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UTILIZATION OF MORPHOLOGICAL AND MOLECULAR MARKERS TO EVALUATE A NEW GENOTYPE RESISTANT TO SOME INSECT ATTACKS IN EGYPTIAN COTTON

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ABSTRACT

Field experiments were conducted during 2007 and 2008 seasons in El-Giza Experimental station, A.R.C. to evaluate a new nectariless cotton genotype, namely Giza 85 Ne which descended from discontinuous backcrossing between the Egyptian cotton variety Giza 85 and a nectariless Upland line, viz., MAR (NLG8CDGP6H-1-95). It was compared with their parents, i.e. Giza 85 and the nectariless line. The Experimental design was a randomized complete block with three replications. Only the Egyptian parent plots were sprayed against insect infestations. Comparative genetic studies among evaluated of genotypes were done using morphological characters related to insect resistance, yield components, fiber quality as well as a histological comparisons among the three evaluated entries. Besides a molecular genetic fingerprinting using RAPD analysis was done. The obtained results can be summarized as follows:

Field and morphological characterization of the new hybrid Giza 85 Ne revealed that nectary was not found on the midrib on the lower side of each leaf and below the bracts when compared with the Egyptian cotton variety Giza 85. The results indicated that insignificant differences were found between Giza 85 Ne and Giza 85 for boll weight, seed cotton yield, lint yield, lint percentage, seed index, micronaire and fiber length. By Contrast, significant differences were reported for Pressley. Besides, leaf anatomy for the thickness of cuticle showed that the upper and lower epidermis were increased in both the nectariless parental line (Ne) and the new hybrid Giza 85 Ne compared with the local variety Giza 85.

The relative resistance of the new cotton genotype to insects was associated with thicker leaf. In the RAPD analysis, ten primers were used and succeeded in the identification and differentiation of the three genotypes. Moreover, accession – specific DNA markers characterized different genotypes and therefore were used to generate unique fingerprint for each genotype. The total number of amplicons was 117 DNA fragments while, ninety bands (76.9 %) were polymorphic amplicons. There were some unique bands in the new hybrid which were not observed in the parental genotypes. With respect to the genetic similarities among the new hybrid Giza 85 Ne and the two parents, the highest similarity was 90.1 % between the hybrid and the Egyptian cotton parent. While, the least similarity was observed between the two parents (53.9 %).

It should be noted, however that there were still some level of polymorphisms between the hybrid Giza 85 Ne and the Egyptian cotton variety Giza 85. Thus, more backcrosses should be done to the Egyptian parent in order to restore the Egyptian genomic background, while, maintaining the new characters for inset resistance.

From the above results, it could be concluded that the new genotype Giza 85 Ne had high yield and yield components, showed desirable fiber quality as well as good level of insect resistance. For that the breeder can use this genotype as good material for insect resistance in the cotton breeding program.

Key Words: ***Insect resistance, Nectariless line MAR, Egyptian cotton, RAPD, Fingerprinting, Fiber traits, Polymorphism, Genetic similarity.***

INTRODUCTION

Cotton is grown for its fiber, mainly to use in textile industry. Yield has top priority in any cotton breeding program. The fiber quality of the Egyptian cotton is the best all over the world where it combines the high length, fineness and highest yarn strength.

Egyptian cotton breeders increased the productivity of new cultivars. However, the total production of Egyptian cotton decreased upon various pest infestations, which reflect critically on the economic outcome. The insects and diseases in cotton cause significant reduction in yield and quality of both lint and seeds and increasing production costs. The pathogens most commonly involved in diseases complex are *Rhizoctonia* Soloni (Mohamed 1990) and *Fusarium* Spp. (Aly *et al.* 1996), The most serious sucking insect pest attacking cotton plants under the Egyptian conditions are the cotton aphid *Aphiis gossypol*, the çotton whitefly *Bemisia tabaci* Genne and the cotton leaf hopper, *Empoacalybica de Ber* (Taneja and Dhindwal 1983 and Simwate *et al.* 1987). Generally, the Egyptian cotton varieties losses ranged from 25.2 to 52.2 % for seed cotton yield due to pests while its losses ranged from 10.7 to 32.2 % for the seed cotton yield due to diseases (Mohamed *et al.* 2006). In the same time the pest control strategy for cotton plants is mainly based on the use of insecticides and fungicides, which gives rise to further problems with respect to their chemical residues in cotton seed oil and to their increasing contributions in the pollution of the surrounding environment.

Genetic resistance to pests is recognized as the most effective economical and reliable means of maintaining healthy plants, reducing crop losses and produce fiber with less trash. Resistance to insect was reported to be associated with several plant morphological traits, such as nectariless. To improve insect resistance of Egyptian cotton, it is necessary to transfer such morphological characteristics from Upland stock or adapted cultivars (Meredith and Wells 1986 and Tang 1987) such trait be incorporated into Egyptian cotton breeding to develop insect resistant cultivars. Inter-specific crosses followed by backcrossing are suggested to be followed in order to combine resistance attributes with agronomic and fiber properties. Improvement of cotton crop through breeding program for resistance to diseases, insect pests and abiotic stresses would require an immediate DNA analysis to find markers that facilitate the scoring of the characteristics across generations. Molecular genetic fingerprinting is also useful tools for phylogenetic studies among different genetic resources.

A number of studies have been conducted to investigate genetic diversity in cotton at the DNA level (Pillay and Myers 1999, Liu *et al* 2000, Hussein *et al* 2002 and Qureshi *et al* 2004).

The aim of the study is to evaluate a promising line (Giza 85 Ne) descended from discontinuous backcrossing between the American cotton (*Gossypium hirsutum*) which characterized by nectariless as the donor parent and the Egyptian cotton cultivar (Giza 85). The new line is to be compared with the long staple cotton variety Giza 85 and with the donor parent in yield and its components, fiber quality, histological studies, detected molecular fingerprints of different genotypes to assess the restoring of Egyptian germplasm upon backcrossing and to determine possible genetic markers linked to insect and diseases resistance genes.

MATERIALS AND METHODS

The materials used in this study consisted of Upland line with nectariless MAR NLG8CDGP5H-1-95 (Ne) of *Gossypium hirsutum* and an Egyptian long staple variety of cotton (Giza 85) belong to *Gossypium barbadense* (L.). They were used as parental genotypes. F_1 derived from crossing the Egyptian cotton with the American line. This F_1 was derived six backcross progenies (BC_6), which were obtained from the cotton breeding program of the Cotton Research Institute.

The present investigation was carried out at the Department of Cotton Breeding Cytology and Genetics Unit, Cotton Research Institute, Agricultural Genetic Engineering Research Institute, Giza, Egypt and Agricultural plant Department, Faculty of Agriculture, Ain Shams University to evaluate a promising line. A randomized complete block trail with three replicates was carried out at Giza Experimental Station in 2007 and 2008 seasons. The parental genotypes were also included in the Experiment as control varieties. Each plot had four rows, 4 meters long and 60 cm apart. Hill were spaced 20 cm a part and thinned to two healthy plants per hill. Only the Egyptian parent was sprayed against insects infestations (such as: plant bugs, whitefly, the pink bollworm and American bollworm) according to recommendations.

The following characters were measured for each cultivar and BC_6 hybrid:

A. Morphological characters at vegetative growth

Nectrariless character related to insect resistance in cotton was recorded.

B. Yield and yield components: These included average of boll weight (BW), seed cotton yield per plant in grams (SCY/P), lint yield per plant in grams (LY/P), lint percentage (L %) and seed index (SI)..

C. Fiber properties: Fiber fineness was measured by the Micronaire apparatus in micronaire units (Mic), Fiber strength was measured by the Pressley apparatus (PI) and fiber length (2.5 % SL).

Analysis of variance and F test were calculated for the studied characters according to Snedecor and Cochran (1981) and Duncan (1988) multiple range test at 5 % level of probability was used for comparison between means.

A. Histological studies

Leaf anatomy: During the vegetative and reproductive growths of cotton plants, samples for anatomical studies were taken as follows: Specimens were taken for histological studies at the age of 120 days after sowing from the leaf lies on the node number four below the apical top. Samples were immersed in a killing solution and fixed at least 48 hours in F.A.A. according to Johanson (1940), Sass (1961) and Willey (1971).

The following measurements and counts were estimated of the histological samples studied: Thickness of upper and lower cuticle and number of hairs / cm^2 by using the electronic microscope.

B. Molecular markers

- ***Genomic DNA extraction and purification***

  DNA easy plant minikit (Quigen Inc., Cat No. 69104, USA) was used for DNA extraction.

- ***RAPD – PCR analysis***

For testing the genetic relationships and detect polymorphism between parental genotypes; Giza 85 and rectrariless line (Ne) and BC_6 hybrid plants, RAPD – PCR reactions were conducted using 10 arbitrary 10 – mer primers with the 5' – 3' sequences as shown in Table (1).

Table 1. Random primer codes and their sequences for RAPD-PCR analysis

	Primer code	Sequences 5' – 3'	GC %
1	OP-A01	CAGGCCCTTC	70 %
2	OP-A02	TGCCGAGCTG	70 %
3	OP-A03	AGTCAGCCAC	60 %
4	OP-A04	AATCGGGCTG	60 %
5	OP-A05	AGGGGTCTTG	60 %
6	OP-A07	GAAACGGCTG	60 %
7	OP-A08	GTGACGTAGG	60 %
8	OP-A09	GGGTAACGCC	70 %
9	OP-A11	CAATCGCCGT	60 %
10	OP-D08	GTGTGCCCCA	70 %

The reaction conditions were optimized and mixtures were prepared (30 µl total volume) consisting of the following: dntps 2.4 µl, $Mgcl_2$ 3.0 µl, Taq (5 µ / µl) 0.2 µl, template DNA (50 µg / µl) 2.0 µl, H_2O (dd) 17.4 µl. Amplification was carried out in a PTC – 200 thermal cycler (MJ Research, Watertown, USA) programmed as flows: denaturation, 94°C for 2 minutes, then for 40 cycles. Each cycle consisted of 1 minute at 94°C, 1 minute at 37°C, 2 minutes and 30 second at 72°C, followed by a final extension time of 12 minute at 72°C and 4°C (infinitive).

- ***Gel electrophoresis***

Gel electrophoresis was applied according to Sambrook *et al.* (1989). Agrose (1.2 %) was used for resolving the PCR products. The run was performed for one hour at 80 volt in pharmacia submarine (20 x 20 cm). Bands were detected on UV transilluminator and photographed by gel documentation, Bio – Rad.

RESULTS AND DISCUSSION

A. Morphological characters at vegetative growth

Presence or absence of nectars in the Egyptian cotton variety Giza 85 compared with Giza 85 Ne hybrid and nectailess line (Ne) was recorded. Giza 85 variety has leaf, extra-floral and floral nectaries. The nectar secreted attracts many insects, and provides an important food for them. A nectary is usually found on the midrib on the lower side of each leaf. Extra-floral nectaries are commonly found below the bracts and also between and inside the bracts. Floral nectaries are located between the sepals and petals. While Giza 85 Ne hybrid and nectaiess line (Ne) plants possesses the antibiosis type of insect resistance, that is, they are nutritionally inferior because they lack the sugar provided in extra-floral nectary secretions. Also nectaries aren't found on the lower side of each leaf below the bracts and between the sepals and petals (**Thaxton *et al* 1998**).

B. Yield and yield components

Mean yield and yield components were presented in Table (2) and the means were follows

1. Boll weight for Giza 85 and Giza 85 Ne were 2.97 and 2.93 g, respectively. These genotypes had lower boll weight than the nectariless Upland cotton, which was 5.20 g. The results showed that nectariless line (Ne) gave significantly differences in boll weight compared with the Giza 85 and Giza 85 Ne hybrid.
2. Seed cotton yield per plant of nectariless line (Ne) gave higher seed cotton yield / plant (78.10 g / plant). Which was significantly higher than seed cotton yield of Giza 85 (50.49) and Giza 85 Ne (49.87).
3. Lint yield for the Upland nectariless line (Ne) was 26.66 g/plant. This donor line was significantly higher to lint yield than either the Egyptian

Table 2. Characters related to yield, its components and fiber properties for Giza 85, Nectariless line (Ne) and Giza 85 Ne hybrid.

Genotypes	Giza 85	Giza 85 Ne	Nectariless (Ne)
Boll weight (g) (BW)	2.97 b	2.93 b	5.20 a
Seed cotton yield/plant (g) (SCY)	50.49 b	49.87 b	78.10 a
Lint yield/plant (g) (LY)	19.43 b	19.13 b	26.66 a
Lint percentage % (LP)	38.49 a	38.37 a	34.11 b
Seed index (g) (SI)	10.23 b	9.40 b	12.00 a
Micronaire reading (Mic)	4.03 b	4.21 b	5.11 a
Pressley index (PI)	10.81 a	10.04 b	8.58 c
Fiber length at 2.5 % span length (2.5 % SL)	31.29 a	30.09 a	27.42 b

cotton variety Giza 85 or the new hybrid Giza 85 Ne, which recorded 19.43 and 19.13 g/plant, respectively.

4. Lint percentage in contrast with the previous character (i.e., lint yield). The nectariless donor line (Ne) showed a significantly lower lint percentage than either Giza 85 or Giza 85 Ne hybrid. The highest mean of lint percentage was for Giza 85 (38.49 %), while the lowest was for the line Ne (34.11 %).
5. Seed index ranged from 9.4 g (Giza 85 Ne) to 12.0 g (line Ne). Higher significant seed index's were found for the nectariless line (Ne).

C. Fiber quality characters

1. **Fiber fineness and maturity (micronaire reading) (Mic):** The means of (Mic) presented in Table (2) showed that the variety Giza 85 and the hybrid Giza 85 Ne had lower values (4.03 and 4.21) while the nectariless line (Ne) recorded 5.11uints. The results showed significant differences between the donor line and each of Giza 85 and Giza 85 Ne hybrid.
2. **Fiber strength (Pressley index) (PI)** Data in Table (2) revealed significant differences among all genotypes. The highest mean of PI was for Giza 85 (10.81), while the lowest mean was for the nectariless line (Ne) (8.58).
3. **Fiber length at 2.5 % span length (2.5 % SL):** No significant differences between Giza 85 and Giza 85 Ne hybrid were detected for fiber length, which recorded 31.29 and 30.09 mm, respectively. While, there were significant differences between the donor nectariless line (Ne) and the other two tested entries (Table 2). The previous results are in agreement with Mohamed *et al.* (2005) and Abd E-Gelil (2006).

Considering all the results of fiber quality characters, it could be concluded that the genotype Giza 85 Ne hybrid is good candidates for selecting higher fiber quality in breeding programs. At the same time, the

hybrid Giza 85 Ne is very similar to the Egyptian cotton variety Giza 85 for yield and its components in one hand and fiber quality characters on the other hand. Therefore, this hybrid produced fiber within limits of the long staple fiber classification group.

D. Histological studies

Leaf anatomy: Different histological feature of the leaf lies on the 4th node from the apical and this histological approach was used in this part of study in case of different applied genotypes. The characters studied were:

The thickness of cuticle: Values of either the upper epidermis or the lowest one are shown in Table (3) and shown in Figure (1). For the upper cuticle, its thickness was decreased with the hybrid Giza 85 Ne and the Egyptian cotton Giza 85, which recorded 0.472 and 0.363 µm, respectively. While, its increase was existed only with the donor nectariless line (1.2 µm). On the other hand, the lower cuticle for Giza 85 Ne and Giza 85 was higher than the upper cuticle (1.2 and 0.714 µm). While, the nectariless line (Ne) was 0.717 µm.

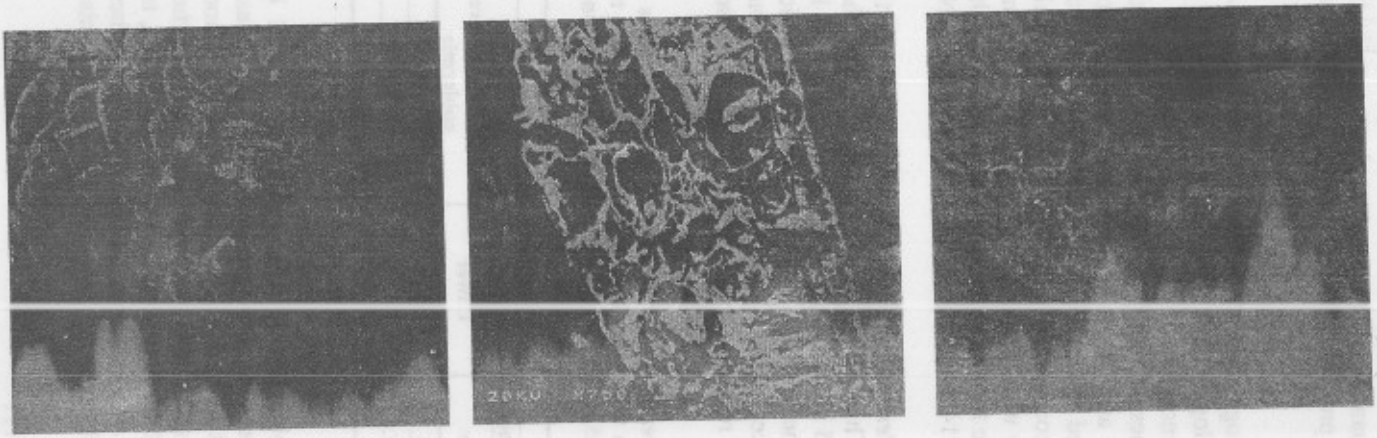
Number of hairs on lower epidermis/mm²: With regard to this trait, the line Ne showed 0.2 hairs / mm². This line had lower number of hairs than the Egyptian cotton variety Giza 85 and Giza 85 Ne hybrid which were 1.8 and 1.2, respectively (Figure 2). The role of cotton leaf thickness of cuticle was focused as host plant resistance. Therefore, thickness could contribute as a sort of non-preference type of resistance representing barriers to insect feeding.

Table 3. Means counts and measurements of certain histological features in transverse section through the Lamina of the 4th apical leaf for the recurrent genotype Giza 85, their hybrid Giza 85 Ne and the donor line nectariless (Ne).

Characters / Genotypes	Lamina		
	Thickness of cuticle (µm)		Number of hairs/mm² of lower epidermis
	Upper	Lower	
Giza 85	0.363	0.714	1.80
Giza 85 Ne hybrid BC_6	0.472	1.200	1.20
Nectariless (Ne)	1.20	0.717	0.20

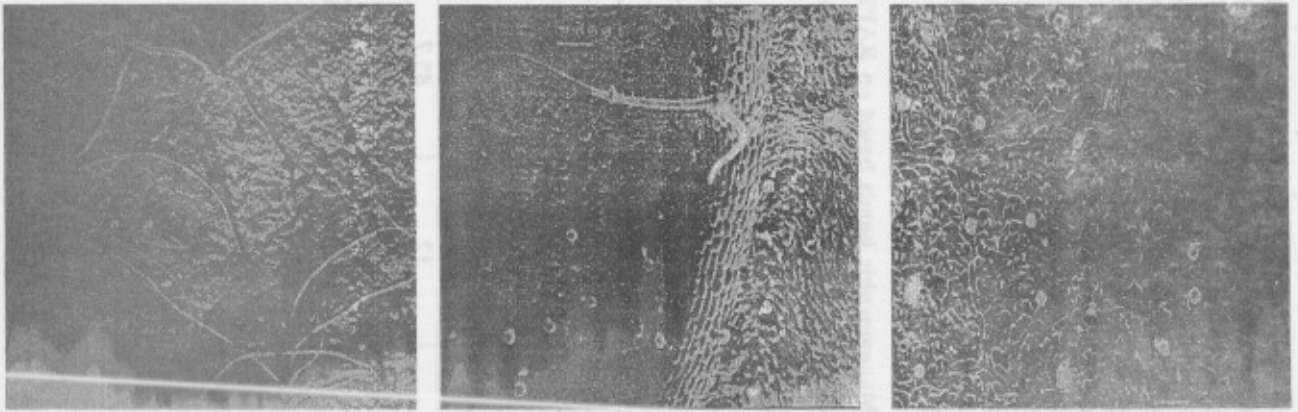
E. RAPD – PCR analysis

Randomly amplified polymorphic DNA (RAPD) analysis would be useful in describing any skewness in the genetic basis, germplasm characterization, fingerprinting and assessing the genetic diversity among cotton genotypes. Out of twenty random decamer primers screened for their capability of amplifying DNA via the polymerase chain reaction (PCR), ten primers generated reproducible and scorable RAPD profiles. These produced multiple band profiles with number of amplified DNA fragments



Giza 85 | Giza 85 Ne hybrid | Nectariless line (Ne)

Fig. (1): The Upper and the lower cuticle for the variety Giza 85, Nectariless line (Ne) and their hybrid Giza 85 Ne.



Giza 85 Giza 85 Ne hybrid Nectariless line (Ne)

Fig. (2): Number of hairs/mm^2 of the lower epidermis for the variety Giza 85, Nectariless line (Ne) and their hybrid Giza 85 Ne.

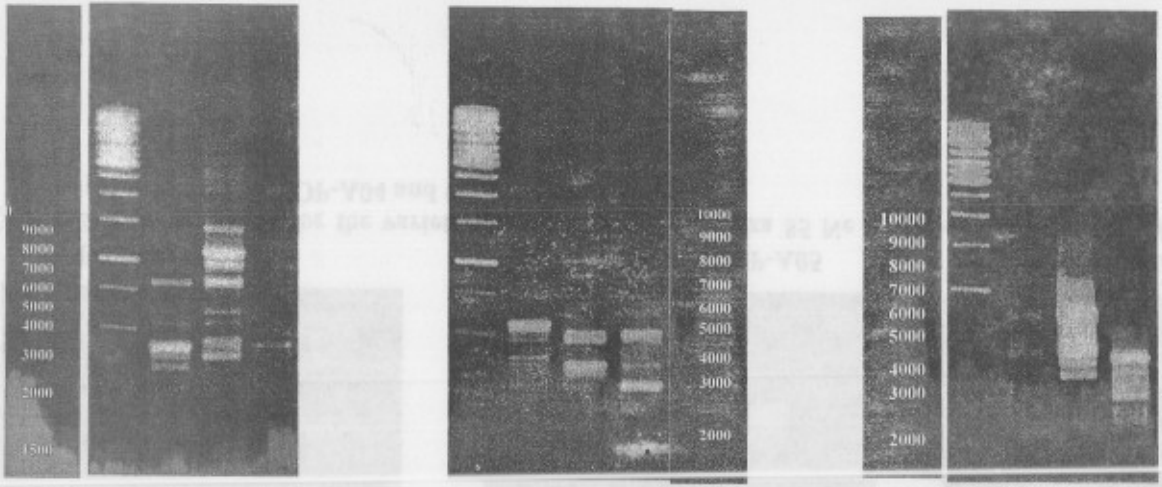
ranging from 9 to 14 (Table, 4 and Figures, 3 - 6). The total number of fragments produced by the ten primers was 117 with an average of 11.7 fragments / primer. Twenty seven bands were monomorphic (common) for all genotypes while, ninety bands (76.9 %) were polymorphic. The highest levels of polymorphism (100 %) were observed in primers OP-A08. However, the lowest level of polymorphism was 61.5 % in primer OP-A03.

In this respect, Tatineni *et al* (1996) studied the level of polymorphism among 19 cotton genotypes using 27 random primers and found that 33.8 % of the primers revealed monomorphic patterns. While, working on 31 *Gossypium* species, three subspecies and one inter-specific hybrid, Khan *et al* (2000) found that the level of polymorphism was 99.8 %. Moreover, Hussein *et al* (2002) used 49 RAPD primers to investigate the genetic diversity among 13 cotton genotypes and detected a level of polymorphism of 30.4 % and Abd El-Salam *et al* (2010) found 67 bands, 85 % were polymorphic among Giza 70 and its off types as revealed by RAPD.

Table 4: Levels of Polymorphism and Monomorphic bands based on RAPD-PCR analysis.

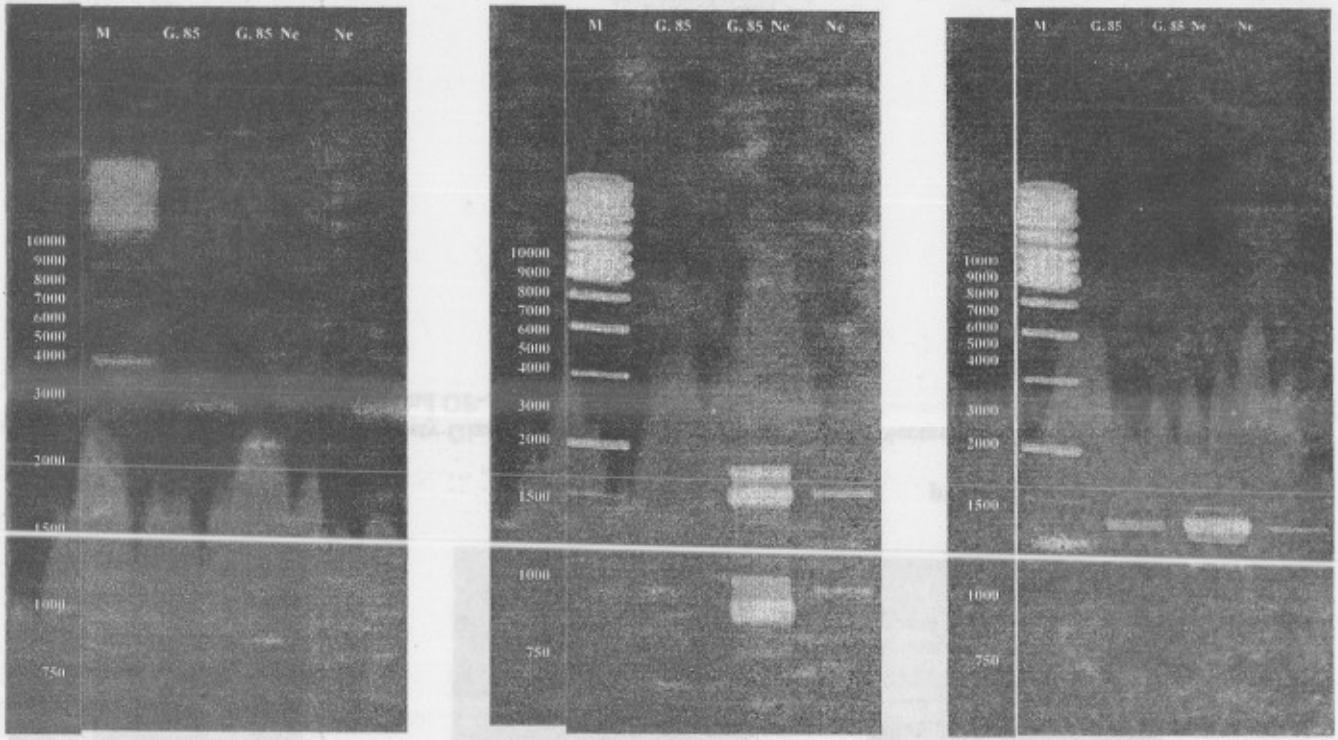
Primer	TB	MB	PB	P %
OP-A01	13	4	9	69.2
OP-A02	13	4	9	69.2
OP-A03	13	5	8	61.5
OP-A04	11	3	8	72.7
OP-A05	14	2	12	85.7
OP-A07	9	1	8	88.9
OP-A08	10	0	10	100
OP-A09	11	3	8	72.7
OP-A11	9	3	6	66.7
OP-D08	14	2	12	85.7
Total	117	27	90	76.9
Average	**11.7**	**2.7**	**9.0**	

TB : Total bands (amplicons), MB : Monomorphic bands (amplicons), PB : Polymorphic bands (amplicons) and P % : Polymorphism %



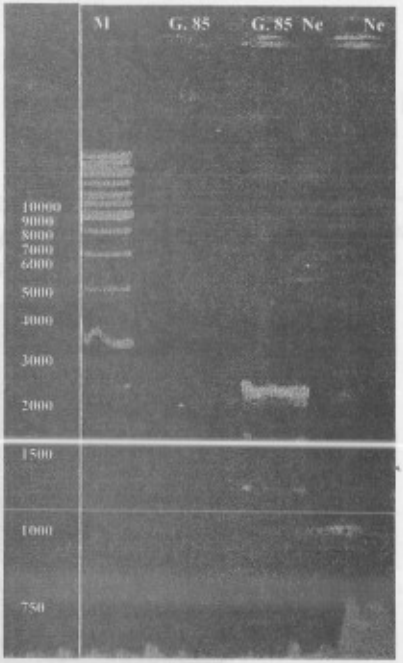
primer OP-A01 primer OP-A02 primer OP-A03

Fig. (3): RAPD-PCR products for the variety Giza 85, the hybrid Giza 85 Ne and the Nectariless line (Ne) using the three primers OP-A01, OP-A02 and OP-A03, respectively.

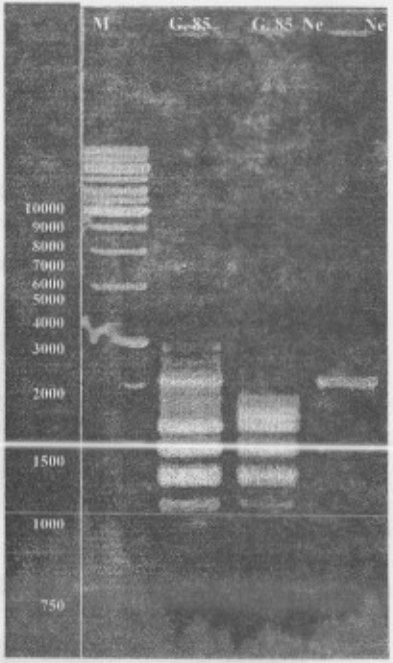


Primer OP-A04 primer OP-A05 primer OP-A07

Fig. (4): RAPD-PCR products for the variety Giza 85, the hybrid Giza 85 Ne and the Nectariless line (Ne) using the three primers OP-A03, OP-A04 and OP-A07, respectivel.

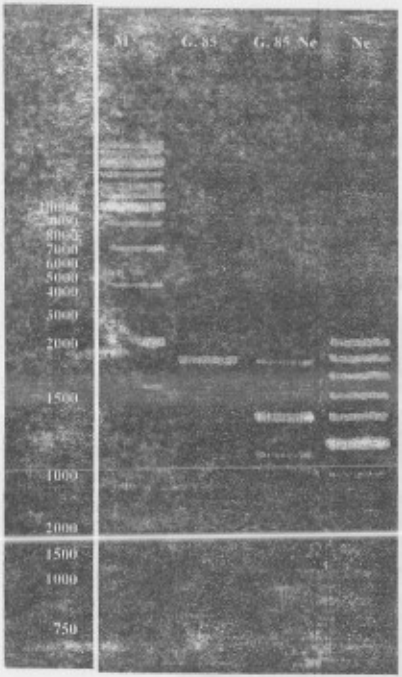


Primer OP-A08

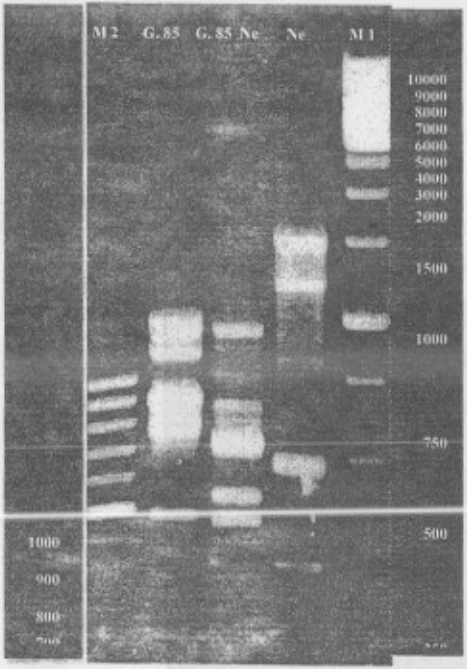


Primer OP-A09

Fig. (5): RAPD-PCR products for the variety Giza 85, the hybrid Giza 85 Ne and the Nectariless line (Ne) using the three primers OP-A08, and OP-A09, respectively.



Primer OP-A11



Primer OP-D08

Fig.(6): RAPD-PCR products for the variety Giza 85, the hybrid Giza 85 Ne and the Nectariless line (Ne) using the three primers OP-A11, OP-A04 and OP-D08, respectiveiy.

Specific markers by RAPD – PCR analysis

Genotypes specific markers generated from RAPD – PCR analysis are shown in Table (5). Ten RAPD – PCR analysis primers were found to be useful as genotypes unique markers. The highest number of RAPD – PCR markers was scored for the nectailess line (Ne) (43 markers). While, the lowest number of RAPD – PCR markers was scored for the Egyptian cotton variety Giza 85 (22 markers). In the meantime, the largest number of genotypes specific markers was generated by the two primers OP-A05 and OP-D08 (12 markers), while the lowest number was generated by the primer OP-A11 (6 markers). As seen from Table (5), there were 90 specific markers for all the genotypes scored and illustrated. These results indicated that the RAPD – PCR exhibited different unique molecular markers, as previously mentioned. For instance, primer OP-A11 could distinguish for all genotypes by distinct bands at MW of about 600 bp for Giza 85, 250, 320 and 450 bp for the hybrid Giza 85 Ne, 700 and 1000 bp for the line nectailess line (Ne).

Genetic distances

Genetic similarities among Giza 85, the hybrid Giza 85 Ne and the nectailess line (Ne) based on RAPD data are shown in Table (6) and dendrogram (Fig. 7). The highest similarity was 90.1 % between Giza 85 and the hybrid Giza 85 Ne, which indicated that the hybrid Giza 85 Ne was closely related with the Egyptian variety. While, each of Giza 85 and the hybrid Giza 85 Ne were genetically distant from the nectariless line nectariless (similarity index 53.9 and 54.4 %, respectively).

From the dendrogram, utilizing RAPD analysis divided the three genotypes into two main clusters. The nectariless line (Ne) was in a separate cluster. The second cluster included two cotton genotypes (Giza 85 and the hybrid Giza 85 Ne) with a similarity 90.1 %. It can be observed that there were some common bands in the Egyptian parent which were found in the hybrid Giza 85 Ne but these bands were not observed in the nectariless line. Our results indicated that the genetic uniformity and similarity between the Egyptian parent Giza 85 and Giza 85 Ne hybrid (BC6) may be due to the use of different new strains of Giza 85. However, it is recommended to make more backcrosses with Giza 85 in order to restore the genome of the recipient cultivar Giza 85, while maintaining the new characters for insect resistance.

Generally, the nectariless hybrid reduced population of pest than the Egyptian cotton variety Giza 85. The decrease in infestation of insects may be referred to chemical and morphological change in leaf structure, which observed in the hybrid Giza 85 Ne. This change may cause a high role on insect resistance as compared with normal line. It could be concluded that the hybrid Giza 85 Ne had good yield and yield components, desirable fiber quality and good level of insect resistance.

Table 5: The genotypes characterized by unique positive and/or negative specific marker, marker size and total number of markers identifying each genotype.

Primer	Giza 85					Giza 85 Ne					Nectariless (Ne)					Total
	Unique positive marker		Unique negative marker		Grand total	Unique positive marker		Unique negative marker		Grand total	Unique positive marker		Unique negative marker		Grand Total	
	Size of the marker band (bp)	Total No. of markers	Size of the marker band (bp)	Total No. of markers		Size of the marker band (bp)	Total No. of markers	Size of the marker band (bp)	Total No. of markers		Size of the marker band (bp)	Total No. of markers	Size of the marker band (bp)	Total No. of markers		
OP-A01	200	1		0	1	1500	1		0	1		0	1100, 1000, 900, 800, 600, 500 and 250	7	7	9
OP-A02	1100, 800 and 600	3	900, 700 and 550	3	6		0	400	1	1	200	1	300	1	2	9
OP-A03		0		0	0	1000, 550 and 450	3	460, 400, 250 and 200	4	7	320	1		0	1	8
OP-A04	250	1		0	1	300	1		0	1		0	1000, 880, 800, 700, 600 and [illegible]	6	6	8
OP-A05	580	1	900, 700, 400, 230 and 200	5	6		0		0	0	300	1	1500, 1200, 950, 550 and 350	5	6	12
OP-A07	350	1	450	1	2	800, 550 and 480	3	900	1	4		0	700 and 250	2	2	8
OP-A08		0	300	1	1	1100 and 450	2		0	2	1000	1	750, 700, 600, 550, 350 and 270	6	7	10
OP-A09	800	1	250	1	2		0	700	1	1	1000	1	600, 550, 500 and 300	4	5	8
OP-A11		0	600	1	1		0	450, 320 and 250	3	3		0	1000 and 700	2	2	6
OP-D08	1600	1	1700	1	2	1000, 550 and 350	3	2000 and 290	2	5	600	1	900, 800, 700 and 500	4	5	12
Total		9		13	22		13		12	25		6		37	43	90

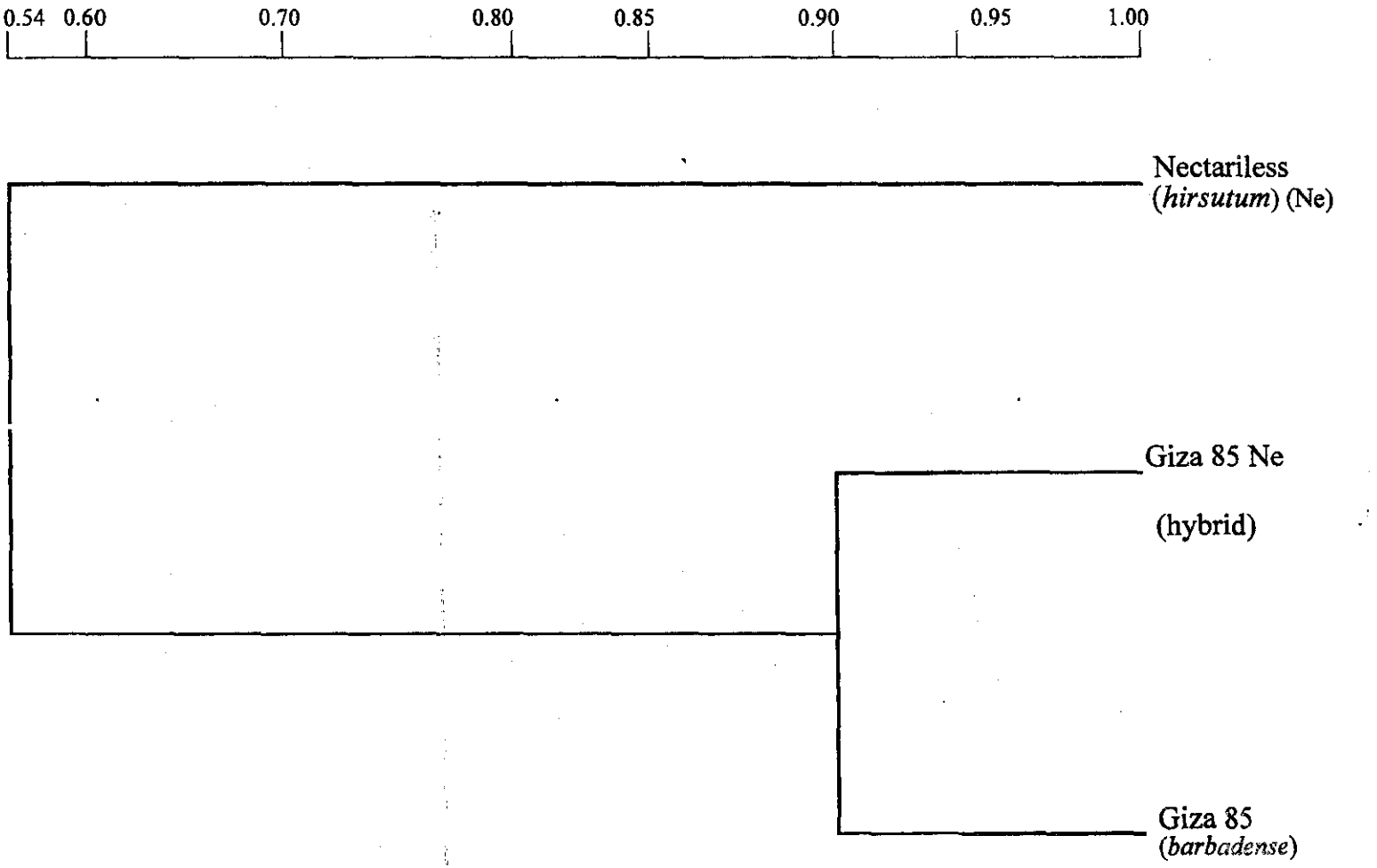


Fig. (7): Dendrogram of Giza 85, Giza 85 Ne and nectariless accession from the RAPD data using un-weighed pari – group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient.

Table 6: Similarity matrices among three genotypes based on RAPD analysis.

Genotypes	Giza 85	Giza 85 Ne (hybrid BC_6)	Nectailess (Ne)
Giza 85	100.0		
Giza 85 Ne	90.1	100.0	
Nectariless (Ne)	53.9	54.4	100.0

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إستخدام الدلائل المورفولوجية والجزيئية لتقييم تركيب وراثى جديد من القطن المصرى المقاوم لبعض الآفات

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أجريت هذه الدراسة فى مزرعة مركز البحوث الزراعية بالجيزة فى موسمى 2007 و 2008م وذلك لتقييم التركيب الوراثى الجديد (الهجين الرجعى السادس) جيزة 85 عديم الغدد الرحيقية (Giza 85 Ne) والذى أنتج بالتهجين الرجعى غير المتصل بين الصنف المصرى جيزة 85 كأب رجعى مع السلالة الأمريكية MAR (NLG8CDGP6H-1-95) التى تتميز بغياب الغدد الرحيقية Nectariless كأب مانح لهذه الصفة فقط. وتم زراعة التركيب الوراثى الجديد جيزة 85 عديم الغدد الرحيقية (Giza 85 Ne) مع كل من الأب المصرى جيزة 85 والسلالة الأمريكية فى تصميم قطاعات كاملة عشوائية مع إجراء رش وقائى ضد الآفات حسب التوصيات للأب

المصرى فقط. وقد تم دراسة الصفات المورفولوجية وعلاقتها بالمقاومة الحشرية ، وكذلك دراسة المحصول ومكوناته وصفات جودة التيلة ، والدراسة التشريحية للأوراق وعمل البصمه الوراثية الجزيئية على الـ DNA على التوالى وكانت أهم النتائج المتحصل عليها ما يلى:

- أظهر الوصف الحقلى والمورفولوجى للتركيب الوراثى الجديد Giza 85 Ne تميز أوراق نباتاته بغياب الغدد الموجوده على السطح السفلى للورقة ، وكذلك غياب الغدد الموجودة فى قاعدة القنابات للبراعم الزهرية وكذلك بين القنابات واللوزة مقارنة بالأب المصرى جيزة 85 (الذى يحتوى على هذه الغدد).
- لوحظ عدم وجود أى فروق معنوية بين التركيب الجديد جيزة 85 عديم الغدد (Giza 85 Ne) والأب المصرى جيزة 85 بالنسبة لوزن اللوزة ، ومحصول النبات من القطن الزهر والشعر ، ومعدل الحليج ، ومعامل البذرة ، ونعومة التيلة (الميكرونير) ، وطول التيلة ، بينما أعطت النتائج فروقاً معنوية لصفة المتانة (معامل البريسلى).
- أظهرت الدراسة التشريحية لأوراق نباتات Giza 85 Ne وجود تغيرات تركيبية بسيطة مع زياده فى سمك طبقة الكيوتيكل للسطح السفلى للورقة مقارنة بالأب المصرى جيزة 85 مما يرجح فاعلية وتأثير سمك هذه الطبقة على مقاومة إمتصاص الحشرات لعصاره النبات.
- أوضحت نتائج التحليل والدراسة على المستوى الجزيئى بطريقة RAPD – PCR نجاح عشرة بادئات فى إمكانية التمييز بين التراكيب الوراثية الثلاثة جيزة 85 ، جيزة 85 عديم الغدد (Giza 85 Ne) والسلالة الأمريكية عديمة الغدد (Nectariless).
- أمكن تحديد واسمات جزيئية مميزة لكل تركيب وراثى ساعدت فى تحديد بصمة وراثية فريدة لكل منها.
- كان العدد الكلى من شظايا الـ DNA الناتجة هو 117 شظية. بينما كان عدد شظايا DNA التى أظهرت تباين بين التراكيب الثلاثة هو 90 شظية . حيث تواجد بعض من الشظايا أو الحزم التى تميز هذه التراكيب عن بعضها البعض كما فى التركيب الوراثى الجديد (الهجين الرجعى) Giza 85 Ne به عدد من الحزم المميزة لغياب الغدد الرحيقية عن التراكيب الأخرى.
- كما أمكن تقدير العلاقات ودرجة القرابة الوراثية بين الأب المصرى جيزة 85 والهجين الرجعى (Giza 85 Ne) وأيضاً السلالة الأمريكية Nectariless.
- سجلت دراسة العلاقة ودرجة القرابة أعلى قيمة تشابه وقرابة وراثية بين الأب المصرى جيزة 85 والتركيب الوراثى Giza 85 Ne بنسبة 90,1 %، بينما كانت قيمة التشابه الوراثى بين التركيب الوراثى الجديد Giza 85 Ne والأب الامريكى 54,4 %.
- ومن درجة التباين بين التركيب الوراثى الجديد مقارنة بالأب المصرى جيزة 85 يمكن القول أنه لايزال هناك أحتياج لعمل مزيد من الهجن الرجعية مع الصف المصرى حتى يتم إسترداد كل مادته الوراثية مع الإستبقاء على الجينات المرتبطة بالمقاومة للحشرات (غيات الغدد الرحيقية).
- من هذه النتائج يتضح أن التركيب الوراثى الجديد Giza 85 Ne لديه المحصول والصفات الجيدة لصفات الجودة مع المقاومة للحشرات.