UTILIZATION OF MORPHOLOGICAL AND MOLECULAR MARKERS TO STUDY COLOUR DETERIORATION OF THE COTTON GIZA 70 IN THE COMMERCIAL SCALE

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Gene frequencies and genotypes frequencies are constant from generation to generation in the absence of migration, mutation and selection in a large random mating population. Although, Egyptian cotton programme depends on elaborate maintenance system but some varieties have shown some changes in their homogeneity or uniformity of some traits such as lint colour, lint quality and seed characters (Naked and Fuzzy). Traditionally, breeder's seed companies and certification agencies determine genetic purity using physical traits expressed by the seed, seedling and mature plant. Recently, continuing advancements in molecular biology techniques provide even greater promise for enhancing the sensitivity of genetic purity determinations (McDonald, 1998).

Very scent studies were conducted to determined genetic changes Abdel-Bary and Bisher (1969) and Lewis (1970) reported that the main causes of genetic changes of a variety were due to mechanical mixing, outcrossing and mutations.

El-Kilany and Youssef (1985) found that lint characteristics started to degenerate in the fifth year of the general use of cotton varieties. Al-Didi (1984) suggested that the various degrees of brown colour existed in the Egyptian cotton variety "Giza 70" was probably raised from either the accumulation of some plus modifiers or as a result of segregation of some minor and major genes. El-Okkia *et al.* (1990) pointed out that the brown lint off type, isolated from the cultivar Giza 70 showed lower lint percent and having shorter, coarser and weaker lint. Tatinei *et al.* (1996) examined the genetic diversity of 16 near-homozygous elite cotton genotypes derived from interspecific hybridization using RAPD procedure and their morphological characters performances. Iqbal *et al.* (1997) investigated varieties belonging to *G. hirsutum* L. and to *G. arboretum* L., using RAPD analysis. Cluster analysis by unweight pair group method of arithmetic means (UPGMA) showed that seventeen genotypes were placed in two-groups with similarity coefficient ranging from 81% to 93%. Therefore, the present investigation was aimed to study the genetic relationships between some off types in the Egyptian cotton variety Giza 70 using some markers i.e. morphological and molecular marker (RAPD).

MATERIALS AND METHODS

Genetic material and field evaluation

Six off types plants for Extra long staple cotton variety Giza 70 were collected from the commercial scale from El beheira governerate. The original parent Giza 70 and their off types were grown and selfed in 2007. These were evaluated in randomized complete block design experiment in three replications in the season of 2008. Row length was 10.0 m and the row spacing was 70 cm. Plant spacing was approximately 25 cm between plants. Normal agricultural practices were used for Sakha region.

Morphological and seed characters

Six studied traits were measured in the field experiments in 2008 on all 7 entries. These traits have been used traditionally to distinguish off type from original G 70. Measurements were made on randomly chosen 10 plants of each entry per plot. Lint percentage (LP), Micronaire reading (M), fiber length (FL), degree of colour reflectance (Rd%) and colour yellowness degree (+b) and uniformity ratio (UR).

All fiber tests were carried out at the Technology Section, Cotton Research Institute, Agricultural Research Center, Giza.

Morphological analysis

Canonical discriminate analysis was used for data analysis according to Hair *et al.* (1987). Canonical discriminate analysis facilitates differentiation of groups by taking into account the interrelationships of the independent variables (traits) and the dependent variables (genotypes). The difference between centroid values of two groups is the D^2 distance and is calculated as $D^2 (X_1-X_2)$, $S^{-1} (X_1-X_2)$ where, X_1 and X_2 are the estimated mean vectors in the respective groups, and S^{-1} is the inverse of the pooled sample variance-covariance matrix (Dillon and Goldstein, 1984). All these computations were performed using Minitab and SPSS computer programs.

Molecular markers

Genomic DNA extraction

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

RAPD- PCR analysis

For testing the genetic purity, RAPD-PCR reactions were conducted using 10 arbitrary 10-mer primers with the 5′→3′ sequences as shown in Table (1).

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, 10 x buffer 3.0 µl, primer (10 um) 2.0 µl, Taq (5u/µl) 0.2 µl, template DNA (50 ng/µl) 2.0 ul, H_2O (dd) 17.4 ul. Amplification was carried out in a PTC-200 thermal cycler (MJ Research, Watertown, USA) programmed 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 minutes at 72°C and 4°C (infinitive).

Gel electrophoresis

Gel electrophoresis was applied according to Sambrook *et al.* (1989). Agarose (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 2000, Bio- Rad.

RESULTS AND DISCUSSION

Canonical discriminate analysis

Significant differences were obseved among the original variety Giza70 and their off types for all traits, except for micronaire reading indicating that there were some degrees of deterioration for the original variety (Table 1). El-Mansy *et al.* (2008) found significant differences between each of the original varieties Giza 70 and Giza 89 with its off-types.

Morphologically the mean values of the original varieties Giza 70 and its off types for all traits are shown in Table (2). Small differences were between T-6 and standard variety, which indicates that the off type-6 was the mostly appear to be morphologically similar to Giza 70. Also, between $T\text{-}_3$ and $T\text{-}_4$, while $T\text{-}_5$ are highly different from Giza 70. The univariate statistical analysis of variance did not show how cultivars differ when all variables are considered together.

Multivariate procedures used in the study revealed genetic divergence among Giza 70 and its off-types groups. The first three canonical functions were significant and accounted for 94.5%. Cumulative variances of the first three canonicals were 99.5% among groups variance as shown in Table (3). Canonical correlation measures the strength of the overall relationships between the canonical discrimination functions and genotypes sets of variables.

Significant canonical correlation indicated that the canonical function can explain the differentiation of the genotypes. The canonical loading reflects to what extent the observed variables shares with the canonical function and could be interpreted in assessing the relative contribution of each variable to each canonical function (Hair *et al*., 1987). The first canonical was the highest of the variance among genotypes. The yellowness degree and lint percentage traits were larger loading than the rest of the traits. While, the second function is dominated by a large loading from lint percentage followed by fiber length. The third function is dominated by a large loading from micronaire value followed by +b.

It could be concluded that degree of colour (+b), lint percentage and fiber length showed higher discrimination among studied genotypes. Therefore,

these characters can be used as indicators for genetic differentiation among original variety and its off-types. The present findings were in harmony with those obtained by El-Okkia *et al.* (1990), Hemaida *et al.* (2006) and El-Mansy *et al.* (2008).

The controid values for the first two canonical discriminate functions for Giza70 and their off types were plotted Fig. (1) and Table (4). The extent of divergence of genotypes was measured by squared distance D^2 (the calculated value of D^2were compared and tested using Chi square at 5% level of significance with 6 degrees of freedom). All distance between original parent (Giza70) and their off-types were significant, while, it was insignificant between (Giza70) and T_{-6}. Also, the genetic distance between the off types were significant except for (T_{-3} and T_{-4}) and (T_{-4} and T_{-5}). On the other hand, the genetic distances between Giza70 and T_{-5} followed by T_{-3} and T_{-4} were more divergent than the rest of the off types (T_{-1} and T_{-2}).

The major differences between the three off-types (T_{-5}, T_{-3} and T_{-4}) and the two off types (T_{-1} and T_{-2}) were in the lint color traits (+b and Rd %). Thus, the creamy lint and naked seed off types were widely divergent from their original (Giza 70). The differences among the original parent (Giza70) and off type may be due to cross pollination between the original and any source but, Al Didi (1984) mentioned that the brown in color of various shades which exited in the variety (Giza70) probably arised from the accumulative number of plus modifiers or develop from the segregation of modifiers as well as the main gene nal parent.

RAPD- PCR analysis

Randomly amplified polymorphic DNA (RAPD) analysis would be useful in describing any skewed in the genetic basis or the purity of our cotton variety Giza 70 and their off types. Out of twenty random decamer primers screened for their capability of amplifying DNA via the polymerase chain reaction (PCR), ten primers were used to test the genetic purity of Giza 70 which generated a total of 78 DNA fragments. Sixty seven bands (85%) were polymorphic. However, eleven bands were monomorphic (common) for all genotypes. The highest levels of polymorphism (100%) were observed in primers OP-A09, OP-B10 and OP-C09, However, the lowest level of polymorphism was 50% in primer OP-B06 as shown in Table (5).

Genotypes specific markers generated from RAPD-PCR analysis are shown in Table (6). Only five out of ten RAPD-PCR primers were found to be useful as genotypes unique markers. The highest number of RAPD-PCR markers was scored for group 2 (5 markers) while, the lowest number of RAPD-PCR markers was scored for group 1 and group 6 (one marker). In the meantime the largest number of genotype specific markers was generated by primer OP-A09 (4 markers) while, the lowest number was generated by the two primers OP-A07 and OP-C09 (2 markers). The primers OP-A04, OP-

A06, OP-BO6, OP-B12 and OP-B20 did not produce any specific markers.

Genetic distances

Genetic similarities among Giza 70 and the six groups based on RAPD data are shown in Table (6) and dendrogram (Fig. 2).The highest similarity was 73% between Giza 70 and group 6, which indicated that group 6 was closely related with the original parent. While, Giza 70 was genetically distant from group 2 (similarity index 51%).

From the dendrogram, utilizing RAPD analysis Fig. (2) divided the seven genotypes into two main clusters. Group 2 was in a separate cluster. The second cluster was composed of two sub clusters with similarity index of 68%, Gize 70 forms the first of them. While, the second sub cluster was divided into two sub sub clusters with similarity index of 73%, the first of them included the off-types 1 and 3 with similarity index 79%, whereas the off-type 4, off-type 6 and off-type 5 formed the other sub sub cluster with similarity index of 76%.

It can be observed that there are some unique bands in the original parent which were not observed in any group such as the band at 265 bp of primer OP-A07 and the band at 179 bp of primer OP-C09. On the other hand, there were some unique bands in group 1 at 301 bp of primer OP-B03, in group 2 at 972 bp of primer OP-A07, at 948, 500, 438 bp of primer OP-A09 and at 864 bp of primer OP-B10, in group 3 at 1066, 845 bp of primer OP-B03, in group 5 at 155 bp of primer OP-A09 and at 1274, 651 bp of primer OP-B10 and one unique band for group 6 at 207 bp of primer OP-C09.

SUMMARY

Since 1996, Egyptian cotton is facing some variables and challenges locally and globally that have influenced its qualities such as discolourition in Giza 70. Six off types plants for Extra long staple cotton variety Giza 70 were collected from the commercial scale from El-Beheira governorate. The original parent Giza 70 and their off types were grown and selfed in 2007. All genotypes were evaluated in randomized complete blocks design with three replications in season 2008.

Multivariate technique using canonical discriminate analysis revealed that the first three functions accounted for about 99.5 for a cumulative variance. The yellowness degree (+b) followed by lint percentage, showed the highest discrimination among genotypes in the first function .While, lint percentage followed by fiber length were the primary source in the second function. Morphologically, the genetic divergence among the original parent and their off-types based on D^2 revealed that the T-5 followed by T-4, T-1, T-3 and T-2 exhibited wide divergence from their original parents, while T-6 was the closest to Giza 70.

Genetic similarities among Giza 70 and the six groups based on RAPD data were between 73% and 51%. The highest similarity was 73% between Giza 70 and

group 6 which means that group 6 was closely related with the original parent. While, the lowest similarity was 51% between Giza 70 and group 2. There were some unique bands in the original parent which were not observed in any other group. On the other hand, there were some unique bands in the different groups.

Our result indicated that, the genetic uniformity between Giza 70 and its off-type T-6 may be due to the use of different commercial strains of Giza 70 for long time. In contrast, genetic distance between Giza 70 and the remaining off-types is thought to be of divergent origin owing to modifier genes or migration into Giza 70 population from another source specially after free marketing.

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

Primer name	Sequence	Primer name	Sequence
OP- A04	5′ AATCGGGCTG 3′	OP-B06	5′ TGCTCTGCCC 3′
OP-A06	5′ GGTCCCTGAC 3′	OP-B10	5′ CTGCTGGGAC 3′
OP-A07	5′ GAAACGGGTG 3′	OP-B12	5′ CCTTGACGCA 3′
OP-A09	5′ GGGTAACGCC 3′	OP-B20	5′ GGACCCTTAC 3′
OP-B03	5′ CAT CCCCCTG 3′	OP-C09	5′ CTCACCGTCC 3′

Table (2): Analysis of variance for yield components and some lint characters among the original variety Giza70 and six off types.

SOV	df	M	F	+b	U	I	Rd
Rep.	2	0.541	0.086	3.263	15.220	0.447	23.68
Geno.	6	0.277	28.073**	12.702**	19.28*	31.353**	53.71**
Error	12	0.153	0.683	0.185	4.08	0.416	2.38

* and ** P > 0.05 and 0.01%.

Table (3): Mean values measured of Giza70 and its off types groups for all character.

Genotypes	Characters					
	L.P	M	F.L	U	+b	Rd %
T_1	30.9	4.5	33.0	96.4	10.9	65.2
T_2	33.5	4.8	28.1	83.3	11.7	64.3
T_3	31.6	4.8	30.6	86.5	13.9	58.8
T_4	31.3	4.5	29.4	84.9	13.1	60.9
T_5	32.0	4.0	27.9	83.5	13.4	59.3
T_6	37.5	4.13	34.8	87.3	9.0	68.9
Giza. 70	38.9	4.47	35.1	88.0	8.9	68.9
CD	1.14	-	1.47	3.59	0.765	2.74

L.P= Lint percentage, M= Micronaire reading, FL=fiber length, U=uniformity, +b= colour yellowness degree, Rd %=degree of colour reflectance.

Table (4): Canonical loadings of the independent variables on the first three canonical discriminate functions of Giza 70 and thier off-types.

Traits	Canonical discriminate functions		
	1	2	3
Micronaire value	1.976	-0.776	-1.712
Fiber length	0.505	-0.862	0.830
+b	-2.966	0.014	1.154
UR	0.348	0.161	-0.186
I.P	2.224	-0.902	0.438
R.D%	-0.690	0.083	-0.311
Eigeno value	171.9	8.10	3.70
Canonical	0.997	0.943	0.887
P level of sign.	**	**	N.S
% of variance	93.1	4.4	2.0
Cumulative variance	93.1	97.5	99.5

Table (5): Levels of polymorphism and unique cultivar-specific bands based on RAPD analysis.

Primer	TB	PB	MB	P%	Unique bands	
					Cultivar	MS
OP-A04	6	5	1	83	-	-
OP-A06	10	6	4	60	-	-
OP-A07	11	10	1	91	T_2, Giza70	972, 265
OP-A09	11	11	0	100	T_2, T_2, T_2, T_5	948, 500, 438, 155
OP-B03	11	10	1	91	T_3, T_3, T_1	1066, 845, 301
OP-B06	4	2	2	50	-	-
OP-B10	9	9	0	100	T_5, T_2, T_5	1274, 864, 651
OP-B12	5	4	1	80	-	-
OP-B20	7	6	1	86	-	-
OP-C09	4	4	0	100	T_6, Giza70	207, 179
Total	78	67	11			

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

Table (6): Similarity matrix among the seven genotypes based on RAPD analysis.

	T-1	T-2	T-3	T-4	T-5	T-6
T-2	0.621					
T-3	0.790	0.644				
T-4	0.771	0.609	0.674			
T-5	0.762	0.667	0.736	0.764		
T-6	0.719	0.653	0.717	0.766	0.758	
Giza70	0.658	0.518	0.608	0.716	0.707	0.736

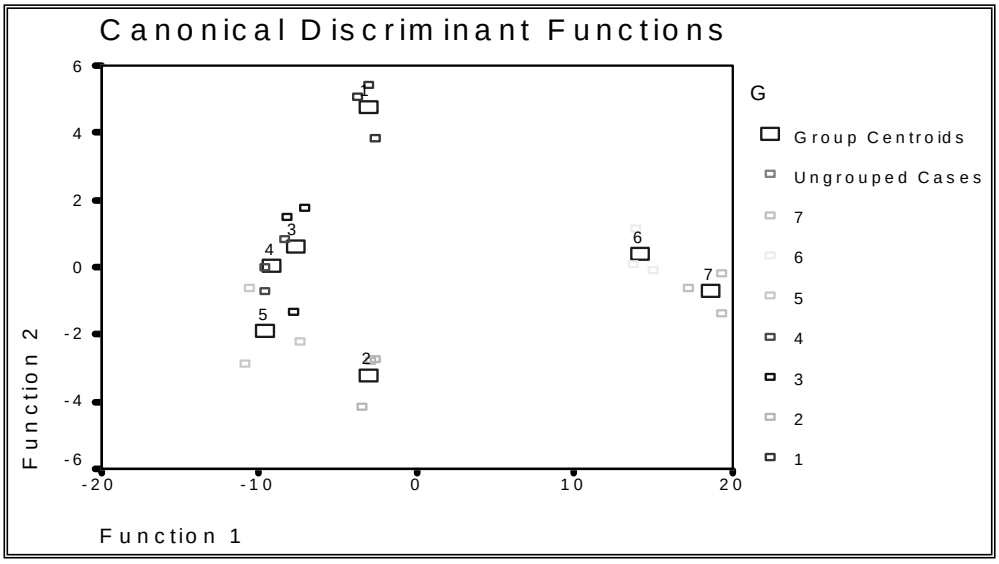

Fig. (1): The controid values for the first two canonical discriminate functions for Giza 70 and their off types. Types 1, 2, 3, 4, 5 and 6 are off types. Type 7 (G70) is origin.

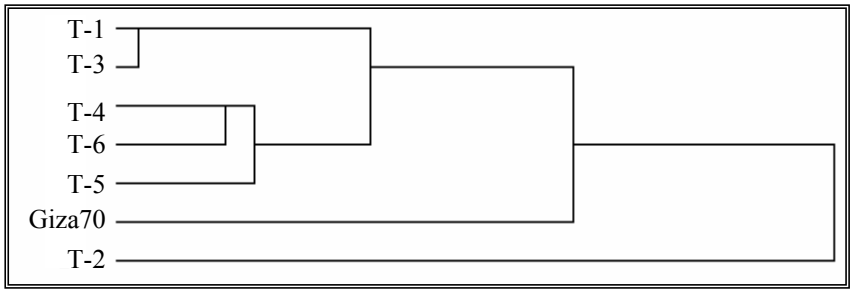

Fig. (2): Dendrogram of the genetic distances between the seven cotton genotypes based on RAPDs analysis.

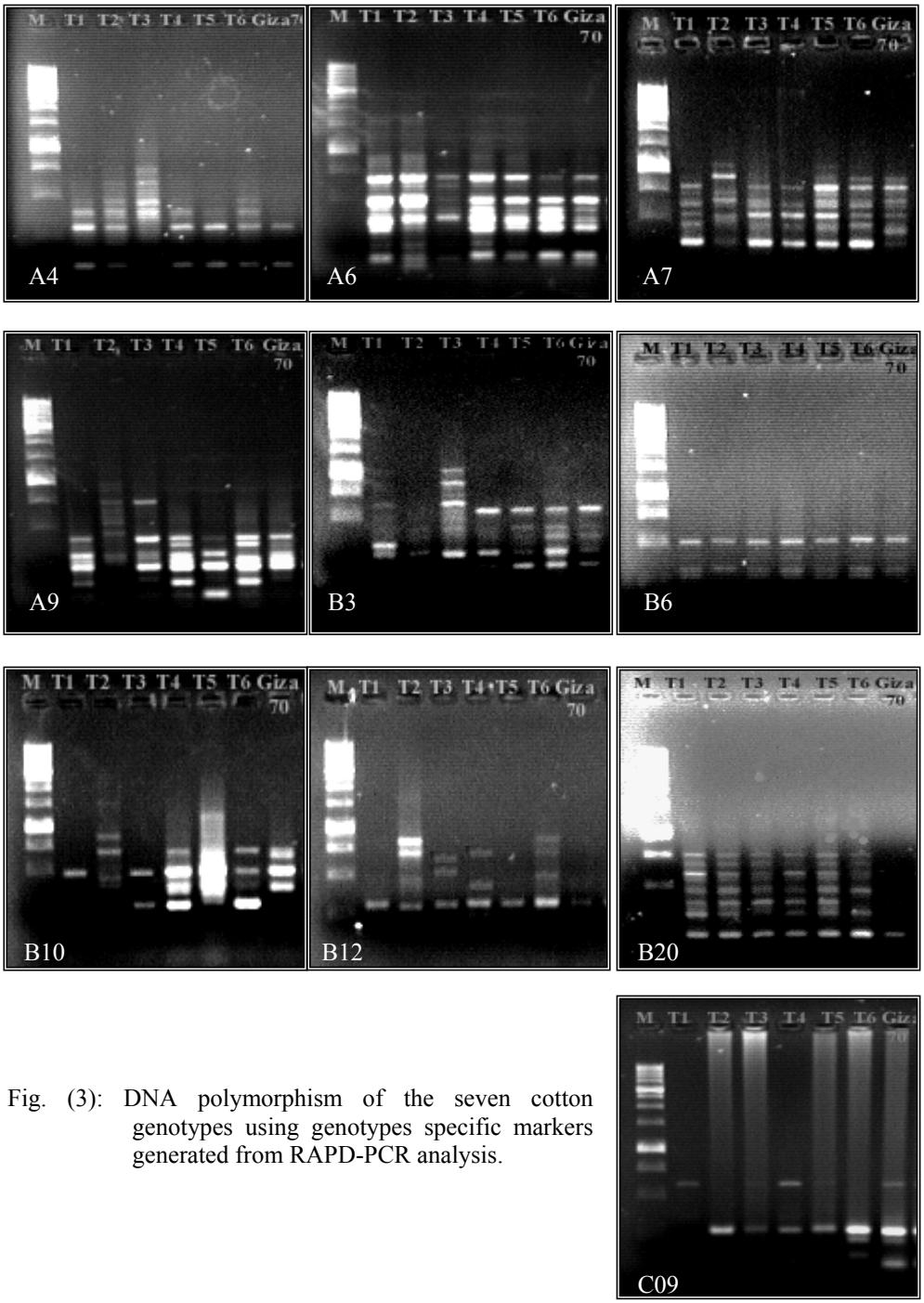

Fig. (3): DNA polymorphism of the seven cotton genotypes using genotypes specific markers generated from RAPD-PCR analysis.