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Research article

STRUCTURE ANALYSIS OF GENETIC DIVERSITY IN TETRAPLOID AND DIPLOID COTTON GENOTYPES

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ABSTRACT: Diploid and tetraploid Cotton cultivars are cultivated in Iran and are considered as an important fiber plant of the country. Due to long time cultivation and selection for specific traits, decrease in genetic diversity occurs and we need to improve genetic diversity of the germplasm available by hybridization among diverse cultivars. Therefore, the present study considers assessment of genetic diversity of some inter-specific cotton hybrids and their parental genotypes in *G. hirsutum* and *G. barbadense* as well as *G. herbaceum* and *G. arboreum* by using thirty homo and hetero ISSR markers. Genetic diversity parameters were determined among the hybrids. AMOVA test showed significant genetic difference among the genotypes and STRUCTURE analysis revealed allele admixture among cultivars. Network analysis of cotton genotypes showed the occurrence of gene exchange/allelic similarity between the genotypes. Some common alleles also were observed between diploid and tetraploid genotypes. Q matrix constructed by STRUCTURE program showed close affinity between diploid genotypes supporting NJ tree. These results indicate that although due to continuous selection of Iranian cotton cultivars we may have narrowed the available cotton gene pool we still find good genetic diversity within both diploid and tetraploid cultivars. A higher level of genetic diversity is present in both inter and intraspecific hybrids.
Key word: Cotton, genetic variation, ISSR, network analysis

INTRODUCTION

Cotton is one of the most important crop plants in the world cultivated in different regions of Iran. The genus *Gossypium* contains about 45 diploid and 5 allotetraploid species [1] of which, four species are cultivated viz. *G. hirsutum* L. [n=2x=26, (AD)1], *G. barbadense* L. [n=2x=26, (AD)2], *G. herbaceum* L. (n=x=13, A1), and. *G. arboreum* L. (n=x=13, A2). Two species of *G. hirsutum* and *G. barbadense* are New World allotetraploid species and dominate worldwide cotton production [2].

G. barbadense is originated in Peru and Ecuador, from where it diffused to eastern South America, the Caribbean, and finally to Central America [3]. *Gossypium barbadense* provides about 9% of world cotton production, and was originally cultivated in the coastal islands and lowlands of the United States, where it became known as Sea Island cotton. Sea Island cottons and likely other forms of *G. barbadense* were then introduced into the Nile Valley of Egypt to produce long-staple fine fibers and known as Egyptian cotton [4] and further developed in regions of the southwestern United States to become 'Pima' Cotton. The center of origin for *G. hirsutum* is Mexico, but diverse forms are spread throughout Central America and the Caribbean [5, 6]. *G. hirsutum* L. and *G. barbadense* L. account for 90% and 8% of the world cotton production respectively.

G. arboreum Asiatic diploid cotton (A- genome) has been successfully cultivated and remains as an important germplasm resource in cotton breeding programs due to its desirable traits for cotton production [7,8]. Another A-genome cotton, *G. herbaceum*, is called Levant cotton a species native to the semiarid regions sub-Saharan Africa and Arabia. It was probably first cultivated in southern Arabia and cultivation spread to Persia, Afghanistan, Turkey, North Africa, Spain, Ukraine, Turkestan and finally to China [9,10]. *G. herbaceum* is considered as landrace cotton with more than 40 ecotypes. These ecotypes along with many other cotton varieties are cultivated in different regions of Iran [11]. The A- genome cottons are a potentially important genetic resource for cotton breeding program [12]. Several changes has occurred in cotton plant due to human selection and continuous cultivation, these changes include an annual plant habit, photoperiod insensitivity, early flowering, larger boll size, yield, and fiber qualities ranging from greater fiber strength, fineness, and length. Continuous selection activities may bring about genetic bottlenecks and loss of genetic diversity in crop plants including cotton [13]. Many investigations showed the presence of limited genetic diversity (18.2%-47.9%) in *G. hirsutum* and *G. barbadense* and even less amount of genetic variation (4.13%-7.9%) among *G. hirsutum* germplasms[13]. Wallace et al [14] discussed anticipated genetic vulnerability for Fusarium wilt Race 4 and the cotton leaf curl virus, and to biotic and abiotic stresses [15], which are associated with increased genetic uniformity. Cotton breeding programs primarily rely on crossing between elite lines of closely related genotypes with high yield and superior fiber qualities and reselection of the existing cultivars [16]. Hybridization is the main breeding strategies in cotton plant with the aim to broaden the genetic diversity available and produce new genotypes [17, 19]. Therefore, making new hybrid combinations and studying the amount of genetic diversity produced is of immediate importance in cotton breeding.

Different molecular markers including RAPD (random amplified polymorphic DNA) and inter- simple sequence repeats (ISSR) are considered as useful molecular markers to study genetic diversity in cotton [20-28, 17, 29-32]. The present study reports ISSR analysis of genetic diversity among inter-specific hybrids and their backcross progenies obtained and try to reveal the genetic structure of these cultivars for the first time. Such information can help us to select the most diverse genotypes for further hybridization and selection.

MATERIAL AND METHODS

Thirty-four cotton genotypes were analyzed by ISSR markers in the present investigation.
These genotypes were intra specific and interspecific hybrids, backcross progenies and F2 plants obtained from hybridization between *Gossypium hirsutum* and *G. barbadense* (tetraploid cotton species) as well as *G. arboretum* and *G. herbaceum* (diploid species) (Table 1).
Since we obtained hybrids by crossing different parental genotypes in order to quantify the level of genetic diversity obtained from these crossing combinations, we identified 7 population groups based on their genetic background as follows: 1- *G. barbadence* parental genotypes including Termez 14 and Barbadens 5595, 2- *G. hirsutum* parental genotypes (No.3 and No.4 genotypes in Table 1) 3- tetraploid interspecific hybrids (*G. barbadence* X *G. hirsutum*) and their F1 and F2 progenies (genotypes No. 5-12 in Table 1), 4 and 5- diploid cotton species (parental genotypes *G. herbaceum* and *G. arboretum*, genotypes No. 13-20 in Table 1), 6- diploid interspecific hybrids (genotypes No. 21-27 in Table 1), 7- diploid species back-cross progenies (genotypes No. 28-34 genotypes in Table 1).

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 Murray & Thompson [33] with modification described by De la Rosa et al [34]. Quality of extracted DNA was examined by running on 0.8% agarose gel.

Inter-Simple Sequence assay

Thirty-five ISSR loci including 20 homo-ISSR as well as fifteen hetero-ISSR (combined) primers were used in this study. UBC807, 810, 811, 823, 834, 849 designed by University of British Colombia, (CA)9GT, (GA)9T, (GA)9A, (GA)9C, (CA)7AT, (CA)7AC, (GT)7TG, (GT)7CA, CAG(GA)7, GCT(GA)7 di-nucleotide repeated motif and (AGC)5GA, (AGC)5GG, (AGC)5GT, (AGC)5GC tri-nucleotide repeated motif were used

Table 1. Thirty four tetraploid and dipoloid cotton genotypes and their hybrids used in this study

Group	Entry	Genotype	Species
1	1	Termez 14 (T14)	*G. barbadens*
	2	Barbadens-5595 (Bar-5595)	*G. barbadens*
2	3	Siokra	*G. hirsutum*
	4	Sahel	*G. hirsutum*
3	5	T14 x Siokra (F1)	*G. barbadence X G. hirsutum*
	6	Bar-5595 x Siokra (F1)	*G. barbadence X G. hirsutum*
	7	Bar-5595 x Sahel (F1)	*G. barbadence X G. hirsutum*
	8	T14 x Sahel (F1)	*G. barbadence X G. hirsutum*
	9	T14 x Siokra (F2)	*G. barbadence X G. hirsutum*
	10	Bar x Sahel (F2)	*G. barbadence X G. hirsutum*
	11	T14 x Sahel (F2)	*G. barbadence X G. hirsutum*
	12	Bar x Siokra (F2)	*G. barbadence X G. hirsutum*
4	13	VTDL(V)	*G. arboreum*
5	14	Sabzevar (Sb)	*G. herbaceum*
	15	Shahreza (Sh)	*G. herbaceum*
	16	Arya	*G. herbaceum*
	17	Bandarabas (Bnd)	*G. herbaceum*
	18	Semnan (Se)	*G. herbaceum*
	19	Kerman (Ker)	*G. herbaceum*
	20	Qom (Q)	*G. herbaceum*
6	21	Semnan X VTDL (Se X V) F5	*G. herbaceum X G. arboreum*
	22	Shahreza X VTDL (RZ x V) F5	*G. herbaceum X G. arboreum*
	23	Bandarabas X VTDL (Bnd x V) F5	*G. herbaceum X G. arboreum*
	24	Arya X VTDL (Arya x V) F5	*G. herbaceum X G. arboreum*
	25	Qom X VTDL (Q x V) F5	*G. herbaceum X G. arboreum*
	26	Kerman X VTDL (Ker x V) F5	*G. herbaceum X G. arboreum*
	27	Sabzevar X VTDL (Sb x V) F5	*G. herbaceum X G. arboreum*
7	28	(Se x V) X Se - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*-second back cross
	29	(Sh x V) X Sh - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross
	30	(Bnd x V) X Bnd - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross
	31	(Arya x V) X Arya - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross
	32	(Q x V) X Q - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross
	33	(Ker x V) X Ker - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross
	34	(Sb x V) X Sb - F5Bc2	*(G. herbaceum X G. arboreum) X herbaceum*- second back cross

PCR reactions were performed for both homo and hetero ISSR loci 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 1 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

ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). The level of intra-population genetic diversity was determined by Nei's gene diversity (H) as well as the Shannon information index (I) [35]. Popgene ver.3.2 and GenAlex ver. 6.0 [36] was used for these analyses.

Several genetic diversity parameters were determined for all loci and also each group of genotypes separately. These parameters include the number of ISSR alleles, the number of unique alleles, expected heterozygosity (H_e) [37], the effective number of alleles (N_e) and Shannon's index. Moreover, Nei genetic distance and Nei genetic identity [38] as well as allele frequency divergence was determined among population groups.

Analysis of Molecular Variance (AMOVA) and the *Fst* pair-wise test (with 10100 permutations) were performed to reveal significant genetic difference between population groups. The population genetic relationship was determined by NJ (Neighbor Joining) tree construction based on Nei and Li genetic distance [39]. For this purpose, ISSR data were used to determine the genetic distance among the populations followed by bootstrapping for 100 times by PhylTools ver. 1.32 [40]. Distance file obtained, was used as input file for Neighbor command of PHYLIP ver. 3.69 [41] to construct NJ tree. The Consensus command of PHYLIP was used to produce bootstrap values for NJ tree. The final tree was observed by TreeVeiw ver. (32) and NJ plot [42]. The populations relationship versus distinctness was test by Principal coordinate analysis plot (PCoA) performed after 999 permutation by GenAlex ver. 6 (2006) and DarWin ver. 5.0 (2009).

Bayesian clustering method as performed in STRUCTURE v. 2.3 [43] was used to elucidate the population's genetic structure. ISSR data were coded as suggested by Falush et al [44] for coding dominant data. The program structure implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The model applied in the analysis assumes the existence of *K* clusters. Applications of this model include demonstrating the presence of population structure, assigning individuals to populations, and identifying migrants and admixed individuals. Individuals in the sample are assigned (probabilistically) to populations, or jointly to two or more populations if their genotypes indicate that they are admixed. We took advantage of an admixture ancestry model under the correlated allele frequency model. The proportional membership of each cluster was estimated for each individual and each population group.

Finally we studied the possible gene exchange of populations by reticulation and networking. For this purpose, the genetic distance determined among populations was used in T-Rex software (Reconstructing and visualizing phylogenetic trees and reticulation networks) [45].

RESULTS

ISSR analysis

Out of thirty-five homo and hetero ISSR primers used, 10 ISSR primers produced bands in both diploid and tetraploid genotypes and their hybrids. In total 163 bands were obtained all of which were polymorphic. UBC810 primer produced the highest number of bands (32). The mean value of effective alleles, Nei's genetic diversity, Shannon index and percentage of polymorphism obtained were 1.233, 0.153, 0.259 and 97.5% respectively. In total 41 specific bands were produced with UBC849 primers showing the highest number of private bands (9).

Some of the genotypes showed presence of specific bands. For example, parental genotype Siokra (*G. hirsutum*) showed a single specific band of the UBC-807 and (GA) 9A primers both having 1400 bp molecular weight. Similarly, Termez14 (*G. barbadense*) had two specific bands with 1200 and 1450 bp molecular weight produced by primer (AGC)5GA and one band produced by GCT(GA)7. The F1 progeny plants of these two parental genotypes (Termrz14 X Siokra), showed the presence of 10 specific bands including ISSR bands with 580 and 680 bp molecular weight from UBC-810, 1480 bp band from UBC-811, 250 bp band from UBC-849, 70 and 800 bp bands from (GA) 9C, 1470 bp band from (CA) 7GT, 750 bp band from (AGC) 5GC and 600 bp band from CAG (GA)7. The F2 progenies of this crossing combination showed the presence of 2 specific bands, 1 band from UBC-849 (200 bp) and 1 band of (AGC) 5GC (400 bp).

Difference in specific bands of different plant generations was also observed among diploid cotton genotypes studied. For instance, parental genotypes VTDL (*G. arboreum*), Arya (*G. herbaceum*) and their hybrid (VTDL X Arya) showed presence of two specific bands of hetero-ISSR primer (GA)9C/(GA)9T (900 and 1000 bps), which were absent in their back cross plants.

Grouping of the genotypes based on Nei's genetic distance by UPGMA and NJ methods produced similar results. NJ tree showed a higher cophenetic correlation value (r = 0.98) and is discussed bellow (Figure 1).

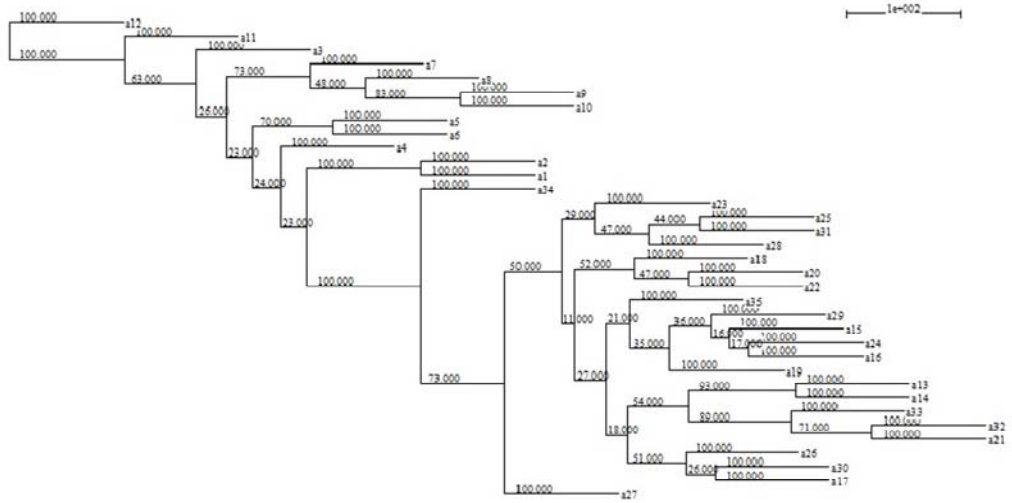

Fig. 1 Neighbor Joining dendrogram based ISSR data. Numbers above the branches are bootstrap values. Genotypes' numbers are according Table 1.

In general 2 major clusters were formed. The first cluster is comprised of tetraploid genotypes including *G. barbadens* and *G. hirsutum* parental genotypes (including genotypes 1-4 in Table 1), and their interspecific hybrids (genotypes 5-12 in Table 1). However, these genotypes show genetic variation among themselves as they are joined each other with some distance and do not form compact clusters. Moreover, some of the branches of NJ tree have low bootstrap values.

Diploid parental genotypes (including *G. arboretum* and *G. herbaceum*) and their interspecific hybrids formed the second major cluster. This cluster also showed different subclusters and indicated genetic difference among the genotypes placed in it. For example, genotypes No. 14-20 (Table 1) are cultivars of *G. herbaceum* but are placed in different subclusters. The same is true for their diploid interspecific hybrids. The genotype No. 27 (Sabzevar X VTDL) and No. 34 (Sb x V) X Sb- Bc2) differed in their genetic content from the other genotypes studied as they stand far from the others with high bootstrap values.

Network analysis (Figure 2) showed the occurrence of gene exchange/allelic similarity between parental genotypes and their progenies. Some common alleles also were observed between diploid and tetraploid genotypes. For instance, allele similarity occurred between Sahel and F1 progeny of T14 X Sahel (Figure 2), while, Barbadence 5595 (tetraploid cotton) showed allele similarity to VTDL genotype (diploid cotton).
Q matrix constructed by STRUCTURE program (Figure 3) showed close affinity between diploid genotypes supporting NJ tree, while tetraploid genotypes were placed far from them. This figure showed common alleles among diploid genotypes while, tetraploid genotypes, differed in their common alleles frequencies. Some specific alleles were observed in tetraploid genotypes (colored black and brown), which were absent in diploid genotypes.

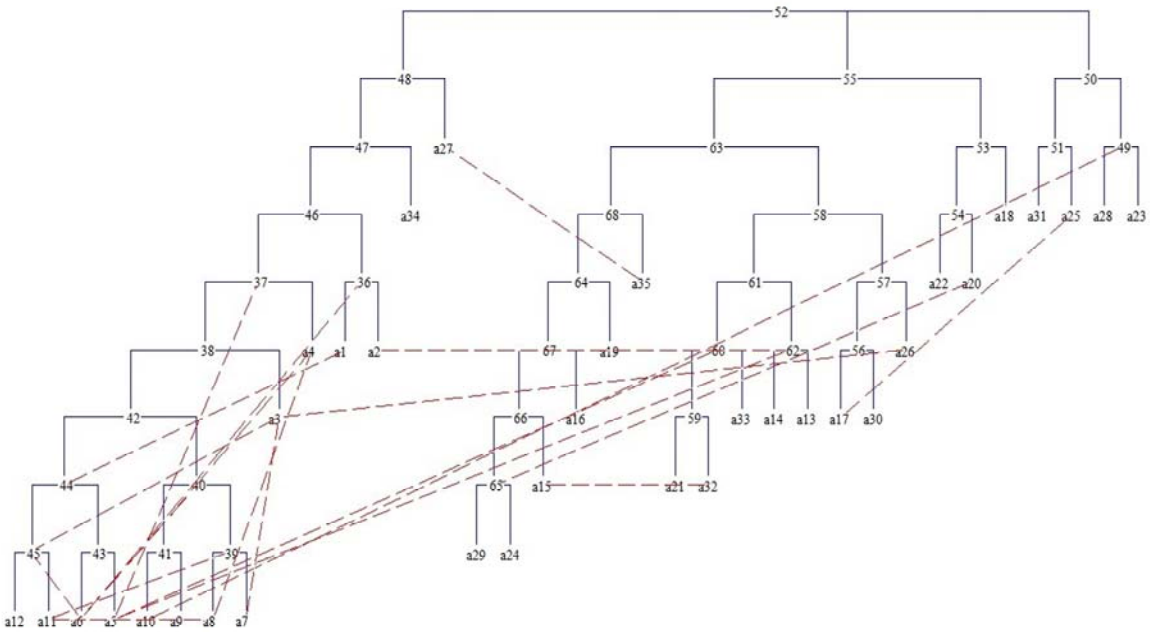

Fig. 2. Network of cotton genotypes. (dashed lines indicate gene exchange events, Genotypes' numbers are according Table 1.

Genetic diversity in population groups

The number of effective alleles, Shannon index, expected heterozygosity and PIC value of population groups are provided in Table 2. The highest number of total bands was observed in population group 3 with 111 bands. The number of effective alleles ranged from 1.00 in *G. herbaceum* parental genotypes to 1.299 in population group 2 (*G. hirsutum* parental genotypes).

Shannon index varied from 0.00 (population group 4) to 0.256 in population group 2. The mean value of polymorphism percentage obtained was 18.93 with the highest percentage occurring in population group 3 (F1 and F2 progenies of tetraploid hybrids, 54.67%). The highest value of expected heterozygosity and PIC occurred in population groups 2 (*G. hirsutum* parental genotypes) 0.175 and 0.440 respectively.

The highest genetic distance (0.349, Table 3) occurred between tetraploid and diploid cotton groups (*G. barbadence* genotypes (group 1) and *G. arboreum* (group 4)) while, the lowest genetic distance (0.007) occurred between backcross of diploid hybrids (group No. 7) and *G. herbaceum* parental genotypes (group No.5).

AMOVA test showed significant difference among the population groups (Table 4) and revealed that 61% of total variation is attributed to among population differences and 39% due to within population variation. Fst pairwise analysis (Table 5) produced more detailed information about population groups genetic difference. For example, significant Fst value was observed between *G. barbadence* x *G. hirsutum* hybrids (population group 3) and population groups 4 (p=0.023), 5 (p=0.003), 6 (p=0.001) and 7 (p=0.001). *G. barbadence* parental genotypes (population group 1) differed significantly from the genotypes included in population groups 5, 6 and 7 (diploid parental genotypes and their interspecific hybrids).

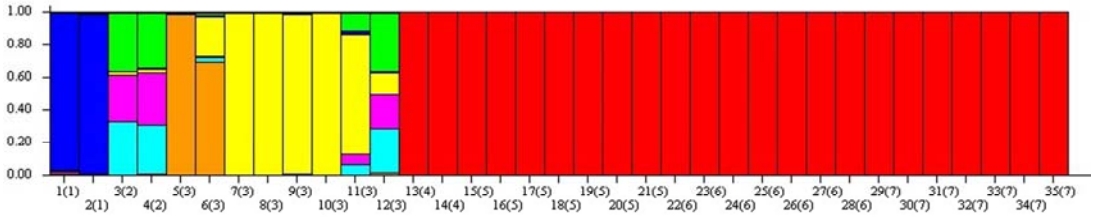

Fig. 3. Q matrix of STRUCTURE program for cotton genotypes studied. Genotypes' numbers are according Table 1.

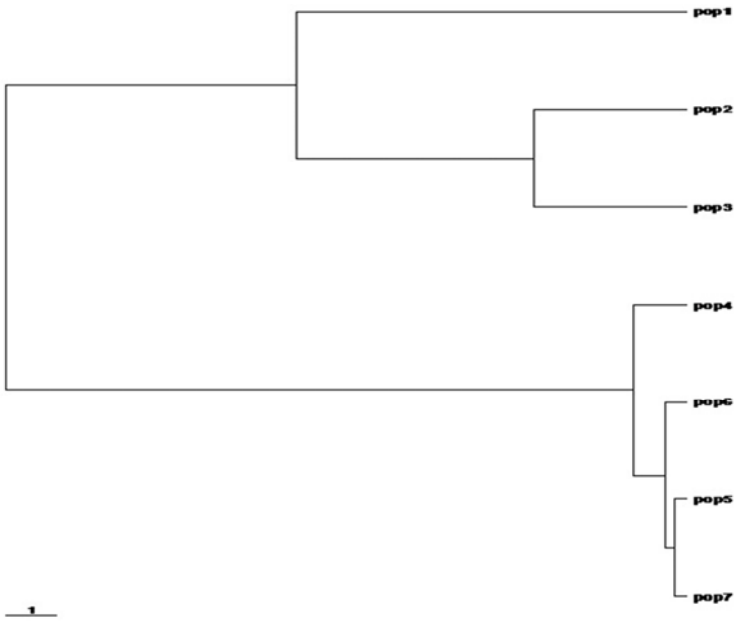

Fig. 4. UPGMA Dendrogram Based Nei's Genetic distance in 7 population groups studied. Population numbers are according Table 1.

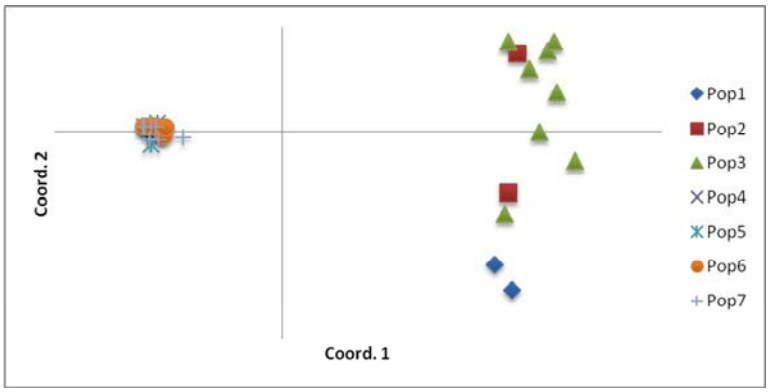

Fig. 5. PCA ordination based on first two components for 7 population groups studied. Population numbers are according Table 1.

UPGMA dendrogram based on Nei's genetic distance distributed 7 population groups in 2 main clusters (Figure 4). *G. barbadence* and G. *hirsutu m* parental genotypes and their F1 and F2 hybrid progenies (population groups 1-3) formed the first main cluster, while *G. arboreum*, *G. herbaceum* parental genotypes and their backcross progenies (population groups 4-7) formed the second main cluster. PCoA analysis with 999 bootstrapping showed that the first 2 PCoA factors comprise about 72% of total variation. Ordination plot based on the first two factors (Figure 5) separated populations groups in 2 distinct groups, very similar to what we obtained in clustering of genotypes as aforementioned. Although, PCoA plot could not differentiate diploid genotypes from each other, it shows differences among tetraploid cultivars as they are placed with some distance from each other. The STRUCTURE plot (Figure 3) showed high allelic similarity among diploid genotypes (population groups 4-7). These genotypes are separated from the others. This plot showed genetic variation among tetraploid genotypes. For example, two genotypes of population group 1 differ almost completely in their allelic composition (colored in blue) from the other tetraploid cultivars and differed from each other in their allele frequency.

Table 2. Genetic parameters obtained in homo and hetero-ISSR primers in 7 group genotypes. Nt: Number of Total bands; Ne: effective alleles; I: Shannon index; H: genetic diversity; PIC: Polymorphic Information Content

Group No.	Nt	Ne	P%	I	He	PIC
1	69	1.130	18.14	0.111	0.076	0.416
2	102	1.299	42.33	0.256	0.175	0.440
3	111	1.265	54.67	0.257	0.165	0.370
4	27	1.000	0.00	0.000	0.000	0.278
5	29	1.034	4.29	0.026	0.018	0.266
6	29	1.035	4.29	0.027	0.019	0.264
7	29	1.048	5.52	0.036	0.025	0.255
Mean	56.57	1.116	18.93	0.102	0.068	0.327

Table 3. Nei genetic distance among population groups. Population numbers are according Table 1

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7
Pop1	0.000						
Pop2	0.216	0.000					
Pop3	0.162	0.103	0.000				
Pop4	0.349	0.294	0.238	0.000			
Pop5	0.345	0.293	0.228	0.019	0.000		
Pop6	0.348	0.291	0.229	0.025	0.011	0.000	
Pop7	0.333	0.280	0.220	0.021	0.007	0.009	0.000

Table 4. AMOVA test among population groups

Source	df	SS	MS	Est. Var.	%
Among Pops	6	362.386	60.398	11.171	61%
Within Pops	28	198.643	7.094	7.094	39%
Total	34	561.029		18.266	100%
Stat	Value	P(rand >= data)			
PhiPT	0.612	0.001			

Table 5. *Fst* pairwise between population groups studied. *Fst* pairwise values in below diagonal and probability values based on 999 permutations are shown above diagonal. Population numbers are according Table 1

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7
Pop1		0.319	0.024	0.334	0.045	0.001	0.021
Pop2	0.175		0.060	0.338	0.057	0.053	0.022
Pop3	0.305	0.155		0.023	0.001	0.001	0.001
Pop4	0.737	0.465	0.501		0.055	0.030	0.079
Pop5	0.886	0.785	0.649	0.519		0.073	0.460
Pop6	0.877	0.777	0.641	0.481	0.105		0.303
Pop7	0.863	0.762	0.636	0.313	0.000	0.046	

The genotypes 3 and 4 differed from most of tetraploid genotypes and also differed from each other in their allele composition. This holds true for 8 genotypes of population group 3 too (tetraploid genotypes). Members of the first 2 populations groups differed in frequency of their common alleles. The third population group (interspecific hybrids) showed higher allelic heterogenity compared to the 2 previous population groups. For example, cultivars 5 and 6 (T14 x Siokra (F1) and Bar-5595 x Siokra (F1)) showed some genetic similarity and were placed close to each other but genotype No. 6 shows allelic similarity to cultivars 7-12 (Table 1) in the same population group. Cultivars No. 11 (T14 x Sahel F2) and 12 although showed similarity in their alleles combination, they differed in their alleles frequencies. These two genotypes showed the presence of some specific alleles (pink color region), which were absent in the other interspecific hybrids of this population group. These alleles are similar to the alleles of the genotypes No. 3 and 4 (Sahel and Siokra cultivars of *G. hirsutum*), and the genotypes No. 1 and 2 (*G. barbadens*). Therefore these are alleles obtained from their parental genomes. However, we should mention that genotype No. 5 is also produced from the same parental combinations (T14 X Siokra crossing) but does not have some of these alleles. The same stands true for genotype No. 6, which is produced by crossing of Bar-5595 X Siokra.

DISCUSSION

Genetic diversity

The presence of ISSR polymorphic bands in the parental and hybrid progenies of diploid and tetraploid cotton genotypes studied indicates the presence of genetic polymorphism in these genotypes which is supported by genetic parameters determined. Degree of genetic variation reported here is in agreement with the other studies performed in cotton. For example, Wei et al [29] 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 could differentiate the genotypes collected from different provinces in China based on ISSR data. Vafaie-Tabar et al [23] reported 79% average genetic similarity among Indian tetraploid cotton cultivars, while Rana and Bhat[46] reported 74% average genetic similarity in some other cotton cultivars from the same country. According to Rana and Bhat [46], the other studies on tetraploid cotton cultivars outside India also show similar range of average genetic similarity.

Rana and Bhat [46] could differentiate the Indian diploid and tetraploid cotton genotypes with the help of RAPD markers and reported the presence of a higher degree of genetic diversity in diploid genotypes compared to tetraploid 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. The result is also supported by the present study. We observed some novel ISSR bands in the hybrid plants not observed in their parental genotypes. Since even single base change at the primer annealing site is manifested as appearance or disappearance of 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 molecular pattern in the offspring [47]. Sushir et al [48] 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 [48].

Tafvizei et al [30] used RAPD markers to analyze different Iranian cotton genotypes (genotypes different from the present investigation) and reported the occurrence of novel RAPD bands in the hybrids which were not observed in their parents. They also noticed absence of some 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 [49] 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.

The combination of results obtained by NJ tree, Network diagram, STRUCTURE plot and PCoA plot illustrate genetic diversity in the cultivars studied and also through light on their genetic affinity and allele exchange. The NJ tree and PCoA plot clearly show genetic distinctness of diploid and tetraploid genotypes studied as they are well separated from each other. Moreover, NJ tree also reveals genetic differences of the genotypes inside these two polyploidy categories. Presence of higher values of polymorphism percentage and expected heterozygosity in the populations group 1-3 (tetraploid genotypes and their interspecific hybrids) compared to those of population groups 4-7 (diploid genotypes and their interspecific hybrids) clearly indicated the availability of higher genetic diversity among tetraploid cotton genotypes. Significant Fst values observed between diploid and tetraploid population groups indicate their genetic distinctness. Allotetraploid nature as well as frequent genetic recombination may be the reason for a higher genetic diversity present in tetraploid cultivars studied. Presence of high within population variance (about 40% of total variation) as indicated by AMOVA indicates genetic differences among cultivars inside both diploid and tetraploid groups. This is also supported by Fst pairwise test within diploids and tetraploid cultivars.

These results indicate that although due to continuous selection and cultivation of Iranian cotton cultivars we may have narrowed the available cotton gene pool, we still find good genetic diversity both within and between diploid and tetraploid cultivars. This genetic diversity is much more in interspecific hybrids. We can use data obtained to plan further hybridization and selection program for cotton in the country.

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