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WORLD COTTON RESEARCH CONFERENCE-7 (WCRC-7)

4-7 October 2022 CAIRO-EGYPT

Cotton researchers and specialists from across the globe will travel to Egypt to present the results of their work, expand and strengthen their personal and professional networks and learn about the most cutting-edge research advances in the cotton research universe.

The next WCRC will not be held until 2026, so make your plans now

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Cotton Research Institute of Iran (CRII)

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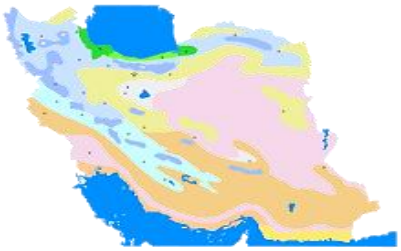
Agriculture in Iran

Iran comprises a land area of over 1.6 million km^2. Only 12% of the total land area is under cultivation but less than one-third of the cultivated area is irrigated; the rest is devoted to dry farming. Almost 20% of Iranian employees are working in agriculture sector, and 15% of gross domestic production, 20% of non-oil export and over 85% of foods of the country are supplied in the agriculture sector.



In most of the country parts, the climatic conditions are arid and semi-arid, while the 3 provinces located on the northern belt connected to the Caspian Sea have a Mediterranean climate. The main crops produced by Iranian farmers are wheat, barley, rice, sugar beets, fruits, nuts and cotton.

Two cotton species (*Gossypium hirsutum* and *G. herbaceum*) are grown in Iran. The total cotton cultivation area in more than 14 provinces is around 90000 ha.

Cotton Research Institute of Iran (CRII) at a glance

Agricultural Research, Education and Extension Organization (AREEO) is the largest responsible body for agricultural research, education and extension in ministry of Agricultur Jahad of Iran.

In 1936-1959, Varamin Plant Breeding Corporation was the only responsible body for cotton research in Iran. After the establishment of Seed and Plant Improvement Institute (SPII) in 1959, the research activities on cotton was moved under the management of SPII in the Cotton Research Department.

Regarding the national importance of cotton in textile and oil industries, in 1997, the Cotton Research Institute of Iran (CRII) was established in the Northern region of Iran, Gorgan, coordinates basic and applied researches along with the development activities on cotton in order to enhanc the productivity and profitability of cotton farmers and the added value chain associated with cotton. CRII has closely cooperated with other institutes in research, farming, industry, and technology.



Missions of the CRII

- Developing cotton research towards the introduction of improved cultivars suitable for different environmental conditions throughout the country
- Development of appropriate farming/ cropping system for different cotton growing zones and their effective management
- Conserving Cotton germplasm
- Development of effective and efficient disease, pest and weed management strategies
- The direction of researches in cotton breeding, agronomy, biotechnology and seed technology
- Development and production of cotton seed nucleus
- Providing training courses and workshops in cotton production and also transferring of research findings to extension agencies, experts and farmers by the cooperation of extension and education services

Achievements of CRII

- Releasing of 12 cotton cultivars including Sepid, Khordad, Golestan, Armaghan, Khorshid, Kashmar, Latif, Shayan, Sajedi, Hekmat, Partov and Taban
- Identification of bio-ecology of cotton pests and diseases
- Monitoring and controlling of quarantined pests and diseases
- Increasing of cotton water productivity by optimizing of irrigation methods
- Preparation of digital soil fertility maps for all Golestan agricultural service centers
- Projecting of semi- mechanized machine of cotton harvester for small farms
- Feasibility of cotton transplanting in Iran
- Development of cotton based on conservation agriculture in Iran
- Enhancement of cotton germplasm collection from 190 to more than 450 germline
- Nucleus seed production for at least 14 cotton cultivars at different stations
- Using peaceful nuclear energy to produce two mutant varieties (Taban and Partov)
- Preparation of agronomic guidelines based on the research results
- Modeling to reduce the effects of abiotic and biotic stress on cotton
- Monitoring and tracing of cotton bacterial blight

- Bio-ecological study of cotton bollworm, spiny worm and red bollworm
- Reduction of 30% in water use by alternate furrow irrigation compared to conventional irrigation

Important Research Topics

- Cotton varieties improvement
- Abiotic stresses (drought and salinity)
- Enhancing water use efficiency
- Pest and diseases resistance
- Cotton yield and quality improvement
- Agronomical, physiological and ecological studies in cotton plants
- Integrated pest management (IPM) strategies Study of epiphytic characteristics of causal agent of cotton bacterial blight in cotton fields
- Study of agronomical methods efficiency on cotton pest, diseases and weeds in cotton fields
- Assessment of new pesticides against cotton pest and diseases
- Assessment of composts on Verticillium wilt disease
- Developing cotton harvesting machines
- Determining water requirement for new cotton cultivars

Structure of the CRII

There are five research departments in CRII headquarters located in Gorgan, namely; Breeding, Agronomy, Plant Protection, Agricultural Engineering and Research Technical Services in Gorgan. It has one research department in Varamin and 14 research stations in the different cotton production areas of Iran. Cotton Research Institute of Iran is responsible for providing all research priorities and activities at the national level. Main station of CRII is Hashemabad Cotton Research Station for breeding research and for studying of cultivar resistant to Verticillium wilt, other disease and pests is Karkandeh Cotton Research Station.

The Breeding department always follows cotton varieties improvement, fundation seed production and cotton germplasm management as three main missions. The main research activity of this department is the evaluation of genetic material to develop adaptable and sustainable varieties with high yield and good fiber quality. The breeding department is responsible for managing, programming, and direct supervision of new cotton varieties improvement, germplasm conservation and nucleus cotton seed development and production at different cotton production of Iran.

The Agronomy Department activities include the study leading to offering the best methods for cultivation and harvesting

like best planting date, planting pattern, nutrition management for the improved varieties and study on another fibrous plant like Kenaf and Flax.

The Plant Protection Department conducts technologies and new approaches to facilitate and lead to reduce damage and high maintaining yield. The main effort followed by plant protection of cotton is focused on monitoring and studying of bio-ecology of insects, diseases and weeds, crop loss assessment and identification of cultivar resistant to biotic agents.

The purposes of the technical and engineering research department are identifying the best method for managing of cotton field's irrigation, enhancing water use efficiency, evaluating new irrigation methods, improving conventional cotton irrigation practices in different cotton production of Iran, designing and optimizing planting, crop operations and harvesting cotton machines, optimum use of residual and cotton waste and its conversion into consumable products.

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Comparative analysis of codon usage pattern of the chloroplast Genomes in diploid and tetraploid species of Gossypium

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Abstract:

Uniparental legacy and profoundly monitored design of the chloroplast genome among angiosperms makes it appropriate for developing quality genealogies and deducing populace accounts in view of these connections. Chloroplast studies enjoy huge benefit of genomics and genome sequencing, and another image is arising of how the chloroplast capacities and speaks with other cell compartments. Cotton is the world's driving material yield and a model framework for investigations of numerous natural cycles and genomics research has progressed quickly in the beyond couple of years. Relative investigation of codon utilization is vital to advance the development of proteins in quality articulation framework. Plastids have exceptional significance because of its little size and high duplicate number of genome. Gossypium spp. is the main fiber crop in the advanced world. In this exploration, the total nucleotide arrangement of the chloroplast genomes of two wild cotton species was examined and dissected with CodonW programming. Equivalent codon utilization shows 57 protein coding genes in chloroplast genome of *G.thurberi* and *G.arboreum* were dissected to figure out the potential variables contributing codon predisposition. All favored interchangeable codons were found to utilize A/T finishing codons as chloroplast genomes are rich in AT. Correspondence analysis and technique for successful number of codon as Nc-plot were led to dissect interchangeable codon use. ENC Vs GC3 plot assembled larger part of the analyzed genes on or just underneath the left half of the normal GC3 curve showing the impact of base compositional requirements in managing codon utilization. As indicated by the corresponding analysis, codon predisposition in the chloroplast genome of *G.thurberi* and *G.arboreum* are connected with their quality length, mutation bias, quality hydropathic level of every protein, gene hydropathic level of each protein, gene function and selection or gene expression only subtly affect codon usage. This study provided insights into the molecular evolution studies.

Keywords:

G.thurberi and *G.arboreum*, Synonymous codon usage, ENC Vs GC3 plot, Codon adaptation index, Correspondence analysis

1. Introduction

Cotton (*Gossypium* spp.), belongs to family Malvaceae and is grown almost all over the world from time immemorial. It is one of the most important cash crops in many countries and is being grown in

warmer regions of the countries. In addition to the lint, cotton seed is used for oil extraction which makes about 80 percent of the national oil production. Cotton is mainly grown for fiber which is an economic component and plays a vital role for uplifting country's economy (Riaz *et al*., 2013). Generally, the alternative codons for any amino acid are not randomly used (Ghosh, T., 2000). Studies of the synonymous codon usage reveal information on molecular evolution of individual genes, which provides data to improve gene recognition algorithms, are utilized to design DNA primers, and detects horizontal transfer events. Most plastidic genomes have four regions, namely large single copy region (LSC, 80 Kb), small single copy region (SSC, 20 kb) and two inverted repeat regions (IR, 25 kb). The single copy region is separated by two IRs. This structural conservation however breaks in some plants such as *Vicia faba* (Kim, K. J., & Lee H. L., 2004). And *Cryptomeria japonica* (Hirao *et al*., 2008 & Saski *et al*., 2005) by loss of an IR, and in *Euglena gracilis* that has three tandem repeats (Hallick *et al*., 1993). Variations among different species provide large information for the phylogenetic studies. Chloroplasts have low mutation rate with great deal of conservation in their genome size and structure, gene content and organization. Few differences have been reported in the same species, but significant differences could be detected between the different species in genome size and gene orientation (Young-Kyu *et al*., 2009). Transplastomics have proved to be a powerful tool to improve the plant genetic architecture with high expression of the foreign protein, low risk of the pollen pollution (Talat, F., 2014 & Ruf *et al*., 2007) and no gene silencing. Therefore, and in addition to phylogenetic analysis based on plastidic genomes, it is imperative to understand the chloroplast genome in order to logically design our next generation transplastomics. Accordingly, chloroplast genomes of many species have been sequenced (Talat, F., 2015 & Diekmann *et al*., 2009). Following a long period of evolution, codons are used in a species-specific manner, a phenomenon known as codon usage bias. In recent years, an ever-increasing body of studies on codon usage bias has been reported for plant breeding (Kawabe *et al*., 2003 & Ravi *et al*., 2008 & Lei *et al*., 2013). Liu and Xue (2005) reported that the chloroplast genome might display characteristics of codon usage that are different from its host nuclear genome. Ruhfel *et al* (2014) put forward that their analyses of the plastid sequence data recovered a strongly supported framework of relationships for green plants. Several articles have also reported the codon usage pattern, and the factors that shape codon usage, for *Zea mays* (Liu *et al*., 2010), *Silene latifolia* (Qiu *et al*., 2011), seven different citrus species (Xu *et al*., 2013), and the *Asteraceae* family (Nie *et al*., 2013). However, the exact codon usage characteristics for single genes of higher plants have not been well explored to date.

2. Material and Methods

Software

Complete chloroplast genome sequence of *G.thurberi*, *G.arboreum*, *G.barbadense* and *G.hirsutum* in FASTA format, with respectively access number and length (NC_015204.1 & 160.264), (HQ_325740.1 & 160.230), (NC_008641.1 & 160.317), (NC_007944.1 & 160,301) downloaded from NCBI (//http:www.ncbi.nlm.nih.gov/nuccore/term). To identify the position of genes in four chloroplast genomes, according to the gene database information for each genome at the NCBI site, a montage of the sequences of each genome with the genes highlighted in the sequence was obtained by word Office 2010. After determining the position of all genes on the corresponding genome sequence, information about each gene from the NCBI site and from the gene bank page for each genome was obtained and written alongside with the corresponding

gene. The introns are distinguished by separated color that this caused till position of the genes, the IGS regions as well as the genes that are shared have also been identified. Additionally, the length of the coding regions (CDS, tRNA, rRNA) and non-coding regions (intron and IGS) for each of the three genomes was obtained separately from this file. Genome structure map and gene distribution drew with giving information about the access number of each genome in OGDRAW (V.1.1) software (http://ogdraw.mpimpgolm.mpg.de). The REPuter software online version (http://bibiserv.techfak.uni-bielfeld.de/reputer) was used to identify duplicate sequences and their position. RSCU of different codon were counted for each gene sample by CodonW in the Mobyle software at the address (http://mobyle.pasteur.fr/cgibin/portal.py). To calculating of the percentage of A, T, C and G nucleotides, as well as AT and GC in genomes in order to making CG table was used Visual Bioanformatics (V.2.1.0) software. SPSS (V.22) and Minitab (V.16) were used for plotting the usage of codon and adaptive analysis charts.

3. Results and Discussions

GC Content

The content of GC is an important feature of the Chloroplasts genome. The GC content for Chloroplast genome of four species are presented in Tables 1, 2, 3 and 4. The results of this study showed that percentage of GC in the *G.thurberi*, *G.arboreum*, *G.barbadense* and *G.hirsutum* genomes are similar and equals 37.2%. Coding and non-coding regions in each of the four genomes had a little GC content and were 40.4%, 33.1% respectively. IR region was richest based on GC, its rate for species was approximately 43%, while GC in SSC and LSC was 31% and 35% respectively. The rRNA genes had the highest GC content about 55.5%, and the protein-coding sequences had the lowest GC that amount was about 37.3%. In the non-coding region, the GC content for IGS and introns was 31.58% and 36.75%.

Table 1. GC contents for Chloroplast genome of *G.thorberi*

-	Coding	Region	-	-	Non	Coding	Region	-	-	-	-
-	Protein	tRNA	rRNA	Total	IGS	Intron	Total	Complete Genome	LSC	SSC	IR
Length	79740	2775	8970	91485	49915	20436	70351	160264	88737	20.271	25628
Proportion	49.76	1.73	5.60	57.08	31.15	12.75	43.90	100.00	55.37	12.65	15.99
T%	31.05	23.14	22.32	30.34	34.32	32.28	33.73	31.83	33.15	34.46	28.52
A%	29.85	24.58	22.17	29.31	34.10	30.97	33.19	30.95	31.67	33.92	28.54
C%	19.28	26.13	27.79	20.56	15.99	19.12	16.90	18.99	18.11	16.54	20.66
G%	18.42	26.16	27.71	19.79	15.58	17.63	16.18	18.23	17.08	15.09	22.29
A+T%	60.90	47.71	44.49	59.65	68.42	63.25	66.92	62.78	64.81	68.38	57.05
C+G%	37.69	52.29	55.51	40.35	31.58	36.75	33.08	37.22	35.19	31.62	42.95

Table 2. GC contents for Chloroplast genome of *G.arboreum*

-	Coding	Region	-	-	Non	Coding	Region	-	-	-	-
-	Protein	tRNA	rRNA	Total	IGS	Intron	Total	Complete Genome	LSC	SSC	IR
Length	79253	2769	8349	90371	493330	20526	69859	160230	88721	20287	25611
Proportion	49.46	1.53	5.21	56.4	30.8	12.8	43.60	100.00	55.37	12.66	15.98
T%	31.4	22.8	22.3	30.2	34.4	32.0	33.7	31.8	33.2	33.9	28.5
A%	30.3	24.0	22.3	29.4	34.0	31.1	33.2	31.0	31.6	34.4	28.5
C%	19.3	27.2	27.7	20.4	16.0	19.0	16.8	18.8	18.1	15.1	21.5
G%	19.0	26.0	27.7	20.1	15.6	17.9	16.3	18.4	17.1	16.6	21.5
A+T%	61.7	46.8	44.5	59.6	68.4	63.1	66.9	62.8	64.8	68.2	57.0
C+G%	38.3	53.2	55.5	40.4	31.6	36.9	33.1	37.3	35.2	31.7	43.0

Table 3. GC contents for Chloroplast genome of *G.barbadense*

-	Coding	Region	-	-	Non	Coding	Region	-	-	-	-
-	Protein	tRNA	rRNA	Total	IGS	Intron	Total	Complete Genome	LSC	SSC	IR
Length	78675	2791	9050	90516	48556	21245	69801	160317	88897	20036	25692
Proportion	49.07	1.74	5.64	56.46	30.28	13.27	43.53	100.00	55.45	12.49	16.03
T%	31.5	23.3	22.3	30.4	34.3	32.0	33.7	31.8	33.1	34.4	28.5
A%	30.2	23.9	22.3	29.3	34.1	31.2	33.2	30.9	31.7	33.8	28.5
C%	19.6	26.7	27.7	20.6	16.0	19.0	16.8	19.0	18.1	16.6	21.5
G%	18.7	26.1	27.7	19.7	15.6	17.8	16.2	18.2	17.1	15.3	21.6
A+T%	61.7	47.2	44.6	59.7	68.4	63.2	66.9	62.8	64.8	68.1	56.9
C+G%	38.3	52.8	55.4	40.3	31.6	36.8	33.1	37.2	35.2	31.9	43.1

Table 4. GC contents for Chloroplast genome of *G.hirsutum*

-		Coding	Region	-	Non	Coding	Region	-	-	-	-
-	Protein	tRNA	rRNA	Total	IGS	Intron	Total	Complete Genome	LSC	SSC	IR
Length	78531	2801	9048	90380	48798	21123	69921	160301	88862	20509	25465
Proportion	48.98	1.74	5.64	56.38	30.44	13.20	43.61	100.00	55.43	12.79	15.88
T%	31.4	22.8	22.3	30.2	34.4	31.9	33.7	31.7	33.2	33.9	28.5
A%	30.4	23.9	22.3	29.4	33.9	31.3	33.2	31.0	31.6	34.4	28.5
C%	19.3	27.1	27.7	20.3	16.0	18.9	16.9	18.8	18.1	15.1	21.5
G%	19.0	26.2	27.7	20.1	15.6	17.9	16.3	18.4	17.1	16.5	21.5
A+T%	61.8	46.7	44.5	59.6	68.4	63.2	66.8	62.8	64.8	68.3	57.0
C+G%	38.2	53.3	55.5	40.4	31.6	36.8	33.2	37.2	35.2	31.7	43.5

NC Graph

The NC graph for each four genomes was drawn and was showed with Figures 1, 2, 3 and 4, and the results for it were almost similar for all species, so describe based on one of them. Figure 1.4 shows that a significant number of points are located on the graph and to a region that is poor from GC, which it is result of a strong nucleotide composition. Most points with low NC values are below the expected graph with high distances. These results show that some genes in *G.thurberi* have a codon application independent of the nucleotide combination (created by mutagenic pressure and other factors), and the combined constraints. Wright, F. (1990) suggested that graph of NC values column to GC3s could be useful for finding the diversity of codon usage among genes. Duret, L. & Mouchiroud, D. (2000), he argued that comparing the actual distribution of genes with the expected distribution would indicate that the tendency of codon preference to be influenced by factors other than combined constraints.

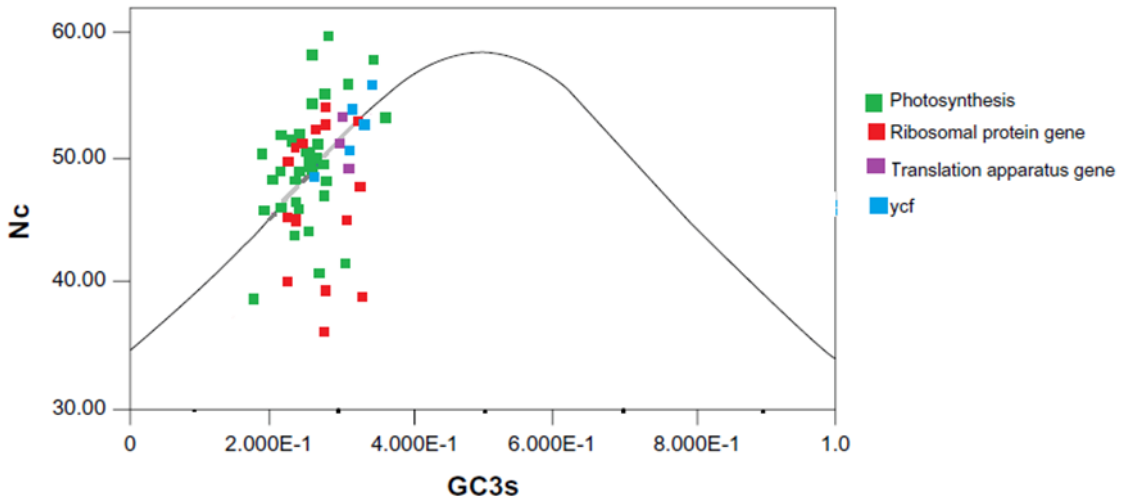

Fig. 1. NC graph of *G.thurberi*

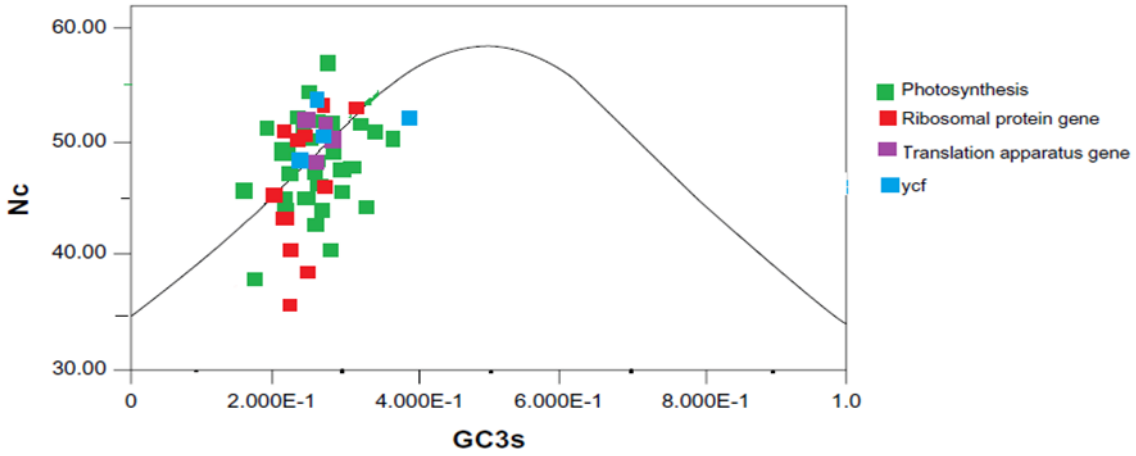

Fig. 2. NC graph of *G.arboreum*

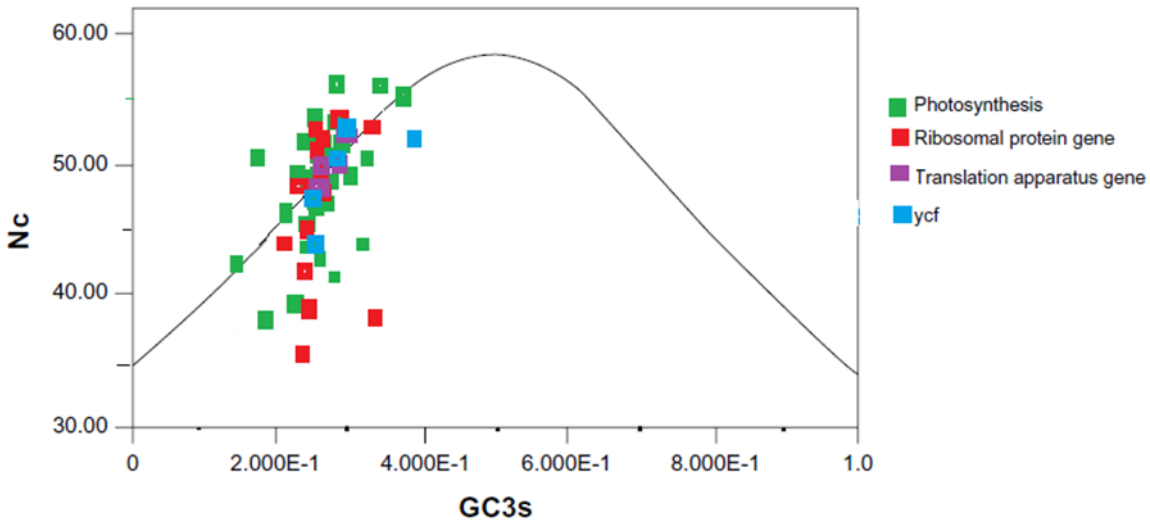

Fig. 3. NC graph of *G.barbadens*

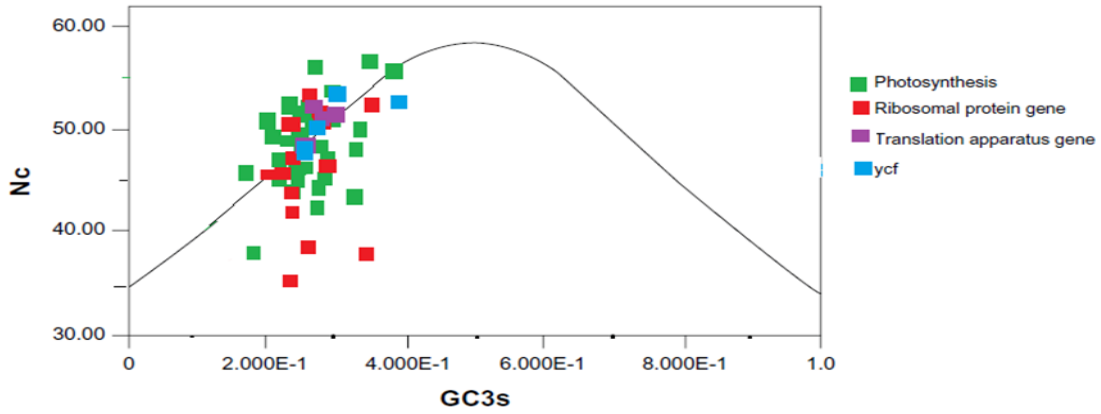

Fig. 4. NC graph of *G.hirsutum*

Comparative Analysis (COA)

Comparative analyses for species this study was conducted to calculate the factors responsible for the distribution of genes in the adaptive analysis chart (COA plot), by determining the correlation between axis4-axis1 with codon preference indices for 57 coding genes with a length of more than 100 codons. The results of analysis for all four genomes were very similar, so, to prevent from repetition of the material, explanations were made based on the table and graph of the *G.thurberi* chloroplast genome. The first axis, 10.3%, and the next three axes expresses 9%, 7.93% and 7.16% of the codon preference variations. The

results showed that the second axis had a positive correlation with C3 (r = 0.374, p <0.01), GC (r = 0.294, p <0.05) and Fop (r = 0.380, p <0.01), the third axis with G3 (r = -0.344, p <0.01) had a positive correlation, but with CAI (r=0.289,p <0.05) had a negative correlation and at the end, the fourth axis had a positive correlation with CAI (r = 0.474, p <0.01) and Fop (r = 0.299, p <0.05), the being of a significant relationship between each nucleotide axes and nucleus shows the probabilistic effect of the nucleotide combination on the formation of codon preference. Considering that in all four species, the number of two axes had a significant relationship with CAI and Fop, it was predicted that the levels of gene expression slightly affected the patterns of codon preference (poor selection), and also because Gravy was meaningful in only one of the axis in the species *G.barbadense* and *G.hirsutum*, the force chosen to affect the codon is poor.

Table 5. Correlations of codon preference indices with gene distribution in *G.thurberi*

-	ENc	CAI	Fop	$T3_s$	$C3_s$	$A3_s$	$G3_s$	$GC3_s$	GC	L sym	GRAVY
Axis1	-0.049	-0.070	0.112	-0.131	0.203	-0.050	-0.087	0.105	0.108	0.092	0.012
Axis2	-0.294*	0.223	0.380**	-0.169	0.374**	-0.085	-0.124	0.252	0.294*	-0.070	-0.261
Axis3	-0.260	0.289*	0.132	0.177	0.007	-0.137	-0.344**	-0.201	0.099	-0.095	0.214
Axis4	-0.001	0.474**	0.299*	0.164	0.227	-0.228	-0.198	0.020	0.180	0.020	0.348**

Table 6. Correlations of codon preference indices with gene distribution in *G.arboreum*

-	ENc	CAI	Fop	$T3_s$	$C3_s$	$A3_s$	$G3_s$	$GC3_s$	GC	L sym	GRAVY
Axis1	0.209	0.358**	-0.372**	-0.010	-0.252	0.112	0.268*	-0.059	-0.264*	0.060	0.141
Axis2	0.189	0.169	0.143	0.370**	-0.314**	0.139	-0.248	-0.559**	-0.28*	-0.143	-0.300
Axis3	0.033	0.526**	0.436**	0.149	0.341*	-0.057	-0.392**	-0.021	0.245	-0.013	0.368**
Axis4	0.552*	0.217	0.335	0.001	0.116	-0.057	-0.290*	-0.085	0.089	-0.161	0.039

Table 7. Correlations of codon preference indices with gene distribution in *G.barbadense*

-	ENc	CAI	Fop	$T3_s$	$C3_s$	$A3_s$	$G3_s$	$GC3_s$	GC	L_sym	GRAVY
Axis1	-0.241	-0.078	-0.252	0.036	-0.408**	0.220	0.036	-0.277*	-0.213	-0.106	0.023
Axis2	0.085	-0.457**	-0.519**	0.043	-0.426**	0.232	0.203	-0.237	-0.399**	0.014	0.109
Axis3	0.463**	-0.253	-0.109	-0.214	0.100	-0.030	0.475**	0.406**	0.046	0.220	-0.194
Axis4	0.078	0.442**	0.304	0.159	0.092	-0.067	0.277*	-0.138	0.123	-0.021	0.364*

Table 8. Correlations of codon preference indices with gene distribution in *G.hirsutum*

-	ENc	CAI	Fop	$T3_s$	$C3_s$	$A3_s$	$G3_s$	$GC3_s$	GC	L_sym	GRAVY
Axis1	-0.185	0.097	-0.033	0.009	-0.240	0.180	-0.030	-0.188	-0.052	-0.093	-0.059
Axis2	0.131	-0.212*	-0.450**	0.083	-0.458**	0.226	0.206	-0.275*	-0.379**	0.005	0.186
Axis3	-0.442**	0.430**	0.229	0.335*	-0.032	-0.084	-0.529**	-0.422**	-0.030	-0.214	0.325*
Axis4	-0.121	-0.431**	-0.377**	-0.105	-0.305*	0.190	0.256	-0.049	-0.243	-0.019	-0.288

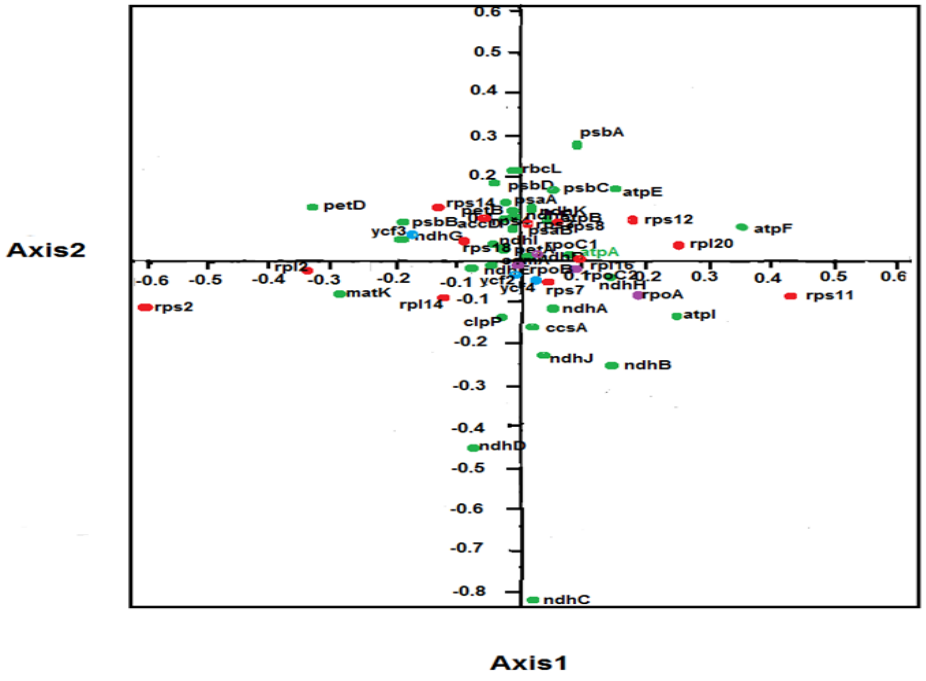

Fig. 5. COA graph of *G.thurber*

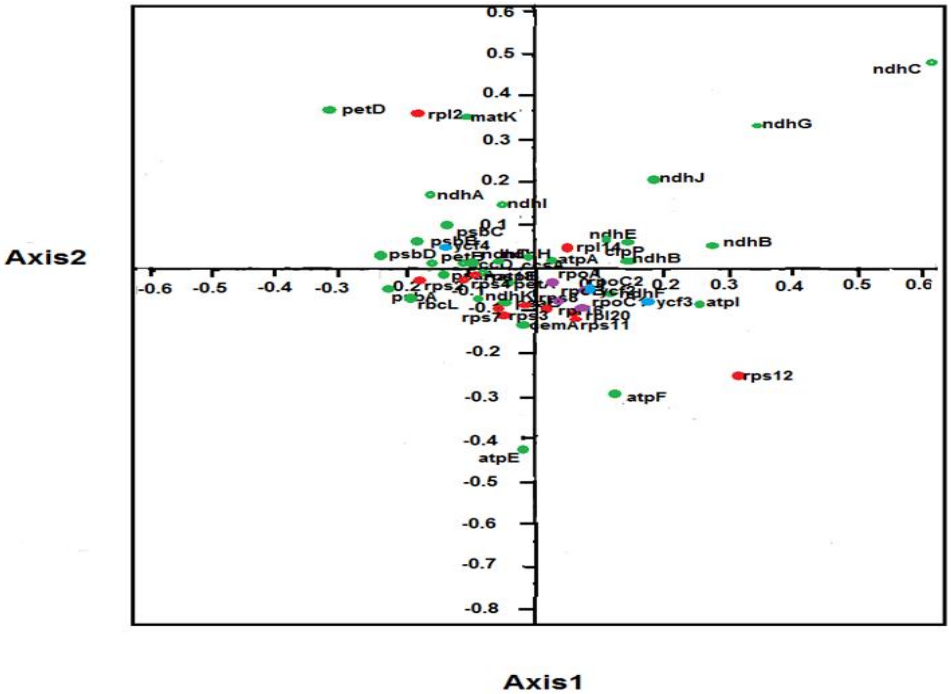

Fig. 6. COA graph of *G.arboreum*

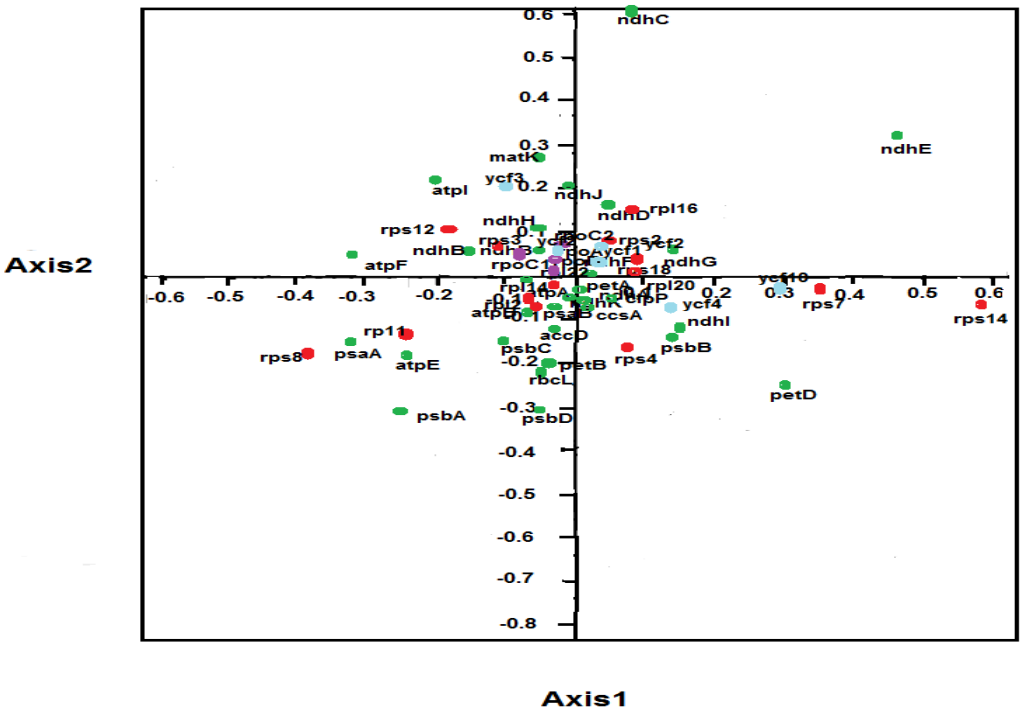

Fig. 7. COA graph of *G.barbadense*

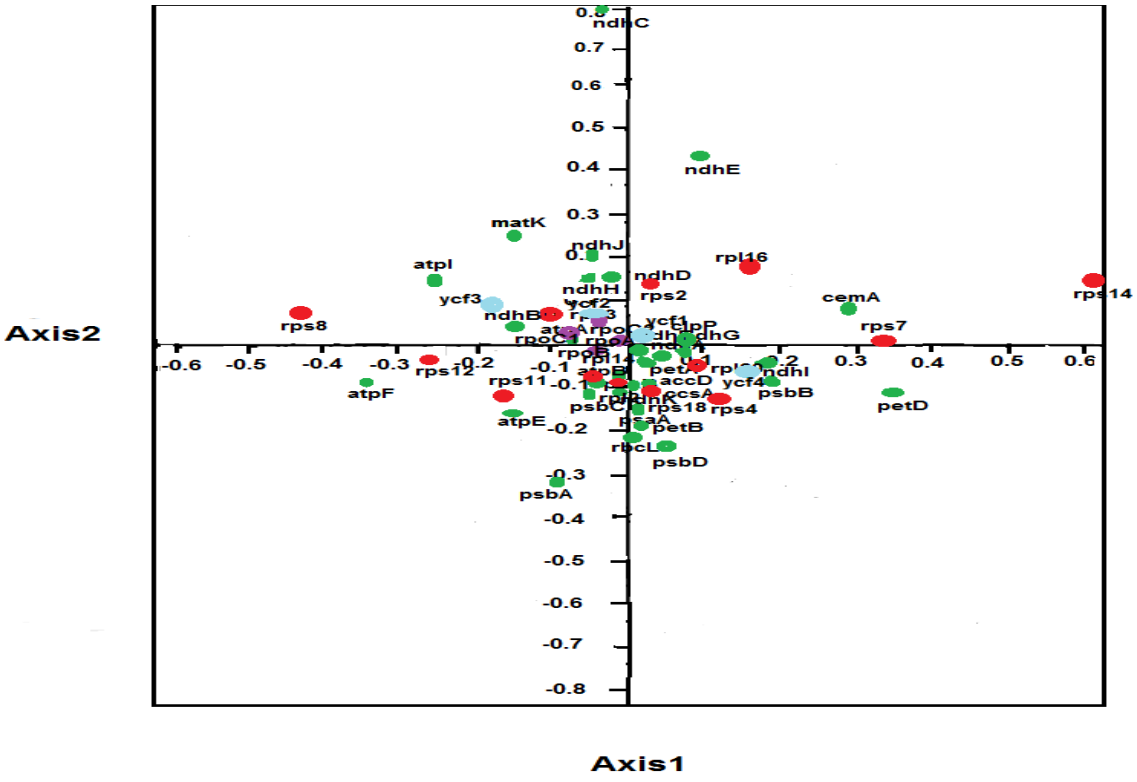

Fig. 8. COA graph of *G.hirsutum*

4. Conclusion

Investigation of codon use is vital to improve the creation of proteins in quality articulation framework. Chloroplast has unique significance because of its little size and high duplicate number of genomes. The genome of chloroplast is the most far-reaching genome in plants, and it has many highlights for advancement examinations because of the one of a kind molecular construction and single-parent inheritance. Chloroplast genomes are valuable in analysis of genetic diversity, in light of the fact that more noteworthy proficiency rather than genomic. Also, and due to the greater level of conservation, the information of the other species can be used to design specific primers for a species with unknown sequence data. This study gave bits of knowledge into the sub-molecular advancement studies.

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Cotton harvesting machine suitable for small farms

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Introduction

Near 40% of the cost of cotton production is manual harvesting. Harvesters can reduce a large percentage of this cost (Saeedi Rad et al., 2017). Difficulty of manual harvesting and lack of labor in recent years, reveal the use of cotton harvesters. Cotton harvesters reduce costs and speeds up harvesting and finally, it will eventually increase income and increase farmers' desire to cotton cultivation (Nowrouzieh et al., 2003). Today, in developed countries, cotton farming is done by machine. This has led to an increase in the area under cotton cultivation and a reduction in production costs. Unfortunately, in Iran, the degree of cotton mechanization in planting operations is about 70%, in growing and protection near 10% and in harvesting is less than 1% (Saeedi Rad et al., 2017).
Although during the golden age of cotton production in Iran, some efforts carried out to use cotton harvesters, these efforts were unsuccessful, and now, except in special cases, the cotton harvest by labors. Limited harvest time and lack of seasonal labor increase the cost of manual harvesting and decrease economic justification to produce cotton in Iran. Also, there are no suitable cotton harvesters Iran and general cotton hand harvesters are very expensive. The use of these harvesters requires large farms, which of course is small farms in Iran (majorities of cotton farmes are 3 hectares) (Rezaei Asl et al., 2013).

Cotton is harvested in October, November, and December. The cotton harvest season coincides with the grain harvest season as well as the harvest of many crops such as saffron and orange. Therefore, lack of labor and workload in this crop season creates problems such as delays in cotton harvesting (Saeedirad et al., 2017). If the cotton harvest is delayed, this product will face a decline in quality and a reduction in the purchase price. The use of cotton harvester can play an effective role in reducing harvesting costs and on the other hand, with timely harvesting, the damage caused by cold and early autumn rainfall can also be reduced (Rahimi et al., 2017). These problems have reduced the area under cotton cultivation in Iran. In this case, there is no choice to use a cotton harvester.

Nazarzadeh Oghaz et al. (2014) conducted a study to evaluate the portable cotton picker machine with the aim of evaluating the portable cotton picking and comparing it with the labor harvesting method. The results of this study showed that the uniformity of fibers and purity of cotton of portable cotton picker were 84.1% and 97.88%, respectively, which is more than 80.9% and 96.15% reported for manual method. Due to the cotton farms are mostly small; the use of large cotton harvesters in these farms is not economic justified. The

purpose of this study is to design a suitable harvester without the limitations of conventional cotton pickers such as incapability of row distance with head distance of cotton picker, the need to use defoliator, the high price of cotton harvester. This machine harvests with maximum efficiency and performance.

Material and methods

The new harvester that designed and made in this project, was a semi-mechanized self-propeller machine with three-wheels that runs on the cotton farm. It has four picking units. The picking unit is moved by labor between the cotton plants and picks up the open bolls. The picking unit consists of a number of fingers that are rotated by an electric motor and pull out seedcotton from the open bolls and transfer them to the basket. For harvesting with this machine do not need to defoliator. Also, row or non-row cultivation does not hinder the work of the harvester. Due to the type of harvest, its field losses are almost zero and it is easy to service and maintenance. The seed cotton is transferred to the basket by pull and push air mechanism. Then seed cotton is collected in large bags and put out at the end of the field. The price of this machine is quite economic justified for farmers who have about 3 hectares of cotton cultivation. This harvester works easily in small farms and transport easily from farm to farm.

This cotton harvester has three parts: picker unites, transportation and electrical. The initial design of the mechanical part of the cotton harvester machine carried out by SolidWorks 2015 software.

This harvester machine was evaluated on two cotton cultivars in Hashemabad cotton research station and the working speed and quality of harvested seedcotton were compared by manual harvesting method. The evaluation was performed in a randomized complete block design with two factors in three replications in the field. The main factor was cultivar (Khorshid and Golestan) and the secondary factor was harvest method (manual and small cotton harvester (SCH)). 4 rows of each cultivar were considered, two rows were harvested by SCH and two rows were harvested manually, and the harvest time and amount in both methods were recorded separately and then analyzed.

Results and discussion

The small cotton harvester was built after the initial design and modifications is shown in Figure 1. This harvester can work with 4 picker units. However, due to the limitations, the prototype was evaluated with two picker units.

Fig 1- 3D model of small cotton harvester created by Solidworks softwear

Field comparison of SCH with manual harvesting showed that cultivar only affects significantly on the performance (hour per hectare) of SCH, which is due to cotton plant form and different number of bolls in the studied cultivars.

The interaction of cultivar and harvest method was not significant on any of the parameters. The average field capacity of both methods in Figure 2 shows that the average material capacity of SCH is about 163.45 (g/min) and the manual harvesting is about 137.72 (g/min).

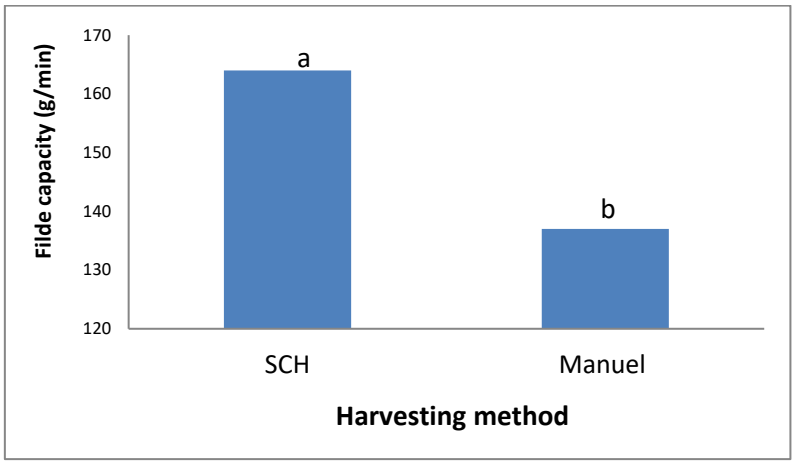

Fig 2- Field capacity comparison of SCH and manual harvesting

In the other words, the capacity of SCH was 18.7% more than the capacity of manual harvesting. Although Rahimi et al. (2014) reported that air suction machines were not able to work and do not harvest clear, this study shows, if the air suction machine was equipped with a picker unit, it increases the field capacity of the air suction machines.

In manual harvesting with two labors, it will take about 117 hours to harvest one hectare of cotton farm, but one hectare of cotton field took about 90 hours with using SCH with two picker units (Fig 3). That's mean 23% of the harvest time was reduced.

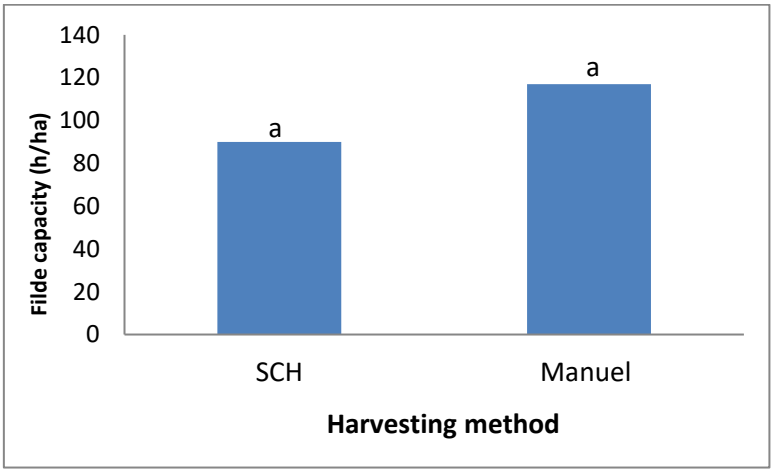

Fig 3- Speed harvesting comparison in SCH and manual harvesting

Seed cotton remaining on the plant in SCH was calculated to be 6.7% and manual method to be 6.2%. The harvester not only reduces the purity percentage, but also increases the purity by about 3%, due to the fingers of picker unite that extracted only the seed cotton. Also, the dust and fine particles in cotton harvested by SCH went out by the pull- push air system in basket. The length of fibers (UHML) that measured by HVI shown in SCH it was 28.2 (mm) and in manual harvesting method was 27.5 (mm).

Due to the simplicity and cheapness of SCH, all cotton farmers can use this machine in cotton fields. Also, SCH will surely satisfy the farmers because the cotton losses are very low. No need for defoliator, non-row harvesting capability, maintaining fiber quality and low price of SCH are the unique features of this harvesting machine.

SCH has been evaluated in different cotton cultivars and has shown that cotton cultivar has no effect on its efficiency.

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Development of high-throughput genotype independent regeneration system from meristem apex culture of Cotton (*Gossypium hirsutum* L)

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Abstract

Development of a plant regeneration protocol for revitalizing recalcitrant species like cotton (*Gossypium hirsutum*. L) from meristem apex culture with higher efficiency will put a spurt on the applications of functional genomics and selective breeding programs using transformation technologies. In this study meristem from 4-5 days old seedlings were transferred into 12 different types of shooting culture media. Based on the results, shooting medium (MS+ B5 vit+ 0.1 mgl-1 α-naphthalene acetic acid (NAA)+ 0.1 mgl-1 6-benzyl amino purine (6-BA) + 30 gl-1 glucose+3gl-1 activated charcoal+2.5gl-1 phytagel) showed the highest regeneration. There was also no significant difference between cultivar's shooting in percent. In order to induce multiple shoots, the seedlings were transferred to 10 multiplication mediums, most branching (11 per explant) was observed in combination 2 mgl-1 (TDZ) and 0.05 mgl-1 NAA. Finally, shoots were transferred into five different rooting media. Statistical analysis of the results showed that the best medium for rooting is MS + 1mg/l IBA + B5 vitamins+ 30g-1glucose+ 2.5gl-1 phytagel+ 3g activated charcoal. In this study we established a rapid, highly efficient genotype independent regeneration method using meristem apex culture commercial cotton cultivars.

Key words: Shoot apex, Cotton, Direct regeneration, α-naphthalene acetic acid, Thidiazuron

Introduction

Cotton (*Gossypium hirsutum.* L) is an economically important 'cash crop' widely used as a source of fiber and edible oil (Yan et al. 2016). Approximately 180 million people across 80 countries of world are dependant on cotton industries (Tsialtas et al. 2016). Cotton is consider to be a sensitive plant compared to other plants as its yield and quality is significantly affected by several biotic and abiotic factors, especially by insect pests and fungal pathogens (Oerke 2006). As per recent study by a group of cotton researchers, it is estimated that more than half of the world's consumption of pesticides is used to control pests of cotton (Juturu et al. 2015a). To develop the genetically improved varieties of cotton with inherent resistance against pest is a need of time to reduce the use of pesticides and herbicides. A limited success has been achieved in this direction via conventional breeding because of two main limitations; time consumption, and dependency on cotton crossable gene pool (Juturu et al. 2015a). To solve these constraints, genetic manipulation is considered to be the most suitable method to overcome this limitation in plant breeding. But a genotype dependent response to tissue culture limits

the application of genetic engineering for developing cotton varieties with better agronomic characteristics.

In the past decade only few successful attempts were made by scientists to develop regeneration methods in Coker, Acala and some Chinese cultivars like Zhongmiansuo-35 and YZ-1, (Jin et al. 2006); standardization of regeneration for commercial cultivars is recently started (Juturu et al. 2015b). The first study on cotton somatic embryogenesis was observed in *Gossypium koltzchianum*, although, there was production of small embryos, roots, and leaves structure, there was no plantlet regeneration (Price and Smith 1979). However, the performance of regeneration of somatic embryos has improved in recent years (Khan et al. 2010), but still there are a lot of problem. The limited success was observed with more responsive Coker lines, most of them are no more in cultivation in present agriculture system. (Aydin et al. 2004). Nevertheless, the regeneration of commercial varieties is very limited and with numerous somatic variations. Success in cotton tissue cultures is dependent on many factors such as type of growth regulators (Michel et al. 2008), composition of the culture medium (Kumar et al. 2015), subculture timing , culture conditions, temperature, number of callus per petri plate, genotype, and explant/donor plant (Juturu et al. 2015a). Among all, genotype plays a crucial role in deciding success of regeneration. The major factor, which influenced SE in cotton has always been the genotype. In fact, all other factors which exert influence on SE would always depend on the genotype used in the study (Michel et al. 2008). Thus, developing genotype independent regeneration protocol in cotton is inevitable to develop transgenic cotton cultivar. The aim of this study was to develop an efficient protocol for genotype independent direct regeneration ensuring lower somaclonal variation and faster regeneration as callus phase is eluded. Our study will thus aid in the faster development of improved cotton plants through gene transformation.

Material and method:

Plant material, sterilization and seed culture

The Iranian cotton (*Gossypium hirsutum.* L) cultivars including Sahel, Varamin, Khorshid, Golestan, Latif, Kashmar and Armaghan were acquired from the Gorgan Cotton Research Institute. Seeds were delinted by using sulfuric acid 96% followed by leaching for 40 minutes. To disinfect, at first seeds were washed with 70% alcohol for a minute and then in a solution of 20% NaClO for 40 minutes. After sterilization, seeds were washed with sterile water 3-5 times, following which four seeds were placed in tubes containing ½ (MS) in the dark with temperature 28±2^{o}C for germination.

Shoot apex isolation and regeneration medium

Isolation of shoot apices was performed using the Ulian protocol (Ulian et al. 1988). Meristems from 3 to 5 days-old seedlings were isolated with the aid of a dissecting microscope. After cutting the cotyledon leaves, two euphyllia were removed using narrow needles and the meristem between the two leaves were isolated. Isolated shoot apices were placed in different regeneration medium (table 1), base of all medium was MS, B5 vitamins, 30g l^{-1} glucose, 3 gl^{-1} charcoal and 2.5gl^{-1} phyta gel. Cultured shoots were then transferred to culture room at 28±1^{o}C, 16 hours photoperiod with fluorescent illumination at 3000 lux and 8 hours of darkness. Shoot growth and development (2-3mm) was observed after 24 hrs.

Table 1. Effect of different phytohormone BAP and NAA on regeneration of shoot apex

BAP (mg/l)	NAA (mg/l)	Days to regeneration	Regeneration %	Shoot length	Number of normal shoot
0	0	20 ^{e}	45.33^{b}	1.02 ± 0.06	74
0	0.1	10 ^{b}	8.43^{e}	1.15 ± 0.31	20
0	0.2	18 ^{e}	8.33^{e}	1.26 ± 1.02	20
0.1	0	10 ^{b}	10.69^{e}	1.83 ± 0.68	40
0.1	0.1	8 ^{a}	99.88^{a}	2.84 ± 1.29	100
0.1	0.2	12 ^{bc}	45.23^{b}	0.84 ± 0.13	75
0.5	0	11 ^{b}	10.36^{e}	0.76 ± 2.42	11
0.5	0.1	13 ^{c}	33.69^{c}	0.87 ± 0.42	12
0.5	0.2	11 ^{b}	38.28^{b}	0.82 ± 1.06	9
1	0	16 ^{d}	22.71^{d}	0.54 ± 1.06	8
1	0.1	11 ^{b}	28.60^{d}	0.42 ± 1.28	8
1	0.2	12 ^{bc}	36.38 ^{b}	0.71 ± 0.30	3

In each column the same letter do not differ at significance level $P < 0.01$ (Duncan's multiple range test)

Multiple shooting

Well-developed regenerated plants were transferred to 12 different types (table 2) of multiple shoot induction media after 2 weeks for inducing multiple shoots. After obtaining the multiple shoots, the shoot branches were separated into individual shoots and transferred to elongation medium (1.0 mg/l BAP and 2.0 mg/l GA3).

Table 2. The effect of TDZ and NAA phytohormones in the induction of multiple shoot induction

TDZ mg/l)	NAA (mg/l)	Mean number of shoot (branching)	Shoot length	Explant response
0	0	00.0	00.0 ± 0.0^{a}	00
0	0.05	00.0^{d}	00.0 ± 0.0^{a}	00
0	0.1	00.0^{d}	00.0 ± 0.0^{a}	00
1	0	2 ± 0.5^{e}	3.7 ± 0.7^{c}	67.5
1	0.05	3.2±1.7^{e}	4.5 ± 0.9^{d}	66.5
1	0.1	3.0 ± 0.7^{e}	2.1 ± 0.6^{b}	60.1
2	0	4.5 ± 1.1^{e}	3.7 ± 0.7^{c}	67.5
2	0.05	11.2±1.7^{ab}	2.2 ± 1.0^{b}	92.5
2	0.1	6.1 ± 0.9^{c}	2.1 ± 0.8^{b}	58.4
3	0	12.7 ± 0.8^{a}	1.5 ± 0.7^{d}	75.4
3	0.05	13.0 ± 1.3^{a}	1.5 ± 1.1^{d}	82.7
3	0.1	12.6 ± 1.4^{ab}	3.7 ± 0.7^{c}	67.2

Rooting

The well developed and elongated shoots were transferred to 5 different combinations of MS rooting medium. Upon successful rooting, proper rooted plants were transferred to pot containing 1:1:1 vermiculite, peat moss and sand as a nurse medium and after hardening, the plants were transferred to bigger pots in green house condition.

Measurements and Statistical analysis

Cultures were observed on a visual basis and regeneration percentage was calculated as the percentage of regenerating explants with a minimum of 1 shoot and 2 roots. The number of days required for plants to reach shooting, the percentage of regeneration, the percentage of normal seedlings, seedling length, number of lateral branches, root length and the percentage of seedlings that showed rooting were evaluated. Test of significance were carried out by analysis of variance (ANOVA) and significant differences among treatments means were compared by Duncan test using the SPSS (17.0) software. Means are the result of three replicates. All the treatments were carried out nine times with three replicates each using a CRD.

Results:

Optimizing an efficient genotype independent protocol for plant regeneration is an essential technique which helps in faster research in tissue culture regeneration and plant genetic engineering. Although numerous reports of somatic embryogenesis of various explants (hypocotyl, cotyledon and meristem) of Coker cultivars cotton is well documented, there are very few reports of regeneration in commercial cultivars. In present study, we developed a genotype independent and highly efficient method for regeneration of commercial Iranian cotton varieties.

Effect of BAP and NAA on shoot regeneration

_ENREF_26 _ENREF_14Meristem of 4-5 days old seedlings were isolated under sterile conditions. Isolated shoot apices were placed vertically on different regeneration media (Fig. 1A,Table 1). In most media, shoot apex swelled and formed leaf-like structures in the center of the meristem after two weeks (Fig. 1B). Results indicated that among the tested hormones combination, (0.1 mg^{-1} BAP + 0.1 mg^{-1} NAA) proved to be most successful for achieving 100% regeneration rate and maximum number of healthy seedlings (Fig. 1C,Table 1). Higher concentration of BAP and NAA resulted in decreased days to regeneration but increased the number of deformed structures (Table 1). Using BAP alone at high concentrations (1 mg^{-1}) caused vitrification, while at lower concentrations (0.1mg^{-1}) regeneration rate was limiting with only 10% success. Similarly, the use of NAA alone in low concentrations (0.1 mg^{-1}) led to reduced regeneration rate i.e. 8 %. Although, there were several reports that high concentrations of NAA increased the efficiency of regeneration (Rauf et al. 2019), but this trend was not observed in our study. Combination of NAA and BAP at low concentrations resulted in the highest possible regeneration and yielded the greatest number of normal healthy seedling (i.e. cent percent). Hormones and hormonal composition also had a significant effect on length of shoot; in (0.1 mg^{-1} BAP + 0.1 mg^{-1} NAA) longest shoots were obtained.

Effect of TDZ on shoot multiplication

Cytokines, especially TDZ has crucial role in the multiple shoot induction (Khawar et al. 2004). To evaluate the effect of different concentration of phytohormones on frequency of shoot formation we used 12 combinations of TDZ and NAA (Table 2). The highest proportion of explants forming adventitious shoots (92.5%) with an average of 11 branches per shoot was obtained with media containing 2mg^{-1} TDZ and 0.05mg^{-1} NAA (Fig. 1D). In higher concentration of TDZ (3mg^{-1}), emerging shoots were forked. Adding TDZ alone at low concentrations (1mg^{-1}) to the medium has led to low branch per explants (67.5%) with an average of 2 branches per shoot (Table 2). Different varieties showed similar multiple shooting patterns in all hormonal composition and hence the effect of varieties on multiple shooting is non-significant. After 3 weeks on multiple

shoot induction medium, individual shoots were separated from each other (Fig 1E, 1F) and transferred to elongation medium (1.0 mg/l BAP and 2.0 mg/l GA3) (Fig 1G) and grown for two weeks. After that seedlings were transferred to five different rooting medium containing different concentrations of IBA (Fig 2A).

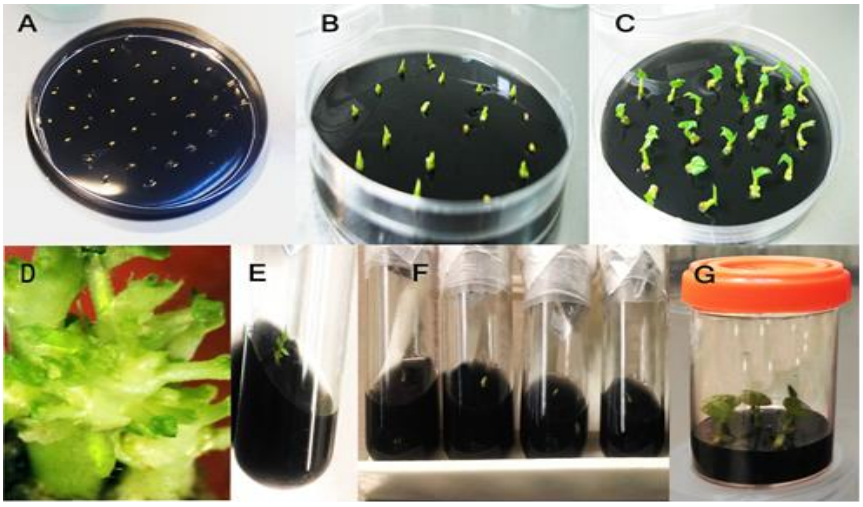

Fig 1. Cotton (Gossypium hirsutum L.) plant regeneration by organogenesis from embryo apex explant. (A) Shoot apex explants from 3-4-d-old seedlings on regeneration medium. (B) Two week later, shoot apex explant turned into green and there are leaves structure. (C) four week later, shoot apex explant turned plantlet. (D) Multiple shoot buds induction media, adventitious shoot buds and leafy structures arising from the central region and sides. (E–G) Multiple shoot excision and transfer to elongation media.

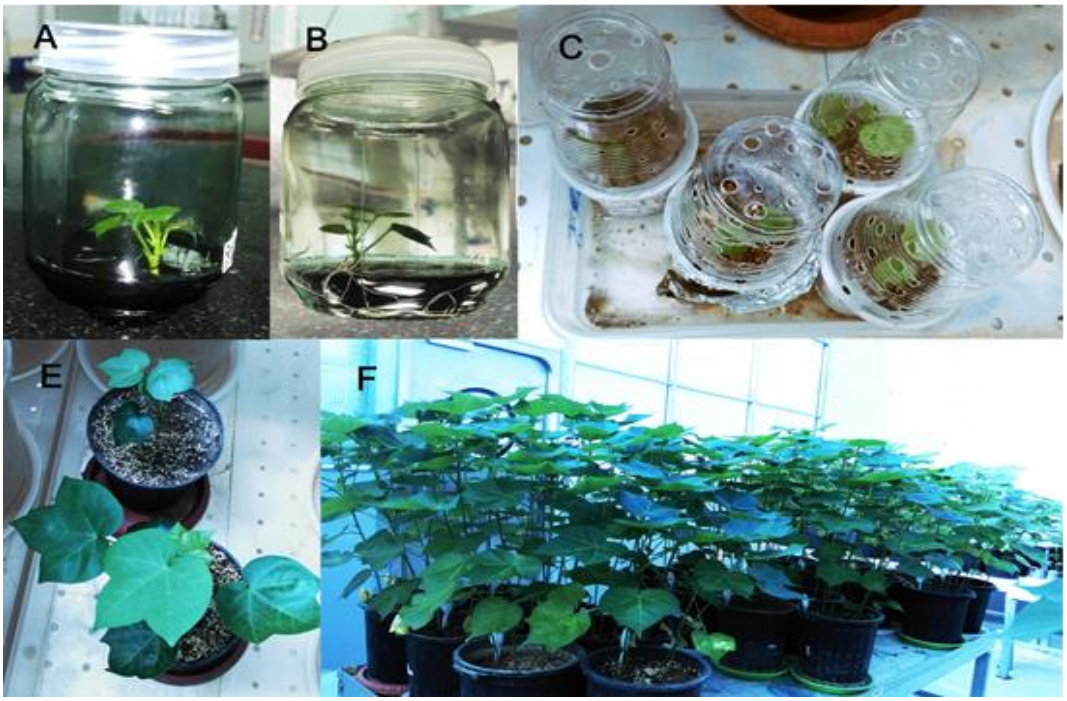

Figure 2. Root development of elongated plantlets and acclimatization. (A-B) Rooting media (C) hardening plastic cup. (E-F) Acclimatization in greenhouse.

Rooting and hardening

The effect of auxin on the rooting is shown in (Table 3). A significant level of rooting was observed in MS, ½MS and MS+1mg/l IBA (Fig 2B). Rooting in these mediums was similar and it was up to 85%. Rooting usually started 10-15 days after transplanting the seedlings to the rooting medium. The highest root length observed was 7.1 cm (fig. 4) in mediums containing MS+1mg/l IBA combination regime. After rooting the plants were transferred to pots containing equal amounts of sand, peat moss and vermiculite (Fig 2C). Each pot was covered by a plastic cup to keep high humidity for 10 days. For maintaining humidity and hardening of plants, an additional hole was made in the cup every alternate day. After 14 days, we removed the cup and plants were transferred to a bigger pot in green house condition (Fig 2D, 2E).

Table 3. Auxin effect on root induction and growth of roots

Treatment	Medium	IBA (mg/l)	Rooting percentage	Root length (cm)
1	1/2MS	0.0	90 [a]	5.1±1.2[a]
2	MS	0.0	90 [a]	5.3± 1.5[a]
3	MS	1.0	95 [a]	0.7[b]±7.1
4	MS	1.5	63 [b]	1.3[c]±3.5
5	MS	2.0	40 [c]	0.5[d]±1

In each column the same letter do not differ at significance level P < 0.01 (Duncan's multiple range test)

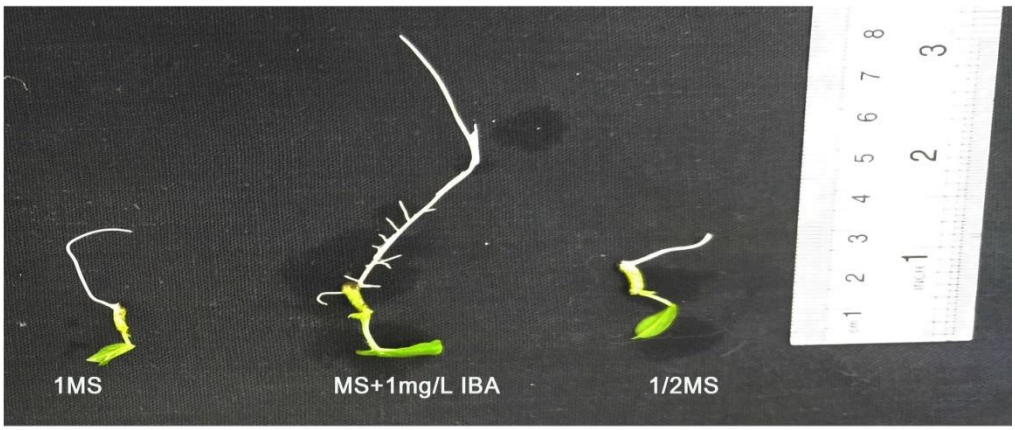

Figure 4. Comparison of the effect of different rooting media on root length.

Discussion:

Incredible progress has been achieved in the development of transgenic cotton through Agrobacterium and direct gene delivery methods since the past two and a half decades Callus induction phase of SE can lead to chromosomal anomalies like aneuploidy and other somaclonal variation. To overcome painstaking SE process in cotton, several studies have been done, the first successful direct regeneration via shoot apex was reported by Gould and Magallanes-Cedeno (1998a). Since then, numerous researches have been reported. After all, the problem of long culture periods and genotype dependence can certainly be overcome by employing meristematic zones such shoot apex and embryonic axis explants. This study provides an efficient protocol for direct regeneration wherein, by the elimination of the callus phase, the time taken for regeneration as well as somaclonal variation was reduced. Medium containing

0.1 mg l^{-1} BAP and 0.1 mg l^{-1} NAA was confirmed as an optimum media for 100% regeneration. Moreover, compared to Zapata et al. (1999), Gould and Magallanes-Cedeno (1998b), the regeneration efficiency was more (100 percent) and it accumulated within a short period (14 days). This medium also produced fewer numbers of vitrified seedlings. This hormone combination has already been reported by (Satyavathi et al. 2002) but in that study, they could obtain only 1 shoot per explant. However, in our study, by sub-culturing the shoot to micropropagation media, we obtained 11 healthy shoots per explant. Using 3 mg/l BAP by Morre et al. (1998) resulted in the formation of 3.4 shoots per embryonic axis in the cotton cultivar Guazuncho. Here, by using different concentrations of TDZ and NAA we developed a method for the micropropagation of meristematic shoots. The combination of TDZ with low auxin concentrations caused branching in the apical meristem explants. Use of TDZ along with low concentrations NAA gave higher number of shoot primordial formation. This result is in harmony with the results reported by (Ouma et al. 2004) and (Divya et al. 2008). Adding TDZ alone at low concentrations ($1mg^{-1}$) to the medium led to lower number of branches per explant, this phenomenon was also reported in *Portulaca grandiflora* (Safdari and Kazemitabar 2010), and *Holarrhena antidysenterica* (Neves et al. 2001) TDZ at higher (3 mg/l) concentration caused bad form and immature structure, higher concentration of TDZ seems to be toxic, this result was also reported by Kehie et al. (2012) in *Capsicum chinense* (Table 2). Effect of TDZ on shoot induction and regeneration depends on the level of internal hormones and this growth regulator increases the level of auxin in the plant (Hutchinson and Saxena, 1996). Using NAA alone at all concentrations has no impact on branching. Proliferation is more influenced by cytokines than auxins, so NAA alone had no effect on proliferation (Band et al., 2013). TDZ and NAA in low concentration produces maximum healthy shoots per explant (11) cytokinin is known to confer morphogenic competence for initiation of shoot proliferation. However, the type and concentration of cytokinin influenced the regeneration frequency, average number of shoots produced per explant, and mean length of the shoots. However, use of TDZ restricted the shoot elongation (Dey et al. 2012), but by sub-culturing the shoots on elongation medium this negative effect of TDZ has been solved. In this study unlike other regeneration, interaction between medium and cultivar was not significant. Large-scale production in a short-term period (approximately 60 days) and the lack or the low unwanted genetic changes are major achievements of this study. Such benefits are useful in the transgenic plant projects involving recalcitrant crops such as cotton. Usually in cotton transgenic studies, researchers transfer the desirable gene to Coker's lines and after achieving the transgenic plants, cross them to commercial cultivars, but, in this protocol, by providing direct, genotype independent method the need for the painstaking process of backcrossing is eliminated. To inhibit phenolic compounds and increase the availability of hormones all mediums used in this experiment were supplemented by activated charcoal (AC) (Pan and Staden 1998). In our study AC in rooting medium reduces the days to rooting. This result is also in line with Suranthran et al. (2011) in *Bacopa monnier*. Adding AC in regeneration and multiplication media could have synergistic effect with hormonal regime for increased regeneration efficiency.

Conclusion

Regeneration in cotton is highly genotype dependent so it is necessary to optimize the protocol which can be universally useful for all the genotypes. This study establishes an efficient and rapid regeneration protocol of *Gossypium*

hirsutum using shoot tip explants. Using the protocol developed in this study, one can achieve *cent percent* of plant regeneration with higher numbers of healthy plant on MS medium containing BAP (0.1 mg l^{-1}) and NAA (0.1 mg l^{-1}). A combination of TDZ (2mgl^{-1}) and NAA (0.01 mg l^{-1}) proved to be superior for multiple shoots. Therefore, the present investigation gives a quick and reliable *in vitro* regeneration protocol in cotton, which can be efficiently utilized for genetic improvement using various genetic engineering techniques.

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INTEGRATED PEST MANAGEMENT PROGRAM- A STEP TOWARDS REVIVAL OF COTTON

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Cotton is "White Gold", an important cash crop and the lifeline of Pakistan's textile industry. Its products account for 55 percent of all foreign exchange earnings in Pakistan. Nearly 26% of growers cultivate cotton, and more than 15 percent of the total cultivated area is devoted to this crop. Approximately, 65 percent of Pakistan's cotton is grown in the province of Punjab, while 35 percent in Sindh, with a negligible area under cotton crop, has been recorded in the Balochistan and Khyber Pakhtunkhwa provinces. Cotton production accounts for 4.5 percent of the value-added in AgGDP and 0.8 percent of GDP. It serves as the raw material for the textile industry, the country's largest agro-industrial sector, employs 17 percent, earns 60 percent of foreign exchange, and contributes 8.5 percent to GDP. Despite its importance, cotton productivity in Pakistan has been underwhelming. Pakistan now ranks 4th in terms of area and ranks 39th in cotton productivity per hectare. (Abdul Wajid Rana, et al., 2020). The cotton area, production and average yield per acre of last three years shown in Table 1.

Table 1: Cotton area, production and yield of South Punjab, during 2019-20, 2020-21 and 2021-22.

Indicators	2019-2020	2020-2021	2021-2022
Cotton area sown (Million acres)	4.199	3.579	2.976
Cotton production (Million bales)	5.714	4.662	4.870
Average Yield (Maunds/acre)	17.5	15.63	19.64

Source: Crop Reporting Services 2021-22

It is clear that the area, production, and average yield decreased significantly during the years 2019-20 and 2020-21 (Table 1). But during 2021-22, although the area under cotton decreased, the production, and yield per acre was increased (CRS-2021-22) significantly. There are so many factors of cotton decline in Pakistan i.e. non-availability of quality seed, low price, climate change (Anonymous, 2015), lack of tolerant varieties, high cost of production, low

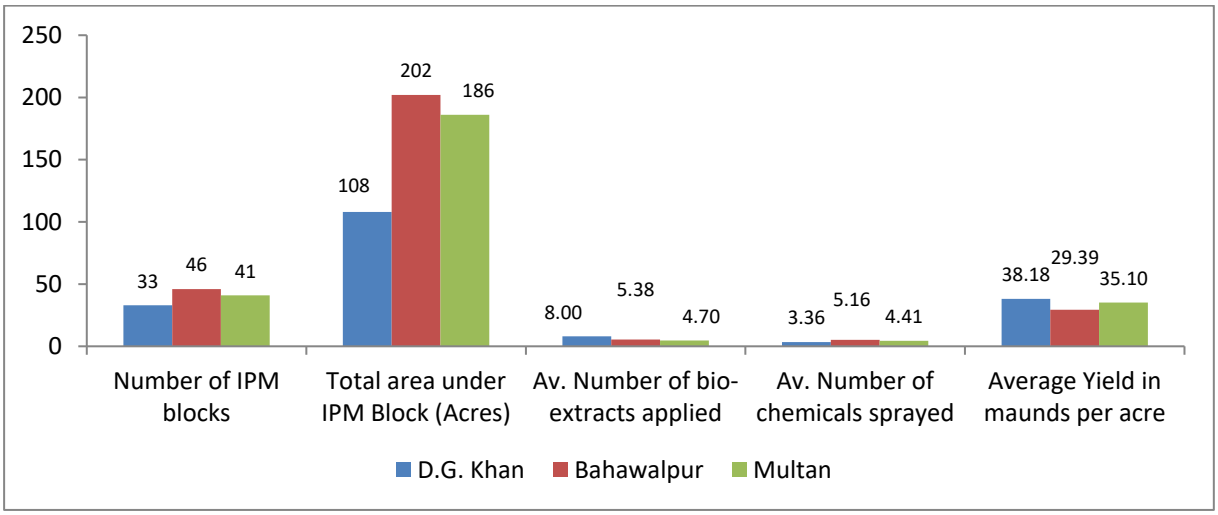
return, heavy attack of insect pests, and lack of proper pesticides etc. After the establishment of South Punjab Agri. Secretariat, it was challenge, for the newly established agri. department to revive the cotton with the consultation of stakeholders. The following step were taken for the revival of cotton

- Preparation of cotton calendar and its implementation
- Selection of Integrated Pest Management (IPM) plots
- Issuance of advisory services
- Creation of South Punjab Ext. and PWQC whatsApp group (for daily update of cotton situation) and
- Regular field visits of Secretariat staff

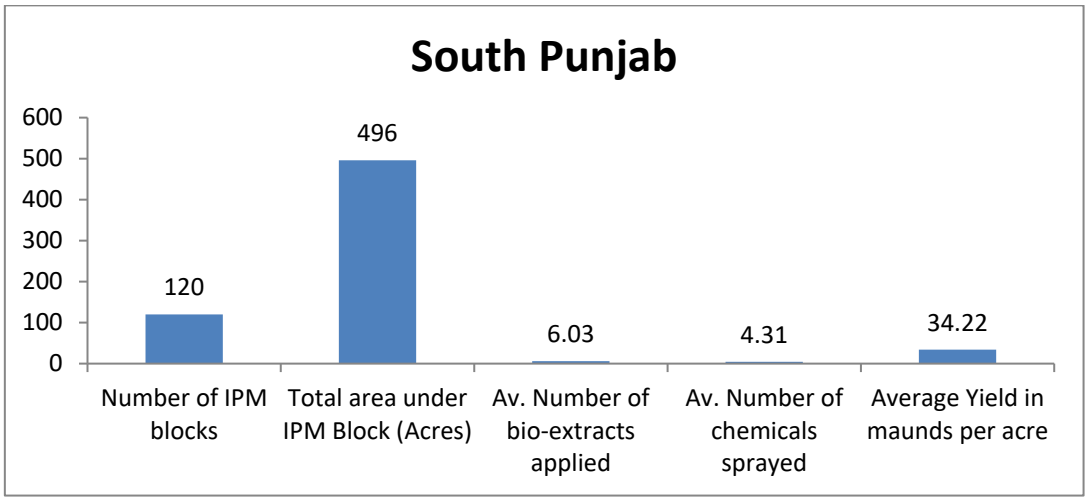
After the selection of IPM plots in South Punjab and then execution of IPM practices on these plots was at top priority. The IPM involves using all existing possible techniques for the management of pests populations with the aim of reducing the chemicals use while maintaining profitability, yield and fibre quality. It is a practice for improving the quality of the environment and the quality of an IPM programme depends on how environmental friendly it is.

The IPM is not a new concept, but the practical execution was made during the cropping season of 2021-22. Total 120 IPM plots were maintained by field formation (Extension and Pest warning and Quality Control of Pesticides) with the willingness of cotton growers. These plots were monitored by the South Punjab Agriculture Secretariat, Officers regularly. On these plots different IPM techniques were applied to overcome the insect pests like botanicals extracts, installation of bio-cards (parasites and predators), pheromones traps, yellow sticky traps and chemicals etc. The chemicals were applied only when the insect pest population reached above the ETL. The advisories were prepared and issued for IPM blocks and cotton growers. Initially, the use of chemicals was delayed for the period of 90 days. During first three months, natural fauna played an important role in the suppression of pests. The growers welcomed advisories messages, in view of the last many year's experience of chemicals application. The messages were disseminated to the cotton growers through farmers gathering, field days, mass sweeping campaign, electronic media as well as individual contact with the growers. The overall impact of these activities resulted in average yield i.e. 34.22 maunds per acre (Saqib Ali Ateel, 2021) of these IPM plots. DAWN, 2 Jan.2022 article, reported that 57% reduction in pesticides was noticed on cotton farms during 2021-22 in Southern Punjab. Such type of IPM practices were highly appreciated by the cotton growers.

IPM Blocks: Among 120 IPM blocks, 33 were maintained in D.G.Khan, 46 in Bahawalpur and 41 in Multan Division, with an area of 108, 202 and 186 acres, respectivelly. A total of number of bio extracts i.e., 8.00, 5.38 and 4.70 were sprayed while 3.36, 5.16 and 4.41, number of chemicals were sprayed during the cropping season of 2021-22. Average yield in maunds per acre was 38.18, 29.39 and 35.10 as shown in graph below.



South Punjab IPM Blocks: In South Punjab, total area was 496 acres under IPM blocks. The average number of bio extracts and chemicals applied were 6.03 and 4.31, respectively. The yield remained 34.22 maunds per acre. The data of IPM blocks was compiled and graphically shown below.



IPM practices:

Bio-cards: Cards of 1x3 inch having eggs of Chrysoperla 16-60 per card and card of 2x3 inch for Trichogramma having 200-250 eggs per card. These cards @ 20 per acre were hanged in the cotton field fortnightly. These effectively control the sucking pests as well as bollworms.

Trichogramma eggs & parasite Chrysoperla eggs card Predators

Botanical Extracts: Kortumma, Neem, Akk and Tobacco @ 600 g dissolved in 100 liter water was used when the population of sucking insect pest recorded above ETL.


Kurtumma


Neem


Akk


Tobacco

Why do we need to develop IPM programs?

Cotton crop in Pakistan is heavily infested by Whitefly (*Bemesia tabaci*), Jassid (*Amrasca devastans*), Thrips (*Thrips tabaci*), Mealy bug (*Phencoccus solenapsis*) and Pink bollworm (*Pectinophora gossypiella*) which are considered as major insect pests. For the management of these pests, cotton growers depend upon the use of synthetic chemicals from growing up to harvesting of the crop. But due to over reliance on chemicals, many problems were appeared including resistance in insect pests, disruption of parasites and predators, outbreak of secondary pests and ecological consequences. So IPM program is considered safe for such type of consequences.

Jassid: It is also known as "leaf hopper". Both the young ones and adults suck the sap from leaves. In case of heavy attack, the leaves become red known as "hopper burn" that impair photosynthetic activity of plants. The 1st and 2nd instars of jassid feed near bases of the leaf veins, later instars get dispersed all over the leaves but feed chiefly on the under surface of leaves. The affected leaves curl downward positions. Severe incidence can lead to the stunting of a cotton plant. The fruiting capacity of the infested plants is affected badly and, in many cases, high infestation can cause the early mortality of cotton plants and leads to reduced yields.

Components of IPM:

- Sowing of tolerant varieties
- Sowing of cotton North-South Directions
- Thinning
- Judicious use of nitrogenous fertilizer
- Flonicamid @ 60 g per 100 liter of water was used (It was noted that less efficacy of bio-extracts against Jassid)


Jassid

Thrips: It is also major sucking pest of cotton crop. Both nymphs and adults suck the cell sap from the lower surfaces of leaves by lacerating the leaves tissue. Thrips also inject saliva during sucking the sap resulted in silvery appearance. Seedlings infested with thrips grow very slow and the leaves become wrinkled, curl upwards and distorted with white shiny patches. During the fruiting phase there is premature dropping of squares, and the crop maturity is delayed that ultimately leads to reduction in seed cotton yield.

Components of IPM:

- Tolerant varieties
- Sowing of cotton North-South Directions
- Thinning
- Breaking of ridge sown cotton beds

- Judicious use of nitrogenous fertilizer
- Chlorfenapyr @ 100ml per 100 liter of water was used (less efficacy was reported against thrips of bio-extracts)

Thrips

Pirate bugs

Cotton Mealy bug:

The cotton mealy bug is a small sap-sucking insect, causes severe economic damage to cotton and other field crops including vegetables and horticultural plants. The young ones are tiny, small and fast-moving crawlers which fix themselves on plants parts. After fixing, mealy powder appears on the insects with the passage of time. In case of severe damage, the affected plant parts completely dried. Mealy bug attacks on almost all organs of cotton plant including main stem, branches and fruit, underdeveloped flowers, bolls of smaller size. The boll opening affected badly resulted in the reduction of yield. Excretion of honeydew by mealy bug attracts ants and also contributes to the development of black sooty mould on leaves as well as on lint. Infested cotton plant shows the symptoms like white fluffy mass on underside of leaves, near growing tips, along leaf veins and on stem, distorted or bushy shoots.

Components of IPM:

- Uprooted the infested parts or plants by covering them with polythene sheet
- Removed alternate host plants
- Encouraged the natural enemies
- Spot spray profenofos @ 70 ml + bleach 200 ml per 20 liter of water was done.

Cotton mealy bug female

Cotton mealy bug male

Predator, Parasite

Pink Bollworm (PBW): The PBW is one of the most destructive pest of cotton known as hidden pest. The young ones of PBW attack the buds and bolls after emerging from the eggs within 30 minutes. The opened flowers look like rosette type in appearance. The larvae feed inside the flowers on pollen grains. After 12-15 days, the larvae along with petals fall on the ground and pupate. The adults emerged after seven days. After mating female select squares, bolls and branches for egg lying. The second generation of larvae enters in 10-12 days old bolls and feed on the developing seeds. Stained lint around feeding areas will be seen in opened bolls. Improper boll opening with damaged seeds are obvious. Small round holes are seen on the septa between locules of open bolls. The infested lint is of inferior quality and known as “Yellow Spot”. PBW spend six months in double seed in left over bolls, until next cropping season. The number of generations of PBW produced in one year are four.

IPM: Plucking of rosette flowers: The rosette flowers were plucked regularly and buried in the soil during pest scouting.

Pheromones traps: Eight pheromones traps per acre were hanged for the monitoring & control of Pink Bollworm.

GRAZING: After the termination of cotton, the left over bolls were grazed by sheeps and goats or by cattles. This practice proved helpful for the management of PBW in next cropping season.

PBW

Pheromones trap

Grazing of Sheep & Goat

Rotavation of cotton ticks

Rosette flower

Alternate host plant: In Pakistan, Okra (lady finger) is recorded as second most important host plant of PBW after cotton (Karar *et al*. 2021). Thus sowing of "okra" was prohibited near cotton field.

Chemical & Botanical Spray:

- Mixture of Tobacco @ 600g+ Gamma-cyhalothrin @ 100ml or
- Tobacco @ 600g+Neem @ 600g+ Gamma-cyhalothrin @ 100ml was sprayed for the control of PBW (Karar, *et al.*, 2021).

Whitefly: This insect has become one of the major pests of cotton. It causes damage to cotton plants in two ways i.e. by sucking the cell sap and by excreting honey dew on which sooty mould grows which affects the photosynthetic activity badly and reduces the yield. Whereas, indirect damage is the lint contamination with honeydew and associated fungi occur during heavy infestations after the boll opening. Excessive/regular usage of pyrethroids against PBW plays an impotent role in the outbreak of whitefly.

Components of IPM:

- Tolerant varieties
- Sowing of cotton North-South Directions
- Thinning
- Judicious use of nitrogenous fertilizer or application of CAN
- Delayed chemical sprays (avoid use of pyrethroids)
- Spray of botanical extracts including Kurtumma, Neem, Akk

and Tobacco @ 600 g per 100 liter of water were sprayed against whitefly and their young ones when whitefly population above ETL

- Yellow sticky traps**:** Eight yellow sticky traps per acre of size 7x11.5 inch treated with German glu were hanged for the control of Adult whitefly.

Whitefly

Yellow sticky traps

Parasite of whitefly

IPM and its role in crop production: IPM has evolved as an economical, environmental and eco-friendly approach to manage insects, diseases, physiological disorders, weeds and rodents that cause economic yield loss and limit the agriculture production (IPM Package for cotton, 2014).

- IPM aims at reducing farmer risks from pesticide poisoning and consumer risks from residues in food chain at community level, low production costs and greater yield savings at farm level, and increased biodiversity especially of productive biota and improved quality of natural resources such as soil and water quality at agricultural ecosystem level.
- IPM aims to reduce pest populations below ETL.
- IPM utilizes the various methods of pest suppression in a compatible manner towards sustainable crop production and eco-friendly environment.

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Improvement in Cotton through Pollen Irradiation and hybridization Technique and its Economic Impact in Pakistan.

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Abstract

The cotton breeding program through seed and pollen irradiation and hybridization technique at NIAB wasinitiated in 1970 and has resulted in the evolution of nineteen varieties with high yield potential, wider adaptability along with desirable fibre quality parameters. Among these five varieties namely; NIAB-846, NIAB-777, NIAB-852, NIAB-IR-824 and NIAB-2008 are developed through pollen irradiation technique. These varieties have been widely planted by farmers community in the country. Along with high yield potential these varieties have desirable fibre quality parameters that are suitable to textile industry in the country. As compared to parents improvement in yield and fibre quality was recorded in the developed mutants. Ginning out turn percentage (GOT) % increased from 37 to 41 %, fibre length 27 to 30.1 mm, fibre fineness 4.7 to 4.6 µg/inch, fiber strength 92 to 98 TPPSI, fiber uniformity and maturity from 84.0 to 85%. Due to the development of these varieties, the total income of the farmers increased with an additional income to the farmer's community. These varieties have contributed in the improvement of socio economic condition of farming community and textile industry.

Introduction

Cotton is an important cash crop and has a global economic impact. In Pakistan being a cash crop it accounts for 4.5% of value added in agriculture and 0.8% of GDP. It sustains the economy by bringing in foreign exchange and creating jobs. Approximately 45% of country's export income comes from textile sector and cotton related industry and 1.5 million farmer's families are directly engaged in cotton production.

The Nuclear Institute for Agriculture and Biology (NIAB) is primarily a research institution involved in goal oriented biological and agriculture research. Utilizing the expertise and facilities available at NIAB, induced mutations breeding work on cotton crop was initiated. The objective was to enhance availability of genetic variability for morphological, yield and quality parameters. Before this many plant research programs related to cotton breeding showed effectiveness of this technique (Carnelius, 1973; Micke *et al*. 1987).

Initial studies show it as important not only in knowing the responses of varieties/hybrids to irradiation but also to find the desirable dose and valuable genetic material for practical mutation breeding work in cotton. With encouraging results of mutation breeding in cotton, it was not only streamlined the use of induced mutations (both seed and pollen) for cotton breeding, but also evolved several mutants/varieties of cotton.

After the emergence of cotton leaf curl virus (CLCuV) disease, a new in our

environment in early 1990's resulted in a decline in cotton production causing a setback to our economy. Hence efforts were intensified for the evolution of cotton varieties possessing attributes i.e. early maturing, high yielding, highly tolerant to virus (old and new strain) and heat, along with fine fiber values coupled with their wider adaptation potential. Hence efforts for creation of useful genetic variability through seed and pollen irradiation were intensified in cotton which resulted in creation of useful variability.

Methodology:

Locally developed and some exotic lines of cotton were used for both seed and male parent pollen irradiation. After through studies radiation doses of 200-400 Gray (Gy) were considered feasible for seed irradiation (Iqbal *et al*. 1991; Iqbal *et al.* 1994) and low doses (05-20 Gy) for pollen irrational. For pollen irradiation @10 Gy of gamma rays before cross pollination was proved very useful. Purpose of all this work was to identify new useful cotton mutants. After male pollen irradiation and hybridization number of crosses was recorded successful and seed thus obtained was used for raising of M_1 and succeeding segregating generations (M_2-M_3).

Selections for mutants were made keeping in view of the important plant traits i.e. early maturity, suitable plant type, acceptable fibre quality traits, excellent seed cotton yield and yield contributing desirable morphological traits etc. along with resistance/tolerance to CLCuV disease. In M_4 breeding behavior of the selected progenies was evaluated and progenies having desirable traits and high yield potential were selected. M_5 generation was evaluated for re-confirmation of selected desirable traits in previous generations.

At last valuable progenies were identified in M_6 and bulked for further evaluation. Number of replicated and essentially required trials e.g. zonal, provincial and national coordinated and adaptability trials were conducted at farmers field, government institutes for further confirmation of seed cotton yield potential, wider adaptability and fibre quality in different environments. Studies like earliness, desirable morphological attributes, tolerance to heat stress, diseases, especially CLCuV disease & insects pests (Haidar *et.al*.2003; Haidar *et. al*. 2007; Akhtar *et. al.* 2004, 2010) were also carried out. The data recorded in different experiments was statistically analyzed according to the methodology of Steel and Torrie (1984).

Our Achievements:

By employing these techniques and subsequently following selection and other studies, in total 19 nineteen varieties/mutants has been released in which (5) five varieties/mutants (Fig-1) namely NIAB-846 (Aslam *et al*., 2018), NIAB-777 (Aslam *et al.*, 2018), NIAB-852 (Haidar *et al*., 2016), NIAB-IR-824 (PakAtom, 2013), and NIAB-2008 (Haidar *et al*., 2016) were developed and released through male parent pollen irradiation technique. These mutants are high yielding, early maturing, insect and disease tolerant with their acceptable/long and fine fiber values. Along with these studies, earlier studies by Aslam and Stelly (1994), Aslam *et al*. (1994) and Aslam (2000) have confirmed the effectiveness of this technique for induction of useful genetic variability in cotton.

These NIAB cotton mutants/varieties have developed an impact on national economy and are planted by farmers' community in the country. These have high yield potential (Table 1), and fibre traits that are suitable to textile industry. The varieties are with improved fibre quality parameters i.e. GOT % increased from 37 to 41 %, fibre length 27 to 29.5 mm, fineness 4.6 to 3.9µg/inch and fiber strength 90 to 94 TPPSI (Table 2) as compared to parent varieties. These varieties have contributed in the improvement of socio economic

condition of farming community and textile industry.

About 6000 kg Breeder Nucleus (BNS) seed of these varieties was produced and provided to about 1000 cotton farmers and seed producing agencies since their approval (Table 3). These varieties are non-GMO type except IR-NIAB-824 in which Bt gene *Cry1Ac* was incorporated through back cross breeding. The non-GMO varieties are included by Government of Punjab Agriculture department in their production plan since their approval and are contributing to 10% area covered by non-GMO varieties and serving as refugee crop as well. Whereas Bt variety IR-NIAB-824 is mostly planted in patches in Punjab province, which is main cotton producing province of Pakistan. The latest Non Bt variety NIAB-2008 is also being planted on vast area in Baluchistan province of Pakistan for production of organic cotton. During 2008-2014, these varieties contributed Rs. 13.5 million additional incomes to the farmers in Punjab. Whereas, during 2014-2020, the three varieties (NIAB-2008, IR-NIAB-824, NIAB-852) were planted on an area of 152 thousand hectors and contributed Rs. 11.4 million additional incomes to the farmers and covered up to 2.0 % acreage under cotton crop in Punjab during their peak period (2017-18). During 2020-21, two hundred and thirty four (234) metric ton quality seed for mutant variety NIAB-2008 was recommended by FSC&RD, Govt of Pakistan for distribution to growers which account 0.54% of total seed supply chain in Pakistan.

Moreover, WWF (World Wide Fund for Nature) Pakistan in collaboration with department of Agriculture Extension, Baluchistan province of Pakistan and CABI are engaged in promotion of organic cotton cultivation in four districts of Baluchistan viz. Barkhan, Lasbela, Khudzar and Kohlu. Therefore, Non-GM cotton seed demand has been increased because of organic cotton cultivation in Baluchistan. One of the NIAB varieties developed through pollen irradiation and hybridization technique i.e. NIAB-2008 has performed very well and planted on approximately 4000 acres during 2020, 2021 and 2022 respectively in the mentioned four districts of Baluchistan province of Pakistan.

Challenges ahead:

In the new scenario our main focus of research is the effective utilization of available germplasm resources in both Non-Bt and Bt background by evolving multi-adversity resistant (MAR) cotton varieties that can withstand under the changing climatic scenario (emerging insect complex, heat, drought and lodging resistant etc) as well as can meet the needs of all cotton related stakeholders (high yielding potential, highly tolerant against virus especially cotton leaf curl virus along with desirable fiber values) and suitable for mechanical picking in the country.

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Table 1: Yield Potential of Cotton Mutants/Varieties Developed Through Pollen Irradiation Technique.

Cotton Mutant	**Method**	**Released/Approved (Year)**	**Yield Potential (Kg. ha^{-1})**
NIAB-846	Pollen irradiation	2008	4300-4800
NIAB-777	Pollen irradiation	2009	5000-6000
NIAB-852	Pollen irradiation	2012	4500-5000
NIAB-IR-824	Pollen irradiation & Bt gene incorporated	2013	4000-4500
NIAB-2008	Pollen irradiation	2016	4500-5000

Table 2: Fiber Characteristics of Cotton Mutants/Varieties Developed Through Pollen Irradiation Technique

Variety	GOT (%)	Fibre Length (mm)	Fibre Fineness (µg/inch)	Fibre Strength (TPPSI)	Uniformity Index (%)	Maturity (%)
NIAB-846	38.49	29.80	4.67	96.10	85.5	83.0
NIAB-777	37.56	28.81	4.67	91.12	81.2	85.0
NIAB-852	38.80	30.10	4.68	94.30	81.3	83.1
NIAB-IR-824	42.70	28.90	4.90	98.30	82.7	83.0
NIAB-2008	37.99	31.16	4.74	92.20	80.5	83.5
NIAB-78 (P)	36.60	27.30	4.70	93.00	84.0	84.0
Reba-288 (P)	36.50	27.40	4.90	92.60	-	-

Table 3: Breeder Nucleus Seed (Kg) Produced and Provided to Framers/Seed Distribution Agencies

Variety/Year	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	Total
NIAB-846 (2008)	633	469	530	170	25	9.0	6	2	0.150	-	-	1844
NIAB-777 (2009)	-	728	847	280	62	29.0	10	2	0.150	-	-	1958
NIAB-852 (2012)	-	-	319	534	394	51.0	10	6	0.150	-	-	1314
IR-NIAB-824 (2013)	-	-	-	16	22	368	111	93	58	10	-	678
NIAB-2008 (2016)							3	205	63	-	10	281
Total	633	1197	1696	1000	503	457	140	308	122	10	10	6074
No. farmers/growers	107	321	314	158	20	94	37	17	6	1	1	1076

Fig: 1: Filed views of cotton mutants developed through pollen irradiation & hybridization technique

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