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Full Length Research Paper

Inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) analyses of genetic diversity in Mehr cotton cultivar and its crossing progenies

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Cotton is cultivated in Iran with diploid and tetraploid forms and hybridization is a means to increase the genetic diversity and obtain new elite cultivars in this crop. This present study considered the molecular genetic diversity in Mehr (*Gossypium hirsutum*) cotton cultivar and its crossing progenies. 21 of 30 random amplified polymorphic DNA (RAPD) primers produced 220 reproducible bands with average of 10.47 bands per primer and 80.12% of polymorphism. OPR02 primer showed the highest number of effective allele (Ne), Shannon index (I) and genetic diversity (H). Some of the cultivars had specific bands, for example the F1 progeny of Mehr X No. 200, Mehr parental genotype and Mehr X Belilzovar hybrid genotype. Results show that some RAPD bands were present in the F1 progenies, but absent in the parental genotypes. Some others were present in the parental genotypes, but were absent in their hybrids. The highest values of genetic diversity in RAPD markers were obtained in Mehr X Sindose and Mehr X Belilzovar hybrids. Nine of ten inter simple sequence repeats (ISSR) primers used produced 113 reproducible bands with average of 54.35% polymorphism. UBC834 locus revealed the highest number of Ne, I and H values. Also, some ISSR bands occurred only in the parental genotype while some others occurred only in the hybrid genotypes. The highest values of genetic diversity in ISSR markers were obtained in Mehr X Sindose and Mehr X Belilzovar hybrids.

Key words: Cotton, genetic diversity, random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR).

INTRODUCTION

Hybridization is the main breeding strategies in cotton plant with the aim to broaden the genetic diversity available and produce new genotypes. Due to continuous artificial selection and cultivation of cotton cultivars, genetic diversity available decreases leading to genetic erosion and possible loss of useful genetic loci, which could result in vulnerability to pests and diseases (Sheidai et al., 2008; Esmail et al., 2008; Wendel et al., 1992). Therefore, making new hybrid combinations and studying the amount of genetic diversity obtained is important. Cotton has two cultivated forms of diploid (*Gossypium herbaceum*) and tetraploid (*Gossypium hirsutum*) whose several genotypes have been cultivated in Iran. Tetraploid cotton (*G. hirsutum*) has a genome constitution of AADD (2n = 52) (Menzel and Brown, 1978) and is one of the world's dominant cotton cultivars.

Different molecular markers have been used to study genetic diversity, hybridization and the occurrence of

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somaclonal variation in cotton. RAPD (random amplified polymorphic DNA) and inter- simple sequence repeats (ISSR) are considered as useful molecular markers for these kinds of investigations (Wajahatollah and Stewart, 1997; Kumar et al. 2003; Vafaie-Tabar et al., 2003; Mehetre et al., 2004; Dongre et al., 2004; Rana et al., 2007; Preetha and Raveendren, 2008; Sheidai et al., 2008; Wei et al., 2008; Tafvizei et al., 2010). For example, Maleia et al. (2010) investigated the genetic divergence through RAPD markers among the introduced African and American cultivars and inbred lines of cotton (*G. hirsutum* L. raça *latifolium* H.) and reported the highest genetic similarity among African commercial cotton cultivars. Also genetic similarity of diploid and tetraploid cotton cultivars was examined using RAPD markers by Chaturvedi and Nag (2010). RAPD analysis has also been utilized for identification of hybrids and assessment of genetic variation in hybrid cotton genotypes by Esmail et al. (2008) and Amjad-Ali et al. (2008). Comparative study of different molecular markers (RAPD, ISSR and SSR) by several research studies revealed that combined molecular data could be reliable tools to evaluate genetic diversity of *Gossypium* species (Wu et al., 2001; Dongre et al., 2004; Ebtissam et al., 2006; Rana et al., 2007; Sapkal et al., 2011). Hence, the present report considers molecular diversity of Mehr cultivar (*Gossypium hirsutum*) and its crossing progenies by using RAPD and ISSR markers for the first time.

MATERIALS AND METHODS

12 cotton genotypes of *G. hirsutum*, including the parental genotypes Mehr, Bellizovar, No. 200, Siokra, Sindose, Sahel and Tabladilla, and their crossing progenies obtained by crossing to Mehr were cultivated. The cotton genotypes were cultivated in three rows of 10 m length with 20 cm interplant distance in the experimental field of Gorgan Cotton Research Center of Iran, according to a completely randomized design (CRD) with three replications. Three to five fresh leaves of each genotype were pooled and used for DNA extraction. The total genomic DNA was extracted using the CTAB method by Murry and Tompson (1980) with modification described by De al Rosa et al. (2002). Quality of extracted DNA was examined by running on 0.8% agarose gel.

RAPD analysis

30 decamer RAPD primers of Operon technology (Alameda, Canada) belonging to OPA, OPB, OPC, OPH, OPI, OPM and OPR sets were used in this study. The PCR reaction mixture consisted of 20 ng template DNA, 1 × PCR buffer (10 mM Tris-HCL pH 8.8, 250 mM KCL), 200 µM dNTPs (Bioron, Germany), 0.80 µM 10-base random primers and 1 unit of *Taq* polymerase (Bioron, Germany), in a total volume of 25 µl. DNA amplification was performed in Techne thermocycler (Germany). Template DNA was initially denatured at 94°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 94°C, primer annealing for 1 min at 36°C and primer extension for 2 min at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion.

The PCR amplified products were separated by electrophoresis on a 2% agarose gels using 0.5 × TBE buffer (44.5 mM Tris/Borate, 0.5 mM EDTA, pH 8.0). The gels were stained with ethidium bromide and visualized under UV light (Sambrook et al., 2001). A 100 bp DNA ladder (GeneRuler, Fermentas) was used as the molecular standard in order to confirm the appropriate RAPD markers. These markers were named by primer origin, followed with the primer number and the size of amplified products in base pairs.

ISSR assay

Nine ISSR primers; UBC810, UBC811, UBC823, UBC834, UBC849, (CA) 7GT, $(GA)_9T$, $(GA)_9A$ and $(GA)_9C$, commercialized by UBC (the University of British Columbia) were used. PCR reactions were performed in a 25 µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, 30 s at 94°C; 1 min at 50°C and 1 min at 72°C. The reaction was completed by final extension step of 7 min at 72°C. Amplification products were visualized by running on 2% agarose gel, following ethidium bromide staining. Fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

RAPD and ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). The level of intra-population genetic diversity of cotton genotypes was determined by Nei's gene diversity (H) as well as the Shannon information index (I) (Nei, 1972). Jaccard's similarity as well as Nei's genetic distance (Nei, 1973), was determined among the cultivars studied and used for the grouping of the genotypes by unweighted paired group method with arithmetic average (UPGMA) and neighbor joining (NJ) clustering methods and ordination based on principal co-ordinate analysis (PCoA) (Podani, 2000; Weising et al., 2005). Cophenetic correlation and bootstrapping (1000 replications) were performed to check the fit of dendrograms obtained. NTSYS Ver. 2.02 (1998) and PAUP ver. 4. b10 (2000) were used for clustering and PCoA analyses.

RESULTS

RAPD analysis

Out of 30 RAPD primers used, 21 primers produced reproducible bands. In total, 220 RAPD bands (loci) were obtained with average of 10.47 bands per primer. The mean percentage of polymorphism was 80.12%. RAPD primers including OPB05, OPM10, OPH19, OPR01, OPI16, OPC12, OPR02 and OPC06 produced the highest polymorphism (100%), while OPC04 produced the lowest percentage of polymorphism (40%). The mean value of effective alleles, Shannon index and genetic diversity of RAPD loci studied were 1.514, 0.435 and 0.294, respectively, while OPR02 primers showed the highest number of effective alleles (1.820), Shannon index (0.622) and genetic diversity (0.437) (Table 1). In

Table 1. Genetic parameters based on RAPD data.

RAPD Loci	Total band	Polymorphism (%)	N_a	N_e	I	H
OPB05	4	100	2.000	1.604	0.514	0.347
OPA04	3	66.67	1.667	1.465	0.401	0.274
OPM10	12	100	2.000	1.397	0.398	0.250
OPA10	11	54.5	1.545	1.282	0.253	0.166
OPH07	14	78.57	1.786	1.593	0.473	0.327
OPA13	11	54.5	1.545	1.314	0.273	0.182
OPA05	11	54.5	1.545	1.453	0.350	0.246
OPC01	13	69.2	1.692	1.526	0.423	0.293
OPI12	10	40	1.400	1.295	0.237	0.163
OPA09	9	77.7	1.778	1.394	0.382	0.247
OPH19	3	100	2.000	1.291	0.318	0.191
OPR01	7	100	2.000	1.691	0.575	0.392
OPC04	10	20	1.200	1.100	0.099	0.064
OPI16	10	100	2.000	1.774	0.617	0.428
OPC12	10	100	2.000	1.467	0.444	0.288
OPR02	14	100	2.000	1.826	0.622	0.437
OPA18	5	80	1.800	1.748	0.541	0.387
OPC06	17	100	2.000	1.627	0.535	0.359
OPR06	15	93.3	1.933	1.640	0.546	0.372
OPR12	16	93.75	1.938	1.454	0.428	0.277
OPC09	14	100	2.000	1.631	0.536	0.361
Mean	10.47	80.12	1.808(0.027)	1.514(0.025)	0.435(0.018)	0.294(0.013)

N_a, Number of different allele; N_e, number of effective allele; I, Shannon's index; H, Nei's genetic diversity; number in parenthesis indicate standard deviation.

primers OPM-10 and OPC-12 showing the highest numbers (4 and 3 bands, respectively). Some of the cultivars showed the presence of specific bands, for example bands no. 2, 4, 5, 11 and 12 (410, 600, 750, 1900 and 2500 bp, respectively), of the primer OPA-10 were specific for the F1 progeny Mehr X No. 200, RAPD bands no. 1 and 3 (350 and 580 bp, respectively) of the primer OPA-10 occurred only in the Mehr parental genotype and band no. 2 (450 bp) of the primer OPA-13 occurred only in the Mehr X Belilzovar hybrid genotype.

Some RAPD bands were also present in all the genotypes except one, for example bands no. 1 and 4 (850 and 1600 bp, respectively) of the primer OPB-05 were only absent in the parental genotype no. 200; band no. 6 (1200 bp) of the same primer was only absent in the Mehr parental genotype; band no. 12 (1750 bp) of the RAPD primer OPH-07 was absent only in the Belilzovar parental genotype; bands no. 3 and 4 (480 and 625 bp, respectively) of the primer OPA-13; bands no. 4, 7 and 9 of the primer OPA-05 (700, 1300 and 625 bp, respectively) were absent only in the Mehr parental genotype; and bands no. 4, 6-8 (900, 1100, 1400 and 1600 bp, respectively) of the RAPD primer OPI-16 were absent only in Hybrid genotype Mehr X Belilzovar.

More also, some RAPD bands were present in the parental genotypes, but were absent in their hybrid. For example, RAPD bands no. 14, 26 and 27 (400, 1000 and 1400 bp, respectively) of the primer OPM-10 were present in the parental genotypes Mehr and Sindose, but were absent in their hybrid Mehr X Sindose. Bands no. 5 to 7 (1000 to 1200 bp) of the primer OPR-01 and bands no. 4, to 7 (900 to 1600 bp) of the primer OPI-16 were present in the parental genotypes Mehr and Belilzovar, but were absent in their hybrid. Similarly, bands no. 3 and 5 of the RAPD primer OPA-10 (580 and 1000 bp, respectively) were present in the parental genotypes Mehr and Cindose but were absent in their hybrid Mehr X Sindose. In addition, some novel RAPD bands were present in the F1 progenies, but were absent in the parental genotypes. For example bands no. 5, 7 and 8 (900 to 1500 bp) of the primer OPM-10 were absent in the parental genotypes of Mehr and Belilzovar, but were

Table 2. Genetic parameters based on ISSR data.

ISSR loci	Total Band	Polymorphism (%)	N_a	N_e	I	H
UBC846	9	22.2	1.222	1.031	0.052	0.027
UBC823	13	69.2	1.692	1.502	0.422	0.290
UBC834	18	77.7	1.778	1.436	0.387	0.257
UBC810	15	46.6	1.467	1.293	0.239	0.163
UBC811	16	43.75	1.800	1.428	0.390	0.256
(GA)9T	13	38.46	1.385	1.300	0.240	0.167
(GA)9A	11	36.3	1.364	1.229	0.207	0.139
(GA)9C	8	75	1.750	1.506	0.416	0.285
(CA)7GT	10	80	1.438	1.277	0.229	0.155
Mean	12.55	54.35	1.549 (0.047)	1.338 (0.036)	0.290 (0.028)	0.196 (0.019)

N_a, Number of different allele; N_e, number of effective allele; I, Shannon's index; H, Nei's genetic diversity; number in parenthesis indicates standard deviation.

present in their hybrid while bands no. 7 to 14 (600 to 1400 bp) of the primer OPA-10 were absent in the parental genotypes of Mehr and Tabladilla but were present in their hybrid.

The mean values of H and I for RAPD markers determined showed 0.30 and 0.43, respectively in the genotypes Mehr, no. 200 and their crossing progeny, 0.24 and 0.35 in Mehr, Siokra and their crossing progeny, 0.27 and 0.39 in Mehr, Tabladilla and their hybrid, 0.31 and 0.45 in Mehr, Sindose and their hybrid and 0.32 and 0.46 in Mehr, Belilzovar and their hybrid. Therefore the highest values of genetic diversity in RAPD markers were obtained in Mehr X Sindose and Mehr X Belilzovar hybrids. Nei's genetic identity and genetic distance for RAPD data determined among the cotton genotypes are given in Table 3. The value of genetic identity varied from 0.41 between Mehr and Mehr X Belilzovar to 0.88 between Mehr X Tabladilla and Mehr X Sindose. The mean value of genetic identity for RAPD markers was 0.64. UPGMA and NJ dendrograms as well as Bayesian clustering of RAPD data produced similar results supported by PCoA ordination plot (Figures 1 and 2). The cophenetic correlation of the NJ tree was higher (r = 0.98) and showed good bootstrap values, therefore it is discussed below.

Mehr parental genotype differed from the other genotypes and stood far from them. Two parental genotypes, no. 200 and Sindose, show similarity in RAPD characteristics and were placed close to each other, while Sahel and Siokra showed affinity. The Belilzovar cultivar joined Sahel and Siokra but at some distance. PCoA analysis of RAPD data showed that the first four factors comprised about 64% of total variance when the first, second and third axis comprised about 25, 16 and 13% of total variance, respectively. Details of similarities versus distances of these genotypes are better understood by a PCoA plot (Figure 2). For example, separation of Mehr and no. 200 genotypes from the others is well documented as they stand on one side of PCoA axis 2, while Sindose stands on the other side of the same axis. The same is true for the cultivar Siokra compared to Sahel and Belilzovar genotypes. The hybrid genotypes were placed in one cluster close to each other and the Tabladilla parental genotype joined them in the NJ tree, but the PCoA plot clearly separated the hybrids from all parental genotypes.

ISSR analysis

The nine ISSR primers used in this experiment produced 113 reproducible bands with 12.55 bands per locus. UBC846 locus showed the lowest polymorphism (22.20%) while (CA)7GT revealed the highest percentage of polymorphism (80%). In total, the average of polymorphism was 54.34% (Table 2). The mean value of effective alleles, Shannon's index and genetic diversity based on ISSR data were 1.338, 0.290 and 0.196, respectively, while UBC823 locus showed the highest number of effective alleles (1.502), Shannon's index (0.422) and genetic diversity value (0.290) (Table 2).

The ISSR band no. 1 (250 bp), band no. 8 (550 bp) of the primer UBC849 and bands no. 4 and 10 (350 and 650 bp, respectively) of the primer UBC810 occurred only in the parental genotype Mehr, while band no. 16 (1200 bp) of this primer occurred only in Tabladilla. Also, band no. 3 (375 bp) of the primer (CA) 7GT occurred only in the hybrid genotype Mehr X Sindose. The ISSR bands no. 1 and 2 (150 and 250 bp, respectively) as well as 8 to 13(650 to 1200 bp) of the primer UBC823 occurred in all the genotypes except in Belilzovar, while band no. 1 (150 bp) of the primer UBC834 was only absent in Sindose and band no. 12 (750 bp) of the same primer was only absent in the hybrid genotype Mehr X Sindose. Similarly, band no. 3 (325 bp) of the ISSR primer UBC810 was absent in Mehr and band no. 13 (900 bp) of the same primer was absent in the hybrid genotype Mehr X Belilzovar.

Table 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for RAPD data among cotton genotypes.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12
1	--	0.7407	0.6296	0.5238	0.7196	0.6508	0.5820	0.5767	0.5714	0.5767	0.6190	0.5556
2	0.3001	--	0.6243	0.6984	0.7143	0.7407	0.6508	0.7090	0.6296	0.6667	0.7302	0.6455
3	0.4626	0.4711	--	0.4921	0.5291	0.4497	0.4233	0.4815	0.4127	0.4180	0.5132	0.4180
4	0.6466	0.3589	0.7091	--	0.7407	0.7672	0.6984	0.6614	0.6878	0.6614	0.6825	0.5767
5	0.3291	0.3365	0.6366	0.3001	--	0.8783	0.7566	0.6667	0.7354	0.7196	0.7196	0.6349
6	0.4296	0.3001	0.7991	0.2650	0.1298	--	0.7937	0.6825	0.7619	0.7778	0.7884	0.6931
7	0.5413	0.4296	0.8597	0.3589	0.2789	0.2311	--	0.7937	0.8201	0.8254	0.8042	0.7090
8	0.5504	0.3439	0.7309	0.4134	0.4055	0.3819	0.2311	--	0.7513	0.7354	0.7249	0.6720
9	0.5596	0.4626	0.8850	0.3742	0.3073	0.2719	0.1983	0.2859	--	0.8677	0.7831	0.7725
10	0.5504	0.4055	0.8723	0.4134	0.3291	0.2513	0.1919	0.3073	0.1419	--	0.8836	0.8413
11	0.4796	0.3145	0.6670	0.3819	0.3291	0.2378	0.2179	0.3218	0.2445	0.1238	--	0.7989
12	0.5878	0.4377	0.8723	0.5504	0.4543	0.3665	0.3439	0.3976	0.2581	0.1728	0.2245	--

Genotypes code from 1 to 12 are respectively No. 200, Sindose, Mehr, Belilzovar, Siokra, Sahel, Tabladilla, Mehr X No. 200, Mehr X Siokra, Mehr X Tabladilla, Mehr X Sindose and Mehra X Belilzovar.

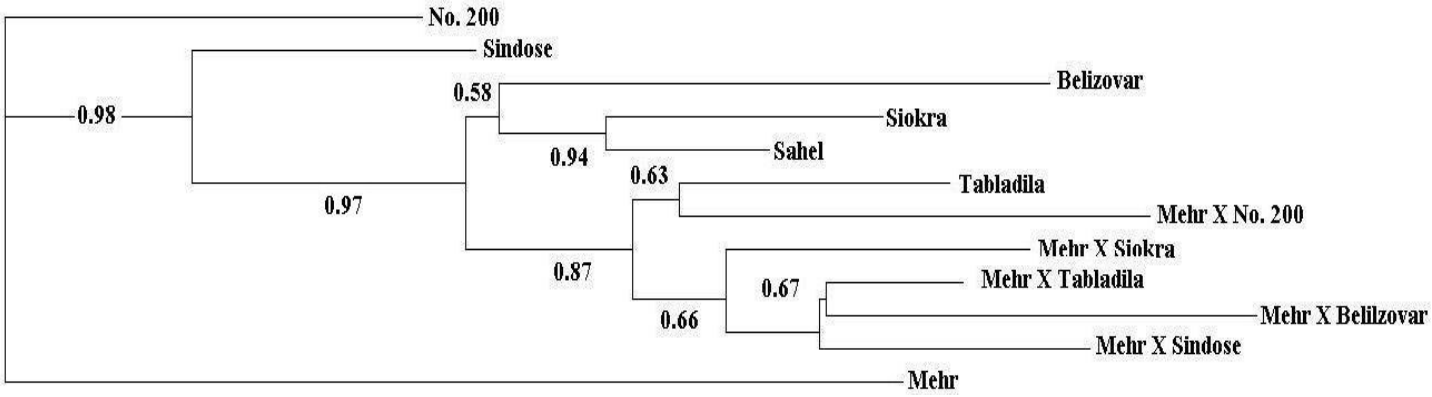

Figure 1. NJ tree of RAPD data (numbers on the tree branches are bootstrap values).

The mean values of gene diversity (H) and I of the different parents and their hybrids for ISSR markers determined were 0.27 and 0.42 in the Mehr, no. 200 and their crossing progeny, 0.22 and 0.32 in Mehr, Siokra and their crossing progeny, 0.27 and 0.35 in Mehr, Tabladilla and their hybrid, 0.31 and 0.45 in Mehr, Sindose and their hybrid and 0.30 and 0.44 in Mehr, Belilzovar and their hybrid. Therefore the highest values of genetic diversity in ISSR markers were obtained in Mehr X Sindose and Mehr X Belilzovar hybrids. Nei's genetic identity and genetic distance for ISSR data determined among the cotton genotypes are given in Table 4. The value of genetic identity varied from 0.39 between Mehr and the Belilzovar cultivars as well as Mehr and Sindose cultivars to 0.88 between Sahel and Siokra cultivars. The mean value of genetic identity obtained for ISSR molecular markers was 0. 63.

UPGMA and NJ dendrograms as well as Bayesian clustering of ISSR data produced similar results supported by PCoA ordination plot (Figures 3 and 4). The cophenetic correlation of NJ tree was higher (r = 0.96) and showed low bootstrap values (not shown) (Figure 3). The parental genotypes Mehr, no. 200 and Belilzovar differ in ISSR characteristics from the other genotypes and were placed far from the others supporting the RAPD tree discussed before. PCoA analysis of ISSR data showed that the first four factors comprised about 61% of total variance when the first, second and third axis comprised about 21, 16 and 12% of total variance respectively. This is better understood by looking at the PCoA plot (Figure 4) showing complete separation of the Mehr genotype on one side of the PCA axis and the Belilzovar cultivar on the other side. Two other parental genotypes, Sahel and Siokra, also show close affinity in ISSR markers supporting the RAPD tree obtained. However the PCA plot still reveals some molecular difference between these two genotypes as they stand on opposite sides of PCoA axis.

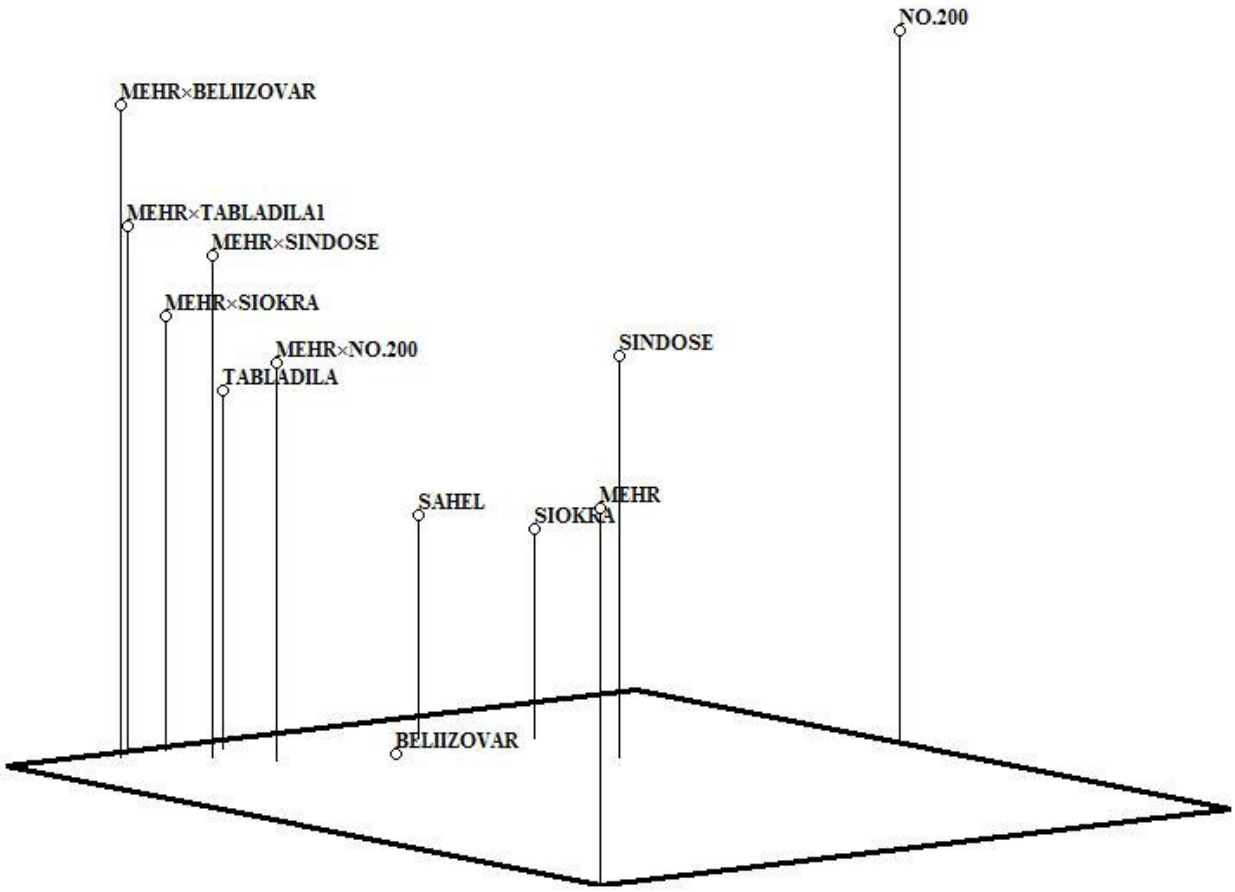

Figure 2. PCoA plot of RAPD data.

The hybrid genotypes Mehr X no. 200, Mehr X Sindose and Mehr X Belilzovar show close affinity in ISSR markers and were placed close to each other in one cluster, while Mehr X Siokra and Mehr X Tabladilla showed affinity and joined each other in another cluster. This is not in complete agreement with the RAPD tree obtained in which all hybrid genotypes were grouped together. The PCoA plot also shows such differences.

DISCUSSION

The presence of RAPD and ISSR polymorphic bands in the parental and F1 progenies of the cotton cultivars studied indicates the presence of genetic polymorphism in these genotypes. Moreover, the occurrence of specific bands/loci only in some of the cultivars illustrates the occurrence of unique insertion/deletion in DNA material of these genotypes which may be used in planning hybridization.

The number of RAPD and ISSR bands and degree of polymorphism obtained in the cotton cultivars studied is mostly in agreement with the other studies performed in cotton. Wei et al. (2008) studied the genetic diversity in 48 cotton accessions of *G. barbadense* and *G. hirsutum* by ISSR markers and obtained 92 reproducible ISSR bands out of which 72 were polymorphic. They also could differentiate the genotypes collected from different provinces in China based on ISSR data. In another study, Vafaie-Tabar et al. (2003) reported 79% average genetic similarity among Indian tetraploid cotton cultivars, while Rana and Bhat (2005) reported 74% average genetic similarity. According to Rana and Bhat (2005), the other studies on tetraploid cotton cultivars outside India also reported similar ranges of average genetic similarity.

The average genetic similarity obtained in this study for RAPD and ISSR markers was 64 and 63% respectively, which is slightly lower than other reports mentioned indicating the presence of slightly higher genetic diversity in the cotton genotypes studied. Such genetic diversity may be used in further hybridization programs. The genetic diversity indices determined for both RAPD and ISSR markers revealed the highest values of genetic diversity in Mehr X Sindose and Mehr X Belilzovar hybrids; therefore we suggest these genotypes be used for further hybridization.

In a similar investigation, Rana and Bhat (2005) could differentiate the Indian diploid and tetraploid cotton

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for ISSR data in cotton genotypes.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12
1	--	0.8095	0.4603	0.6190	0.7778	0.7937	0.7619	0.7143	0.7619	0.8095	0.6984	0.7619
2	0.2113	--	0.3968	0.6190	0.7143	0.7619	0.7302	0.7778	0.7302	0.8095	0.7937	0.8254
3	0.7758	0.9243	--	0.3968	0.5556	0.5079	0.5079	0.4603	0.5079	0.5238	0.3810	0.4444
4	0.4796	0.4796	0.9243	--	0.6825	0.6667	0.6032	0.6508	0.7619	0.7460	0.6032	0.6984
5	0.2513	0.3365	0.5878	0.3819	--	0.9206	0.7619	0.8413	0.8254	0.8413	0.7619	0.7302
6	0.2311	0.2719	0.6774	0.4055	0.0827	--	0.7460	0.8254	0.7778	0.8254	0.7778	0.7778
7	0.2719	0.3145	0.6774	0.5055	0.2719	0.2930	--	0.7619	0.6825	0.7302	0.6508	0.7143
8	0.3365	0.2513	0.7758	0.4296	0.1728	0.1919	0.2719	--	0.7937	0.8095	0.8254	0.8254
9	0.2719	0.3145	0.6774	0.2719	0.1919	0.2513	0.3819	0.2311	--	0.8889	0.7143	0.8095
10	0.2113	0.2113	0.6466	0.2930	0.1728	0.1919	0.3145	0.2113	0.1178	--	0.6984	0.7937
11	0.3589	0.2311	0.9651	0.5055	0.2719	0.2513	0.4296	0.1919	0.3365	0.3589	--	0.8095
12	0.2719	0.1919	0.8109	0.3589	0.3145	0.2513	0.3365	0.1919	0.2113	0.2311	0.2113	--

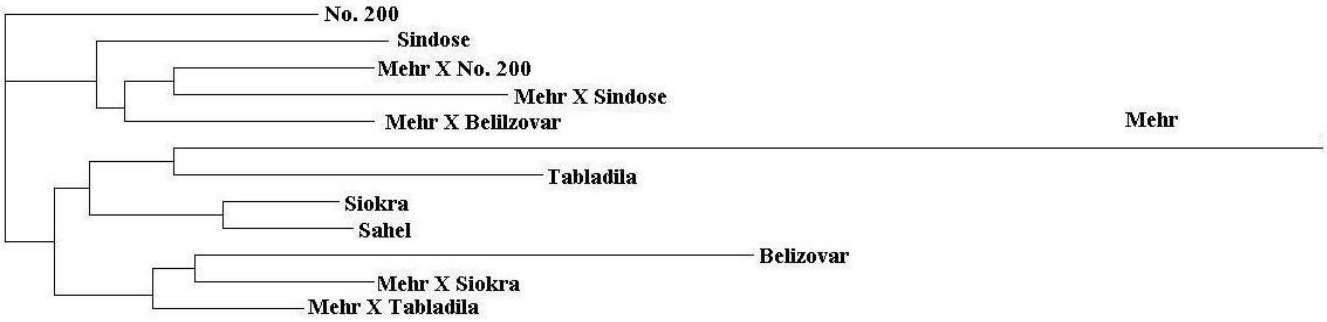

Figure 3. NJ tree of ISSR data.

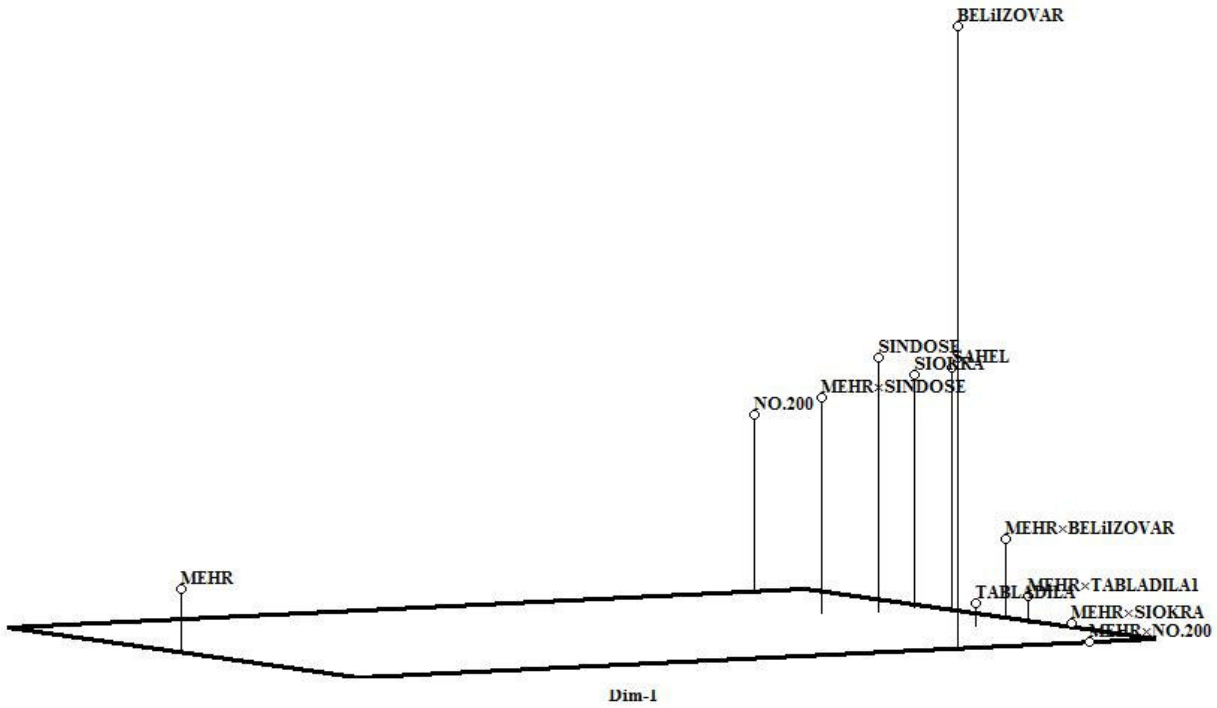

Figure 4. PCoA plot of ISSR data.

genotypes with the help of RAPD markers identifying a higher degree of genetic diversity in diploid genotypes compared to tetraploid cultivars.

Dendrograms obtained in both RAPD and ISSR markers are partly in agreement, indicating the genetic distinctness of the cotton genotypes, particularly Mehr and Belilzovar parental cultivars. Furthermore, the hybrids obtained from different crosses showed different molecular affinities not necessarily close to their parental genotypes, possibly due to further molecular changes occurring in them. This is also supported by the observations that some novel RAPD and ISSR bands occurred in the hybrid plants that were absent in their parental genotypes. Since even single base change at the primer annealing site is manifested as appearance or disappearance of RAPD and ISSR bands, these bands may indicate the occurrence of genetic changes in the genome of the progenies either through the loss or rearrangement of some of their nucleotides. Chromosomal crossing over during meiosis may result in loss of primer attachment pair sites in the offspring leading to novel RAPD pattern in the offspring (Smith et al., 1996).

Sushir et al. (2008) carried out cytogenetic and RAPD analysis of F1 and F2 progenies of the interspecific cross between *Gossypium arboreum* X *G. anomalum* and reported that among nine F2 segregates, F2-1 progeny plants showed one additional band than F1, and F2-5 progeny plants showed the recombination event. On the contrary, in plants F2-6 and F2-8 loss of priming sites happened showing that recombination between A and B genomes of *G. arboreum* and *G. anomalum,* respectively is possible (Sushir et al., 2008). Tafvizei et al. (2010) also used RAPD markers to analyze different cotton genotypes and reported the occurrence of novel RAPD bands in the hybrids not observed in their parents and also reported the absence of RAPD bands in the hybrids which were present in their parents. This phenomenon is not confined to cotton and occurs in other plant species too. For example, Wang et al. (2004) reported the presence of some RAPD bands in the parental genotypes of *Fagopyrum* that were not observed in their hybrid, and also noticed the appearance of some novel RAPD bands in the hybrids which were not present in the parental genotypes.

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