

MOLECULAR GENETIC CHARACTERIZATION OF SOME OFF-TYPES FROM COTTON VARIETY GIZA 90

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ABSTRACT

Off-type plants of Giza 90 cotton cultivar were used in the present investigation to study some of their economic traits and their effects on varietal deterioration. Plants of Giza 90 variety as well as its off-type plants were grouped, characterized and evaluated in the field and the laboratory throughout three successive seasons. Also, molecular genetic fingerprinting using RAPD-PCR analysis technique was used for setting up a convenient and standard protocol to identify and differentiate among Giza 90 variety and its off-types. Field characterization of both standard and off-type cotton indicated that the off-type plants were taller, of larger leaves, with different boll shapes and seeds were naked. Significant difference between the standard type of Giza 90 and its off-type plants were found for boll weight, seed cotton yield, lint yield, lint percentage, Micronaire, fiber length, degree of yellowness and reflection degree. On the other hand, insignificant differences for Pressley and uniformity ratio were detected. In RAPD analysis, ten primers out of the tested thirteen oligomers succeeded to test the genetic purity of Giza 90 and its off-type which generated a total number of amplicons of 67 DNA fragments while, 44 bands (65.7 %) were polymorphic amplicons. There were unique bands in the standard type Giza 90 which were not observed in the two off-types. On the other hand, there were some unique bands in the off-type. Genetic similarities among Giza 90 and the its off-type were between 73.6 % and 59.1 %. The largest genetic distance was 40.9 % between Giza 90 and Giza 90 T₁, while the lowest distance was 26.4 % between Giza 90 and Giza 90 T₂. The genetic distance between Giza 90 and the two off-type is thought to be of divergent origin owing to modifier genes or migration into Giza 90 variety from another source especially after free marketing. Our results indicated that, existence of the off-type plants among the true type variety is dangerous. Therefore, removing the off-type plants out of the farmer field and minimizing the period of production and handling the certified seeds by the farmers would be helpful to avoid the occurrence of varietal deterioration.

Key Words: *Off-types, Egyptian cotton, RAPD, Fingerprinting, Fiber traits, Polymorphism, Genetic similarity.*

INTRODUCTION

Cotton is the most important fiber crop in the world as well as in Egypt. Increasing cotton production and quality is an urgent national goal to meet the consistent demand for this crop. Homogeneity of such characters represent the practical criteria for identification and judging the purity of the cotton. In Egypt, cotton cultivars are exposed to different factors which cause deterioration such as mechanical mixing, natural crossing, mutations and the change of gene frequency by genetic drift and natural selection. Consequently changes in the homogeneity and uniformity could eventually occur due to some off-type plants that are spontaneously induced.

Description of the off-type plants and the study of their economic characters are very helpful to impede the varietal deterioration. El-Shazly (1987) and Kamal *et al* (1988) 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 and concluded that the Egyptian cotton variety including off-type cotton locks would cause lack of color uniformity, depression of waste in spinning processes. Abo-Arab *et al* (1990), Fayed *et al* (1990) and Abo-Arab *et al* (1992) studied the lint colour of off-types isolated from Giza 70, Giza 76 and Giza 77 as well as F₁ and F₂ progenies derived from crossing these off-types with their original varieties. They found that these changes in lint colour appeared to be under polygenic nature and additive genetic effects. 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, while the discoloration type of Giza 83 exhibited later maturity and coarser fiber compared to the standard type. Evaluation of the off-type plants for some economic characters compared to the true type of Giza 90 would require an immediate DNA analysis to find markers that facilitate the scoring of the characteristics. Molecular genetic fingerprinting is quite useful tools for phylogenetic studies among different genetic resources (Suiter 1988, Percy and Wendel, 1990, Wendel *et al* 1991, Zhang *et al* 1998 and Jinggao *et al* 1999). The randomly amplified polymorphic DNA (RAPD) is used identify genotypes and to detect molecular markers linked to a certain gene of interest, (Williams *et al* 1990, Brubaker and Wendel 1994, Multani and Lyon 1995, Iqbaleta 1997, Liu *et al* 2000, Abdel Ghany and Zaki 2003, Qureshi *et al* 2004, Hussein *et al* 2006 and Abd El-Salam *et al* 2010).

The main objectives of the present study were to identify and differentiate the standard cotton variety Giza 90 and its off-types for yield and fiber quality traits and molecular characterization as well as to determine some specific markers at the molecular level.

MATERIALS AND METHODS

Materials

The present investigation was carried out at the farm of Sids Agricultural Research Station in Beni-Suef Governorate, the Department of Cotton Breeding, Cytology and Genetics Unit, Cotton Research Institute and Agricultural Genetic Engineering Research Institute, Agricultural Research Center, Giza, Egypt during the three seasons of 2008, 2009 and 2010. The materials used in this investigation were the Egyptian long staple variety of

cotton "Giza 90" belonging to *Gossypium barbadense* L. as well as its two off-types.

The off-type plants were taller than the standard type "Giza 90", isolated from seed production area in Sohag Governorate (Table 1). The first off-type (Giza 90 T₁), produces white lint cotton. The second off-type (Giza 90 T₂) has dark creamy lint and its seeds were naked.

Methods

The off-type plants of Giza 90 were picked from different farmer fields in 2007 season. They were cultivated in seasons 2008 and 2009. During this period some characters were recorded and laboratory examination for lint and seed type was made. In 2010 season, the standard variety "Giza 90" and its two off-types (Giza 90 T₁ and Giza 90 T₂) were included in a comparison experiment; it was a randomized complete block design with three replications. Each plot contained three rows, four meters long, 60 cm apart and intra hill spacing was 20 cm. The hills were thinned to two plants per hill. All agricultural practices were applied according to the recommendations.

Table 1. Characteristics of the standard and off-type cotton plants of Giza 90.

Characters	Giza 90 S	Giza 90 T ₁	Giza 90 T ₂
Plant height	Ranged from 120 – 130 cm	Ranged from 150 – 180 cm.	Ranged from 150 – 180 cm.
Leaf	Small in size and the lobes are wrapped up the lower surface has 2 – 3 nectar glands.	The leaves are larger than Giza 90 S and has 1 – 2 nectar glands at the lower surface.	The leaves are of similar size to those of Giza 90 S and has 1 – 2 nectar glands at the lower surface.
Boll	Small conical shape, dark green and one nectar gland is found mostly at the base of bracts.	Conical shape with 1 - 2 nectar glands at the base of the bracts.	Conical size, shiny green and 1 - 2 nectar glands at the base of bracts.
Seed type	Small size, dark brown and most seeds are tufted to 1/4 fuzz.	Dark brown naked seeds	Seeds are naked
Lint color	Light creamy.	White lint	Dark creamy

Giza 90 S = the standard cultivar Giza 90

Giza 90 T₁ = the first off-type.

Giza 90 T₂ = the second off-type.

Yield components and fiber properties

A representative sample of ten guarded plants of each type as well as the control was Taken from each plot to estimate boll weight (BW), seed cotton yield / plant (SCY/P), lint yield / plant (LY/P), lint percentage (LP) and seed index (SI). The micronaire value (Mic), Pressley index (PI), fiber length (FL), uniformity ratio (UR), yellowness (+b) and reflectance ratio (Rd %) were measured as individual plants in Cotton Technology Research Division, Cotton Research Institute, Giza.

Analysis of variance and F test were performed for the studied characters. Duncan's (1988) in multiple range test was conducted to determine the significant differences among the means at 5 % level of probability (Snedecor and Cochran 1981).

Molecular genetic analysis

Genomic DNA extraction and purification

DNA extraction was done according to Porebski *et al* (1997), the genome was isolated from young leaves following the CTAB and purified by the cesium chloride density gradient centrifugation method (Sambrook *et al* 1989) with some modified as described by Hussein *et al* (2003).

Estimation of DNA concentration

The concentration of DNA was measured according to the following equation:

$$\text{DNA conc. } (\mu\text{g} / \mu\text{l}) = \text{O.D 260} \times \text{dilution} \times 0.05$$

The purity of DNA is determined from the ratio:

$$\text{Pure DNA} = \text{O.D 260} / \text{O.D 280} = 1.7 - 2.0$$

Preparation of PCR reactions:

Random amplified polymorphic of DNA from cotton samples was carried out according to the procedure given by Williams *et al* (1990) with some modifications. The amplification reaction was carried out in a volume of 50 μl containing 250 ng of genomic DNA as a template. The PCR mixture per genotype consisted of the following:

Component	Amount for one PCR reaction
2 mM dNTPs	5 μl
50 mM MgCl ₂	2 μl
10 x PCR buffer	5 μl
10 μM primer	4 μl
5 U / N1 Taq. Pol, neraz	1 μl
250 ng / μl template DNA	6 μl
H ₂ O (d.w)	27 μl
Total volume	50 μl

PCR program and temperature profile

Amplification of the DNA was performed by placing the tubes containing the reactions in Perkin Elmer thermal cycler 2400 RAPD-PCR performed in 50 µl reaction volumes for 40 cycles. After the reaction mixture was mixed with DNA loading buffer and electrophoresed on 1.5 % agarose gel. The program was as follows: 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 (infinite).

Gel electrophoresis

The amplification products were resolved by electrophoresis in a 1.5 % agarose gel at 80 V for about 2 hours. PCR products were visualized by staining gel ethidium bromide. Bands were detected on UV transilluminator and photographed by gel documentation.

RAPD analysis

The banding patterns generated by RAPD analysis using 13 primers (Table 2) were compared to determine the genetic relatedness of the standard Giza 90 and its off-types. Clear and distinct amplification products were scored as (+) for presence and (-) for absence of bands. The genetic similarity and similarity matrix among genotypes were estimated according to Sneath and Sokal 1973.

Table 2. The sequences of primers used for RAPD analysis and the percentage of GC content.

	Primer code	Sequences 5' – 3'	GC %
1	OP-A06	GGTCCCTGAC	70 %
2	OP-A13	CAGCACCCAC	70 %
3	OP-C01	TTCGAGCCAG	60 %
4	OP-C02	GTGAGGCGTC	70 %
5	OP-C05	GATGACCGCC	70 %
6	OP-C06	GAACGGACTC	60 %
7	OP-C08	TGGACCGGTG	70 %
8	OP-C09	CTCACCGTCC	70 %
9	OP-D06	ACCTGAACGG	60 %
10	OP-D09	CTCTGGAGAC	60 %
11	OP-D12	CACCGTATCC	60 %
12	OP-G14	GGATGAGACC	60 %
13	OP-M14	AGGGTCGTTC	60 %

RESULTS AND DISCUSSION

Morphological characteristics

As shown in Table (1), some characters of the standard cultivar Giza 90 were compared with its corresponding off-type plants. It could be noticed that, the plant height, leaf size, boll shape, seed type and lint color characters are of prime importance to characterize the off-type plants. Giza 90 T₁'s leaves were larger than those of Giza 90 S of dark brown naked seeds and white lint. While, Giza 90 T₂'s lint was dark creamy.

Mean yields and yield components are presented in Table (3). Data showed significant differences among Giza 90 and its off-type plants for all studied characters. Concerning boll weight, results showed that the off-type plants; Giza 90 T₁ and Giza 90 T₂ bear smaller bolls compared with the normal type Giza 90 S. With respect to the other yield components, namely seed cotton yield, lint yield and lint percentage, considerable reductions regarding these traits were found in Giza 90 T₁ of about 21.4 g decrease in SCY, 10.8 g decrease in LY and 9.5 % decrease in LP. These results illustrate that regarding Giza 90 S, the out crossing with both off-types may incur high reduction in these traits. Therefore, these yield components are very important for detecting the varietal degeneration. El-Okkia *et al* (1990), Hemaida (2000) and Hemaida *et al* (2006) obtained similar results in their study of Giza 70, Giza 80 and Giza 83 varieties, respectively, they showed a considerable reduction of the off-type plants for yield components.

Means of some fiber properties are given in Table (3). The results showed significant differences for micronaire value among Giza 90 S and its off-type plants. The two off-types, exhibited coarser fiber than the standard type. Concerning the Pressley index and uniformity ratio, results showed that the standard type Giza 90 S produced stronger lint and larger uniformity ratio than its two off-type plants. Also, data showed significant differences for fiber length (FL) among Giza 90 S and its off-type. The off-type plants Giza 90 T₁ and Giza 90 T₂ gave less fiber length compared with the normal type Giza 90 S. With respect to the lint color properties, it is apparent that the discolored type Giza 90 T₁ had relatively higher reflectance (RD %) and lower chroma (+b) values than the standard type Giza 90 S.

On the other hand, the second off-type Giza 90 T₂ had considerably lower reflectance (Rd %) and higher chroma (+b) values than the corresponding standard type Giza 90 S. The present findings were in harmony with those obtained by El-Mansy *et al* (2008) and Abd El-Salam *et al* (2010). They found inferior quality characters associated with the discoloration of cotton.

Table 3. Mean of yield, its components and fiber properties for Giza 90 and its off-types.

Traits	Genotypes	Giza 90 S	Giza 90 T ₁	Giza 90 T ₂
Boll weight (g) (BW)		3.0 a	2.2 b	2.5 ab
Seed cotton yield / plant (g) (SCY)		45.8 a	24.1 c	33.6 b
Lint yield / plant (g) (LY)		17.9 a	7.1 c	11.7 b
Lint percentage % (LP)		38.9 a	29.4 c	34.9 b
Seed index (g) (SI)		9.8 b	11.1 a	10.2 b
Micronaire reading (Mic)		4.0 b	4.7 a	4.4 a
Pressley index (PI)		10.2 a	9.2 a	9.4 a
uniformity ratio % (UR)		87.4 a	84.7 a	86.9 a
Fiber length at 2.5 % Span length (FL)		30.6 a	27.5 b	29.6 ab
Yellowness (+b)		11.9 b	10.5 c	12.6 a
Reflectance percentage % (Rd)		67.3 b	71.5 a	62.7 c

From the previous results, it could be concluded that the existence of off-type plants in the commercial cultivar is dangerous and had very bad effect on the varietal purity beside plant heterogeneity. These off-types had poor yield characteristics and led to inferior fiber properties. 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 handling the certified cotton seeds would be very helpful in maintaining the Egyptian cultivars from deterioration.

RAPD – PCR analysis

Thirteen decamer RAPD primers (Table 2) were screened with the DNA of the standard type Giza 90 and its off-types. Any fragment thought to be artifact or difficult to be scored was not included in the data set. Ten primers generated reproducible and scorable RAPD profiles. These produced multiple band profiles with a number of amplified DNA fragments ranging from 4 to 9 (Table, 4 and Figures, 1 three 4). The total number of fragments produced by the ten primers was 67 with an average of 6.7 fragments / primer. While, the number of polymorphic fragments ranged from 1 to 8. A maximum number of 9 amplicons was amplified with primers OP-C09 and OP-D06, while the minimum number of fragments (4) was amplified with primer OP-C01. The highest number of polymorphic bands (8) was obtained with primers OP-C09 and OP-D06, which exhibited the highest percentage (88.9 %) of polymorphism. However, the lowest level of polymorphism was 20 % in primer OP-D12. Also, Table (4) revealed that the total number of polymorphic amplicons obtained by the ten studied primers was 44. This corresponds to a level of polymorphism of 65.7 % and an average number of polymorphic fragments / primer of 4.4. In

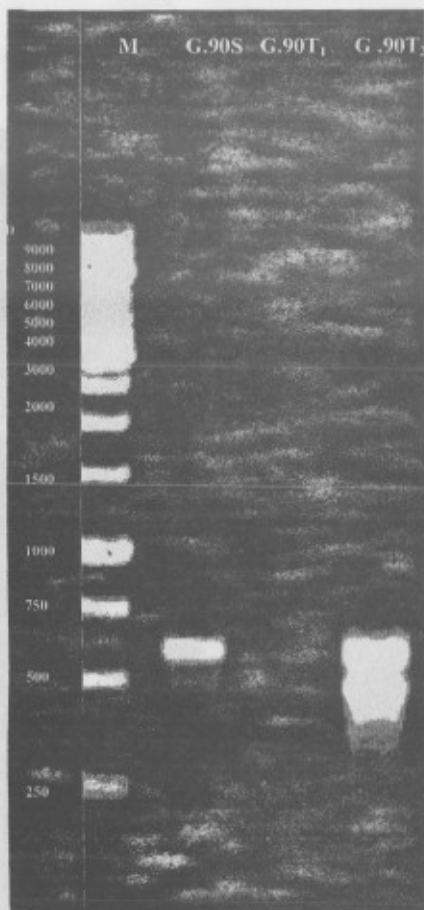
Table 4. Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD-PCR analysis among the standard type Giza 90 and its off-types.

Primer	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphic
OP-C01	4	2	2	50
OP-C02	7	2	5	71
OP-C05	6	3	3	50
OP-C06	7	5	2	28.6
OP-C08	5	1	4	80
OP-C09	9	1	8	88.9
OP-D06	9	1	8	88.9
OP-D09	8	2	6	75
OP-D12	5	4	1	20
OP-M14	7	2	5	77.4
Total	67	23	44	65.7
Average	6.7	2.3	4.4	

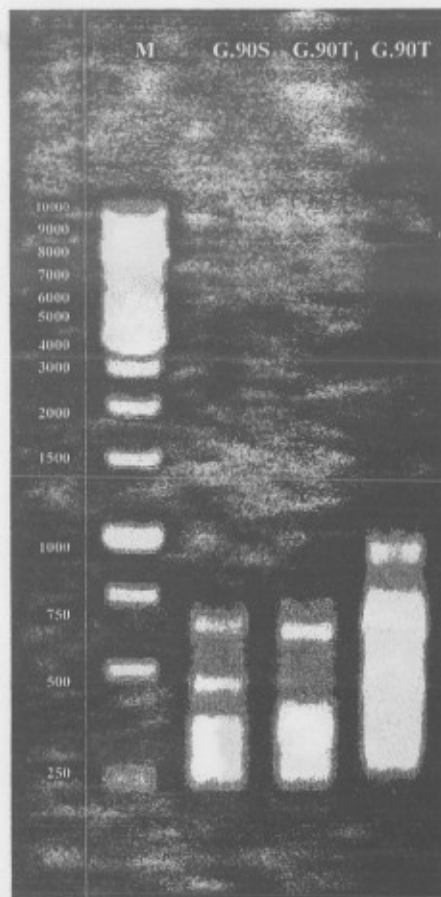
this respect, Tatineni *et al* (1996) studied the level of polymorphism among 19 cotton genotypes using 27 random primers and found that 33.8 % of the primers revealed monomorphic patterns. On the other hand, working on 31 *Gossypium* species, three subspecies and one inter-specific hybrid, Khan *et 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 %, Abd El-Salam *et al* (2010) found 67 bands, 85% were polymorphic among Giza 70 and its off-types as revealed by RAPD.

Specific markers for Giza 90 and its off-types by RAPD- PCR analysis

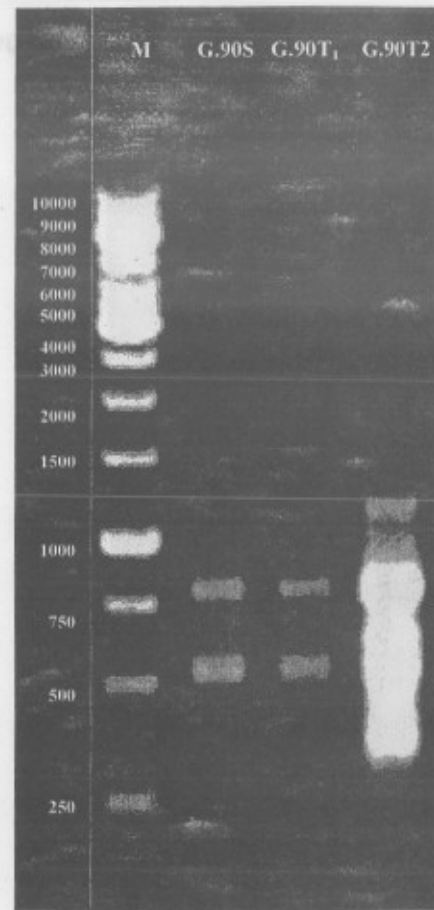
The data gained from the RAPD- PCR analysis, differential between off-type plants and the variety Giza 90 through the studied criteria. Some molecular markers were detected for some of the studied genotypes. When the data of the used 10 primers were combined, complete identification was obtained for the studied genotypes. Some of these primers were more successful such as OP-C02, OP-C08, OP-C09, OP-D06, OP-D09 and OP-M14, where they generated a high number of RAPD markers, (Table 5). The largest number of specific markers was generated by the two primers OP-C09 and OP-D06 (8 markers) while, the lowest number was generated by primer OP-D12 (1 marker).



primer OP-C01

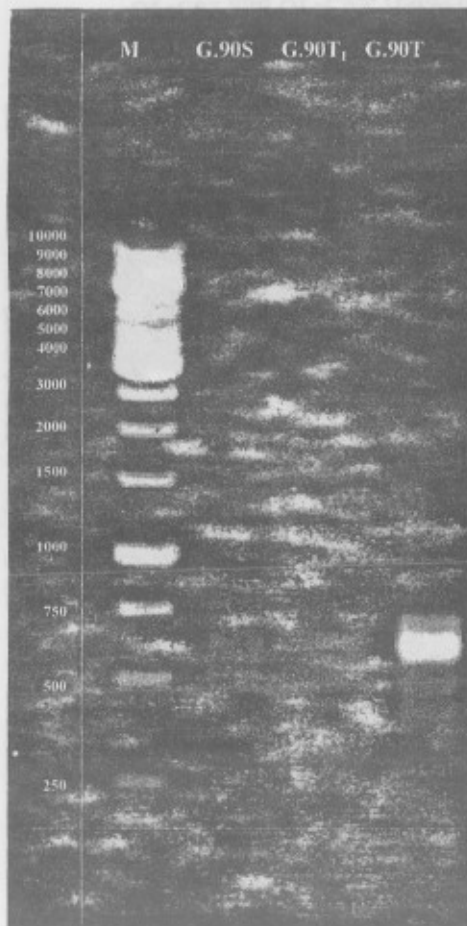


primer OP-C02

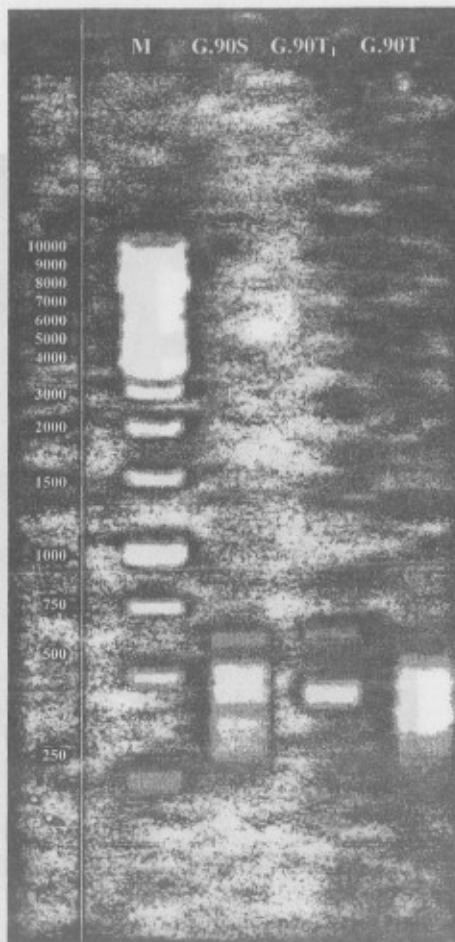


primer OP-C05

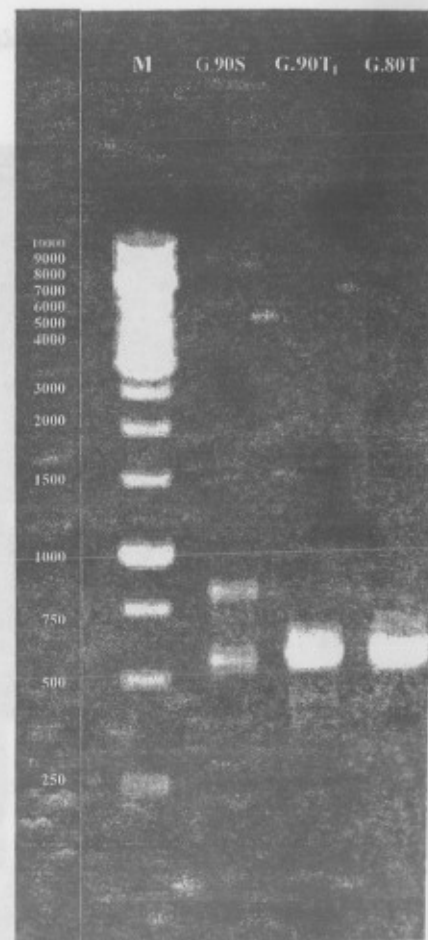
Fig.1. DNA polymorphism based on (RAPD) analysis for the standard variety Giza 90 and its off-types against primers OP-C01, OP-C02 and OP-C05, respectively.



Primer OP-C06

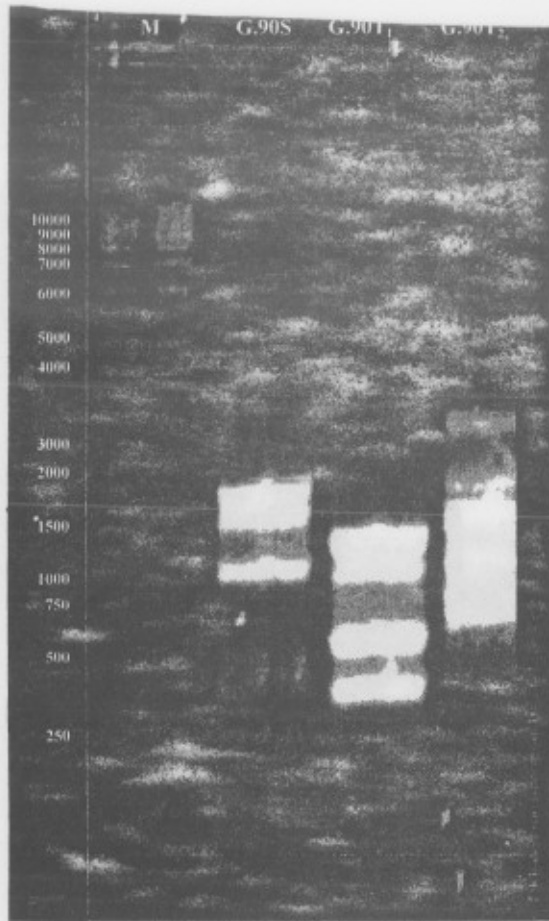


primer OP-C08

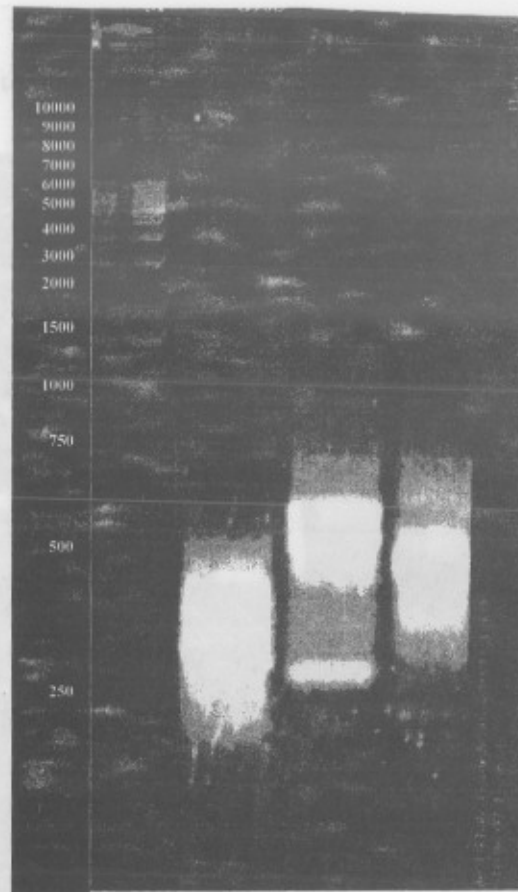


primer OP-C09

Fig.2. DNA polymorphism based on (RAPD) analysis for the standard variety Giza 90 and its off-types against primers OP-C06, OP-C08 and OP-C09, respectively.

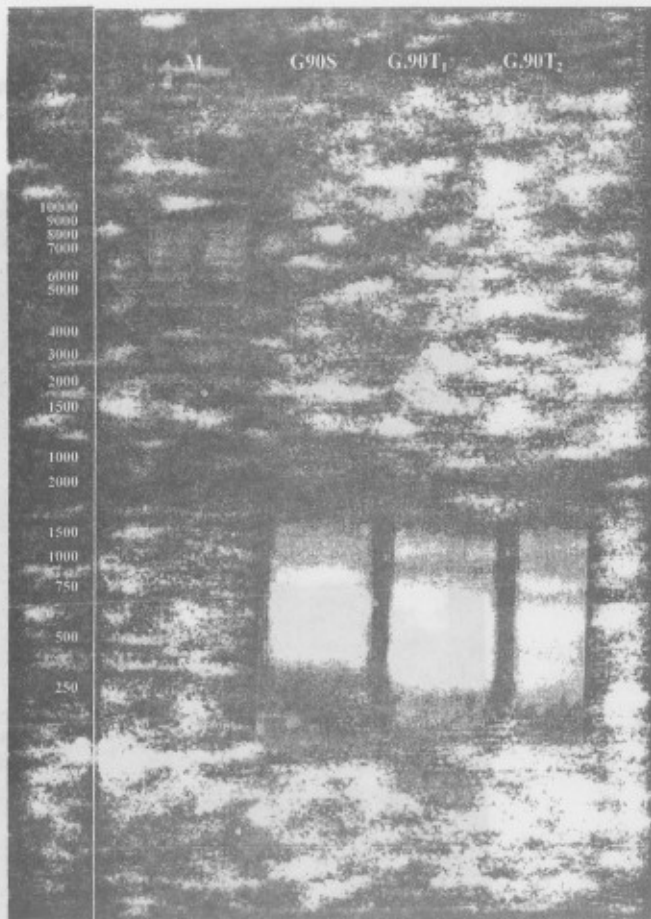


Primer OP-D06

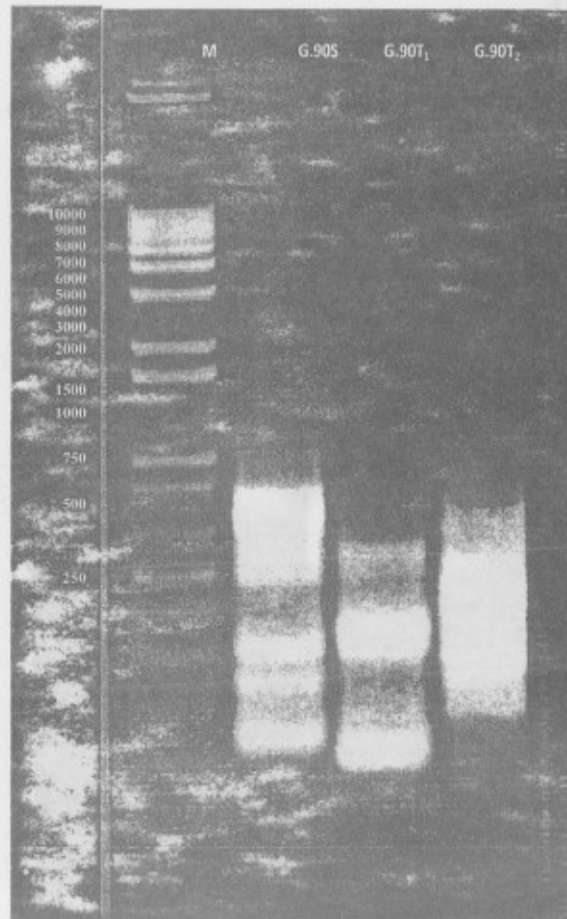


Primer OP-D09

Fig.3. DNA polymorphism based on (RAPD) analysis for the standard variety Giza 90 and its off-types against primers OP-D06 and OP-D09, respectively.



Primer OP-D12



Primer OP-M14

Fig4. DNA polymorphism based on (RAPD) analysis for the standard variety Giza 90 and its off-types against primers OP-D12 and OP-M14, respectively.

As seen from Table (5), there were 44 specific markers for Giza 90 and its off-types scored and illustrated. These results indicated that the RAPD-PCR exhibited different unique molecular markers, as previously mentioned. For instance, primer OP-C08 could distinguish between Giza 90 S and its off-type plants by distinct bands at Mw of about 300 and 500 bp for Giza 9, 400 bp for Giza 90 T₁ and 650 bp for Giza 90 T₂.

Table 5. Molecular specific markers and their MW for the standard type Giza 90 and its off-types produced by different primers of RAPD-PCR analysis.

Genotypes Primers	Giza 90 S	Giza 90 T ₁	Giza 90 T ₂
OP-C01	250 (-)	500 (-)	-
OP-C02	-	500 (-)	200 (-) 250 (-) 300 (+) 1000 (+)
OP-C05	-	-	300 (+) 600 (+) 1000 (-)
OP-C06	-	-	700 (+) 900 (+)
OP-C08	300 (+) 500 (+)	400 (-)	650 (-)
OP-C09	600 (-)	200 (+) 350 (+) 400 (+)	800 (-) 900 (+) 1000 (+) 1100 (-)
OP-D06	350 (+)	300 (+) 400 (+) 800 (+) 900 (-) 1000 (-)	500 (+) 1500 (+)
OP-D09	1000 (-)	200 (-) 300 (+) 500 (-) 750 (+) 800 (+)	-
OP-D12	-	300 (+)	-
OP-M14	1200 (+)	400 (-) 800 (+) 1000 (-)	300 (-)

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

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

- : No marker detected.

Genetic distances

Genetic similarities among Giza 90 and its off-types based on RAPD data are shown in Table (6) and dendrogram (Fig. 5). The highest similarity was 73.6 % between Giza 90 S and Giza 90 T₂, while Giza 90 was genetically distant from Giza 90 T₁ (similarity index of 59.1 %).

From the dendrogram, utilizing RAPD analysis Fig (5) divided the three genotypes into two main clusters. Giza 90 T₁ was in a separate cluster. The second cluster included two cotton genotypes (Giza 90 S and Giza 90 T₂) with a similarity 73.6 %. 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 distances between the standard Giza 90 and its the two off-types Giza 90 T₁ and Giza 90 T₂ which were 40.9 % and 26.4 %, respectively.

Table 6. Genetic similarity matrices among the standard type Giza 90 and its off-types as computed according to Dice coefficient from RAPD.

Genotypes	Giza 90 S	Giza 90 T ₁	Giza 90 T ₂
Giza 90 s	100.0		
Giza 90 T ₁	59.1	100.0	
Giza 90 T ₂	73.6	68.2	100.0

The complete identification was obtained for data gained from the RAPD analysis, yield components and fiber properties.

In general, from the previous results, it could be concluded that the source of Giza 90 off-type plants especially Giza 90 T₁ which remarkably differed from the standard type Giza 90 for most studied characters, may be a result of mixture by impure seeds, while the type Giza 90 T₂ which exhibit slight differences from the standard Giza 90 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 the commercial cultivar Giza 90 was dangerous and had very bad effects on the varietal purity beside plant heterogeneity. These off-type plants had poor yield characteristics and led to inferior fiber properties.

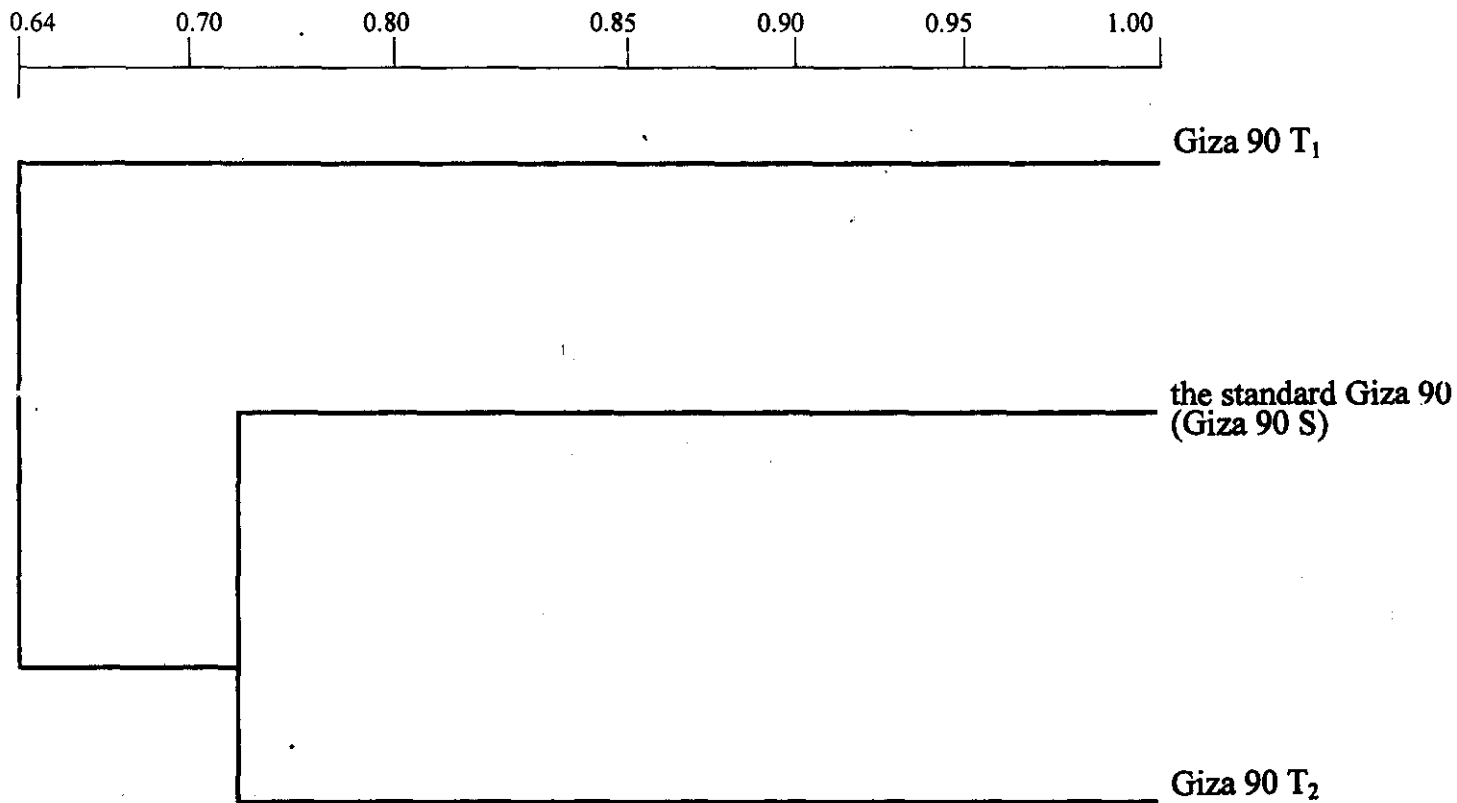


Fig. 5. Dendrogram of the genetic distances between the standard variety Giza 90 and its off-types based on RAPD analysis.

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التوصيف الوراثي الجزئى لبعض الشوارد المغايرة فى صنف القطن جيزة ٩٠

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- أشتملت هذه الدراسة على صنف القطن المصرى جيزة ٩٠ وكذلك الشوارد المغايرة له (اثنين) بهدف دراسة صفاتهم الإقتصادية وأثر ذلك على تدهور صفات المحصول والجودة. وقد أجرى التوصيف الحظي وتقييم صفاتهم المحصولية والتكنولوجية خلال ثلاثة مواسم بمزرعة محطة البحوث الزراعية بسمنس.
- كما استخدمت طريقة التضخيم العشوائى لـ DNA عن طريق جهاز تفاعل البلمرة المتسلسل على المستوى الجزئى لتقييم الاختلافات والتباين فى كل من الصنف التجارى جيزة ٩٠ والشوارد المغايرة له.
- أظهر الوصف الحظي للشوارد المغايرة تميز نباتاتها بأنها أكثر طولاً والأوراق أكبر حجماً ، وكذلك سجلت اختلافات فى شكل اللوزة ، ونسب توزيع الزغب على البذور عنها فى الطرز القياسى (جيزة ٩٠) محل الدراسة.
 - وأشارت نتائج تحليل التباين وجود فروق معنوية بين الصنف التجارى جيزة ٩٠ والنباتات المغايرة له فى معظم الصفات محل الدراسة كوزن اللوزة ، ومحصول الزهر والشعر ، ومعدل الطولج ، والميكروثير (تعومة التيلة) ، وطول التيلة ، ودرجة الأصفرار والإعكاس ، وعلى الجانب الآخر وجود فروق غير معنوية لصفى المتانة (البرسلى) ومعامل الإنتظام للشعيرات.
 - أوضحت نتائج التحليل والدراسة على المستوى الجزئى بطريقة RAPD - PCR نجاح عشرة بائكات من الثلاثة عشر بادئ المختبره لتقييم نقاوة الوراثة للصنف التجارى جيزة ٩٠ الشوارد المغايرة له. حيث كان العدد الكلى من الشظايا لـ DNA للنتيجة هو ٦٧ شظية. بينما كان عدد شظايا DNA التى أظهرت تباين بين جيزة ٩٠ والطرز المغايرة هو ٤٤ شظية. كما توجد بعض من الحزم فى صنف جيزة ٩٠ لم تكن موجودة فى الشوار $G. 90 T_1$ أو $G. 90 T_2$.
 - كما أمكن تقدير العلاقات ودرجة القرابة الوراثية بين الصنف التجارى جيزة ٩٠ والشوارد $G. 90 T_1$ ، $G. 90 T_2$ حيث كانت نسبة التشابه والقرابه الوراثية ٥٩,١ % و ٧٣,٦ % على التوالي.
 - لقد سجلت أعلى قيمة إختلاف وتباين بين جيزة ٩٠ والطرز المغاير $G. 90 T_1$ بنسبة ٤٠,٩ % ، بينما كان أقل تشابه ورثى بين الصنف القياسى جيزة ٩٠ والمغاير التالى $G. 90 T_2$ بنسبة ٢٦,٤ % . وهذا التباين الوراثى قد ينتج من حدوث تحورات للجينات الخاصة بالصنف القياسى من مصادر مختلفة نتيجة لتسويقه وتوزيعه على نطاق تجارى.
 - ومن هذه النتائج يتضح مدى خطورة توجود هذه الشوارد المغايرة والمختلفة وراثياً عن نباتات الصنف المنزرع جيزة ٩٠ ، وذلك فإن إزالة النباتات المخالفة من مساحات إكثار تقوى القطن للتعبئة وتقليل مدة تداول السلالات عند المزارعين سوف يساعد فى المحافظة على نقاوة التقوى ومنع تدهور الأصناف.