Australian Journal of Crop Science AJCS
AJCS 5(7):815-821 (2011) ISSN:1835-2707

Evaluation of genetic diversity among Iranian apple (*Malus* × *domestica* Borkh.) cultivars and landraces using simple sequence repeat markers

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Abstract

In current research work, genetic relationships among apple cultivars and landraces from several geographical regions of Iran evaluated using simple sequence repeat (SSR) markers. Forty five alleles were generated at 16 SSR loci. Polymorphism information content (PIC) was varied from 0.18 to 0.76. The mean PIC value for all loci was 0.49. Markers with high PIC values such as CH03c02, CH03g12z, CH05d04, Hi01d06y and Hi02d04 could be effectively used in genetic diversity studies of apple. Jaccard's similarity coefficient among apple cultivars and landraces ranged from 0.19 to 0.79 which indicated a broad genetic base. Maximum and minimum similarity coefficients were observed between 'Salmas4' and 'Dirras-e Mashhad' genotypes, 'Meshki-e Damavand 2' and 'Sifeshirin', respectively. Cluster analysis based on Jaccard's similarity coefficient and UPGMA method distinguished apple genotypes into two groups. Results confirm that SSR is a reliable DNA marker that could be used for exact genetic diversity studies in apple breeding programs.

Keywords: AMOVA, cluster analysis, genetic variability, molecular markers, principal co-ordinates analysis, SSR.
Abbreviations: amplified fragment length polymorphisms (AFLPs), analysis of molecular variance (AMOVA), genetic similarity (GS), polymerase chain reaction (PCR), polymorphism information content (PIC), random amplified polymorphic DNA (RAPDs), principal co-ordinates analysis (PCoA), simple sequence repeats (SSRs), un-weighted pair-grouped method with arithmetic average (UPGMA).

Introduction

Apple (*Malus*× *domestica* Borkh.) is one of the most widespread and popular fruit trees in the world (Janick et al., 1996). About 59 species and 7500 cultivars were identified in all over the world. Apple is an ancient fruit crop in Iran (Janick et al., 1996) and there is an extremely abundant germplasm resource for it. There is high level of genetic diversity in Iran's cultivated apple due to closely distance to apple origin in Central Asia. To be most efficiently managed and effectively utilized, germplasm collections must be well characterized. In most cases, the identification of cultivars, lines and hybrids is based on morphological traits. But number of these traits is limited, they are unstable and they do not always enable to distinguish between closely related accessions or cultivars (Konarev et al., 2000). The emergence of PCR-based molecular markers, such as random amplified polymorphic DNA (RAPDs), microsatellite or simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), has created the opportunity for fine-scale genetic characterisations of germplasm collections (Powell et al., 1996; Kumar, 1999; Agarwal et al., 2008). In apple, RFLP (Watillon et al., 1991) and RAPD (Koller et al., 1993; Mulcahy et al., 1993; Tancred et al., 1994) markers have been employed to identify cultivars and to group them according to their genetic similarity. SSRs markers, due to their high polymorphism, random distribution and co-dominant Mendelian inheritance, are the most reliable markers for cultivars identification and genetic diversity studies. SSRs constitute the current marker system of choice for characterising apple germplasm (Hokanson et al., 1998; Gianfranceschi et al., 1998; Hokanson et al., 2001; Silfverberg-Dilworth et al., 2006; Song et al., 2006; Xuesen et al., 2007; Gharghani et al., 2009; Treuren et al., 2010). Hokanson et al. (1998) used eight microsatellite markers developed in the cultivar "Golden Delicious" to characterise 66 apple accessions. Through this screening, they were able to determine the genetic diversity among the accessions, although seven pairs of accessions could not be differentiated due to being sport mutations or closely related genotypes. Similarity analysis clustered the accessions in agreement with their putative pedigree. In another work, Hokanson et al. (2001) analysed 142 accessions of 23 *Malus* species, hybrids and cultivars with the eight microsatellite primers. This set of primers was enough to distinguish all. Gharghani et al. (2009) investigated the relationships of a collection of 159 accessions of wild and domesticated apples including Iranian indigenous apple cultivars and landraces, selected wild species, and old apple scion and rootstock cultivars from different parts of the world with nine simple sequence repeat (SSR) loci. They concluded that microsatellite genotyping of apple appears to be an efficient tool in the management of collections and in variety identification. With the advent of high-density SSR maps for apple (Liebhard et al., 2003; Silfverberg-Dilworth et al., 2006), it is now feasible to estimate genetic diversity with a large number of markers that

Table1. Name and origin of 44 Iranian apple genotypes used for SSR fingerprinting.

Code	Accession	Origin	Code	Accession	Origin
01	'Kochkine'	Sanandaj	23	'Kokla'	Kermanshah
02	'Golab-e Nemati Yazd'	Yazd	24	'Torsh-e Sefid'	Zanjan
03	'Golab-e Damavand 2'	Damavand	25	'Sheikh Ahmad'	Bane
04	'Tabestan-e Rostami'	Mahallat	26	'Sor-e Paeize'	Kamyaran
05	'Shahriar 2'	Tehran	27	'Ilam 4'	Ilam
06	'Boshgabi-e Talegan'	Talegan	28	'Ferdous Shahriar'	Tehran
07	'Chaharmahal-e 5'	Sahrkord	29	'Damavand 1'	Damavand
08	'Zanjane 5'	Zanjan	30	'Golden-e Asiaei'	Foriegn
09	'Salmas 4'	Salmas	31	'Hamadan-e 3'	Hamadan
10	'Zanjane 14'	Zanjan	32	'Meshki-e Germez'	Tabriz
11	'Panbei Domaze'	Yasooj	33	'Torsh Alma'	Urmia
12	'Israeili Malayer'	Malayer	34	'Manouchehri'	Arak
13	'Germez-e Gilan'	Rasht	35	'Moroti-e Shemiran'	Tehran
14	'Tokhmemorghi-e Sarein'	Ardabil	36	'Shahrood 15'	Sahrood
15	'Dirras-e Mashhad'	Mashhad	37	'Boshghabi-e Torsh'	Karaj
16	'Golab-e Damavand 3'	Damavand	38	'Sifeshirin'	Sagez
17	'Paeiz-e Boomi- Ahar'	Ahar	39	'Sib Golab-e Germez'	Esfahan
18	'Chaharmahal-e 3'	Shahrkord	40	'Beigi'	Gazvin
19	'Sattari-e Jonoob'	Shiraz	41	'Shahrood 19'	Shahrood
20	'Zanjan 7'	Zanjan	42	'Ilam 2'	Ilam
21	'Golden- Canada'	Foriegn	43	'Talkh-e Arak'	Arak
22	'Meshki-e Damavand 2'	Damavand	44	'Sor- e Derige'	Sanandaj

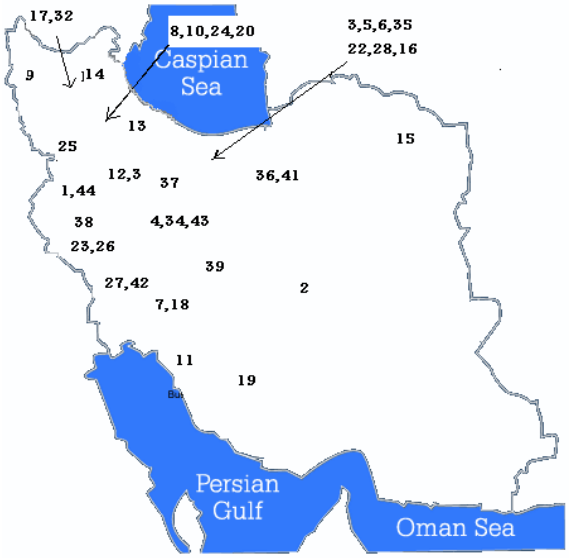

Fig 1. Geographical distribution of the 44 Iranian apple genotypes used to evaluate genetic diversity using 16 simple sequence repeat (SSR) markers. Numbers in the map shows the apple genotype codes. For genotype names corresponding to each code see Table1.

are well distributed across the apple genome. The advantage of using markers with known map positions instead of a random sample is that there is control over the coverage of the genome. It is thus possible to avoid overrepresentation of certain regions of the genetic map that can produce inaccurate estimates of genetic similarities among individuals. This study was conducted to analyze genetic diversity in Iranian domestic apples via SSR marker.

Results and discussion

The sixteen pairs of SSR markers out of sixty markers with high polymorphism applied to fingerprinting of 44 Iranian native apple genotypes (Table 1, Figure1). Results revealed that applied markers had various total amplified bands. According to Table 2, SSR loci analyzed in this study displayed 2 to 5 alleles per locus with an average of 2.8. The maximum number of alleles was observed at CHO3Co2 locus

Table2. Primer sequences, linkage group, annealing temperature, number of alleles and polymorphic information content (PIC) of the 16 simple sequence repeat (SSR) loci applied to 44 apple genotypes.

Primer	Reverse Sequence (5′→3′)	Forward Sequence (5′→3′)	Linkage group[2]	Annealing temperature	Allele no.	[3]PIC
CH02h11a	CGTGGCATGCTTATCATTTG	CTGTTTGAACCGCTTCCTTC	04	50	2	0.28
CH03d12	GCCCAGAAGCAATAAGTAAACC	ATTGCTCCATGCATAAAGGG	06	50	3	0.31
CH03e03	GCACATTCTGCCTTATCTTGG	AAAACCCACAAATAGCGCC	06	53	2	0.48
CH03g12z	GCGCTGAAAAAGGTCAGTTT	CAAGGATGCGCATGTATTTG	01	50	3	0.63
CH04a12	CAGCCTGCAACTGCACTTAT	ATCCATGGTCCCATAAACCA	11	50	3	0.50
CH05d04	ACTTGTGAGCCGTGAGAGGT	TCCGAAGGTATGCTTCGATT	12	50	3	0.62
CH05d11	CACAACCTGATATCCGGGAC	GAGAAGGTCGTACATTCCTCAA	12	51	3	0.55
CH05e03	CGAATATTTTCACTCTGACTGGG	CAAGTTGTTGTACTGCTCCGAC	02	51	3	0.57
Hi01d06y	GGAGAGTTCCTGGGTTCCAC	AAGTGCACCCACACCCTTAC	16	53	3	0.65
Hi02d04	TGCTGAGTTGGCTAGAAGAGC	GTTTAAGTTCGCCAACATCGTCTC	10	51	3	0.60
Hi03a03	ACACTTCCGGATTTCTGCTC	GTTTGTTGCTGTTGGATTATGCC	06	51	3	0.18
Hi03e03	ACGGGTGAGACTCCTTGTTG	GTTTAACAGCGGGAGATCAAGAAC	03	53	2	0.41
CH03c02	TCACTATTTACGGGATCAAGCA	GTGCAGAGTCTTTGACAAGGC	12	51	5	0.76
II	AAGACTCACAAACTAGCTGTCAAAT	TGCTCCTCTCTAGCTATTGCATAAT	-	53	2	0.35
III	CACCTGACCTTCTCTCTACCTCTAC	CAACTCCCCTTATTCTTCTTCTCTC	-	53	3	0.63
[1]Md-Exp7	CATAGAAGGTGGCATGAGCA	TTTCTCCTCACACCCAAACC	-	51	2	0.36

[1]Costa F, Van de Weg W E, Stella S, Dondini L, Pratesi D, Musacchi S, Sansavini S (2008) Map position and functional allelic diversity of Md-Exp7, a new putative expansion gene associated with fruit softening in apple (*Malus* × *domestica* Borkh.) and pear (*Pyrus communis*). Tree Genetics and Genomes 4(3), 575-586. [2]http://www.hidras.unimi.it/HiDRAS-SSRdb/pages/ExtractDB.php?term=Gel%20picture. [3]PIC: Polymorphism information content

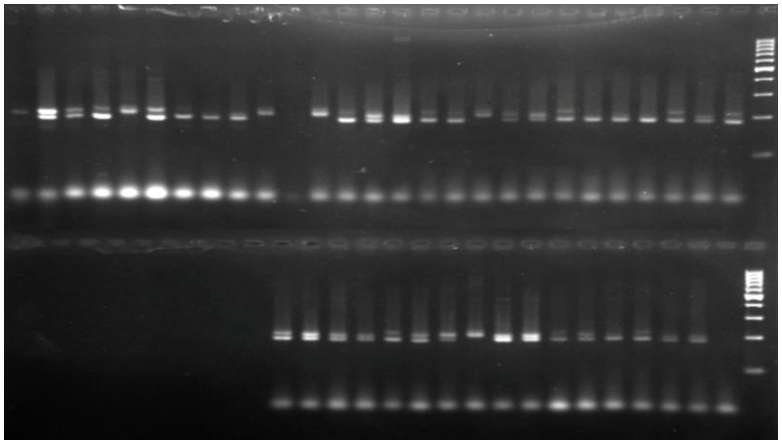

Fig 2. SSR fingerprint of 44 Iranian apple genotypes produced by CHO3eO3 locus

Table 3. Comparison of different methods for constructing similarity matrices and dendrograms.

Similarity coefficient	Cluster algorithm	
	UPGMA	Complete linkage
D	r = 0.63	r = 0.54
H	r = 0.62	r = 0.52
J	r = 0.65	r = 0.56
Phi	r = 0.62	r = 0.52
SM	r = 0.62	r = 0.52
Un1	r = 0.60	r = 0.51

D: Dic (Nei, and Li, 1979); H: Hamann (Hamann, 1961); J: Jaccard (Jaccard, 1908); Phi: Pearson's Phi coefficient (Sokal and Sneath, 1963); SM: Simple Matching (Sneath and Sokal, 1973); Un1: 'Unnamed' coefficient no. 1 (Rohlf, 2000). UPGMA: un-weighted pair-grouped method with arithmetic average.

(Table2) and its size ranged from 116 to 136 bp. SSR fingerprint of 44 Iranian apple genotypes produced by CHO3eO3 locus are presented in Figure2. The set of microsatellite markers used have showed a low level of polymorphism among the genotypes investigated (Table 2) that agreed with others such as Silfverberg-Dilworth et al. (2006) and Guilford et al. (1997), but it was too lower than the reported amount in the other studies for apple inbred lines and hybrids (Hokanson et al., 1998; Goulão and Oliveira, 2001; Zhang et al., 2007; Gharghani et al., 2009). Our estimates are similar to values reported for self- pollinating or annual crops such as tomato, with estimates ranging from 1.5 to 3.1 mean alleles per locus (Broun and Tanksley, 1996; Smulders et al., 1997); wheat with estimates of 3.8 (Devos et al., 1995); sorghum 2.3 (Brown, 1995); cucumbers and melons 2.6 and 2.9 (Katzir et al., 1996) and watermelons with 2.0 alleles per locus (Jarret et al., 1997). The lower value obtained in this research may be due to use agarose-gel electrophoresis for the screening of the microsatellites. Compared to polyacryl-amide-gel electrophoresis or automated analysis, agarose-gel electrophoresis is the most-appropriate technology for routine analysis of these kinds of markers. Agarose-gel is non-toxic and safe to handle. However, an automated detection system would be able to resolve allelic variation at a finer scale than gel-electrophoresis analysis. The lower value obtained in this research may be also due to some reasons such as low number of markers used in the present study. The distribution power of each marker was estimated by the polymorphism information content (PIC) value. PIC value ranged from 0.18 for the Hi03a03 locus to 0.76 for the CH03c02 locus. The mean PIC value for all loci was 0.49. Markers with high PIC values such as CH03c02, CH03g12z, CH05d04, Hi01d06y and Hi02d04 could be effectively used in genetic diversity studies of apple. Meanwhile in previous researches using 28 SSRs primers on 27 Iranian apple varieties, the average of PIC value and mean allele frequency were 0.68 and 5.4, respectively (Nagshin et al., 2008). Different methods were used for constructing similarity matrices and dendrograms (Table 3). The cophenetic correlation, a measure of the correlation between the similarity represented on the dendrograms and the actual degree of similarity, was calculated for each dendrogram (Table 3). Among the different methods, the highest value (r= 0.65) was observed for UPGMA based on Jaccard's coefficient (Table 3). Therefore, the dendrogram constructed based on this method was used for depicting genetic diversity of genotypes (Figure 3). The average pair-wise genetic similarities was 0.54 and it ranged from a maximum of 0.79 between 'Salmas 4' and 'Dirras-e Mashhad' genotypes to a minimum of 0.19 between 'Meshki-e Damavand 2' and 'Sifeshirin' genotypes (Table 4). This is implying that there is high level of genetic variation between studied apple genotypes. Based on AMOVA, all apple genotypes were placed in two groups. Group one comprises 3 genotypes ('Panbei Domaze', 'Boshgabi-e Torsh', and 'Sifeshirin'). In this group the highest Jaccard's similarity value (GSj = 0.56) was observed between, 'Panbei Domaze' and 'Boshgabi-e Torsh' genotypes. The 'Panbei Domaze' and 'Sifeshirin' genotypes belong to the west of Iran. It seems that, 'Boshgabi-e Torsh' have had the common origin with them and during the time it has transferred to central parts of Iran. The majority of studied genotypes located in group two. This is might be due to ancestor relationships among them. In group two, highest value of Jaccard's similarity (GSj = 0.79) was between 'Salmas4' and 'Dirras-e Mashhad' genotypes. According to reports of Alizadeh (2006), these two genotypes have some common properties such as semi sensitivity to drought stress and low sensitivity to chlorosis. It can result that the majority of the Iranian native apples are very similar together, as they can assume as one population. This is in agreement with formerly study on Iranian apples genotypes by Gharghani et al. (2009). Our results also revealed that geographical distribution of genotypes could not be used as a base to cross parent to obtain high heterosis and therefore it must be carry out by exact genetic studies. Considering to importance of apple production in Iran and the role of Persia in apple domestication during the history, a general goal of such studies will be the use of molecular markers which are linked to traits to improve breeding lines through marker assisted selection.

Material and methods

Plant material

Forty-four local and commercial Iranian apple genotypes from different geographical and/or agroclimatic locations in Iran were used in this study (Table 1; Figure 1). Sixteen microsatellite markers (Table 2) were used for DNA fingerprinting. SSR markers were screened out of sixty markers existing in our laboratory. The choice of SSR markers was based on the degree of polymorphism and their known genetic locations in order to give a uniform coverage of the apple genome (Liebhard et al., 2002, 2003; Silfverberg-Dilworth et al., 2006).

DNA extraction and PCR amplification

Total genomic DNA was extracted from young leaves taken from a single plant per genotype using method of Dellaporta et al. (1983). Genomic DNA was re-suspended in 100 µL TE (10 mM Tris, 1 mM EDTA). The concentration of each DNA sample was determined spectrophotometrically at 260 nm (BioPhotometer 6131; Eppendorf, Hamburg, Germany). The quality of the DNA was checked by running 1 µl DNA in 0.8% (w/v) gels in 0.5X TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). DNA samples that gave a smear in the gel were rejected. Each PCR amplifications was carried out in 25 µl containing 1X reaction buffer [200 mM Tris–HCl, pH 8.55, 160 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 2.0 mM $MgCl_2$, 0.4 mM of dNTPs (dATP, dCTP, dGTP and dTTP) (CinnaGen Inc., Tehran, Iran), 0.16 µM of each SSR primer (CinnaGen Inc., Tehran, Iran), 1.0 Units Taq DNA polymerase (CinnaGen Inc., Tehran, Iran), and 25 ng of genomic DNA template. DNA

Table4. Jaccard′s coefficient matrix for apple genotypes based on SSR data

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
2	0.4																																										
3	0.52	0.66																																										
4	0.41	0.59	0.56																																									
5	0.41	0.48	0.42	0.53																																								
6	0.38	0.5	0.42	0.64	0.57																																						
7	0.3	0.37	0.36	0.59	0.37	0.56																																					
8	0.33	0.73	0.52	0.57	0.42	0.55	0.47																																				
9	0.4	0.6	0.46	0.7	0.41	0.55	0.61	0.75																																			
10	0.4	0.57	0.42	0.4	0.4	0.39	0.28	0.55	0.45																																		
11	0.33	0.38	0.37	0.29	0.44	0.38	0.29	0.38	0.37	0.29																																	
12	0.45	0.51	0.46	0.39	0.69	0.48	0.29	0.5	0.42	0.54	0.39																																
13	0.2	0.44	0.39	0.43	0.4	0.47	0.52	0.57	0.47	0.33	0.26	0.33																															
14	0.3	0.51	0.44	0.46	0.53	0.5	0.45	0.61	0.55	0.4	0.48	0.5	0.43																														
15	0.38	0.5	0.39	0.55	0.43	0.53	0.56	0.68	**0.79**	0.37	0.4	0.4	0.5	0.5																													
16	0.34	0.51	0.37	0.48	0.41	0.69	0.5	0.57	0.62	0.3	0.39	0.44	0.56	0.51	0.6																												
17	0.32	0.4	0.33	0.44	0.46	0.48	0.5	0.47	0.59	0.3	0.36	0.4	0.45	0.59	0.56	0.6																											
18	0.33	0.36	0.33	0.23	0.52	0.3	0.26	0.33	0.25	0.4	0.37	0.66	0.34	0.47	0.27	0.42	0.37																										
19	0.38	0.5	0.42	0.43	0.5	0.41	0.33	0.46	0.46	0.36	0.44	0.48	0.32	0.57	0.45	0.51	0.58	0.52																									
19	0.37	0.46	0.36	0.41	0.48	0.32	0.48	0.45	0.55	0.32	0.43	0.36	0.44	0.55	0.6	0.42	0.53	0.31	0.51																								
21	0.39	0.58	0.48	0.43	0.45	0.42	0.34	0.56	0.56	0.27	0.46	0.43	0.48	0.51	0.6	0.61	0.46	0.4	0.54	0.61																							
22	0.37	0.54	0.4	0.46	0.48	0.5	0.35	0.66	0.6	0.41	0.3	0.44	0.44	0.5	0.6	0.56	0.46	0.4	0.56	0.45	0.66																						
23	0.6	0.48	0.46	0.43	0.41	0.41	0.37	0.46	0.51	0.45	0.37	0.43	0.42	0.46	0.53	0.48	0.39	0.42	0.58	0.51	0.58	0.56																					
24	0.4	0.5	0.41	0.45	0.55	0.48	0.33	0.39	0.43	0.24	0.31	0.37	0.46	0.48	0.46	0.54	0.51	0.37	0.6	0.53	0.64	0.53	0.5																				
25	0.4	0.55	0.48	0.56	0.47	0.54	0.44	0.54	0.59	0.41	0.39	0.44	0.43	0.5	0.67	0.51	0.43	0.28	0.55	0.54	0.55	0.5	0.55	0.57																			
26	0.47	0.55	0.44	0.57	0.42	0.46	0.5	0.6	0.7	0.42	0.44	0.35	0.45	0.6	0.61	0.53	0.47	0.32	0.61	0.65	0.55	0.53	0.68	0.55	0.68																		
27	0.44	0.5	0.53	0.44	0.53	0.59	0.38	0.43	0.38	0.41	0.44	0.44	0.38	0.38	0.4	0.45	0.35	0.34	0.36	0.39	0.37	0.35	0.36	0.46	0.52	0.5																	
28	0.52	0.57	0.61	0.43	0.56	0.46	0.34	0.48	0.46	0.46	0.5	0.58	0.37	0.53	0.48	0.48	0.55	0.5	0.58	0.48	0.53	0.48	0.51	0.54	0.47	0.5	0.58															
29	0.57	0.48	0.46	0.39	0.45	0.42	0.33	0.51	0.46	0.32	0.36	0.5	0.39	0.46	0.5	0.48	0.51	0.39	0.45	0.42	0.54	0.05	0.48	0.41	0.4	0.43	0.41	0.63													
30	0.38	0.55	0.42	0.34	0.34	0.42	0.37	0.42	0.37	0.34	0.34	0.34	0.43	0.42	0.4	0.5	0.45	0.3	0.32	0.43	0.41	0.3	0.4	0.41	0.36	0.39	0.41	0.48	0.6												
31	0.26	0.44	0.37	0.39	0.48	0.48	0.43	0.42	0.37	0.29	0.44	0.44	0.43	0.53	0.44	0.55	0.52	0.45	0.5	0.43	0.45	0.38	0.44	0.51	0.36	0.39	0.36	0.53	0.45	0.58											
32	0.38	0.38	0.37	0.46	0.48	0.55	0.44	0.35	0.35	0.32	0.32	0.41	0.52	0.35	0.41	0.57	0.4	0.4	0.32	0.28	0.41	0.4	0.46	0.39	0.45	0.32	0.38	0.4	0.38	0.37	0.42										
33	0.46	0.62	0.56	0.44	0.5	0.43	0.34	0.59	0.48	0.65	0.46	0.57	0.4	0.59	0.51	0.45	0.5	0.45	0.6	0.43	0.41	0.48	0.55	0.42	0.52	0.57	0.46	0.66	0.5	0.46	0.46	0.43									
34	0.41	0.68	0.46	0.45	0.48	0.43	0.32	0.64	0.58	0.45	0.37	0.53	0.42	0.53	0.58	0.5	0.37	0.37	0.46	0.48	0.56	0.58	0.41	0.47	0.57	0.46	0.38	0.46	0.45	0.41	0.33	0.39	0.62								
35	0.3	0.42	0.42	0.35	0.62	0.37	0.37	0.39	0.37	0.39	0.5	0.56	0.34	0.57	0.41	0.38	0.54	0.5	0.41	0.55	0.46	0.31	0.35	0.43	0.42	0.44	0.48	0.65	0.45	0.5	0.42	0.33	0.51	0.41							
36	0.32	0.55	0.52	0.53	0.56	0.5	0.41	0.57	0.51	0.45	0.4	0.5	0.45	0.53	0.66	0.46	0.59	0.33	0.48	0.43	0.5	0.53	0.46	0.41	0.63	0.42	0.4	0.61	0.48	0.37	0.44	0.61	0.65	0.6	0.46						
37	0.38	0.4	0.33	0.38	0.51	0.37	0.44	0.37	0.41	0.29	0.56	0.38	0.37	0.41	0.43	0.31	0.4	0.29	0.35	0.51	0.48	0.34	0.39	0.33	0.43	0.35	0.32	0.42	0.36	0.27	0.35	0.41	0.36	0.41	0.42	0.5					
38	0.31	0.45	0.32	0.3	0.35	0.29	0.25	0.36	0.34	0.31	0.36	0.37	0.24	0.25	0.33	0.27	0.43	0.26	0.37	0.33	0.25	**0.19**	0.31	0.3	0.29	0.25	0.32	0.41	0.37	0.36	0.36	0.24	0.33	0.31	0.26	0.32	0.4				
39	0.37	0.57	0.5	0.51	0.51	0.5	0.4	0.61	0.55	0.45	0.5	0.53	0.41	0.61	0.48	0.46	0.56	0.41	0.62	0.45	0.46	0.5	0.51	0.39	0.5	0.53	0.3	0.57	0.51	0.42	0.42	0.35	0.59	0.48	0.53	0.55	0.41	0.4			
40	0.34	0.5	0.57	0.52	0.37	0.44	0.45	0.56	0.56	0.35	0.29	0.4	0.52	0.38	0.46	0.4	0.47	0.2	0.34	0.39	0.44	0.44	0.35	0.37	0.45	0.45	0.42	0.44	0.51	0.36	0.3	0.33	0.42	0.42	0.29	0.42	0.35	0.3	0.5		
41	0.29	0.46	0.41	0.45	0.34	0.5	0.47	0.59	0.56	0.36	0.34	0.31	0.52	0.45	0.64	0.52	0.41	0.23	0.31	0.41	0.51	0.46	0.5	0.37	0.5	0.47	0.35	0.35	0.46	0.45	0.4	0.42	0.37	0.39	0.28	0.5	0.3	0.32	0.44	0.47			
42	0.26	0.37	0.3	0.3	0.35	0.34	0.29	0.41	0.34	0.26	0.36	0.37	0.24	0.41	0.37	0.32	0.36	0.3	0.37	0.37	0.48	0.37	0.4	0.25	0.33	0.32	0.19	0.28	0.37	0.36	0.3	0.28	0.28	0.31	0.41	0.41	0.31	0.33	0.52	0.25	0.5		
43	0.3	0.44	0.52	0.4	0.35	0.39	0.43	0.58	0.5	0.36	0.31	0.36	0.5	0.46	0.62	0.46	0.5	0.34	0.41	0.43	0.53	0.51	0.44	0.4	0.56	0.38	0.29	0.5	0.46	0.37	0.35	0.46	0.48	0.5	0.48	0.76	0.34	0.27	0.56	0.41	0.54	0.4	
44	0.45	0.5	0.65	0.34	0.37	0.34	0.29	0.45	0.38	0.42	0.38	0.42	0.4	0.54	0.37	0.42	0.43	0.45	0.54	0.42	0.42	0.37	0.58	0.46	0.48	0.54	0.38	0.57	0.42	0.4	0.4	0.38	0.6	0.4	0.5	0.5	0.26	0.33	0.56	0.34	0.37	0.32	0.63

Genotype codes: see Table1.

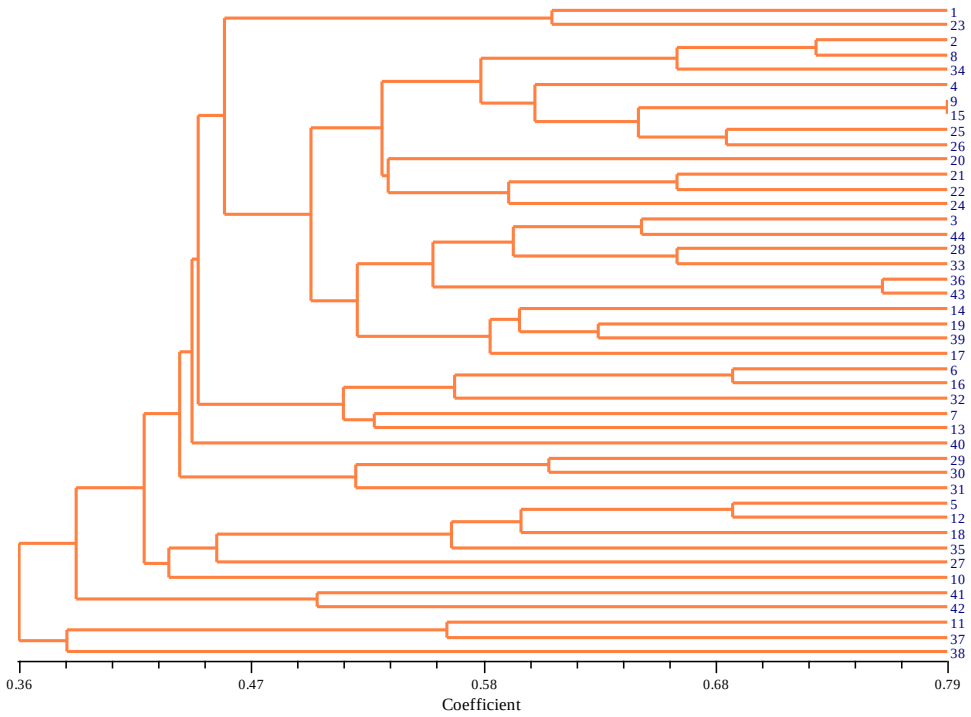

Fig 3. Dengrogram of 44 Iranian apple germplasm generated by the UPGMA clustering method based on a Jaccard's coefficients of similarity matrix. Numbers in the dengrogram shows the apple genotype codes. For genotype names corresponding to each code see Table1.

amplifications were performed in a Gene Amp PCR System 9700 Thermocycler (PerkinElmer–Applied Biosystems) programmed for a preliminary step of 95 ºC for 2 min, followed by 35 cycles of 93 ºC for 45 s, 50-55 ºC for 60 s and 72 ºC for 2 min. A final extension was done for 10 min at 72 ºC. Then samples were held at 4ºC until the SSR fragments were separated by electrophoresis using 3% agarose gel in 0.5X TBE buffer and visualized with ethidium bromide (1.0 μgml^{-1}) under UV light.

Data analysis

Banding profiles generated by SSR assays were separately compiled into a data matrix on the basis of presence (1) or absence (0) of bands. The binary matrices were used to estimate DNA polymorphisms and genetic relatedness of apple genotypes. Different methods used for constructing similarity matrices and dendrograms. The efficiency of clustering algorithms and their goodness of fit were determined based on the cophenetic correlation coefficient. Analysis of molecular variance (AMOVA) was carried out using GENALEX version 6 software (Peakall and Smouse, 2006) to determine the number of clusters. In addition to cluster analysis, principal co-ordinates analysis (PCoA) was used to confirm the results of cluster analysis. PCoA is low-dimensional graphical plot that use to depict the relationships among studied genotypes. Data analyses were performed by the NTSYS-pc version 2.11 software (Rohlf, 1998). Allelic polymorphism information content (PIC) was calculated as described by Anderson et al. (1992):

$$PIC = 1 - \sum_{i=1}^{n} P_i^2$$

Where P_i is the proportion of the population carrying the i^{th} allele, calculated for each microsatellite locus.

Acknowledgments

We would like to thank Dr A. Alizadeh the manager of Kahriz agriculture station Centre, Urmia, Iran, for providing the plant materials. The authors are gratefully thanked to the Institute of Biotechnology, Urmia University, Urmia, Iran, for the financial support of this work. The reviewers are kindly acknowledged for their helpful suggestions.

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