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Genetic diversity evaluation of some elite cotton varieties by RAPD analysis

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Abstract Random amplified polymorphic DNA (RAPD) analysis was used to evaluate the genetic diversity of elite commercial cotton varieties. Twenty two varieties belonging to *Gossypium hirsutum* L. and one to *G. arboreum* L. were analyzed with 50 random decamer primers using the polymerase chain reaction (PCR). Forty nine primers detected polymorphism in all 23 cotton varieties, while one produced monomorphic amplification profiles. A total of 349 bands were amplified, 89.1% of which were polymorphic. Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) showed that 17 varieties can be placed in two groups with a similarity ranging from 81.51% to 93.41%. *G. hirsutum* L. varieties S-12, V3 and MNH-93 showed a similarity of 78.12, 74.46 and 69.56% respectively with rest of the varieties. One variety, CIM-1100, showed 57.02% similarity and was quite distinct. The diploid cotton *G. arboreum* L. var. Ravi was also very distinct from rest of its tetraploid counterparts and showed only 55.7% similarity. The analysis revealed that the intervarietal genetic relationships of several varieties is related to their center of origin. As expected, most of the varieties have a narrow genetic base. The results obtained can be used for the selection of possible parents to generate a mapping population. The results also reveal the genetic relationship of elite commercial cotton varieties with some standard "Coker" varieties and the diploid *G. arboreum* L. var. Ravi (old world cotton).

Key words Cotton varieties · RAPD · Genetic similarities

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Introduction

A majority of the present-day commercial cotton varieties belong to *Gossypium hirsutum* L. (upland cotton); a few belong to *G. barbadense* L. (8% of total world production) and some to diploid species (Lee 1984). Breeders have evolved these varieties through selection based on morphological and physiological features (yield, fiber quality, resistance against certain pests and diseases etc.). Pakistan is among the top-three cotton producing countries with an annual production of nearly 12 million bales. A large number of cotton varieties grown in Pakistan originated from intraspecific crosses of *G. hirsutum* L. at various research centers around the country. These hybridization practices resulted in a narrow genetic base for the new varieties. Any crop with a narrow genetic base is more prone to natural disasters, such as the outbreak of a disease. Epidemics of cotton leaf curl virus disease in Pakistan since 1991 is a typical example and none of the varieties was found to be resistant.

Morphological features are indicative of the genotype but are represented by only a few loci because there is not a large enough number of characters available. Moreover, they can also be effected by environmental factors and growth practices. To have an accurate and reliable estimate of genetic relationships and genetic diversity, a large number of polymorphic markers are essentially required. Biochemical markers such as isozymes have been used to distinguish between homozygous and heterozygous individuals and to estimate the level of genetic variability in plant populations (Melchinger et al. 1991). Wendel et al. (1992) studied the genetic distances of a large number of accessions of upland cotton from different locations by isozyme analysis. However, isozyme analysis has certain limitations due to the availability of a limited number of marker loci, a general lack of polymorphisms for these loci in elite breeding materials, and the chance of variability in banding patterns being due to plant development (Tanksley et al. 1989).

The random amplified polymorphic DNA (RAPD) technique of Williams et al. (1990) provides an unlimited number of markers which can be used for various purposes. In addition to the technical simplicity and speed of RAPD methodology (Gepts 1993), its level of genetic resolution is equivalent to restriction fragment length polymorphism (RFLP) for determining genetic relationships among *Brassica oleracea* L. genotypes and *B. napus* L. breeding lines (Dos Santos et al. 1994; Hallden et al. 1994). RAPD markers have been

successfully used for the estimation of genetic similarities and the cultivar analysis of various plant species including rice (Yu and Nguyen 1994; Mackill 1995), broccoli and cauliflower (Hu and Quiros 1991), banana (Kaemmer et al. 1992; Howel et al. 1994), celery (Yang and Quiros 1995), *Brassica* (Demeke et al. 1992; Jain et al. 1994), *Triticum* (Vierling and Nguyen 1992; Chandrashekhar and Nguyen 1993), *Medicago* L. (Yu and Pauls 1993), *Coffea* L. (Orozco-Castillo et al. 1994), and *Lycopersicon* Miller (Williams and St-Clair 1993).

Multani and Lyon (1995) studied 14 Australian cotton varieties by RAPD markers using silver staining and demonstrated that very closely related varieties can also be distinguished. The genetic distances obtained from RAPD markers of 16 elite US cotton varieties were compared with the taxonomic distances measured from morphological characters. The classification of varieties based on the two methods produced similar results (Tatineni et al. 1996).

The objective of present work was to evaluate the level of genetic similarities of some elite cotton varieties and their genetic relationships with some exotic and/or standard varieties which will help in the identification of diverse parents and the possible use of genetic fingerprinting for varietal identification and classification.

Materials and methods

Plant material

Seeds of the cotton varieties used for analysis were obtained from various breeders and are shown in Table 1. Plants were grown in pots in a glasshouse.

DNA isolation

For individual plant analysis, DNA was isolated from three to four young leaves. For intervarietal studies, 3 g of young leaves (bulked from four to six different plants) were ground to a very fine powder in liquid nitrogen and transferred into a 50-ml centrifuge tube. Then, 15 ml of hot (65°C) 2 × CTAB [2% cetyldimethyl triethylammonium bromide, 1.4 M NaCl, 20 mM EDTA (pH 8.0), 0.1 M Tris-HCl (pH 8.0), 1% polyvinyl pyrolidone (PVP), 1% 2-mercapto-ethanol] was added and incubated for 30 min at 65°C with occasional swirling. The mixture was emulsified with an equal volume of chloroform: isoamyl alcohol (24:1) and spun at 4000 rpm for 10 min. The upper phase was again emulsified with an equal volume of chloroform: isoamyl alcohol (24:1) and spun for 10 min at 4000 rpm. The aqueous phase was removed and DNA was precipitated with a 0.6 vol of isopropanol. DNA was pelleted at 4000 rpm for 4 min and the supernatant was discarded. After adding 10 ml of 70% ethanol to the DNA pellet, it was kept on a slow shaker for 20 min and then spun at 4000 rpm for 2 min. The above wash step was repeated and then the sample was spun at 4000 rpm for 10 min. The pellet was air dried (20 min) and resuspended in 0.5 ml of 0.1 × TE buffer. After treatment with RNase, the DNA concentration was measured using a UV-visible spectrophotometer. DNA was diluted in sterile distilled water to a concentration of 12.5 ng/μl for use in PCR analysis.

PCR and primers

Random decamer primers (Operon Technologies Inc., Alameda, Calif. USA) were dissolved in sterilized distilled water at a concentration of 15 ng/μl. Fifty primers belonging to Operon kits OPI (20 primers), OPR (20 primers) and OPZ (10 primers, OPZ-01 to OPZ-10) were used for PCR amplifications.

Amplifications were carried out in a 50-μl reaction volume containing 10 mM Tris -HCl (pH 8.3 at 25°C), 50 mM KCl, 3.0 mM $MgCl_2$, 0.1 mM each of dATP, dGTP, dCTP and dTTP, 1 unit of *Taq* DNA polymerase (Perkin Elmer, Norwalk, Conn.), 0.001% gelatin (Sigma, St-Louis, Mo.), 50 ng of template DNA and 30 ng of primer. The reaction mixture was overlaid with two drops of mineral oil in order to avoid evaporation. The amplifications were carried out in a Perkin Elmer Thermal Cycler 480, programmed for a first denaturation step of 3 min at 94°C followed by 40 cycles of 94°C for 1 min, 36°C

Table 1 List of cotton varieties used in the genetic analysis studies

S. no.	Variety	Parental information	Year	Center of origin
1	MNH-93	C158 × (MS 39 × MEX 12)	1980	CCRI, Multan
2	Siokra-324	Exotic Australia	Not available	
3	Coker-304	Exotic, USA	Not available	
4	Coker-312	Exotic, USA	Not available	
5	CIM-109	NIAB-78 × A-89/FM	1990	CCRI, Multan
6	CIM-240	CIM-70 × W 1106	1992	CCRI, Multan
7	CIM-1100	492/87 × CP-15/2	Under approval trials	CCRI, Multan
8	NIAB-78	AC 134 × Delta Pine 16	1983	NIAB, Faisalabad
9	NIAB-86	H 1-9-6-2 × SP-16	1990	NIAB, Faisalabad
10	S-12	MNH-93 × Arizona 7203-14-4	1988	CRS, Multan
11	S-14	AC-134 × Paymaster	1988	CRS, Multan
12	FH-87	Not available	1988	CRI, Faisalabad
13	FH-682	Not available	1992	CRI, Faisalabad
14	Karishma	NIAB-86 × W83-29MEX	Under approval trials	NIAB, Faisalabad
15	Ravi	(Selection from D-465)	1982	CRI, Faisalabad
16	V_3-NCVT	Not available	Under approval trials	
17	SLH-41	S-178/68 (209F × Mysor American) × 149 F × MEX-68 × 2267/70	1984	CRS, Sahiwal
18	SLS-1	Not available	1995	CRS, Sahiwal
19	B-557	268 F × L-5	1976	CRS, Bahawalpur
20	BH-36	BS-1 × Texas Brocham-76-C	1992	CRS, Bahawalpur
21	RH-1	LH-62 × W-1104	1990	CRS, Rahim Yar Khan
22	AEM-52	Not available	Not available	
23	AENS-18/87	Not available	Not available	

for 1 min and 72°C for 2 min. After the completion of 40 cycles, the reactions were kept at 72°C for 4 min and then held at 4°C until the tubes were removed. PCR products were separated on a 1.2% agarose gel with ethidium bromide in the gel using 0.5 × Tris Borate EDTA (TBE) buffer.

Analysis of amplification profiles

Amplification profiles of 22 cotton varieties were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data for all the 50 primers was used to estimate the similarity on the basis of the number of shared amplification products (Nei and Li 1979). A dendrogram based on similarity coefficients was generated by using the unweighted pair group method of arithmetic means (UPGMA).

Results and discussion

The amplification profiles of 23 cotton varieties were polymorphic with 49 primers and can be used for varietal identification. The banding patterns produced with one primer (OPI-03) were not polymorphic for any of the 23 varieties. Individual plants of two varieties (S-12 and Karishma) were also studied for intravarietal genetic polymorphisms. DNA of individual seedlings/young plants were isolated separately and used for PCR amplifications. Twenty individual plants of S-12 produced consistent amplification profiles with 13 primers. There is very little cross-pollination in cotton and the variety S-12 is quite homogeneous, so the results are not surprising and can be used to evaluate seed purity. In the case of the variety Karishma, 15 individual plants were studied with nine primers. Polymorphisms were observed with two out of these nine primers. In this variety, the response of individual plants to cotton leaf curl virus (CLCuV) was variable. The morphological features of individual plants in the field were also not uniform (data not shown). The intravarietal polymorphisms detected by RAPD analysis suggest that the variety is not homogeneous and segregation might still be occurring in the population. The polymorphisms of amplification profiles of individual plants have earlier been reported in rye "*Secale cereale* L var. Balboa" (Iqbal and Rayburn 1994).

The fifty primers studied amplified a total of 349 DNA fragments. Out of these 349 amplified fragments, 38 (10.9%) were not polymorphic. The rest of the bands

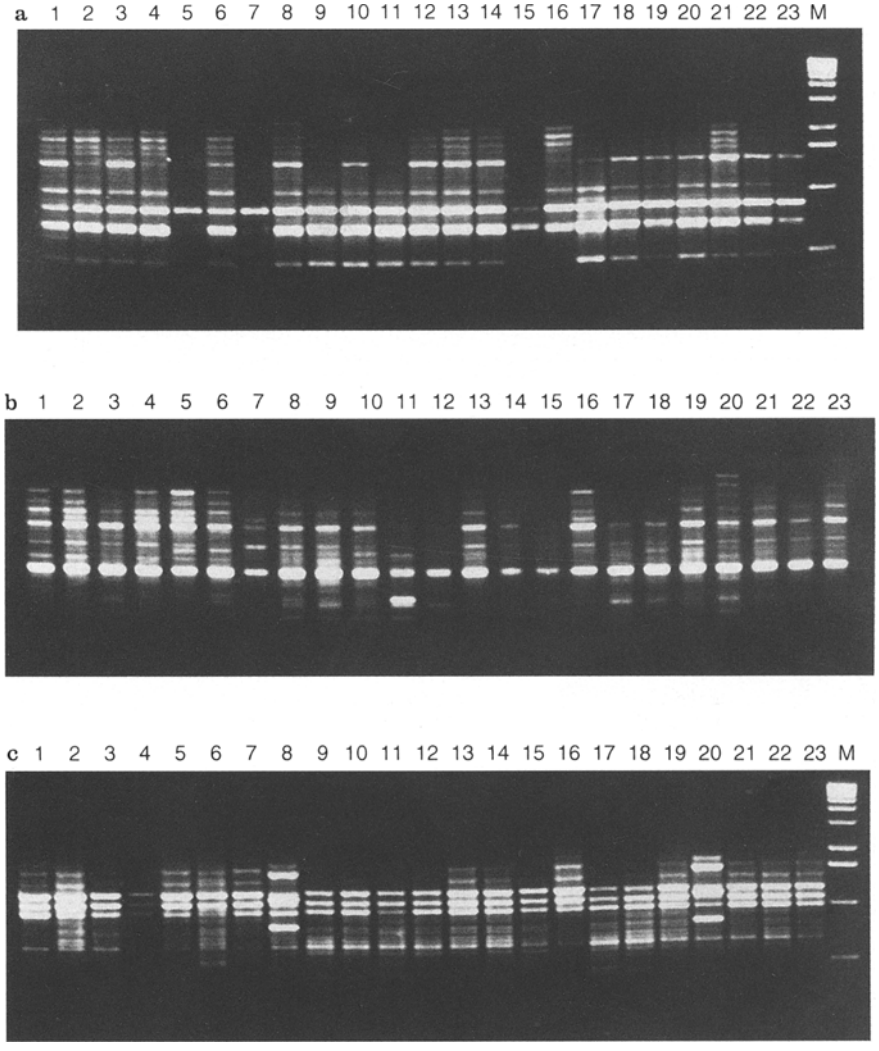

Fig. 1 Amplification profiles of 23 cotton varieties with **a** = OPZ-04, **b** = OPZ-06 and **c** = OPZ-10 primers. *Lane* *1* = MNH-93; *2* = Siokra-324; *3* = Coker-304; *4* = Coker-312; *5* = CIM-109; *6* = CIM-240; *7* = CIM-1100; *8* = NIAB-78; *9* = NIAB-86; *10* = S-12; *11* = S-14; *12* = FH-87; *13* = FH-682; *14* = Karishma; *15* = Ravi; *16* = V_3-NCVT; *17* = SL-41; *18* = SLS-1; *19* = B-557; *20* = BH-36; *21* = RH-1; *22* = AEM-52; *23* = AENS-18/87; *M* = size marker (1-kb DNA ladder)

(89.1%) were polymorphic in one or other of the 23 varieties. Even though the amplitude of polymorphisms was high (89.1% bands were polymorphic and 98% of the primers produced polymorphic profiles), there was not a single primer (out of 50 studied) which could differentiate clearly between all the varieties. This might be indicative of a narrow genetic base for some of the cotton varieties studied.

The levels of polymorphism were different with different primers among different varieties (Fig. 1a, b, c). The diploid cotton, *G. arboreum* var. Ravi (Fig. 1a, lane #15), can be clearly distinguished from its tetraploid counterparts. Only 150 fragments were amplified from Ravi genomic DNA with 50 primers as compared to the total of 349. But the lowest number of amplified fragments were 138 from CIM-1100. The number of amplified fragments from the rest of the varieties ranged from 200 to 300 with a maximum of 301 bands from MNH-93. The number and size of amplified fragments also varied with different primers. A maximum of 16 fragments were amplified with primer OPI-06 and a minimum of three bands with primers OPI-03 and OPR-08. The size of amplified fragments also varied with different primers. A 4.5-kb fragment was amplified from 15 varieties with primer OPI-08 while the smallest-size (0.3 kb) fragment was amplified by primer OPR-10.

The main objective of the present study was to estimate the genetic similarities of elite cotton varieties. The similarity matrix obtained after multivariant analysis using Nei and Li's (1979) coefficient is shown in Table 2. These similarity coefficients were used to generate a dendrogram (Fig. 2) by UPGMA analysis in order to determine the grouping of different varieties. From the similarity matrix, the least similar variety is Ravi. Its similarity ranges from 48.63% (with S-12) to 64.46% (with SLS-1). The low similarity of Ravi with other varieties is due to the fact that it is a diploid cotton (*G. arboreum* L.) and belongs to the old world. In the dendrogram, Ravi does not cluster with any other variety tested and is easily distinguishable. CIM-1100 is also quite distinct from rest of the tetraploid varieties. Its similarity coefficient varies from 51.48% with MNH-93 to 67.39% with CIM-109. The parents of CIM-1100 are different from rest of the varieties and it also has some distinct morphological characters. This variety is reported to be tolerant to CLCuV disease (personal communication) while the others (except Ravi) are susceptible. Although the number of fragments amplified from Ravi and CIM-1100 are 150 and 138, they are only 55.47% similar which means that more than 50% of the amplified fragments were polymorphic. These results are a good indication of the reliability of the RAPD technique for the evaluation of genetic similarities.

Similarity matrix data revealed that varieties FH-87 and FH-682 are 93.41% similar while SL-41 and SLS-1 are 91.10% similar. Interestingly, the first two varieties have been developed at one breeding center and the latter two at another research station. On the basis of the RAPD data their genetic base looks very narrow. The

Table 2 Similarity matrix for Nei and Li's coefficient of 22 cotton varieties obtained from RAPD marker analysis. Numbers on the top of the table represent the same varieties as listed in the first vertical column

Variety		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	MNH-93	1																					
2	Coker-304	0.778	1																				
3	Coker-312	0.789	0.897	1																			
4	CIM-109	0.791	0.886	0.859	1																		
5	CIM-240	0.770	0.881	0.884	0.885	1																	
6	CIM-1100	**0.514**	**0.609**	**0.582**	**0.673**	**0.604**	1																
7	NIAB-78	0.751	0.865	0.800	0.818	0.873	**0.623**	1															
8	NIAB-86	0.739	0.859	0.824	0.822	0.832	**0.620**	0.890	1														
9	S-12	0.654	0.796	0.776	0.736	0.770	**0.614**	0.821	0.817	1													
10	S-14	0.687	0.859	0.829	0.796	0.811	**0.603**	0.847	0.885	0.790	1												
11	FH-87	0.734	0.886	0.857	0.834	0.861	**0.592**	0.900	0.900	0.824	0.883	1											
12	FH-682	0.758	0.891	0.870	0.845	0.887	**0.608**	0.892	0.883	0.823	0.858	0.934	1										
13	Karishma	0.720	0.856	0.829	0.791	0.844	**0.567**	0.814	0.801	0.771	0.802	0.881	0.856	1									
14	Ravi	**0.567**	**0.584**	**0.529**	**0.561**	**0.589**	**0.554**	**0.593**	**0.634**	**0.486**	**0.604**	**0.604**	**0.594**	**0.555**	1								
15	V3	0.700	0.819	0.816	0.831	0.841	**0.649**	0.791	0.756	0.713	0.734	0.816	0.832	0.772	**0.572**	1							
16	SLH-41	0.688	0.844	0.792	0.774	0.808	**0.591**	0.837	0.870	0.795	0.857	0.868	0.847	0.753	**0.609**	0.728	1						
17	SLS-1	0.705	0.840	0.779	0.756	0.804	**0.564**	0.850	0.885	0.787	0.857	0.887	0.852	0.783	**0.644**	0.739	0.911	1					
18	B-557	0.749	0.840	0.814	0.807	0.831	**0.636**	0.867	0.884	0.788	0.838	0.886	0.873	0.779	**0.603**	0.772	0.883	0.870	1				
19	BH-36	0.706	0.836	0.824	0.786	0.810	**0.590**	0.871	0.892	0.797	0.848	0.885	0.869	0.813	**0.602**	0.757	0.864	0.888	0.878	1			
20	RH-1	0.742	0.883	0.870	0.813	0.853	**0.621**	0.896	0.878	0.813	0.843	0.902	0.909	0.861	**0.622**	0.808	0.873	0.865	0.895	0.881	1		
21	AEM-52	0.708	0.860	0.877	0.791	0.827	**0.585**	0.849	0.850	0.801	0.820	0.892	0.863	0.817	**0.566**	0.780	0.833	0.846	0.872	0.863	0.904	1	
22	AENS-18/87	0.741	0.851	0.843	0.850	0.847	**0.593**	0.857	0.837	0.777	0.820	0.883	0.891	0.832	**0.574**	0.792	0.853	0.849	0.862	0.862	0.883	0.873	1

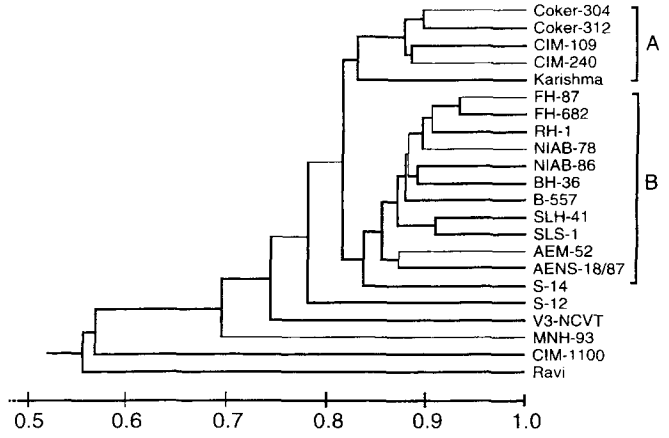

Fig. 2 Dendrogram of 22 cotton varieties generated from RAPD data using the unweighted pair group method of arithmetic means (UPGMA). The scale is based on Nei and Li's coefficients of similarity

coefficient of similarity of most of the other varieties ranges between 70 and 90%. 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. In the present study the genetic similarity of 17 varieties ranges between 81.51 and 93.41%. The *G. hirsutum* L. cotton varieties are least similar (48.63–64.46%) to the *G. arboreum* L. From the dendrogram, two clusters represented as A and B are very distinct. The two American varieties Coker-304 and Coker-312, along with CIM-109, CIM-240 and Karishma, are in cluster A. The varieties CIM-109, CIM-240 and Karishma has American parents and the results fit to the known pedigree. In cluster B, there are 12 varieties and their similarity ranges from 93 to 83%. The similarity between the two clusters is also more than 80% (81.51%). There are only four tetraploid varieties in addition to the diploid "Ravi" which have less than 80% genetic similarity. These results are in accordance with the known genetic make up (pedigree) of the varieties.

The genetic relatedness measured from the analysis of RAPD patterns generated after bulking the DNA of alfalfa populations has been found to be very similar to the index of genetic distances (IGD) calculated after a comparison of individuals (Yu and Pauls 1993). Tatineni et al. (1996) studied 19 cotton genotypes with eight primers and compared the genetic distance obtained from RAPD data with the taxonomic data. In their studies 8 out of 27 primers studied (33.8%) did not produce any polymorphisms while in our study 98% of the primers produced polymorphic patterns but the level of genetic similarity was quite high. Brubaker and Wendel (1994) also demonstrated that the level of RFLP diversity was low in *G. hirsutum* L. cultivars as compared to the other reported taxa. The genotypes used in the present study are elite commercial varieties in addition to some standard Coker (American) varieties and a diploid variety. The results are indicative of their genetic relationships and are in accordance with the earlier studies. Intravarietal studies of S-12 describe the usefulness of the technique for the evaluation of seed purity and of the genetic similarity of the cotton varieties. Moreover, the genetic similarities obtained from the analysis can be used for the selection of parents to generate a mapping population and will also help in the selection of parents for breeding purposes.

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