Molecular characterization and genetic relationships among cotton genotypes 1- RAPD, ISSR and SSR analysis

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ABSTRACT

Cotton is the world's leading fiber crop and the second important oil seed crop. Recent advances in genomics research have provided new tools, such as molecular markers, that will assist breeders in the improvement of this important crop. Use of molecular markers in genome analysis, mapping of agriculturally important traits and marker-assisted selection have been greatly advanced by the development of PCR-based markers. The present study is a part of a cotton genomics project that addresses the use of different PCR-based molecular markers (RAPD, ISSR and SSR) for germplasm characterization, fingerprinting and assessing the genetic diversity among some of the accessions available at the Cotton Research Institute, ARC, Egypt. Twenty-one cotton accessions were assayed using 28 RAPD and 12 ISSR primers, in addition to 24 SSR specific primer pairs. The total number of amplicons detected by RAPD, ISSR and SSR was 323, 125 and 62, respectively. While, the number of polymorphic amplicons was 191, 62 and 39, respectively. Thus, the level of polymorphism among the 21 accessions as revealed by RAPD, ISSR and SSR was 59.1%, 49.6 and 62.9 respectively. The genetic relationships among the 21 accessions were estimated in terms of similarity using the Dice coefficient .The topology of the dendrograms derived from the different marker types was unique, however, with evident similarities. All dendrograms clearly clustered the accessions belonging to G. hirsutum in one group and those of G. barbadense, expect Pima Early American, in another group. One out of the 28 RAPD primers produced 21 different banding patterns, thus, identifying each of the 21 accessions by a unique banding profile. Two ISSR primers (S1 and S2) revealed 18 banding patterns each and together made it possible the identification of all the genotypes. Moreover, accession-specific DNA markers characterized different genotypes and therefore were used to generate unique fingerprint for each genotype. The RAPD, ISSR and SSR detected 4, 8 and 5 unique positive and/or negative markers characterizing 3, 4 and 4 accessions, respectively. Furthermore, five SSR alleles ($M8_{280}$, $M8_{290}$, $C11_{250}$, $C11_{260}$ and $M13_{150}$) could the discriminate between accessions belonging to the species G. barbadense and those of G. hirsutum. Thus, they were considered as species-specific markers.

Keywords: *Cotton, Gossypium barbadense, G. hirsutum, RAPD, ISSR, SSR, Fingerprinting, Molecular characterization, Unique markers, Genetic similarity.*

INTRODUCTION

Cotton is an economic plant of world importance. It is the world`s leading fiber crop and the second most important oil seed crop. It belongs to the genus Gossypium which comprises about 50 diploid and tetraploid species indigenous to Africa, central and south America, Asia, Australia, the Galabago and Hawaii (Fryxell, 1992). The majority of the worldwide present-day commercial cotton varieties belong to *Gossypium hirsutum* L. (n=2X=26), while a few (10% of the total world production) belong to *Gossypium barbadense* (n=2X=26) and some to diploid species (*G. arboreum* L. and *G. herbaceum*).

Egyptian cotton (*Gossypium barbadense* L.) has a worldwide reputation because of its fiber length and fineness. In Egypt, plant breeders are making major contributions to improve the productivity and quality of this crop through breeding and selection of new cultivars. However, the majority of these cultivars are virtually derived from crosses between relatively few numbers of accessions. This has led to decreasing the genetic variation among Egyptian cotton germplasm. Some attempts have been made to increase genetic variation through induction of mutations with of limited success (Elenani, 1970, and Ahmad, 1985).

In recent years, the field of genomics, one of the major fields of molecular biology, has provided tools suitable for rapid and detailed genetic analysis of organisms, which could assist breeders in their breeding programs for crop improvement, particularly when dealing with complex genetic traits or QTLs. The most fundamental of these tools are DNA markers, which portray genomic information carried by different individuals. Therefore, DNA markers can be used to increase the efficiency of breeding programs by providing a fingerprint for each cultivar and permitting the estimation of the relatedness between different genotypes (Qureshi *et al.*, 2004). Consequently, they provide the means for better use of existing germplasm resources and to predict which crosses might produce new and superior genic combinations.

Various DNA profiling methods are currently available, some are "hybridization-based" (e.g. RFLPs, Botstein *et al.*, 1980) while others are "PCR-based" assays (e.g. RAPD, Williams *et al.*, 1990, SSRs, Litt and Lutty, 1989, and AFLPs, Zabeau and Vos, 1993). PCR-based markers share a number of general advantages over RFLP technology. The major advantages are the speed with which results are generated, low amount of genomic DNA required for PCR and the ability to share information on primer sequences without the need to exchange DNA (Dongre and Parkhi 2005). Although genomic technologies, through the use of molecular markers, have revolutionized plant genetics and breeding (Tanskley *et al.*, 1989,), cotton genomics is far behind other crops of comparable values.

A number of studies have been conducted to investigate genetic diversity in cotton at the DNA level (Brubaker and Wendel, 1994, Multani and Lyon, 1995, Tatineni *et al.*, 1996, Pillay and Myers, 1999, Liu *et al.*, 2000, Abdallah *et al.*, 2001, Hussein *et al.*, 2002 and Qureshi *et al.*, 2004). These studies in general, revealed that the cultivated cotton displays a very low level of genetic diversity. Therefore, the need for accession-specific DNA markers in cotton breeding programs still exists.

In the present investigation, RAPD and two microsatellite-based markers (ISSR and SSRs) were employed to estimate the genetic polymorphism among 21 cotton accessions of

the collection available at the Cotton Research Institute, Egypt. An attempt has been made to identify unique DNA markers characterizing each accession and to determine the genetic relatedness among these accessions.

MATERIALS AND METHODS

Plant materials and DNA extraction

Twenty one cotton accessions (Table 1) were selected from the germplasm available at the Cotton Research Institute, ARC, Egypt and included in this study. 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 (1): List of the cotton accessions.

Serial No.	Accessions	Abbreviations	Species
1	TAMCOT CABC	Tc	*G .hirsutum*
2	TAMCOT CAMD E	Tk	*G. hirsutum*
3	Okra leaf	Ol	*G. hirsutum*
4	Turpan zining	Tz	*G. hirsutum*
5	YUNANI	Yu	*G .hirsutum*
6	Giza 70	G. 70	*G .barbadense*
7	Giza 90	G. 90	*G. barbadense*
8	Pima Early American	Pe A	*G. barbadense*
9	Pima high yield	Phy	*G. barbadense*
10	24111 (Aust.)	A24111	*G. hirsutum*
11	24240 (Aust.)	A21240	*G .hirsutum*
12	Australian deeply divided	Ad	*G .hirsutum*
13	Russian 6022	R 6022	*G. hirsutum*
14	Giza 72	G. 72	*G. barbadense*
15	Giza 72 x Delcero (BC9)	G. 72 x Delc	
16	Delcero	Delc	*G.hirsutum*
17	Giza 83	G. 83	*G.barbadense*
18	Giza 83 x Delta pine (BC7)	G. 83 x Dp	
19	Delta pine	Dp	*G.hirsutum*
20	TAMCOT LUXOR	TL	*G.hirsutum*
21	Giza 45	G. 45	*G.barbadense*

Molecular analysis

RAPD

RAPD amplification was performed as described by Williams *et al.* (1990) with minor modifications. A set of 25 random 10 mer primers (Table 2) was used in the RAPD analysis. The amplification reaction was carried out in 25 µl total volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 10µM primer, 1 U Taq DNA polymerase and 25 ng templates DNA. PCR amplification was performed in a Perkin-Elmer/Gene Amp PCR

system 9700 (PE Applied Biosystem). The PCR program was as follows: an initial denaturation step at 94°C for 5 min, followed by 40 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension cycle of 7 min at 72°C. The amplification products were resolved by electrophoresis in 1.5 % agarose gels.

ISSR analysis

Twelve oligonucleotides composed of defined short tandem repeat sequences and representing different microsatellites (di- and tri-repeats) (Table 3) were used as generic primers in PCR amplification of inter-simple sequence repeat regions as described by Hussein *et al* (2003). PCR was performed in 25µl reaction volume containing 1x PCR buffer, 1.75 mM $MgCl_2$, 5 mM of each dNTPs, 40µM oligonucleotide primer, 25 ng genomic DNA and 1 U Taq DNA polymerase. A high stringency touchdown and hot start thermocycling profile was employed as follows: one cycle for 5 min at 94°C,10 touchdown cycles (94°C/30sec, 65-55 °C/45 sec , 72°C / 1 min), followed by 35 cycles (94°C/30sec, 55°C 45 sec , 72°C / 1 min) and a final extension cycle at 72°C for 7 min. The PCR products were separated on 1.5 % agarose gels.

SSR analysis

SSR assays were performed as described by Hussein *et al.* (2003). DNA amplification was carried out using twenty-four SSR primer pairs derived from Cotton Database (Table 4). The PCR reaction was conducted in 25µl reaction volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM of each dNTPs, 1µM of forward and reverse primers, 1 U hot start Taq polymerase and 25 ng genomic DNA. Hot start and touchdown profile was used as follows: an initial denaturation step at 94°C for 15 min, followed by 35 cycles at 94°C for 30 sec, annealing temperature (Ta) for 30 sec and primer elongation at 72°C for 1 min., then a final extension at cycle at 72°C for 7 min. The PCR products were detected by electrophoresis on polyacrylamide non-denaturing gels stained with ethidium bromide.

Data analysis

The banding patterns generated by RAPD and microsatellite-based markers analyses were examined to determine the level of polymorphism and the genetic relatedness among the 21 cotton accessions. The amplified fragments were scored as present (1) or absent (0). The genetic similarity and similarity matrix among genotypes were estimated according to Dice coefficient: GS(ij)= 2a/(2a+b+c), where GS(ij) is the measure of genetic similarity between individuals i and j , (a) is the number of bands shared by i and absent in j , and (c) is the number of bands absent in i and in j (Sneath and Sokal, 1973).Cluster analysis was based on similarity matrix obtained with unweighted pair group method using arithmetic average (UPGMA), and the relationships between accessions were displayed as dendrogram.

Table (2): Total number of amplicons, number of polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers for 21 cotton accessions.

Primer name	Total no. amplicons	No. Polymorphic amplicons	Polymorphism %	No. banding Patterns
OP - A02	10	3	30.0	6
OP - A08	14	9	64.0	21
OP - A09	12	6	50.0	12
OP – A10	20	11	55.5	16
OP - A11	18	16	88.8	20
OP - A16	12	6	50.0	13
OP - B03	10	6	60.0	14
OP - B07	7	5	71.4	12
OP - B08	13	9	69.2	18
OP - B09	12	8	66.7	12
OP – B11	8	7	87.5	12
OP – B12	10	8	80.0	12
OP – B17	15	11	73.3	17
OP - B20	7	5	71.4	8
OP - D09	8	4	50.0	7
OP – D12	14	9	64.3	17
OP - O02	12	9	75.0	11
OP – O14	10	8	80.0	15
OP - P01	13	5	38.5	9
OP - P02	12	8	66.7	15
OP - P03	16	9	56.2	20
OP - P04	11	6	54.5	18
OP - P06	8	3	37.5	5
OP – P16	7	4	57.1	6
OP – Z03	11	3	27.3	5
OP – Z07	6	3	50.0	5
OP – Z11	7	6	85.7	8
OP – Z17	10	3	30.0	14
Total	**323**	**191**	**59.1**	**348**
Average	**11.5**	**6.8**		**12.4**

RESULTS AND DISCUSSION

RAPD analysis

A total of 40 decamer primers were initially screened against DNA of 21 cotton accessions. Only the primers that generated repeatable and easily scrorable polymorphic RAPD markers were selected for accession identification. The amplification profiles of the 21 accessions produced by the 28 selected primers revealed a total of 191 polymorphic bands out of 323 reproducible products (Table 2). This corresponds to a level of 59.1% polymorphism. The number of amplicons /primer ranged from 6 (OP-Z07) to 20 (OP-A10), whereas the number of polymorphic bands per primer ranged from 3 (OP-P06, OP-Z03, OP-Z07 and OP-Z17) to 16 (OP-A11). (Fig. 1 A and B). The 28 primers yielded 348 banding patterns (12.4 per primer). The number of banding patterns per primer varied considerably and ranged from 5 (OPP-06, OPZ-03 and OPZ-07) to 21 (OPA-08). Therefore, only one out of the 28 primers could differentiate clearly between all accessions. This might be indicative of a narrow genetic base for some of the cotton accessions studied. In this respect, Tatineni *et al.* (1996) studied the level of polymorphism among 19 cotton genotypes using 27 random primers and found that 33.8% of the primers revealed monomorphic patterns. On the other hand, working on 31 *Gossypium* species, three subspecies and one inter-specific hybrid, Khan *et al.* (2000) found that the level of polymorphism was 99.8%. Moreover, Hussein *et al.* (2002) used 49 RAPD primers to investigate the genetic diversity among 13 cotton genotypes and detected a level of polymorphism of 30.4%. This discrepancy in the level of polymorphism recorded in the different studies could be attributed to the use of accessions belonging to the same or different cotton species.

ISSR analysis

Inter simple sequence repeat is an alterative technique to study polymorphism based on the presence of microsatellite throughout genomes (Bornet and Branchard, 2004). ISSR markers are DNA sequences delimited by two inverted SSR composed of the same units which are amplified by a single PCR primer, composed of few SSR units with or without anchored end (Bornet and Branchard, 2001, 2004). Twelve ISSR primers revealed discernible amplification profiles, therefore, employed to investigate the genetic polymorphism among the 21 cotton accessions (Fig. 1 C and D). A total of 62 polymorphic amplicons out of 125 reproducible products were obtained from the 12 primers (Table 3). The average number of polymorphic amplicons was 5.2 fragment/ primer. The highest number of amplified products was produced by primer S1 (17 amplicons), while the lowest (4 amplicons) was detected by primer I5. The number of polymorphic amplicons ranged from 0 (primer I5) to 9 (primer I8 and S1), with a level of polymorphism ranging from 0.0% to 70.0% and an average of 48.8%. Moreover, the 12 ISSR primers revealed variable number of banding patterns ranging from 1 (primer I5) to 18 (primers S1 and S2). A combination of the latter two primers made it possible to differentiate among all accessions. In this respect, several authors demonstrated that ISSR-PCR gives multilocus patterns which are very reproducible, abundant and polymorphic in plant genomes (Bornet and Branchard, 2001, 2004, Hussein *et al.*, 2003, 2005).

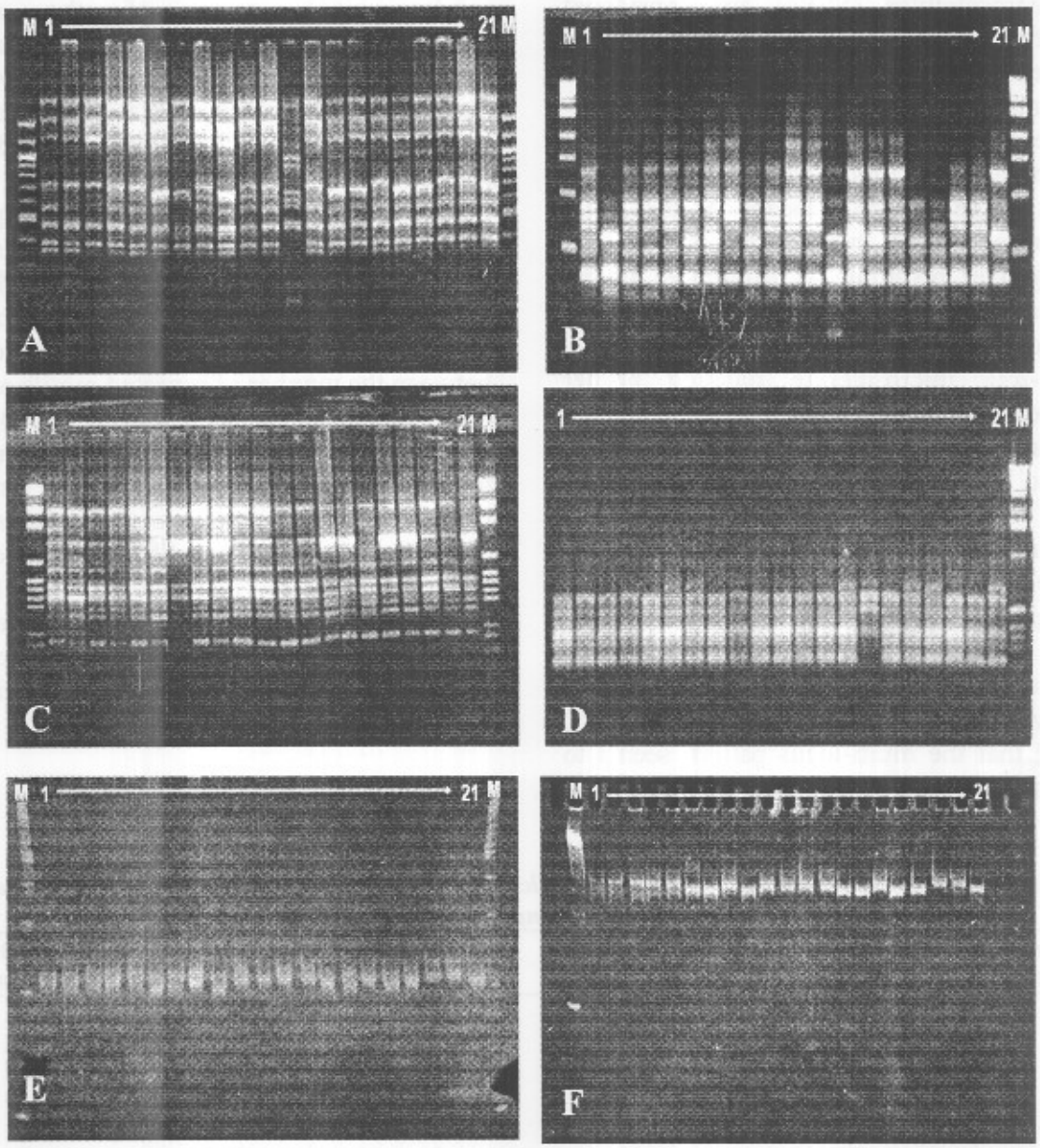

Fig (1): Profiles of the 21 cotton accessions as revealed by RAPD OP-P3 and OP-P2 (B), ISSR S2(c) and I2(D) and SSR C4(E)and M8(F).

Simple sequences repeat (SSR) analysis

Microsatellites (SSRs) are considered one of the markers of choice for fingerprinting and genome mapping because they are codominant, usually multiallelic, and hyper-variable and randomly dispersed throughout the genome. The microsatellite variation is thought to be due to slippage of the DNA polymerase during replication or unequal crossing over resulting in differences in the copy number of the core nucleotide sequence (Yu and Kohel, 1999, Qureshi *et al.*, 2004). Twenty-four microsatellite flanking primer pairs were employed to investigate the genetic polymorphism. As shown in Table (4) and Fig. (1 E and F), the number of alleles per primer ranged from 1 to 5, while the number of polymorphic alleles varied from 0 to 5 and the average level of polymorphism was 62.9. In this regard, Buteler *et al.* (1999) pointed out that the multi-locus amplification of the SSR is particularly common in species with allopolyploid origin. Rallo *et al.* (2000) claimed that the multi-locus pattern seems to be associated with compound microsatellite. This is not in agreement with the present results, since many of the simple microsatellites produced multi-locus patterns (Table 4). Similar findings were previously obtained by Hussein *et al.*(2002). On the other hand, Fisher *et al.* (1998) reported that polymorphism is though to be related to the number of repeats. In the present study, primer pairs SH1 and SH5 comprised 17 and 18 repeats, respectively, and exhibited only one allele across the 21 cotton genotypes. Therefore, we can deduce no clear relationship between the number of repeated motifs and the level of polymorphism. Liu *et al.* (2000) used 65 SSR primer pairs and obtained 71 markers loci in 13 monosomic genotypes and 27 monotelodisomic cotton cytogenetic stocks. They stated that most SSRs revealed two alleles, one for *G. hirsutum* and one for *G. barbadense*. Moreover, they showed that most SSR loci tend to be multi-allelic and polymorphic for repeat number, which is easily scored and used for genotyping.

Table (3): ISSR primers, total number of amplicons, number of polymorphic amplicons, level of polymorphism and the number of banding obtained for each primer.

Primer code	Sequence	Total no. amplicons	No.polymorphic amplicons	Polymorphism %	No. banding Patterns
I1	$TAT(CA)_7$ C	15	3	20.0	4
I2	$CAC(TCC)_5$	7	4	57.1	4
I4	$CAT(CA)_7T$	10	7	70.0	13
I5	$CGA(AT)_7$	4	0	00.0	1
I6	$(GA)_8CG$	7	4	57.1	9
I7	$ATTA(CA)_7$	10	7	70.0	10
I8	$(AG)_8CT$	13	9	69.2	16
I9	$AAC(TG)_7T$	9	4	44.4	8
I10	$(TCC)_5AC$	10	6	60.0	13
I13	$(GAA)_3$	9	3	33.3	12
S1	$(GTG)_3G$	17	9	52.9	18
S2	$(CA)_6AG$	14	6	42.8	18
Total		**125**	**62**	**49.6**	**126**
Average		**10.4**	**5.2**		**10.5**

Table (4): SSR loci, observed size, repeat unit, number of alleles and number of banding patterns obtained for each SSR locus.

Code No.	SSR locus	Observed allele size (bp)	Repeat unit	No. alleles	Polymor. alleles No.	Polymor. alleles %	No. banding Patterns
M 8	JESPR-8R	295 ,290 ,280	(GAA)12	3	3	100	3
M 15	JESPR-15R	50	(GAA)7	1	0	0	1
M 16	JESPR-16R	170 , 190 , 195	(AT)4 , TGA(AT)3	3	0	0	1
M 17	JESPR-16R	195 , 215	(GAT)6	2	0	0	1
M 23	JESPR-23R	100 , 190	(CTT)9	2	1	50	2
M 26	JESPR-26	100 , 140 , 290	(CAA)9	3	0	0	1
C 4	BNL - 3558 R - 3558 F	210 , 200 , 220 , 240 , 245	AAGCAAATC$(ATG)_2$ AACATACG	5	5	100	7
C 7	BNL - R 3359 - F 3359	200 , 210	$(TTG)_3GGA(ATG)_2GA$	2	2	100	2
C 11	BNL - 3563 R - 3563 F	260 , 250	AAGCTAAAACTTGA CACAAGCC	2	2	100	2
SH 6	BNL - 272 R - 272 F	170 , 175 , 180 , 195	(AT)8(AG)12(GA)5	4	4	100	6
SH 8	BNL - 258 R - 258 F	260 , 250	(GCT)20	2	2	100	2
SH 1	BNL - 113 R - 113 F	50	(AG)17	1	0	0	1
SH 5	BNL - 418 R - 418 F	60	(AG)18	1	0	0	1
SH 3	BNL - 219 R - 219 F	105 , 110 , 130	(AG)12	3	3	100	3
SH 2	BNL - 134 R - 134 F	250	(T)6(AT)10(T)16	1	0	0	1
SH 4	BNL - 300 R - 300 F	140, 120, 100 , 90 , 80	(AT)12(AG)12	5	4	80	5
M 19	JESPR-19	210, 200 , 190 , 180	(GAA)20	4	4	100	7
M 11	JESPR-11	300 , 250 , 200	(GAA)10	3	2	66.7	4
M 55	JESPR-55	200 , 150 , 90	(GAA)23	3	1	33.3	2
M 14	JESPR-14	190 , 220 , 300	(CTT)7	3	1	33.33	2
M 13	JESPR-13	150 , 170 , 180 , 210	(CTT)19	4	1	25	2
M 20	JESPR-20	310 , 420	(CTT)9	2	2	100	4
M22	JESPR-22	90	(GAA)12	1	0	0	1
M 12	JESPR-12	220 , 230	(CTT)7	2	2	100	2
Total				**62**	**39**	**62.9**	**63**
Average				**2.6**	**1.6**		**2.6**

Genetic relationships among cotton accessions

The genetic relationships among the 21 cotton accessions examined using the Dice coefficient to compute the similarity matrices. These similarity matrices were employed to generate dendrograms using the UPGMA method. Based on the RAPD, ISSR and SSR data, the genetic similarities ranged from 72.1% to 95.7%, from 79.1% to 95% and from 72.1% to 96.6, respectively. In this respect, Wu *et al.*, (2001) evaluated the similarity coefficient between 36 upland cotton cultivars using SSR, ISSR and RAPD markers and found that the pairs of similarity coefficients ranged from 57.5% to 92.9%. The RAPD based dendrogram separated the accessions Giza 45 from all the other accessions, which were clustered into two clusters, one containing all the accessions belonging to the species *G. barbadense,* except Pima Early American which was clustered with the accessions belonging to *G. hirsutum* in a second group (Fig. 2 A). While, the dendrograms deduced from the ISSR and SSR data divided the 21 accessions into two clusters, one including the *G. barbadense* accessions, while the other comprised the *G. hirsutum* accessions in addition to Pima Early American (Fig. 2 B and C).

Although the dendrograms constructed on the basis of the different markers types revealed great similarities, however, each dendrogram showed a unique topology. The reshuffling in the position of some of the accessions in the different dendrograms could be attributed to the narrow genetic background of the accessions belonging to each of the cotton species and/or to the nature of the evolutionary mechanisms underlying the variation measured by the different types of markers (Hussein *et al.* 2002). Therefore, a dendrogram was constructed for the 21 cotton accessions based on the combined data from RAPD, ISSR and SSR analyses (Fig. 2 D). The combined dendrogram assigned the cotton accessions into two clusters, one corresponding to *G. barbadense* and the other to *G. hirsutum* except Pima Early American. Moreover, within each species, the different accessions formed sub groups which corresponded to their origin and pedigree. In this respect, Multani and Lyon (1995) studied a number of Australian cotton cultivars and found 92.1 -98.9% genetic similarity among nine cultivars of *G. hirsutum* L., while *G. barbadense* L. var. Pima S-7 showed about 57% similarity with the *G. hirsutum* L. varieties. Wu *et al.* (2001) indicated that the classification of 36 upland cotton cultivars, based on the pairs of similarity coefficients deduced from RAPD, SSR and ISSR data, was mostly consistent with known pedigree information. Hussein *et al.* (2002) constructed a dendrogram based on the combined data from RAPD, ISSR, SSR and AFLP analysis of 13 cotton genotypes and demonstrated that the Hindi off-type genotype (*G. hirsutum*) was clearly separated from the Egyptian cotton genotypes belonging to the *G. barbadense*.

Identification of unique DNA markers

Identification of unique DNA markers characterizing the various cotton genotypes by unique fingerprints could have a number of potential applications including the determination of cultivar purity, efficient use and management of genetic resources collection and the establishment of property rights.

Table (5), shows three cotton genotypes which were characterized by four positive and/or negative RAPD markers. In addition, four genotypes were identified by eight ISSR markers, while five SSR markers identified four genotypes. Therefore, the different marker types (RAPDs, ISSRs and SSRs) were

successful in characterizing 10 out of the 21 cotton accessions by unique positive and/or negative markers.

Furthermore, the SSR assay permitted the discrimination between the two cotton species (*G. hirsutum* and *G. barbadense*) through revealing five species-specific markers (Table 5). Multani and Lyon (1995) identified 69 RAPD markers unique to *G. barbadense* cv. Pima S-7 and characterized 10 *G. hirsutum* cultivars based on cultivar-specific RAPD markers. Khan *et al.* (2000) reported that despite the strong homology exhibited by many of the cotton genotypes, RAPD analysis was able to characterize very closely related species, subspecies and genotypes. Hussein *et al.* (2002) detected 26 RAPD, 16 ISSR and 2 SSR cotton variety-specific DNA markers. The results of the present investigation indicated the usefulness of the molecular markers in characterizing the cotton accessions by one technique or another. Since the different markers systems differ in the mechanism of detecting polymorphism, genome coverage and the ease of application, therefore, could be complementary to each other.

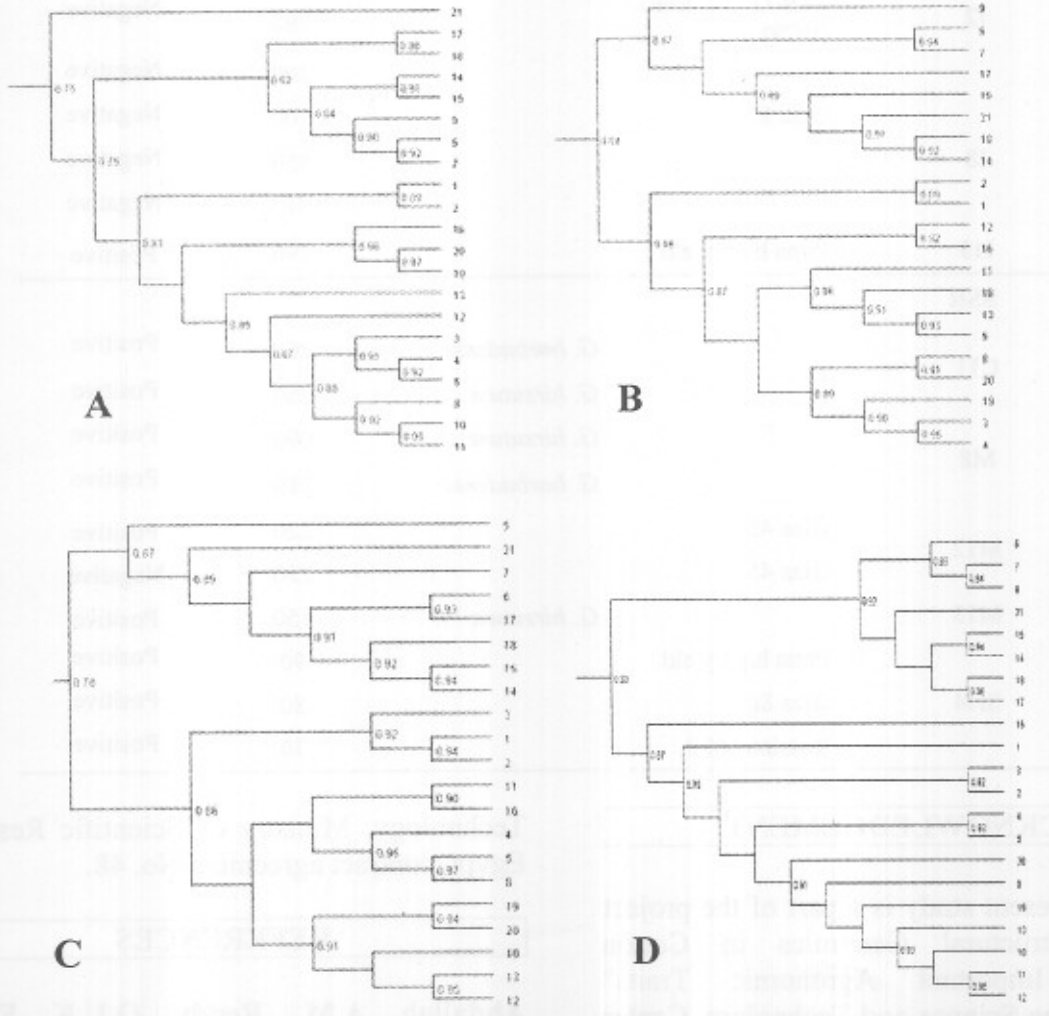

Fig (2): Dendrograms of the 21 cotton accessions as revealed by RAPD (A), ISSR (B), SSR (C) and Co mbined data (D).

Table (5): Unique genotype- and species-specific RAPD, ISSR and SSR markers and their corresponding sizes.

Marker	Genotype	Species	Marker size	Marker type
RAPD				
OP - B09	Giza 83 x Deltapine (BC7)		300	Negative
OP - P03	Aust. Deeply divided		700	Positive
			170	Negative
OP – P04	Giza 72		400	Positive
ISSR				
I1	Giza 45		200	Positive
	Giza 45		300	Negative
I2	Giza72 x Delcero (BC9)		298	Negative
			200	Negative
	Giza 83		700	Negative
I8			550	Negative
			517	Negative
I13	Pima high yield		396	Positive
SSR				
C11		*G. barbadense*	260	Positive
		G. hirsutum	250	Positive
M8		*G. hirsutum*	290	Positive
		G. barbadense	280	Positive
M12	Giza 45		220	Positive
	Giza 45		230	Negative
M13		*G. hirsutum*	150	Positive
	Pima high yield		90	Positive
SH4	Giza 80		80	Positive
	Russian 6022		70	Positive

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الملخص العربي

التوصيف الجزيئي وعلاقات القرابة بين تراكيب وراثية مختلفة من القطن
1- باستخدام تقنيات الـ SSR ,ISSR,RAPD

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يعتبر القطن محصول الألياف الرئيسي كما يحتل المرتبة الثانية ضمن محاصيل الزيوت .وحديثا أدي التقدم في أبحاث الجينوميا الي توفير طرق جديدة من بينها الواسمات الجزيئية التي سوف تساعد المربي في تحسين هذا المحصول الهام .

وقد أدي استخدام الواسمات الجزيئية التي تعتمد علي تقنية الـ PCR الي تقدم سريع في تحليل الجينومات وتحديد المواقع الخريطية للصفات الاقتصادية الهامة والانتخاب غير المباشر باستخدام الواسمات الجزيئية . وتمثل الدراسة الحالية جزء من مشروع لدراسة جينوم القطن علي نطاق واسع حيث يتناول هذا البحث استخدام ثلاثة أنواع مختلفة من الواسمات الجزيئية التي تعتمد علي تقنية الـ PCR وهي تقنيات SSR,ISSR,RAPD لتوصيف الأصول الوراثية وتحديد البصمة الوراثية لها وتحديد مستوي التباين الوراثي بين التراكيب الوراثية المختلفة المتوفرة في معهد بحوث القطن بمركز البحوث الزراعية – مصر . وقد تمت دراسة 21 تركيب وراثي من القطن باستخدام 28 بادئ عشوائي في تقنية الـ RAPD و 12 بادئ في تقنية ISSR بالاضافة الي 24 زوج من بادئات الـ SSR . وكان العدد الكلي لشظايـــــــا الـ د ن أ الناتجة هو 323 ، 125 ، 62 في تقنيات الـ SSR,ISSR,RAPD علي التوالي . بينما كان عدد شظايا الـ د ن أ التي أظهرت تباين هو 191 ، 62 ، 39 علي التوالي وبهذا يكون مستوي التباين ما بين الـ 21 تركيب وراثي الذي أظهره الـ SSR,ISSR,RAPD هو 59ر1% ، 49. 6% ، 62.9% علي التوالي . وقد تم تقدير درجة القرابة الوراثية ما بين الـ 21 تركيب وراثي باستخدام معامل Dice . وأظهرت الدندروجرامات الناتجة من أنواع الواسمات الجزيئية الثلاثة منفرده درجة عالية من التشابه . حيث وقعت جميع التراكيب الوراثية التابعة لنوع G. hirsutum في مجموعة واحدة والتراكيب الوراثية التابعة لنوع *G.barbadense* في مجموعة ثانية ما عدا *Pima Early American* . وقد أظهر بادئ واحد من بين الـ 28 بادي من نوع RAPD 21 نمط لشظايا الـ د ن أ مما أدي الي تمييز كل من الـ 21 تركيب وراثي تحت الدرسة كما أظهر كل من اثنين من بادئات الـ ISSR (S1,S2) 18 نمطا مختلفا لشظايا الـ د ن أ مما أدي الي امكانية تمييز التراكيب الوراثية المختلفة . كما أمكن تحديد واسمات جزيئية مميزة لكل تركيب وراثي ساعدت في تحديد بصمة وراثية فريدة لكل منها حيث كان عدد الواسمات الجزيئية الفريدة التي أظهرها الـ SSR,ISSR,RAPD هو 4 ، 8 ، 5 ميزت 3 ، 4 ، 4 تركيب وراثي علي التوالي بالاضافة الي ذلك فقد نجحت خمسة اليلات من الـ SSR هي ($M8_{280}$, $M8_{290}$, $C11_{250}$, $C11_{260}$, $M13_{150}$) في التمييز ما بين التراكيب الوراثية التابعة لنوع *G.hirsutum* وتلك التابعة لنوع *G.barbadense* وبذلك تعتبر واسمات جزيئية مميزة للنوع .

الكلمات الدالة : القطن ، *Gossypium barbadense* , *G.hirsutum* ، SSR,ISSR,RAPD ، تحديد البصمة الوراثية ، التمييز الجزيئي ، واسمات فريدة ، التشابة الوراثي .