GENETIC CHARACTERIZATION AND RELATIONSHIPS AMONG EGYPTIAN COTTON VARIETIES AS REVEALED BY BIOCHEMICAL AND MOLECULAR MARKERS

B. E. ABDEL-FATTAH

Genetics Department, Faculty of Agriculture, Assiut University, Egypt

Cotton is an economic plant of world importance. It is the world's leading textile fiber crop and it is also a source of secondary products such as oil, live-stock feed (cotton seed cake) and cellulose (Anderson, 1999 and Frelichowski *et al.,* 2006).

Assessment of genetic markers and diversity form an integral part of any successful breeding program. Morphological features are indications of the genotype but are represented by only a few loci because there are not a large enough number of characters available. Moreover, they can also be affected by environmental factors and growth practices. To overcome the limitations associated with morphological markers, various biochemical and molecular marker techniques have come up in recent years. Biochemical markers such as isozymes have been used to study the genetic distances and estimate the level of genetic variability of cotton varieties and accessions (Wendel *et al.,* 1989; Percy and Wendel, 1990; Abdel-Tawab *et al.,* 1990 & 1993; Melchinger *et al.,* 1991; Wendel *et al.,* 1992; Sukumar and Allan, 1998; Farooq *et al.,* 1999). However, isozyme analysis has certain limitations due to the availability of a limited number of marker loci, a general lack of polymorphism for these loci in elite breeding materials, and the chance of variability in banding patterns being due to plant development (Tanksley *et al.,* 1989). Protein markers have also been used to identify different cotton species, varieties and lines (Khan, 1991; Goyal, 1993; Renata *et al.,* 2004; Murtaza *et al.,* 2005; Yunuskhanov *et al.,* 2007; Kurbanbaev *et al.,* 2008)

DNA based molecular markers such as RFLP, AFLP, SSR, ESTs, SNP and RAPD have been widely used in genetic analyses, breeding studies and investigations of genetic diversity and the relationship between cultivated species and their wild parents. They have several advantages, including high polymorphism and independence from effects related to environmental conditions and the physiological stage of the plant. Among these marker techniques, the randomly 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 sequences for the design of primers (Williams *et al.,* 1990; Welsh and McClelland, 1990). The RAPD markers have already been used in cotton for the assessment of genetic vari-

ability, diversity and fingerprinting cotton genotypes (Pillay and Myers, 1999; Jing *et al.,* 2000; Hussein *et al,.* 2002, 2006, and 2007; Muhammad *et al.,* 2009; Zahid *et al.,* 2009) as well as for the detection of variation between closely related cultivars (Multani and Lyon 1995; Rahman *et al.,* 2002; El-Defrawy *et al.,* 2004; Masoud *et al.,* 2007).

The objectives of this investigation were to: (1) determine the genetic differences between nine Egyptian cotton varieties using six isozyme systems, protein patterns and 10 RAPD markers, (2) identify variety specific RAPD markers in the tested genotypes, and (3) assess the genetic distance and relationships among these varieties.

MATERIALS AND METHODS

Plant Materials

Nine Egyptian cotton varieties (*Gossypium barbadense* L.), namely, Dandara, Giza 75, Giza 83, Giza 85, Giza 86, Giza 88, Giza 89, Giza 90 and Giza 91, were used in the present investigation. The code and origin of these varieties are shown in Table (1).

Germination condition of seed

All seeds were surface sterilized with 40% Clorox (5.2% v/v) sodium hypochlorite solution for 10 minutes and washed three time with sterile water, the seeds were germinated on filter paper in Petri dishes and incubated at room temperature in the dark.

Protein and isozymes analyses

Nonsoluble protein, and esterase (EST), peroxidase (PRX), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), glutamate-oxalacetate-transaminase (GOT) and superoxide dismutase (SOD) isozymes were extracted from the nine cotton varieties using bulked samples (5 seedlings) from each of the nine varieties. The extraction buffer consisted of 0.1 M Tris-HCl and 2.0 mM EDTA, pH 7.8. Protein electrophoresis was carried out according to Laemmli (1970) method with 12% SDS polyacrylamide gels under denaturing conditions. Gels were stained for detecting protein bands with Commassie Blue R. Then, gels were destained by repeated immersions in a mixture of methanol: acetic acid: water (1:1:8, by volume). The molecular weight of protein bands were determined against protein markers which consisted of 96, 67, 48.1, 30 and 14.0 KD using GS 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version). For isozyme analyses, samples were electrophoreased on 7.5 % polyacrylamide gels under nondenaturing conditions. The activities of the enzymes were stained according to Tanksley and Orton (1986) method.

Random amplified polymorphic DNA (RAPD) analysis

DNA extraction

Total genomic DNA was isolated from fresh leaves, bulked from 5 different plants per genotype using CTAB protocol

for plants (Murray and Thompson, 1980; Saghai-Maroof *et al.,* 1984; Kumar *et al.*, 2003) with some modifications. Fresh leaves were ground in liquid nitrogen using a mortar and a pestle, then, 800 µl of 60°C extraction buffer (100mM Tris-HCl, 1.4M NaCl, 20mM EDTA, 2% cyl-tri-methyl-ammonium bromide (CTAB), adjusted to pH 8.0 and 50 µl β-mercaptoethanol were added to the samples, mixed by gentle inversion and incubated at 60°C in water-bath for 30 minutes. An equal volume of chloroform: isoamylalcohol (24:1) was added to the cooled mixture. Emulsion was mixed gently and centrifuged at 5000 rpm for 20 minutes at 10°C. The supernatent was transferred to a new Eppendorf tube. An equal volume of chilled isopropanol was added to precipitate the DNA. Precipitated DNA was centrifuged at 10000 rpm for 5 minutes at 4°C to make the pellet. The supernatant was poured-off and the pellet was washed with cold 70% ethanol (v/v) and centrifuged thereafter at 10000 rpm for 2 minutes at 4°C. The pellet was dried in a 37°C incubator (under vacuum). The pellet was thereafter dissolved in 300-500µl TE buffer. Dissolved pellet was then treated with 3µl RNase and incubated in 37°C for 30 minutes. Samples were then treated with 3µl proteinase-K and incubated at 37°C for 30 minutes. DNA dilutions were made to detect the optimum concentration for RAPD-PCR analysis.

RAPD-PCR reactions

Polymerase chain reaction (PCR) was conducted using ten arbitrary 10 mer primers (Operon Tech., Inc) (Table 2). PCR reactions were conducted according to Williams *et al*. (1990). The reaction conditions were optimized and mixtures (25 µl total volume) were composed of 11.0 µl dH_2O, 3.0 µl 10X reaction buffer, 3.0 µl dNTP's mix, 2.0 µl primer, 4.0 µl $MgCl_2$, 0.3 µl Taq DNA polymerase and 1 µl Template DNA. Amplification condition were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, UK) with the following specification: initial denaturation for 3 minutes at 85°C (1^{st} step), 40 cycles of 1 minute at 85°C, 2 minutes at 33°C and 2 minutes at 72°C (2^{nd} step), 10 minutes at 72°C (3^{rd} step), then followed by a final hold at 4°C. The amplification products were separated by loading 14 µl of each reaction onto a 1.4% ultra pure agarose gel containing 0.01% ethidium bromide in 1X concentration TBE buffer (89 mM Tris-borate, 2.5 mM EDTA). Electrophoresis was carried out under constant voltage of around 60V for approximately 3-3.5 hours. The patterns were visualized on a Transilluminator (Ultra-Violet Product, Upland, CA, USA).

Data analyses

RAPD-based molecular markers were scored visually using the software package MVSP (Multi-Variate Statistical Package) and DNA bands were scored as present (1) or absent (0). The pairwise comparisons between the tested isolates were used to calculate the coefficient of genetic similarity matrix (Gs) according

to Nei and Li (1979). To convert the genetic similarity into genetic distance, logarithmic transformation (-Ln Gs) was computed to linearize the distance measure. Cluster analysis was presented as the dendrogram based on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Isozyme analysis

Isozymes are not only quicker and less labor intensive than traditional methods but are also more reliable since the expression of isozymes loci are co-dominant (Arus, 1983, Ryan and Scowcroft, 1987). In the present investigation, six isozyme systems of peroxidase (PRX), esterase (EST), malate dehydrogenase (MDH), acid phosphatase (ACP), glutamate-oxalo-acetate transaminase (GOT) and superoxide dismutase (SOD) were used to detect the genetic variability among nine cotton (*Gossypium barbaradense* L.) varieties namely: Dandara, Giza-75, Giza-83, Giza-85, Giza-86, Giza-88, Giza-89, Giza-90 and Giza-91 (Fig. 1).

No differences were found among the nine genotypes in the isozyme patterns of GOT, MDH, EST, ACP and PRX. All tested genotypes revealed similar patterns of two GOT bands, two ACP bands, two MDH bands, seven EST bands and four PRX bands (Fig. 1). These results indicated that these enzymatic systems could not be used alone to differentiate between the tested genotypes.

Meanwhile, isozyme differences in SOD bands were evident for the tested genotypes. One positive and three negative enzymatic bands were detected in the SOD zymograms (Fig. 1). All tested genotypes displayed the positive SOD band. Three negative SOD bands were observed in Giza-75 and Giza-89 while the other genotypes displayed two negative bands.

Concerning this Farooq *et al.* (1999) used 6 isozyme systems to differentiate among cotton varieties and found monomorphic patterns for esterase and superoxide dismutase isozymes in all tested varieties.

Protein pattern analysis

Gel electrophoresis provided a simple method for assaying variation in soluble proteins. The protein banding-pattern of each organism represents a biochemical genetic fingerprint to that organism and each band in the pattern reflects a separate transcriptional event. Most of the proteins assayed by electrophoresis (under denaturing conditions) are encoded by single gene locus. The gel proteins can, then, be interpreted as single-locus genotypes and the genetic information can be readily obtained. In the present investigation, except the differences in band intensity, Fig. (1) revealed low quantitative differences between the nine cotton varieties. The 70.0 KD protein band was detected only in the cotton varieties Giza 83,

Giza 85, Giza 89 and Giza 91. In addition, one protein band at molecular weight 46 KD was only expressed in the varieties Dandara, Giza 75, Giza 86, Giza 90 and Giza 91. These few variations in protein patterns resulting from cotton seedlings could not be used alone to differentiate between the tested varieties. Khan (1991) found considerable differences in protein banding patterns of nine varieties of American upland and American-Egyptian cotton. In 8X8 full diallel cross, Murtaza *et al.* (2005) found that the F_2 progenies differed from each others in their protein patterns, but their patterns were identical to the respective maternal parents. Protein patterns have also been used to identify different cotton species, varieties and lines (Goyal, 1993, Renata *et al.,* 2004, Yunuskhanov *et al.,* 2007 and Kurbanbaev *et al.,* 2008).

Random amplified polymorphic DNA (RAPD)

In the present investigation, 10 random primers were used to study the genetic differences and relationships among the nine cotton (*Gossypium barbaradense* L.) varieties (Table 3 and Figs. 2, 3). The 10 primers amplified a total of 151 DNA fragments from all tested varieties with an average of 15.1 bands/primer and ranged in size from 1621bp (OPW-15) to 106 bp (OPA-15) (Table 3 and Fig. 2). Each of OPU-7, OPD-2 and OPI-9 amplified a maximum of 19 bands, while a minimum of 6 bands were amplified with the primer OPA-2. The two varieties, Giza 90 and Giza 91, displayed the higher number of DNA fragments (129 bands), while variety Dandara revealed the least number of bands (118 bands). These variation in the number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome (Kernodle *et al.,* 1993).

Out of the 10 primers surveyed, 8 primers detected polymorphism among the all tested varieties, while the OPA-2 and OPA-8 primers displayed monomorphic patterns. A total of 151 DNA bands were amplified by the 10 primers from all tested varieties and 55 of these fragments showed polymorphism (36.4%). The rest of these bands (63.6%) were common between the tested varieties.

The monomorphic bands are constant and cannot be used to study the diversity while polymorphic bands revealed differences and could be used to examine and establish systematic relationships among the genotypes (Hadrys *et al.,* 1992). The highest number of polymorphic bands was amplified by the OPS-19 (78.6%), while the OPC-18 primer generated the lowest number of polymorphic bands (21.4%) (Table 4). In Egyptian cotton, different levels of RAPD polymorphism were detected by a number of researchers using different primers and different genotypes. In this instance, Hussein *et al.* (2002) used 49 RAPD primers and detected 30.4% polymorphism among 13 cotton genotypes. El-Defrawy *et al.* (2004) reported that 49.3% out of 71 bands amplified by 6 primers were poly-

morphic in six Egyptian cotton cultivars. Hussein *et al.* (2006) assayed twenty-one cotton accessions using 28 RAPD primers and found that the level of polymorphism among the 21 accessions was 59.1%. Furthermore, Hussein *et al.* (2007) found that 63.2% of bands generated from 11 cotton genotypes by 15 primers were polymorphic. Esmail *et al.* (2008) subjected 21 cotton genotypes to RAPD analysis using 53 UBC 10-mer RAPD primers. They detected a total of 113 bands, among which 96 bands (84.95%) were polymorphic. Zahid *et al.* (2009) measured the genetic distance among 20 different species of *Gossypium* using 63 random 10-mer primers and they observed that 310 out of a total of 370 RAPD bands (83.8%) were perceived polymorphic.

Unique DNA fragments with different sizes were detected in particular genotype but not in the others using different primers. The presence of a unique band for a given genotype is referred as positive marker while the absence of a common band served as negative marker. Such bands could be used as DNA markers for genotype identification and discrimination. In this respect, one DNA fragment in the variety Giza 75 [106 bp (OPA-15)], three bands in Giza 85 [1231 bp, 1185 bp and 1058 bp (OPS-19)], two bands in Giza 86 [1248 bp (OPU-7) and 1066 bp (OPS-19)], one band in Giza 88 [415 bp (OPD-2)], two bands in Giza 90 [669 bp (OPW-15) and 330 bp (OPI-9)] and three bands in Giza 91 [765 bp, 710 bp and 576 bp (OPS-19)] were varieties-specific positive markers (Table 5). Varieties -specific negative markers were also recorded for Giza 85 [1143 bp (OPU-7), 1091 bp (OPI-9) and 119 bp (OPA-15)], Giza 86 [625 bp (OPU-7)], Giza 88 [1621 bp, 1462 bp, 1282 bp, 315 bp and 275 bp (OPW-15) and 656 bp (OPU-7)] and Giza 90 [442 bp (OPA-15)]. Meanwhile, no unique markers were detected for Dandara, Giza 83 and Giza 89. The largest number of RAPD-PCR specific markers was scored for Giza 88 (7 markers) followed by Giza 85 (6 markers), while the lowest specific markers (1 marker) was scored for Giza 75. In the meantime, the higher number of RAPD varieties -specific markers was generated by the primer OPS-19 (7 markers), followed by OPW-15 (6 markers). On the other hand, the least number of RAPD specific markers was generated by the primer OPD-2 (1 marker) (Table 5).

In the present investigation, 23 varieties -specific markers (12 positive and 11 negative) were detected indicating that they could be used as markers for all studied varieties, except Dandara, Giza 83 and Giza 89. Hussein *et al.* (2002) identified 101 genotype specific DNA markers for Hindi off type (*G. hirsutum*) and 38 markers for Giza 45 (*G. barbadence*), when applying RAPD, ISSR, SSR and AFLP. El-Defrawy *et al.* (2004) found five positive and eight negative bands in Giza-45, two positive bands in Giza-90, one positive and one negative bands in Giza-75 and one positive band in Dandra were unique DNA markers for these varieties. Adawy (2007) detected 11 SSR and EST unique specific markers identify-

ing 9 out of 14 cotton genotypes. The present investigation revealed that the six Egyptian varieties (Giza 75, G-85 G-86, G-88, G-90 and G-91) could be characterized individually based upon variety-specific RAPD markers, thus making it possible to differentiate closely related varieties by molecular markers. These results suggested that the RAPD approach showed considerable potential for identifying and discriminating cotton varieties. Similar conclusion was also reported by Hussein *et al.* (2002).

The presence/absence data of the ten primers was analyzed using the software package MVSP program according of Nei and Li (1979) method to estimate the genetic similarity (Gs) (Table 6). The genetic similarity among the nine cotton varieties were converted to genetic distance estimates using the formula D = -ln Gs (Swofford and Olson 1990). The genetic similarity among the nine cotton varieties was high, ranging from 87.3 % to 96.1 %. The highest similarity (96.1%) and shortest genetic distance (0.04) were scored between Giza-89 and Giza-75 (Table 6). The high similarity between these two varieties was due to the contribution of Giza-75 in the parents of Giza-89 (Table-1). The lowest genetic similarity (87.3%) and longest genetic distance (0.136) were found between Giza-85 and Giza-86. These results were substantiated by the fact that only 107 out of 137 bands were common between these two genotypes which may be due to the absence of common parents between them (Tables 1and 3). Powell *et al.* (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured. Based on the RAPD analysis, Hussein *et al.* (2007) found that the genetic similarities ranged from 63.8% to 95.9% in 8 Egyptian cotton (*G. barbadense*) and 3 genotypes of *G. hirsutum*).

Cluster dendrogram (Fig. 4) based on similarity matrix obtained with unweighted pair group method using arithmetic means (UPGMA) showed that the variety Giza 88 was separated in a single branch from the other varieties within 89.4% branched-off genetic similarity, reflecting a relatively longer genetic distance from the other varieties. In fact, such genotype did not have any common parent with the other varieties (Table 1). Genetically distinct varieties were identified that could be potentially important sources of germplasm for cotton improvement (Rana and Bhat, 2005).

The dendrogram also showed that both Giza-89 and Giza-75 were clustered together firstly with 0.961 genetic similarity while, the second cluster included Giza-83 and Giza-91 at 0.953 similarity. The variety Giza-90 was clustered singly with the first cluster at similarity of 0.934 and with the second cluster at 0.932 similarity. These varieties were clustered with Giza-86 (0.922 similarity) followed by Dandara (0.916 similarity) and Giza-85 (0.905

similarity). These results were substantiated previously by the involvement of Giza-67 as a common parent between Giza 75, Giza 83 and Giza 85 as well as the contribution of Giza 75 in the gene pool of Giza 86 and Giza 89 (Table 1). Using 6 RAPD primers, El-Defrawy *et al.* (2004) reported that the varieties Giza-83 and Giza-85 followed by Giza-90 were clustered together within 96.0 – 97.3% genetic similarity, then they clustered with Dandara (93.9% similarity) followed by Giza-75 (84.7% similarity). Hussein *et al.* (2007) analyzed the RAPD, SSR, EST, AFLP and combined data, and found that the dendrograms clustered the 11 cotton genotypes into two main clusters: one containing the 8 Egyptian varieties belonging to *G. barbadense* and the other one contained the 3 genotypes belonging to *G. hirsutum*. Rana and Bhat (2005) clustering 59 cotton varieties using UPGMA cluster analysis, and showed that all *G. barbadense* cultivars grouped in one cluster and having maximum similarity while, the second cluster contained all *G. hirsutum* except cultivars CPD 423, CP 15/2, Laxmi and G. Cot12 which did not cluster with the rest of the *G. hirsutum* cultivars.

In the present investigation, six isozyme systems, protein patterns and 10 RAPD primers were used to study the genetic variability among nine Egyptian cotton varieties (*Gossypium barbadense* L.). Polymorphism was not evident in all tested isozymes, except SOD, and it was low in protein patterns, while it was very high in all RAPD markers. Polymorphisms due to RAPD-PCR analysis could be caused by differences in nucleotide sequences at the priming sites (such as point mutations), or by structural rearrangements within the amplified sequence, (e.g., insertions, deletions, inversions) (Welsh and McClelland, 1990).

Twenty-three variety-specific markers were detected in the present study for 6 out of 9 studied varieties. These variety-specific markers would be useful for introgression studies where plant breeders want to transfer some desirable traits from one cultivar into another. Localization of these markers on the chromosomes would be useful for keeping track of important traits that need to be transferred. Similar conclusion was also reported by Rana and Bhat (2005).

The high level of polymorphism and the determination of RAPD specific markers detected in the present investigation confirmed the efficiency of RAPD molecular markers in detecting polymorphism among cotton varieties, estimation of relatedness and identifying varieties by unique fingerprints. Polymorphism also considered as a useful selection tool in monitoring alien genome introgression in cotton breeding programs. Similar conclusions were also obtained in different cotton genotypes by Jing *et al*. (2000), Hussein *et al.* (2002, 2006 and 2007), El-Defrawy *et al.* (2004), Rana and Bhat (2005), Esmail *et al.* (2008) and Zahid *et al.* (2009).

SUMMARY

Six isozymes systems peroxidase (PRX), esterase (EST), malate dehydrogenase (MDH), acid phosphatase (ACP), glutamate-oxalo-acetate trancaminase (GOT) and superoxide dismutase (SOD), Protein patterns and 10 RAPD markers were used to detect the genetic variability and relationships among nine cotton varieties (*Gossypium barbaradense* L.) namely: Dandara, Giza-75, Giza-83, Giza-85, Giza-86, Giza-88, Giza-89, Giza-90 and Giza-91. Polymorphism was not evident in all tested isozymes, except SOD. All tested genotypes revealed similar patterns of 2 GOT bands, 2 ACP bands, 2 MDH bands, 7 EST bands and 4 PRX bands. Protein analysis revealed low quantitative differences between the nine cotton varieties was observed. One protein band at 70.0 kD was detected only in Giza 83, Giza 85, Giza 89 and Giza 91, while the 46 kD protein only expressed in Dandara, Giza 75, Giza 86, Giza 90 and Giza 91.

The results of RAPD analysis showed that 8 out of the 10 tested primers displayed polymorphism among the all tested varieties, while 2 primers were monomorphic. 36.4% out of 151 bands amplified were polymorphic. Unique DNA fragments with different sizes were detected in Giza 75, Giza 85, Giza 86, Giza 88, Giza 90 and Giza 91 varieties, while no unique markers were detected for Dandara, Giza 83 and Giza 89. The genetic similarity among the nine cotton varieties was high, ranging from 87.3 % to 96.1 %. The highest similarity and shortest genetic distance were found between Giza-89 and Giza-75. While the lowest genetic similarity and longest genetic distance were found between Giza-85 and Giza-86. The UPGMA Cluster analysis showed that the variety Giza 88 was separated in a single branch from the other varieties within 89.4% branched-off genetic similarity, reflecting a relatively longer genetic distance from the other varieties. The other varieties were cultured together within a range of 0.905 - 0.961 genetic similarity. The investigation suggested that the RAPD approach showed considerable potential for identifying and discriminating cotton varieties.

REFERENCES

Abdel-Tawab, F. M., E. M. Fahmy, M. A. Rashed, A. El-Soudy and M. O. Ismael (1990). Use of seed protein PAGE, esterase isozyme patterns and immunodiffusion analysis to differentiate between cotton cultivars of two different species. Egypt. J. Genet. Cytol., 19: 37-46.

Abdel-Tawab, F. M., E. M. Fahmy, M. O. Ismail and F. M. El-Domyati (1993). Application of electrophoretic analysis in cultivar identification in cotton (*Gossypium barbadense* L.). 4th Conf. Agric. Dev. Res., Ain-Shams Univ., Cairo, Feb. 13-18, Annals Agric. Sci., Sp. Issue, 2, 489-503.

Adawy, S. S. (2007). An Evaluation of the utility of Simple Sequence Repeat loci (SSR), Expressed Se-

quence Tags (ESTs) and Expressed Sequence Tag Microsatellites (EST-SSR) as Molecular Markers in Cotton. Journal of Applied Sciences Research, 3: 1581-1588.

Anderson, C. G. (1999). Cotton marketing. In: Smith, C.W, Cothren, J.T.(eds) Cotton: Origin, History, Technology and Production. Wiley, New York, pp: 659-679 (C.F. Frelichowski *et. al.,* 2006).

Arus, P. (1983). Genetic purity of commercial seed lots. In: Tanksley, S. D. and Orton, T. J. (Eds.) Isozymes in plant genetics and breeding, Part A, pp. 415-423. Elsevier Sci. Publishers B. V., Amsterdam.

El-Defrawy, M. M., Mervat M. Hashad and E. N. Elsayed (2004). Molecular polymorphism in egyptian cotton (*Gossypium barbadense* L.). Assiut Journal of Agricultural Science, 35: 83-96.

Esmail R. M., J. F. Zhang and A. M. Abdel-Hamid (2008). Genetic Diversity in Elite Cotton Germplasm Lines Using Field Performance and Rapd Markers. World Journal of Agricultural Sciences, 4: 369-375.

Farooq, S., N. Iqbal and A. A. Zaidi (1999). Isozyme markers in cotton breeding 1. Standardization of different isozyme systems for identification of different cultivars of cotton (*Gossypium hirsutum*). Pakistan Journal of Botany, 31: 5-20.

Frelichowski, Jr., M. B. Palmer, D. Main, J. p. Tomkins, R. G. Cantrell, D. M. Stelly, J. Yu, R. J. Kohel and M. Uloa (2006). Cotton genome mapping with new microsatellites from Acala "Maxxa" BAC-ends. Mol. Gen. Genomics, 275: 479-491.

Goyal, K.C. (1993). Identification of some cotton cultivars using PAGE. Indian J. plant physiol., 35: 405-407.

Hadrys, H., M. Balick and B. Schiewater (1992). Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol., 1: 55-60.

Hussein, Ebtissam H. A., A. Amina Mohamed, S. Attia and S. S. Adawy (2006). Molecular characterization and genetic relationships among cotton genotypes 1- RAPD, ISSR and SSR analysis. Arab J. Biotech., 9: 313-328.

Hussein, Ebtissam H. A., M. H. A. Osman, M. H. Hussein and S. S. Adawy (2007). Molecular Characterization of Cotton Genotypes using PCR-based Markers. J. Appl. Sci. Res., 10: 1156-1169.

Hussein, Ebtissam H. A, M. Sh.Al-Said, H. A. El-Itriby, and M. A. Madkour (2002). Genotyping Egyptian Cotton Varieties (*G. barbadense*)

using molecular markers. (Poster) Biotechnology and Sustainable Development Voices of the South and North Conf. Held at the Bibliotheca Alexandrina Conference Center, March 16-20, Alexandria, Egypt.

Jing, K. Z., S. Ji-Zhong, Z. Jin-Fa, N. Yi-Chun and L. Jin-Lan (2000). Genetic diversity evaluation of some Chinese elite cotton varieties with RAPD markers. Acta Genetica Sinica, 27: 817-823.

Kernodle, S. P., R. E. Cannon and J. G. Scandalios (1993). Rapid and simple phage DNA isolation. Biotechniques, 14:360:362.

Khan, I. A. (1991). Electrophoretic analysis of seed proteins of various cotton varieties of different geographic areas. Pak. J. Agri. Sci., 28: 110-116.

Kumar, A., P. Pushp-Angadan and S. Mehrotra (2003). Extraction of High-Molecular-Weight DNA From Dry Root Tissue of *Berberis lycium* Suitable for RAPD. Plant Molecular Biology Reporter, 21: 309a-309d.

Kurbanbaev, I. Zh., D. Yu. Ataev, and Sh. Yunuskhanov (2008). Protein Markers for Identification of Different Species and Varieties of Cotton. Applied Biochemistry and Microbiology, 45: 432-437.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Masoud, S., Zahra, H. Shahriari, H. Rokneizadeh and Zahra Noormohammadi (2007). RAPD and cytogenetic study of some tetraploid Cotton (*Gossypium hirsutum L.*) cultivars and their hybrids. Cytologia, 72: 77-82.

Melchinger, A. E., M. M. Messmer, M. Lee, W. L. Woodman and K. R. Lamkey (1991). Diversity and relationships among US maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci., 31: 669-678.

Muhammad, A., M. Ur. Rahman, J. I. Mirza and Y. Zafar (2009). Parentage confirmation of cotton hybrids using molecular markers. Pak. J. Bot., 41: 695-701.

Multani, D. S. and B. R. Lyon (1995). Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome, 38: 1005-1008.

Murray, M. G. and W. F. Thompson (1980). Rapid isolation of high molcular weight plant DNA. Nucleic Acids Research, 8: 4321-4325.

Murtaza, N., M. Kitaoka and G. M. Ali (2005). Genetic differentiation of cotton cultivars by polyacrylamide

gel electrophoresis. Journal Central Europe Agriculture, 6: 69-76.

Nei, M. and W. H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Percy, R. G. and J. F. Wendel (1990). Allozyme envidence for the origin and diversification of (*Gossypium barbadense* L.). Theor. Appl. Genet., 79: 529-542.

Pillay, M. and G. O. Myers (1999). Genetic diversity in cotton assessed by variation in ribosomal RNA genes and AFLP markers. Crop Sci., 39: 1881-1886.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Rahman, M., D. Hussain and Y. Zafar (2002). Estimation of divergence among elite cotton cultivars-genotypes by DNA fingerprinting technology. Crop Sci., 42: 2137-2144.

Rana, M. K. and K. V. Bhat (2005). RAPD markers for genetic diversity study among India cotton cultivars. Current Science, 88: 1956-1961.

Renata, S. M., M. D. G. G. C. Vieira, E. N. Mann and M. C. Alves (2004). Varietal diversity in cotton based on seed protein profile extracted at high temperature. Revista Ciência Agronômica, Vol. 35, Número Especial, Out., 227-231.

Ryan, S. A. and W. R. Scowcroft (1987). A somaclonal variant of wheat with additional B-amaylase isozymes. Theor. Appl. Genet., 73: 459-464.

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen and R. W. Allard (1984). Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci., 81: 8014-8018.

Sukumar, S. and Z. Allan (1998). Genetic diversity and phylogenetic relationships in cotton based on isozyme markers. Journal of Crop Production, 1: 79-93.

Swofford, D. L. and G. J. Olson (1990). Phylogeny reconstruction. In: Hillis DM, Moritz C (eds) Molecular Systematics. Sinauer Associates, Sunderland, Massachusetts.

Tanksley, S. D., N. D. Young, A. H. Paterson and M. W. Bonierbale (1989). RFLP mapping in plant breeding: new tools for an old science. Biotechnology, 7: 257-264.

Tanksley, S. D. and T. J. Orton (1986). "Isozymes in plant genetics and breeding". Part A (eds.), Elsevier Sci. Puplishers B. V., New York, pp. 500.

Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Wendel, J. F., J. M. Stewart and J. H. Retting (1989). Genetic diversity, introgression and independent domestication of old world cultivated cottons. Amer. J. Bot., 67: 1795-1806.

Wendel, J. F., C. L. Brubaker and A. E. Percival (1992). Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. American Journal of Botany, 97: 1291-1310.

Williams, J. G. K., A. R. Kublik, K. J. Livak, J. A. Rafaliski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Yunuskhanov, Sh. Yu., D. Yu. Ataev and I. Zh. Kurbanbaev (2007). Species-specific Features of the Protein Patterns of Diploid Cotton Seeds with A and D Genomes and of Some Amphidiploids. Russian Journal of Genetics, 43: 404-410.

Zahid M., F. Raheel, A. S. Dasti, S. Shahzadi, M. Athar and M. Qayyum (2009). Genetic diversity analysis of the species of *Gossypium* by using RAPD markers. African Journal of Biotechnology, 8: 3691-3697.

Table (1): The code number and origin of the nine cotton varieties used in the present investigation.

Code number	Common name	Origin
1	Dandara	Selected from Giza-3
2	Giza 75	Giza 67 x Giza 69
3	Giza 83	Giza 67 x Giza 72
4	Giza 85	Giza 67 x Giza 58B
5	Giza 86	Giza 75 x Giza 81
6	Giza 88	Giza 77 x Giza 45B
7	Giza 89	Giza 75 x 6022
8	Giza 90	Dandara x Giza 83
9	Giza 91	Giza 81 x Giza 83

Table (2): Primer sequences and codes used.

Serial No.	Primer codes	Sequence (5’ to 3’)
1	OPA02	5'-TGCCGAGCTG-3'
2	OPI09	5'-TGGAGAGCAG-3'
3	OPA08	5'-GTGACGTAGG-3'
4	OPA15	5'-AGATGCAGCC-3'
5	OPC18	5'-TGGGGGACTC-3'
6	OPY05	5'-GGCTGCGACA-3'
7	OPU07	5'-CCTGCTCATC -3'
8	OPS19	5'-GAGTCAGCAG-3'
9	OPW15	5'-ACACCGGAAC-3'
10	OPD02	5'-GGACCCAACC-3'

Table (3): Survey of the RAPD-DNA fragments of the ten primers in nine cotton varieties.

No	Primers	Bp	Dandara	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91	No	Primers	Bp	Dandara	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91
1	OPW-15	1621	1	1	1	1	1	0	1	1	1	55	OPI-9	1212	1	1	1	1	0	0	1	1	1
2		1462	1	1	1	1	1	0	1	1	1	56		1091	1	1	1	0	1	1	1	1	1
3		1282	1	1	1	1	1	0	1	1	1	57		925	1	1	1	1	1	1	1	1	1
4		1119	1	1	1	1	1	1	1	1	1	58		853	1	1	1	1	1	1	1	1	1
5		938	1	1	1	1	1	1	1	1	1	59		789	0	0	0	1	0	0	0	1	0
6		866	1	1	1	1	1	1	1	1	1	60		715	1	1	1	1	1	1	1	1	1
7		805	1	1	1	1	1	1	1	1	1	61		633	1	1	1	1	1	1	1	1	1
8		723	1	1	1	1	1	1	1	1	1	62		592	1	1	1	1	1	1	1	1	1
9		669	0	0	0	0	0	0	0	1	0	63		550	1	1	1	1	1	1	1	1	1
10		634	1	1	1	1	1	1	1	1	1	64		518	1	1	1	1	1	1	1	1	1
11		584	1	1	1	1	1	1	1	1	1	65		498	1	1	1	1	1	1	1	1	1
12		534	0	0	1	1	0	0	0	1	1	66		448	1	1	1	1	1	1	1	1	1
13		496	1	1	1	1	1	1	1	1	1	67		410	1	1	1	1	1	1	1	1	1
14		435	1	1	1	1	1	1	1	1	1	68		363	0	1	0	1	0	1	1	1	1
15		391	1	1	1	1	1	1	1	1	1	69		330	0	0	0	0	0	0	0	1	0
16		350	1	1	1	1	1	1	1	1	1	70		311	1	1	1	1	1	1	1	1	1
17		315	1	1	1	1	1	0	1	1	1	71		270	1	1	1	1	1	1	1	1	1
18		275	1	1	1	1	1	0	1	1	1	72		235	1	1	1	1	1	1	1	1	1
19	OPU-7	1407	0	1	1	1	1	0	1	1	1	73		161	0	1	0	0	1	1	1	1	0
20		1248	0	0	0	0	1	0	0	0	0	74	OPD-2	1231	0	1	1	0	1	0	1	1	1
21		1143	1	1	1	0	1	1	1	1	1	75		1108	0	1	1	0	1	0	1	1	1
22		1071	1	1	1	1	1	1	1	1	1	76		997	0	1	1	0	1	0	1	1	0
23		1014	1	0	1	1	1	1	1	0	0	77		896	1	1	1	1	1	1	1	1	1
24		905	1	1	1	1	1	1	1	1	1	78		824	1	1	1	1	0	0	1	0	0
25		809	1	1	1	1	1	1	1	1	1	79		750	1	1	1	1	1	1	1	1	1
26		747	1	1	1	1	1	1	1	1	1	80		686	1	1	1	1	1	1	1	1	1
27		707	0	1	0	0	0	0	1	1	1	81		620	1	1	1	1	1	1	1	1	1
28		656	1	1	1	1	1	0	1	1	1	82		585	1	0	0	0	0	0	1	0	1
29		625	1	1	1	1	0	1	1	1	1	83		515	0	0	0	0	0	1	0	1	0
30		584	1	1	1	1	1	1	1	1	1	84		534	1	1	1	1	1	1	1	1	1
31		552	1	1	1	1	1	1	1	1	1	85		489	1	1	1	1	1	1	1	1	1

Table (3): Cont.

No	Primers	Bp	Dandara	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91
32		489	1	1	1	1	1	1	1	1	1
33		399	1	1	1	1	1	1	1	1	1
34		372	1	0	0	0	0	1	0	0	0
35		301	1	1	1	1	1	1	1	1	1
36		271	1	1	1	1	0	0	1	1	1
37		230	1	1	1	1	1	1	1	1	1
38		1298	1	1	1	1	1	1	1	1	1
39		1096	1	1	1	1	1	1	1	1	1
40		978	1	1	1	1	1	1	1	1	1
41		839	1	1	1	1	1	1	1	1	1
42	OPA-8	716	1	1	1	1	1	1	1	1	1
43		609	1	1	1	1	1	1	1	1	1
44		501	1	1	1	1	1	1	1	1	1
45		455	1	1	1	1	1	1	1	1	1
46		363	1	1	1	1	1	1	1	1	1
47		309	1	1	1	1	1	1	1	1	1
48		245	1	1	1	1	1	1	1	1	1
49		891	1	1	1	1	1	1	1	1	1
50		822	1	1	1	1	1	1	1	1	1
51	OPA-2	748	1	1	1	1	1	1	1	1	1
52		716	1	1	1	1	1	1	1	1	1
53		687	1	1	1	1	1	1	1	1	1
54		565	1	1	1	1	1	1	1	1	1

No	Primers	Bp	Dandara	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91
86		451	1	1	1	1	1	1	1	1	1
87		415	0	0	0	0	0	1	0	0	0
88		393	1	1	1	1	1	1	1	1	1
89		368	1	1	1	1	1	1	1	1	1
90		331	1	1	1	1	1	1	1	1	1
91		297	1	1	1	1	1	1	1	1	1
92		267	1	1	1	1	1	1	1	1	1
93		1260	1	1	1	1	1	1	1	1	1
94		1123	0	1	0	0	0	1	1	1	0
95		953	1	1	1	1	1	1	1	1	1
96		830	1	1	1	0	1	1	0	1	1
97		751	1	1	1	1	1	1	1	1	1
98		668	0	1	1	1	0	1	1	1	0
99		592	1	1	1	1	1	1	1	1	1
100		509	1	1	1	1	1	1	1	1	1
101	OPA-15	473	0	1	1	1	0	1	1	1	1
102		442	1	1	1	1	1	1	1	0	1
103		387	1	1	1	1	1	1	1	1	1
104		348	1	1	1	1	1	1	1	1	1
105		325	1	1	1	1	1	1	1	1	1
106		226	1	1	1	1	1	1	1	1	1
107		167	1	1	1	1	1	1	1	1	1
108		119	1	1	1	0	1	1	1	1	1
109		106	0	1	0	0	0	0	0	0	0

Table (3): Cont.

No	Primers	Bp	Dandara	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91
110	OPC-18	1327	1	1	1	1	1	1	1	1	1
111		1263	1	0	0	1	1	1	1	1	0
112		1203	1	1	1	1	1	1	1	1	1
113		1107	1	1	1	1	1	1	1	1	1
114		1000	1	1	1	1	1	1	1	1	1
115		908	1	1	1	1	1	1	1	1	1
116		840	1	1	1	1	1	1	1	1	1
117		796	0	1	1	1	0	1	1	0	1
118		739	1	1	1	1	1	1	1	1	1
119		689	0	0	1	1	0	0	0	0	1
120		617	1	1	1	1	1	1	1	1	1
121		573	1	1	1	1	1	1	1	1	1
122		529	1	1	1	1	1	1	1	1	1
123		490	1	1	1	1	1	1	1	1	1
124	OPY-5	999	1	1	1	1	1	1	1	1	1
125		918	1	1	1	1	1	1	1	1	1
126		825	1	1	1	1	1	1	1	1	1
127		757	1	1	1	1	1	1	1	1	1
128		700	1	1	0	1	0	0	1	0	0
129		664	1	1	1	1	1	1	1	1	1
130		619	1	1	1	1	1	1	1	1	1
131		578	1	1	1	1	1	1	1	1	1
132		535	0	0	1	0	1	1	0	1	1
133		502	1	1	1	1	1	1	1	1	1
134		453	1	1	0	1	0	0	1	0	0
135		414	1	1	1	1	1	1	1	1	1
136		381	0	0	1	0	1	1	1	1	1
137		345	0	0	1	0	1	1	0	1	1

No	Primers	Bp	Dandra	Giza-75	Giza-83	Giza-85	Giza-86	Giza-88	Giza-89	Giza-90	Giza-91
138	OPS-19	1231	0	0	0	1	0	0	0	0	0
139		1185	0	0	0	1	0	0	0	0	0
140		1058	0	0	0	1	0	0	0	0	0
141		1066	0	0	0	0	1	0	0	0	0
142		930	0	1	1	1	0	1	0	0	0
143		892	1	1	1	1	1	1	1	1	1
144		823	1	1	1	1	1	1	1	1	1
145		765	0	0	0	0	0	0	0	0	1
146		710	0	0	0	0	0	0	0	0	1
147		677	1	0	0	0	0	0	1	1	1
148		637	1	1	1	1	1	1	1	1	1
149		576	0	0	0	0	0	0	0	0	1
150		553	0	1	1	1	0	1	0	0	1
151		434	0	1	1	1	1	1	0	0	1

Table (4): Number of amplified DNA-fragments and polymorphic bands in nine cotton varieties investigated with ten RAPD primers.

Primers Code	No. of amplified bands									Total amplified bands	No. of polymorphic bands	% of polymorphic bands
	Dandara	Giza 75	Giza 83	Giza 85	Giza 86	Giza 88	Giza 89	Giza 90	Giza 91			
OPW-15	16	16	17	17	16	11	16	18	17	18	7	38.9
OPU-7	16	16	16	15	15	14	17	16	16	19	9	47.4
OPI-9	15	17	15	16	15	16	17	19	16	19	6	31.6
OPD-2	14	16	16	13	15	14	17	16	15	19	7	36.8
OPC-18	12	12	13	14	12	13	13	12	13	14	3	21.4
OPA-15	13	17	15	13	13	16	15	15	14	17	7	41.2
OPA-8	11	11	11	11	11	11	11	11	11	11	0	0.0
OPA-2	6	6	6	6	6	6	6	6	6	6	0	0.0
OPY-5	11	11	12	11	12	12	12	12	12	14	5	35.7
OPS-19	4	6	6	9	5	6	4	4	9	14	11	78.6
TOTAL	118	128	127	125	120	119	128	129	129	151	55	36.4

Table (5): Cotton varieties characterized by unique positive and/or negative RAPD markers, marker size and total number of markers.

Primer	Giza 75		Giza 85		Giza 86		Giza 88		Giza 90		Giza 91		Positive marker	Negative marker	Total markers
	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker			
OPW-15								1621 1462 1282 315 275	669				1	5	6
OPU-7				1143	1248	625		656					1	3	4
OPI-9				1091					330				1	1	2
OPD-2							415						1		1
OPA-15	106			119						442			1	2	3
OPS-19			1231 1185 1058		1066						765 710 576		7	0	7
Total	1		3	3	2	1	1	6	2	1	3		12	11	23
	1		6		3		7		3		3				

Table (6): Genetic similarity (above the diagonal) and distance (below the diagonal) values calculated from 151 DNA fragments generated with ten primers in nine cotton varieties.

Genotypes	Dandara	Giza 75	Giza 83	Giza 85	Giza 86	Giza 88	Giza 89	Giza 90	Giza 91
Dandara	--	0.084	0.09	0.09	0.088	0.121	0.059	0.106	0.098
Giza 75	0.919	--	0.052	0.078	0.093	0.106	0.04	0.077	0.077
Giza 83	0.914	0.949	--	0.074	0.063	0.093	0.069	0.073	0.048
Giza 85	0.914	0.925	0.929	--	0.136	0.131	0.087	0.126	0.108
Giza 86	0.916	0.911	0.939	0.873	--	0.101	0.084	0.079	0.088
Giza 88	0.886	0.899	0.911	0.877	0.904	--	0.115	0.111	0.12
Giza 89	0.943	0.961	0.933	0.917	0.919	0.891	--	0.06	0.077
Giza 90	0.899	0.926	0.930	0.882	0.924	0.895	0.942	--	0.073
Giza 91	0.907	0.926	0.953	0.898	0.916	0.887	0.926	0.930	--

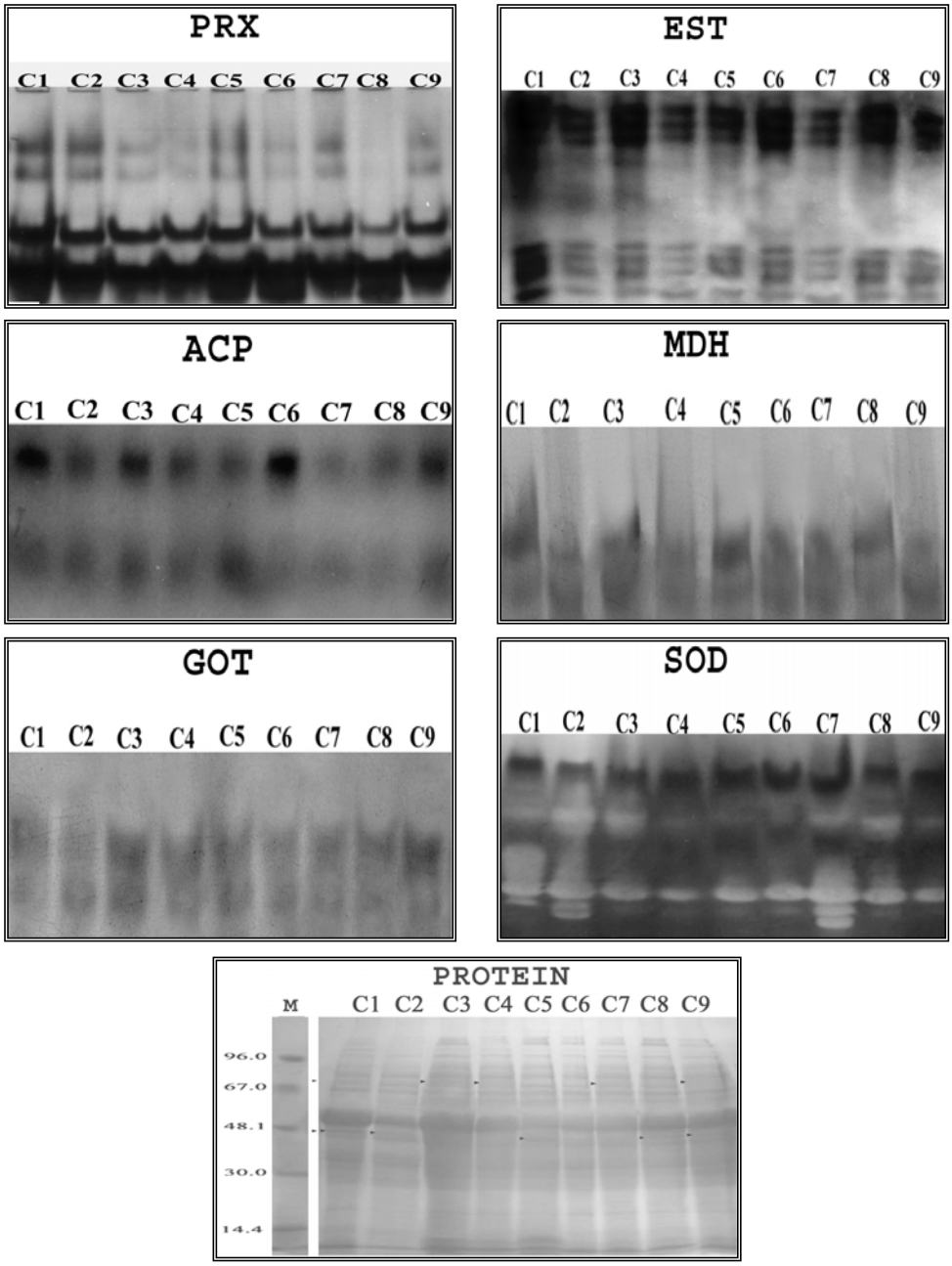

Fig. (1): Electrophoretic patterns of esterase (EST), peroxidase (PRX), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), glutamate-oxalacetate-transaminase (GOT), superoxide dismutase (SOD) isozymes and protein patterns detected in cotton varieties (C1) Dandara, (C2) Giza 75, (C3) Giza 83, (C4) Giza 85, (C5) Giza 86, (C6) Giza 88, (C7) Giza 89, (C8) Giza 90 and (C9) Giza 91.

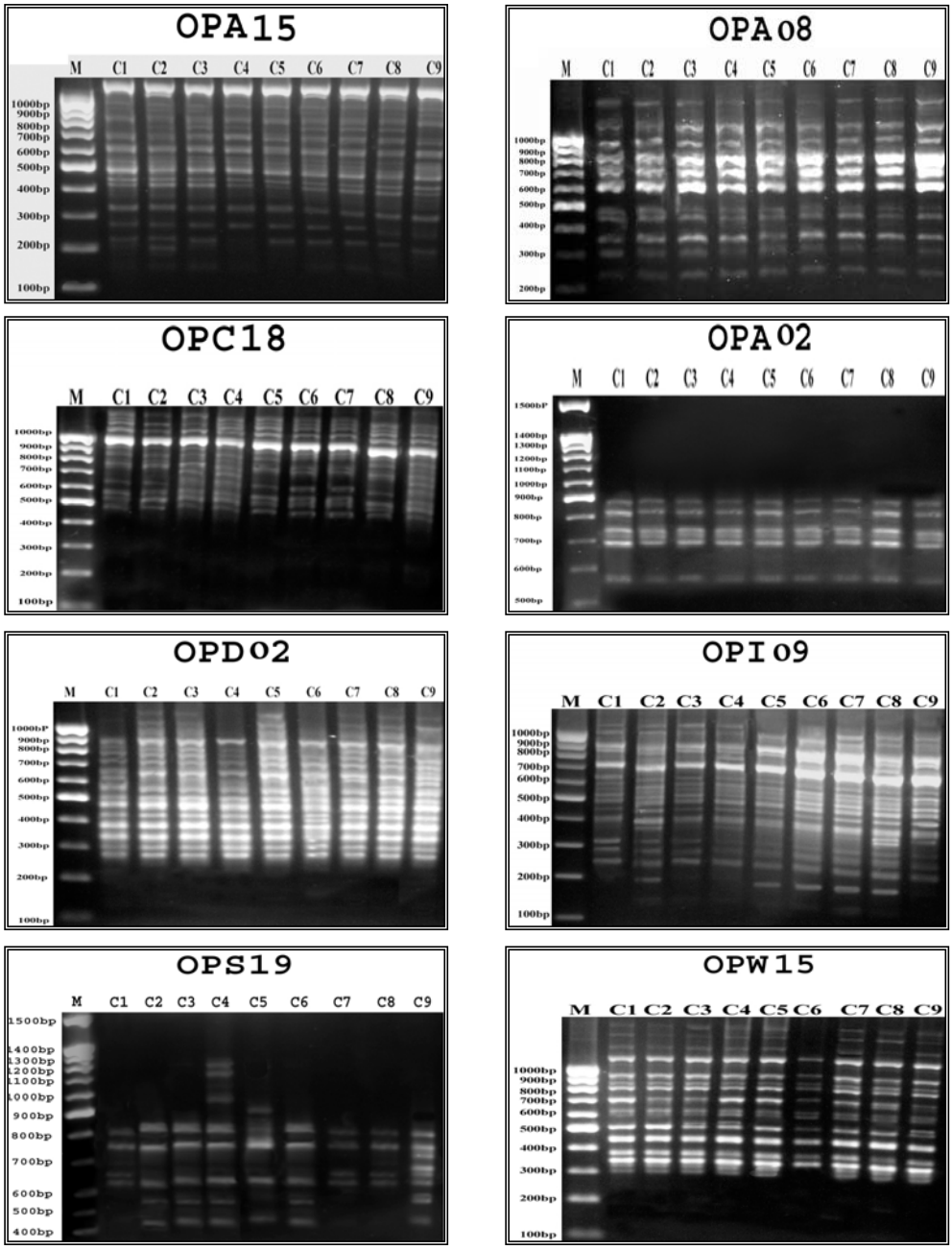

Fig. (2): Agarose gel electrophoresis of RAPD profile in the cotton varieties (C1) Dandara, (C2) Giza 75, (C3) Giza 83, (C4) Giza 85, (C5) Giza 86, (C6) Giza 88, (C7) Giza 89, (C8) Giza 90 and (C9) Giza 91.

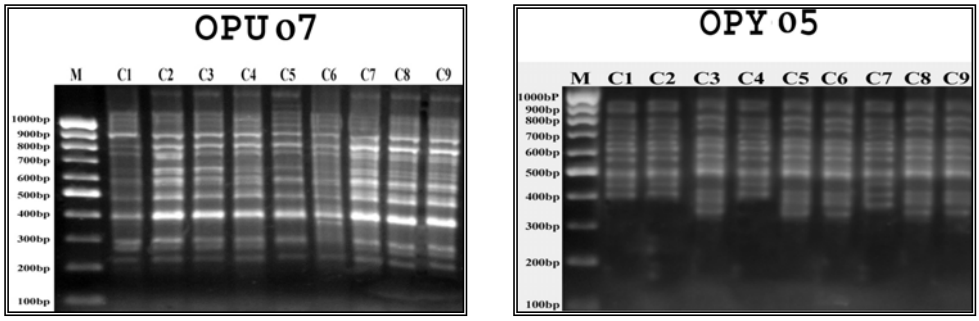

Fig. (3): Agarose gel electrophoresis of RAPD profile in the cotton varieties (C1) Dandara, (C2) Giza 75, (C3) Giza 83, (C4) Giza 85, (C5) Giza 86, (C6) Giza 88, (C7) Giza 89, (C8) Giza 90 and (C9) Giza 91.

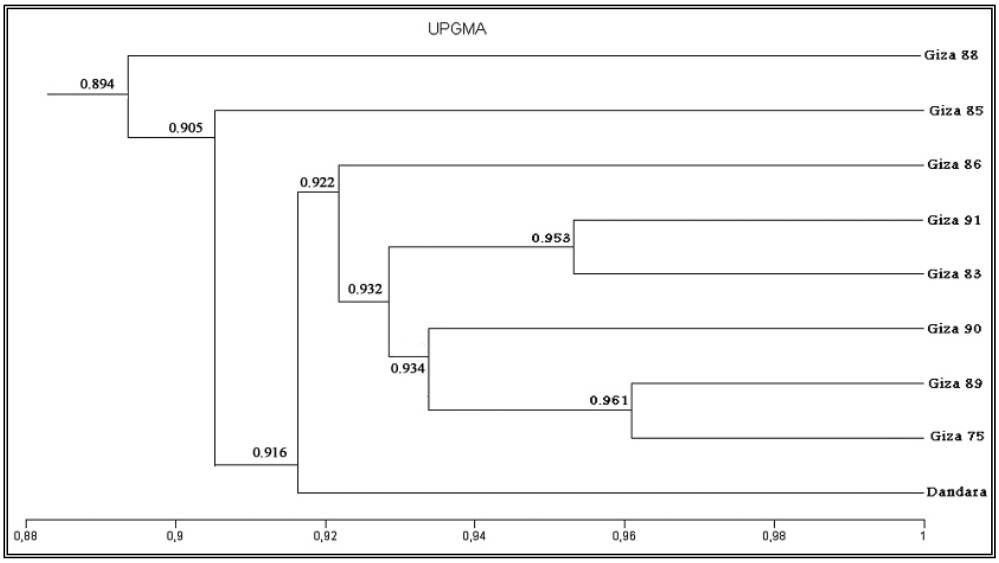

Fig. (4): Dendrogram of nine cotton varieties developed from RAPD data using UPGMA analysis. The scale is based on Nei and Li coefficients of similarity.