Molecular characterization and genetic relationships among cotton genotypes
2- AFLP Analysis

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ABSTRACT

The high-resolution genotyping method of amplified fragment length polymorphism (AFLP) was used to study the genetic relationships among 21 cotton genotypes from two different species G. barbadense and G. hirsutum. Sixteen AFLP primer combinations were used to selectively amplify the DNA fragments that matches the primer-extension sequence to investigate the genetic polymorphism among the 21 cotton genotypes. The 16 AFLP primer combinations produced 940 bands among which 474 were polymorphic, thus, representing a level of polymorphism of 50.4% among the 21 cotton genotypes. The amplification of AFLP templates resulted in a number of reproducible fragments ranging from 31 to 90 per primer with a size range of 60 bp to 780 bp. Fifteen primer combinations detected unique specific markers identifying 8 out of the 21 genotypes. A dendrogram was generated from the AFLP information that revealed two main clusters. All the genotypes belonging to G. barbadense except one (Pima Early American) were grouped in one cluster, while the accessions representing G. hirsutum constituted the second cluster, thus, confirming the results previously obtained by RAPD, ISSR and SSR analysis on the same cotton genotypes. To evaluate the efficiency of the different marker systems, the sum effective number of alleles (SENA), the average expected heterozygosity for polymorphic markers (Hav(p)), the effective multiplex ratio (E) and marker index (MI) were calculated. The AFLP exhibited considerably high SENA (318.2) compared to RAPD, ISSR and SSR (127.7, 46.0 and 22.3, respectively). The average heterozygosity values were comparable for the different marker systems (0.39, 0.36, 0.39 and 0.34 in AFLP, RAPD, ISSR and SSR, respectively). The MI was 182.2 in AFLP's, while it was 73.6, 26.3 and 12.7 in RAPD, ISSR and SSR, respectively. Thus, the results indicated that AFLP is more effective in detecting high level of polymorphism. The correlation coefficient was considerably higher between SSR and ISSR (0.61), and it was lower between RAPD's and AFLP's (0.26) than that between AFLP and ISSR (0.44) and AFLP and SSR (0.49). The results confirmed that different marker systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application. Therefore, they could complement each other to draw more accurate conclusions.

Keywords: *Cotton, Gossypium species, AFLP, fingerprinting, cluster, diversity, structural genomic, molecular markers, SENA, expected heterozygosity for polymorphic loci (Hav(P)).*

INTRODUCTION

Assessment of genetic diversity forms an integral part of any successful breeding program. Conventionally, breeders have been employing morphological markers for genetic diversity estimation; however, the number of morphological descriptors in various crops is in vogue for characterization purposes. To overcome the limitations associated with morphological markers, a large number of molecular markers have come up in recent years. These molecular markers can increase the speed and precision of breeding programs.

Cotton, the leading natural fiber crop and the second oil important crop, is largely produced by two primary cultivated allotetraploid species known as Upland or American cotton (*Gossypium hirsutum* L.) and Egyptian cotton (*Gossypium barbadense* L.). These allotetraploid species (2n=52) diverged from each other and from their diploid progenitors (2n=26) through selection and domestication. Upland cotton, *G. hirsutum* dominates the world's cotton fiber production accounting for approximately 90% of the total world production. The second most cultivated species, *G. barbadense*, includes superior extra-long, strong and fine cottons. In Egypt, cotton is considered one of the most important cash crops. However, cultivated cotton has a narrow genetic base, so there is little variation available for use in the development of new, higher yielding cotton cultivars. Knowledge of genetic diversity and relationships among breeding materials could have a significant impact in cotton improvement. One of the major limitations to the application of genomic technology in cotton is the paucity of informative DNA markers. Technological developments have expanded the range of DNA polymorphism assays for genetic mapping, marker assisted plant breeding, genome fingerprinting and for investigating genetic relatedness. The most widely used of these technologies are restriction fragment length polymorphism or RFLP (Botstein *et al.*, 1980), random amplified polymorphic DNA or RAPD (Williams *et al.*, 1990), simple sequence repeat markers or microsatellites (SSR) (Tautz, 1989) and amplified fragments length polymorphism or AFLP (Zabeau and Vos, 1993). AFLP is a powerful fingerprinting technique, which detects polymorphism on the level of restriction enzymes sites. It is based on PCR amplification of restriction enzymes and oligonucleotides adaptors of few nucleotide bases. This method generates a large number of restriction fragment bands facilitating the detection of polymorphism. Therefore, AFLP markers combine the advantages of RFLP's and PCR-based markers. This technology has been adopted for fingerprinting and mapping of different plants (Reddy *et al.* 1996; Cervera *et al.*, 1998; Saliba-Colombani *et al.* 2000; Adawy *et al.* 2005; Hussein *et al.* 2005).

The present study has been carried out with the main objective to explore the efficiency of the AFLP technology in identifying molecular markers useful for the assessment of genetic diversity among 21 cotton genotypes. This study is part of a wider project aimed at the assessment and interpretation of diversity and fingerprinting at the molecular level of the cotton genotypes available at the Cotton Research Institute, ARC, and Egypt. Moreover, the data obtained from AFLP were compared to those deduced in a previous work from the analysis of RAPD, ISSR and SSR (Hussein *et al.*, 2006). This information will help the breeders to predict which matings might produce new and superior gene combinations and, therefore, fastening breeding programs with higher accuracy.

MATERIALS AND METHODS

The plant material used in this study consisted of 21 cotton genotypes (Tamkot Kabc, Tamkot Kamde, Okraleaf, Turpan Zining, Yonany, Giza 70, Giza 90, Pima Early American, Pima High Yield, 24111(Aust.), 24240 (Aust.), Australian deeply divided, Russian 6022, Giza 72, Giza72 x Delcero (BC9), Delcero, Giza 83, Giza 83x Delta Pine(BC7), Delta Pine, Tamcot Luxor and Giza 45). The seeds were kindly provided by Cotton Research Institute (CRI), Agricultural Research Center (ARC), and Giza, Egypt.

Table (1): AFLP primer combinations, total number of amplicons, number of polymorphic amplicons and level of polymorphism detected by different primer combinations for the 21 cotton genotypes.

Primer combination	Total no. of amplicons	Polymorphic amplicons	Polymorphism %
1/3 E AAC /M CAG	90	38	42.2
1/7 E AAC /M CTG	71	20	28.2
1/8 E AAC /M CTT	85	29	34.1
3/3 E ACA /M CAG	60	24	40.0
4/6 E ACC /M CTC	32	25	78.1
5/1 E ACG /M CAA	59	27	45.7
5/3 E ACG /M CAG	39	13	33.3
5/4 E ACG /M CAT	31	26	83.9
5/5 E ACG /M CTA	57	50	87.7
5/6 E ACG /M CTC	43	24	55.8
6/5 E ACT /M CTA	37	21	56.8
6/6 E ACT /M CTC	60	56	93.3
7/1 E AGC /M CAA	87	24	27.6
7/3 E AGC /M CAG	50	26	52.0
8/3 E AGG /M CAG	83	42	50.6
8/8 E AGG /M CTT	56	29	51.8
Total	**940**	**474**	**50.4**

DNA extraction

Total genomic DNA was isolated from young leaf material following the CTAB procedure of Porebski *et al,* (1997) with some modifications as described by Hussein *et al.* (2003).

Table (2): The cotton genotypes characterized by unique positive and/or negative AFLP markers, marker size and total number of markers identifying each genotype.

	Tam. kabc		**Tam.Kam.**		**Okraleaf**		**Tur.zining**		**Pima High Yield**		**Delcero**		**G. 83**		**Tam. l ux**	
Primer comb.	+ bp	- bp	+ bp	- bp	+ bp	- bp	+ bp	- bp	+ bp	- bp	+ bp	- bp	+ bp	- bp	+ bp	- bp
6 – 5								70								
1 – 3					123							600				
5 – 3												295				
4 – 6		102		450												
5 – 4															450	
1 – 8												235 250 290				
3 – 3					510							350				
8 – 8												620 700	380 415			
1 – 7		285 310 370										182 340 500				
6 – 6												250 380 450				
8 - 3												250 300 305				
7 – 1	360 430 440	173	190	290								235 255 450				
7 – 3		210	240								270 560	285 600				
5 – 6						185				135	202					
5 – 1											375					
Sub-total	3	6	2	2	2	1		1		1	4	22	2		1	
Total	**9**		**4**		**3**		**1**		**1**		**26**		**2**		**1**	

Table (3): Unique species-specific AFLP markers and their corresponding sizes.

Primer Comb.	Positive markers size *G.hirsutum*	Positive markers size *G.barbadense*	Total
1/3	125, 134, 217	136, 215, 310, 320, 292, 450, 500	10
1/7	50,175, 480	75	4
1/8	178	310, 360	3
3/3	325, 315	158, 162, 205, 322	6
5/1	168, 199	167, 198, 260, 265	6
5/3	------	250	1
5/4	205	98, 150, 168, 178, 197, 280, 297	8
5/5	126	68, 103, 110, 112,170, 236, 245, 310	9
5/6	160	113, 121, 196	4
6/5	162, 164	167, 169	4
6/6	105, 120, 123	166, 175	5
7/1	-------	105, 172, 180, 185, 225, 260	6
7/3	450	------	1
8/8	60, 175	103, 180, 275	5
Total	**22**	**50**	**72**

AFLP fingerprinting

The AFLP procedure was performed, with minor modifications, according to the protocol of Vos *et al.* (1995) that is supplied with the AFLP Analysis System I (Gibco BRL, USA, Cat. No. 10544). Approximately 400 ng DNA of each of the (21 cotton genotypes) was digested simultaneously with *Eco*RI and *Mse*I at 37°C for 2 h. A small aliquot of the digested DNA was run on a 1.5% (w/v) agarose gel to check if the DNA digestion was complete. The digested samples were incubated at 70°C for 15 min to inactivate the restriction endonucleases. *Eco*RI and *Mse*RI adaptors were ligated to the digested DNA samples to generate template DNA for amplification. The ligation products were diluted 10 fold in TE buffer and 5 ul added to preamplification reaction. Preamplification was carried out with 16-primer combinations each carrying one selective nucleotide in a thermocycler for 20 cycles set at 94°C denaturation (30 sec), 56°C annealing (60 sec) and 72°C extensions (60 sec). The

amplification products were diluted 50 folds in TE buffer and stored at -20°C. Selective AFLP amplification was carried out with *Eco*RI and *Mse*I primers each carrying three selective nucleotides and 5 uL of the diluted PCR products from the preamplification product. The PCR selective amplification temperature profile was as follows: one cycle at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 60 sec; followed by 12 cycles of touchdown PCR in which the annealing temperature was decreased by 0.7°C every cycle until a 'touchdown' annealing temperature of 56°C was reached. Once reached, another 23 cycles were conducted as described above for preamplification. Two µl of the reaction product was mixed with an equal volume of formamide loading buffer (98% [v/v] formamide, 10 mM EDTA, 0.005% [v/v] of each of xylene cyanol and bromophenol blue), denatured by incubating at 90°C for 5 min and quickly cooled on ice. The products were analyzed on 6% (w/v) denaturing polyacrylamide gels. The gel was run at constant power (50–55 W) until the xylene cyanol was about two-thirds down the length of the gel. The gel was silver stained according to the protocol described by the manufacturer (Promega Corp., USA, Silver Sequence DNA Staining Reagents and Cat. No. Q4132).

Data analysis

The genetic similarity coefficient, similarity matrices and cluster analysis were estimated as mentioned in Hussein *et al.* (2005). The sum of effective number of alleles (SENA) was calculated from the formula: $\text{SENA} = \sum [(1 / \sum p_i^2) - 1]$, where the p_i is the frequency of the *i*-th allele (Powell *et al.*, 1996). The observed heterozygosity was calculated as the ratio between heterozygous genotypes and the total analyzed genotypes for each locus. However, the arithmetic mean of expected heterozygosity for the polymorphic loci ($H_{av(p)}$) was calculated for each marker class as follows: $H_{av(p)} = \sum H_p/n_p$ and the fraction of polymorphic loci (β) is calculated as: $\beta = n_p / (n_p + n_{np})$, where n_p is the number of polymorphic loci and n_{np} is the number of non-polymorphic loci. Then, the average heterozygosity is calculated as follows: $H_{av} = \beta (\sum H_p/n_p)$ (Powell *et al.*, 1996). The number of polymorphic markers per gel lane, called marker index (*MI*), is simply the product of effective multiplex ratio and the average expected heterozygosity for the polymorphic markers and calculated from the formula: $MI = EH_{av(p)}$, where the effective multiplex ratio (E) is the number of loci polymorphic in the germplasm set of interest analyzed per experiment and calculated from the formula: $E=n\beta$ (Powell *et al.*, 1996).

The softwares used through this study were SPSS 10.0, POPGEN 3.2, XLSTAT-Pro 7.1, and Microsoft EXCEL.

RESULTS AND DISCUSSION

Polymorphism detected by AFLP analysis

The aim of the present study was the assessment and interpretation of diversity and fingerprinting at the molecular level of the 21 cotton genotypes using AFLP and comparing the data with the previously obtained results using RAPD, ISSR and SSR markers (Hussein *et al.*, 2006). Brubacker and Wendel (1994), Tatineni *et al.* (1996) and Rahman *et al.* (2002) have conducted a number of molecular studies to investigate genetic diversity in different cotton cultivars. Generally, these studies indicated a very low level of genetic variation. Investigation of cultivar-specific DNA markers in cotton is highly required for breeding programs, cultivar identification and plant variety protection.

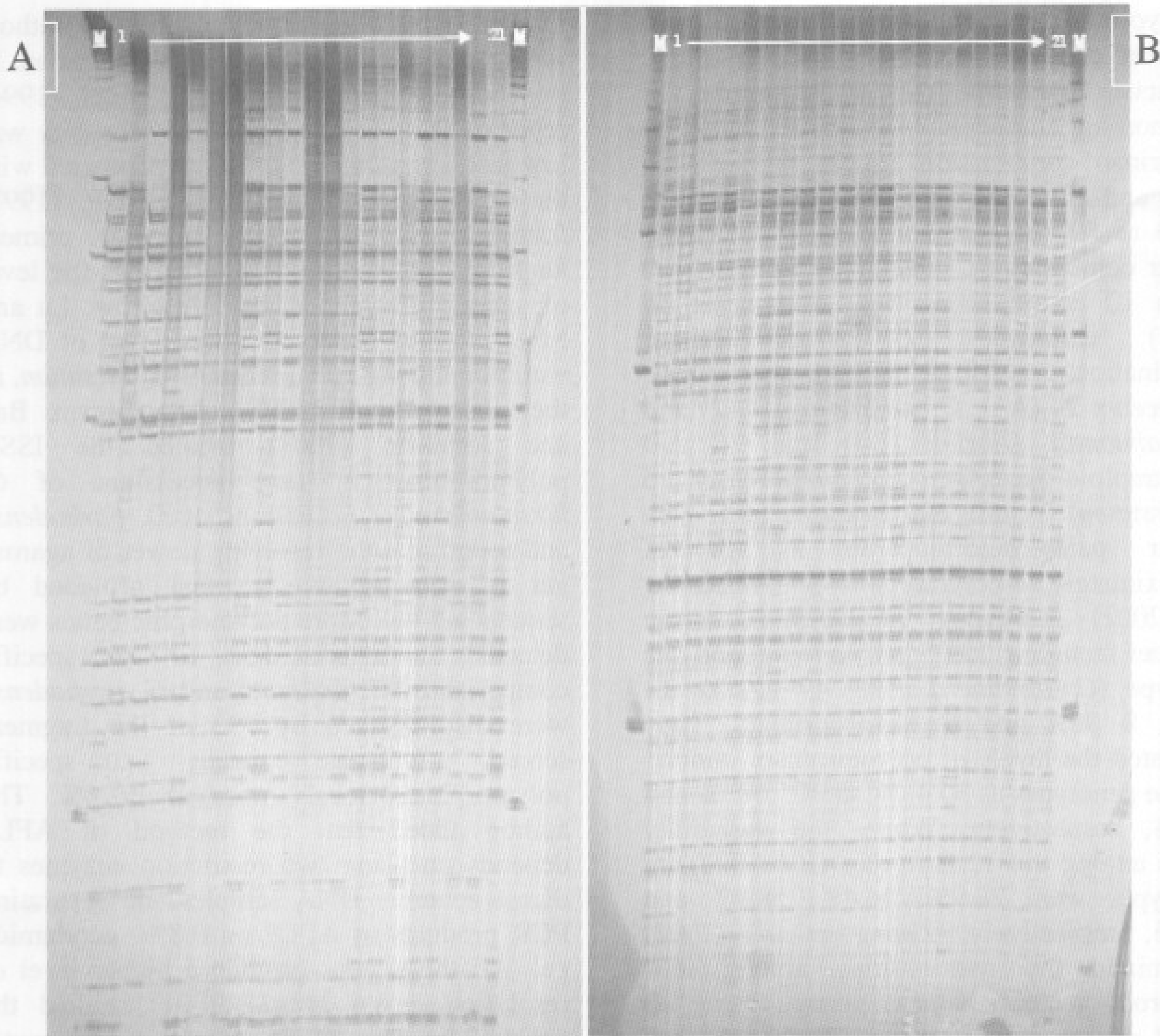

Fig (1): AFLP profiles of the 21 cotton genotypes as revealed by primer combinations 5/5 (A), 7/1 (B). (Tamkot Kabc, Tamkot Kamd, Okra leaf, Turpan Zining, Yonany, G. 70, G. 90, Pima Early American, Pima High Yield, 24111(Aust.), 24240 (Aust.), Australian deeply divided, Russian 6022, G. 72, G. 72 x Delcero (BC9), Delcero, G. 83, G. 83xDelta Pine(BC7), Delta Pine, Tamcot Luxor, G.45.).

The cotton genotypes assayed in the present work comprised 12 genotypes belonging to *G. hirsutum*, 7 *G. barbadense* genotypes and 2 hybrids (G. 83x Delta Pine Bc 9 and G.72x DelceroBc7). Sixteen AFLP primer combinations were used to analyze the different genotypes. As shown in Table (1), a total of 940 bands were amplified from the 16 primer combinations. The bands ranged in size from 60 to 780 bp (Fig. 1). The number of amplified DNA fragments per primer ranged from 31 bands (primer combination 5/4) to 90 bands (primer combination 1/3) and the percentage of polymorphism ranged from 27.6% (primer combination 7/1) to 93.3% (primer combination 6/6). Across the cotton

genotypes, the primer combination 6/6 produced the highest number of polymorphic products (56), while the lowest number of polymorphic amplicons (13) was exhibited by the primer combination 5/3. In this respect, Pillay and Myers (1999) found that an average of 40 to 80 scorable bands were detected / primer combinations and ranged from 50-500 bp in 10 cotton accessions. Abdalla *et al.* (2001) employed 16 AFLP primer combinations on three diploid species, *G. herbaceum* L. (A1*), G. arbareum* L.(A2) and *G. raimondii Ulbrich* (D5) and 26 AD allotetraploid accessions (*G. barbadense* and *G. hirsutum*). Within the tetraploid taxa, each primer pair detected an average of approximately 12 polymorphic loci. Hussein *et al.* (2002) genotyped 13 Egyptian cotton varieties including 12 (*G. barbadense*) and one off type (*G. hirsutum)* using 49 RAPD, 14 ISSR, 8 SSR and 6 AFLP primers. They estimated the levels of polymorphism among all the genotypes as 30.4 %, 53%, 68 % and 56.3%, respectively. While, the variability levels among the 12 Egyptian *G. barbadense* genotypes were 24.9%, 44.4%, 58.9% and 43.1%, respectively. Zhong *et al.* (2002) determined the relationships among four backcross generations and parents using 43 AFLP primer combinations, which produced 251 polymorphic markers among the five parents and 91 to 129 polymorphic markers within each of the five populations of the four sets of crosses and backcrosses. Zhang *et al.* (2005) proposed the use of frequent restriction enzymes in combinations with AFLP to cleave the AFLP fragments, using four upland cotton genotypes (*G. hirsutum*) and three Pima cotton (*G. barbadense*). This is because, in certain plant species including cotton (*G. hirsutum* L. or *G. barbadense* L.), the level of molecular markers is relatively low, limiting its utilization in the development of genome-wide linkage maps. Moreover, other molecular markers have been used by different authors for investigating genetic diversity in cotton. In this respect, Brubaker and Wendel (1994) reported that the level of RFLP diversity was low in *G. hirsutum* cultivars as compared with the other reported taxa. Iqbal *et al.* (1997) found that 98% of the RAPD primers amplified polymorphic patterns, but the level of genetic divergence was quite low. Lu and Myers (1999) observed a low level of DNA variation among 10 varieties of *G. hirsutum,* as they observed only 13.5% polymorphism. Bao and Jonathan (2001) studied the ISSR polymorphism in nine accessions of *G. hirsutum* and 2 accessions of *G. barbadense* and found that the resolving power of agarose gel is poor relative to that provided by sequencing gel. More polymorphic bands were detected in inter– than in intra-specific comparisons. *G. hirsutum* and *G. barbadense* were distinguished by 135 of the fragment scored, whereas mean intra-specific polymorphism levels were about 6%. The author added that the method of AFLP depends on using two restriction enzymes to digest genomic DNA samples and separating PCR products of AFLP on 0.8% acrylamide gel. All these steps provide a higher level of resolution. Wu *et al.* (2001) studied the genetic diversity of 36 domestic and exotic Upland cotton (*G. hirsutum* L.) cultivars using 81 SSR, 7 ISSR and 53 RAPD primers, which produced 282 polymorphic bands. Rahman *et al.* (2002) used 50 RAPD primers to amplify the genomic DNA extracted from 60 old cotton plants. The banding patterns produced with five primers was monomorphic and the remainder primers amplified polymorphic fragments among a few genotypes. They added that there was no single primer that could detect DNA polymorphism among all the genotypes. Rana and Bhat (2005) estimated the genetic diversity in 59 cotton cultivars belonging to four cultivated species

of cotton. In 41 *G. hirsutum* cultivars, the average genetic similarity was 74%. More genetic diversity was observed in diploid than in tetraploid cotton cultivars. They attributed the high percentage of polymorphism to the nature of the diverse genetic material, which belonged to four different cultivated species in cotton.

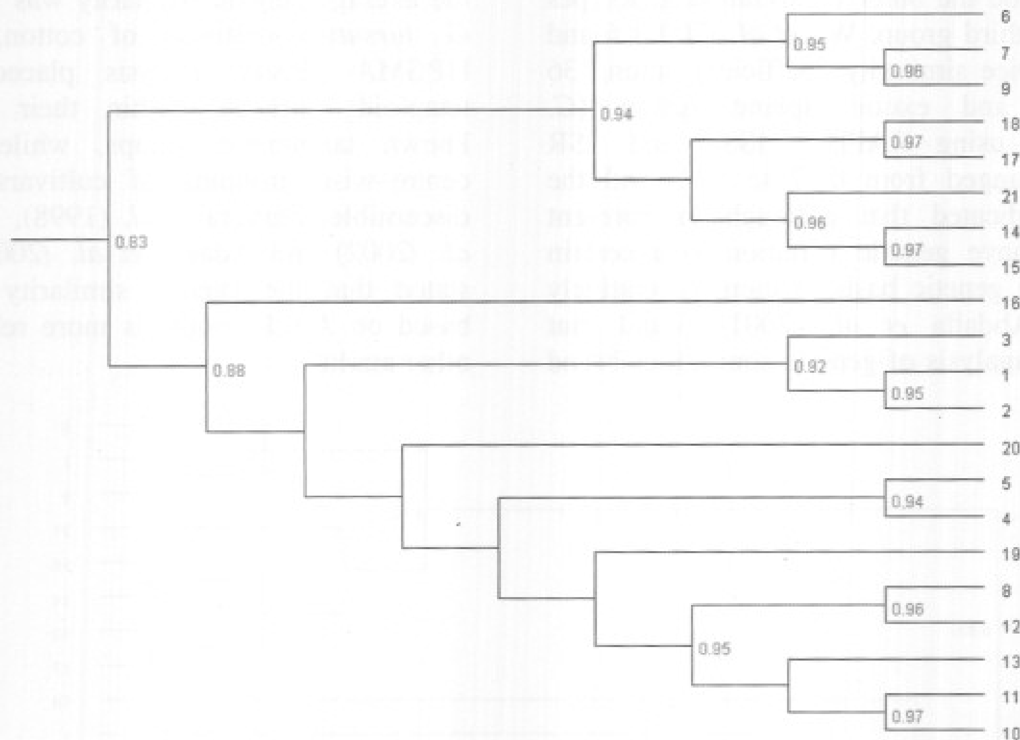

Fig (2): Dendrogram of 21 cotton genotypes as revealed by AFLP data.

Genetic similarity and relationships among cotton genotypes

In this study, the genetic similarity among the 21 cotton genotypes ranged from 97.3 to 79.7 (Data not shown). The highest similarity (97.3) was scored between G83 and G83xDelta Pine and the lowest similarity (79.7) was between Okra leaf and G83xDelta Pine. The AFLP dendrogram (Fig. 2) assigned the cotton genotypes into two main clusters. All the genotypes belonging to *G. barbadense* expect one (Pima Early American) were grouped in one cluster, while the genotypes representing *G. hirsutum* constituted the second cluster. Thus, confirming the results obtained by RAPD, ISSR and SSR analysis on the same cotton genotypes (Hussein *et al.*, 2006). In this regards, Multani and Lyon (1995) found in Australian cotton that the genetic similarity ranged from 92.1 – 98.9% among nine cultivars of *G. hirsutum* and also found that *G. barbadense* (v. Pima S-7) showed about 57% similarity with the *G. hirsutum* varieties is in agreement with our results. Tatineni *et al.* (1996) assessed the genetic diversity among 19 cotton genotypes with eight primers, and found that the RAPD data were comparable to the taxonomic data. Pillay and Myers (1999) used four AFLP primer combinations in 10 cotton accessions

(*Gossypium* spp.) and showed that the UPGMA cluster analysis of similarity coefficients grouped the diploid species into one unit, some of the *G. hirsutum* were clustered with *G. barbadense* into another group, while the other *G. hirsutum* genotypes formed a third group. Wu *et al*. (2001) found that the Dice similarity coefficient among 36 domestic and exotic upland cotton (*G. hirsutum*) using RAPD , ISSR and SSR markers ranged from 0.57 to 0.93 and the results indicated that although the present cultivars have genetic variation to a certain extent, the genetic basis of them is relatively narrow. Abdalla *et al.* (2001) found that UPGMA analysis of genetic similarities based on 368 polymorphic markers placed 25 AD tetraploid cotton cultivars into two distinct monophyletic clodes that are in complete agreement with the traditional taxonomic arrangement. Rana and Bhat (2005) found that the average genetic similarity was 74% in 41 *G. hirsutum* cultivars of cotton, and the UPGMA cluster analysis placed all the tetaploid cultivars within their respective known taxonomic groups, while breeding centre–wise grouping of cultivars was not discernible. Cervera *et al*. (1998), Hussein *et al.* (2002) and Adawy *et al.* (2004b; 2005) stated that the genetic similarity measured based on AFLP results is more reliable than other markers.

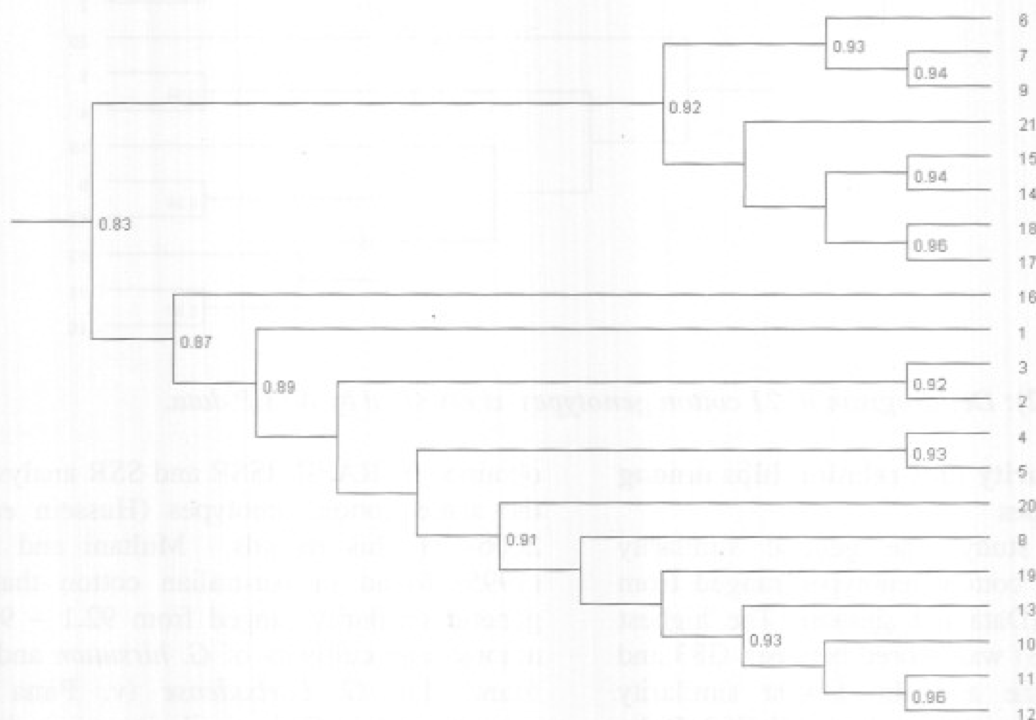

Fig (3): Dendrogram of 21 cotton genotypes as revealed by combined data.

Cultivar identification by unique markers

Fifteen primer combinations detected unique specific markers identifying 8 out of the 21 cotton genotypes. Each of these primer combinations revealed unique markers characterizing one or more genotypes. The total number of unique bands across the 21 cotton genotypes was 47 including 14 positive

unique markers (UPM) and 33 unique negative markers (UNM). The number of UPM ranged from 1 to 4 and the number of UNM ranged from 1 to 22 in the different genotypes .The genotype (Delcero) was characterized by the highest number of UPM and UNM, (4 and 22, respectively).The positive unique markers were detected by primers (7/3 and 5/6) while the negative markers were obtained by primers (1/3, 5/3, 1/8, 3/3, 8/8, 1/7, 6/6, 8/3, 7/1 and 7/3). Cultivar Tamcot Kabc was characterized by 3 UPM and 6 UNM, where the primer combination 7/1 identified the 3 UPM, while the 6 UNM were detected by primer combinations 4/6(1), 1/7 (3), 7/1(1) and 7/3(1). Cultivar Tamcot Camde was characterized with 2 UPM and 2UNM.The UPM were detected by primer combination 7/1and 7/3, while the UNM were observed with combination 4/6 and 7/1.Okraleaf cultivar was characterized with 3 unique markers (2 UPM and 1 UNM), the UPM in combinations 1/3 and 3/3, while, the UNM in combination 5/6. The cultivar G.83 was characterized by 2 UPM in combination 8/8.Each of the remaining cultivars i.e., Pima High Yield, Turpan Zining and Tamcot Luxor was characterized by only one UPM or UNM (Table2). In this context Hussein *et al*. (2002) identified genotype specific DNA markers when applying RAPD, ISSR, SSR and AFLP on 12 cotton varieties are belonging to (*G. barbadence*) and one off type Hindi belonging to (*G.hirsutum).* They stated that the Hindi off type was characterized by the highest number of unique markers (101) followed by G.45 (38).Moreover, in the present investigation, 14 out of the 16 selected primer combinations distinguished the *G.hirsutum* genotypes from the *G.barbadense* genotypes.The number of species-specific AFLP markers ranged from one to ten in the different primer combinations with a total number of 72 markers (Table 3).

Comparison of RAPD, ISSR, SSR and AFLP data

In a previous work, the authors used different molecular markers, i.e., RAPD, ISSR and SSR on the same genotypes (Hussein *et al.,* 2006). This work has been extended in the present study to fingerprinting these accessions with AFLP markers. Therefore, data of the four marker systems were combined to obtain more coverage of the genome. Similarity matrices based on combined data using Dice coefficient revealed that the highest genetic similarity value (96.5) was between genotypes G.83 and G.83 x Delta Pine and the lowest genetic similarity value (79.2) was between genotypes Tamkot Kabc and G. 83 Delta Pine. The dendrogram constructed on the basis of the combined data from RAPD, ISSR, SSR and AFLP analysis (Fig. 3) clearly assigned the cotton accessions into two clusters, one corresponding to *G. barbadense* and the other to *G. hirsutum*, while the genotype Pima Early American occupied a position within the *G. hirsutum* cluster. The dendrogram constructed on the basis of the combined data from the four analysis showed the same grouping pattern as generated by AFLP (Fig. 3) and thus confirming that AFLP is effective in discriminating closely related taxa in cotton and provides sufficient numbers of polymorphic markers in a few experiments. Moreover, the grouping of the different genotypes in the two clusters was in agreement with the traditional taxonomic arrangement.

Table (4): Effectiveness of RAPD, ISSR, SSR and AFLP markers in detecting polymorphism in cotton genotypes.

	RAPD	ISSR	SSR	AFLP
Total bands	323	125	62	940
Polymorphic band	191	62	39	474
% of polymorphism	59.1	49.6	62.9	50.4
No. of primers used	28	12	24	16
Average polymorphism pe	6.8	5.2	1.6	29.6

Table (5): Sum effective number of alleles (SENA), Expected heterozygosity for polymorphic products ($H_{av(p)}$), Effective multiplex ratio (E), and Marker Index (MI) of the different marker systems.

Marker type	SENA	($H_{av(P)}$)	E	MI
AFLP	318..2	0.39	470	182.2
RAPD	127.7	0.36	204	73. 6
ISSR	46.0	0.39	68	26.3
SSR	22.3	0.34	37	12.7

Table (6): Correlation coefficients among the RAPD, ISSR, and AFLP similarity matrices.

Marker type	AFLP	RAPD	ISSR	SSR
AFLP	1			
RAPD	0.258	1		
ISSR	0.444	0.456	1	
SSR	0.499	0..500	0.613	1

A summary of the effectiveness of the different markers is given in Table (4). AFLP showed 29.6 as average polymorphism /primer combination compared with 6.8, 5.2 and 1.6 in RAPD, ISSR and SSR, respectively. While, the percentage of total polymorphism detected was 50.4 in AFLP compared with 59.1% , 49.6% and 62.9 in RAPD, ISSR and SSR, respectively. This reveals that AFLP is a useful technique for detecting markers for genetic studies in cotton. In addition, banding patterns obtained with AFLP were highly reproducible when the same sample DNA is used in independent experiments. In agreement with Mackill *et al.,(* 1996), Russell *et al.(*1997), Marsan *et al.* (1998) and Adawy *et al.* (2004c), who pointed out that the comparative studies of currently available DNA marker systems (RAPD, RFLP, AFLP) in rice (*Oryza stiva* L.), barley and maize, also indicated that a higher degree of polymorphism can be detected by AFLP. Other workers compared the effectiveness of different marker systems for revealing patterns

of relationships with varying results. Studies carried out by Hussein *et al*. (2002) on cotton, (2003) on citrus and Adawy *et al*. (2005) on date palm confirmed that different marker systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application. Therefore, they could complement each other to draw conclusions that are more accurate. Table (5) shows the efficiency of the four different marker systems RAPD, ISSR, SSR and AFLP on cotton germplasm.

The sum effective number of alleles (SENA), the average expected heterozygosity for polymorphic markers (Hav (e)), the effective multiplex ratio (E), and marker index (MI) were calculated. The highest SENA (318.2) was detected by AFLP compared with 127.7, 46 and 22.3 in RAPD, ISSR and SSR, respectively. The value of Hav (p) was very close in the different types of markers (0.39, 0.36, 0.39 and 0.34 in AFLP, RAPD, ISSR and SSR, respectively). Moreover, AFLP scored MI value of 182.2 compared to 73.6, 26.3 and 12.7 in RAPD, ISSR and SSR, respectively. Finally, the E was higher also in AFLP (470) compared to 204, 68 and 37 in RAPD, ISSR and SSR, respectively. Thus, the results indicated that the AFLP is more effective in detecting high level of polymorphism. In accordance with our results, Powell *et al*. (1996) reported that Hav values calculated in soybean for AFLPs and RAPD showed no significant differences. This is in agreement with Adawy *et al*. (2005), who found that AFLP showed SENA of 205.7, Hav(p) of 0.93, E of 301 and MI of 117.3 in date palm. While, these values were lower in RAPD and ISSR, i.e., SENA was 45.1 and 17.8, Hav (p) was 0.36 and 0.35, E was 71 and 30 and MI was 25.9 and 10.4 in RAPD and ISSR, respectively. Table (6) represents the compatibility and the degree of correlation among the similarity matrices revealed by RAPD, ISSR, SSR and AFLP. The estimated correlation coefficients were considerably high between ISSR and SSR (0.60), followed by between SSR and RAPD (0.50), then between SSR and AFLP (0.49), ISSR and RAPD (0.46) and AFLP and ISSR (0.40). While, the lowest correlation coefficient was between RAPD and AFLP (0.26). Virk *et al*. (2000) studied the polymorphism among *Oryza sativa* germplasm using average heterozygosity (Hav) and effective number of alleles (SENA) and found that Hav gave similar results for each marker system (0.36, 0.33, 0.34 and 0.31 in AFLP, RAPD, ISSR and isozymes, respectively). Hussein *et al*. (2003) on citrus also revealed that the correlation coefficient between RAPD and AFLPs was lower than that obtained between AFLP and microsatellites. In agreement with our findings. Similarly, Adawy *et al*. (2005) on date palm found that the correlation coefficient between RAPDs and AFLPs (0.23) was lower than that obtained between AFLP and ISSR (0.44). In conclusion, this study demonstrated that all four types of marker assays have different properties and could complement each other.

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المستخلص العربى

التوصيف الجزيئي وعلاقات القرابة بين تراكيب وراثية مختلفة من القطن

2- باستخدام تقنية الـ AFLP

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نجحت 16 توليفة من بادئات AFLP لدراسة علاقات القرابة بين 21 صنف من القطن يتبعوا نوعان من القطن *G. barbadense* و *G. hirsutum*. و أظهر الـ AFLP 940 شظية من بينهم 474 شظية أظهرت اختلافات وراثية بين الأصناف المدروسة و بنسبة تابين 50.42% و يتراوح عدد الشظايا لكل زوج من البادئات من 31 – 90 شظية حيث كان طول الشظايا يتراوح ما بين 60 – 780 قاعدة نيتروجينية – كما أمكن تمييز ثماني تراكيب وراثية بواسمات جزيئية فريدة بواسطة 15 زوج من البادئات و أظهر دندروجرام الـ AFLP أن التراكيب الورثية تحت الدراسة تقع في مجموعتان اساسيتان تضم المجموعة الأولى كل اصناف القطن التي تتبع نوع *G. barbadense* فيما عدا بيما مبكر أمريكي وتضم المجموعة الثانية كل أصناف القطن الأخرى التي تتبع نوع *G. hirsutum* وهذة النتائج اتفقت مع النتائج المتحصل عليها سابقا لنفس التراكيب الوراثية باستخدام تقنية SSR, ISSR, RAPD و أظهرت النتائج المجمعة لكلا من RAPD, SSR, ISSR and AFLP تطابق دندروجرام توزيع التراكيب الوراثية في مجموعتها مع دندرؤجرام AFLP كذلك تم مقارنة كفاءة هذة التقنيات الأربع في تحليل جينوم القطن وذلك بتقدير مجموع العدد الفعال من اليلات (SENA) وحساب متوسط الـ Heterozygosity و الـ Multiplex ratio و معامل الواسم (MI) لكل واسم حيث أظهرت واسمات الـ AFLP مجموع عالي للعد الفعال من اليلات 318.2 مقارنة بـ SSR, ISSR, RAPD 127.7 و 46 و 22.3 على التوالي. بينما أظهرت واسمات الـ AFLP, SSR, ISSR and RAPD نسبة Heterozygosity متقاربة . أما قيمة الـ MI كانت 182.2 في AFLP مقارنة بـ 73.6 و 26.3 و 12.7 في RAPD , SSR , ISSR و كانت أقل ما يكون بين الـ AFLP , RAPD (0.26).وقد اكدت النتائج أن الأنظمة المختلفة الأربعة المستخدمة تختلف في ميكانيكية اكتشاف التباين و تغطية الجينوم لذلك فهي مكملة لبعضها وبالتالي يمكن أن تساهم في حفظ الأصول الوراثية وبرامج التربية .