

# GENETIC RELATIONSHIPS AMONG SOME EGYPTIAN COTTON GENOTYPES AS REVEALED BY RAPD'S ANALYSIS

By

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## ABSTRACT

The present study was undertaken to evaluate genetic divergence among nine Egyptian cotton cultivars (*Gossypium barbadense*) random amplified polymorphic DNA analysis( RADP-PCR) and to assess the relationship between Euclidean distance and Nei's genetic distance.

Screening of ten random primers with nine genotypes of cotton indicated that eight primers produce different polymorphic bands and amplified fragment DNA and generated a total of 123 amplification products, among which 118 were found to be polymorphic, with 95.90% polymorphism. All primers produced polymorphic amplification products, however, the extent of per cent polymorphism varied with each primer (75.0 to 100%).

Genetic relationships among a number of genotypes can be summarized using cluster analysis to place similar genotypes into phenotypic groups. Relationships among the nine Egyptian cotton cultivars based on showed the dissimilarity matrix of the euclidean distance using six fiber quality traits between all pairs of cotton cultivars ranging from 0.324 between Giza69 and Giza81 to 6.014 between Giza76 and Giza80;the average of distances among cultivars was 3.169.

Nei's genetic distance between all pair of cotton cultivars ranged from 0.278 between Dendera and Giza 83 to 0.704 between Giza77 and Giza80 ; the average of similarity among cultivars was 0.491.

UPGMA cluster analysis placed all the Egyptian cotton cultivars within their respective known taxonomic groups, while breeding centre-wise grouping of cultivars was not discernible.

It indicates that breeders have not been working in isolation, and that breeding material for the development of these cultivars has been shared between the breeding stations and cotton improvement programmes.

## INTRODUCTION.

Cotton (*Gossypium barbadense*) represents one of the most important economical crops in Egypt. Molecular genetics markers studies of cotton cultivars offer possibility for use in improving cotton cultivars(Rao *et al.*,1990; Khalil *et al.*,1998; Barakat and El Ham, 2004).

This would help the advancement of breeding material through consistent progress; DNA markers technology has provided plant breeders with a tool to select desirable plants directly on the basis of genotype instead of phenotype; conventional breeding of the cotton plant generally aims to improve agronomically relevant, otherwise interesting traits, by combining characters present in different parental lines of cultivated species or their wild relatives(Welsh *et al.*,1990;Tatineni *et al.*,1996;Lu and Myers, 2002).

Molecular markers, however, facilitate all these processes, and can accelerate the generation of new varieties and allow connection of phenotypic characters with the genomic loci responsible for them, both of these properties make molecular markers indispensable for cotton plant improvement(Popove *et al.*, 2002 ).

Morphological and physiological features of plants have been used by plant scientists to understand genetic diversity; morphological characters are very few in the cotton plant compared to biologically active genes; in closely related cotton plant varieties and species, there are very few morphological differences, which as a matter of fact do not represent the true genetic differences at the DNA level(Kumar *et al.*, 2003;Vafaie *et al.*,2003 ).

Moreover, in most cases a plant genome has large amounts of repetitive DNA, which is not expressed and does not contribute to the physiological and morphological appearance of the plant. Therefore, there is a need to study polymorphism at the DNA level, to indicate genetic diversity in cotton.

The use of molecular markers enables cotton breeders to connect the gene action underlying a specific phenotype with the distinct regions of the genome in which the gene resides, e.g., the phenotypic expression of fiber quality is confined to domesticate species ( Pendse *et al.*,2001).

The genetic advances in fiber quality can be considered as indicator of the existence of genes that contribute to fiber quality in germplasm that does not express the phenotype. Molecular markers could provide the opportunity to use precision in identifying the phenotype of these traits and allow direct selection for genotypes, thereby providing a more efficient means of selection for fiber properties.

Molecular markers provide an opportunity to identify and isolate the genes relating to fiber characters by map-based cloning and once markers for an interesting trait are established, these should allow prediction of fiber characters, yield or resistance of individual offspring derived from a cross, solely by the distribution pattern of markers in the offspring genome and improvement of the agronomic value of cotton by breeding for quantitatively inherited traits, such as yield, drought and cold tolerance (Stewart *et al.*, 1994; George and Daryl, 1998; Semagn *et al.*, 2006 ).

## MATERIALS AND METHODS

This research was conducted at Sakha Experimental Farm, Agricultural Research Station, ARC and Genetics Department, Faculty of Agriculture at Moshtohor, Benha University, during summer season of 2006.

### Plant Material:

Nine cotton cultivars (*Gossypium barbadense*) of Egyptian cotton germplasm including Giza69, Giza75 and Giza81 as long stable cultivars (LS) and Giza76 and Giza77 as extra long Stable (ELS) and extra fine cultivars seeded in delta; and Giza80, Giza90 and Dendera as long stable cultivars seeded in upper Egypt were used in this study. The names and pedigrees of these cultivars are shown in Table(1).

Table (1): Names and pedigrees of the nine Egyptian cotton genotypes.

Code.	Name	Classification	Pedigree
P <sub>1</sub>	Dendera	Long Stable	Selection from Giza3
P <sub>2</sub>	Giza69	Long Stable	Giza51 A x Giza30
P <sub>3</sub>	Giza75	Long Stable	Giza67 x Giza69
P <sub>4</sub>	Giza76	Extra Long Stable	Menoufi x Pima S-2
P <sub>5</sub>	Giza77	Extra Long Stable	Giza70 x Giza68
P <sub>6</sub>	Giza80	Long Stable	Giza66 x Giza73
P <sub>7</sub>	Giza81	Long Stable	Giza67 x H 10867/63
P <sub>8</sub>	Giza83	Long Stable	Giza72 x Giza67
P <sub>9</sub>	Giza90	Long Stable	Giza80 x Giza83

### **Field Experiment:**

The following, nine genotypes were distributed in the field, following a complete block design with three replicates under the field conditions and applied as recommended for cotton production. 2.5% span length (mm) (2.5% SL), pressely index (PI), reflectance % (Rd %), yellowness degree(+b), micronaire reading(MR), elongation%(E%) were studies for the fiber quality traits of the all genotypes using HVI system, Cotton Research Institute at Giza.

### **DNA Extraction:**

Young leaves were collected in 1.5 ml eppendorf tube, quickly frozen in liquid nitrogen and ground with konte pestles into fine powder.

DNA was extracted according to (**Paterson et al., 1993**) mini preparation protocol. The purity of extracted DNA was tested on 1% agarose gel using 0.5x TE (Tris EDTA) buffer and stained with 10 mg/ml ethidium bromide. The gel was exposed to UV-light and photographed. Optimizations of the working dilutions were made using various dilution ratios. Finally, the dilution that produced amplification with the RAPD primer and three samples for screening was 1:1000 after determining the concentration with a TD-700Fluorometer.

### **RAPD-PCR:**

RAPD-PCR was carried out according to (**Williams et al., 1990; Welsh and McClelland, 1990**) the primers used were 10 –mer oligonucleotide; ten primers were selected as potentially useful and the codes and sequences of the used primers are shown in Table(2).

PCR reaction were optimized and mixtures (25µl total volume) were composed of dNTPs (200µM), MgCl<sub>2</sub> (1.5mM), 1x buffer, primer(0.2µM), DNA (50ng), Taq DNA polymerase (2units).

Amplification was carried out in a thermo Cycler programmed for 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min and elongation at 72°C for 1 min. The 40th cycle was followed by an extended primer extension step at 72°C for 4 min and then being held at 4°C until electrophoresis.

Amplification products (15µl) were mixed with 3µl loading buffer and separated on 1.3% agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 1kb plus DNA ladder marker.

Table (2): Name and sequences of the used primers with RAPD molecular markers.

Code.	Primer name	Nucleotide sequence 5' to 3'
1.	OPERON -B01	5'-GTTTCGCTCC-3'.
2.	OPERON -B05	5'-TGCGCCCTTC-3'.
3.	OPERON -B06	5'-TGCTCTGCCC-3'.
4.	OPERON -B07	5'-GGTGACGCAG-3'.
5.	OPERON -B10	5'-CTGCTGGGAC-3'.
6.	OPERON -B14	5'-TCCGCTCTGG-3'.
7.	OPERON – AD 20	5'-TGCGCTCCTC-3'.
8.	OPERON - AF 20	5'-CTCCGCACAG-3'.
9.	OPERON – AE 03	5'-CATAGAGCGG -3'.
10.	OPERON -AC 13	5'-TCTTGCCCTC-3'.

### Data Analysis:

The obtained data of RAPD analysis was entered in a computer file as binary matrices were 0 stands for the absence of a band and in each individual sample. Similarity coefficients were calculated according to dice matrix (Nei and Li, 1979; Rohlf, 1993). Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

## RESULTS AND DISCUSSION

### 1.Evaluation of genotypes:

Represents the means the summer season of 2006 of the nine genotypes for fiber quality traits in Table(3).

Highly significant differences among genotypes were detected for 2.5%SL, PI, MR,E% ,Rd%, MR and +b.

In this table observed that Giza76 and Giza77 were the highest cultivars in 2.5% span length, pressely index and the lowest

micronaire reading (most finest ones), these results confirm the classification of these cultivars as extra long and extra fine cultivars from this collection; the cultivars Dendera, Giza77, Giza80, Giza83 and Giza90 showed the brownest cultivars and in the same time the lowest brightness ones( the highest +b and lowest Rd % ones), nevertheless Giza69, Giza75, Giza76 and Giza81 showed the opposite trend.

Table (3):Performance of nine cotton genotypes means for fiber quality traits.

Genotypes	2.5 % (SL)	(PI)	(MR)	(E%)	(Rd%)	(+b)
<b>Dendera</b>	29.9	9.3	3.9	8.4	66.5	12.2
<b>Giza 69</b>	30.7	9.9	4.1	6.6	76.4	9.3
<b>Giza 75</b>	30.9	10.6	4.5	6.2	74.6	9.4
<b>Giza 76</b>	35.5	11.0	3.7	6.6	75.9	9.4
<b>Giza 77</b>	34.5	11.0	3.7	6.6	66.5	12.0
<b>Giza 80</b>	30.8	9.7	4.3	7.2	62.5	13.0
<b>Giza 81</b>	31.0	10.3	4.1	6.8	75.9	9.6
<b>Giza 83</b>	30.0	9.3	4.2	7.0	64.6	12.1
<b>Giza 90</b>	30.1	9.3	4.0	7.9	65.0	11.6
<i>LSD<sub>05</sub></i>	0.095	0.055	0.110	0.110	0.387	0.173
<i>LSD<sub>01</sub></i>	0.131	0.075	0.159	0.151	0.533	0.239

## 2. Genetic relationship using fiber quality traits:

### a. Euclidean distance.

Genetic relationships among a number of genotypes can be summarized using cluster analysis to place similar genotypes into phenotypic groups. Relationships among the nine cotton cultivars based on standardized values of the six quantitative characters in table(4) showed the dissimilarity matrix of the euclidean distance using fiber quality traits between all pairs of cotton cultivars ranging from 0.324 between Giza69 and Giza81 to 6.014 between Giza76 and Giza80;the average of distances among cultivars was 3.169 .

Table (4): Euclidean Distance matrix of nine Egyptian cotton genotypes using seven fiber quality traits.

Genotypes	Dendera	Giza 69	Giza 75	Giza 76	Giza 77	Giza 80	Giza 81	Giza 83	Giza 90
Dendera	0.0								
Giza 69	4.295	0.0							
Giza 75	3.682	0.827	0.0						
Giza 76	4.721	2.028	1.992	0.0					
Giza 77	2.136	4.493	3.792	4.003	0.0				
Giza 80	1.789	5.879	5.184	6.014	2.349	0.0			
Giza 81	4.081	0.324	0.626	1.869	4.224	5.653	0.0		
Giza 83	0.973	4.968	4.292	5.299	2.128	1.006	4.761	0.0	
Giza 90	0.696	4.790	4.133	5.123	2.099	1.254	4.579	0.460	0.0

#### b. Cluster analysis:

The dissimilarity matrix was used to generate a dendrogram of the nine genotypes (Fig.1); the cluster dendrogram showed a genetic variation pattern and the nine cultivars formed three groups. The first group was divided into two clusters at a distance of about ( 0.6 ) ; the first subcluster included Giza69 and Giza81 at genetics distances of about (0.630):making this a more divergent (0.2), the second subcluster included one cluster included an individual cultivars Giza75 at a genetic distances about (3.23): making this more divergent (0.6).

The second group includes one cluster included an individual cultivars Giza76 at a genetic distances about (108.35): making this more divergent (4.5).

The third group was divided into four clusters at a about (5.0); the first subcluster included two cultivars Giza83 and Giza90 at a genetic distances of about (1.27): making this a more divergent (0.2), the second, third and fourth subcluster included an individual cultivars Dendera, Giza80 and Giza77 at a genetic distances of about (11.57 and 27.82.): making this a more divergent(0.6, 1.3 and 2.0), respectability.

The differences between fiber quality traits in groups lead to the formation of the three main groups.

		Euclidean Distance Cluster Combine					
C A S E		0	1	2	3	4	5
Label	Num	+-----+	+-----+	+-----+	+-----+	+-----+	+-----+
GIZA69	2	-+---+					
GIZA81	7	+ I +-----+					
GIZA75	3	-----+		II			+-----+
GIZA76	4	-----+					+-----+
GIZA83	8	-+---+					
GIZA90	9	+ +-----+				III	
DENDERA	1	-----+	+-----+				
GIZA80	6	-----+		+-----+			
GIZA77	5	-----+					

Fig.(1):Dendrogram obtained from UPGMA cluster based on fiber quality data from the nine cotton genotypes.

### 3.Genetic relationship using random amplified polymorphic DNA analysis( RADP-PCR):

#### a. RAPD-PCR analysis.

In the present study, the genetic variability among different genotypes of Egyptian cotton cultivars based on RAPD-PCR analysis has been studied.

Screening of ten random primers with nine genotypes of cotton indicated that eight primers produce different polymorphic bands and amplified fragment DNA as shown in table (5,6) and Fig.(2).The ten primers used in the study generated a total of 123 amplification products, among which 118 were found to be polymorphic, with 95.90% polymorphism. All primers produced polymorphic amplification products, however, the extent of per cent polymorphism varied with each primer (75.0 to 100%).

The PCR products of primer OPB-01 ranged from five bands in Dendera to 13 bands in Giza80 (table 5,6 and Fig.2A); this primer produced five monomorphic bands in all genotypes; 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 among them. For instance Giza69 has two positive unique band at Molecular Weight (*M.W.*) of 551.52 and 213.64bp;also, Giza 77 and Giza90 exhibited one positive unique band of 581.82 to 190.91bp, respectively.



The PCR products of primer OPB-05 and analysis of these products are illustrated in Fig.2A and table(5,6); this primer produced 3-10 bands for the studied genotypes; there were five monomorphic bands in all genotypes and one unique positive bands was found in Giza76 with *M.W.* of 551.52bp.

The result of RAPD analysis using primer OPB-06 were illustrated in Fig.2B and table(5,6);the total number of bands varied with a lowest number of three bands in Dendera and the highest number of 12 bands in Giza90; they was one monomorphic band in all genotypes and three unique positive bands were found in Giza69 and Giza90, respectively.

The PCR products of primer OPB-07 ranged from one band in Giza77,Giza80 and Giza90 to four bands in Giza75 and Giza76 (table 5,6 and Fig.2B); this primer produced three monomorphic bands in all genotypes; some genotypes had some specific bands and could be used to distinguish among them; for instance Giza75 has two positive unique bands at *M.W.* of 160.55 and 109.36bp.

The PCR products of primer OPB-10 and analysis of these products are illustrated in Fig.2C and table(5,6); this primer produced 4-5 bands for the studied genotypes. One monomorphic band in all genotypes and five unique positive bands were found in Dendera, Giza69 and Giza 83 with *M.W.* of (377.27, 295.47bp), (510.23,190.91bp) and (218.18bp), respectively.

The result of RAPD analysis using primer OPB-14 were illustrated in Fig.2C and table(5,6);the total number of bands varied with a lowest number of three bands in five cultivars and the highest number of eight bands in Giza75; there were two monomorphic band in all genotypes and two unique positive bands were found in Giza75 with *M.W.* 594.74 and 572.73bp.

The result of RAPD analysis using primer OPAD-20 were illustrated in Fig.2D and table(5,6);the total number of bands varied from the lowest number of four in Giza76 bands and a highest number of nine bands in Giza69, Giza77and Giza83.

One monomorphic band in all genotypes and three unique positive bands were found in Giza75 and Giza83 with *M.W.* (515.15, 510.23bp) and 863.09bp, respectively.

Table(5): Number of amplified fragments and specific markers of nine cotton genotypes based on RAPD-PCR analysis with eight primers.

Genotypes	RAPD Primers								
		B01	B05	B06	B07	B10	B 14	AD 20	AF 20
	<i>TAF</i>	23.00	20.00	16.00	10.00	13.00	13.00	19.00	22.00
	<i>MP</i>	5.00	5.00	1.00	3.00	1.00	2.00	1.00	0.00
	<i>PB</i>	18.00	15.00	15.00	7.00	12.00	11.00	18.00	22.00
	<i>PB%</i>	78.26	75.00	93.75	70.00	92.31	84.62	94.74	100.0
<b>P<sub>1</sub></b>	<i>AF</i>	5.0	8.0	3.0	3.0	3.0	5.0	7.0	6.0
	<i>SM</i>	0.0	0.0	0.0	0.0	2.0	0.0	0.0	1.0
<b>P<sub>2</sub></b>	<i>AF</i>	5.0	7.0	4.0	3.0	3.0	6.0	9.0	10.0
	<i>SM</i>	2.0	0.0	1.0	0.0	2.0	0.0	0.0	0.0
<b>P<sub>3</sub></b>	<i>AF</i>	5.0	8.0	6.0	2.0	4.0	6.0	6.0	7.0
	<i>SM</i>	0.0	0.0	0.0	2.0	0.0	2.0	0.0	1.0
<b>P<sub>4</sub></b>	<i>AF</i>	8.0	8.0	4.0	4.0	4.0	5.0	4.0	8.0
	<i>SM</i>	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>P<sub>5</sub></b>	<i>AF</i>	11.0	10.0	4.0	1.0	4.0	3.0	9.0	9.0
	<i>SM</i>	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
<b>P<sub>6</sub></b>	<i>AF</i>	13.0	8.0	5.0	1.0	5.0	3.0	6.0	10.0
	<i>SM</i>	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0
<b>P<sub>7</sub></b>	<i>AF</i>	8.0	3.0	5.0	3.0	5.0	3.0	6.0	9.0
	<i>SM</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>P<sub>8</sub></b>	<i>AF</i>	7.0	9.0	7.0	3.0	4.0	3.0	8.0	10.0
	<i>SM</i>	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0
<b>P<sub>9</sub></b>	<i>AF</i>	8.0	7.0	10.0	1.0	4.0	3.0	5.0	7.0
	<i>SM</i>	1.0	0.0	2.0	0.0	0.0	0.0	0.0	1.0
<b>Total</b>	<i>AF</i>	70.0	68.0	48.0	21.0	36.0	37.0	60.0	78.0
	<i>SM</i>	4.0	1.0	3.0	2.0	5.0	2.0	3.0	5.0

TAF=Total amplified fragment., MP= Monomorphic bands.,

PB=Polymorphic bands. AF=Amplified fragment, SM=Specific marker.

The PCR products of primer OPAF-20 ranged from seven bands in Dendera and found 11 bands in Giza80 in table 5,6 and Fig.2D; this primer don't produce any monomorphic bands in all genotypes; some genotypes had some specific bands and could be used to distinguish among them. For instance Dendera, Giza75,Giza77, Giza80 and Giza90 have five positive unique bands at *M.W.* of (515.15, 566.67, 493.94, 854.31*bp* ),respectively.

Table (6 ):Trait specific RAPD markers in the nine cotton cultivars.

Molecular Marker	Band Number	M.W (bp)	Trait	Parents
B01	1	551.52	L, RD.	Giza 69
	9	213.64		
	3	581.82	EL, SL, PI, +b.	Giza 77
	13	190.91	L, E.	Giza 90
B05	4	551.52	L,SL,PI,RD.	Giza 76
B06	5	213.64	L, RD.	Giza 69
	5	515.15	L, E.	Giza 90
	11	510.23		
B07	6	160.55	L,PI, MR.	Giza 75
	7	109.36		
B10	2	377.27	L,E.	Dendera
	5	295.47		
	1	510.23	L, RD.	Giza 69
	5	190.91		
	5	218.18	L, +b, MR.	Giza 83
B14	1	594.74	L, PI, MR.	Giza 75
	2	572.73		
AD20	8	515.15	L, +b, MR, E.	Giza 80
	9	510.23		
	1	863.09	L, +b, MR	Giza 83
AF20	7	515.15	L.	Dendera
	6	566.67	L,PI, MR.	Giza 75
	8	493.94	EL, SL, PI, +b.	Giza 77
	4	854.31	L, +b, MR, E.	Giza 80
	3	854.31	L,E.	Giza 90

We found that all the studied primers produced polymorphic amplification products. **Iqbal et al.1997** also observed that 98% of the primers in their study produced polymorphic profiles. A high level of polymorphism (97.2%) was observed in our study.

**Lu and Myers,2002** observed a low level of DNA variation among ten varieties of *G. hirsutum*, as they observed only 13.5% polymorphism. **Iqbal et al. 1997** found 89.1% polymorphism among 23 *G. hirsutum* cultivars and **Rahman et al.2003** observed 66.2% polymorphism in 27 cotton varieties.

Conflicting reports on the extent of observed polymorphism in cotton in different studies could be attributed to the nature of the genetic material under investigation and the high degree of polymorphism in our study compared to other reports, is due to the more diverse material which belonged to different cultivated of cotton.

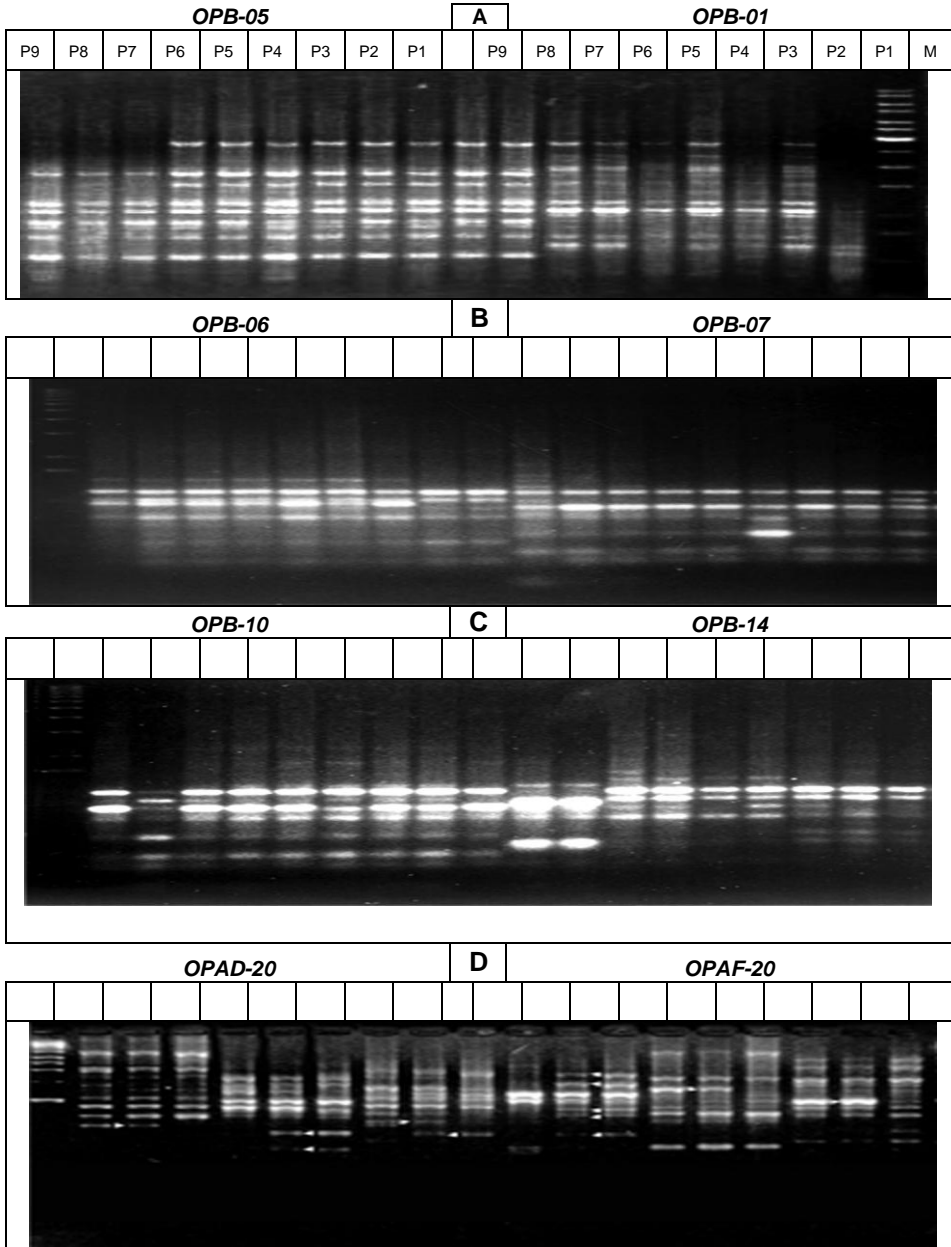


Fig.(2):DNA polymorphism of the nine cotton genotypes using RAPD\_PCR with four primers.

Species-specific markers could be useful for introgression studies where plant breeders want to transfer some desirable traits from one species into another. Localization of these markers on the chromosomes would be useful for keeping track of important traits that need to be transferred. Genetically distinct cultivars are potentially important sources of germplasm for cotton improvement

#### **b. Jaccard's similarity coefficient.**

Similarity between all pair of cotton is shown in table (7); the data showed that the similarity matrix of genetic distance ranged from 0.278 between Dendera and Giza 83 to 0.704 between Giza77 and Giza80 ; the average of similarity among cultivars was 0.491.

Using RAPD markers greater diversity was observed in tetraploid cotton cultivars. This observation has been documented **Kumar, et al.,2003**. Prevalence of greater diversity necessitates further collections and conservation of tetraploid cotton plant genetic resources in the country.

In Indian tetraploid *G. hirsutum* cotton cultivars, **Vafai, et al.2003** observed 79% average genetic similarity. In nine *G. barbadense* cultivars in our study average genetic similarity was 49%. Similarity ranges of 92.1–98.9%( **Multani, et al.,1995**); 70–90%7 and 81.6–94.9%(**Rahman, et al.,2003**) for genetic kinship in elite *G. barbadense* cultivars have been reported in the exotic materials.

This highlights the point that the most commonly cultivated species have a comparatively narrow genetic base, which might be due to the fact that common parents are usually employed in the pedigrees for the development of new cultivars.

Thus, there is potential for broadening the genetic base of the most commonly cultivated cottons by way of introgression breeding using the less commonly cultivated species.

Table (7): Genetic distances among the nine Egyptian cotton genotypes based on Jaccard's coefficient, using RAPD data.

Genotypes	Dendera	Giza 69	Giza 75	Giza 76	Giza 77	Giza 80	Giza 81	Giza 83	Giza 90
<b>Dendera</b>	1.0								
<b>Giza 69</b>	0.424	1.0							
<b>Giza 75</b>	0.531	0.515	1.0						
<b>Giza 76</b>	0.355	0.433	0.452	1.0					
<b>Giza 77</b>	0.394	0.342	0.441	0.680	1.0				
<b>Giza 80</b>	0.394	0.382	0.531	0.620	0.704	1.0			
<b>Giza 81</b>	0.400	0.344	0.452	0.520	0.500	0.556	1.0		
<b>Giza 83</b>	0.278	0.343	0.400	0.400	0.484	0.533	0.680	1.0	
<b>Giza 90</b>	0.364	0.438	0.412	0.323	0.324	0.364	0.519	0.500	1.0

### c. Cluster analysis.

Nei's genetic distance (Fig.3) showed that the genetic distance for each genotype combination ranged from 0.4 to 0.7 and the studied cultivars formed two main clusters; the first main cluster separated at genetic similarity of 0.45 and created four sub clusters, the first subcluster included an individual cultivars Giza76 at genetic similarity of 0.65, the second subcluster included Giza77 and Giza80 at genetic similarity of 0.71, the third subcluster included Giza81 and Giza83 at genetic similarity of 0.68 and the forth subcluster included an individual cultivars Giza90 at genetic similarity of 0.51.

In reaction to second main cluster separated at genetic similarity of 0.47 and created two sub clusters, the first subcluster included two cultivars Giza75 and Dendera at genetic similarity of 0.53, the second subcluster included an individual cultivars Giza69 at genetic similarity of 0.47.

The differences between fiber quality characters in groups lead to the formation of the two main groups.

Separate clusters have been observed for cultivars of *G. hirsutum* and *G. barbadense*. This discrepancy might be due to different parts of the cotton genome being scanned, since the oligonucleotides employed in the two studies were unique except for two primers (Tatineni, et al.,1996; Rana and Bhat,2002).

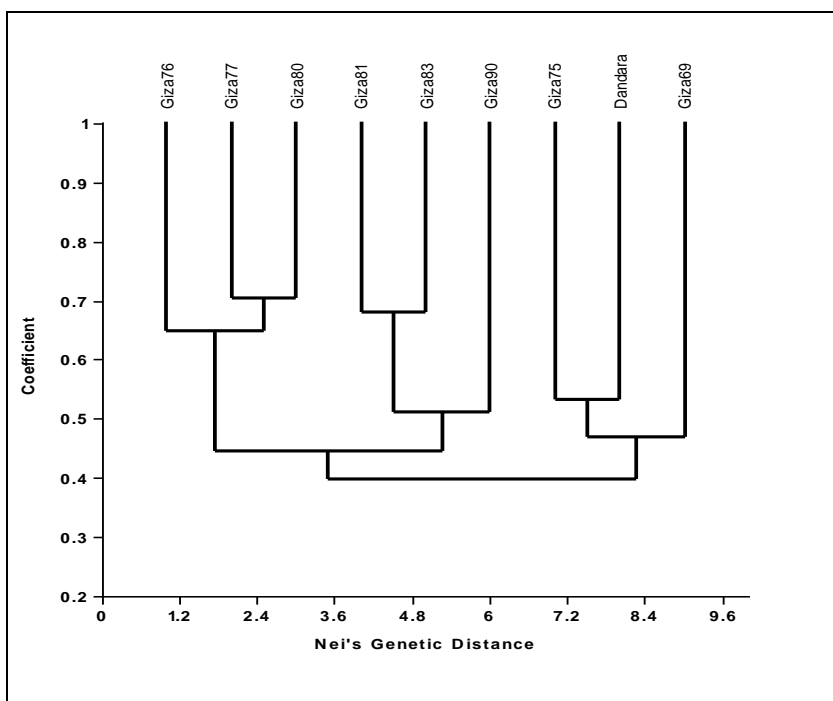


Fig.(3):Dendrogram obtained from UPGMA cluster based on RAPD data from the nine cotton genotypes.

This analysis revealed that most of the variation could be attributed to within breeding-centre variance, while variance among breeding centers was little. It indicates that breeders have not been working in isolation, and that breeding material for the development of these cultivars has been shared between the breeding stations and cotton improvement programmes.

From the above study it can be concluded that RAPD markers were found to reveal sufficient genetic diversity and a high level of genetic polymorphism in tetraploid cultivars had more genetic diversity.

UPGMA cluster analysis placed all the tetraploid cultivars within their respective known taxonomic groups, while breeding centre-wise grouping of cultivars was not discernible.

This study recommends the use of RAPD as rapid and accurate method of identification to facilitate classification of fiber quality

traits for morphological studies and germplasm management and genetics improvement program in Egyptian cotton.

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