3
T11 – Drip+0.5 IW/CPE+15gm C-SAP	106.7	1.33	18.5	28.1	52.0
T12 – Drip+0.5 IW/CPE+20gm C-SAP	104.7	1.67	17.3	28.5	52.8
T13 – Drip+0.5 IW/CPE+25gm C-SAP	105.5	1.60	17.5	26.7	49.5
T14 – Drip+0.5 IW/CPE+30gm C-SAP	109.8	1.57	17.6	27.5	51.0
T15 – Drip+0.5 IW/CPE + Control, No C-SAP	102.4	1.40	16.8	27.2	50.4
T16 – Drip+0.8 IW/CPE + Control, No C-SAP	102.7	1.47	16.7	26.8	49.6
Mean for drip (D)	106.2	1.50	17.6	27.7	51.4
For comparison of RxD					
SE(m)	1.7	0.09	0.2	0.9	1.7
C.D at 5%	11.1	NS	1.2	NS	NS
For comparison of treatments (from T_1 to T_{16})					
SE(m)	4.0	0.21	0.7	1.2	2.3
C.D at 5%	11.7	NS	1.9	NS	NS

Table 3: Effect of different C-SAP treatments on seed cotton yield and yield components

Treatments	Seed cotton yield/plant (g)	Single boll weight (g)	Seed cotton Yield (Kg/ha)
RAINFED			
T1 – Control	102.8	5.08	1,710
T2 – RDF+5gm C-SAP	106.4	5.52	2,073
T3 – RDF+10gm C-SAP	109.6	5.42	1,976
T4 – RDF+15gm C-SAP	107.5	5.37	2,089
T5 – RDF+20gm C-SAP	117.9	5.43	2,277

T6 – RDF+25gm C-SAP	107.5	5.28	1,884
T7 – RDF+30gm C-SAP	106.7	5.25	1,884
T8 - RDF+35gm C-SAP	106.5	5.43	1,901
Mean for rainfed (R)	108.1	5.35	1,974
DRIP (0.5 IW/CPE)			
T9 – Drip+0.5 IW/CPE+5gm C-SAP	105.9	5.38	2,163
T10 – Drip+0.5 IW/CPE+10gm C-SAP	115.0	5.57	2,347
T11 – Drip+0.5 IW/CPE+15gm C-SAP	106.9	5.23	2,191
T12 – Drip+0.5 IW/CPE+20gm C-SAP	111.9	5.37	2,198
T13 – Drip+0.5 IW/CPE+25gm C-SAP	109.5	5.53	2,151
T14 – Drip+0.5 IW/CPE+30gm C-SAP	113.5	5.50	2,188
T15 – Drip+0.5 IW/CPE + Control, No C-SAP	102.7	5.53	2,065
T16 – Drip+0.8 IW/CPE + Control, No C-SAP	113.5	5.67	2,126
Mean for drip (D)	109.9	5.47	2,179
For comparison of RxD SE(m)	3.3	0.05	49
C.D at 5%	NS	NS	NS
For comparison of treatments (from T_1 to T_{16})			
SE(m)	5.3	0.15	89
C.D at 5%	15.0	NS	259

Table 4: Effect of C-SAP application on fiber quality parameters of cotton

Treatments	Fiber length (cm)	Fiber Strength (g/tex)	Micronaire value	Uniformity index	Maturity ratio	Elongation percentage
T_1 – Control	27.6	28.1	3.17	84.1	0.83	7.43
T_2 – RDF+5gm C-SAP	27.7	27.9	3.07	85.4	0.82	7.10
T_3 – RDF+10gm C-SAP	27.6	27.2	3.38	84.7	0.83	6.97
T_4 – RDF+15gm C-SAP	27.2	26.9	3.17	84.5	0.82	7.30
T_5 – RDF+20gm C-SAP	27.4	28.0	3.31	85.2	0.83	7.03
T_6 – RDF+25gm C-SAP	27.5	26.9	3.46	84.5	0.84	7.10
T_7 – RDF+30gm C-SAP	27.4	27.8	3.36	84.9	0.84	6.40
T_8 - RDF+35gm C-SAP	27.4	27.1	3.26	84.2	0.83	7.20
T_9 – Drip+0.5 IW/CPE + 5gm C-SAP	28.3	28.0	3.25	84.9	0.83	7.57
T_{10} – Drip+0.5 IW/CPE + 10gm C-SAP	27.4	27.8	3.38	84.1	0.83	7.33
T_{11} – Drip+0.5 IW/CPE + 15gm C-SAP	27.8	27.9	3.36	84.4	0.83	7.33
T_{12} – Drip+0.5 IW/CPE + 20gm C-SAP	27.7	28.1	3.64	83.7	0.84	7.33
T_{13} – Drip+0.5 IW/CPE + 25gm C-SAP	27.7	28.7	3.47	84.4	0.83	7.13
T_{14} – Drip+0.5 IW/CPE + 30gm C-SAP	27.8	27.4	3.82	85.6	0.85	6.93
T_{15} – Drip+0.5 IW/CPE + Control, No C-SAP	27.6	28.1	3.48	85.0	0.83	7.27
T_{16} – Drip+0.8 IW/CPE + Control, No C-SAP	27.5	28.1	3.31	83.9	0.82	7.97
SE(m)	0.3	0.8	0.12	0.5	0.003	0.29
C.Dat 5%	NS	NS	0.34	NS	NS	NS

4. Effect of C-SAP on fiber quality: Observations on fiber quality are given in Table 4 A comparison revealed that fiber quality parameters viz, Fiber length, Fiber strength, Uniformity index, Maturity ratio and Elongation percentage were not significantly affected by C-SAP application rates. Micronaire value showed significant difference among the treatments but rates of application of C-SAP did not show any

clear trend of relation with dose of C-SAP applied. Application of C-SAP does improve the quality of the fibers

Table 5: Soil chemical properties as affected by C-SAP application in cotton

Treatments	pH of the soil	EC of the soil M mho/sec	P in soil Kg /ha	K in soil	S in soil
T_1 – Control	8.09	0.17	90.0	398.0	142.2
T_2 – RDF+5gm C-SAP	8.02	0.17	105.9	378.7	164.2
T_3 – RDF+10gm C-SAP	8.14	0.18	88.4	394.1	129.1
T_4 – RDF+15gm C-SAP	8.04	0.14	86.4	427.5	190.5
T_5 – RDF+20gm C-SAP	8.08	0.16	77.7	480.9	122.0
T_6 – RDF+25gm C-SAP	8.13	0.17	32.2	735.4	141.7
T_7 – RDF+30gm C-SAP	8.56	0.19	35.5	463.4	170.0
T_8 - RDF+35gm C-SAP	7.89	0.17	55.9	388.3	129.8
T_9 – Drip+0.5 IW/CPE+ 5gm C-SAP	8.10	0.14	21.8	349.9	162.3
T_{10} – Drip+0.5 IW/CPE + 10gm C-SAP	8.38	0.15	44.8	384.1	174.8
T_{11} – Drip+0.5 IW/CPE + 15gm C-SAP	7.98	0.15	62.7	342.3	160.7
T_{12} – Drip+0.5 IW/CPE + 20gm C-SAP	8.39	0.19	29.3	685.0	150.4
T_{13} – Drip+0.5 IW/CPE + 25gm C-SAP	8.04	0.11	45.4	367.0	146.5
T_{14} – Drip+0.5 IW/CPE + 30gm C-SAP	7.88	0.15	50.7	365.7	171.6
T_{15} – Drip+0.5 IW/CPE + Control, No C-SAP	8.15	0.13	38.3	359.5	175.8
T_{16} – Drip+0.8 IW/CPE + Control, No C-SAP	8.20	0.14	61.9	378.5	161.4
SE(m)	0.19	0.02	18.1	89.7	19.9
C.D at 5%	NS	NS	NS	NS	NS

5: Effect of C-SAP on Soil properties: Data on different soil property parameters presented in Table 5 indicates that for all the above parameters viz., pH, EC, P, K and S content of soil there were no significant differences among different treatments. It shows that the different rates of C-SAP applications had no influence on the soil nutrient status (above nutrients). Soil micronutrient status was also studied in all the treatments, and it was observed that the content of all these micronutrients was unaffected by C-SAP application in cotton. Therefore, C-SAP does not have any harmful impact on the soil and is safe to use.

Table6: Soil micronutrient status as affected by C-SAP application in cotton

Treatments	Ca in soil Meq/ 100g	Mg in soil	Zn in soil PPM	Fe in soil	Mn in soil	Cu in soil
T_1 – Control	56.5	20.2	0.70	3.54	9.2	2.56
T_2 – RDF+5gm C-SAP	53.2	24.3	1.34	3.18	216.3	5.32
T_3 – RDF+10gm C-SAP	53.7	18.8	0.80	3.34	12.0	2.32
T_4 – RDF+15gm C-SAP	56.3	16.7	8.86	3.21	222.2	4.86
T_5 – RDF+20gm C-SAP	59.0	22.5	1.12	3.38	11.6	3.06
T_6 – RDF+25gm C-SAP	55.7	21.2	1.24	3.62	182.5	4.96
T_7 – RDF+30gm C-SAP	51.3	23.7	1.24	2.39	177.1	4.62
T_8 - RDF+35gm C-SAP	61.7	23.0	0.93	3.16	12.0	2.40
T_9 – Drip+0.5 IW/CPE+ 5gm C-SAP	55.8	20.5	0.87	2.90	131.7	3.62
T_{10} – Drip+0.5 IW/CPE + 10gm C-SAP	49.8	17.5	1.71	2.69	192.6	4.75
T_{11} – Drip+0.5 IW/CPE + 15gm C-SAP	56.0	25.3	0.78	4.02	35.4	2.50
T_{12} – Drip+0.5 IW/CPE + 20gm C-SAP	53.7	22.8	1.45	2.29	181.7	4.93
T_{13} – Drip+0.5 IW/CPE + 25gm C-SAP	53.7	36.0	4.60	3.41	14.3	2.27
T_{14} – Drip+0.5 IW/CPE +	57.3	21.2	1.02	3.70	10.0	2.56

30gm C-SAP						
T_{15} – Drip+0.5 IW/CPE + Control, No C-SAP	57.3	17.3	1.23	2.95	183.4	4.83
T_{16} – Drip+0.8 IW/CPE + Control, No C-SAP	58.0	23.5	0.70	3.06	9.0	2.32
SE(m)	3.6	3.2	1.98	0.46	13.9	0.30
C.D at 5%	NS	NS	NS	NS	40.4	NS

Manganese in soil showed larger variation but the differences were not significant. Based on these observations it can be concluded that the soil fertility was not affected adversely due to different rates of C-SAP applications.

Table 7: Monthly Rainfall at Agricultural Research Station, Dharwad

Months	Average Normal (Average of 30 years)		2021		Deviation in rainfall Excess/ Deficit	
	Rainfall (mm)	No. of rainy days	Rainfall (mm)	No. of rainy days	In mm	Excess/ Deficit in percentage
January	1.8	0	55.2	3	53.4	2905.4
February	2.5	1	13.4	2	10.9	433.2
March	8.0	1	0.0	0	-8.0	-100.0
April	45.8	3	79.2	7	24.2	53.0
May	74.0	4.5	138.0	9	64.0	86.6
June	121.9	9.1	182.0	12	60.1	49.3
July	128.0	11.4	182.8	14	54.8	42.8
August	114.2	11.3	37.0	7	-77.2	-67.6
September	113.7	7.2	38.6	8	-75.1	-66.1
October	125.4	7.1	90.2	9	-35.2	-28.1
November	31.6	1.8	121.2	7	89.6	283.9
December	8.4	1	40.6	2	32.2	383.3
Total	775.3	58.4	978.2	80	202.9	26.2

Conclusion: The research on possible influence of C-SAP in soil indicates that cotton on soil moisture, is enhanced at different stages of crop growth up to 30% and this is capable of increasing seed cotton yield by 15 to 20% under rainfed situation followed by limited irrigated situation (0.5 IW/CPE). Fiber properties did not show any noticeable change due to application of C-SAP. Further application of C-SAP does not seem to adversely affect different soil properties studied and there is no difference in terms of micronutrient content. Beneficial effects of biological SAP have been reported by many studies [(Agaba *et.al*., (2010), Demitri *et. al*., (2013), Thombare *et.al*., (2018), Hou *et.al*., (2018), Kong *et.al*., (2019), Ostrand *et al*., (2020)]. C-SAP application does not have any harmful side effects on soil properties. When total rainfall is higher, the +differences caused by C-SAP may be masked and hence the results are expected to be more pronounced when C-SAP is tested under moisture stress situations.

The aim of the series of research experiments done at HKRITA, Hong Kong, Shahi Exports and University of Agricultural Sciences Dharwad, India is to put cotton waste back into nature to grow new cotton more efficiently, improve farmers' income and livelihood of cotton farmers and contribute to a potential win-win-win for industry, farmers, and the environment. These initial results indicate that CSAP may be targeted for use even under drought so that cotton yield can still be guaranteed, ensuring farmers a stable income.

References

1. Agaba, H. L. J., Orikiriza, B., Francis, O, E, J., Obua, J., Kabasa, D. and Huttermann, A, (2010). Effects of hydrogel amendment to different soils on plant available water and survival of trees under drought conditions. Clean – Soil, Air, Water 38: 328–35.
2. Demitri, C., Scalera, F., Madaghiele, M., Sannino, A. and Maffezzoli, A, (2013). Potential of cellulose-based superabsorbent hydrogels as water reservoir in agriculture, Int. J. Polym. Sci., 435073.
3. Chen, X., Mao, X., Lu, Q., Liao, Z. and He, Z, (2016). Characteristics and mechanisms of acrylate polymer damage to maize seedlings. Ecotoxicology and Environ. Safety, 129: 228–34.
4. Han, Y, G., Yang, P. L., Luo, Y. P., Ren, S, M., Zhang, L. X. and Xu. L, (2010). Porosity change model for watered super absorbent polymer-treated soil. Environl. Earth Sci., 61:1197–205.
5. Chang, L., Xu, L., Liu, Y. and Qiu, D, (2021). Superabsorbent polymers used for agricultural water retention. Polymer Test., 94: 107021
6. Megan, S., Ostrand, DeSutter, T. S., Aaron, L. M., Daigh., Limb, R. L. and Steele, D. D, (2020). Superabsorbent polymer characteristics, properties and applications. Agrosyst. Geosci. Environ., 3:e-20074.

7. Thombare, N., Mishra, S., M. Siddiqui, Z., Jha, U., Singh, D. and Mahajan, G. R, (2018). Design and development of guar gum based novel, superabsorbent and moisture retaining hydrogels for agricultural applications. Carbohydr. Polym., 185 169–178.
8. Kong, W., Lia, Q., Lia, X., Sua, Y., Yue, Q. and Gao, B, (2019). A biodegradable biomass-based polymeric composite for slow release and water retention. J. Environ. Manag., 230: 190–198.
9. Hou, X., Li, R., He, W., Dai, X., Ma, K. and Liang, Y, (2017). Superabsorbent polymers influence soil physical properties and increase potato tuber yield in a dry-farming region. J. Soils Sediments 18: 816–826.
10. Yang, L., Yang, Y., Chen, Z ., Guo, C. and Li, S. (2014). Influence of super absorbent polymer on soil water retention, seed germination and plant survival for rocky slopes eco-engineering. Ecol. Engineering 62: 27–32.

Odor Control in Cotton Fabrics Treated with Silver Nanoparticle in Aqueous Extract from Banana Peel

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Abstract

Background: Body odors are the result of bacterial activity and can be described by their chemical compounds. Odor which is "Perm stink" the unpleasant smell that won't wash out of textile products has become a topic of conversation for consumers and brands alike. The type of odor treatment usually uses heavy metals like silver, zinc, sometimes copper and other chemicals. Consumers love the odor control, they don't like bacteria, but they also do not like chemicals.

Results: The innovation is to use biosynthesis of silver nanoparticles (AgNPs) in combination with natural plant extracts from Banana peel and thus, can be an economic and efficient alternative for the large-scale synthesis of nanoparticles.

Conclusion: The produced new composite shows a clear microbial resistance against different species of Bacteria. The treatments on cotton not only improve its antimicrobial efficiency but also natural biocidal effect, which forms in cotton textiles, so it's green and its odor control at the same time.

Keywords: Banana peel, silver nanoparticle, Cotton, odor control, microbial resistance

Introduction

Cotton is the most widely used textile fiber in the world. According to Fibre2Fashion's market analysis tool TexPro, global cotton production was 26.43 million metric ton in market year (MY) 2019-20. in MY 2020-21, it is expected to raise to 26.52 million metric ton in MY 2021-22. Cotton has a high absorbency rate and holds up to 27 times its own weight in water (Meenaxi et al. 2009). However, the moist cotton can be easily attacked by bacteria. Antimicrobial textiles with improved functionality have a variety of applications in health and hygiene products, especially the garments worn close to the skin, (Menezes, E., 2002). With increasing world population and the spread of disease, the number of antibiotic resistant microorganisms is rising along with the occurrence of infections from these microorganisms. Consumers worldwide are looking for clothing which provide greater comfort and remains fresh and odor -free in use. Clothes and other textiles materials can act as carriers for microorganisms such as pathogenic or odor generating bacteria and moulds. Vast evidence of research has seen in the area of finishing of textiles to impart functional properties such as anti-odor or fragrance finishing, antimicrobial finishing, cosmeto-textiles for skin care and so on, (Holme, I. 2007). The term body odor means odors generated as a result of natural functioning of human body. Odor produced by microorganisms of the skin through decomposition of skin secretions, urine and other body odor. Such odors are mainly organic compound, which contain different functional group and chemical structure. Such as amine, alcohols, aldehyde ketone phenols etc, (Prada et al. 2014). At present, many of the plants used for dye extraction are classified as medicinal, and some of these have been shown to possess significantly antimicrobial effect. The antimicrobial activities of some of these dyes are reported as potent owing to the existence of phenol, tannin and quinone in their extracts. The antimicrobial effects of some plants used in dye industries contribute to the longer life of the products are used, (Mehrabian et al. 2000). Banana peel was evaluated as a multi-functional antibacterial and UV protective agent on the cotton substrate (Salah M. 2012). The preparation of nanoparticles by plant extracts gives many advantages as it doesn't need elaborate processes for example, intracellular formation or multiple purification process or the protection of microbial cell cultures (Mohanpuria et al. 2008). The preparation of silver nano-particles by green method such as synthesis from honey bee (El-Bisi et al. 2013), Citrullus Colocynthis, and, sugar (El- Bisi et al. 2015, Fouda et al. 2013). All this natural and biomaterials

can be used as a reducing and stabilizing at the same time to produce silver nanoparticles (AgNPs) and at the same time they don't have harmful effect. Salah M, and Yasser (2015) studied biosynthesis of silver AgNPs by banana leaf extracts. Plant extracts are not expensive and ecofriendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. The AgNPs formed by reaction of banana leaf extracts with aqueous solutions of silver nitrate ($AgNO_3$). The synthesized nanoparticles were confirmed by using UV–Vis absorption spectroscopy, X-Ray diffraction (XRD) and Transmission Electron Microscope (TEM). Cotton textiles such as underwear, T-shirts and outerwear are prone to developing odors, as are bed linen and towels. Active odor control gave cotton textiles long-lasting freshness. In the present work, the natural Banana peel in combination with AgNPs was directly applied onto cotton fabric to evaluate odor control and antibacterial activity against different bacterial strains.

Materials

Cotton fabric

The Egyptian cotton Giza 94 variety of fiber characteristics: UHM (33.5), UI (87.2), Elongation % (6.2), cN/tex (43.0), and Micronaire value (4.1) was used to produce cotton carded roving with 0.25 Ktex linear density in the Spinning and Weaving Company, Alexandria. The yarn samples were tested in the Spinning Research Department, Cotton Research Institute, under standard conditions. Physical properties of the yarn used were measured using the Statimat ME and Uster Tester 3 as represented in Table 1. Carded ring yarn of Ne 30/1 was produced as they are widely used for woven fabrics and used to produce plain woven fabrics on a Texmaco shuttle loom by Cairo Secondary School for spinning and weaving. The construction of fabric was kept constant at 24 ends and 22 picks per centimeters (i.e.,60 ends × 56 picks per inch) for single 30Ne yarns. Table 2. showed the physical and mechanical tests for the produced fabric in weft and warp direction after conditioning of the fabrics for 24 hours under the standard atmospheric conditions.

Precursor Materials

All chemicals used were of analytical grade using doubly distilled water (18.5 MΩ.cm-1). NaOH was analytical grade (Koch-Light Co.), Hydrogen peroxide (30% LR grade) from Aldrich. Sodium carbonate (LR grade), sodium silicate (136°Tw, 27% SiO2), the wetting agent was the commercially Triton X 100, and $AgNO_3$ were supplied by Merck.

Table 1. Physical properties of spun on ring spinning system (30/1s)

Yarn Properties Spinning system	Ring
Tenacity (cN\tex)	17.63
Hairiness (H)	4.81
Elongation (E%)	5.89
CVm%	15.78
No. of Neps / Km	293

Methods

Scouring and bleaching

Scouring of the fabric samples was performed by the pad-steam technique by padding the fabric with 3% NaOH containing 1.5 to 2% of the wetting agent in a two-bowel padding mangle adjusting the squeeze pressure to enable 100% wet pick-up of the fabric and subsequently steamed in a laboratory steamer at 100℃ for 10 min. The scoured fabric was washed with water, neutralized with dilute acetic acid, further

washed with water, and finally dried in air. The scoured fabrics were immersed in alkaline bleach liquor (180 ml H_2O), containing Na_2CO_3 (0.2 g/l), NaOH (1.5 g/l), SiO_2 (0.4 g/l), $MgSO_4$ (0.2 g/l), Triton 100 (0.5 g/l), and H_2O_2 (10 ml-1) were added to the bleaching liquor. The samples were removed from the liquor and neutralized with aqueous solution containing 0.1% acetic acid followed by a through hot water (80 to 85℃) to ensure removal of residual chemicals. Samples were dried in an oven at 100℃ for 60 min.

Table 2. Physical properties of the produced Fabric

Fabric properties			
Air Permeability cm3/cm2.sec		76.33	
Pilling Res. Grade		4	
Breaking force Kg/F		65.5	64.3
Warp	Weft		
Elongation %		8.6	8.9
Warp	Weft		
Thickness mm		0.44	
Thickness Loss after 1000 Abrasion cycle (%)		2.45	

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Banana peel extraction

The extraction of Banana peel was carried out with the procedure obtained by (salah M, 2012). Banana peel was collected and dried in an open environment for 7 days under shade. The peel was then ground into a fine powder. About 20 g of peel powder was boiled in 1L of NaOH (0.1%) until the solution be 500 ml. The solution was allowed to stand wherein a yellow supernatant forms at the top. The entire slurry was then filtered and any solid material discarded. The extracted liquor was used as the foundation of the dye and used as reducing agent for Synthesis of AgNPs.

Synthesis of AgNPs

AgNPs was prepared according to our previous work, (Salah M. and Abd El-Baset, Y. A, 2015), with minor adaptation by adding volumes of the Banana peel extracts in a range between 25 ml to 100 ml with 50 ml of $AgNO_3$ solution (10^{-3} M). Then, the volume was mixed with de-ionized water until to reach a final volume of 200 ml. This mixture was subjected to microwave irradiation for 90 sec. at 200 Watt. The solution allowed to stand in a dark bottle to avoid oxidation for 24 h.

Cotton fabric treatment technique

Coating and dyeing were carried out by padding the cotton fabric in the Banana peel extracted solution used as natural dye with synthesized AgNPs (DI) and without synthesized AgNPs, (DII) using exhaust method at 40°C and liquor to material ratio of 50:1. After 1h, the temperature was increased to 80°C for 30 min. the treated fabric samples were thoroughly washed, neutralized and dried in air. The curing technique was carried out using microwave for 90 sec. at 500 Watt.

Characterization of the cotton fabric

Antibacterial assessment (anti-odor)

Antibacterial assessment (anti-odor) against Staphylococcus aureus (gram positive), Klebsiellapn eumoniae (gram negative) and Pseudomonas aeruginosa was carried out according to modified KSK 0693-2006 standard method (Assessment of antibacterial activity). The concentrations of the cultures were adjusted using spectrophotometer (λ660nm) to 1.3 ×105 colony forming unit (CFU) per ml. The bacteriostatic reduction rate was estimated by the standard equation:

$$\text{Reduction (\%)} = [(A-B) / A] \times 100 \quad [1]$$

Where A and B is the bacteria colonies of untreated and treated fabrics (D1, and DII), respectively.

Dyeability Measurements

The color strength (K/S) of the treated samples using the untreated samples as blank was determined using Perkin Elmer Spectrophotometer, Model Lambda 35 equipped with integrated sphere with applying the Kubelka-Munk equation, (1931).

$$K/S = [(1-R)\ 2/\ 2R]$$

The color parameters L*(lightness-darkness), a*(red-green), b*(blue-yellow component, and ΔE were measured by using the Win lab software CIE TC1-29 proposed color difference equation.

Physical properties

Air permeability was determined by Prolific air Permeability tester (FX3300 SDL) as per the standard procedure ASTM D737-04 (2008). five readings were taken for the fabric sample and then the mean was calculated. Pilling resistance test was calculated with ASTM-D3512 standard method. The tensile test was done according to ASTM D5034. Five fabric strips in both warp and weft directions were subjected to the load and the average force was expressed as the fabric breaking strength and elongation% were measured according by the following Equation;

$$E\ \% [(L-L_0)/ L_0]\ 100$$

where 'L_0' is the initial length of strip (strain gauge = 20 cm), 'L' is the length of strip at the rupture point

Results and discussions

Banana peel contains flavonoids (Luteolin and Apigenin) which have bactericidal and antioxidant activity, and contribute to the Ag ion reduction process. Fig. 1 showed the UV-Vis absorbance peaks at around 430 nm which confirmed the preparation of AgNPs, and the solution turned from yellowish to dark brown. The bright colors attributed to the collective oscillation of the free conduction electrons induced by an electromagnetic field. These oscillations depend on the particle size, and with increasing size the Plasmon absorption maximum is shifted to longer wavelength and the band width increases. The maximum absorbance of $AgNO_3$: Banana peel extract reached when the ratio was 1:1.

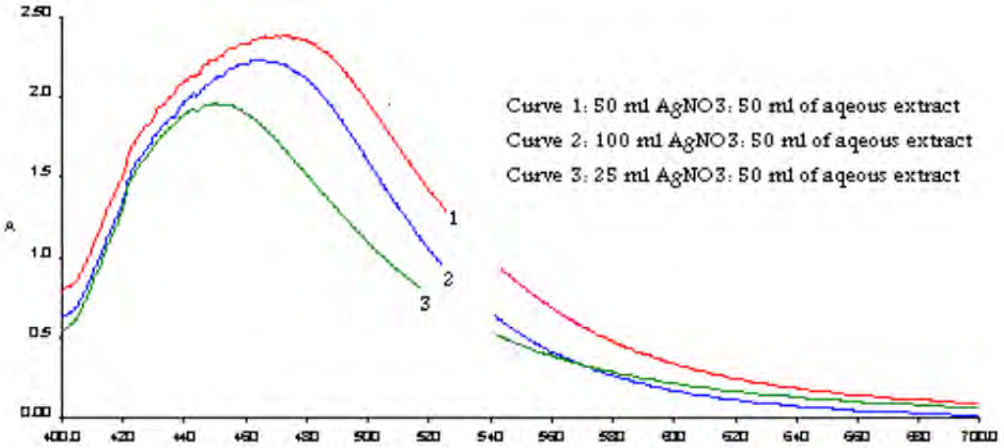

Fig.1. UV-Vis absorbance of the synthesize AgNPs with aqueous extract of Banana peel

Antimicrobial assessment (anti-odor)

The antibacterial activity (the bacteriostatic reduction rates in percentage) of control, DI, DII against Staphylococcus aureu, Escherichia coli, and Pseudomonas aeruginosa was illustrated in Fig.2. DI sample displayed outstanding antibacterial activities in the range 90.5, and 91.9, and 93.4 against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa respectively. The antibacterial activity of DI samples might be attributable to luteolin which is one of the more common flavones a known antibacterial and antioxidant present in the banana peel. These results were accordance with our previous work Salah M, and Yasser, (2015). On the other hand, DII have an excellent antimicrobial activity and displayed outstanding antibacterial activities in the range 97.5, and 98.9, and 97.4 against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa respectively. It has been noted that the AgNPs improved the antibacterial activity. These results indicated that AgNPs inhibit the growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. AgNPs acts as synergism effect with the presence of luteolin as natural inhibitor. These results indicated that the AgNPs and luteolin inhibit the growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Liao et al., 2019, pointed out that AgNPs can accumulated in the pits that form on the cell wall after they anchor to the cell surface. This accumulation can cause cell membrane denaturation. Because of their nanoscale size, AgNPs, have the ability to penetrate bacterial cell walls and subsequently change the structure of the cell membrane, Zhou X, et al. (2019). In addition, AgNPs, can dephosphorylate tyrosine residues on the peptide substrates be involved in bacterial signal transduction. Fig. 3 demonstrated that AgNPs penetrate the fabric surface and will cause physical changes in the bacterial membrane, like the membrane damage, which can lead to cellular contents leakage and bacterial death. Ag^{+} as a heavy metal ion can cause the increase of cellular oxidative stress in microbes, which is another antibacterial mechanism. The released Ag+ from AgNPs was suggested to interact with respiratory chain proteins on the membrane, interrupt intracellular O_2 reduction, and induce ROS production. The results were accordance with the data obtained by long et al. 2017.

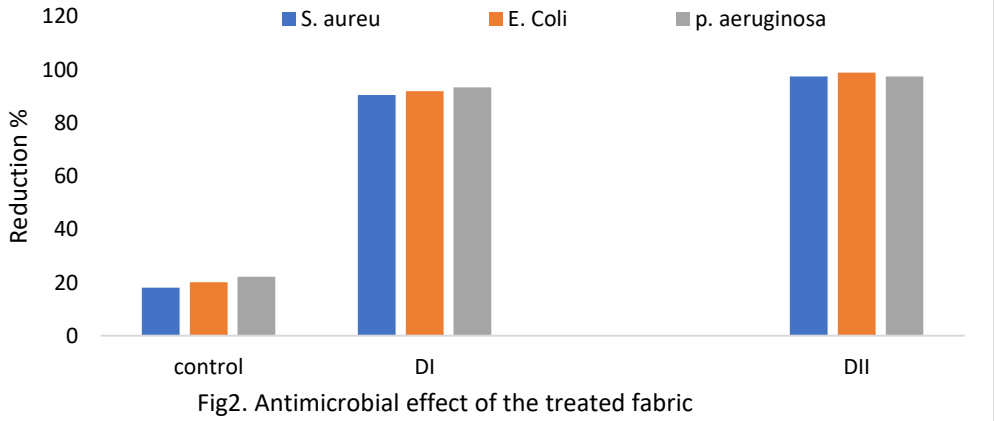

Fig2. Antimicrobial effect of the treated fabric

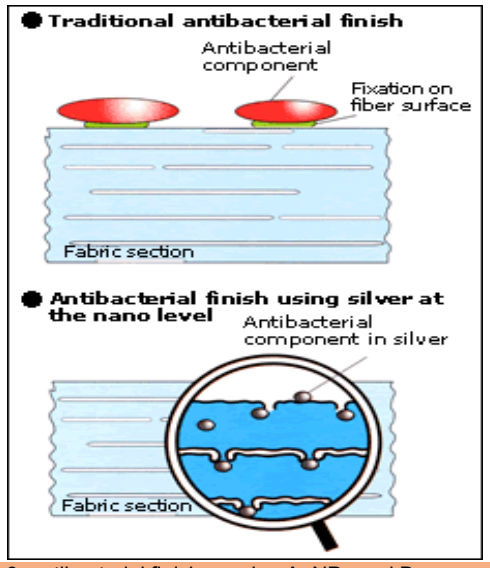

Fig.3. antibacterial finishes using AgNPs and Banana peel

Dyeability measurements

The K/S values of the treated samples were found to be higher than the corresponding untreated samples of cotton as shown in Table 3. The higher K/S values of treated samples indicate that the presence of AgNPs increased the dye affinity towards the material. The AgNPs in the fabric thus acted as mordant. It was observed that AgNPs gave a stronger yellowish brown. The negatively charged dye anions got attracted towards the fabric probably due to the polarity developed in the metal particles by induction which resulted in better bonding between the dye and the fabric. ΔE gave its maximum value which is mainly attributed to the lower values of L*, due to the high stability formation of the complex between the AgNPs and luteolin as a phenolic compound. ΔE was very obvious for DII compared with the control and DI. Ag^{+} metal ion is well known for its ability to form coordination complexes, and readily chelated with the dye. Such a strong coordination tendency enhances the interaction between the fiber and the dye, resulting in high dye uptake. The results obtained revealed that the values of L*, a*, and b* have been changed by the addition of the AgNPs which shift the color of the dye due to the reaction between the metal ion and negatively charged on
the fabric surfaces.

Table 3. Color measurements of the fabric samples

M. S.	K/S	L	a	b	ΔE
Control	0.13	85.3	1.3	0.69	2.3
DI	2.45	73.3	-7.43	44.8	10.23
DII	3.30	66.8	-6.01	45.92	16.6

Where M Measurements, and S. Samples

Physical properties of the treated fabric

The effects of the treatment fabric on the physical properties were examined and presented in Table. 4. It is demonstrated from the results that the control sample have the highest air permeability followed by DI and finally DII. This reduction was attributed to pore filling action of both lotluin and AgNPs. Tensile strength of DII sample increased more than that DI and the control. This improvement was attributed that the small size of AgNPs can enter in between the polymer molecules and perhaps act as fillers or crosslinking agents which also contribute to the load sharing phenomenon during load application to the material. Unlike chemical crosslinking which causes an improvement in tensile strength, and decrease the elongation % of the fabric used. The results in the work of Jeong (2009) reported that higher concentration of Ag

nanoparticles in the finishing solution resulted in lower tensile strength. Pilling resistance of DII increased due to the pore/void volume filling action of n AgNPs. This result confirmed with the work of Dastjerdi et al.,

Table 4. Physical properties of the treated fabric

Fabric properties		Control		DI		DII	
Air Permeability $cm^3/cm^2.sec$		76.33		80.1		82.3	
Pilling Res. Grade		4		3/4		3	
Breaking force Kg/F		65.5	64.3	66.1	65.4	68	66.2
Warp	Weft						
Elongation %		8.6	8.9	8.4	8.2	8.1	7.9
Warp	Weft						

Conclusion

The natural Banana peel in combination with AgNPs was directly applied onto cotton fabric to evaluate odor control and antibacterial activity against different bacterial strains. Banana peel used as reducing agent for biosynthesis of AgNPs and in the same time acts as natural dye for the cotton fabric. The obtained results revealed of the treated fabric sample acquired excellent antimicrobial, anti-odor, high color strength, and breaking force. On the contrary, the treated fabric showed decrease in air permeability, elongation% and pilling resistance.

References

1. Dastjerdi R, Montazer M, Shahsavan S, A new method to stabilize nanoparticles on textile surfaces. Colloids and Surfaces A-Physicochemical and Engineering Aspects 2009, 345 (1-3): 202-210
2. El- Bisi, M.K., Allam, Abeer, O.G., El-hadi, A. and Hassanein, H.D., Citrullus colocynthis nanosilver-hybrid as antimicrobial agent. International Journal of Advanced Research, 3, 5, 1549-1559(2015).)
3. El-Bisi, M.K, El-Rafie H.M, El-Rafie, M. H. and Hebeish, A., Honey bee for eco-friendly green synthesis of silver nanoparticles and application to cotton textile, Egyptian Journal of Chemistry, 2013, 56 (3), 187-198.
4. Fibre2Fashion's market analysis tool TexPro, www.fibre2fashion.com/news/textile
5. Fouda MMG, Abdel-Halim ES, Al-Deyab SS (2013) Antibacterial modification of cotton using nanotechnology. Carbohydr Polym 92 (2): 943-954.
6. Holme, I.: Innovative technologies for high performance textiles. Coloration Technology 123, 59–73 (2007). (1994).
7. Jeong KM, Bae HS (2009) Surface Characteristics and Physical Properties of Cotton Fabric for Patient Clothing Material through a Chitosan/Nanosilver Colloidal Solution. Journal of the Korean Society of Clothing and Textiles 33 (12): 1873.
8. Kubelka, P. and Munk, F. Z. (1931). Tech. Phys. 12, 593
9. Li L, Li L, Zhou X, et al. Silver nanoparticles induce protective autophagy via Ca 2+ /CaMKKβ/AMPK/mTOR pathway in SHSY5Y cells and rat brains. Nanotoxicology. 2019;13(3):369–391. doi:10.1080/17435390.2018.1550226
10. Liao C, Li Y, Tjong SC. Bactericidal and cytotoxic properties of silver nanoparticles. Int J Mol Sci. 2019;20(2):449. doi:10.3390/ ijms20020449
11. Long YM, Hu LG, Yan XT, et al. Surface ligand controls silver ion release of nanosilver and its antibacterial activity against Escherichia coli. Int J Nanomedicine. 2017; 12:3193–3206.
12. Meenaxi Tiwari, Archana Singh., Alka Ali., "Resist printing on cotton fabric with vat dye using different resisting agents", Asian Dyer 2009, 32 - 35.
13. Mehrabian S, Majd A, Majd I, Antimicrobial effects of three plants Rubia Tinctorum, Carthamus Tinctorius and Juglans Regia) on some airborne microorganism. Aerobiologia, 2000,16(3-4): 455-458).
14. Menezes, E., "Antimicrobial finishing for specialty textiles", Clothesline. 5, 2002, 96– 99.]
15. Mohanpuria, P, Rana, N.K. and Yadav, S.K., J. Nanopart. Res. 2008, 10, p.507.
16. Prada PA, Curran AM, Furton KG, Characteristic human scent compounds trapped on natural and synthetic fabrics as analyzed by SPMEGC/MS. J Forensic Sci Criminol 2014, 1(1):1–10. https ://doi.org/10.15744 /2348-9804.1. S101]
17. salah M [Antibacterial Activity and UV Protection Property of Some Egyptian Cotton Fabrics Treated with Aqueous Extract from Banana Peel, International Journal of Clothing Science 2012, 1(1): 1-6].
18. Salah M, and Yasser, Eco-Friendly Synthesis of Silver Nanoparticles obtained from Extracts of Banana Leaf and its Application on Cotton Fabric for Microbial Resistance, Int. J. Adv. Sci. Eng. Vol. 1 No. 4 53-58 (2015)
19. Salah M. 2012, (Antibacterial Activity and UV Protection Property of Some Egyptian Cotton Fabrics Treated with Aqueous Extract from Banana Peel. International Journal of Clothing Science 2012, 1(1): 1-6)