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UTILIZATION OF MORPHOLOGICAL AND MOLECULAR MARKERS FOR ISOLATION AND CHARACTERIZATION OF OFF TYPES OF GIZA 86 EGYPTIAN COTTON

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ABSTRACT

Homogeneity and uniformity of yield and lint quality characters represented the practical criteria for identification and judging the purity of cotton cultivars. Thus, morphological and molecular markers were used to assess the genetic changes isolated from general farm from Giza 86 commercial variety. Eighteen spontaneous changes were isolated and selfed and thus evaluated with the original variety at Sakha Agriculture Research Station. Significant difference were obtained among Giza 86 standard variety and their off types for most studied characters, indicating the presence a lot of genetic variability .Moreover the off types differ among them . The original variety Giza 86 surpassed all the off types for all fiber characters and lint percentage. All the off types were inferior in lint percentage and all fiber quality characters, which showed decreased in lint percentage and sharply decreased in fiber length accompanied by decreasing in length uniformity with coarser and weaker lint. Some off types showed changed in lint color. The differences among the original Giza 86 variety and their off types were mainly affected by two factors, the first factor was due to the cultivar and their off types, and the second factor was concerning the ability of characters that might exhibited discrimination. The first six canonical varieties were significant (P < 0.01) and accounted for 98.2% of the among genotypes variance. The first canonical discriminate function which represented 78.1% of the total variability among genotypes with the largest Eigen value (95.126) is dominated by a large loading from degree of yellowness followed by fiber strength and fiber reflectance. The second function was largely affected by uniformity ratio which showed negative loadings and accounted for about 8.2% of the total variance among genotypes. Lint percentage followed by boll weight and lint strength showed the highest discrimination among genotypes at the third function. It is obvious that the genetic composition of Giza 86 compared to their off types chiefly differed in some characters such as degree of yellowness, fiber length, strength, lint percentage and micronaire reading. On besides, some characters showed high discrimination than the other. The standard variety Giza 86 and their off types, were grouped into ten major clusters according to the relative dissimilarity among them and contribution of the evaluated characters. The original variety Giza 86 formed a unique group with a wide divergent distance from the other off types. Twenty four out of 67 bands generated from all RAPD primer pairs were polymorphic and representing 35.82% of the total generated bands with an average 2.4 polymorphic bands per primer. The similarity coefficient matrix based on RAPD markers among the 19 genotypes ranged from 0.54 to 0.98. The dendrogram separated all genotypes into two major groups. The first group contained three off types, while the second groups consisted of the other off types with the original variety Giza 86 and can also be separated into eight sub clusters. Results from morphological measurements and DNA, RAPDs, markers are complementary factors for each other in studying and identifying the genetic variability and genetic diversity among genotypes and both gave essential information for understanding genetic variability in Egyptian cotton germplasm and provided a useful guide for conserving elite cotton

germplasm and eliminate any spontaneous changes from commercial varieties during the multiplicities stages to maintain the uniformity and homogeneity of Egyptian cotton.

Key Words: ***Cotton, Off types, Deterioration, Multivariate analysis, Discriminate function, RAPD marker.***

INTRODUCTION

The international reputation of Egyptian cotton has been achieved by its unique technological and fiber properties characteristics such as length, strength and fineness's, in addition to high homogeneity and high uniformity between the qualities make international yarn factories prefer it over other cottons. Manufacturing thus reduces production costs with high quality products resulting in ultimately maximizing competitiveness.

The propagation areas of cotton are exposed annually to many mechanical and genetic mixing factors that have effect on the genetic purity of cultured cultivars and lead to changes in the homogeneity and uniformity as well as eventually some off types are spontaneously existed through late segregations (Hemaida *et al* 2006).

Such off types include changes in seed characters, naked and fuzzy seeds, or/and lint color, brown to reddish and inferior in lint quality, shorter, weaker and coarser lint (El-Mansy *et al* 2008) with decrease in yield characters especially lint percentage with very late in maturity (El-Mansy 2000 and Abd El-Salam *et al* 2015). This could lead to degeneration of the Egyptian cotton and market rejection of such varieties, if they haphazardly multiplicated(Ramadan 2015). Maintaining of genetic purity among cotton genotypes offers a measure of protection against degeneration of yield potentials and quality.

Changes on the Egyptian cottons were studied by several researcher (Hemmaida *et al* 2006, El-Mansy*et al* 2008, Abd El-Salam *et al* 2010, El lawendey *et al* 2010, Abd El-Salam *et al* 2015 and Ramadan 2015) who confirmed the dangerous effects of spontaneous changes on Egyptian cottons degeneration.

Due to the narrow genetic bases of cotton germplasm that cotton breeders have been utilizing low efficiency of traditional selection methods, cultivar improvement and isolation or identification of any changes which lead to degeneration. Analysis of genetic diversity and relatedness among genotypes is useful in breeding programs because it provides a tool for accurate organization of germplasm, efficient parental selection and isolate any changes or dangerous types. During the past few decades, a number of

molecular techniques have been recruited to complement traditional methods for the evaluation of biodiversity, estimation of relatedness and genotype identification Rana *et al* (2005) and Tyagi *et al* (2014). They have several advantages including high polymorphism and independence from effects related to environmental conditions and physiological stage of the plant. Random amplified polymorphic DNA (RAPD) markers generated by polymerase chain reaction (PCR) is technically the simplest, less expensive, fast and does not require prior knowledge of the target sequence for the design of primer. The RAPD markers have been already used in cotton to assess the genetic variability, diversity and detection variation by Abd El-Salam *et al* (2010), Abd El-Fattah (2010), El-Mansy *et al* (2012) and Abd El-Latif *et al* (2012).

The main objective of the present work is to find the best technique to maintain the genetic purity of Egyptian cotton. In the analysis presented here, canonical discriminant analysis and cluster analysis were used to study the differences among the original cultivar Giza 86 and their off types based on morphological and molecular markers and to study the dangerous effect of such off types if they haphazard multiplication in later generation.

MATERIALS AND METHODS

Eighteen spontaneous changes plants were isolated from general planting and propagation fields of Giza 86 cotton cultivated cultivar based on their morphological characters. These off type plants were planted and self-pollinated for many years. In 2016 growing season the 18 off types were sown with the originated cultivar Giza 86 in a randomized complete block design with three replicates. Each replicate consisted of 19 rows, one row for each off type with one row of Giza 86. Data were recorded on lint percentage% (LP%), seed index (SI), lint index (LI), fiber length (FL), fiber strength (gm/tex), fiber fineness (FF), degree of yellowness (+b) and lint reflectance (Rd%). All fiber properties were measured in the laboratories of the Cotton Technology Research Division, C.R.I.

Molecular marker (DNA extraction): The nineteen cotton genotypes (18 off types and Giza 86 standard cultivar) were used for DNA isolation. Total genomic DNA was extracted from seedlings by the easy extraction kit (EZ-10 Spin Column Genomic DNA Minipreps Kit, plant. BIOBASIC INC) followed by the quantification. Qualification of the extracted DNA was determined on 0.8% agarose gel stained with ethidium

bromide. Random amplified polymorphic DNA (RAPD) was used to characterize genetic variations of studied genotypes. A set of ten 10-mer random primers was used for RAPD-PCR. PCR amplification reactions were carried out in 25 µl reaction volume according to instruction supporting with My Taq^{TM} Red Mix, 2x (BIOLINE). The amplification runs through four min at 94 ˚C then 35 cycles of 1 min at 94˚C, 1 min at 32 or 36 ˚C (according to the primer) and 1 min at 72 ˚C, followed by a final extension at 72 ˚C for 5 min using (My Gene ® –MG96G) programmable thermal cycler. Fifteen µl of PCR amplified product were loaded into 1.5 % agarose gel supplemented with ethidium bromide. The TBE buffer 1X was used as a running buffer and 1Kb DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 3 h. Gels were then visualized and photographed under UV-trans illuminator by digital camera with UV filter adaptor.

The used primer codes, sequences and annealing temperatures

Primer code	**Sequence 5'⟶ 3'**	**Primer code**	**Sequence 5' 3'**
P1	AGG GGT CTT G	**P7**	**GGT GAC GCA G**
P2	CAA TCG CCG T	**P8**	**GAT GAC CGC C**
P3	CTG CTG GGA C	**P9**	**TGC TGC AGG T**
P4	GTG AGG CGT C	**P10**	**CCA GCA GCT T**
P5	TTG GCA CGG G	**P12**	**GTG ATC GCA G**

Statistical analysis

The data were subjected to the analysis of variance of all genotypes for every characters separately. This analysis provides a test of significant among genotypes. After this step, multivariate technique (Haire *et al* 1987) was conducted by using: (i) canonical discriminate analysis. This is a dimension-reduction technique related to principal component analysis and canonical correlation. It facilitates differentiation of groups by taking into account the interrelationships of the independent variables (traits) and the dependent (genotypes). An important property of canonical variables is that they are uncorrelated even though the underlying quantitative variables may be highly correlated; (ii) hierarchical clustering was then carried out on each data set using Ward's minimum variance method, which minimize within cluster sum of squares. The results from clustering analysis are presented as

dendrogrames. The dendrogram is constructed on Euclidean distance basis according to Nei (1973) and developed by Johnson and Wichern (1988).All these computations were performed by using SPSS Computer Procedures (1995). All gels of molecular markers were scored as 0/1 for absence / presence of the bands, respectively and the resulting scored band were analyzed using PAST program according to Hammer *et al* (2001). The data matrix was used to calculate genetic similarity based on Accord's Similarity Coefficients to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative nested clustering.

RESULTS AND DISCUSSION

Significant difference were obtained among Giza 86 standard cultivar and their off types for most studied characters, indicating the presence of a lot of genetic variability that could be assessed by means of these genotypes (Table 1 and 2).

The data presented in Table (3) showed that the original cultivar Giza 86 surpassed all off types for all fiber characters and lint percentage. However some off types surpassed the original cultivar for some yield characters with inferior in all fiber quality characters. All off types were inferior in lint percentage and all fiber quality characters, which showed decreased in lint percentage and decreased in fiber length with coarser and weaker lint.

On the same time some off types showed changed in lint color, light to dark creamy lint (Table 3). This was undesirable phenomenon in cotton production since uniformity in lint color is one of the main objects of cotton breeders in Egypt .Lack of color uniformity was essentially responsible for market rejection of several Egyptian cotton varieties El-Mansy *et al* (2012). Most off types showed sharply decreased in fiber length accompanied by decreasing in length uniformity. The uniformity trait is very important for consumer market of cotton, since the higher index, the lower the losses in spinning processes (Araujo *et al* 2012).

The previous results assured the differences among the original cultivar Giza 86 and their off types. These differences were mainly affected by two factors, the first factor was due to the cultivars and their off types, and the second factor was concerning the ability of characters that might exhibited discrimination (Hemaida *et al* 2006).

Table 1. Distinguishing morphological features of Giza 86 original and their off types.

Characters	Giza 86	Off types
Vegetative characters	Color greenish, balanced between top and bottom large plant size with medium internodes with one or two vegetative branches.	Pale green and red stem "shiny green" very large with a lot of vegetative branches "abundant branching".
Seedling	Moderate in size with small cotyledon leaves, thin and very limited pall red spots.	Large with shiny green cotyledon leaves, thicker with longer internodes and smooth with diffused and dense red spots.
Flower	-Large coarser leaves with slight-lobed, ding green 1/2 cut lobbed with larger medium lobs. There are large nectar gland at the lower surface, very limit with very pall red spots. -Small tubular yellow flower with pale red spot on the base of petals. -The staminal column is long which anthers are compactly arranged. The anther filament is of same length with three lobbed stigma. -Bracts are medium in size which cover the flower bud completely and the bud is very small size and pale-yellow.	-Large and profusely leaves, smooth, shiny green with deep lobbed over one nectar gland at the lower surface diffused and dense red spots. -Large and somewhat cube flower with pale yellow and very dark red spots on the base of petals. -The staminal column is somewhat long to short. The anthers are loosely arranged on the stamina column. The stigma lobbed ranged from triple to quadruple. -Bracts are large and apparent (clear) and completely cover the bub. Bud flower is large size and dark yellow color.
Nectar glands of bracts	Somewhat presence of one nectar gland	Presence of two or three and dark red color
Bolls	Tapering, canonical shape, medium size, rough, deeply pitted and glandes often with dark green usually 3 loculi.	Dark to shiny green somewhat globular, moderately round to tapering with tite on the tip. Smooth to somewhat smooth, many types had 3 to 4 loculi.
Seeds	Moderate size, brownish to black color and 1/3 – 1/2 fuzzy. Fuzz is homogeneity green color.	Small to large seeds, dark brown to black color, naked to somewhat naked with snake at the top. Fuzz is brownish, brown to gray.
Lint	Long staple with shiny white lint somewhat fine and strong lint.	Inferior of lint quality. Short, coarser and weak lint.Lint color varied from white to dark brown.

Table 2. Analysis of variance for the studied characters among Giza 86 original cultivar with their off types.

SOV	df	Boll weight (g)	Seed cotton yield (g)	Lint yield (g)	Lint percentage (%)	Seed index (g)	Micronaire reading
G	17	0.3976**	136.5**	16.72**	15.42**	0.9691**	0.193**
Rep.	2	0.20519	56.01	5.604	2.31	0.0319	0.03722
Error	34	0.08499	47.12	5.661	2.011	0.3103	0.03056
SOV	df	Fiber length	Uniformity ratio	Fiber strength (g/tex)	Pressely index	Degree of reflectness	Degree of yellowness
G	17	8.599**	29.42**	26.11**	1.63**	43.36**	10.03**
Rep.	2	11.6646	12.272	2.568	0.0985	3.515	0.3635
Error	34	0.8282	1.3	0.936	0.1275	1.667	0.1467

**** Highly significant at 1% probability level.**

Table 3. Mean values of the original Giza 86 variety and their off types.

Genotypes	Boll weight (g)	Seed cotton yield (g)	Lint yield (g)	Lint percentage (%)	Seed index (g)	Micronaire reading
Giza86	3.63	54.6	21.9	39.97	11.4	4.37
2	3.37	61.17	22.57	36.83	10.73	4.97
3	2.8	54.83	17.63	31.87	10.6	4.97
4	2.77	72.5	23.53	32.43	10.2	4.57
5	2.63	60.67	19.43	31.8	10.47	4.97
6	3.07	44.1	14.17	32.13	10.47	5.2
7	2.53	60.37	19.1	31.87	11.33	5.17
8	2.67	58.17	18.33	31.77	11.47	5
9	3.03	59.77	19.47	32.5	10.67	5.23
10	2.53	64.73	20.6	31.63	11.73	5.37
11	3.43	55.27	19.6	35.43	11.13	5.03
12	3.17	65.13	23.23	35.7	11	5.37
13	2.23	59.17	18.5	31.27	10.2	4.9
14	2.83	52.53	17.6	33.5	10.6	4.8
15	2.77	63.33	21.03	33.17	9.8	5.07
16	3.3	62.33	20.77	33.27	10.33	5.23
17	2.77	53.73	18.8	34.9	10.53	5.03
18	3.1	68.43	22.93	33.8	9.67	5.07
LSD 0.05	0.402	9.477	3.285	1.958	0.769	0.241

Table 3. Cont.

Genotypes	Fiber length	Uniformity ratio	Fiber strength (g/tex)	Pressely index	Degree of reflectness	Degree of yellowness
Giza86	**33.47**	**86.4**	**44.3**	**10.53**	**76.4**	**8.2**
2	**28.97**	**83.4**	**34.6**	**8.1**	**60.67**	**14.9**
3	**29.63**	**84.03**	**34.03**	**8.57**	**64.67**	**12.97**
4	**31.1**	**84.5**	**36.33**	**9.6**	**68.8**	**9.43**
5	**30.33**	**84**	**35.23**	**8.77**	**68.3**	**11.1**
6	**27.8**	**79.4**	**33.93**	**8.47**	**63.7**	**14.07**
7	**29.47**	**81**	**36.47**	**9.37**	**66.23**	**12.17**
8	**30.67**	**85.3**	**37.1**	**9.7**	**69.33**	**9.63**
9	**28.63**	**78.57**	**33.53**	**8.27**	**64.67**	**12.7**
10	**29.47**	**85.17**	**35.5**	**8.47**	**69.97**	**9.9**
11	**27.83**	**83.2**	**33.57**	**8.1**	**70.83**	**10.23**
12	**28.67**	**80.33**	**35.23**	**8.6**	**71.47**	**10.47**
13	**29.17**	**80.77**	**37.53**	**8.67**	**67.87**	**11.6**
14	**31.33**	**84.23**	**37.9**	**9.8**	**69.43**	**11.17**
15	**27.47**	**83.57**	**33.27**	**8.37**	**63.3**	**13.13**
16	**26.67**	**78.43**	**31.63**	**7.8**	**62.9**	**14**
17	**30.03**	**80.5**	**32.27**	**9.07**	**66.87**	**11.53**
18	**27.13**	**74.1**	**31.73**	**8.03**	**64.8**	**12.97**
LSD 0.05	**1.256**	**1.574**	**1.336**	**0.493**	**1.783**	**0.529**

**** significant at 0.05 level of probability.**

Thus canonical discriminant analysis simultaneously examines differences in the morphological variables and indicates the relative contribution of each variable to cultivar discrimination (Vaylay and Santen 2002 and El Mansy 2015). Multivariate procedures based on, morphological and agronomic characters have been used in assessment of genetic variability and genetic diversity among standard cultivar Giza 86 and their spontaneous off types.

In an analysis with 12 variables, 12 functions were existed. However, only those which exhibited multivariate variations were

considered. The first six functions accounted for about 98.2% of multivariate variation among all genotypes.

Differentiation of genotypes

The first six canonical varieties were significant ($P < 0.01$) and accounted for 98.2% of the among genotypes variance (Table 4). Each canonical variate (genotypes) is the linear combination of the independent variables (traits) and is orthogonal to the other. Thus, the maximal amount of variation is shown in the first function, the highest Eigen values were recorded in the first function and the second in the second function, these values could measure the explained variance associated with each variable (Haire *et al* 1987).

Table 4. Canonical loading of the independent variables on the first six canonical discriminant function of Giza 86 and their off types.

Variables	Function 1	Function 2	Function 3	Function 4	Function 5	Function 6
Degree of yellowness	0.531934	-0.29233	0.444719	0.341792	-0.2264	0.399744
Lint percentage (%)	-0.06297	0.177029	0.688033	-0.16533	0.089583	0.323545
Fiber tsrength (g/tex)	-0.29727	-0.09668	0.372786	0.766059	-0.08143	0.207251
Micronaire reading	0.105308	-0.09305	-0.16366	-0.15672	0.643741	-0.34587
Pressely index	-0.1998	0.112346	-0.13137	0.445615	-0.08124	0.693972
Uniformity ratio	-0.1987	-0.49308	-0.02209	-0.16248	-0.18193	0.569129
Boll weight (g)	-0.00147	0.125532	0.531375	-0.20504	0.044456	0.127137
Lint yield (g)	-0.01201	0.053177	0.089221	-0.11601	-0.10839	-0.05351
Seed cotton yield (g)	-0.00141	0.023833	-0.04664	-0.08441	-0.13295	-0.11891
Seed index (g)	-0.07296	-0.11974	0.033393	-0.02967	0.472674	0.118292
Micronaire reading	-0.32357	0.231713	0.052926	-0.03815	0.426699	-0.41758
Fiber length	-0.1412	0.022721	-0.04032	0.209991	-0.06809	0.477792
Eigenvalue	95.12646	9.935829	5.062468	4.544804	2.794851	2.216635
% of Variance	78.0775	8.155088	4.155151	3.730265	2.293946	1.819361
Cumulative %	78.0775	86.23259	90.38774	94.11801	96.41196	98.23132
Canonical Correlation	0.994785	0.953183	0.913811	0.905346	0.858187	0.83013

Canonical correlation measure the strength of the overall relationships between the linear composites of predictor (canonical discriminant variate, characters, and criterion of predictor, genotypes, sets of variables). The significant ($P < 0.01$) canonical correlation between the genotypes with the first six canonical (Table 4) indicated that the canonical

variates can explain the differentiation of genotypes. Similar results were reported by El Mansy *et al* (2012) and Abdel Salam *et al* (2015).

Canonical loadings measure the simple linear correlation between the characters and the functions, genotypes. Thus, the canonical loading reflecting the variance that the observed variables share with the canonical variate, and it can be interpreted in assessing the relative contribution of each variable to each canonical function (Haire *et al* 1987). Thus, each character was an important source of variance in, at least, one discriminant function, and some characters may have greater importance in determining plant phenotypes than others (El-Mansy 2014). The first canonical discriminant function which represented 78.1% of the total variability among genotypes with the largest Eigen value (95.126) is dominated by a large loading from degree of yellowness followed by fiber strength and fiber reflectance (Table 4). The genotypes which possess high values of yellowness showed negative values of other characters with increases of coarser fibers (inferior values). The second function is largely affected by uniformity ratio which showed negative loadings and accounted for about 8.2% of the total variance among genotypes. The third function was highly affected by LP% followed by boll weight and fiber strength. It also showed positive discrimination. The variances explained by the fourth, fifth and six functions were 3.7%, 2.3% and 1.8%, respectively with eigen values more than unity. The fourth function showed positive loading and was highly affected by fiber strength, pressely index, degree of yellowness. However functions five and six were largely dominated by micronaire reading and seed index, fiber length, respectively (Table 4).

It is evident that the genetic composition of the original variety Giza 86 and their off types chiefly differed in some characters such as degree of yellowness, fiber length, strength, lint percentage and micronaire reading. On the other hand, some characters showed high discrimination than the others. Thus, the cotton breeder could predict and discover changes or deterioration in cultivars when some of them were deviation from the standard type of characters such as lint color and lint percentage. Hemaida *et al* (2006) cleared that lint percentage followed by lint index revealed high discrimination within Giza 83 and its off types. While El-Mansy *et al* (2008) showed that lint color followed by fiber length and percentage showed high multivariate variance among Giza 70, Giza 89 and their off types.

According to the first two function, the eighteen genotypes were plotted (Figure 1). It is clear that the first function separated the original cultivar Giza 86 at the separate group and widely distance from the other off types according to differentiation of characters which largely affected. In that respects, Abd-El-Salam *et al* (2015) cleared that the first and second functions were effective to separate off types from their original cultivar Giza 88.

According to the relative dissimilarity among them and contribution of the evaluated characters (Figure 2). It is clear that the original cultivar Giza 86 formed unique group and at wide divergent distance from the other off types. On the other hand, the off types number 6, 16 and 18 showed divergent distance from the other off types and they were nearly related with each other. The divergent distance between the original cultivar Giza 86 and five off type's number 6, 16, 18, 4 and 15 might assure the occurrence of double spontaneous alternations. Such alternation might be induced simultaneously in seed and lint characters after some times. These results are in harmony with those obtained by El-Mansy *et al* (2008).

The maximum inter-cluster distances (Table 5) were observed between cluster number 4 and cluster 6 followed by cluster 1 and 5 and cluster 5 and 6. However, the minimum inter cluster distance was observed between cluster 3 and 7 followed by cluster 7, 8 and 2 and 9 respectively nearly related. Finally, all plant breeders must have through knowledge about variability in their crop, and all have an intuitive feel for how different genetic groups relative to one another when considering many traits simultaneously. Generally, canonical discriminant function analysis is useful in identifying the genetic variation and the most influential traits affecting genetic variation of plant populations. Vaylay and Santon (2002) canonical loadings of morphological and agronomic traits of an individual cultivar indicate the magnitude of genetic variation. Knowledge of genetic variation of traits among various type of variability which existed spontaneously in the standard varieties in response to natural selective forces will be useful for plant breeders by focusing attention on such particular traits and could safety condense selection to eliminate such off types easily from the original cotton cultivars.

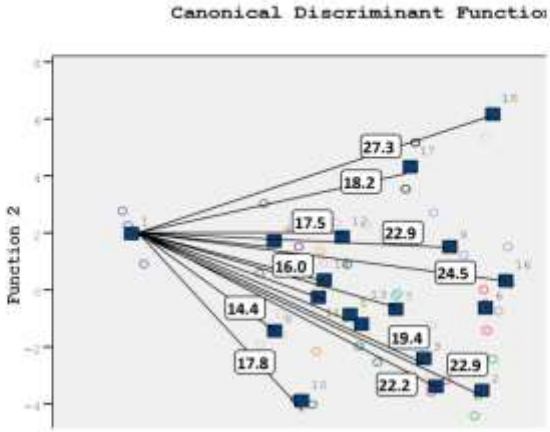

Fig. 1.The centroid values for the two canonical functions for Giza 86 and their off types

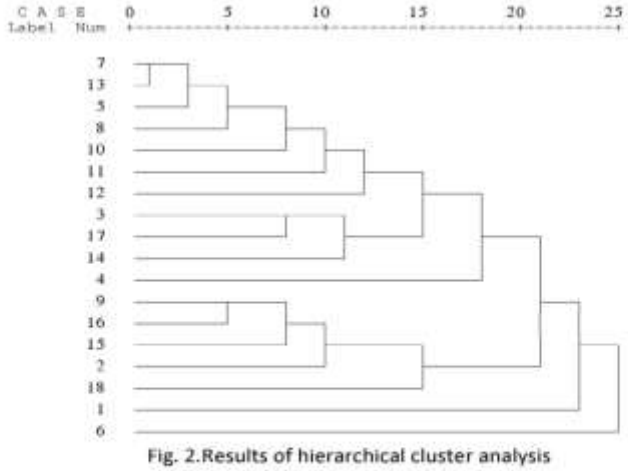

Fig. 2.Results of hierarchical cluster analysis

The results of canonical discriminate function analysis and cluster analysis appeared to be of complete accordance. The canonical analysis could provide no clear grouping but gave a special idea for genetic variability and most influential characters however, cluster analysis could efficiently describe the characteristics of groups of different genotypes and both gave a sensible and useful integration of the data. However, more extensive molecular data are needed in order to interpret the best general conclusion about the relationship among the Giza standard variety and their off types.

Table 5. Distance between the ten clusters.

Cluster	2	3	4	5	6	7	8	9	10
1	**21.82**	**17.79**	**22.69**	**27.34**	**24.94**	**13.45**	**15.00**	**22.48**	**16.96**
2		**9.94**	**16.01**	**13.50**	**20.30**	**13.65**	**13.16**	**6.85**	**11.34**
3			**17.05**	**15.16**	**14.39**	**5.80**	**6.74**	**6.93**	**8.17**
4				**13.98**	**31.41**	**18.13**	**18.70**	**14.55**	**9.85**
5					**26.61**	**19.79**	**17.88**	**9.55**	**12.79**
6						**15.52**	**15.69**	**18.87**	**22.28**
7							**6.62**	**12.13**	**9.37**
8								**11.01**	**9.13**
9									**8.39**

Polymorphism analysis as detected by RAPD analysis

Data in Table (6) revealed that 67 bands were generated from all RAPD primer pairs. Twenty four of them were polymorphic and representing 35.82% of the total generated bands with an average 2.4 polymorphic bands per primer. The number of amplicons/primer ranged from 3 (P5) to 10 (P3-P4). The primer pairs P6 gave the highest percentage of the polymorphic bands (100%) while the primer P1 produced the lowest percentage of polymorphic bands (0%). No unique bands for all tested primers with all studied genotypes were detected. The lowest number of generated bands (40) occurred in genotype 9, while genotype 6 presented the greatest bands.

Table 6. Number of amplified DNA fragments and polymorphic% of studied genotypes investigated with ten RAPD primers.

Primer Code	Range of fragment size (bp)	Total No. of fragments	Mono morphic fragments	Poly morphic fragment	Unique fragments	Polymorphism %
P1	240- 1885	9	7	2	0	22.22%
P2	545-1805	8	4	4	0	50.00%
P3	178- 1756	10	9	1	0	10.00%
P4	178- 1750	10	8	1	0	10.00%
P5	377- 861	3	3	0	0	0.00%
P7	190- 1448	6	0	6	0	100.00%
P8	365- 1545	4	2	2	0	50.00%
P9	262- 2626	4	2	2	0	50.00%
P10	341-1657	8	4	4	0	50.00%
P12	213- 1384	5	3	2	0	40.00%
Total	190- 2626	67	42	24	0	35.82%
Average		6.7	4.2	2.4	0	

Genetic relationship among cotton genotypes

Based on RAPD data analysis, the similarity matrix among the 19 genotypes ranged from 0.54 to 0.98. The highest similarity value revealed was between 1 and 2 (0.98), while the lowest similarity value showed between 8 and 13.

RAPD markers were assayed for their usefulness in assessing molecular diversity and genetic relatedness in 18 cotton genotypes, original cultivar Giza 86 and their 18 off types. Data summarized in Table (7) showed that 67 bands were generated from all RAPD primer pairs. Only 42 out of these 67 were polymorphic and representing 35.82% of the total generated bands with an average 2.4 polymorphic bands per primer reflecting considerable amount of genetic variability among those genotypes (Fig.3). Most primers were found to produced polymorphic amplification products though the extent of polymorphism viewed with each primmer. The number of amplicons / primer ranged from 3 (P5) to 10 (P3 – P4). The pairs (P7) showed the highest percentage of the polymorphic bands (100%). While the primer (P1) produced the lowest percentage of polymorphic bands (0%). No unique bands were detected all tested primers with all studied genotypes. One reason for most primer to bepolymorphic could be due to the difference between the original cultivar Giza 86 and their off types. Beside, some off types were in late segregation while another in the primary stage. Earlier, using RAPD markers Rana *et al* (2005) found high level polymorphism (67%) in 32 cotton cultivars while, El-Mansy *et al* (2012) found that 44 out of 52 microsatellite markers were polymorphic and accounted for 84.6% of the total number of generated bands with an average of 7.3 bands/primer. To estimate the genetic similarities of the cotton genotypes a similarity matrix was obtained using Jaccord's Similarity Coefficient and showed in Figure 4. Among the cotton genotypes similarity values ranged from 0.54 to 0.98. The highest similarity value was observed between the off types 1 and 2 (0.98), while the lowest similarity value showed between off types 8 and 13. These similarity coefficients were used to generate a dendrogram (Figure 4) by UPGMA analysis to grouping different genotypes.

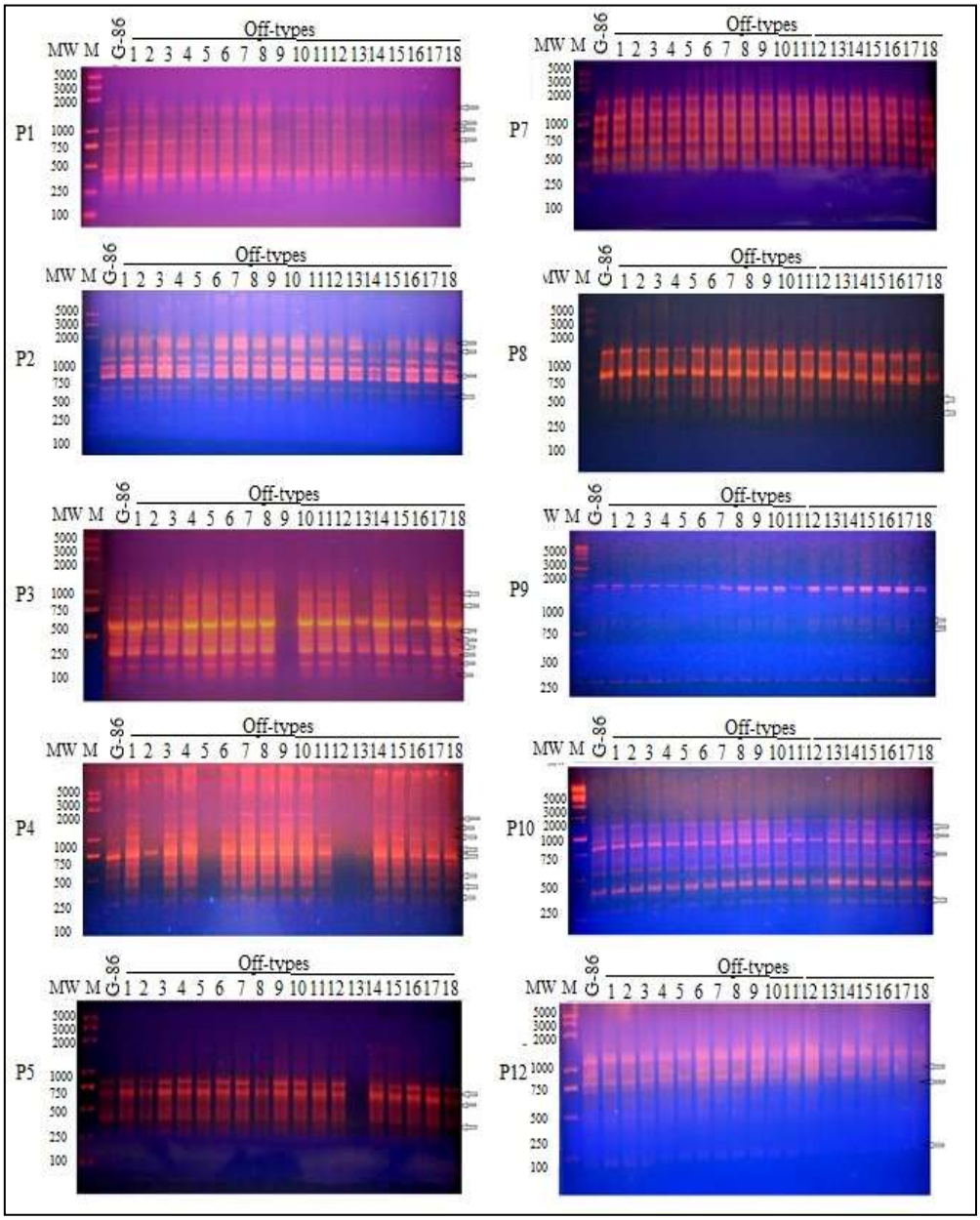

Fig. 3. RAPD amplification of 18 cotton genotypes.

The dendrogram (Figure 4) assigned the genotypes into groups which correspond well with their centers or sub clusters of release and / or pedigree relationship. The dendrogram separated all genotypes into two major groups. The first group contained three off types, while the second groups consisted of the other off types with the original variety Giza 86 and can also separate into eight sub clusters. The three off types in cluster 1 are more closely related and more distance from the other off types. On the other side the original cultivar Giza 86 formed unique cluster and divergent distance from the other off types. Similar findings have proved the successful application of RAPDs for the estimation of genotypic variability (Rana *et al* 2005, and Badeaa Mahmoud *et al* 2018), where the information obtained is useful in breeding programs.

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Data obtained from figure 4 cleared greater genetic distinctiveness among genotypes as measured by average dissimilarity. This suggests greater distinctiveness of the genetic background for the genotypes. This was clearly obvious among the various off types and the original cultivar Giza 86. This may reflect the consequence of the sufficient effort in the maintenance of genetic purity by condense of selection on the early stage to eliminate any changes and effort for eliminate such spontaneous changes in the later multiplicities in general farm. The relative measure of genetic distinctiveness could provide useful information for maintenance of genetic purity of the commercial cultivars.

Finally, results from morphological measurements and DNA, RAPDs, markers are complementary factors for each other in studying and

identifying the genetic variability and genetic diversity among genotypes and both gave essential information for understanding genetic variability in the Egyptian cotton germplasm with providing a useful guide for conserving elite cotton germplasm and eliminate any spontaneous changes from commercial cultivars during the multiplicities stages to maintenance the uniformity and homogeneity of the Egyptian cotton.

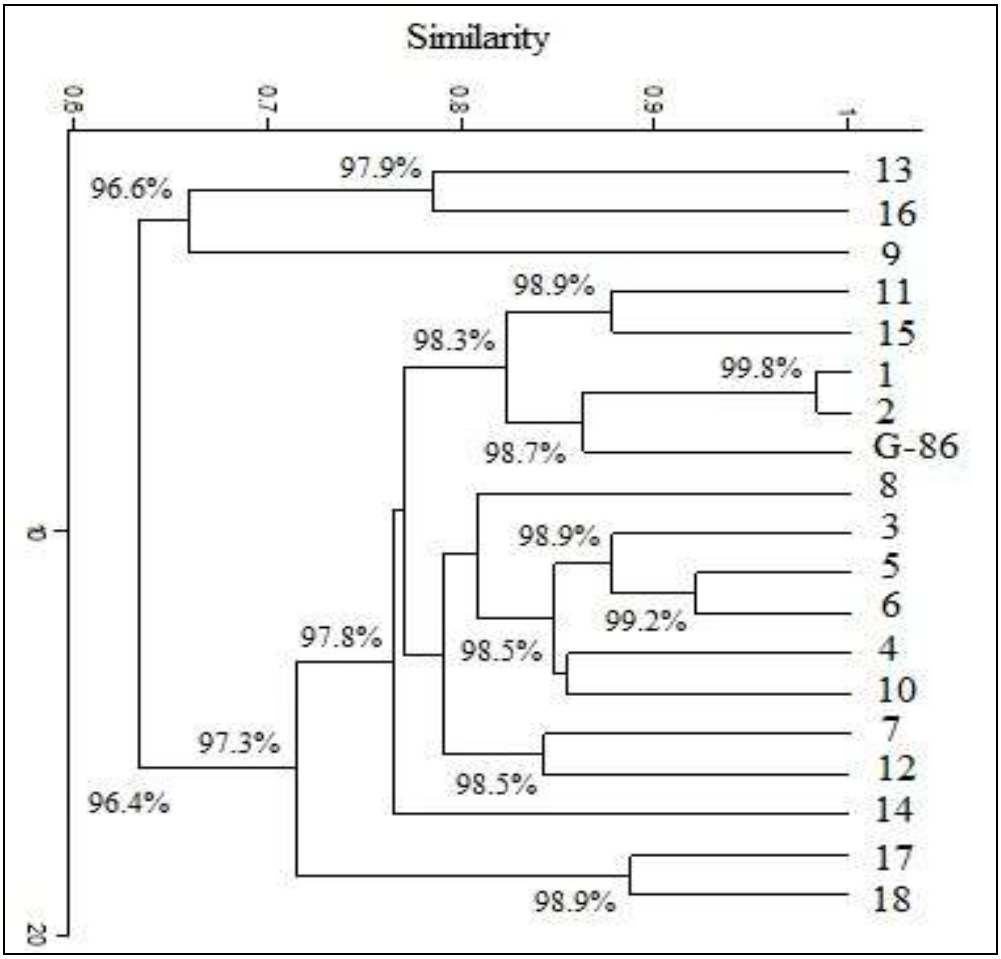

Fig. 4. The dendrogram of 19 cotton genotypes developed from twelve RAPD primers.

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استخدام المعلمات المورفولوجية والجزيئية لعزل وتوصيف النباتات المغايرة لصنف القطن المصرى جيزة ٨٦

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يعتبر التجانس والتماثل للصفات المحصولية وصفات جودة التيلة أحد المعايير الأساسية لتوصيف وتحديد النقاوة الوراثية لأصناف القطن المنزرعة. وعلى هذا تم استخدام المعلمات المورفولوجية والجزيئية لتقييم التغيرات الوراثية والتى تم عزلها من الزراعة العامة لصنف القطن جيزة ٨٦. تم عزل ثمانية عشر طراز من الزراعة العامة لصنف القطن جيزة ٨٦ وعمل التلقيح الذاتى لها وتم تقييمها مع الصنف الأصلى فى مزرعة محطة البحوث الزراعية بسخا موسم ٢٠١٨. وكانت أهم النتائج المتحصل عليها ما يلى:- أظهر تحليل التباين وجود اختلافات معنوية بين الصنف الاصلى والطرز المغايرة لمعظم الصفات تحت الدراسة مما يدل على وجود كمية كبيرة من الاختلافات الوراثية كما اختلفت الطرز المغايرة عن بعضها البعض. تفوق الصنف القياسى جيزة ٨٦ عن الطرز المغايرة لصفات معدل الحليج وكل صفات جودة التيلة. فى حين أظهرت الطرز المغايرة

انخفاضاً ملموساً لصفات تصافى الحليج مع انخفاض حاد لصفات طول التيلة مصحوبة بإنخفاض فى تجانس الطول مع درجة عالية من الخشونه وانخفاض المتانة الذاتية جم/تكس. كما أظهرت بعض الطرز المغايرة تغيرات فى لون التيلة حيث تراوح لون الشعر من اللون الفاتح الى الكريمى الداكن. أوضحت الدراسة أن الاختلافات بين الطرز المغايرة والصنف الأصلى تأثرت بعاملين أساسيين العامل الأول هو عامل الصنف والعامل الثانى هو ما يتعلق بقدرة الصفات على التمايز بين التراكيب المختلفة. باستخدام طريقة تحليل التمايز Function analysis Discriminate أمكن ترتيب الصفات طبقاً لقدرتها على التمايز وإظهار الأختلافات بين التراكيب الوراثية. أوضح تحليل التمايز أن العوامل السته الأولى كانت معنوية وتحصر حوالى ٢ر٩٨% من التباين الكلى بين التراكيب الوراثية كما وجد أن العامل الأول والذى يحتوى على ١ر٧٨% من التباين الكلى مع أكبر قيمة للتباين المرتبط اكثر تأثراً بصفات درجة الاصفرار يتبعها صفات متانة التيلة ونسبة الإنعكاس بينما كان العامل الثانى أكثر تأثراً لصفات درجة التجانس للطول والتى أظهرت قيماً سالبة وكانت تمثل ٢ر٨% من التباين الكلى بين التراكيب الوراثية. فى حين أظهرت صفات معدل الحليج يتبعها وزن اللوزة ومتانة الشعر وقراءة الميكرونير قيماً أعلى للتميز بين التراكيب الوراثية على المحور الثالث. من الواضح أن التركيب الوراثى للصنف جيزة ٨٦ يختلف بشكل أساسى عن الطرز المغايرة فى الكثير من الصفات مثل درجة الاصفرار ومتانة وطول التيلة ومعدل الحليج وقراءة الميكرونير ومن الناحية الأخرى أظهرت بعض الصفات قدرة عالية على تميز التراكيب الوراثية عن الأخرى وهذا يمكن المربى من التنبؤ واكتشاف التدهور أو التغير فى الأصناف التجاريه المنزرعه وذلك بملاحظة انحراف بعض صفات الأصناف عن الصفات القياسية مثل لون التيلة ومعدل الحليج. تم توزيع التراكيب الوراثية الثمانية عشر (الصنف الأصلى والطرز المغايرة)على عشرة مجاميع كبيرة بناءاً على عدم التشابه النسبى بينها والمساهمة النسبية للصفات المختلفة فى التمايز بينها. كما أن من الواضح أن الصنف الأصلى جيزة ٨٦ كون مجموعة منفصلة وبعيده من بقية الطرز المغايرة. هذا التباعد بين الصنف الأصلى والطرز يؤكد حدوث طفرات مزدوجة أو تغيرات مزدوجة مثل هذه التغيرات تحدث بطريقة تلقائية فى صفات البذره والشعر. من بين ٦٧ حزمة أظهرت ٢٤ حزمة اختلافات فيما بينها أى ما يقرب من ٣٥،٨% من الحزم الكليه كما أظهر تحليل التشابه على أساس RAPD أن معامل التشابه تراوح بين – ٥٤% الى – ٩٨%. تم توزيع التراكيب الوراثية الى مجموعتين كبيرتين على أساس التشابه النسبى بينها (المجموعة الأولى تضم ٣ طرز مغايرة والمجموعة الثانية أمكن تقسيمها الى تحت ٨ مجاميع) فى حين أن الصنف جيزة ٨٦ وقع فى مجموعة منفصلة . اوضحت النتائج المتحصل عليها ان كلا من القياسات المورفولوجية والجزيئية مكملتان لبعضها البعض فى دراسة وتحديد نوع التباين الوراثى والتباعد الوراثى وكلاهما يقدم معلومات مفيدة وجيدة لفهم الاختلافات الوراثية فى الأقطان المصرية والحفاظ على صفوة السلالات من التدهور وامكانية التخلص من هذه التغيرات خلال مراحل الاكثار للحفاظ على تماثل وتجانس اصناف القطن التجاريه المنزرعه.