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Comparative Genetic Studies on Some Off-Type Cotton Plants of Giza 80 Cultivar and Their Effects on Varietal Deterioration

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Abstract

The present work was conducted at Agricultural Research Station of Sids during the two seasons, 2008 and 2009. The materials used included the off-type plants derived from the Egyptian cotton variety Giza 80 which cultivated in a large scale of the Middle Egypt to study some of their economic traits and their effects in reducing yield and lint quality of Giza 80. Giza 80 variety as well as its off-type plants were characterized in the field and in the laboratory, growth habits, fruiting behavior yield and yield components, intensity of fuzz distribution on the seeds and fiber properties. Also, the RAPD technique was used as a tool for setting up a convenient and standard protocol to determine the difference and variation between plants of the variety Giza 80 and its off-type plants.

Field characterization of the off-types showed, taller plants, longer leaves, different boll shapes and intensity of fuzz distribution on the seeds as compared to their standard type Giza 80. The results showed that the off-type plants of Giza 80 gave considerably lower values for boll weight, seed cotton yield, lint yield, lint percentage, Pressley index, uniformity ratio and fiber length traits, while they gave higher values for micronaire compared with the normal type of Giza 80.

As in results of RAPD analysis, ten primers out of the tested thirteen oligomers succeeded to generate polymorphic DNA. Also, revealed that the total number of polymorphic amplicons was (29). This corresponds to level of polymorphism of (29.3 %). There were some specific markers at the molecular level, which could differential between Giza 80 cultivar and its off-types. These results showed differences in size and number of the amplified fragments per primer, indicating a high degree of variability between them. Based on the obtained data of PCR products and a phylogenetic tree, i.e. dendrogram was constructed for Giza 80 and its off-types. There are some degree of the genetic divergences between Giza 80 cultivar and its three off-types, Giza 80 T_1, Giza 80 T_2 and Giza 80 T_3 which were 20.3 %, 29.2 % and 29.9 %, respectively.

It could be concluded that the existence of the off-type plants among the true type variety was

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dangerous. Therefore, the successive production of pure seeds by the maintenance program, removing the off-type plants out of the general farms and minimizing the period of production and handing the certified seeds by the farmers would be helpful to avoid the occurrence of varietals deterioration.

Introduction:

Cotton is an economic crop of world importance. It is the world's leading fiber crop. Egyptian cotton (*Gossypium barbadense* L.) has a worldwide reputation because of its fiber length and fineness. In Egypt, yield and lint characteristics of cotton are considered the main properties in the cotton production and industry. Homogeneity and uniformity of such characters represent the practical criteria for identification and judging the purity of cotton cultivars. However, cotton cultivars production in the general farms, might be mistakenly, mixed by strange seeds or out-crossed by different genotypes, consequently changes in the homogeneity and uniformity and eventually some off-type plants are spontaneously induced the off-types are inferior cotton plants exist occasionally among commercial cotton varieties throughout the long period of their culture.

The importance of this study, as one of the main research point in the maintaining genetic purity among cotton genotypes was to recognize and study the off-type cotton plants, in which offer information of protection against degeneration of yield potentials and fiber quality. El-Shazly (1987) and Kamal *et al.* (1988), they found that the discolored cotton was associated with deterioration in fiber quality and lower yield components. El-Okkia *et al.* (1990) studied the variation between the standard type of Giza 70 and its off-type (Giza 70 brown locks). They concluded that the Egyptian cotton varieties included of-type cotton locks would cause lack of color uniformity depression of yield and quality, reduction of yarn strength and increment of waste in spinning processes. Abo-Arab *et al.* (2000), studied some Egyptian cotton varieties as well as their off-types (three off-types for each), they found that the results showed significant differences between the original parents and their derived off-types for fiber characters in both varieties, indicated that these changes appeared to be genetic alterations, one or more, mutant genes. Hemaida (2000) and Hemaida *et al.* (2006), studied the differences among the standard types of Giza 80 and Giza 83 with their off-type plants. The results showed that the off-type plants of Giza 80 gave considerably lower values for boll weight, lint percentage, seed index and fiber strength characters.

Molecular studies are useful tools for phylogenetic studies among different genetic resources (Suiter, 1988; Percy and Wendel, 1990; Wendel et al,

1991; Zhang et al., 1998; Jinggao, 1999). The randomly amplified polymorphic DNA (RAPD) [Welsh and McClelland, 1990] is used to identify genotypes and to detect molecular markers linked to certain gene of interest. Consequently used to detect the variations in molecular level between Giza 80 and its off-types.

The main objectives of the present investigation was to study the depression in yield and quality as affected by the existence of the off-type plants in the cotton variety Giza 80.

Materials and Methods

The present study was carried out during the two growing seasons of 2008 and 2009 at the farm of Sids Agricultural Research Station in Beni-Suef Governorate.

Materials:

The materials used in this study were the Egyptian cotton variety Giza 80 belonging to *Gossypium barbadense* L. as well as its off-types. The characteristics of the studied materials are shown in Table (1).

In 2007 growing season, random samples of seed cotton off-type plants of Giza 80 were picked from different general farms. During season 2008, the standard cultivar Giza 80 and its off-types were self-pollinated. In 2009 season, the standard variety and its three off-types were grown in a randomized complete block design with three replications. Each plot was three rows, the row was four meters long, 60 cm apart and inter hill spacing was 20 cm. Seedlings were thinned to two plants per hill. The recommended cultural practices were adopted throughout the growing season.

Methods:

A. Yield components and fiber properties:

A random sample of ten guarded plants of each type as well as the control (Giza 80) were chosen from each plot to determine boll weight (BW), seed cotton yield / plant (SCY/P), lint yield / plant (LY/P), lint percentage (L.P.) and seed index (SI). The fiber properties i.e., micronaire reading (Mic), Pressley index (PI), fiber length (FL), uniformity ratio (UR), yellowness (+b) and reflectance (Rd %) were measured at the Cotton Technology Research Division, Cotton Research Institute, Giza.

Table (1): Characteristics of the studied materials

Characters	Giza 80 S	Giza 80 T_1	Giza 80 T_2	Giza 80 T_3
Plant	Normal in size (plant height ranged from 130 – 145 cm) and medium internodes.	Tallest type with longest internodes (plant height ranged from 150 – 180 cm).	Taller than standard type and has long internodes (plant height ranged from 200 – 250 cm).	Tallest type with long-est inter-nodes (plant height ranged from 200 – 250 cm)
Leaf	Shiny green, the lobes are wrapped up with 2 – 3 nectar glands at the lower surface.	Normal area and with 2 – 3 nectar glands at the lower sur-face.	Green, large area and flatted lobs with 1 – 2 nectar glands at the lower sur-face.	Green, large area and flatted lobs with 2 – 3 nectar glands at the lower surface.
Boll	Large size, conical shape, shiny green and there are three large nectar glands at the base of the bracts.	Large size and with 2 - 3 nectar glands at the base of the bracts.	Small, globular shape , green and without nectar glands at the base of the bracts.	Small, shiny green and some-times any nectar glands at the base of the bracts.
Seed types	Big in size, dark brown and most seeds are tufted.	Completely fuzzy seeds.	Small, naked, black and thorny top seeds.	Small and naked to tufted seeds.
Lint color	Dark creamy lint	creamy lint	Dark cream lint	Light creamy lint

Giza 80 S = the standard cultivar Giza 80
Giza 80 T_1 = the first off-type.
Giza 80 T_2 = the second off-type.
Giza 80 T_3 = the third off-type.

Analysis of variance and Duncan's (1988) Multiple Range Test were conducted to determine the significant differences among the means at 5 % level of probability (Snedecor and Cochran, 1981).

B. Molecular genetic study:

B.1. *Extraction and purification of genomic DNA*:

Genomic DNA was isolated from young leaves following the CTAB according to Dellaporta *et al.* (1993) as modified by Porebski *et al.* (1997) and purified by the cesium chloride density gradient centrifugation method (Sambrook *et al.*, 1989).

B.2. *Estimation of DNA quantity and quality*:

DNA concentration was determined by diluting the DNA 1 : 5 in H_2O. The DNA samples were electrophoretacally analyzed in 0.7 % agarose gel against 10 µl of DNA size marker. This marker covers a range of DNA fragment size and a range of concentration. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of unknown DNA band with the different bands in the DNA size marker.

B.3. *Random amplified polymorphic DNA (RAPD)*:

RAPD – PCR Reactions and Thermo Cycling Profile and Detection of the PCR products:

RAPD amplification was performed as described by Williams *at al.*, (1990) with minor modifications. A set of 13 random 10 mer primers was used in the RAPD analysis (Table, 2). The amplification reaction was carried out in 25 µM total volume containing 1 x PCR buffer, 1.5 mM $MgCl_2$, 2m M dNTPS, 10 µl primer, 1 ul Taq DNA polymerase and 25 ng templates DNA. PCR amplification was performed in a perkin-Elmer / Gene Amp PCR system 9700 (PE Applied Biosystem). [The PCR program was follows:

An initial de-naturation step at 94°C for 5 min, followed by 40 cycles at 94°C for 1 min, 36°C for 1 min and 72° C for 2 min and a final extension cycle of 7 min at 72 °C. The amplification products were resolved by electrophoresis in 1.5 % agarose gels].

B.4. *Data analysis*:

The banding patterns generated by RAPD were examined to determine the level of polymorphism and genetic relatedness among the four cotton genotypes (Giza 80 and its off-types). The amplified fragments were scored as present (+) or absent (-). The genetic

Table (2): The primers codes, sequences and the percentage of the GC content.

	Primer code	Sequences 5' – 3'	GC %
1	OPA-03	AGTCAGCCAC	60 %
2	OPA-04	AATCGGGCTG	60 %
3	OPA-10	GTGATCGCAG	60 %
4	OPB-09	TGGGGGACTC	70 %
5	OPB-14	TCCGCTCTGG	70 %
6	OPB-15	GGAGGGTGTT	60 %
7	OPB-16	TTTGCCCGGA	60 %
8	OPB-17	AGGGAACGAG	60 %
9	OPB-18	CCACAGCAGT	60 %
10	OPB-19	ACCCCCGAAG	70 %
11	OPC-02	GTGAGGCGTC	70 %
12	OPG-14	GGATGAGACC	60 %
13	OPP-05	CCCCGGTAAC	70 %

similarity and similarity matrix among genotypes were estimated according to Dice Coefficient: GS (ij) = 2a / (2a + b + c), where GS (ij) is the measure of genetic similarity between individuals (i) and (j), (a) is the number of bands shared by (i) and absent in (j) and (c) is the number of bands absent in (i) and in (j) (Sneath and Sokal, 1973). Cluster analysis was based on similarity matrix obtained with unweighted pair group method using arithmetic average (UPGMA), and the relationships between accessions were displayed as dendrogram.

Results and Discussion

The results can be discussed as follows:

A. Morphological characteristics:

Field characters of standard cultivar Giza 80 compared with its corresponding off-type plants are illustrated in Table (1). It could be noticed that plant height, leaf size, boll shape, seed type and lint color characters are of prime importance to distinct the off-type plants. For instance, all off-type plants are taller than standard typ . Giza 80 T_2's leaves are green large area and flatted lobs. Its bolls are small, globular shape, green and without nectar glands at the base of the bracts. Giza 80 T_3's lint color is light creamy. While, Giza 80 standard's bolls are large size, conical shape, shiny green and its lint is dark creamy.

B. Yield and yield components:

Average of yield and its components are presented in Table (3). There were significant differences among Giza 80 and its off-type plants for most studied characters.

Table (3): Average performances of yield, its components and fiber properties for Giza 80 and its off-types.

Traits \ Genotypes	Giza 80 S	Giza 80 T_1	Giza 80 T_2	Giza 80 T_3
Boll weight (g)	3.5 a	3.2 b	2.6 c	2.8 c
Seed cotton yield / plant (g)	41.13 ab	41.02 ab	18.20 c	36.28 bc
Lint yield / plant (g)	15.64 ab	15.56 ab	5.06 c	11.92 b
Lint percentage %	38.02 a	37.93 a	27.80 c	32.86 b
Seed index (g)	11.6 ab	12.5 a	11.9 ab	11.2 b
Micronaire reading	4.2 c	4.6 bc	4.9 ab	5.2 a
Pressley index	9.6 a	9.1 b	8.6 c	8.4 c
uniformity ratio %	83.8 a	83.6 a	83.6 a	81.3 b
Fiber length at 2.5 % Span length	32.1 a	32.2 a	31.3 a	29.4 b
Yellowness	13.1 a	12.9 a	13.0 a	11.3 b
Reflectance percentage %	62.3 b	63.8 b	62.8 b	67.7a

Results showed that the off-type plants Giza 80 T_2 and Giza 80 T_3 gave bear smaller bolls compared with the normal type Giza 80 S. Giza 80 T_2 and Giza 80 T_3 gave the lowest means of seed cotton yield and lint yield compared with the standard Giza 80 S. Data of lint percentage (L.P.) revealed that the off-type cotton plants gave less lint percentage compared with the normal type Giza 80 S.

C. Fiber quality characteristics:

Results showed significant differences for micronaire value (Mic) and Pressley index among Giza 80 S, Giza 80 T_2 and Giza 80 T_3. The two off-types exhibited coarser fiber than the standard type Giza 80 S. However, uniformity ratio (U.R.) and fiber length (2.5 % SL) traits, results showed that the third type Giza 80 T_3 produced lower uniformity ratio and shorter lint than all the other genotypes. With respect to the lint color properties, it is apparent that the discolored type Giza 80 T_3 had relatively higher reflectance (Rd %) and lower chroma (+b) values than the corresponding standard type Giza 80 S.

Generally, the results showed that the three off-types of Giza 80 gave the lowest means of yield, yield components and fiber quality compared with the standard Giza 80. In the same time, the third off-type had shorter and coarser fiber compared with other types.

D. Molecular genetic studies:

Plant molecular geneticists currently use RAPD markers routinely to identify genetic variations (Keil and Griffin, 1994; Perron *et al.*, 1995 Lashermes *et*

al., 1996; Irwin *et al.*, 1998; Sun *et al.*, 1998; Hussein *et al.*, 2002 and 2006.

In the present study, the genetic variability among different genotypes of *Gossypium barbadense* (standard type Giza 80 and its off-types) based on RAPD analysis has been studied. Initial screening of 13 random primers that produced informative and polymorphic products resolvable by agarose gel electrophoresis was done as shown in Figures (1 – 4) and Tables (4 – 7). The number of polymorphic fragments ranged from 0 to 6. A maximum number of (13) amplicons were amplified with primer OPP-05, while the minimum number of fragments (7) was amplified with primer OPB-17. The highest number of polymorphic bands (6) was obtained with primer OPP-05, which exhibited the highest percentage (46.2 %) of polymorphism. Also, revealed that the total number of polymorphic amplicons obtained by the ten studied primer was (29). This corresponds to a level of polymorphism of 29.3 % and an average number of polymorphic fragments / primer of 2.9.

The comparison between the standard type Giza 80 and its off-types showed differences in the sizes and number of the amplified fragments per primer for genotypes, indicating a high degree of variability between them. The number and size of RAPD markers depend on the complementary of sequence of particular primer and the template DNA (Williams *et al.*, 1993).

Primer A-03:

It produced four common bands in all genotypes at MW of 400, 550, 650 and 800 bp. The other bands were polymorphic as they were present in some genotypes and absent in the others. Some genotypes had some specific bands and could be used to distinguish them. For instance Giza 80 S had two unique bands at MW 450 and 700 bp. Also, Giza 80 T_1 could be distinguishing from the other genotypes by the existence of one unique band at MW 350 bp. These unique bands specify the corresponding genotypes and each is said to be a positive molecular marker.

Primer A-04:

Results of RAPD analysis showed some genotypes had some specific bands and could be used to distinguish among them. For instance Giza 80 S and Giza 80 T_1 exhibited one unique band at MW 350 and 500 bp respectively, these bands were absent while present in all genotypes. Meanwhile, Giza 80 S could be distinguished from the other genotypes by the existence of two unique bands at MW 550 and 650 bp. There were 4 common bands in all genotypes at MW of about 300, 450, 700 and 750 bp.

Primer B-14:

This primer produced three common bands in all genotypes with MW of 400, 750 and 850 bp. Giza 80 T_3 showed two bands at MW of about 1000 and 1500 bp were absent while, present in

all other genotypes. The absence of these bands can be considered as negative genotypes markers which can be used to specify each genotype.

Table (4): The total number of RAPD-PCR fragments generated by a battery of 10 primers, total number of amplicons, polymorphic amplicons, percentage of polymorphism and their amplification efficiency in the standard cultivar G. 80 and its off-types.

Primer	Number of fragments in different genotypes				Total of DNA fragments	Total of amplicons	Polymorphic amplicons	% of polymorphism
	Giza 80 S	Giza 80 T_1	Giza 80 T_2	Giza 80 T_3				
OPA-03	8	7	4	4	23	9	3	33.3
OPA-04	10	8	6	6	30	11	4	36.4
OPB-14	8	8	6	3	25	9	3	33.3
OPB-15	7	9	8	7	31	10	1	10.0
OPB-16	3	4	7	6	20	8	2	25.0
OPB-17	4	5	7	6	22	7	0	0.0
OPB-18	9	10	8	7	34	11	2	18.2
OPB-19	7	3	5	5	20	9	4	44.4
OPC-02	10	8	8	10	36	12	4	33.3
OPP-05	5	8	7	4	24	13	6	46.2
Total	71	70	66	58	265	99	29	29.3
Average	**7.1**	**7.0**	**6.6**	**5.8**	**26.5**	**9.9**	**2.9**	

Primer B-15:

Using primer B-15 resulted in detecting a total number of 31 bands, six bands at MW 480, 650, 750, 850, 1100 and 1500 bp were found in all genotypes. One unique positive band was found in Giza 80 T_1 with MW of 550 bp.

Primer B-16:

Its results indicated that three common bands in all genotypes with MW of 400, 600 and 700 bp. Some genotypes had some specific bands which could be used to distinguish them. Each of Giza 80 T_1 and Giza 80 T_2 shared one positive unique band at MW of 850 and 300 bp, respectively. The total number of fragments in different genotypes is 20 bands.

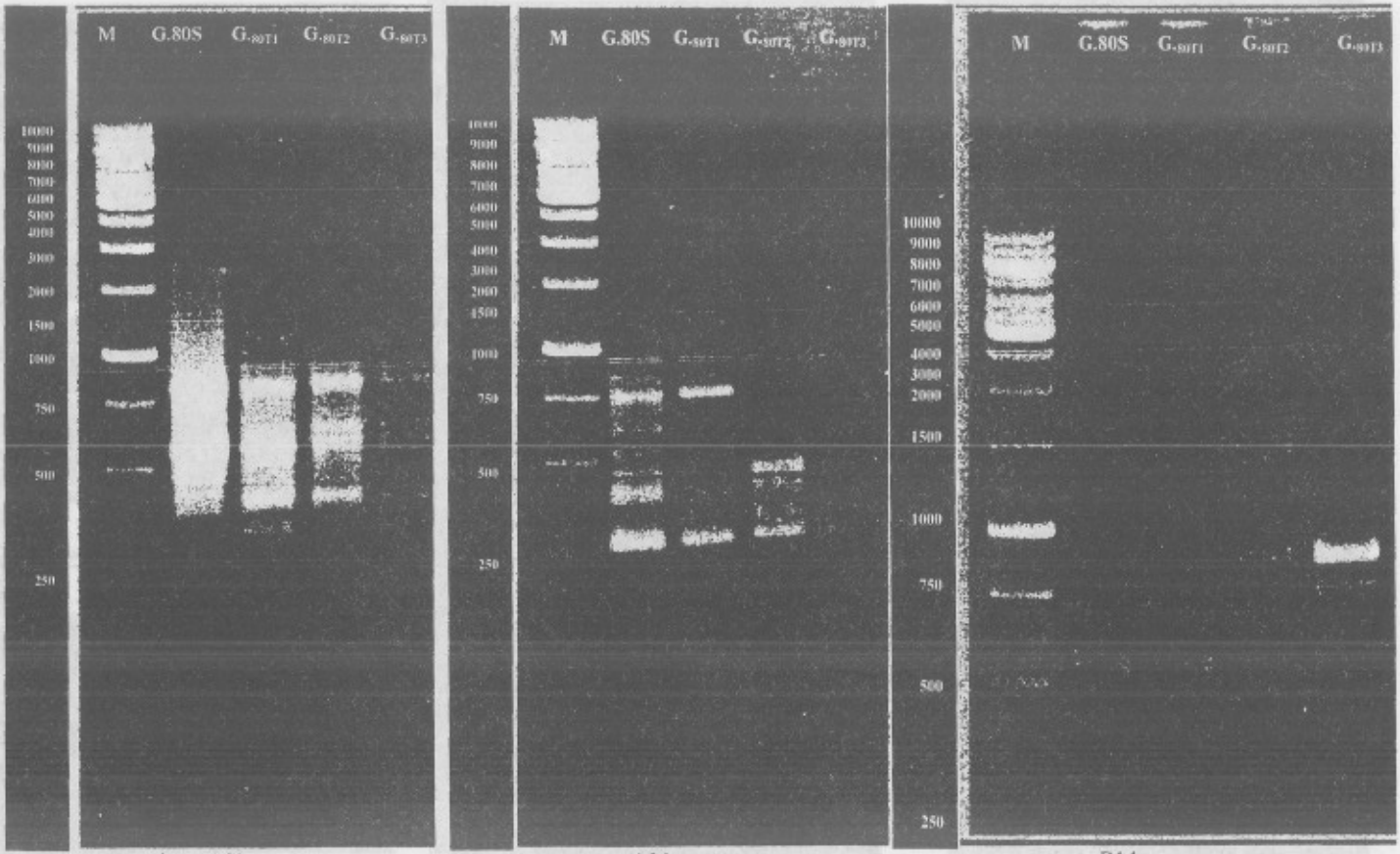
Primer B-17:

The results indicated that there were four common bands in all genotypes with MW of 450, 500, 550 and 700 bp. This primer produced a total number of 22 polymorphic bands in all genotypes. The minimum number was 4 bands in Giza 80 S and the maximum was 7 bands in Giza 80 T_2 with molecular weights range from 250 – 1300 bp.

Table (5): Molecular weights (in base pairs) of amplified DNA fragments that were produced by A03, A04, B14 and B15 primers for the standard cultivar Giza 80 and its off-types.

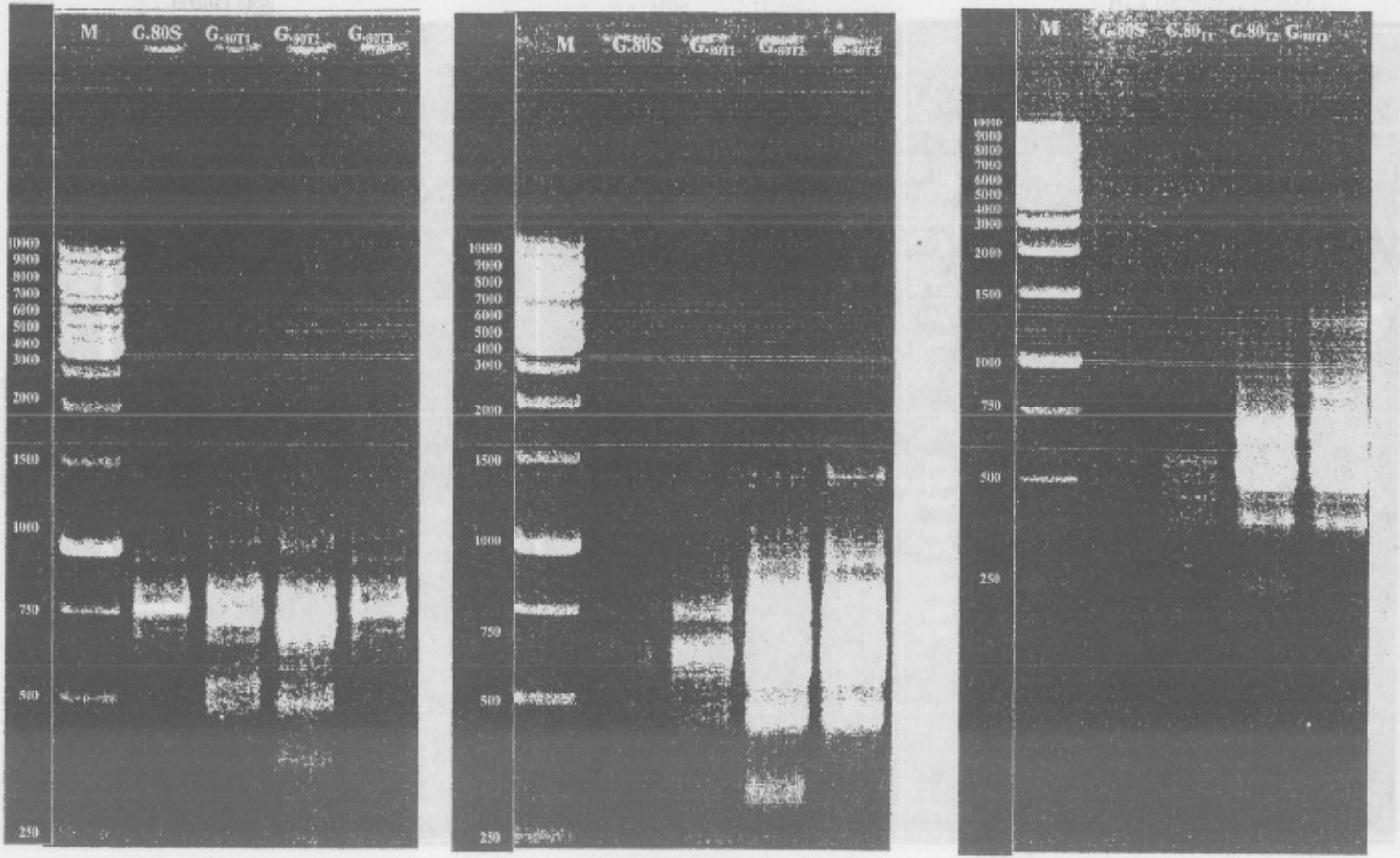
A03					A04				
MW (bo)	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**	**MW (bo)**	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**
800	+	+	+	+	1200	+	+	-	-
700	+	-	-	-	1000	+	+	-	-
650	+	+	+	+	800	+	+	-	-
550	+	+	+	+	750	+	+	+	+
500	+	+	-	-	700	+	+	+	+
450	+	-	-	-	650	+	-	-	-
400	+	+	+	+	550	+	-	-	-
350	-	+	-	-	500	+	-	+	+
300	+	+	-	-	450	+	+	+	+
B14					350	-	+	+	+
					300	+	+	+	+
2000	+	+	-	-	B15				
1500	+	+	+	-	1500	+	+	+	+
1000	+	+	+	-	1300	-	+	+	-
850	+	+	+	+	1100	+	+	+	+
750	+	+	+	+	1000	+	-	-	+
600	+	+	-	-	850	+	+	+	+
500	+	+	-	-	750	+	+	+	+
400	+	+	+	+	650	+	+	+	+
320	-	-	+	-	300	-	+	+	-

MW: Molecular weights (in base pairs).
Giza 80 S: The standard type Giza 80.
+ : Present. Giza 80 T_1: The first off-type.
- : Absent Giza 80 T_2: The second off-type.
Giza 80 T_3: The third off-type



primer A03 A04 B14

Figures (1): DNA polymorphism based on (RAPD) analysis for four genotypes against primers A03, A04 and B14, respectively.



Primer B15 primer B16 primer B17

Figures (2): DNA polymorphism based on (RAPD) analysis for four genotypes against primers B15, B16 and B17, respectively....

Primer B-18:

It produced six common bands in standard cultivar Giza 80 and its off-types at MW of about 350, 500, 750, 850, 1000 and 1200 bp. Some genotypes had some specific bands and could be used to distinguish them. For instance Giza 80 T_3, one positive (at MW of 900 bp) and one negative (at MW of 650 bp) markers were generated.

Primer B-19:

This primer produced one common band for Giza 80 and its off-types at MW of 650 bp. Standard cultivar Giza 80 could be distinguished from the other genotypes by the existence of two RAPD markers MW 450 and 550 bp. These unique bands specify the corresponding genotype and each is said to be a positive molecular marker. While, some genotypes exhibited of some specific bands and could be used to distinguish among them. For instance in Giza 80 T_3 two bands of MW of about 350 and 400 bp were absent, while present in all other genotypes.

Primer C-02:

The obtained results of primer C-O2, observed that the lowest number was eight bands in Giza 80 T_1 and Giza 80 T_2 while, the highest number was ten bands (for Giza 80 S and Giza 80 T_3). There were six common bands in Giza 80 and its off-types with MW of 150, 300, 400, 500, 650 and 700 bp. Four RAPD markers were generated for Giza 80 T_1 and Giza 80 T_3 in which two of them were negative. For Giza 80 T_1, one positive (at MW of 450 bp) and two negative (at MW of 250 and 350 bp) markers were generated. While, only one marker (at MW of 800 bp) was generated, that happened to be positive.

Primer P-05:

Using this primer produced two common bands between Giza 80 and its off-types. Six RAPD markers were generated for Giza 80 T_1, Giza 80 T_2 and Giza 80 S. For Giza 80 T_1 three positive (600, 1200 and 1500 bp) markers for Giza 80 T_2 two positive (250 and 350 bp) markers were generated. While, only one marker (1300 bp) was generated for Giza 80 S that happened to be positive.

D.1. *Specific markers for standard Giza 80 and its off-types by RAPD – PCR analysis:*

On the molecular level, RAPD – PCR exhibited variations between the standard Giza 80 and its off-types. From the observed results, it could be concluded that group B was more successful than any other genotype, for matching with the studied cotton genotypes (Table 8). Moreover primer B-16 was found to be the most efficient primer since it could distinguish two genotypes Giza 80 T_1 and Giza 80 T_2 by the existing of unique specific markers at MW of 850 and 300 bp, respectively. Also, primer B-19 could distinguish for the standard Giza 80 from the other genotypes (its off-types) by the existence of two unique bands were considered as posi-

tive markers at MW (450 and 350 bp), while Giza 80 T_3 could be distinguished by two unique specific negative markers at MW (350 and 400 bp). The obtained results of primer P-05, some genotypes had some specific bands and could be used to distinguish them. For instance, at MW of about 1300 bp for the standard Giza 80, at MW 600, 1200 and 1500 bp for Giza 80 T_1, also at MW 250 and 350 bp for Giza 80 T_2. These unique bands specify the corresponding genotypes and each are said to be a positive molecular markers.

As seen from Table (8), there were 29 specific markers for cotton genotypes scored and illustrated. These results indicated that the RAPD - PCR exhibited different unique molecular markers, as previously mentioned. For instance, primer A-03can distinguish two genotypes by distinct bands at MW of about 450 and 700 bp for the standard Giza 80 and 350 bp for Giza 80 T_1.

D.2. Genetic similarity and phylogenetic tree for RAPD – PCR analysis:

The genetic similarity and phylogenetic tree have categorized the standard Giza 80 and its off-types into two groups (Table, 9 and Figure 5). The first group contains the two genotypes Giza 80 and Giza 80 T_1 with similarity of 79.7 %, which are in the same cluster.

The second group was includes Giza 80 T_2 and Giza 80 T_3 with similarity of 82.9 %, this close similarity between these two different genotypes should be a matter of future research to explain the closely relationship between these genotypes which fall in

Table (6): Molecular weights (in base pairs) of amplified DNA fragments that were produced by B16, B17 and B18 primers for the standard cultivar Giza 80 and its off-types.

B16					B17				
MW (bo)	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**	**MW (bo)**	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**
1500	-	-	+	+	1300	-	-	+	+
950	-	-	+	+	900	-	-	+	+
850	-	+	-	-	700	+	+	+	+
700	+	+	+	+	550	+	+	+	+
600	+	+	+	+	500	+	+	+	+
550	-	-	+	+	450	+	+	+	+
400	+	+	+	+	250	-	+	+	-
300	-	-	+	-					
B18									
1200	+	+	+	+					
1100	+	+	-	-					
1000	+	+	+	+					
900	-	-	-	+					
850	+	+	+	+					
750	+	+	+	+					
650	+	+	+	-					
500	+	+	+	+					
400	+	+	-	-					
350	+	+	+	+					
250	-	+	+	-					

MW: Molecular weights (in base pairs).

\+ : Present.

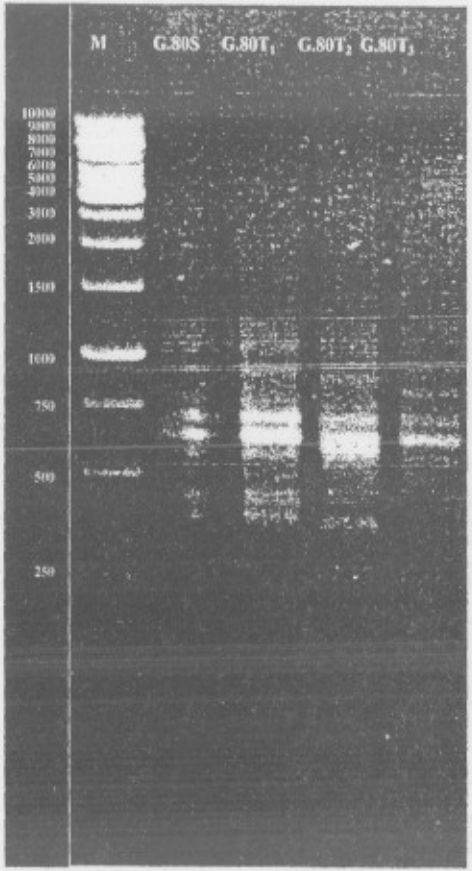
\- : Absent.

Giza 80 S: The standard type Giza 80.

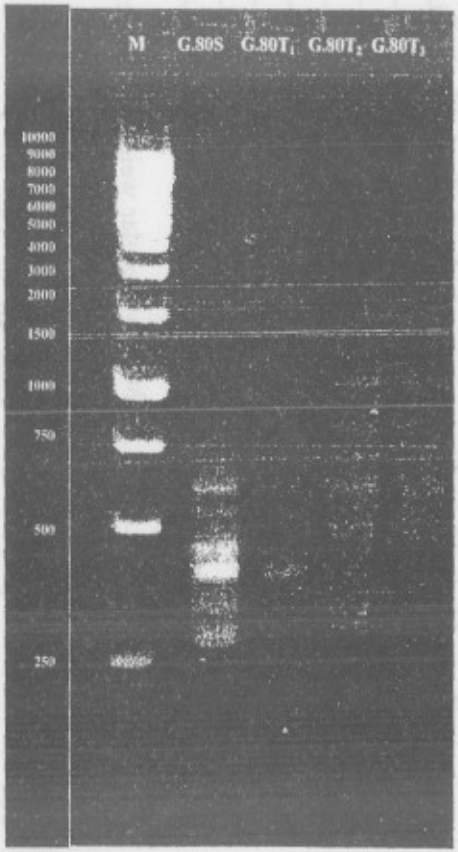
Giza 80 T_1: Giza 80 T_1: The first off-type

Giza 80 T_2: The second off-type

Giza 80 T_3: Giza 80 T_3: The third off-type.



Primer B18



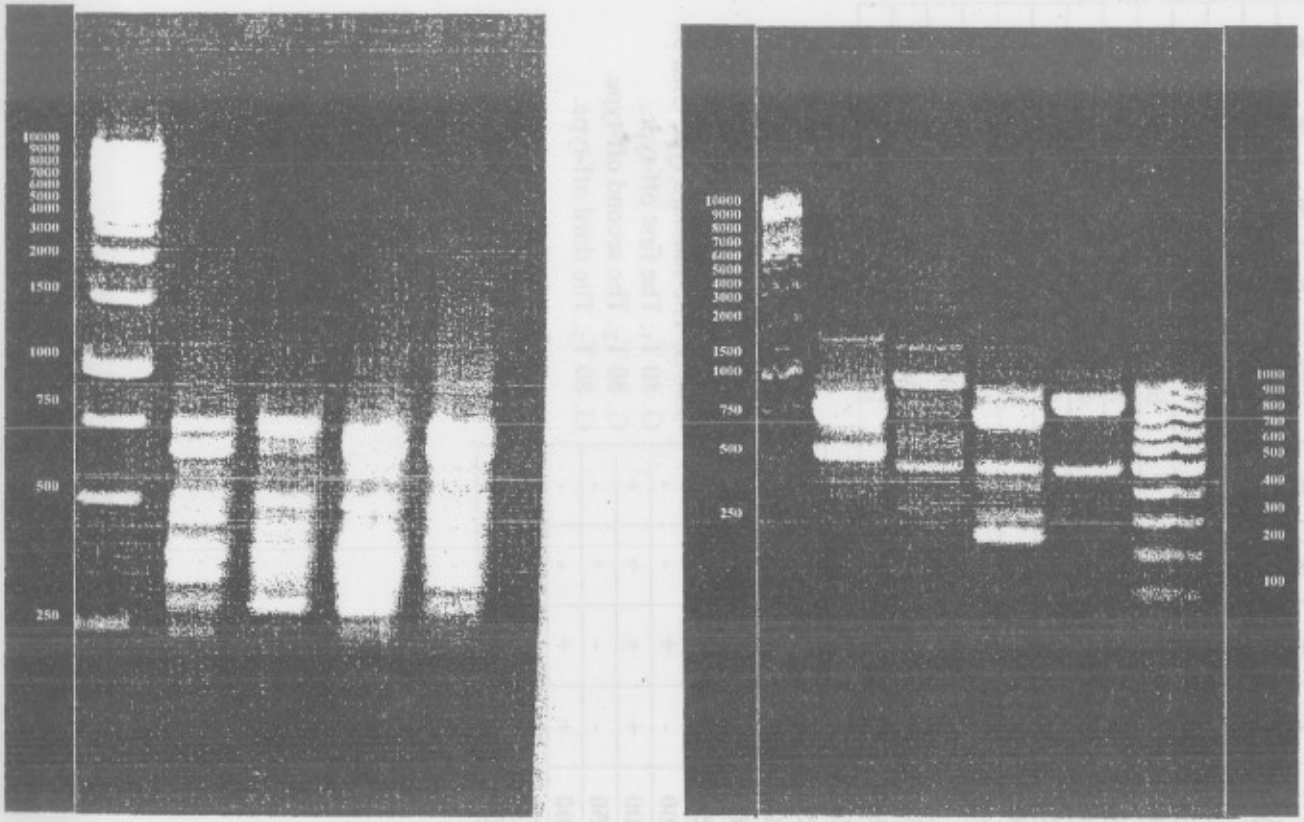
Primer B19

Figures (3): DNA polymorphism based on (RAPD) analysis for four genotypes against primers B18 and B19, respectively.

Table (7): Molecular weights (in base pairs) of amplified DNA fragments that were produced by B19, C02 and P05 primers for the standard cultivar Giza 80 and its off-types.

B19					C02				
MW (bo)	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**	**MW (bo)**	**G. 80 S**	**G. 80 T_1**	**G. 80 T_2**	**G. 80 T_3**
1000	-	-	+	+	800	-	-	-	+
650	+	+	+	+	750	+	+	-	-
550	+	-	-	-	700	+	+	+	+
500	-	-	+	+	650	+	+	+	+
450	+	-	-	-	500	+	+	+	+
400	+	+	+	-	480	+	-	-	+
350	+	+	+	-	450	-	+	-	-
300	+	-	-	+	400	+	+	+	+
200	+	-	-	+	350	+	-	+	+
P05					300	+	+	+	+
1500	-	+	-	-	250	+	-	+	+
1300	+	-	-	-	150	+	+	+	+
1200	-	+	-	-					
950	-	+	-	+					
900	-	-	+	+					
800	+	+	+	+					
750	+	-	+	-					
600	-	+	-	-					
500	+	+	+	+					
350	-	-	+	-					
300	+	+	-	-					
270	-	+	+	-					
250	-	-	+	-					

MW: Molecular weights (in base pairs).
G.80 S: The standard type Giza 80
G. 80 T_1: The first off-type.
G. 80 T_2: The second off-type.
G. 80 T_3: The third off-type.



Primer C02 Primer P05

Figures (4): DNA polymorphism based on (RAPD) analysis for four genotypes against primers C02 and P02.

different Egyptian cotton clusters. In Table (8) the genetic similarity showed the lowest value of 63.6 % between Giza 80 T_1 and Giza 80 T_2. The highest value recorded between Giza 80 S and its off-type Giza 80 T_1 was 79.7 %. Also the results observed that the genetic similarity among standard Giza 80 and its off-types population could be determined, which recorded ranged from 70.1 to 79.7 %.

Table (8): Molecular specific markers and their MW for standard type Giza 80 and its off-types produced by different primers of RAPD-PCR analysis.

Genotypes / Primers	Giza 80 S	Giza 80 T_1	Giza 80 T_2	Giza 80 T_3
A03	450 (+) 700 (+)	350 (+)	-	-
A04	350 (-) 550 (+) 650 (+)	500 (+)	-	-
B14	-	-	320 (+)	500 (-) 600 (-)
B15	-	550 (+)	-	-
B16	-	850 (+)	300(+)	-
B17	-	-	-	-
B18	-	-	-	650 (-) 900 (+)
B19	450 (+) 550 (+)	-	-	350 (-) 400 (-)
C02	-	250 (-) 350 (-) 450 (+)		800 (+)
P05	1300 (+)	600 (+) 1200 (+) 1500 (+)	250 (+) 350 (+)	

(+) : Positive marker, which is absent in all genotypes and present in one.

(-) : Negative marker, which is absent in one genotypes and present in all genotypes.

- : No marker detected.

Considering all the data gained in the present study from the RAPD-PCR analysis it can be concluded that there was some degrees of the genetic divergences between the standard Giza 80 and its three off-types Giza 80 T_1, Giza 80 T_2 and Giza 80 T_3, which were 20.3 %, 29.2 % and 29.9%, respectively. The complete identification was obtained for data gained from the RAPD–PCR analysis, yield components and fiber properties.

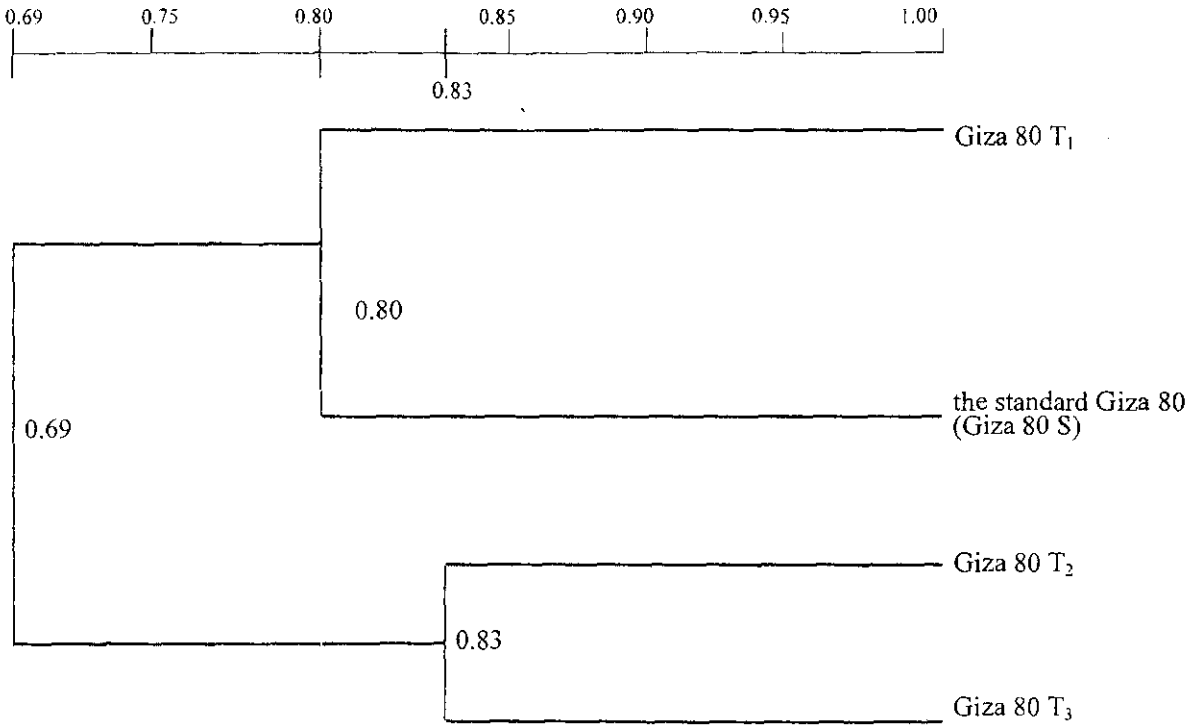


Figure 5 : Dendrogram demonstrating the relationship among four cotton genotypes (G. 80 and its off-types) across RAPD – PCR.

Generally from the previous results, it could be concluded that the source of Giza 80 off-types plants especially Giza 80 T_2 and Giza 80 T_3 which remarkably differed from the standard type Giza 80 S for most studied characters, might be a result of mixture by impure (strange) seeds, while the type Giza 80 T_1 which exhibit slightly differences from the standard type Giza 80 might be due to a late segregation of– out crossing with impure seeds or effect of mutation.

Also, it could be concluded that the existence of the off-type plants in commercial cultivar Giza 80 was dangerous and had very bad effects on the varietal purity beside field heterogeneity. These off-type plants had poor yield characteristics and led to inferior fiber properties. At the same time, the differences among Giza 80 with its off-type patterns were mainly affected by two factors; the first one was attributed to be cultivar and its off-type groups and second factor was concerning the ability of characters that might exhibit discrimination.

On the other hand, the production and renewal of pure seeds annually, rouging the off-type plants before and after blooming, to avoid the out crossing and minimizing the duration of production and handing the certified cotton seeds would be very helpful in maintaining Giza 80 cotton cultivar from deterioration.

Table (9): Matrix of the genetic similarity estimated among the studied four cotton genotypes (standard type Giza 80 and its off-types) based on RAPD-PCR analysis.

Genotypes	**Giza 80 S**	**Giza 80 T_1**	**Giza 80 T_2**	**Giza 80 T_3**
Giza 80 s	100.0			
Giza 80 T_1	79.7	100.0		
Giza 80 T_2	70.8	63.6	100.0	
Giza 80 T_3	70.1	73.5	82.9	100.0

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دراسات وراثية مقارنة لبعض الطرز المغايرة لصنف القطن جيزة ٨٠ وأثرها على تدهور صفات المحصول والجودة

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أجريت هذه الدراسة فى محطة البحوث الزراعية بسدس خلال الموسمين الزراعية ٢٠٠٨ ، ٢٠٠٩. وقد أستخدم فى هذه الدراسة الطرز المغايرة لصنف القطن المصرى جيزة ٨٠ والذى يزرع فى مصر الوسطى وذلك بهدف دراسة الصفات الاقتصادية لهذه الطرز وأثر ذلك على نقص وتدهور صفات المحصول والجودة للصنف جيزة ٨٠ .

وقد أجرى التوصيف الحقلى والمعملى للصنف جيزة ٨٠ والطرز المغايره له من حيث النمو ، والتزهير والمحصول ومكوناته ، وتوزيع الزغب على البذور بالإضافة إلى تقييم صفاتها المحصولية والتكنولوجية.

كما أستخدمت طريقة التضخيم العشوائى لـ DNA عن طريق جهاز تفاعل البلمرة المتسلسل RAPD-PCR باستخدام ثلاثة عشر بادئات عشوائية (كل منها مكون من عشر قواعد نيتروجينية) لتحديد الاختلاف والتباين وكذلك التغيرات الناتجة على مستوى الـ DNA بين نباتات صنف جيزة ٨٠ ونباتات الطرز المغايرة لهذا الصنف.

- اظهر التوصيف الحقلى لنباتات الطرز المغايرة أنها أطول وذات أوراق كبيرة الحجم ، كما وجد اختلاف فى شكل اللوزة ونسب توزيع الزغب على البذور عنها فى الطرز القياسى جيزة ٨٠ محل الدراسة.
- وقد أشارت النتائج أن نباتات الطرز المغايرة الثلاثة كانت أقل فى وزن اللوزة ، ومحصولى القطن الزهر والشعر ، ومعدل الحليج ، والمتانة (البرسلى) ، ومعامل الانتظام للشعيرات ، وطول التيلة ، بينما أعطت قيماً عالية فى قراءة الميكرونير (أقل نعومة) بالمقارنة بالصنف جيزة ٨٠.
- أوضحت الدراسة على المستوى الجزيئى بطريقة RAPD – PCR نجاح عشرة بادئات من الثلاثة عشر بادئات المختبره فى إنتاج وتقييم الاختلافات فى عدد الحزم للـ polymorphic DNA وكان أعلى عدد من الـ polymorphic (٦ حزم) نتيجة استخدام البادئ OPP-05 محققاً أعلى نسبة للـ polymorphism (٤٦,٢ %) وأن العدد الكلى للـ polymorphic amplicons كان ٢٩ حزمة بنسبة polymorphism ٢٩,٣ %. كما تمكنت هذه البادئات فى إيجاد بعض المعلمات الجزئية الخاصة ذات الحزم المميزة لبعض التراكيب والتى أظهرت اختلافات بين صنف القطن جيزة ٨٠ والطرز المغايرة له.
- كما أظهرت النتائج الاختلافات فى عدد وحجم الحزم الـ DNA الناتجة عن كل بادئ وهذا يشير إلى وجود درجة عالية من التباين بين التراكيب الوراثية محل الدراسة.
- وباستخدام النتائج المتحصل عليها من التباين ، ومن RAPD – PCR وشجرة القرابة أمكن تحديد العلاقات ودرجة القربة بين الصنف التجارى جيزة ٨٠ والطرز المغايرة له. فقد وجد أن درجات الإختلافات والتباعد الوراثى بين الصنف جيزة ٨٠ والثلاثة طرز المغايرة له G. 80 T_1 ، G. 80 T_2 ، G. 80 T_3 كانت ٢٠,٣ % ، ٢٩,٢ % ، ٢٩,٩ % على التوالى.
- ومن هذه الدراسة يتضح مدى خطورة تواجد هذه الطرز المغايرة والمختلفة وراثياً عن نباتات الصنف المنزرع جيزة ٨٠ ، ولذلك فإن إنتاج التقاوى النقية عن طريق برنامج المحافظة على النقاوة الوراثية لأصناف القطن المنزرعة وتجديد وإنتاج التقاوى النقية سنوياً ، وإزالة النباتات المخالفة فى صفاتها لطراز الصنف القياسى من مساحات إكثار تقاوى القطن النقية وتقليل مدة تداول السلالات عند المزارعين سوف يساعد على المحافظة على نقاوة التقاوى ومنع تدهور الأصناف.