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ISSR, RAPD and agronomic study in some F1 and F2 cotton genotypes

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ABSTRACT Cotton is an important economic crop plant with diploid and tetraploid cultivars. Hybridization is one of the main breeding strategies in cotton breeding producing new genotypes and also increasing the genetic diversity in cotton germplasm available. The present study considers agronomic and molecular study of genetic variations in thirteen F1 and F2 cotton genotypes (*Gossypium hirsutum*) obtained by crossing the cotton cultivars Bellizovar, No. 200, Siokra, Sindose and Tabladilla. Out of 30 RAPD primers used 19 primers produced 191 reproducible bands/loci out of which 63 bands were polymorph. Eight ISSR primers used produced 86 reproducible bands, out of which 27 bands were polymorph and 59 bands were monomorph. Some bands were present in the F1 progenies but absent in the F2 progenies of the same genotype. The mean values of gene diversity (H) and Shannon's Information Indices (I) for ISSR markers in the F1 progenies were 0.27 and 0.40 respectively, while the same values in F2 progenies were 0.18 and 0.26 respectively. Similarly the mean values of H and I of the F1 progenies for RAPD markers were 0.07 and 0.11 respectively, while the same values in F2 progenies were 0.09 and 0.13 respectively. UPGMA and NJ dendrograms grouped the F1 and F2 progenies of Siokra X Bellizovar together, standing from the other genotypes due to their genetic differences. The use of present finding in planning future hybridization is discussed.

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KEY WORDS

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Hybridization is one of the main breeding strategies in cotton breeding producing new genotypes and also increasing the genetic diversity in cotton germplasm available. The genetic variations induced by hybridization may lead to the formation of unique gene combinations necessary for new superior cultivars. This is important when we consider the fact that the extensive cultivation of closely-related cultivars by producers could result in vulnerability to pests and diseases due to loss of genetic diversity which brings about the genetic erosion (Sheidai et al. 2008; Van Esbroeck and Bowman 1998).

Cotton is an important economic and fiber crop, grown in 70 countries in the world including Iran. Both diploid (*Gossypium herbaceum*) and tetraploid (*G. hirsutum*) cotton cultivars are cultivated in the country. Tetraploid cotton (*Gossypium hirsutum*) has genome constitution of AADD (2n = 52) (Menzel and Brown 1978) and is one of the world dominating cotton cultivars.

Different molecular markers including RAPD (Random Amplified polymorphic DNA) as well as ISSR (Intersequence Simple Repeats) have been used for studying genetic diversity, hybridization and the occurrence of somaclonal variation in cotton (Wajahatollah and Stewart 1997; Kumar et al. 2003;

Vafaie-Tabar et al. 2003; Mehetre et al. 2004; Dongre 2007; Rana et al. 2007; Preetha and Raveendren 2008; Sheidai et al. 2008; Wei et al. 2008; Tafvizei et al. 2010). The present report considers molecular diversity of F1 and F2 progenies of five cotton cultivars not studied before by us.

Materials and Methods

Plant materials

For homogeneity of parent genotypes' seeds, selfing crosses have been carried out for two continues generations/years before future studies. Thirteen F1 and F2 cotton genotypes (*Gossypium hirsutum*) obtained by crossing the cotton parental genotypes Bellizovar, No. 200, Siokra, Sindose and Tabladilla, 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 3 replications.

Total genomic DNA was extracted from fresh leaves using the CTAB method by Murry and Tompson (1980) with the modification described by De la Rosa et al. (2002).

RAPD analysis

Thirty decamer RAPD primers of Operon technology (Alameda, Canada) belonging to OPA, OPB, OPC, OPH, OPI and

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OPM sets were used in this study. The PCR reaction mixture consisted of 20 ng template DNA, 1 x PCR buffer (10 mM Tris-HCL pH 8.8, 250 mM KCL), 200 µM dNTPs, 0.80 µM 10- base random primers and 1 unit of *Taq* polymerase, in a total volume of 25 µl. DNA amplification was performed on a palm cyclor GP-001 (Corbet, Australia). Template DNA was initially denatured at 92°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92°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 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH 8.0) or 6% polyacrylamide gels. 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

Eight ISSR primers used are UBC807, UBC810, UBC811, UBC823, UBC832, UBC834, UBC849 and (CA)9GT commercialized by UBC (the University of British Columbia). 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; 0.2 µM of a single primer; 20 ng genomic DNA and 3 unit of *Taq* DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techno thermocycler (Germany) with following program: 5 min initial denaturation step 94°C, 30 s at 94°C; 1 min at 50°C, 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 base pairs (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). Jaccard similarity as well as Nei,s genetic distance (Nei 1973) was determined among the cultivars studied and used for grouping of the genotypes by UPGMA (Unweighted Paired Group with Arithmetic Average) and NJ (Neighbor Joining) clustering methods and ordination based on principal coordinate analysis (PCO) (Podani 2000; Weising et al. 2005). The fit of dendrograms obtained were checked by bootstrapping using 100 replications. Bayesian clustering was also performed on RAPD and ISSR data by using Markov chain Monte Carlo (MCMC) method (Hall 2001; Weising et

al. 2005). NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) were used for clustering and PCO analyses. Bayesian clustering was performed by MrBayes ver. 3.1 (2005).

In total 8 agronomic characters (Table 1) were studied which were subjected to the analysis of variance (ANOVA) followed by the Least Significant Test (LSD) to show significant difference among the cotton genotypes.

Results

RAPD analysis

Out of 30 RAPD primers used, 19 primers produced reproducible bands. In total 191 RAPD bands (loci) were obtained out of which 63 bands were polymorph while 128 bands were common in the cotton genotypes studied. Among the primers used OPI-12 and OPA-05 produced the highest number of bands (17 & 16 respectively), while primers OPA-11 produced the lowest number of bands (2). The Primers OPM-19 and OPH-07 produced the highest number of polymorphic bands (8 and 7 respectively). The primers OPI-18, OPA-09, OPH-07, OPC-12, OPA-05, OPA-13, OPM-19 and OPC-08 produced 1 unique band while, the other primers produced no unique band at all.

Some of the cultivars showed the presence of specific bands, for example band No. 5 (1250 bp) of the primer OPA-09 was specific for the F1 progeny of No. 200 X Belilzovar, band No. 1 (3000 bp) of the primer OPM-19 was specific for the F2 progeny of Sindose X Belilzovar, band No. 5 of the primer OPI-18 (900 bp) was specific for the F1 progeny of Siokra X Tabladilla, while band No. 1 of the primer OPA-05 (2300 bp) occurred only in the F1 progeny of No. 200 X Belilzovar.

Some bands were present only in two genotypes, for example band No. (1600 bp) of the primer OPM-10 occurred in the F1 and F2 progenies of No. 200 X Belilzovar, band No. 2 (750 bp) of the primer OPI-16 occurred in F2 progeny of Sindosa X Belilzovar and F1 progeny Tabladilla X Belilzovar, while bands No. 1 and 2 (750 and 1000 bp) of the primer OPC-09 occurred in the F1 progeny Tabladilla X Belilzovar and F2 progeny of No. 200 X Tabladilla.

Some bands were present in all the genotypes except one, for example bands N0. 6 (1200 bp) of the primer OPA-09 was only absent in the F1 progenies of Siokra X Tabladilla and bands No. 2, 3 and 4 (2500, 2100 and 2000 bp respectively) of the primer OPM19 were absent only in the F2 progenies of No. 200 X Tabladilla.

Some RAPD bands were present in the F1 progenies but absent in the F2 progenies of the same genotype. For example bands No. 1 and 2 (1800 and 1400 bp respectively) of the primer OPI-16 and bands No. 7 and 13 (1000 and 400 bp respectively) of the primer OPA-11 occurred in the F1 progenies of Siokra X Belilzovar but were absent in the F2 progenies. On the other hand band No. 2 (1700 bp) of the primer OPC-O4 was absent in the F1 progenies of Siokra X

Table 1. Agronomic characters in cotton genotypes studied.

		Pod weight gram	Pod No.	Yield Kg/hectare	Kill%	Kill length mm	Microner mg/inch	Strength g/tex	Elongation%
N x Bel F2	Mean	138.10	14.93	3016.66	39.34	26.60	5.03	30.53	6.30
	N	3	3	3	3	3	3	3	3
	Std. Deviation	22.70	4.98	500.53	1.23	1.20	0.28	1.06	0.10
N x SK F2	Mean	136.46	12.20	2886.66	41.37	30.10	4.83	30.33	6.50
	N	3	3	3	3	3	3	3	3
	Std. Deviation	5.02	1.56	735.27	2.97	1.58	0.30	0.90	0.20
N x Tab F2	Mean	150.10	14.10	3103.33	39.26	29.36	4.56	31.60	6.60
	N	3	3	3	3	3	3	3	3
	Std. Deviation	10.82	5.40	746.48	3.04	1.33	0.25	1.22	0.10
Sin x Bel F2	Mean	128.96	14.46	3220.00	42.82	27.90	4.96	29.33	6.40
	N	3	3	3	3	3	3	3	3
	Std. Deviation	9.83	1.13	888.43	3.62	0.45	0.25	2.60	0.00
SK x Bel F2	Mean	131.26	13.80	2631.66	41.79	27.60	5.03	28.76	6.20
	N	3	3	3	3	3	3	3	3
	Std. Deviation	5.78	2.27	210.73	2.89	2.26	0.23	0.72	0.10
SK x Tab F2	Mean	146.96	14.00	3106.66	40.46	30.30	4.96	29.80	6.50
	N	3	3	3	3	3	3	3	3
	Std. Deviation	9.30	3.90	1188.87	3.38	2.71	0.11	0.98	0.01
Tab x Bel F2	Mean	150.26	13.06	3520.00	39.60	29.36	4.86	29.60	6.66
	N	3	3	3	3	3	3	3	3
	Std. Deviation	71.13	0.61	633.79	2.97	2.34	0.11	2.19	0.56
N x Bel F1	Mean	144.53	13.40	2780.00	38.69	25.53	5.00	27.43	6.13
	N	3	3	3	3	3	3	3	3
	Std. Deviation	10.55	2.47	985.95	1.32	1.13	0.20	1.96	0.25
N x SK F1	Mean	148.10	11.96	2900.00	39.82	27.53	5.030	30.83	6.43
	N	3	3	3	3	3	3	3	3
	Std. Deviation	6.33	3.39	807.27	2.13	5.77	0.15	0.30	0.23
N x Tab F1	Mean	141.43	13.20	2523.33	39.95	28.53	5.02	29.83	6.36
	N	3	3	3	3	3	3	3	3
	Std. Deviation	14.3	2.30	614.35	1.82	1.06	0.17	0.83	0.15
Sin x Bel F1	Mean	125.26	18.10	2793.33	42.40	28.30	4.93	29.96	6.33
	N	3	3	3	3	3	3	3	3
	Std. Deviation	13.78	2.15	780.14	2.69	2.26	0.005	1.50	0.20
SK x Bel F1	Mean	134.33	13.63	2483.33	39.41	26.10	4.86	25.83	5.93
	N	3	3	3	3	3	3	3	3
	Std. Deviation	13.87	2.08	431.08	0.53	1.05	0.25	1.90	0.28
SK x Tab F1	Mean	146.23	12.63	2970.0	40.200	26.86	4.96	28.13	6.13
	N	3	3	3	3	3	3	3	3
	Std. Deviation	10.11	3.35	431.08	2.77	1.56	0.15	2.30	0.25
Tab x Bel F1	Mean	142.10	14.30	2970.00	40.22	26.23	5.00	28.83	6.10
	N	3	3	3	3	3	3	3	3
	Std. Deviation	4.03	3.70	648.61	1.30	2.19	0.17	3.11	0.55
Total	Mean	140.29	13.84	2911.30	40.38	27.88	4.94	29.34	6.32
	N	42	42	42	42	42	42	42	42
	Std. Deviation	12.21	2.95	690.36	2.40	2.03	0.22	2.03	0.30

Abbreviations: N = No. 200 Bel = Belilzovar, SK = Siokra, Tab = Tabladila and Sin = Sindose.

Belilzovar but was present in the F2 progenies.

In Sindose X Belilzovar, band No. 1 (3000 bp) of the RAPD primer OPM-19 and band No. 4 and 17 (1500 and 300 bp respectively) of the primer OPA-05 occurred in the F1 progenies but was absent in the F2 progenies while, bands No. 3 (750 bp) of the RAPD primer OPI-16, band No. 8 (250 bp) of the primer OPB-12 and bands No. 8 and 9 (1400 and 750 bp respectively) of the primer OPC-12 were absent in the

F1 progenies but occurred in the F2 progenies.

The mean values of gene diversity (H) and Shannon's Information Indices (I) of the F1 progenies for RAPD markers were 0.07 and 0.11 respectively, while the same values in F2 progenies were 0.09 and 0.13 respectively. These indices determined in the crosses in which Siokra genotype was the pollen parent were 0.08 and 0.11, in the crosses in which No. 200 genotype was the pollen parent were 0.08 and 0.12, in the

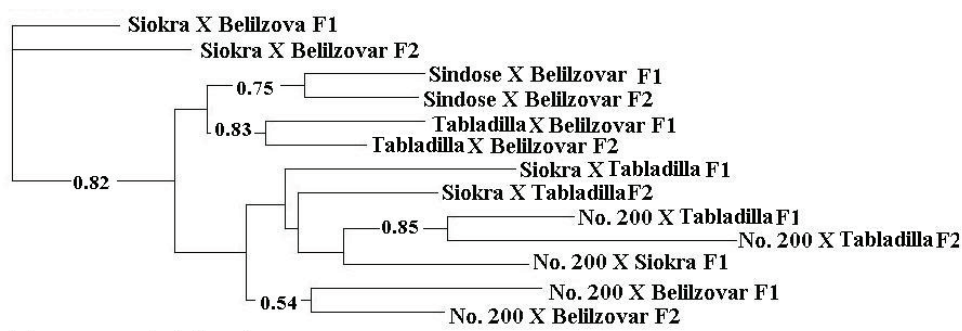


Figure 1. NJ tree of RAPD data. (numbers on the tree branches are bootstrap values).

crosses in which Sindose genotype was the pollen parent were 0.02 and 0.04, and in the crosses in which Tabladilla genotype was the pollen parent were 0.02 and 0.03 respectively.

The genetic diversity indices determined for RAPD markers in the crosses in which Belizovar was the female parent showed almost similar values of H and I in all crosses, *i.e.* Siokra X Belizovar (H=0.08, I=0.12), Sindose X Belizovar (H=0.07, I=0.09), Tabladilla X Belizovar (H=0.07, I=0.11) and No. 200 X Belizovar (H=0.11, I=0.15) compared to that of other crosses.

Similarly in the crosses in which Tabladilla was the female *i.e.* Siokra X Tabladilla, H value was 0.12 and I=0.16 while, in No. 200 X Tabladilla H value was 0.12 and I=0.17.

UPGMA and NJ dendrograms as well as Bayesian clustering of RAPD data produced similar results supported by PCO ordination plot (Fig. 1). The Cophenetic correlation of NJ tree was higher ($r=0.98$) and showed almost good bootstrap values, therefore it is discussed below.

In general 3 major clusters were obtained. The F1 and F2 progenies of Siokra X Belizovar formed the first major cluster standing far from the other genotypes. The second major cluster is comprised of two subclusters in which, F1 and F2 progenies of Sindose X Belizovar formed the first subcluster, while F1 and F2 progenies of Tabladilla X Belizovar formed the second subcluster.

The second major cluster is comprised of 3 subclusters, F1 and F2 progenies of Siokra X Tabladilla formed the first subcluster, while F1 and F2 progenies of No. 200 X Tabladilla formed the second subcluster and F1 and F2 progenies of No. 200 X Belizovar formed the third subcluster.

The branch lengths of NJ tree based on RAPD markers were longer for the F1 progenies compared to that of F2 progenies in Tabladilla X Belizovar, Siokra X Tabladilla and No. 200 X Belizovar.

ISSR analysis

The eight ISSR primers used in this experiment produced 86 reproducible bands, out of which 27 bands were polymorph and 59 bands were monomorph. The highest number of

polymorphic bands was obtained for the primer UBC849 while ISSR primers UBC807 and UBC823 produced only 1 polymorphic band. The ISSR primer UBC832 was the only one primer producing a single specific band.

The ISSR band No. 4 (1900 bp) of the primer (CA)7GT occurred only in two genotypes of F2 progeny of Siokra X Belizovar and F1 progeny of Siokra X Tabladilla, while the ISSR bands No. 6 and 8 (800 and 600 bp respectively) of the primer UBC832 occurred only in the F1 progeny of No. 200 X Siokra.

The ISSR band No. 7 (1500 bp) of the primer (CA)7GT and band No. 5 (800 bp) of the primer UBC811 occurred in all the genotypes except in F2 progeny Siokra X Tabladilla and No. 200 X Tabladilla.

Some ISSR bands were present in the F1 progenies but absent in the F2 progenies of the same genotype, for example bands No. 3 and 5 (1000 and 800 bp respectively) of the ISSR primer UBC811 and bands No. 1 and 2 (200 and 900 bp respectively) of the primer UBC834 occurred in the F1 progenies of Siokra X Belizovar but were absent in the F2 progenies of this cross. On the other hand, band No. 3 (1500 bp) of the ISSR primer UBC807, band No. 1 (1500 bp) of the primer UBC832 and bands No. 4 and 13 (1900 and 1000 bp respectively) of the primer (CA)7GT were absent in the F1 progenies of Siokra X Belizovar but were present in the F2 progenies of this cross.

Similarly in Sindose X Belizovar band No. 3 (1500 bp) of the ISSR UBC832 and band No. 3 (1500 bp) of the primer UBC807 occurred in the F1 progenies but were absent in the F2 progenies. Band No. 1 (1600 bp) of the primer UBC810 occurred in the F2 progenies but was absent in the F1 progenies. In Tabladilla X Belizovar also bands No. 6 (1200 bp) of the ISSR primer UBC842, band No. 3 (1300 bp) of the primer UBC832 occurred in the F1 progenies but were absent in the F2 progenies, while band No. 3 (1500 bp) of the ISSR UBC807 was only present in F2 progenies of the same genotype.

The mean values of gene diversity (H) and Shannon's Information Indices (I) for ISSR markers in the F1 progenies

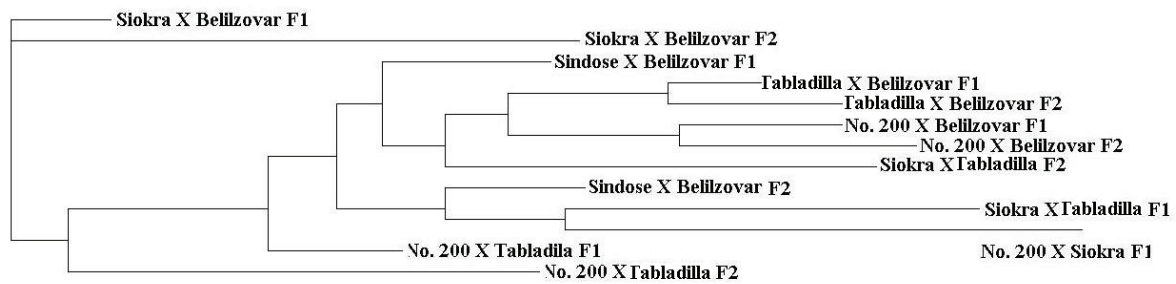


Figure 2. NJ tree of ISSR data.

were 0.27 and 0.40 respectively, while the same values in F2 progenies were 0.18 and 0.26 respectively.

These genetic diversity indices determined in the crosses in which Siokra genotype was the pollen parent were 0.22 and 0.32 respectively, in the crosses in which No. 200 genotype was the pollen parent were 0.23 and 0.34, in the crosses in which Sindose genotype was the pollen parent were 0.06 and 0.09, and in the crosses in which Tabladilla genotype was the pollen parent were 0.07 and 0.09 respectively.

These genetic diversity indices determined for ISSR markers in the crosses in which Belizovar was the female parent showed very low and almost similar values of H and I in all crosses i.e. Siokra X Belizovar (H=0.04, I=0.05), Sindose X Belizovar (H=0.03, I=0.04), Tabladilla X Belizovar (H=0.02, I=0.03) and No. 200 X Belizovar (H=0.02, I=0.03).

Similarly in the crosses in which Tabladilla was the female parent, i.e. Siokra X Tabladilla the H value was 0.04 and I = 0.06 while, in No. 200 X Tabladilla, H value was 0.03 and I = 0.04.

UPGMA and NJ dendrograms as well as Bayesian clustering of ISSR data produced similar results supported by PCO ordination plot. The Cophenetic correlation of NJ tree was higher ($r=0.96$) and showed about 65% bootstrap values, therefore it is discussed below (Fig. 2).

In general 3 major clusters were obtained. The F1 and F2 progenies of Siokra X Belizovar formed the first major cluster standing far from the other genotypes, supporting RAPD tree result. The second major cluster is comprised of F1 and F2 progenies of Tabladilla X Belizovar, F1 and F2 progenies of No. 200 X Belizovar and F1 and F2 progenies of Sindose X Belizovar as well as Siokra X Belizovar. The third cluster is formed by F1 and F2 progenies of No. 200 X Tabladilla, supporting RAPD tree result.

The branch lengths of NJ tree based on ISSR markers were longer for the F2 progenies compared to that of F1 progenies in Siokra X Belizovar, Tabladilla X Belizovar, No. 200 X Belizovar and No. 200 X Tabladilla.

Agronomic data

The ANOVA followed by LSD test showed significant difference for most of the agronomic characters studied, for

example, pod weight differed significantly ($P<0.05$, Table 1) between F2 progenies of No. 200 X Tabladilla and F1 and F2 progenies of Sindose X Belizovar, it also differed between F2 progenies of Tabladilla X Belizovar and F1 and F2 progenies of Sindose X Belizovar. Similarly the mean No. of pods differed significantly ($P<0.05$) between the F1 progenies of Sindose X Belizovar and F1 and F2 progenies of No. 200 X Siokra, and also with F1 plants of Siokra X Tabladilla. The mean kill length differed significantly ($P<0.05$) between the F2 progenies of No. 200 X Belizovar and F2 progenies of No. 200 X Siokra and also with the F2 progenies of Siokra X Tabladilla.

UPGMA dendrogram of agronomic data showed the highest value of Cophenetic correlation ($r=0.98$) and is discussed here. In general 4 major clusters are formed. In the first cluster the F2 progeny of the Sindose X Belizovar stands alone far from the other genotypes. This genotype showed the highest values of pod weight and number, yield, percentage of kill and strength compared to those of other genotypes (Table 1).

In the second major cluster, the F1 plants of Siokra X Belizovar, Sindose X Belizovar and No. 200 X Belizovar as well as F2 progeny of Siokra X Belizovar join each other due to similarity in micronaire and elongation percentage. The first two genotypes show more similarity and join each other with a lesser distance compared to the other two genotypes of this cluster.

The F2 progenies of No. 200 X Tabladilla and Siokra X Tabladilla also show similarity in the pod No., yield, micronaire and elongation percentage forming the third major cluster. In the fourth major cluster, three genotypes i.e. F1 and F2 plants of Tabladilla X Belizovar and F1 plants of Siokra X Tabladilla show more similarity and join each other, the F1 plants of No. 200 X Siokra and No. 200 X Tabladilla show join each other, while the F2 progeny of No. 200 X Belizovar joins the other genotypes in this cluster with some distance. The members of this cluster show similarity in kill %, micronaire and elongation percentage.

The grouping obtained by agronomic tree partly agrees with RAPD and ISSR trees. For example, F1 and F2 progenies of Siokra X Belizovar show close affinity in RAPD and

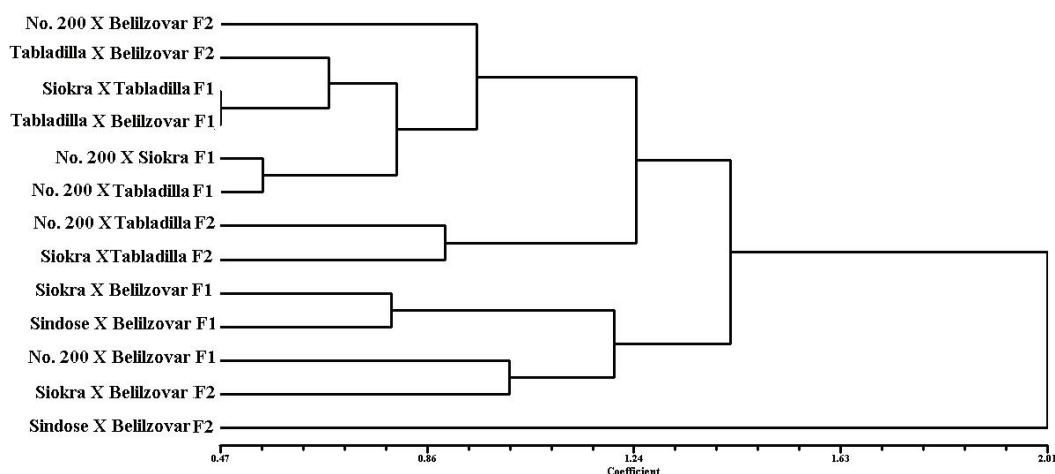


Figure 3. UPGMA tree of agronomy data.

ISSR tree and also join each other with some distance in the agronomic tree. The same is true for F1 and F2 progenies of Tabladilla X Bellizovar and No. 200 X Tabladilla as well as Siokra X Tabladilla genotypes.

Discussion

The presence of RAPD and ISSR polymorphic bands in F1 and F2 progenies of the cotton cultivars studied indicates the presence of genetic polymorphism in these genotypes which may be used in planning hybridization in cotton. 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.

The number of RAPD and ISSR bands and degree of polymorphism obtained in F1 and F2 progenies of cotton cultivars studied is almost in agreement with the other studies performed in cotton. For example, Wei et al. (2008) used ISSR markers to study genetic diversity in 48 cotton accessions including *G. barbadense* and *G. hirsutum*, obtaining 92 ISSR bands out of which 72 bands were polymorph. UPGMA tree of ISSR data separated different genotypes of cotton collected from different provinces in China. Similarly Rana and Bhat (2005) studied genetic diversity among different Indian diploid and tetraploid cotton cultivars by RAPD markers and showed that diploid cultivars show greater genetic diversity than tetraploid cultivars. 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. Other studies on tetraploid cotton cultivars outside India reported similar ranges of average genetic similarity (Rana and Bhat 2005). The average genetic similarity obtained in the present study for ISSR markers is 80% while the same value for RAPD markers is 85% indicating the presence of greater genetic similarities among

the cotton genotypes studied, indicating the presence of a narrower genetic diversity in these cultivars which should be considered for future hybridization program. We may use more distant genotypes for future hybridization to improve the degree of genetic polymorphism available in Iran. The relatively low values of gene diversity (H) and Shannon's Information Indices (I) in RAPD and ISSR markers in both F1 and F2 progenies also support the above said conclusion. However, the genetic diversity values obtained are much higher in the crosses with Siokra and No. 200 genotypes as the pollen parents and are very low in the crosses in which the Bellizovar and Tabladilla genotypes are the female parents. Therefore these points should also be considered in the future hybridization program. Chen et al. (2004) also reported the effects of reciprocal differences in morphological and RAPD characters in interspecific hybridization in *Cucumis*.

A longer branch length of NJ tree based on molecular data indicates the occurrence of a higher degree of molecular changes in these genotypes, which seems in some cases it occurs in F1 plants but surprisingly in some cases it occurs in the F2 plants. We usually expect a higher degree of genetic variation in F1 progenies compared to that of F2 plants, but in case of RAPD markers a higher degree of molecular changes occur in F2 plants of Tabladilla X Bellizovar, Siokra X Tabladilla and No. 200 X Bellizovar and in the case of ISSR markers, it happened for F2 plants of Siokra X Bellizovar, Tabladilla X Bellizovar, No. 200 X Bellizovar and No. 200 X Tabladilla. This may happen due to frequent genetic recombination, molecular insertion/deletion, etc. in the F2 progenies. As we can see the crosses showing this phenomenon in both RAPD and ISSR sequences are mainly Tabladilla X Bellizovar and No. 200 X Bellizovar, therefore we may consider these crosses as more vulnerable to molecular changes.

Dendrograms obtained in both RAPD and ISSR markers almost are in agreement, indicating the genetic distinctness

of the F1 and F2 progenies of each hybrid genotype as they each form a separate cluster and also show that the F1 and F2 progenies of Siokra X Belizovar differ greatly from the other genotypes in both RAPD and ISSR loci. These are also partly supported by the agronomic tree obtained. It is expected to see some difference between agronomic/ morphological dendrogram and that of molecular data as agronomic/ morphological characteristics are mainly of polygenetic nature affected greatly by environmental factors (Preetha and Raveendren 2008).

As stated before there were bands which occurred in the F2 progenies but were absent in the F1 progenies of the genotypes studied. 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 plant 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). Similarly Tafvizei et al. (2010) reported the occurrence of RAPD band in the hybrids not observed in their parents and also absence of RAPD bands in the hybrids which were present in their parents in cotton hybrids. Wang et al. (2004) also reported the occurrence of RAPD bands in the parental genotypes of *Fagopyrum* which were not observed in the hybrid obtained. They also observed the appearance of some RAPD bands absent in the hybrids which were not present in the parental genotypes due to genetic rearrangements. Therefore the present study reveals genetic differences of the cotton genotypes obtained by hybridization and also identify some of the parental genotypes which can be used as pollen parents in the further hybridization program and cotton breeding.

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