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Climate Smart Breeding in Cotton

Zulqurnain Khan and Zarmeen Zafar

Institute of Plant Breeding and Biotechnology, MNS-University of Agriculture, Multan, Pakistan

Correspondence: zulqurnain.khan@mnsuam.edu.pk

Abstract:

Climate change is posing a potential threat to agricultural crop production. Abiotic stresses are yield-limiting factors. Biotic stresses are also getting worse in the form of pest pressure owing to changing climatic conditions. There is a need to develop and use modern approaches to cope with this situation and feed the masses in the future when global population is going to touch 10 billion by 2050. New breeding techniques employing molecular markers and genome editing may provide pragmatic, promising and sustainable solutions to the current problems. Cotton crop is facing major issues due to climate change, especially in Pakistan. This short review will give a brief description of the current status and impact of climate change on cotton and new breeding tools which may be used for genetic improvements of crop plants.

Keywords: Abiotic stress, climate change, cotton, impact of change in climate, breeding techniques, genome-editing tools

Introduction

Climate changes have disastrous impact on human health, natural systems and agricultural yield (Arunanondchai et al., 2018). There is direct relation between the rapidly growing global population and an increase in food consumption owing to stability of global ecosystem. The performance of agriculture is significantly impacted by soil fertility, the amount of water available and air pollution (Noya et al., 2018). Due to sudden changes in the environment, direct or indirect abiotic stress is intensifying on plant production. The greenhouse effect and rising average world temperatures are mostly caused by the emission of hazardous gases, particularly CO_2 (Vaughan et al., 2018). Pakistan is also experiencing problems from climate change, due to its agricultural sector. Pakistan is ranked seventh among the countries most vulnerable to climate fluctuations (Kreft et al., 2013). Climate change have significantly contributed to this decline, making it difficult for Pakistan's agricultural economy to achieve food security and reduce poverty (A. Ali & Erenstein, 2017). Crops of different variety are susceptible to changing climatic conditions. Cotton is widely acclimated to growing in temperate, tropical and subtropical conditions worldwide; however, its growth may be limited by future climate fluctuations (Bange et al., 2016). Climate change has both positive and negative impacts on cotton as described in Figure 01.

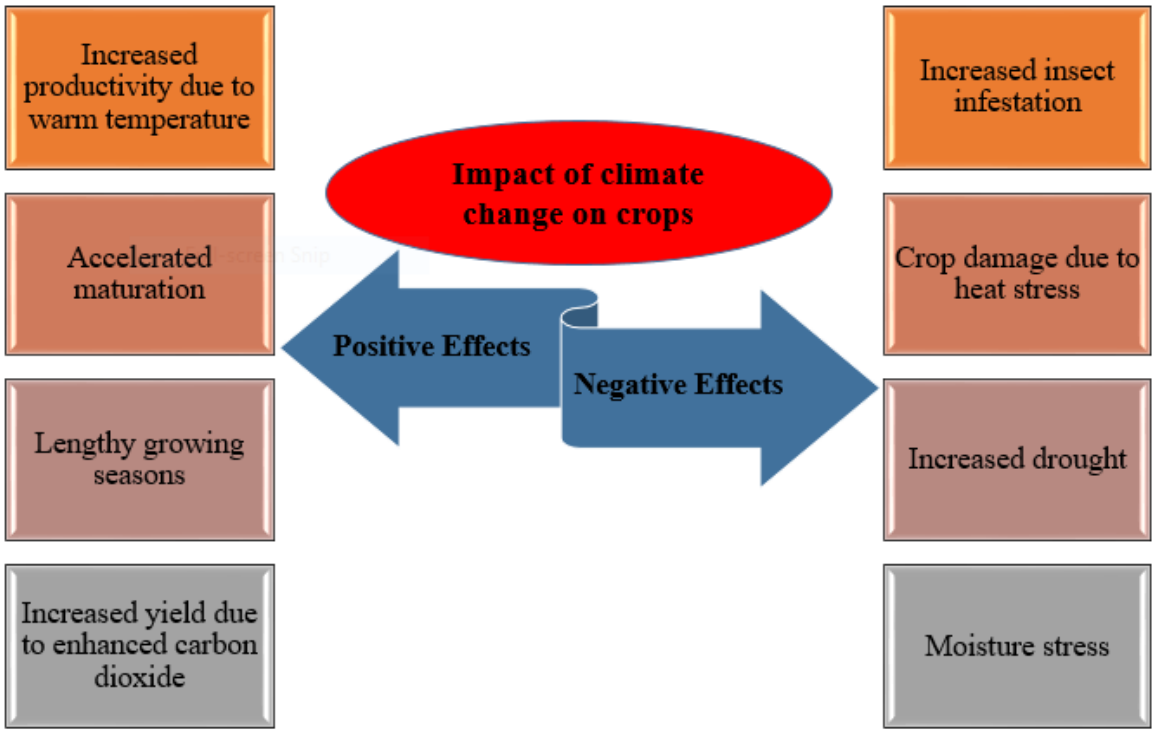

Figure 01: Positive and negative impacts of climate change on cotton

Climatic change for instance temprature affects cotton plant growth by effecting different organs and different stages of plant development (Hu, 2019). In underdeveloped countries, poor climatic conditions primarily affect agricultural productivity therefore extreme heat and excessive CO_2 led scientists to discover novel solutions to less predicted problems (Rosenzweig et al., 2014). Climate smart breeding has been applied to determine how cotton productivity would change as a result of varying climatic circumstances. Climate-smart breeding is the practice of breeding plants or altering their genomes to help them adapt to the changing climatic circumstances, which includes rising temperatures, rising carbon dioxide levels, and shifting patterns of precipitation. Major objectives of climate-smart breeding relies on:

- Productivity
- Adaptation
- Mitigation

Modern plant breeding, which involves tools of genetic engineering and/or biotechnology, is required to satisfy these demands since traditional breeding techniques cannot keep up with the needs of a changing environment.

Classical cotton breeding techniques:

Before the formal beginning of plant breeding and genetics as a scientific discipline, cotton breeding has a history (Khan et al., 2022). In traditional cotton breeding, main techniques employed were introduction, selection, and hybridization.

- Introduction entailed the export of new genetic material to another country, where testing would be run in various environments and times of the year to look for the necessary phenotypic features. The germplasm that produces the desired outcomes would subsequently be made available for purchase. This process required acclimating different cotton cultivars to different environments.
- Second, cotton breeders employed the selection process to produce cotton. The fundamental phenomenon driving cotton's selective breeding was spontaneous mutations. In certain cases, these

spontaneous mutations result in modified plant having recognizable traits. The cotton breeders later discovered this plant, and after growing the new offspring apart from the original plant's seed and undergoing the necessary screening processes, the cotton variety would be prepared for commercialization.

- The goal of the hybridization technique was to mutagenically introduce desirable variabilities into cotton plants.

The adoption of less time-consuming and more effective breeding tools for cotton breeding is now necessary. It has become essential to use genomic and biotechnological technologies in cotton breeding. All these variables inspired scientists to think differently, which led to the development of cutting-edge cotton breeding technologies that improved cotton breeding's effectiveness and methodology.

Marker-assisted selection (MAS)

One of the most promising and often utilized methods for breeding cotton nowadays is marker-assisted selection (MAS). Molecular markers have evolved from low throughput restriction fragment length polymorphisms (RFLPs) (Tanksley et al., 1989) to single nucleotide polymorphism (SNP) markers based on NGS methods (Varshney et al., 2009). The identification of cotton varieties, creation of genetic maps, study of QTLs, and MAS for bacterial blight resistance have all been accomplished using RFLP (Jalloul et al., 2015; Tang et al., 2015). According to (Bakht et al., 2017; Noormohammadi et al., 2013; Sultana & Alam, 2016) RAPDs have been used in the genetic diversity, genetic mapping, and phylogenetic investigations. In cotton breeding, AFLP markers have been employed to characterize the germplasm (Badigannavar et al., 2012; Mokrani et al., 2012). Additionally, using AFLP, linkage mapping was carried out in cotton (Badigannavar, 2010; Fang et al., 2013). SCAR markers were employed to improve the cotton fibre quality. They were also effectively used to restore fertility (Feng et al., 2021). Using SSR markers, it was possible to determine the genetic diversity of several cotton species (Shim et al., 2019; W. Zhao et al., 2019). The cotton fibre quality attributes were also improved by SSR (Zhou et al., 2020). For the development of economic features, characterization of the germplasm, and linkage mapping in cotton breeding, ISSR markers have been utilized effectively (Modi et al., 2020). Using ISSR, the genetic diversity of cotton germplasm was also investigated (Khan et al., 2022). Hybrid cotton was improved using STS markers, which have already been utilized to improve cotton (Hayat et al., 2020). To identify markers in cotton, ESTs markers were utilized (Ashraf et al., 2016). 10 KASP studies that may be applied in MAS to increase Verticillium wilt resistance have been effectively verified in cotton (Y. Zhao et al., 2021). Cotton's genetic map was created using SNPs (I. Ali et al., 2018).

Genome editing

Genome editing is the most recent and advanced breeding technology. This technology has the unprecedented ability to create a new genetic variation at target genetic loci with high accuracy. Within a very short span of time, genome editing technology has been adopted and deployed in a diverse arena of plant breeding, including climate-smart breeding (Gao, 2021). Genome-assisted breeding can considerably lessen the consequences of climate change on future agricultural scenarios, even if the affects of climate change on crop resistance may vary depending on the environment and crop and might be difficult to forecast. Zinc Finger Nuclease (ZFN), Transcription activator-like effector nucleases (TALEN), and CRISPR-Cas are the three available genome editing tools, among which CRISPR-Cas has gained rapid popularity due to its versatility and ease of use. ZFN, TALEN, and CRISPR-Cas tools can create a targeted double-strand break in the genome. The creation of genomic DSB induces cellular repair pathways and the formation of random insertions or deletions (indels). These indels

are novel genetic variation that can be artificially created with genome editing tools. If indels are generated in a gene coding sequence, the gene function is often disrupted. Therefore, genome editing tools greatly facilitate us to disrupt undesired genes for crop improvement. In CRISPR-Cas, with the help of a small guide RNA and a Cas nuclease, one can generate genetic variation precisely at a desired location of the crop genome (Molla, Karmakar, et al., 2020). Figure: 02 is illustrating phenomena of CRISPR-Cas system

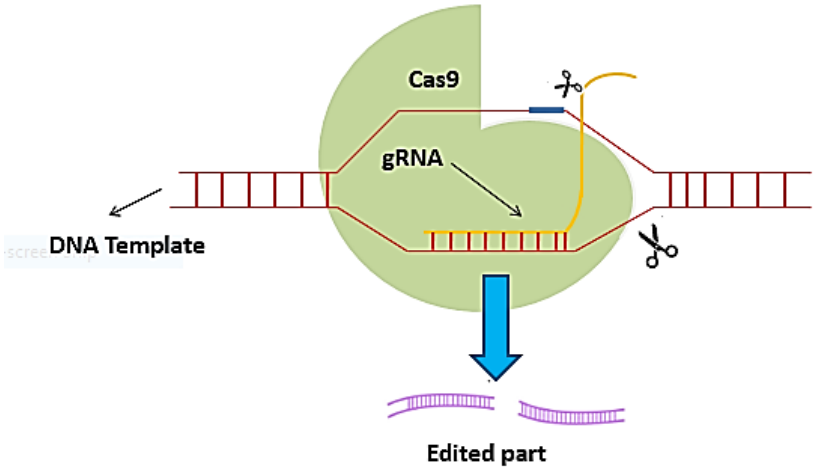

Figure: 02 CRISPR-Cas 9 based genome editing

Although Cas9-induced indels can be predicted uncontrollable (Allen et al., 2021). Therefore, for generating precise indels, conventional ZFN, TALEN, and CRISPR-Cas tools are inefficient. Recently developed base editing tools can perform targeted swapping of one nucleotide with another in the genome ((Molla & Yang, 2019); (Molla, Qi, et al., 2020)). Prime editing technology enables us to install precisely small insertion, deletion, substitution, and a combination thereof in the genome (Molla et al., 2021).Conventional ZFN, TALEN, CRISPR-Cas along with base editors and prime editors empowered us to perform innumerable types of manipulations in crop genome for climate-smart breeding. Remarkably, the fruit of genome editing technologies has been realized very rapidly in the form of released crop varieties. A TALEN-facilitated genome-edited soybean has been commercialized in the USA, while CRISPR-Cas9-edited tomato has been recently marketed in Japan. The power of these technologies is being harnessed for breeding climate-resilient crop varieties all over the world. For example, CRISPR-Cas9 generated variants of the ARGOS8 gene enhanced maize yield under drought conditions (Shi et al., 2017). Drought and salt tolerance of rice plants has been enhanced through the editing of the DST gene (Santosh Kumar et al., 2020) . CRISPR-Cas9-mediated knock-out of the SlAGL6 gene has increased the thermo-tolerance of tomato plants (Klap et al., 2017). Base editing and prime editing tools made it easier to breed non-transgenic herbicide-tolerant crop varieties, which would greatly help us to mitigate CO2 emission through reduced tilling (Molla et al., 2021). Wild crop relatives have many desirable traits including abiotic and biotic stress tolerance. However, transferring them into cultivars is tedious and many times impossible. Genome editing assisted rapid de novo domestication can facilitate in generating new crop varieties with desired traits removing the unwanted characters (Zsögön et al., 2018).Table 01 shows genome editing in cotton against various climatic stress

Table 01: Improvements of cotton using genome editing tools

Abiotic stress	Target gene	References
Cold stress resistance	AmDUF1517	(HAO et al., 2018)
Drought resistance	MATE	(Lu et al., 2019)
	Beta	(Lv et al., 2007)
Herbicide resistance	2,4-D mono-oxygenase	(Bayley et al., 1992, p. 2)
	AHAS	(Rajasekaran, 1996)
	Bar	(Kumar & Timko, 2004)
Salt resistance	AVP1	(Pasapula et al., 2011)
	SNAC1	(Liu et al., 2014)
	AtEDT1/HDG11	(Yu et al., 2016)

Overall, genome editing technologies provide us with excellent opportunities to rapidly breed crop varieties suitable for a changing climate.

Conclusion

All climate smart breeding technologies rely on successful exploitation/deployment of genetic variation (natural/novel) and need to be easy and cost-effective for rapid adoption. Rapid and precise identification of superior lines, both at the phenotypic and genotypic levels, and rapid generation development are the keys to climate-smart breeding. Integration of genome editing and speed breeding with other technologies will increase the breeding pace like never before. Rapid deployment of the available technologies could help us to reduce the negative impact of climate change on agriculture.

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Exogenous application of bio-chemicals mitigates water deficit stress in cotton crop

Noor Mohammad*, Fiaz Ahmad, Asia Perveen and Ahmad Waqas

Physiology/Chemistry Section, Central Cotton Research Institute, Multan-66000, Pakistan Correspondence:
*Correspondence: noor.1272@gmail.com

Abstract:

Purpose: Deficit water stress is a common abiotic stress during the cotton-growing season in Pakistan and it causes negative effects on cotton growth and yield. Application of certain bio-chemicals cans potentially strengthen plant's defense systems against water deficit stress.

Methods: A field trial was conducted at Central Cotton Research Institute, Multan, Pakistan ($30^0 08' 55.0'' N\ 71^0 26' 22.2'' E$) to evaluate the effect of bio-chemicals i.e. IAA, calotrope leaf extract, salicylic acid, $AgNO_3$ and acetic acid on cotton crop under artificially imposed water stress conditions. Cotton was sown on19thApril in a randomized complete block design with split-split plot arrangement. Bio-chemicals were applied through foliar sprays; first application starting from squaring stage (30 days after planting) and subsequent two applications after 20 days interval each. Two cotton genotypes CIM-785 and CIM-775 were used as test crop. The NPK fertilizers were applied to soil @ 150 N, 50 P_2O_5, 50 K_2O kg ha^{-1}. Standard production and management practices of the area were employed for the crop.

Results: Plant structural development, proline content, relative water content, chlorophyll content, number of bolls, boll weight and seed cotton yield were studied. Maximum proline contents were observed where calotrope leaf extract was applied and maximum chlorophyll contents were observed where acetic acid and IAA was applied.In normal irrigation and water stress condition maximum yield was obtained in the treatment where salicylic acid was applied in CIM-785, while in CIM-775 maximum yield was observed where acetic acid was applied.

Conclusion: Foliar application of bio-chemicals enhanced the yield and yield attributing factors in normal irrigation and water stress conditions. These also improved cotton plant structural development, physiological parameters i.e. proline content, relative water contentand chlorophyll content. It is therefore, concluded that water deficit stress can be mitigated through foliar application of bio-chemicals on cotton plant.

Keywords: drought, salicylic acid, acetic acid, leaf extract.

Introduction

Increasing scarcity of irrigation water is a principal threat to sustainable production of cotton in Pakistan. Water-deficit stress is a major environmental factor restricting more than one third of the arable land for cultivation across the world (1). Drought is a common abiotic stress during the cotton-growing season, which causes a series of negative effects on cotton plant growth, yield and fiber quality (2). Cotton is dreadfully drought sensitive crop and is prone to yield reduction by drought, because drought stress is a complex phenomenon that affects the

physiology of cotton plant. In addition, cotton is a very susceptible plant to the quantity of irrigation water and therefore, irrigation management is very complicated. The flowering and boll-forming stage is the key yield determinant period of cotton plants(3). Water stress occurring during this stage seriously affects cotton plant development and final productivity. During the last decade, the foliar application of plant growth regulators and biomolecules, such as brassinosteroids and polyamine have become an established procedure in crop production to increase yield and quality of the crop under abiotic stresses including drought (4).

Foliar application of plant growth regulator, at low concentrations, plays an important role in plant's defense systems against biotic and abiotic stresses (5). These bio-chemicals are involved in the regulation of plant physiological processes including stomatal closure, chlorophyll and protein synthesis, nutrient uptake, transpiration, and photosynthesis. Studies have indicated that exogenous application of bio-chemicals may lead to improvements in morpho-physiological traits that are involved in determination of plant yield. Furthermore, application of bio-chemicals induces the degradation of reactive oxygen species and increases the activity of antioxidant enzymes especially under water stress. The objective of the present work was to investigate the possible role of bio-chemicals in mitigating water deficit stress and quantification of parameters that improve cotton productivity.

Results and Discussion

Plant height varied significantly among biochemical treatments, between genotypes, water levels and their interactions were also significant, while numbers of nodes on main stem and inter-nodal length varied non-significantly (Table 2). Maximum plant height in CIM-785 was observed where salicylic acid was sprayed under non-stressed (NS) and water-stressed (WS) condition these finding are line with previous studies (7, 8). Similarly, in CIM-775 maximum plant height was observed where calotrope leaf extract was sprayed under NS and WS condition similar results were also observed in tomato crop (9).

Table 2: Plant structure at maturity in different treatments under no stress and water stress condition in two genotypes

Treatments	Water level	Plant Height (cm)		Node		Inter-nodal	
		CIM-785	CIM-775	CIM-785	CIM-775	CIM-785	CIM-775
Control	NS	121	141	43	40	2.82	3.53
	WS	109	133	39	39	2.80	3.41
$AgNO_3$	NS	123	154	43	49	2.86	3.14
	WS	112	124	40	41	2.80	3.02
Salicylic Acid	NS	150	182	50	48	3.00	3.76
	WS	140	166	47	45	2.98	3.71
Acetic acid	NS	131	160	46	44	2.85	3.66
	WS	100	151	41	42	2.44	3.59
Calotrope Leaf Ext.	NS	149	209	55	55	2.73	3.79
	WS	140	178	52	48	2.71	3.70
IAA	NS	134	166	43	45	3.11	3.68
	WS	100	150	39	41	2.56	3.67
Biochemical Treatment (BioTr)		*		*		*	
Genotype (G)		*		*		ns	
Water level (WL)		**		ns		*	
BioTrXG		*		ns		*	
BioTrxWL		*		*		ns	
WLxG		*		ns		ns	
BioTrXGXWL		*		ns			

Proline content, relative water content (RWC) and chlorophyll (SPAD) values varied significantly among different treatments and between genotypes under normal irrigation and water stress condition. The proline content varied from 8.86 to 11.57 µg g^{-1} FW, RWC from 73 to 89 % and chlorophyll (SPAD) values from 48 to 58 in CIM-785, while in CIM-775 the proline content varied from 8.22 to 11.22 µg g^{-1} FW, RWC from 73 to 91% and chlorophyll (SPAD) values from 46 to 58 in different treatments, irrespective of water regimes. Maximum proline contents were observed where calotrope leaf extract was applied and maximum chlorophyll SPAD values were observed where both acetic acid and IAA were applied (Table 3). These results are in line with the findings that RWC decreased and proline contents increased in cotton under stressed condition (10, 11). Foliar application of bio-chemicals improved the stress tolerance in crops by adjusting the plant metabolisms as observed in above mentioned results. Similar findings have been reported earlier (12, 13).

Table 3: Proline content, relative water content and chlorophyll (SPAD) values in different treatments under no stress and water stress condition in two genotypes

Treatments	Water level	Proline (µg g^{-1} FW)		RWC (%)		Chlorophyll (SPAD) values	
		CIM-785	CIM-775	CIM-785	CIM-775	CIM-785	CIM-775
Control	NS	8.92	8.60	85	84	51	50
	WS	9.65	9.09	73	73	48	52
$AgNO_3$	NS	8.86	8.22	89	87	57	55
	WS	9.89	9.67	74	75	49	48
Salicylic Acid	NS	9.79	8.80	89	84	51	52
	WS	11.19	10.04	80	78	51	52
Acetic acid	NS	9.09	9.29	87	88	57	58
	WS	9.62	9.57	74	79	52	46
Calotrope Leaf Ext.	NS	9.39	9.15	86	91	49	56
	WS	11.57	11.22	77	76	55	50
IAA	NS	9.69	8.74	85	88	58	56
	WS	9.69	9.02	79	76	52	52
Biochemical Treatment (BioTr)		**		*		**	
Genotype		*		ns		**	
Water level (WL)		**		**		**	
BioTrXG		*		ns		**	
BioTrxWL		ns		ns		**	
WLxG		**		**		**	
Bio TrXGXWL		**		ns		**	

Seed cotton yield and yield contributing factors differed significantly among various biochemical treatments and between genotypes, and their interactions under normal irrigation and water stress condition. In normal irrigated plots (NS), bolls per plant varied from 24 to 37, boll weight from 2.37 to 3.36 g and seed cotton yield from 3071to 3748 kg ha^{-1} in CIM-785, while in CIM-775 bolls per plant varied from 34 to 44, boll weight from 2.28 to 2.76g and seed cotton yield from 3140 to 3640 kg ha^{-1} in different treatments. However, in water stressed plots (WS), bolls per plant varied from 22 to 30, boll weight from 2.21 to 2.95 g and seed cotton yield from 2852 to 3467 kg ha^{-1} in CIM-785, while in CIM-775 bolls per plant varied from 31 to 35, boll weight from 2.16

to 2.39 g and seed cotton yield from 2662 to 3008 kg ha^{-1} in different treatments(Table 4). Foliar application of bio-chemicals enhanced the yield and yield attributing factors both in normal irrigation and water stress condition as compared to control, however in normal irrigation and water stress condition maximum yield was obtained in the treatment where salicylic acid @ 20 mM was applied in CIM-785. These finding are in line with the previous studies (7, 14), while in CIM-775 in normal irrigation and water stress condition maximum yield was obtained in the treatment where glacial acetic acid @ 50 mM was applied, inconformity with the previous studies (15, 16).

Table 4: Seed cotton yield and yield attributing factors in different treatments under no stress and water stress condition in two genotypes

Treatments	Water level	Bolls per plant		Boll weight (g)		Yield (kg ha^{-1})	
		CIM-785	CIM-775	CIM-785	CIM-775	CIM-785	CIM-775
Control	NS	24	34	2.79	2.28	3071	3140
	WS	22	31	2.54	2.16	2852	2662
$AgNO_3$	NS	33	39	3.36	2.69	3463	3389
	WS	29	34	2.88	2.29	3139	2704
Salicylic Acid	NS	37	39	2.72	2.45	3748	3157
	WS	30	32	2.52	2.31	3467	2711
Acetic acid	NS	27	40	3.10	2.76	3455	3640
	WS	25	35	2.95	2.39	3067	3008
Calotrope Leaf Ext.	NS	31	44	2.37	2.44	3370	3291
	WS	28	31	2.21	2.30	3084	2816
IAA	NS	26	37	3.16	2.38	3453	3408
	WS	23	33	2.37	2.29	3156	2882
Treatment (BioTr)		*		*		*	
Genotype		**		*		**	
Water level (WL)		ns		ns		*	
BioTrXG		**		ns		ns	
BioTrxWL		ns		ns		ns	
WLxG		ns		**		ns	
BioTrXGXWL		ns		ns		ns	

Conclusion:

From this study, it is concluded that foliar application of bio-chemicals improved the plant growth and development as observed by increased plant height in treated plots over untreated plots both under normal irrigated and water stress condition. Furthermore, application of bio-chemicals improved metabolism of cotton under NS and WS conditions as evidenced by improvement in proline contents, RWC and chlorophyll SPAD values and ultimately resulting in enhanced seed cotton production and its contributing factors.

Materials and methods

Two cotton genotypes CIM-785 and CIM-775 were used as test crop.The crop was sown on April 19, 2021 in a randomized complete block design with split-split plot arrangement. Water stress applied at squaring stage on the basis of leaf water potential (LWP) i.e. -1.6 $\pm$ 0.2 MPa Ψ_w (normal irrigation; NS) and -2.4 $\pm$ 0.2 MPa Ψ_w (water stressed; WS). Bio-chemicals were applied through foliar sprays; first application starting from squaring stage and subsequent two applications after 20 days interval each. Recommended NPK fertilizers were applied

to soil @ 150 N, 50 P_2O_5, 50 K_2O kg ha^{-1}. Standard production and management practices were adopted. The detail of different treatments including bio-chemicals applied along with their concentrations is given in Table 1.

Table 1: Bio-chemicals and their concentration applied through foliar spray in two cotton genotypes

Bio-chemicals	Water Regimes	Genotype	
IAA @150 µM	No Stress (on what basis)	CIM-785	CIM-775
	Water stress (on what basis)	CIM-785	CIM-775
*Calotrope leaf extract @ 50 ml/L	No Stress	CIM-785	CIM-775
	Water stress	CIM-785	CIM-775
Salicylic acid @ 20 mM	No Stress	CIM-785	CIM-775
	Water stress	CIM-785	CIM-775
$AgNO_3$ @40 µM	No Stress	CIM-785	CIM-775
	Water stress	CIM-785	CIM-775
Acetic acid @ 50 mM	No Stress	CIM-785	CIM-775
	Water stress	CIM-785	CIM-775
Control (Water alone)	No Stress	CIM-785	CIM-775
	Water stress	CIM-785	CIM-775

*Calotrope leaf extract was prepared by grinding 250 g fresh calotrope leaves. Added one liter distilled water in crushed calotrope leaves and heated till boiling point. After cooling to room temperature, the solution was filtered by using cotton cloth. Calotrope leaf extract was used @ 1:20 (leaf extract:water) ratio for foliar application.

Plant height was measured with scale and numbers of nodes on main stem were counted at maturity. Inter-nodal length was measured by using the following formula:

Inter-nodal length = Plant height at maturity/ Numbers of node on main stem

Chlorophyll SPAD values were determined by Chlorophyll meter Minolta 502.

Proline content was determined by following methods described by Bates, et al. (6).

Relative water content (RWC) in leaf samples was determined by using the following formula:

RWC= (fresh leaf weight –leaf dry weight)/(leaf saturated weight – leaf dry weight) × 100

Boll weight was determined by picking100 bolls from each plot and taking the mean weight. Seed cotton yield was determined after final picking.

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Feeding ethogram of *Dysdercus koenigii* (Pyrrhocoridae: Hemiptera) to Cotton Seeds

Rabia Saeed[a**#], Qandeel Fatima[a#], Waqar Jaleel[a**#], Zahid Mahmood[b], Syed Ishfaq Ali Shah[a,b]

[a] *Entomology Section, Central Cotton Research Institute, Multan, Pakistan.*

[b] *Cytogenetics Section, Central Cotton Research Institute, Multan, Pakistan.*

[#] Equal contribution

****For correspondence**

1. waqar4me@yahoo.com, waqarjaleel@ccri.gov.pk
2. civilservisesgop@gmail.com

Abstract

Cotton is considered as an economical crop worldwide. In Pakistan it is a very important cash crop. Its major usage is in textile industries and therefore it is a very demandable crop, but it has been attacked by many chewing and sucking pests among them *D. koenigii* is becoming a destructive cotton pest. At first it was considered as a minor pest but recent studies have shown that it has been one of the major causes of loss in yield of cotton crop. It sucks the cell sap and transfers fungi and then feed on the inside of bolls. Integrated management of *D. koenigii* is the need of time. Ethogram study of an insect is very important to develop the bait that are environmentally safe. Therefore, this study explains the first-time feeding ethogram of third instar of red cotton bug were studied on cotton seeds (CIM-620). The results verified that antenna is a very important olfactory organ in red cotton bug while antenna and proboscis have gustatory sense.

Key words: *Dysdercus koenigii*, fitness, red cotton bug, ethogram.

Introduction

The red cotton bug (RCB), *Dysdercus koenigii* F. (Hemiptera: Pyrrhocoridae), is an economic pest in cotton growing areas of the world (Sarmad *et al.*, 2020, Jaleel *et al.*, 2014, Jaleel *et al.*, 2013). The *D. koenigii* was thought to be a minor insect pest of cotton crop, but it has recently emerged as a significant insect pest of cotton crops in Pakistan and India, causing severe lint discoloration (Karar *et al.*, 2021). It is found in many countries some of which are Pakistan, India, China, Afghanistan etc. (Hussain *et al.*, 2021). The *D. koenigii* (Fabricius) is an economically important pest in Pakistan's cotton agro-ecosystem (Saeed *et al.*, 2020). The adults and nymphs of red cotton bug damage cotton lint by sucking the sap from the leaves and green bolls that causes extreme damage such as it sheds the young bolls, rotten green bolls etc (Lanbiliu *et al.*, 2020). When it sucks the cell sap, it transfers the fungi that causes slimy wet to dry rot and then it feeds on the inside of balls (Saeed *et al.*, 2016). In order to commence eating, *D. koenigii* nymphs demonstrated a sequence of behavioral patterns (Kayesth & Gupta, 2016).

Insects are particularly exposed to heat stress in their natural environment.(Sarmad *et al.*, 2020). To minimize its population, insecticides are one of the basic strategies, whereas resistance to

insecticides occur because of the continuous application of insecticides. (Saeed *et al.*, 2018). In Pakistan, imidacloprid, a neonicotinoid pesticide, is frequently used alone or in combination with other insecticides to combat *D. koenigii*, a potential cotton pest.(Saeed & Abbas, 2020). To best authors knowledge, the antennal, proboscis and tarsal gustatory sense of red cotton bugs to cotton seeds are still unknown. Therefore, this study first time explains the feeding ethogram of 3rd instar of red cotton bugs on cotton seeds.

Results and Discussion

In the tenure of pre-feeding the significant difference was observed that the ratio of nymphs using their antenna to sense the seed was more as compared to nymphs using their foreleg or both antenna and foreleg (Fig. 5).

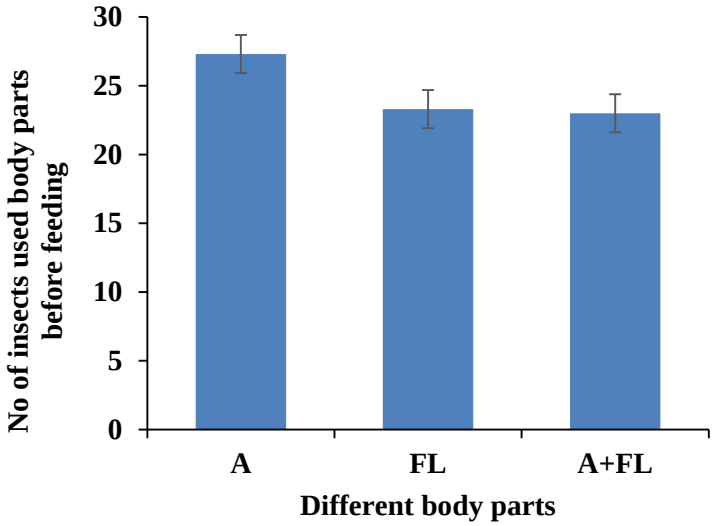

Fig. 5. No. of 3rd instar of red cotton bugs used body parts before feeding on cotton seed.
A: Antenna; FL: Foreleg; A+FL; Antenna +Foreleg

In the feeding time it was recorded that ratio of insects those sensed through both of the antenna and forelegs was more as compared to ones those used only their antenna or foreleg in particular (Fig. 6).

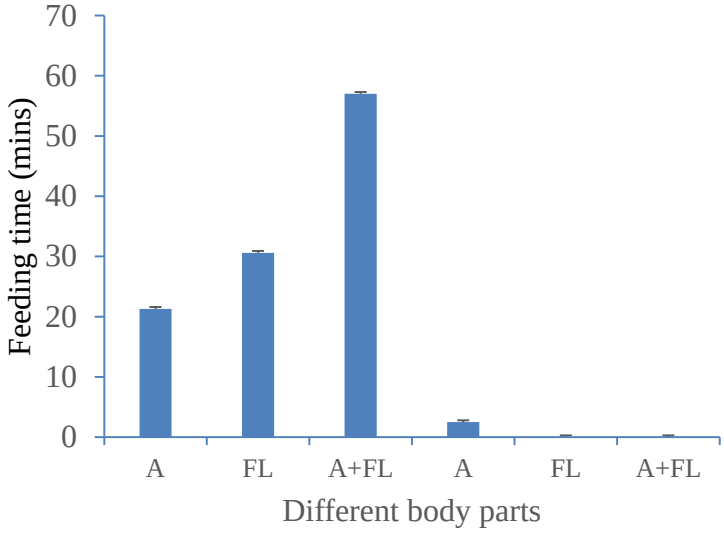

Fig. 6. Feeding time (mins) of 3rd instar red cotton bugs
A: Antenna; FL: Fore leg; A+FL: Antenna + Fore leg

Feeding Ethogram

Ethogram of feeding behavior in red cotton bug was categorized into two main steps (Fig. 7), first olfactory sense and second gustatory sense. Gustatory sense was further categorized into three steps. First type used their antennae to sense the seed, second used their foreleg tarsi, and third used their antenna and foreleg tarsi both. Our ethogram result showed that, most of the nymphs od 3rd instar red cotton bug used their foreleg tarsi and antenna both to taste the treated seed as compared to antennae and foreleg in particular.

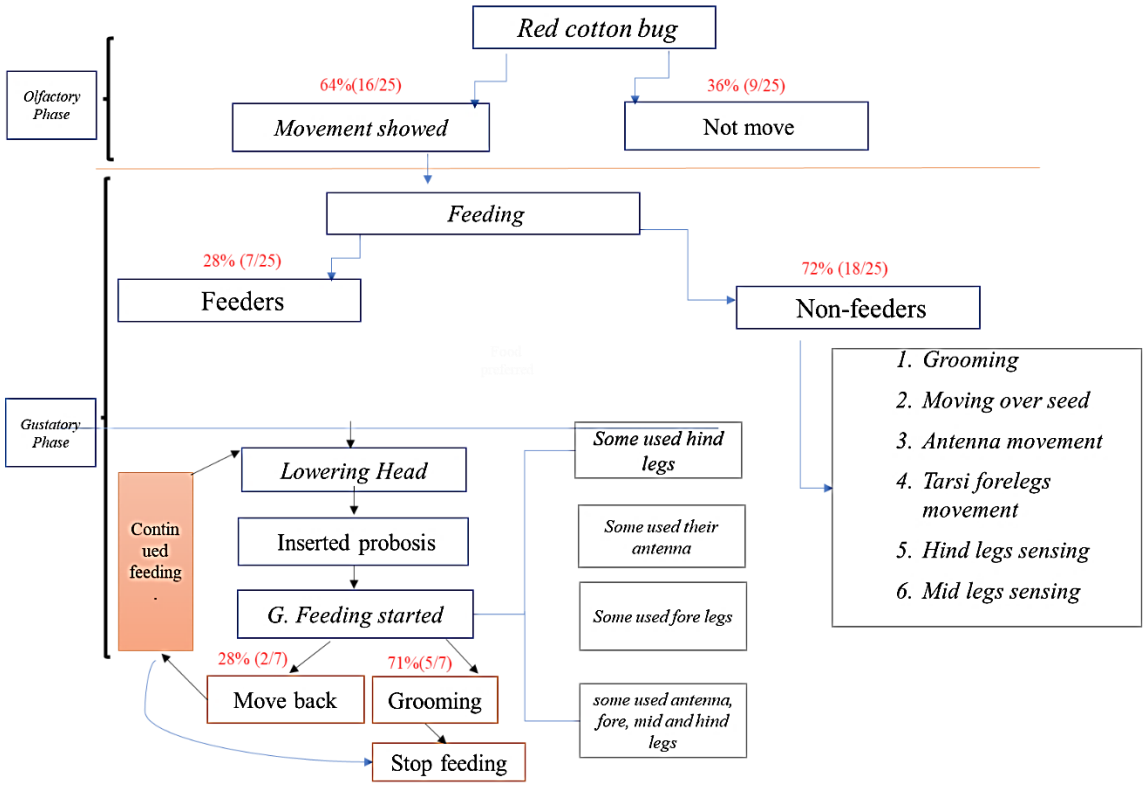

Fig.7. Flow chart showing feeding ethogram of 3rd instar of red cotton bug.

Several insects use their tarsal sensilla for taste of food (Jaleel *et al.*, 2021b, Jaleel *et al.*, 2021a, Yosano *et al.*, 2020). While in our study, the olfactory sense of 3rd instar of red cotton bug was observed in antennae and foreleg tarsi, while the main gustatory sense in proboscis, and gustatory behaviour reported in legs tarsi.

Conclusion

Ethogram study shows that the antenna was the most important olfactory sense on cotton seeds while the gustatory sense of 3rd instar of *D. koenigii* observed in both antenna and foreleg tarsi.

Methodology

The experiment for sucking pest of cotton (RBC) to conduct its rearing, to check the feeding behaviour of red cotton bugs on cotton seeds in the Entomology Section, Central Cotton Research Institute (CCRI), Multan, Pakistan.

Rearing of D. koenigii

The adults were reared in 16 × 20 cages with plastic screen top, front and netted sheets on the sides for aeration by following the methodology of Jaleel et al. (2013). For the rearing, the cages were prepared in such a way that soil was spread on the base of cages and water was provided to settle it down

(Fig. 1). Adults were collected from the fields of cotton crop in CCRI. Generation 49 and 51 were reared on CIM-620 seeds and maintained at temperature and relative humidity of ± 25°C and 50 ± 5% respectively. During its rearing it was observed that female's life span was 21-22 days whereas male's life span was 27-28 days. Oviposition occurred after 6-7 days of pairing. The cages were cleaned on daily basis and water was supplied on the surface of soil placed in cages using a syringe to maintain humidity as well as to provide water to insects. Seeds were changed after every 3 days. The eggs laid were collected in petri dishes settled with the soil on the base and after coming to the 2nd instar they were shifted in glass jars. These jars were also set with the soil on the base and a cloth on the top to cover the opening.

Fig. 1. Rearing cages of red cotton bugs

Fig. 2. Jars where nymphs of red cotton bugs were kept

Fig. 3. 1st instar of red cotton bugs

Feeding Ethogram

The purpose of the experiment is to find out the behavioural sequence of insect' detecting and deciding to feed on the preferable food (Jaleel *et al.*, 2021a, Jaleel *et al.*, 2021b). An individual worker Jaleel et al. (2021), was transferred into a Petri dish (3 × 1.5 cm) by a fine and soft forceps for feeding pattern on sucrose. The worker was allowed to acclimatize the environment of the Petri-dish for at least 48 h, and then the Petri dish was placed in the dark black condition for 2 h (de Brito Sanchez *et al.*, 2014). A droplet (10 mL) of 20% sucrose solution was dispensed by a micropipette (1–20 mL) into the centre of the Petri dish. Its foraging behaviour was observed until touching a droplet up to 20 min. If a worker would not access to the droplet, then it was discarded. Observations were done for 3 h under a Keyence VHX-5000 digital microscope (Jaleel *et al.*, 2018, Pozuelo *et al.*, 2015).

Fig. 4. Feeding of 3rd instar red cotton bug on cotton seed.

Statistical Analysis

The frequency and percentage in feeding ethogram of third instar were calculated in MS excel 2019. The pre-feeding and feeding time of *D, koenigii* was differentiated using One-Way ANOVA in statistics software. The LSD test was used to comparison the pre-feeding and feeding time of *D. koenigii*.

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In Silico Analysis and Expression Profiling of *Expansin A4, BURP Domain protein RD22- like* and *E6-like* Genes Associated with Fiber Quality in Cotton

Farzana Ashraf, Asif Ali and Zahid Mahmood

Abstract

Background: Wild *Gossypium* species and races are rich source of genetic polymorphism due to environmental dispersal and continuous natural selection. These genetic resources hold mass of outclass genes that can be used in cotton improvement breeding programs to exploit possible traits such as fibre quality, abiotic stress tolerance, and disease and insect resistance. Therefore, use of new molecular techniques such as genomics, transcriptomics and bioinformatics is very important to utilize the genetic potential of wild species in cotton improvement programs.

Methods: Interspecific lines and *Gossypium* species used in the study were grown at Central Cotton Research Institute (CCRI), Multan. After retrieving DNA sequence of the genes from NCBI, the primers for gene expression and full-length gene sequence were designed. Expression profiling of *Expansin A4*, *BURP Domain protein RD22-like* and *E6-like* fibre genes was performed through Real Time PCR. BLAST and DNA sequence alignment was conducted for sequence comparison of interspecific lines and *Gossypium* species. Different *in silico* analysis were used for characterization of fibre genes and identification of cis acting promoter elements in promoter region.

Results: Variable expression of genes related to fibre development was observed at different stages. BLAST and DNA sequence alignment exhibited resemblance of interspecific lines with *G. hirsutum*. *In silico* analysis on the sequence data also confirmed the role of *Expansin A4*, *BURP Domain protein RD22-like* and *E6-like* fibre genes in fibre development. Similarly, several stress tolerant and light responsive cis acting elements were identified through promotor analysis, which may contribute for fibre development in the breeding programs.

Conclusion: *Expansin A4*, *BURP Domain RD22-like* and *E6-like* have positive role in fibre development with variable expression at fiber length and strength associated stages.

Keywords: DNA sequencing, Expression analysis, Fibre genes, *In silico* analysis, Cotton

Introduction

Globally, synthetic fibre consumption is continuously increasing and projected to reach at 130 million tons by 2030. The consumption of synthetic fibre is 62.7% compared to 24.3% cotton fibre consumption [1]. Competition of cotton fibre with polyester is creating negative influence on the demand of cotton. Genetic improvement of cotton for fibre traits is very crucial to meet the challenges of the textile industry. So, there is need to devise clear-cut policies for cotton breeding program to enhance the quality cotton production. In a breeding program, germplasm collection, its conservation and utilization, trait specific screening programs and modern genomics have key role in variety development [2]. Cotton genetic resources have been extensively studied over the last many decades to introduce valuable traits in cotton [3-5]. These genetic resources include wild *Gossypium* germplasm, innovative

cytogenetic stocks with specific chromosomes additions or deletions in different species, large mapping families, recombinant inbred lines, near isogenic lines and interspecific lines. While there are some queries about narrow genetic base of these cultivars and most breeders would admit that in breeding programs maximum utilization of genetic diversity within their material should be ensured. Breeders will have to utilize wild cotton relatives, as well as advance lines or cultivars to develop cotton varieties with superior traits.

At the cellular level, cotton fibre development is supported by several genes which facilitates the elongation process, for example, *Expansins* are involved in fibre elongation at various development stages [6]. High transcript abundance of *GhEXP1* was observed in cotton fibre during the elongation phase of fibre development, which steadily decreased from 16 to 20 DPA [7,8]. In cotton, *GhEXPA1* along with *GhRDL1* showed an increase in fiber length and an enlargement of endopleura cells of ovules [9]. The BURP Domain is a plant-specific protein characterized by repetitive units of amino acid [10]. This protein is mainly involved in promoting the fibre cells elongation when over-expressed. Because *GhRDL1* directly interacts with cotton α-*Expansin* fibre gene therefore, *Expansin*s mediate *GhRDL1's* effect on overall fibre cell enlargement [9,11]. It was suggested that E6 protein is involved in fibre development, but no support was present to justify this hypothesis as no conclusive evidence was presented [12]. When E6 antisense suppression construct was used, there was knockdown to uncover a phenotype *E6-like*. E6 proteins play a comprehensive role in cell wall, and are deposited during fibre elongation, which give high transcripts in fibre cell during transcriptomic analysis [13].

Transcriptional profiling is a unique tool to gain knowledge about gene mechanisms, regulatory pathways, and gene expression [14,15]. Number of techniques are used for specific gene expression studies but Real Time PCR is the most reliable technology for absolute and comparative quantification of the gene transcription [16]. This comprehensive wide-ranging gene expression study is supportive to sightsee the role of genes, which are up regulated, entirely expressed, or down regulated during different cotton fibre development stages. Through transcriptomic data, one can explain the fibre expansion process and can discover highly expressed genes for the development of transgenic cotton varieties with superior fibre traits. Profiling of fibre genes in interspecific lines will enable us to unravel variable expression pattern of selected fibre genes.

Application of *in silico* methods along with expression profiling is important for characterization of fibre genes. DNA sequence of interspecific lines and *Gossypium* species were aligned to have information about differences and similarities. Diploid and tetraploid genomes of various *Gossypium* species have repeatedly sequences making their entire genome sequences. These valuable repeatedly sequenced data revealed the evolutionary history of the cotton with polyploidization and decaploidization leading to the of the formation of genus *Gossypium* [17]. Multiple sequence alignment approaches envisage algorithmic explanation about evolutionarily sequences alignments. Fibre genes were subjected to BLAST analysis for expression validation and multiple DNA sequence alignment for similarities and differences of interspecific lines and parent species. Genomics combines recombinant DNA technology, DNA sequencing and bioinformatics sequence to analyze the structure and function of genes [18]. Bioinformatics is a systematic field that utilizes advance approaches for computational analysis of biological data [18]. Bioinformatics also aids to recognize different promoters involve in fibre yield and quality, abiotic stress tolerance and disease resistance. Strength and specificity related

character of promoter sequence can be exhibited through expression profiling. Strong promoters predict high expression and vice versa. Fibre genes protein *E6*, *Expansin A4* and BURP *Domain RD22-like* also have strong promoters, which can be used in future breeding program.

Cotton breeders have extensively carried out interspecific hybridization for utilization of desirable genes from wild species to cultivated cotton and developed interspecific cotton varieties. Among them, a lot of upland cotton lines with improved traits including fibre quality and insect pest resistance have been developed [19-22]. All these upland cotton lines are designated as introgression lines of interspecific hybridization. These interspecific lines with their practical value in cotton breeding program have changed genetic basis from narrow line to a wide broad base in the present upland cotton germplasm and have broken the bottlenecks of breeding. However, the full potential of interspecific lines have not yet been obtained for beneficial traits exploitation in traditional and advanced breeding programs [23]. Therefore, this study was designed to evaluate the expression of fibre genes in diverse interspecific lines and *Gossypium* species and their role in different fibre development stages. Results of this study will be directive for development of high-quality cotton varieties.

Results

Expression profiling of Expansin A4, BURP Domain protein RD22-like and E6-like

Overall expression of *Expansin A4* gene was remarkably high in rapid elongating fibre during 10 DPA in all interspecific lines and *Gossypium* species. Maximum transcripts were found in SL-19 (Fig. 1). Expression of *BURP Domain protein RD22-like* was almost remained constant from 10-20 DPA fibre in all genotypes except in *Gossypium anomalum*. Transcripts of *BURP Domain protein RD22-like* gene were maximum in 10 DAP fibre as compared to 5 DPA. In all three interspecific lines, highest expression was detected at 15 and 20 DPA fibre stages in SL-19, SL-79 and SL-369 respectively (Fig. 2). Expression pattern of *E6-like* showed that high expression was detected at 10 and 15 DPA fibre stages predicting its main role in fibre elongation. In interspecific lines, transcripts of *E6-like* gene were varied from 0 DPA till 20 DPA. In SL-19, expression of fibre gene starts to increase from 0 DPA and reached at maximum level at 15 DPA and after that slightly decreases at 20 DPA (Fig. 3).

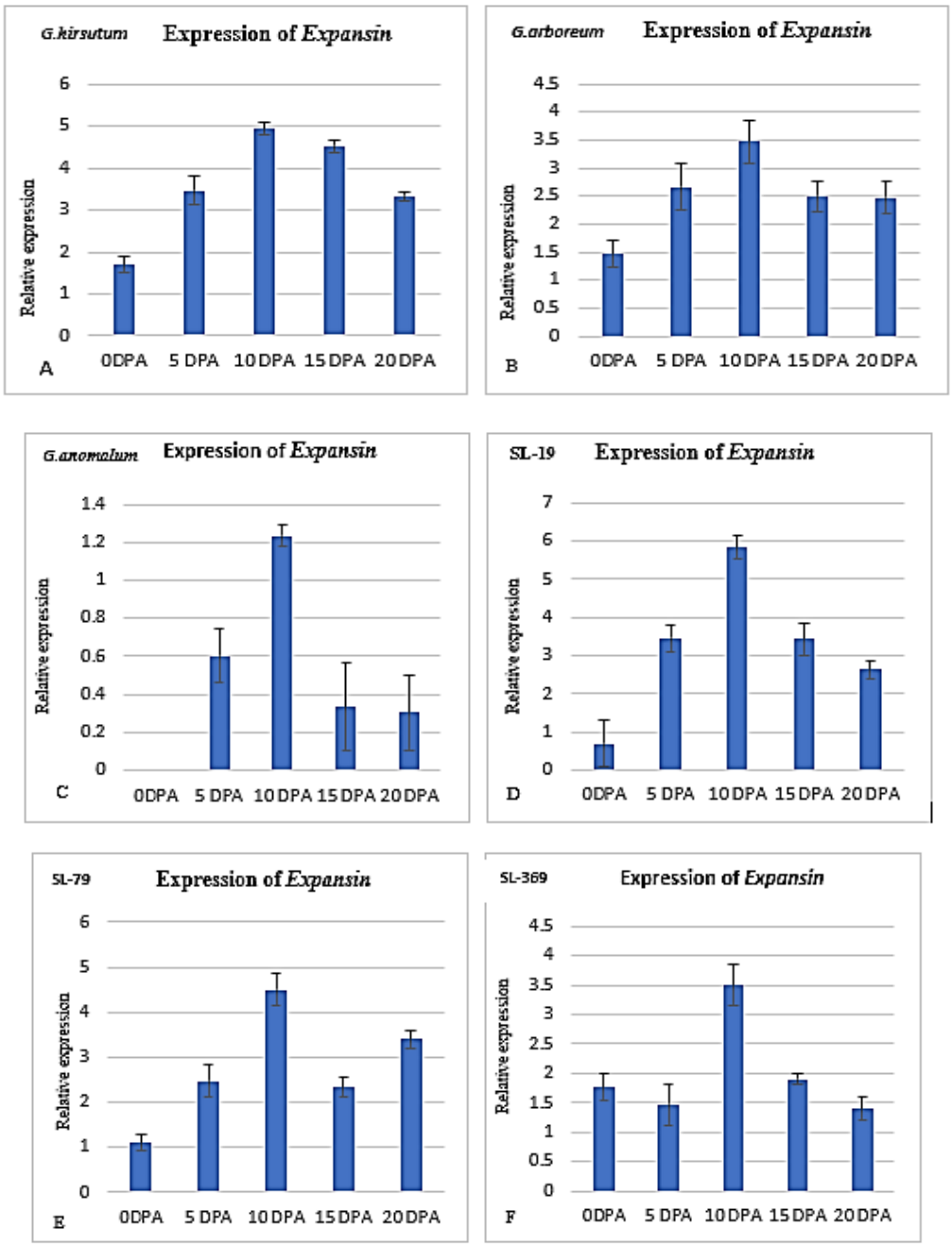

Fig. 1 Expression profiling of *Expansin A4* in *Gosssypium* species and interspecific lines: **A** (Expression of *Expansin A4* in *G. arboreium*), **B** (Expression of *Expansin A4* in *G. hirsutum*), **C** (Expression of *Expansin A4* in *G. anomalum*), **D** (Expression of *Expansin A4* in SL-19) **E** (Expression of *Expansin A4* in SL-79), **F** (Expression of *Expansin A4* in SL-369).

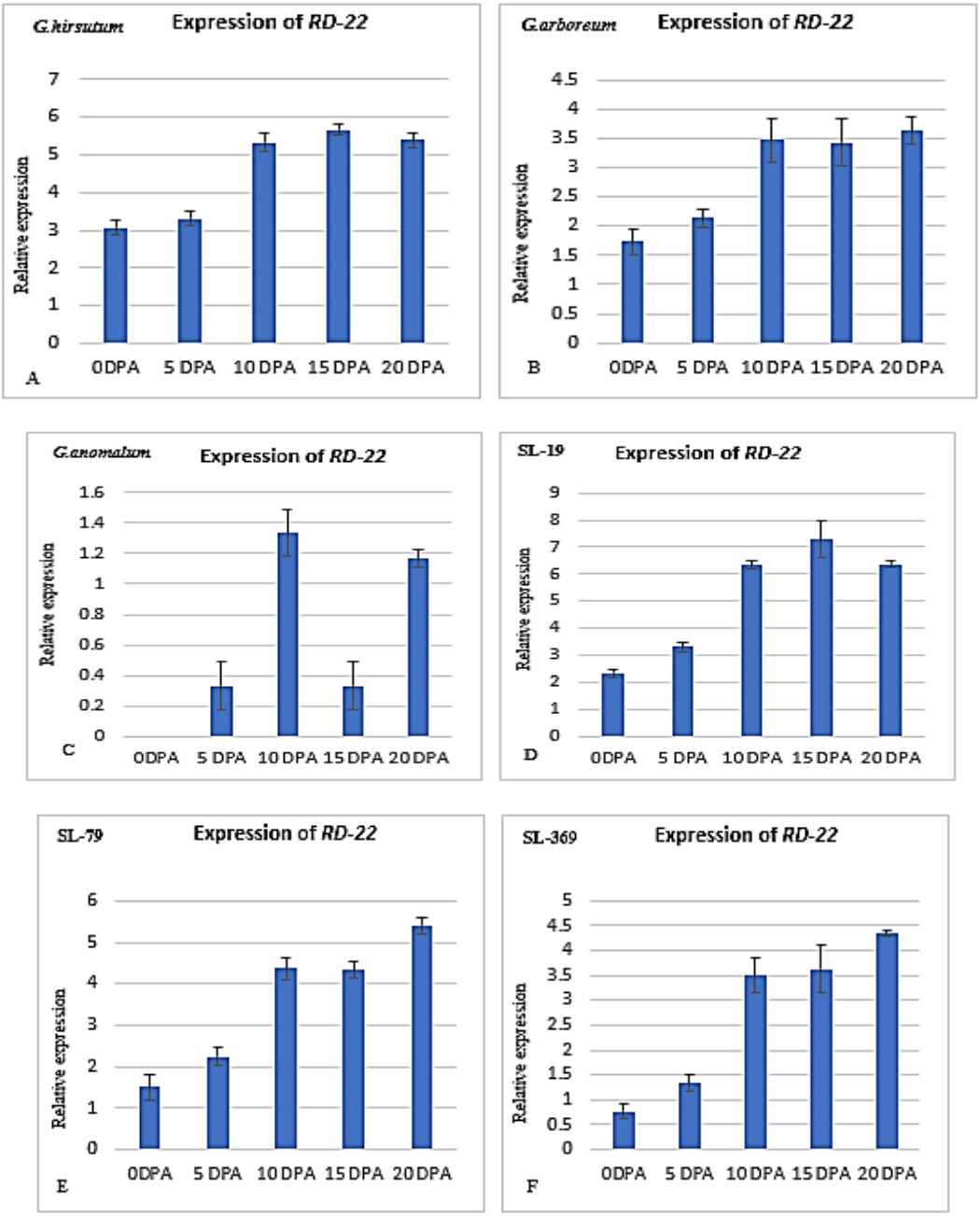

Fig. 2 Expression profiling of *BURP Domain RD-22* in *Gosssypium* species and interspecific lines: **A** (Expression of *RD-22* in *G. arboreium*), **B** (Expression of *RD-22* in *G. hirsutum*), **C** (Expression of *RD-22* in *G. anomalum*), **D** (Expression of *RD-22* in SL-19) **E** (Expression of *RD-22* in SL-79), **F** (Expression of *RD-22* in SL-369).

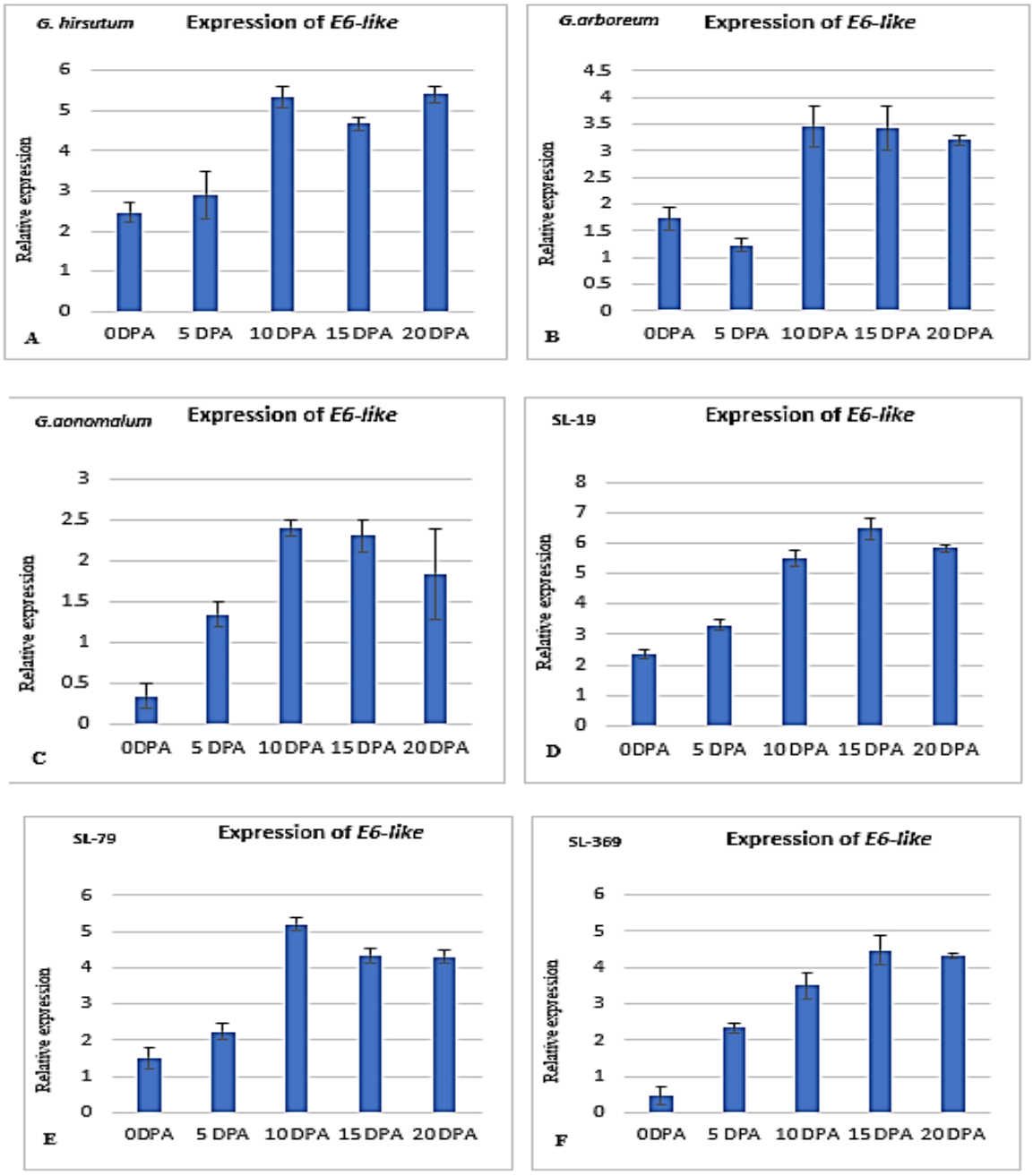

Fig. 3 Expression profiling of *E6-like* in *Gosssypium* species and interspecific lines: **A** (Expression of *E6-like* in *G. arboreium*), **B** (Expression of *E6-like* in *G. hirsutum*), **C** (Expression of *E6-like* in *G. anomalum*), **D** (Expression of *E6-like* in SL-19) **E** (Expression of *E6-like* in SL-79), **F** (Expression of *E6-like* in SL-369).

To validate expression results, the target gene transcriptomic profiles (E6*-like*, *Expansin A4* & *BURP Domain protein* RD22-like) were validated by using existing RNA-seq data on Cotton FGD. The results of available fibre specific genes were generally similar with our expression analysis results. Heat map was created on the basis of RNA-seq data of related expressed in transcript per Million (TPM) during different fibre development stages. *E6-like*, *Expansin A4* and *BURP Domain RD22-like* showed similarity with gene Gh-D05G160200, Gh_A10G149600 and Gh_D05G052400 respectively. (Fig. 4). An expression trend of gradual increasing from 5 DPA to 10 DPA were identified, while similar tendencies were also observed in our experiment.

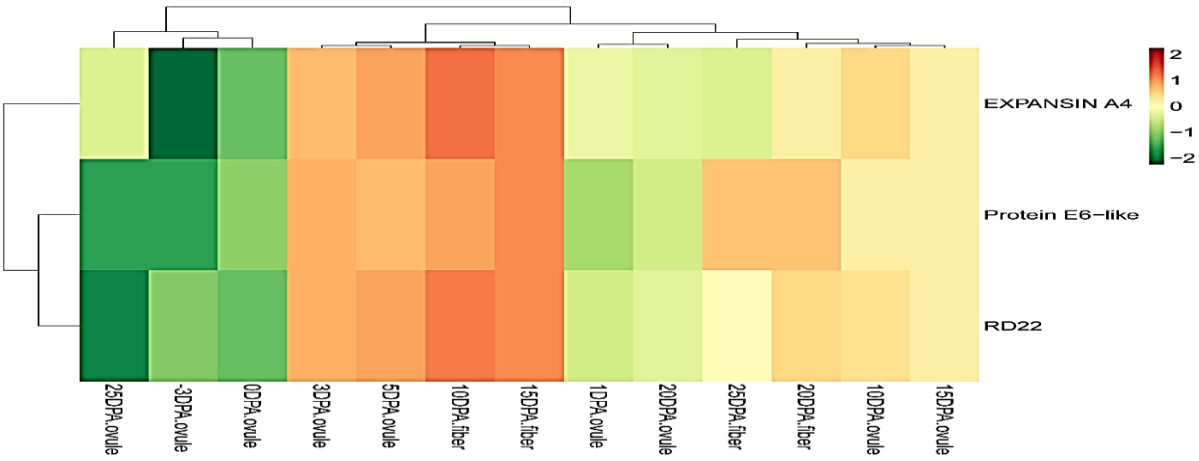

Fig. 4 Heat map of expression levels (log-transformed transcript per kilobase million (TPM) values). Figure was generated based on available RNA-seq data of *BURP Domain RD2-like-2*, *Expansin* and *E6-like* submitted bio projects from cotton FGD data base. Red indicates high expression, yellow indicates intermediate expression and green indicates no expression. It is straightforward to identify highly expressed genes in specific tissues from this figure. Tissues are labeled with Days After post anthesis (DPA). Rows indicates the fibre gens and column show the fibre stages (250DPA ovule -25 DPA fibre). The data denotes the logarithm-transformed values of log2, day post anthesis and fragments per kilobase of transcript per million mapped reads**.**

Sequence comparison of interspecific lines and species

In *E6-like* DNA sequencing, all interspecific lines exhibited sequences more similar to *G. hirsutum* as depicted at nucleotide positions 213, 217 and 221-226. In *Expansin A4*, interspecific lines were also more closely related to *Gossypium hirsutum* predicted at 390, 393, 507, 519 & 657bp which also confirm its breeding history. In B*BURP Domain RD22-like*, it was also predicted that almost all dissimilar nucleotide (241-300, 301-360, 361-420) were observed in *G. anomalum* as compared to other species of cotton (Fig. 5).

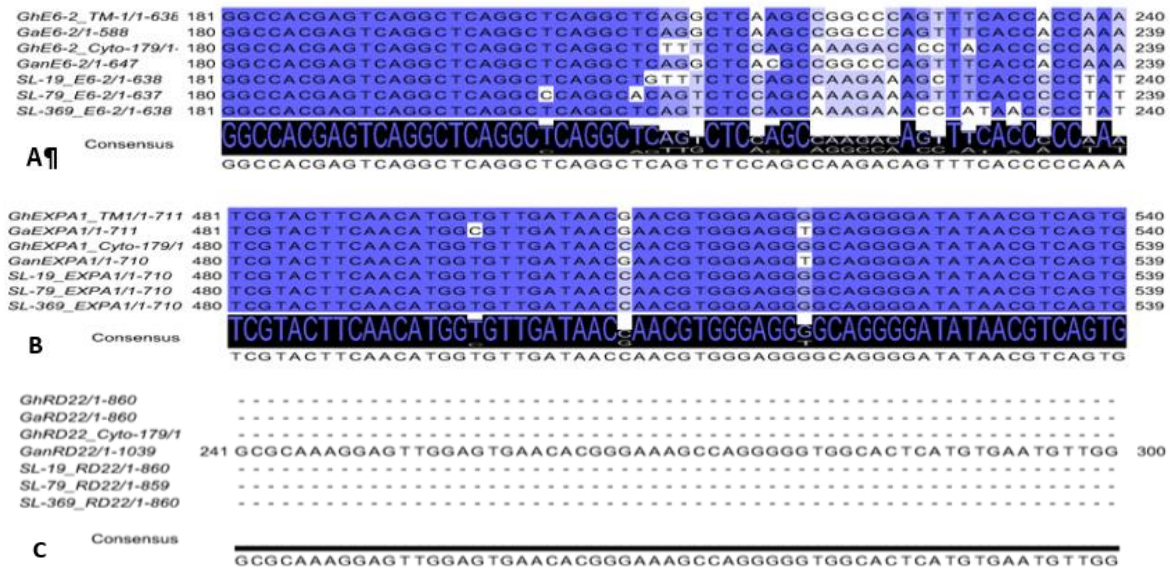

Fig. 5 DNA sequence alignments of fibre genes. A (*E6-like*), B (*Expansin A4*), C (*BURP Domain RD22-like*). White shadings indicate the polymorphic nucleotides. Interspecific lines and *Gossypium* species names are indicated in the left and number of bases depicted in each line is marked by the number shown at the top right of each section.

In silico analysis of E6-like, Expansin A4 and BURP Domain RD-22

Physicochemical properties

Expasy's Protpam analysis of predicted protein showed that Protein *E6-like* and *RD-22* was characterized as unstable as value of instability index was 47.75 and 44.72 respectively (Table 1). *Expansin A4* was characterized as a stable protein with value of instability index of 29.01.

Table 1 Physicochemical properties of fibre genes

Physicochemical properties	*E6-like*	*Expansin A4*	*BURP Domain RD-22*
Number of amino acids	241	258	335
Total negatively amino acid charged residues (Asp + Glu)	37	13	35
Total positively amino acid charged residues (Arg + Lys)	25	16	34
Molecular weight	28223.37	27936.46	36595.05
Theoretical pI	5.00	8.36	6.89
Aliphatic index	32.37	62.83	75.64
Grand average of hydropathicity (GRAVY)	-1.356	-0.090	-0.266
Instability index (II)	47.75	29.01	44.72

Subcellular Localization

DeepLoc analysis designated that protein. Proteins E6-like, Expansin A4 *and* BURP Domain RD22-like *were a membrane soluble protein family. Location in different organelles with the approximate values (Table 2) predicted the probability of protein location in different organelles. Highest Extracellular values of Proteins* E6-like, Expansin A4 *and* BURP Domain RD22-like *(0.819, 0.729 and 0.843 respectively) showed that these proteins are extracellular.*

Table 2 *Predicted subcellular localization of* E6-like, Expansin A4 *and* BURP Domain RD-22

Fibre gene	Extracellular	Lysosome	Endoplasmic reticulum	Cell membrane	Golgi apparatus	Cytoplasm
E6-like	0.8195	0.1706	0.0083	0.0013	0.0002	0.0002
Expansin A4	0.7293	0.2373	0.0329	0.0005	0	0
BURP Domain RD-22	0.8435	0.1316	0.0237	0.0008	0	0.0003

Signal peptide analysis

In E6-like, Expansin-A4 *and BURP Domain RD22 were 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 3) that shows that peptide signal is present.*

Table 3 *Signal peptide Analysis of* E6-like, Expansin A4 *and* BURP Domain RD22-like

Fibre gene	Measure	Position	Value	Cut Off	Signal Peptide
E6-like	max.C	26	0.792		
	max.Y	26	0.840		
	max.S	15	0.941		
	Mean S	1-25	0.891		
	D	1-25	0.868	0.450	Yes
Expansin A4	max.C	30	0.427		
	max.Y	30	0.586		
	max.S	9	0.950		
	Mean S	1-29	0.821		
	D	1-29	0.713	0.45	yes
BURP Domain RD22-like	max.C	30	0.427		
	max.Y	30	0.586		
	max.S	9	0.950		
	Mean S	1-29	0.821		
	D	1-29	0.713	0.450	Yes

Promoter sequence Analysis

Sequence analysis of cotton *E6-like*, *Expansin A4* and *BURP Domain protein RD22-like*promoter using PlantCARE predicted many vital motifs in this region related to gene expression (Fig. 4). 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. There were other vital core promoter elements required for promoter activity including TATA box and CAAT box (Table 4). Cis-acting essential element for the abscisic acid reaction (*Hordeum vulgare*), light response elements (*Arabidopsis thaliana*), gibberellin-enhancer element (*Brassica oleracea*) and element for variation of the palisade mesophyll cells (*Arabidopsis thaliana*) were present in *E6-like* promoter region. Similarly, in *Expansin A4* various cis acting premotor elements were identified. Abscisic acid responsiveness elements were identified in *Arabidopsis thaliana*, light responsiveness in *Zea mays*, element responsive for transcription start in *Brassica oleracea* and MeJA-responsiveness in *Hordeum vulgare*. In *BURP Domain RD22-like*, elements essential for light responsiveness were present in *Petroselinum crispum* while promoter and enhancer regions were identified in *Arabidopsis thaliana*. MYBHv1 binding site, MeJA and anaerobic induction responsive elements were present in *Hordeum vulgare* and *Zea mays* respectively.

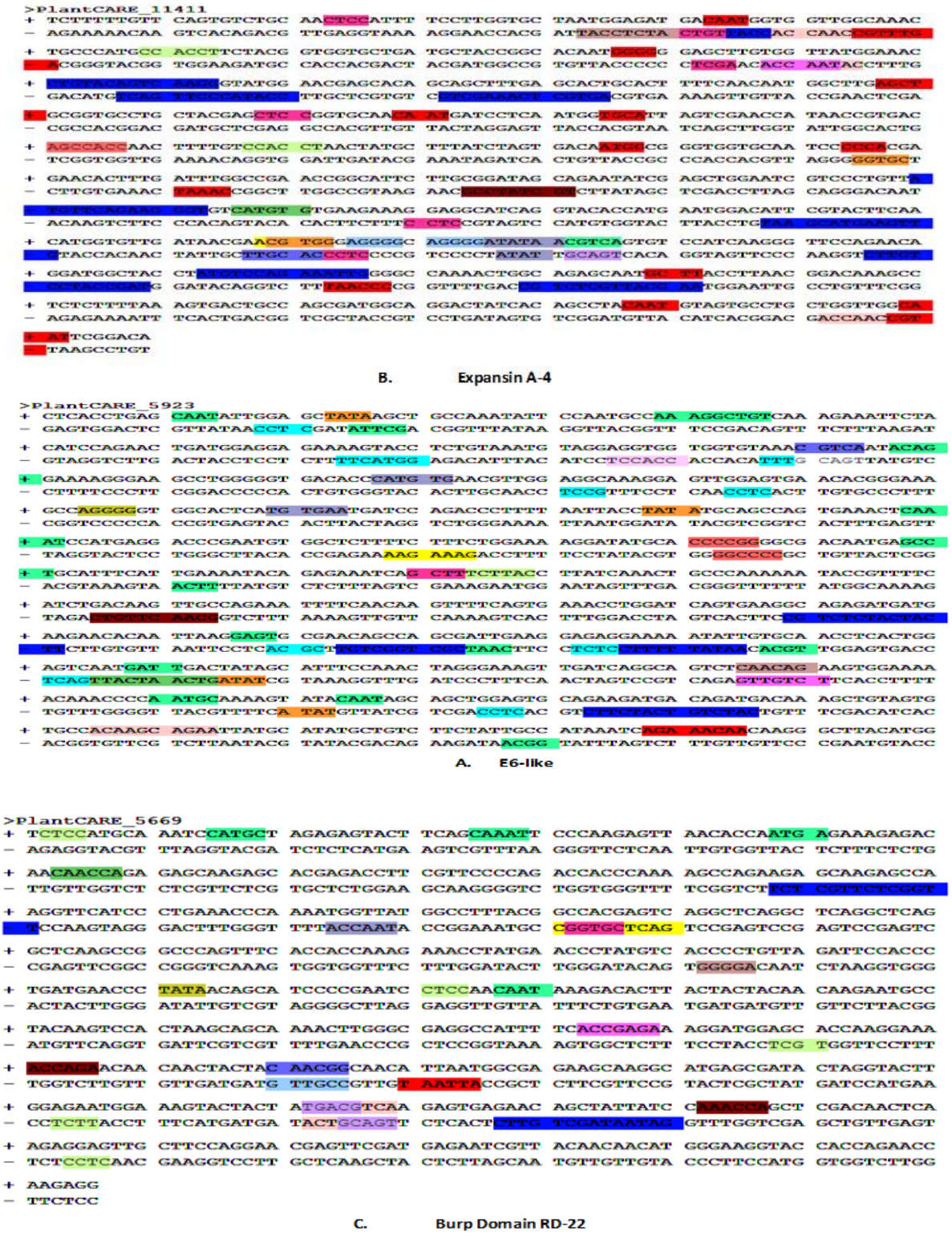

Fig. 6 *In silico* analysis of promoter sequences of fibre genes. **A** (cis-acting regulatory elements in *E6-like*)-like, **B** (cis-acting regulatory elements in *Expansin A4*), **C** (cis-acting regulatory elements in *BURP Domain RD-22*) Highlighted regions show cis regulatory motifs present in the promoter regions with specific function.

Table 4. Cis acting promoter elements in promoter region

Site Name	Organism	Position	Strand	Score.	Sequence	Function
			E6-like			
ABRE	*Hordeum vulgare*	425	-	9	GCAACGTGTC	cis-acting element involved in the abscisic acid responsiveness
AE-box	*Arabidopsis thaliana*	748	+	8	AGAAACAA	part of a module for light response
CAAT-box	*Arabidopsis thaliana*	638	+	5	CCAAT	common cis-acting element in promoter and enhancer regions
CAAT-box	*Pisum sativum*	852	-	5	CAAAT	common cis-acting element in promoter and enhancer regions
GARE motif	*Brassica oleracea*	615	-	7	TCTGTTG	gibberellin-responsive element
HD-Zip 1	*Arabidopsis thaliana*	564	-	8	CAAT(A/T) ATTG	element involved in differentiation of the palisade mesophyll cells
TATA-box	*Arabidopsis thaliana*	575	-	4	TATA	core promoter element around -30 of transcription start
TC-richrepeats	*Nicotiana tabacum*	380	+	9	GTTTTCTTAC	cis-acting element involved in defense and stress responsiveness
TCT-motif	*Arabidopsis thaliana*	384	+	6	TCTTAC	part of a light responsive element
			Expansin A-4			
G-Box	*Pisum sativum*	507	-	6	CACGTT	cis-acting regulatory element involved in light responsiveness
ABRE	*Arabidopsis thaliana*	508	+	5	ACGTG	cis-acting element involved in the abscisic acid responsiveness
ABRE	*Arabidopsis thaliana*	508	+	5	ACGTG	cis-acting element involved in the abscisic acid responsiveness
ATC-motif	*Zea mays*	384	-	9	TGCTATCCG	part of a conserved DNA module involved in light responsiveness
CAAT-box	*Pisum sativum*	361	-	5	CAAAT	common cis-acting element in promoter and enhancer regions
CAAT-box	*Arabidopsis thaliana*	581	-	8	CCCAATTT	common cis-acting element in promoter and enhancer regions
CAAT-box	*Petunia hybrida*	694	-	7	TGCCAAC	common cis-acting element in promoter and enhancer regions
TATA-box	*Arabidopsis thaliana*	527	-	4	TATA	core promoter element around -30 of transcription start
TGACG-motif	*Hordeum vulgare*	532	-	5	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
			BURP Domain RD22-like			
ABRE	*Triticum aestivum*	181	-	9	GACACGTGGC	cis-acting element involved in the abscisic acid responsiveness
ARE	*Zea mays*	542	+	6	AAACCA	cis-acting regulatory element essential for the anaerobic induction
Box 4	*Petroselinum crispum*	450	-	6	ATTAAT	part of a conserved DNA module involved in light responsiveness
CAAT-box	*Arabidopsis thaliana*	55	+	5	CCAAT	common cis-acting element in promoter and enhancer regions
CCAAT-box	*Hordeum vulgare*	440	+	6	CAACGG	MYBHv1 binding site
CGTCA-motif	*Hordeum vulgare*	515	+	5	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness
TATA-box	*Arabidopsis thaliana*	291	+	4	TATA	core promoter element around -30 of transcription start
TGACG-motif	*Hordeum vulgare*	512	+	5	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
TGACG-motif	*Hordeum vulgare*	515	-	5	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness

Discussion

Realistic genetic resources are accessible for innovative cotton breeders to make more perfection in crop improvement. Transcriptomic analysis of interspecific lines and *Gossypium* species for fibre traits identified in this study will improve our understanding of fibre genes that have key role in fibre development. Transcriptomic analysis simplifies the breeding through expression profiling of highly expressed genes. Transcriptomic analysis was performed for the identification of differentially expressed genes at different fibre growth stages in interspecific lines and three *Gossypium* species. Our study predicts

expression analysis of selected fibre genes during 0, 5, 10, 15 and 20 DPA fibre stages. High level variable regulation of genes encoding for fibre development was observed at different stages. Transcriptomic profiling has been effectively used for gene identification in cotton crop [27-31]. Here, we describe transcriptome profiling of genes in cotton fibre through quantitative Real Time PCR.

This is the initial comprehensive expression profiling that identified the differentially expressed genes with different stages contributing to fibre development in contrasting interspecific lines of cotton. Real Time PCR results predicted high expression levels specifically in the interspecific lines SL-19 (long staple line) as compared to parent species (Fig. 1-3) envisaging that when genome of two different species merge with each other, its progenitors possess more DNA content, which can be associated with fibre elongation and amplified size of single-celled fibres. It was also concluded that transgressive segregates are possible with hybrid vigor because of different genome groups of *Gossypium*, which make it possible to get interspecific lines with good fibre length, fibre strength and fibre fineness [32-35].

Expression profiling was compared with RNA sequence data submitted in different bio projects on FGD (Fig. 5). In *Expansin A 4*, our results were according to PRJNA490626 project in which transcripts were detected in 5 experiments including fibre development at various stages (0-25 DPA). Maximum expression was at 10 DPA which was similar to our results. GhEXPA4a and GhEXPA4b are specific fibre related genes that exhibited high expression during the fibre initiation and elongation stages (0 to 15 DPA). Over-expression of *GhEXPA8* predicted that these genes have ability to improve the fibre length and fineness in cotton crop [6]. Expansin proteins indorse the spillage between different microfibrils by Hemicellulose and cellulose cleavage [36]. Moreover, our data also suggested that Expansin protein has essential role in cotton fibre development by enlargement of fibre cells through sliding apart cellulose micro fibrils. Expression levels for *E6-like* genes was also compared. *E6-like* gene has similarity with genes Gh-D05G160200 for fibre related gene. It also plays its role fibre development. *E6* gene was firstly recognized as fibre gene with high expression during cotton fibre development and similar *E6*-like was predicted in Angiosperms [13].

BURP Domain proteins are known as important proteins that has significant roles in plant growth and stress responses [37,38]. Number of BURP proteins have been recognized and characterized on the basis of sequences features. However, different members from different subfamilies predicted variable expression patterns. In our findings, *BURP Domain RD22-like* genes actually execute main function in fibre elongation and maturation. Although low copy number of TPM of *BURP Domain RD22-like*gene were observed but this has a role in fibre development. The cotton fibre related gene (AtRD-22-Like) with over expression in elongating fibre cells, translates a BURP Domain-containing protein [9]. Cotton plants with high expression of GhRDL1 and *GhEXPA1* give more number of bolls, resulting up to 40% more lint yield plant^{-1} without disturbing fibre quality and non-reproductive growth. [9].

It is further concluded from the study that there is a direct association between *Expansin A4, E6-like, BURP Domain protein RD22-like* and fibre quality traits. Thus, these are key target for improving the fibre characteristics. Transformation of these highly expressed genes in local cotton varieties can fulfill the mechanized textile industry requirements. Moreover, genetically modified cotton produced by over expression of these genes will be the best source for use as a long staple variety or use as a parent in breeding program.

Biological sequences comparison in molecular biology and bioinformatics has been an imperative approach to supports analysis, such as prediction of protein sub-cellular localization [39], Physio chemical properties [40] and the field of taxonomy [41]. *E6-like* was characterized as unstable as value of instability index was 47.75. 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. Similarly, *Expansin A4* was characterized as a stable protein with value of instability index of 29.01. An imperative step on this mode is prediction of subcellular localization of each protein. *E6-like*, *Expansin A4* and *BURP Domain RD22-like* were characterized as a membrane soluble protein family. *In silico* analysis also confirm the role of genes in fibre elongation, *Expansin*-A4, *BURP Domain protein RD22-like-like*and *E6-like* play its main role in rapid elongation and also with predominantly effect in transition stage of elongation supporting to secondary cell wall synthesis.

DNA sequence alignment is a criterion for almost all comparative genomic analyses, including documentation of well-preserved sequence motifs and investigation of genes and species historical relationships [42]. *E6-like*, *Expansin A4* and *BRUP Domain RD22-like* PCR amplified full length gene was sequenced and subjected to BLAST analysis followed by multiple sequence alignment of DNA sequence and protein sequence for similarities and differences of interspecific lines and parent species (Fig. 5). It was concluded from the sequence comparison of interspecific lines and species of cotton that tri-species introgression lines are more closely related to *Gossypium hirsutum* as compared to *Gossypium arboreum* and *Gossypium anomalum* depicted. This confirms its back crossing with *Gossypium hirsutum* for yield improvement. These interspecific lines were also originate from BC_4S_5 population {*G. hirsutum* × 2(*G. arboreum* × *G. anomalum*) developed at Cytogenetics Section, CCRI, Multan [22]. In interspecific hybrids of *Gossypium*, a greater proportion of female gametes than male gametes is generally useful with few exceptions [43], hence backcross breeding should be subjugated. Review of backcrossing with distinct reference to cotton traits improvement exhibited that during repeated backcrossing one set of chromosomes retained with genes balanced. This technique has been used successfully in crosses of different *Gossypium* species [44-46].

In silico analysis tries to find proteins with consistent annotations about their interaction and functions in the cellular machinery. An imperative step on this mode is prediction of subcellular localization of each protein. *E6-like*, *Expansin A4* and *BURP Domain RD22-like* were characterized as a membrane soluble protein family. In *E6-like*, *Expansin-A4* and *BURP Domain RD22-like* were characterizes as extracellular membrane that's why signal peptide was present in protein coding. As validation of specific genes for crop improvement programs is also becoming popular engendering novel properties [47-49].Promoter regions *In silico* analysis of fibre related gene could be used to predict gene expression profiles in cotton plant. Many stresses resistant, light responsive which can contribute for fibre development were present in *E6-like*, *Expansin A4* and *Burp Domain RD22-like* (Fig. 6 and Table 6). To explore the molecular mechanisms regulating cotton fibre development, promoters of several cotton fibre genes have been identified. *E6* was the first of such genes to be reported, and the *E6* promoter has been used for engineering cotton fibre quality [50]. *GhRDL1*, a gene highly expressed in cotton fibre cells at the elongation stage, encodes a BURP domain-containing protein [51], and the *GaRDL1* promoter exhibited a trichome-specific activity in transgenic *Arabidopsis* plants [52]. The aim of our analysis was to predict promoter and regulatory elements of genes encoding useful stress responsive leading to fibre production. In cotton, basic

information related to different cis acting elements was generated to support the effort of improving cotton plant for a stress resistant with more fibre production.

Conclusion

The SL-19 appeared to be a promising source for cotton quality improvement with maximum expression for all fibre genes. To address the negative correlation between yield and fibre quality, use of genetic engineering is recommended to break this linkage by transferring *E6-like*, *Expansin A4* and *BURP Domain RD22-like* genes in local cotton cultivars.

Materials and methods

DNA Sequence retrieval and primer designing

DNA sequences of selected fibre genes (*Expansin A4, BURP Domain protein RD22-like and E6-like*) were retrieved from NCBI website https://www.ncbi.nlm.nih.gov/. RT-PCR Primers were designed using PRIMER 3.0 software (Table 5).

Collection of fibre tissues

Three interspecific lines (SL-19, SL-79 and SL-369) of varying fibre length categorized as long fibre (34.7mm), medium fibre (28.5 mm) and short fibre (24.0 mm) 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 days after anthesis). 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.

Plant RNA extraction and cDNA synthesis

RNA was extracted following Gynidium isothiocynate method [24,25]. 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. 18S rRNA constitutive gene primers were used as data normalizer in this assay.

Table 5 Primers used for Real Time qPCR Assay

Gene annotation	Primer pair	Primer sequence (5'-3')	Primer length	Product length (bp)	Accession No.
18S rRNA	RT18S -F	AAACGGCTACCACATCCAAG	20	153	**U42827.1**
	RT18S R	CCTCCAATGGATCCTCGTTA	20		
E6-like	RTE6-F	ATGGCTTCCTCACCAAAACTCTTCT	25	211	**DQ023519**
	RTE6-R	TTTCAGGGATGAACCTTGGCTCTT	24		
Expansin A4	RT EXPF	ATGGCAACCAAAACGATGATGT	22	220	**DQ204495**
	RT EXPR	AAGCTGCTGTGCTCGTTCCAT	21		
BURP Domain RD22-like-like	RTRD22-F	ATGAAGGTTCTCTCCCCAATTCT	23	198	**XM_016894801**
	RTRD22-R	GACGTTTACACCACCACCTCCT	22		

Full length gene specific primer designing

Full length primers (Table 6) were retrieved from phytozome https://phytozome.jgi.doe.gov/pz/portal.html.

Table 6 Detail of full-length fibre genes

Gene	Accession No	Size	5'F	5'R
E6-like	DQ023519	726	ATGGCTTCCTCACCAAAACTCTTCT	TCAGGGTTCGAACTCTTCCTCGCTT
Expansin A4	DQ204495	777	ATGGCAACCAAAACGATGATGT	TTAAAACTGGCCTCCTTCAAAAGT
RD-22	XM_016894801	1008	ATGAAGGTTCTCTCCCCAATTCT	TTACTTAGGGACCCAAACAATGT

Sequencing of PCR product

PCR products of full-length primers were sent to Macrogen Korea for Sanger sequencing. Sequencing PCR was performed using gene specific forward primers.

Sequencing comparison of interspecific lines and species

Multiple alignment of predicted DNA sequences and phylogenetic tree analysis was performed at https://www.ebi.ac.uk/Tools/msa/clustalo [26].

In silico analysis of fibre genes

Sequence of Sus gene was taken from NCBI database **(**https://www.ncbi.nlm.nih.gov/) by searching accession number in all data bases. Coding sequences 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.

Theoretical computation of physicochemical properties

Basic physiochemical properties and hydropathy index of protein sequences were computed through Expasy's ProtParam Proteomic server (http://web.expasy.org/protparam/).

Functional annotation of protein

For Subcellular Location DeepLoc-1.0 (http://www.cbs.dtu.dk/services/DeepLoc) databases was used. Moreover, SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP/) was used to check existence of signal peptide.

Promoter sequence analysis

Promoter analysis was carried out at http://bioinformatics.psb.ugent.be/webtools/plantcare/html/.

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Effect of Roller Gin Types and Seed Cotton Grade on Ginning Efficiency and Fiber Properties of Some Egyptian Cotton Varieties

Ibrahim, A. E. Ibrahim[1]; Beheary, M.G. I.[1]; Aly A. A. El-Banna[1] and Monzer A. A. Sharaf El-Deen[2]

[1] Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt
[2] Al Wadi Company for Trade and Cotton Ginning, Egypt

Abstract:

This investigation was carried out by Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria Egypt. The experiments were conduct at El-Wadi Ginning Company and Cotton Research Institute to investigate the effect of roller gin types and seed cotton grade on ginning efficiency and fiber properties of some Egyptian cotton varieties. Two types of roller gins (McCarthy and Rotary) using four Egyptian cotton varieties namely; Giza 86, 96, 94 and Giza 95. Four seed cotton grades i.e., (Good/Folly Good, Good +1/4, Good and Good -1/4). The completely randomized design with three replicates was used. The obtained results revealed that the Rotary gin stand surpassed in lint cotton grade, less ginning time (hr/kentar), gin stand capacity (kentar/hr), fiber elongation (%), fiber brightness (Rd%), nep count/g and less trash area and trash count. While, the McCarthy gin stand outperformed in lint percentage, ginning time (hr/kentar), upper half mean length (mm), fiber strength (g/tex), trash area, less nep/g count and trash count and the spinning consistency index (g/tex). Likewise, the highest seed cotton grade (Good/fully good) recorded the best results in lint grade and fiber properties. The first and the second order interactions were significant effect in all characters under study.

Keywords: cotton, lint grade, McCarthy and Rotary gin stand.

Introduction

Cotton is one of the most important fiber crops in the world; it ranked first in some countries, including Egypt. Egypt has traditionally been the leading source of extra-long staple cotton, (ELS). Today, it remains the second largest exporter of ELS cotton to the world markets. Likewise, Egypt is producing cotton in a wide range of quality and staple length from 29 up to 38 mm. The area of cotton cultivation in world is 78,296 acres, the yield of the crop is 682 Ibs, and the productivity is 111,174 bales, according to The International Cotton Advisory Committee Bulliten.

Cultivated cotton varieties in Egypt belong to either extra-long staple ELS (33.4 to 34 mm) i.e., Giza 45, Giza 87, Giza 96 and Giza 94 or long staple (LS) (31.5 % to 33.4 mm) i.e., Giza 86 and Giza 95. All Egyptian cotton is hand- picked and ginned by roller gin to preserve its unique fiber characteristics and maintain its high quality.

Ginning is an important process to produce fibers for spinning and textile industries. The development and new innovations in the ginning industry have helped to spread the cultivation of cotton and increase the cultivated area. The main objective of the ginning process is separating

fibers from seeds and the perfect ginning operation would be performed without the slightest injury to either seed or fiber. Ginning mills must become increasingly sophisticated with the use of recent technology to improve gin-stand capacity, reduce labor operating costs and preserving fiber quality. There are three of conventional roller gin-stand being used in Egypt, Single Roller Gin (McCarthy), Double Roller Gin (Indian) and Rotary knife Gin. In 2007, modification was done to produce a new generation called high capacity rotary roller gin-stand with a productivity of twenty kentar/hr of lint cotton, to reduce a lot of operating cost. It is the time to start attempts to develop the conventional roller gin stand, used in Egypt, to maximize the productivity and reduce ginning costs. Hossam El-Din (2001), Jadhav *et al.* (2003), Murali *et al.* (2004) showed that most operating parameters of the roller gin-stand were not affected by the stationary knife design, the only items that were affected by the knife design were fiber length, micronaire, strength, trash levels in the lint before lint cleaning. Patil *et al.* (2007 b), Sharma (2008), Estur and Gergely (2010) mentioned that the type of ginning technology also has an impact on lint quality and as roller ginning is less damaging to the fiber than saw ginning.

A comparison study between effect of the developed gin-stand and McCarthy gin roller on physical fiber quality parameters Abdel-Hameed *et al.* (2012), illustrate that the developed gin-stand could be considered the best for ginning Egyptian seed cotton samples. The developed gin stand had maximum value of ginning efficiency of 81.28 % at a drum speed of 0.79 m/s, feed rate of 8 kg/h and clearance of 3 mm. Sharma (2014 b) reported that the cost of processing of cotton plays a vital role in making it competitive and acceptable, thus every effort should be made to achieve the target of preserving inherent qualities of fiber at lowest cost in the ginning process. El-Banna (2019 a) concluded that the ginning efficiency of the cotton variety, was found to be more affected with gin stand type as well as the seed cotton levels. The gin stand type and seed cotton level were the most contributors to gin stand capacity (G.S.C.) and ginning time. As the seed-cotton levels decreased, the gin stand capacity decreased and *vice versa*. The seed cotton level had an effect on the most fiber properties and lint cotton grade.

The present investigation aimed to study the effect of roller gin types and seed cotton grade on ginning efficiency and fiber properties of some Egyptian cotton varieties.

RESULTS AND DISCUSSION

1- Lint grade:

Data showed in Table (1) indicated that the highest lint cotton grade was recorded with the Rotary gin compared with the McCarthy roller gin. Giza 95 recorded the highest lint grade followed by other cotton varieties. Regarding the effect seed cotton grades could be noticed that the highest lint cotton grade was attained from the highest seed cotton grade, (Good/fully good).

The first and the second order interactions between ginning types, (S), cotton varieties (V) and seed cotton grade (G), were significant effect on this trait and will be discussed the second order interaction (S x V x G) only in this study. Data showed in Table (2) cleared that the highest lint cotton grade was recorded from the rotary gin stand and the highest seed cotton grade (G/FG) with all cotton varieties under study.

These results are in agreement with Ibrahim (2010 b), he summarized that the highly significant interaction was found between gin stand type and seed cotton grade for lint grade.

2- Ginning efficiency parameters, (lint %, ginning time and the gin stand capacity):

The results in Table (1) indicated that the McCarthy gin stand produced the highest mean values of lint % (36.68 %) and ginning time (1.96 hr/kentar). On the contrary the rotary gin stand was recorded the highest gin stand capacity (2.90 kentar/hr) with the lowest ginning time (0.391 hr/kentar).

The results showed that Giza 95 was recorded the highest lint cotton grade (28.4), lint % (36.45%), the biggest gin stand capacity (2.369 kentar/hr) and the lowest ginning time (0.998 hr/kentar) compared with other cotton varieties in this study.

The highest seed cotton grade good/fully good recorded the highest mean values of lint cotton grade, lint %, the gin stand capacity and the lowest ginning time compared with the lowest seed cotton grade (G-1/4).

Regarding the second order interaction (S x V x G) data presented in Table (2) indicated that the highest lint cotton grade was attained from the highest seed cotton grade with the rotary gin stand with all cotton varieties, while the lowest lint cotton grade was showed from the lowest seed cotton grade with the McCarthy gin stand. Concerning the lint % data cleared that the seed cotton grade (Good/Fully Good) of Giza 95 cultivar with McCarthy recorded the highest lint % (39.83 %), while the lowest mean value for the same trait was found from (Good- ¼ x Giza 96 x Rotary gin stand) with the mean value of (30.19%). For ginning time, the results indicated that the seed cotton grade, (Good - ¼) took the longest ginning time with all cotton cultivars with McCarthy gin stand. While, the seed cotton grade (Good/fully good) with Giza 95 cultivar and the Rotary gin stand recorded the lowest ginning time with the mean value of (0.15 hr/kentar). The highest gin stand capacity (5.71 kentar/hr) was ginned with the Good/fully good x Rotary gin stand using the cotton variety, Giza 95, while the lowest gin stand capacity (0.35 kentar/hr) was ginned with Good- ¼ x McCarthy gin stand using the cotton variety, Giza 86.

These results are in agreement with those obtained by Batisha (2005), concluded that all studied ginning efficiency parameters were significantly affected by seed cotton level. Frig (2002), Abdel-Aal (2007) and Soliman (2016), stated that the all studied ginning efficiency parameters were significantly affected by the seed cotton level, the highest seed cotton level (G/FG) gaves the highest mean value of the gin stand capacity. Also, Ibrahim (2010 b) and Soliman (2016), concluded that the highest values of the gin-stand capacity (kg/inch/hr) was recorded with the highest seed cotton grade, (Good + ¼).

3- H.V.I. fiber properties, (Upper half mean length (mm), uniformity Iindex (%), short fiber (%), fiber strength (g/tex), fiber elongation (%), micronaire reading and maturity ratio (%)

With regard to data shown in Table (3), it could be noticed that there were significant differences among the two ginning types for upper half mean length, fiber strength and fiber elongation percentage only. The highest mean values for fiber length and fiber strength were recorded from the McCarthy gin stand with the mean values of (33.25 mm and 43.1 g/tex, respectively) compared with the Rotary gin stand.

The cotton variety Giza 96 gave the highest mean values for fiber length, uniformity index and fiber strength with the mean values of (35.03 mm, 88.6 and 45.2 g/tex, respectively) and the lowest mean

values for both short fiber percentage (5.5%) and fiber maturity (0.860 %). While, Giza 95 recorded the lowest mean values for fiber length and fiber uniformity index and recorded the highest mean values for short fiber %, fiber elongation % and micronaire value.

The highest mean values for UH.M.L. (33.19 mm), uniformity index 87.5 %, fiber strength (43.1 g/tex), micronaire value (4.26), the fiber maturity ratio (0.871) and the lowest short fiber (6 %) and fiber elongation (5.8 %) were attained from the highest seed cotton grade (Good/Fully Good) compared with the lowest seed cotton grade (Good-1/4).

Respecting the second order interaction (S x V x G), the results in **Table (4)** showed that the interaction between (any cotton variety in this study x the highest seed cotton grade (G/FG) x using the McCarthy gin stand) recorded the highest mean values for the fiber properties, except short fiber % and fiber elongation % compared with the treatments with the rotary gin stand.

These results are in agreement with those obtained by **Ibrahim (2010 b)** he recorded that the highest mean values of gin stand capacity, ginning out-turn, lint grade, micronaire value, fiber elongation (%) and reflectance degree (Rd %) were obtained from the highest seed-cotton level, Good to Fully Good (G/FG).

Also, these results are in agreement with those obtained by ICAC (2001), Batisha (2005) and Abdel-Aal (2007), concluded that hair weight bundle strength was significantly affected by the cotton cultivar. Also, Ibarhim (2010 b) and Patil and Vaishali (2010), stated that the fiber properties had a significant variation of the elongation with different roller speeds. Also, these results are in accepted with those obtained by Hossam El-Din *et al.* (2003) and Yehia (2003), summarized that fiber maturity parameters, elongation was highly significantly affected by the cotton cultivar (Kamal *et al*., 2002, Fouda, 2004, Eman, Batish, 2005 and Ibrahim, 2013) agreement for those results. In brief, lint grade affected significantly the fiber properties and the best values averages were obtained from classer grade Good to Fully Good for all traits of fiber (**El-Banna,** 2019 b).

Likewise, Yehia (2003), summarized that fiber maturity parameters, bundle strength was highly significantly affected by the cotton cultivar (Kamal *et al*., 2002, Asal, wali, 2003, Fouda, 2004, Mohamed, 2005 and Ibrahim, 2013) agreement for those results.

4- H.V.I. fiber properties, (fiber color, trash attributes, spinning consistency index and nep counts):

Data presented in Table (5) cleared that the best fiber characters i.e., the highest fiber brightness (Rd %), the lowest trash attributes were showed from the rotary gin stand compared with the McCarthy gin stand. While, the McCarthy gin stand recorded the highest spinning consistency index and the lowest nep counts compared with rotary gin stand.

Concerning the cotton variety effect, could be noticed that the variety Giza 96 gave the highest mean values of fiber Rd %, spinning consistency index and nep counts, while the cotton variety Giza 95 recorded the lowest men values of fiber Rd %, trash attributes and spinning consistency index.

The highest seed cotton grade Good/fully good recorded the highest mean values of both fiber Rd % and the spinning consistency index, meanwhile, the same seed cotton grade (G/FG) recorded the lowest mean values for fiber +b, trash attributes and nep counts and vice versa for the effect of the lowest seed cotton grade (Good-1/4) on the same traits.

Table (6) illustrated the second order interaction (S x V X G) and could be concluded that the highest fiber Rd % and the lowest trash attributes were ginned with the highest seed cotton grade from Giza 96 using the rotary gin stand. Also, the highest spinning consistency index and the lowest nep counts were attained from the highest

seed cotton grade (G/FG) from Giza 96 with the McCarthy gin stand.

These results are in agreement with those obtained by Fouda (2004) and Batisha (2005), summarized that highly significant difference in was found among studied cotton varieties in fiber reflectance degree (Rd) and yellowness (+b). These results are in accepted with those obtained by El-Feki *et al.*, 2005, El-Mansy *et al.*, 2008 and Ibrahim (2010 a and b).

These findings accepted with those reported by GTZ, (2003), Abdel-Aal, (2007), Armijo and Gillum, (2007) and Gérald and Nicolas, (2010). Also, these results are in accepted with those obtained by Abudullahi and Ayele (2008) and Ibrahim (2013), summarized that fiber quality were highly significantly affected by the cotton cultivar (Kamal *et al*., (2002), Fouda, (2004), El-Feki *et al*., (2005) and Adanacioglu and Olgun, (2010) agreement for those results.

MATERIALS AND METHODS

This investigation was carried out by Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria Egypt. The experiments were conduct at El-Wadi Ginning Company and Cotton Research Institute, Agriculture Research Center, Giza, Egypt to investigate the effect of roller gin types and seed cotton grade on ginning efficiency and fiber properties of some Egyptian cotton varieties. Two types of roller gins (McCarthy gin stand and Rotary knife gin stand) were used. Four Egyptian cotton varieties namely; Giza 86, 96, 94 and Giza 95 were used. Four seed cotton grades i.e., (Good/Fully Good, Good +1/4, Good and Good -1/4) with three replicates (20 kg/rep.) belong to each cotton variety as determined by an Experts Committee from Cotton Arbitration and Testing General Organization (CATGO).

Studied characters:

1. **Lint grade:** It was determined by a committee of three cotton expert classers of the Cotton Arbitration and Testing General Organization, (CATGO).
2. **Ginning efficiency parameters**: These parameters were calculated, according to the following equations, proposed by Chapman and Stedronsky (1959):

2-1. Gin stand capacity (G.S.C.): as the lint weight in kg per inch per hour, as follows:

$$\text{Gin stand capacity (G.S.C.)} = \frac{60 * \text{weight of ginned lint (kg)}}{\text{Ginning time (min)} * \text{Length of roller (inch)}} = (\text{kg/inch/hr.})$$

2-2. Ginning time (G.T.):

$$\text{Ginning time (G.T.)} = \frac{\text{Ginning time (minute)} * 157.5}{\text{Seed-cotton weight (Kg)} * 60} = (\text{hr/Kentar})$$

2-3. Ginning out-turn (G.O.T.): as a percentage, as follows:

$$\text{Lint (\%)} = \frac{\text{Lint weight (kg)}}{\text{Seed-cotton weight (kg)}} * 100 = (\%)$$

3. **HVI fiber properties as follows:**
   - Upper half mean length (mm), (U.H.M.L).
   - Uniformity index (%) (UI).
   - Short fiber (%) (SF) (<12.7mm).
   - Fiber strength (g/tex) (Str).
   - Fiber elongation (%) (Elg).
   - Micronaire reading (MIC).
   - Maturity ratio (%) (Mat).
   - Color attributes: Fiber brightness (Rd %) and Degree of yellowness (+b).
   - Trash attributes: Trash area (%), (Tr Ar) and Trash count, (Tr cnt).
   - Spinning consistency index, (SCI).

4. **Nep count**: were determined by Nep tester at the laboratory of Cotton Arbitration and Testing General organization, (CATGO), Alexandria, Egypt.

Statistical procedures:

The completely randomized design with three replications was used to outline this work. The attained data was statistically analyzed as a factorial experiment according to Steel and Torrie (1980). Statistical analysis was done by, ANOVA, F-test, least significant differences (L.S.D) at 0.05 level of probability were used to compare the treatments means using SAS software package (version 9.13, 2007).

Conclusion: use the appropriate ginning type and the seed cotton grade affects the fiber quality characteristics. The best lint grade and ginning efficiency (ginning time and gin stand capacity) were attained by using the rotary gin stand. While the McCarthy gin stand outperformed in lint percentage. The rotary gin stand excelled in trash area, trash count, fiber elongation % and fiber Rd %. The McCarthy gin stand excelled in nep count, spinning consistency index, upper half mean length and fiber strength. The best results in lint grade and fiber properties with the highest of seed cotton grade good/fully good. There were insignificant differences between ginning types in some fiber properties which uniformity index, short fiber %, micronair reading and maturity ratio.

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Table (1): Mean performance of lint grade, lint %, ginning time and gin stand capacity as affected by the ginning types (S), cotton varieties (V), seed cotton grades (G) and their interactions

Traits / Variables	Lint grade	Lint %	Ginning time (hr/kentar)	Gin stand capacity (kentar/hr)
Ginning types (S)				
The McCarthy gin stand	27.6 b	36.684 a	1.962 a	0.646 b
The Rotary gin stand	28.2 a	33.441 b	0.391 b	2.90 a
L.S.D. (0.05)	**0.02**	**0.147**	**0.018**	**0.053**
Cotton varieties (V)				
Giza 96	27.7 b	34.364 c	1.228 a	1.565 bc
Giza 94	27.7 b	35.308 b	1.249 a	1.609 b
Giza 86	27.7 b	34.125 d	1.230 a	1.532 c
Giza 95	28.4 a	36.454 a	0.998 b	2.396 a
L.S.D. (0.05)	**0.03**	**0.208**	**0.026**	**0.075**
Seed cotton grades (G)				
Good/Fully Good	29.8 a	37.447 a	0.810 d	2.579 a
Good+1/4	28.4 b	35.849 b	0.967 c	1.947 b
Good	27.5 c	34.155 c	1.247 b	1.517 c
Good–1/4	25.8 d	32.801 d	1.681 a	1.061 d
L.S.D. (0.05)	**0.03**	**0.208**	**0.026**	**0.075**
Interactions				
S * V	*	*	*	**
S * G	*	*	*	**
V * G	*	*	*	**
S*V*G	*	*	*	**

Means within each column followed by the same letter are not significant difference at 0.05 level of probability.
*** and ** : Significant difference at 0.05 and 0.01 levels of probability, respectively.**

Table (2): The interaction between cotton varieties, seed cotton grades and ginning types (S x V x G) for lint grade, lint %, ginning time and gin stand capacity

Variables			Traits			
Ginning types (S)	**Cotton varieties (V)**	**Seed cotton grades (G)**	**Lint grade**	**Lint %**	**Ginning time (hr/kentar)**	**Gin stand capacity (kentar/hr)**
The McCarthy gin stand	**Giza 96**	**Good/Fully Good**	29.5	38.758	1.422	0.851
		Good+1/4	28.0	36.334	1.655	0.672
		Good	27.0	34.900	2.144	0.510
		Good–1/4	25.0	33.184	2.887	0.361
	Giza 94	**Good/Fully Good**	29.5	39.027	1.422	0.888
		Good+1/4	28.0	37.569	1.707	0.725
		Good	27.0	36.330	2.166	0.555
		Good–1/4	25.0	35.388	2.950	0.396
	Giza 86	**Good/Fully Good**	29.5	38.407	1.426	0.848
		Good+1/4	28.0	35.417	1.655	0.673
		Good	27.0	34.323	2.145	0.504
		Good–1/4	25.0	32.747	2.888	0.356
	Giza 95	**Good/Fully Good**	30.0	39.830	1.185	1.059
		Good+1/4	28.5	39.190	1.353	0.839
		Good	28.0	39.197	1.904	0.633
		Good–1/4	26.5	37.047	2.476	0.472
The Rotary gin stand	**Giza 96**	**Good/Fully Good**	30.0	35.623	0.284	3.873
		Good+1/4	28.5	34.137	0.379	2.704
		Good	27.5	31.787	0.448	2.088
		Good–1/4	26.0	30.187	0.607	1.463
	Giza 94	**Good/Fully Good**	30.0	35.517	0.290	3.819
		Good+1/4	28.5	34.267	0.379	2.808
		Good	27.5	32.693	0.451	2.186
		Good–1/4	26.0	31.370	0.627	1.500
	Giza 86	**Good/Fully Good**	30.0	35.640	0.292	3.587
		Good+1/4	28.5	34.247	0.371	2.671
		Good	27.5	31.513	0.446	2.077
		Good–1/4	26.0	30.709	0.613	1.545
	Giza 95	**Good/Fully Good**	30.0	36.772	0.157	5.711
		Good+1/4	29.0	35.330	0.232	4.482
		Good	28.5	33.493	0.272	3.583
		Good–1/4	27.0	31.777	0.403	2.392
L.S.D (0.05)			**0.07**	**0.589**	**0.073**	**0.150**

Table (3): Mean performance of upper half mean length, uniformity index, fiber strength, fiber elongation, micronaire reading and maturity ratio affected by the ginning types (S), cotton varieties (V), cotton grades (G), and their interactions on trash properties and nep count.

Traits / Variables	Upper half mean length (mm), (U.H.M.L)	Uniformity index (%) (UI)	Short fiber (%) (SF)	Fiber strength (g/tex)	Fiber elongation (%)	Micronaire reading	Maturity ratio (%)
	Ginning types (S)						
McCarthy gin	33.25 a	86.9	6.3	43.1 a	5.7 b	4.13	0.872
Rotary gin	32.68 b	86.5	6.1	40.8 b	6.2 a	4.13	0.869
L.S.D.(0.05)	**0.27**	**NS**	**NS**	**0.7**	**0.2**	**NS**	**NS**
	Cotton varieties (V)						
Giza 96	35.03 a	88.6 a	5.5 c	45.2 a	5.6 c	3.84 d	0.860 b
Giza 94	34.83 a	88.0 a	5.4 c	41.9 b	5.7 bc	4.05 c	0.869 a
Giza 86	32.96 b	86.7 b	6.2 b	44.4 a	6.0 b	4.21 b	0.871 a
Giza 95	29.04 c	83.6 c	7.6 a	36.1 c	6.6 a	4.42 a	0.866 ab
L.S.D.(0.05)	**0.39**	**0.8**	**0.3**	**0.9**	**0.3**	**0.09**	**0.008**
	Seed cotton grades (G)						
Good/Fully Good	33.19 a	87.5 a	6.0 b	43.1 a	5.8 c	4.26 a	0.871 a
Good+1/4	33.03 ab	86.9 ab	6.1 ab	42.1 b	5.8 bc	4.19 a	0.868 ab
Good	32.90 ab	86.5 bc	6.3 a	41.7 bc	6.1 ab	4.10 b	0.864 ab
Good–1/4	32.74 b	86.1 c	6.4 a	40.9 c	6.2 a	3.96 c	0.862 b
L.S.D.(0.05)	**0.39**	**0.8**	**0.3**	**0.9**	**0.3**	**0.09**	**0.008**
	Interactions						
S * V	*	*	*	*	*	*	*
S * G	*	*	*	*	*	*	*
V * G	*	*	*	*	*	*	*
S*V*G	*	*	*	*	*	*	*

Means within each column followed by the same letter are not significant difference at 0.05 level of probability.
*** : Significant difference at 0.05 level of probability.**

Table (4): The interaction between cotton varieties, seed cotton grades and ginning types (S x V x G) for upper half mean length, uniformity index, fiber strength, fiber elongation, micronaire reading and maturity ratio

Variables			Upper half means length (mm), (U.H.M.L).	Uniformity Index (%) (UI)	Short fiber (%) (SF)	Fiber strength (g/tex)
Ginning types (S)	**Cotton varieties (V)**	**Seed cotton grades (G)**				
The McCarthy gin stand	**Giza 96**	**Good/Fully Good**	36.83	89.5	5.4	48.2
		Good+1/4	36.17	88.7	5.4	47.8
		Good	35.85	88.5	5.4	47.5
		Good–1/4	35.58	88.5	5.4	47.4
	Giza 94	**Good/Fully Good**	35.22	89.5	5.3	44.0
		Good+1/4	34.82	88.6	5.4	43.7
		Good	34.70	88.4	5.4	43.6
		Good–1/4	34.70	86.7	5.5	41.9
	Giza 86	**Good/Fully Good**	33.48	88.0	6.0	46.0
		Good+1/4	33.44	86.4	6.1	45.1
		Good	33.39	86.0	6.4	43.9
		Good–1/4	32.66	87.4	6.9	43.1
	Giza 95	**Good/Fully Good**	28.58	84.2	7.6	38.5
		Good+1/4	28.99	84.0	7.9	36.4
		Good	28.89	83.6	7.8	36.5
		Good–1/4	28.72	83.2	8.1	35.8
The Rotary gin stand	**Giza 96**	**Good/Fully Good**	34.27	89.2	5.5	43.9
		Good+1/4	33.92	88.7	5.7	43.5
		Good	33.79	88.2	5.9	42.0
		Good–1/4	33.85	87.7	5.6	41.4
	Giza 94	**Good/Fully Good**	35.21	88.8	5.4	41.5
		Good+1/4	34.72	88.4	5.4	40.3
		Good	34.65	88.2	5.4	40.7
		Good–1/4	34.59	86.7	5.5	39.7
	Giza 86	**Good/Fully Good**	32.82	86.8	5.9	45.9
		Good+1/4	32.80	86.7	6.1	44.2
		Good	32.62	86.6	6.1	43.9
		Good–1/4	32.48	85.9	6.2	43.4
	Giza 95	**Good/Fully Good**	29.10	83.9	6.8	36.6
		Good+1/4	29.35	83.6	7.1	35.7
		Good	29.32	83.5	7.8	35.2
		Good–1/4	29.34	82.6	7.8	34.2
L.S.D (0.05)			**1.10**	**2.2**	**0.8**	**2.6**

Table (4): Continuous

Variables			Fiber elongation (%)	Micronaire reading	Maturity ratio (%)
Ginning types (S)	Cotton varieties (V)	Seed cotton grades (G)			
The McCarthy gin stand	Giza 96	Good/Fully Good	4.8	3.77	0.866
		Good+1/4	5.0	3.76	0.863
		Good	5.2	3.67	0.860
		Good–1/4	5.4	3.57	0.856
	Giza 94	Good/Fully Good	5.1	4.20	0.880
		Good+1/4	5.1	4.19	0.873
		Good	5.5	4.14	0.863
		Good–1/4	5.7	3.92	0.863
	Giza 86	Good/Fully Good	5.9	4.39	0.870
		Good+1/4	5.8	4.19	0.870
		Good	5.8	4.04	0.870
		Good–1/4	6.2	3.95	0.860
	Giza 95	Good/Fully Good	6.5	4.64	0.873
		Good+1/4	6.6	4.62	0.870
		Good	6.6	4.55	0.870
		Good–1/4	6.6	4.48	0.870
The Rotary gin stand	Giza 96	Good/Fully Good	5.7	4.12	0.870
		Good+1/4	6.0	4.05	0.860
		Good	6.2	3.88	0.856
		Good–1/4	6.3	3.87	0.853
	Giza 94	Good/Fully Good	5.5	4.08	0.870
		Good+1/4	5.9	4.00	0.870
		Good	6.3	3.94	0.860
		Good–1/4	6.3	3.94	0.876
	Giza 86	Good/Fully Good	5.9	4.45	0.876
		Good+1/4	6.0	4.30	0.873
		Good	6.0	4.34	0.873
		Good–1/4	6.2	3.99	0.873
	Giza 95	Good/Fully Good	6.7	4.47	0.866
		Good+1/4	6.4	4.42	0.866
		Good	6.7	4.25	0.863
		Good–1/4	6.9	3.95	0.850
L.S.D (0.05)			**0.8**	**0.24**	**0.023**

Table (5): Mean performance of color attributes, trash attributes, spinning constant and nep count as affected by the ginning types (S), cotton varieties (V), seed cotton grades (G) and their interaction

Traits / Variables	Color attributes		Trash attributes		Spinning constant index (SCI)	Nep count
	Fiber brightness (Rd%)	Degree of yellowness (+b)	Trash area (%) (Tr Ar)	Trash count. (Tr cnt)		
	Ginning types (S)					
McCarthy gin	73.9 b	10.1	0.60 a	52 a	203 a	101 b
Rotary gin	74.5 a	10.2	0.46 b	40 b	194 b	124 a
L.S.D. (0.05)	**0.3**	**NS**	**0.09**	**6**	**4**	**5**
	Cotton varieties (V)					
Giza 96	77.6 a	9.8 b	0.50 ab	41 bc	224 a	150 a
Giza 94	75.7 b	9.1 c	0.60 a	48 ab	210 b	112 b
Giza 86	75.5 b	9.2 c	0.54 ab	54 a	206 b	85 d
Giza 95	68.1 c	12.5 a	0.47 b	40 c	153 c	104 c
L.S.D. (0.05)	**0.5**	**0.3**	**0.12**	**8**	**6**	**7**
	Seed cotton grades (G)					
Good/Fully Good	75.2 a	9.8 b	0.36 c	34 c	203 a	108 b
Good+1/4	74.6 a	10.2 a	0.48 bc	45 b	199 ab	111 b
Good	73.8 b	10.2 a	0.58 ab	49 ab	197 b	113 ab
Good–1/4	73.2 c	10.4 a	0.70 a	55 a	194 b	118 a
L.S.D. (0.05)	**0.5**	**0.3**	**0.12**	**8**	**6**	**7**
	Interactions					
S * V	*	*	*	*	*	*
S * G	*	*	*	*	*	*
V * G	*	*	*	*	*	*
S*V*G	*	*	*	*	*	*

Means within each column followed by the same letter are not significant difference at 0.05 level of probability.
*** : Significant difference at 0.05 level of probability.**

Table (6): The interaction between cotton varieties, seed cotton grades and ginning types (S x V x G) for color attributes, trash attributes, spinning constant and nep count

Traits			Color attributes		Trash attributes		Spinning constant index (SCI)	Nep count
Ginning types	Cotton varieties	Seed cotton grades	Fiber brightness (Rd%)	Degree of yellowness (+b)	Trash area (%)	Trash count		
The McCarthy gin stand	Giza 96	Good/Fully Good	78.0	9.3	0.20	31	243	87
		Good+1/4	77.6	9.5	0.53	46	235	105
		Good	76.9	9.9	0.74	53	234	108
		Good–1/4	76.0	10.0	0.77	55	232	109
	Giza 94	Good/Fully Good	75.6	8.6	0.58	49	220	104
		Good+1/4	75.0	9.3	0.61	55	214	106
		Good	75.1	9.2	0.64	57	211	106
		Good–1/4	74.4	9.4	0.91	62	208	110
	Giza 86	Good/Fully Good	77.1	9.2	0.50	44	212	76
		Good+1/4	76.3	9.2	0.52	60	213	88
		Good	74.8	9.3	0.57	62	211	94
		Good–1/4	73.7	9.4	0.68	69	202	107
	Giza 95	Good/Fully Good	68.2	12.2	0.54	34	157	100
		Good+1/4	68.0	12.2	0.58	45	156	102
		Good	68.0	12.3	0.62	50	153	107
		Good–1/4	67.3	12.7	0.67	52	150	108
The Rotary gin stand	Giza 96	Good/Fully Good	78.7	9.9	0.17	18	214	205
		Good+1/4	78.1	10.0	0.45	36	214	192
		Good	77.6	10.0	0.55	40	211	190
		Good–1/4	77.4	10.0	0.57	50	211	206
	Giza 94	Good/Fully Good	76.6	8.4	0.32	29	214	113
		Good+1/4	76.6	9.2	0.40	39	206	116
		Good	76.4	9.2	0.58	43	204	117
		Good–1/4	75.8	9.3	0.80	53	204	121
	Giza 86	Good/Fully Good	77.2	9.8	0.40	42	208	90
		Good+1/4	76.3	9.1	0.39	51	202	81
		Good	73.8	9.1	0.60	48	200	74
		Good–1/4	74.8	9.4	0.66	58	198	70
	Giza 95	Good/Fully Good	70.0	12.5	0.17	28	158	87
		Good+1/4	69.2	12.5	0.35	30	153	101
		Good	67.9	12.6	0.36	38	153	110
		Good–1/4	66.3	12.7	0.55	41	146	116
L.S.D (0.05)			1.6	0.7	0.34	24	16	19