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Preliminary Evaluation of Genetic Diversity among Iranian Red Fleshed Apples Using Microsatellite Markers

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ABSTRACT

Red fleshed apples have high levels of anthocyanins in their flesh. Iran enjoys a large variety of these apples due to its location in Central Asia. In the present study, 20 genotypes including eight Iranian red fleshed and 12 commercial Iranian and foreign apples were selected for the study of genetic diversity of red fleshed apples. We used a set of 11 microsatellite markers (SSRs) to determine genetic diversity and the linkage between these SSRs and red fleshed color. Seven SSRs were amplified and revealed adequate performance. On the whole, 56 alleles were detected ranging from 3 to 11, with an average of 8 alleles per locus. Cluster analysis was performed by the UPGMA algorithm and Dice similarity coefficient through NTSYS-pc ver. 2.02 software. The obtained dendrogram classified the studied genotypes into seven categories. Heterozygosity and Shannon Index were estimated using POPGEN 1.32 software. The genetic diversity for the two populations (Iranian and foreign) were calculated using Gene Alex ver. 6.3 software. These findings can be helpful for conservation and selection of these genetic resources and future breeding programs.

Keywords: Dice similarity coefficient, Diversity, Malus, Red fleshed apple, SSR.

INTRODUCTION

Anthocyanins are an important group of natural antioxidants that have potential health benefits such as anti-inflammatory nature, ability to prevent cancer and heart diseases, etc. (Eberhard *et al.,* 2000; Kahkonen *et al.,* 2001; Wolfe *et al.,* 2003; Lee *et al.,* 2003; Butelli *et al.,* 2008).

Red fleshed apples have red to pink color in their flesh (Espley *et al.,* 2009) because of anthocyanin production (Mazza and Velioglu, 1992) (Figure 1). Entire tree, leaves, wood, flowers and fruits have the red pigment (Espley *et al.,* 2009). The red color is anthocyanin accumulation and is regulated by developmental, hormonal, and light signals (Ubi *et al.,* 2006). While many steps have been described in anthocyanin biosynthetic pathway (Inagaki *et al.,* 1994), several reports show regulation of anthocyanin bio-synthesis by MYB transcription factors in diverse plant species (Allan *et al.,* 2008). Flesh color is controlled by a gene called MYB10 that is expressed in higher amounts in the red fleshed apples than the white fleshed (Espley *et al.,* 2009).

The point in apple breeding is usually to make better-quality apples. Red fleshed apples have striking red flesh, but their fruits are small. Therefore, a recent objective of apple breeding programs is introducing the red fleshed apple cultivars with better qualities. Genetic diversity is desirable for long-term crop improvement and reduction of vulnerability of plants to

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Figure 3. Two genotypes of Iranian red- fleshed apple.

important crop stresses (White and Walker, 1997). The findings of genetic diversity can be used in breeding programs for increasing the genetic variation in base populations by crossing cultivars with a high level of genetic distance as well as for the introgression of exotic germplasm. To be most efficiently managed and effectively utilized, germplasm must be well characterized. But, the number of these characteristics is limited, they are unstable, and do not always distinguish between closely related cultivars (Konarev., 2000). Hence, genetic diversity estimations based on molecular marker data yield a minimum genetic distance which indicates that two cultivars are not essentially derived (Lefebvre *et al.,* 2001). This analysis will be useful in the selection of parental genotypes for mapping populations and breeding programs. Extensive research has indicated that the number of DNA markers used for genetic studies in plants varies with the total number of genotypes assessed (Thottapilly *et al.,* 1996). Microsatellite markers or SSRs (Simple Sequence Repeats) are one of the most informative and appropriate markers for plant genome analysis (Perera *et al.,* 2000). The valuable attributes of all SSR markers are co-dominance, technical and analytical simplicity, sensitivity, and uniform dispersion throughout genome with a frequency of every 10^3 Kb. Thus, SSR markers are ideal tools for many genetic applications (Moyib *et al.,* 2007). These markers are abundant and uniformly dispersed throughout the apple genome (Morgan and Richards, 2002). In recent years, SSRs have been broadly used for classification of apple varieties (Guilford *et al.,* 1997; Goulão and Oliveira, 2001; Liebhard *et al.,* 2002; Zhang *et al.,* 2007; Zhang *et al.,* 2009; Mingyang and Fengwang, 2012). There are a great number of reports available on suitability of SSRs for genetic studies in apple genotypes (Hokanson *et al.,* 1998; Gianfranceschi *et al.,* 1998; Harris *et al.,* 2002; Goulão and Oliveira, 2001; Mac an Tsaoir *et al.,* 2006; Silfverberg- Dilworth *et al.,* 2006, Mingyang and Fengwang, 2012). Red fleshed apple is an extraordinary fruit that merits more extensive research. The aim of our research was to study genetic diversity of some Iranian red fleshed apple genotypes using SSRs.

MATERIALS AND METHODS

Plant Material and DNA Extraction

The plant materials used in this study consisted of eight genotypes of red fleshed apples (Haji Qermez, Gousht Qermez, Shahroud-10, Arous Gousht Qermez, B.9, Qazvin-1, Qazvin-2, Qazvin-3), and 12 commercial apple cultivars (Shafiei, Golab-e kohanz, Jonathan, Golden Delicious, Granny Smith, Soltani Shabestar, Fuji, Red

Delicious, Heydar Zadeh, Golab-e sahneh, Gala, and Shafi Abadi) that were selected from Seed and Plant Improvement Institute, Karaj, Iran (Table 1). Genomic DNA was extracted from young fresh leaves of each apple genotype using the CTAB method described by Doyle and Doyle (1987). The quality of genomic DNA samples were verified by 1% agarose gel electrophoresis and quantified by Nanodrop spectrophotometer (Thermo scientific, Wilmington, USA).

SSR Primers and PCR

A total of 11 SSRs were used to study diversity among 20 apple cultivars. Primers previously described by Chagné *et al.* (2007) to determine the genetic diversity (Table 2). PCR was carried out on 1 µl of genomic DNA (100 ng μl^{-1}) in 25 µl of reactions with 0.2 µM of each primer, 0.2 mM of dNTP, 0.5 unit of Taq polymerase, 2mM $MgCl_2$, 10 mM of Tris- HCl (pH 8.5), and 19.1 µl of distilled H_2O; (Taq polymerase, $MgCl_2$ buffer, dNTPs, and primer pairs were bought from Cinnagen Company). The PCR reactions were performed using a touchdown PCR protocol described by Gianfranceschi *et al.* (1998) with some modification in annealing temperature (Table 2). Polymorphism was detected by a 6% vertical polyacrylamide gel electrophoresis. Polyacrylamide gels were silver stained, as described by Bassam *et al.* (1991).

Data Analysis

Bands on silver stained gels were scored as 1 (presence) or 0 (absence). Data were entered in a binary matrix as discrete variables and analyzed with POPGEN ver. 1.31 software. Observed alleles (n_a), effective alleles (n_e), expected heterozygosity (H_e) (Nei, 1973) and Shannon's Information index (Shannon and Weaver, 1949) was obtained for all loci. The studied individuals classified into two groups; Iranian (13 individuals) and Foreign

Table 1. Apple genotypes used in this study.

Code of each genotype	*Malus*. sp. or cultivars	Place
1	Haji Qermez [a]	Ardebil [b]
2	Gousht Qermez [a]	Tehran [b]
3	Shahroud -10 [a]	Semnan [b]
4	Arous Gousht Qermez [a]	Tehran [b]
5	B.9 [a]	Russa [b]
6	Qazvin-1 [a]	Qazvin
7	Qazvin-2 [a]	Qazvin
8	Qazvin-3 [a]	Qazvin
9	Shafiei	Unknown [b]
10	Golab-e Kohanz	Karaj [b]
11	Jonathan	USA [b]
12	Golden Delicious	USA [b]
13	Granny Smith	Australia [b]
14	Soltani Shabestar	Azarbaijan [b]
15	Fuji	Japan [b]
16	Red Delicious	USA [b]
17	Heydar Zadeh	Mashhad [b]
18	Golab-e Sahneh	Kermanshah [b]
19	Gala	New zealand [b]
20	Shafi Abadi	Tehran [b]

[a] The genotypes that are red-fleshed apple cultivars.
[b] Collected from Horticultural Research Station at Kamal-Abad (Karaj, Iran).
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Table 2. SSR primers sequence used in this study.[a]

Primer name[a]		Sequence	Tm[b]
MdMYB17	F	*5- TGCTCCTCTCTAGCTATTGCATAAT-3*	60-56
	R	*5- AAGACTCACAAACTAGCTGTCAAAT-3*	
MdBHLH3	F	*5- CAACTCCCCTTATTCTTCTTCTCTC-3*	60-56
	R	*5- CACCTGACCTTCTCTCTACCTCTAC-3*	
MdMYB12	F	*5- CTCGGCAATCGGTAAAGCTA-3*	60-56
	R	*5- TATGAACAGTGAAACCCTAACCCTA-3*	
NZmsPa18	F	*5- GGCACAAGCACAAGGAAACA-3*	60-56
	R	*5- GTTTGAGCCAGTCCATTTTTCCCTAT-3*	
NzmsPa113	F	*5- TCGATGAACAAGGCCCAAAG-3*	60-56
	R	*5- GTTTAGGGGGACGGAAGGAACAAG-3*	
NzmsPa136	F	*5- CCTCAACAAATATAAGACTCTCTC-3*	60-56
	R	*5- GTTTCTCCACTCTGTCCGTACATT-3*	
NzmsPa145	F	*5- AAAACCCAACACCACAGC-3*	60-56
	R	*5- GCTTCTTTGGATTCTGGATG-3*	
NzmsPa151	F	*5- GATTTTCTGATAATCCTGCC-3*	60-56
	R	*5- GTTTAAACAACTCCAGCTCTGC-3*	
NzmsPa192	F	*5- GTTTCTGGTTTCACTGGCATT-3*	60-56
	R	*5-GTTGCCACATTTTCACCATA-3*	
NzmsPa1213rd	F	*5- CAACCTTTCTCTCATTCACTC-3*	60-56
	R	*5- GTTTCCTTTTATGTACCGGCTT-3*	
NZmsPal443ird	F	*5- AAAAGCTCTCTCACTCCC -3*	60-56
	R	*5- GTTTATCTGCAAATCTGAGACC-3*	

[a] MdMYB17, MdBHLH3 and MdMYB12 were selected from Hemmat *et al.* (2003); the rest of SSRs were chosen from Changné (2007). F: The abbreviation of forward primer; R: The abbreviation of reverse primer, Tm: The abbreviation of melting temperature.
[b] Touchdown PCR by reducing 1°C for 4 steps.

(7 individuals). number of observed alleles (n_a), expected alleles (n_e), expected heterozygosity (H_e), Shannon index (I), polymorphic information content (PIC) and percentage of polymorphism (P%) were evaluated for this classification using Gene Alex ver. 6.3 software..

Cluster analysis was constructed based on Un-weighted Pair Group Method with Arithmetic Means (UPGMA) algorithm and Nei's genetic distances using NTSYS-pc ver. 2.02 software.

RESULTS

SSR Polymorphism

Seven out of the 11 primers used to measure genetic diversity of the 20 genotypes showed polymorphism. Three SSRs MdMYB17, NZmsPa151, and NzmsPa151 did not yield any amplification products, and primer NZmsPa192 was not polymorphic, i.e. it produced two alleles of the same size for all genotypes. A total of 56 alleles were detected by the seven polymorphic SSRs. The highest number of amplicons were obtained for the two primers MdBHLH3 (Figure 2) and NZmsPa113 (11 alleles), whereas the primer NZmsPa145 resulted in the lowest number of amplicons (3 alleles), with an average of 8 alleles per locus (Table 3). The highest effective alleles (n_e) were 10.25 (MdBHLH3).

The NZmsPa145 and MdBHLH3 SSRs revealed the highest and lowest values for H_e (0.47 and 0.07, respectively). The lowest (0.25) and highest (1.00) H_o values were obtained for MdMYB12 and NZmsPa1213, respectively. The average of H_o values was 0.60 across the loci. The H_e values ranged

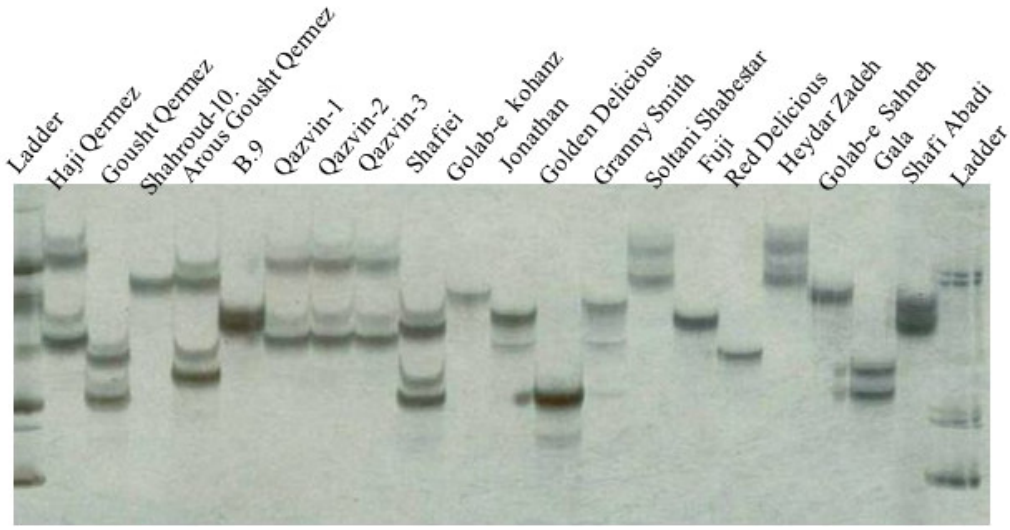

Figure 2. Six percent acrylamide gel electrophoretic silver stained patterns of the apple genotypes amplified with microsatellite MdBHLH3 (11 alleles).

from 0.52 for NZmsPa145 to 0.92 for MdBHLH3, with an average of 0.77 across loci (Table 3). The highest and lowest Shannon's Index values were obtained for MdBHLH3 and NZmsPa145 as 2.35 and 0.88, respectively. The average of Shannon's Index values was 1.75 across the loci.

Genetic diversity for the two populations (Iranian and Foreign) were calculated and showed a significant diversity, with *Na*=1.82 , *Ne*= 1.27, *I*= 0.35, *He*= 0.19, *PIC*= 0.28 and *p%*= 91.07% for Iranian population (Table 4).

Partitioning the variation within and between populations using an analysis of molecular variance (AMOVA) showed that 1% of the total genetic variation existed among populations (Table 5).

Genetic Relationships Based on SSRs Data

UPGMA cluster analysis of the 20 apple genotypes using the polymorphic loci resulted in the dendrogram shown in Figure 3. Seven groups were distinguished by the

Table 3. Number of observed alleles (n_a), expected alleles (n_e) per locus, observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon index (I).

Locus	N_a [a]	H_o [b]	H_e [c]	I [d]	PIC [e]
MdBHLH3	11	0.55	0.92	2.35	0.25
MdMYB12	5	0.25	0.54	1.03	0.23
NZmsPa18	8	0.90	0.85	1.92	0.35
NZmsPa113	11	0.75	0.88	2.15	0.33
NZmsPa136	8	0.30	0.83	1.84	0.26
NZmsPa145	3	0.45	0.52	0.88	0.26
NZmsPa1213	10	1.00	0.87	2.07	0.37
Mean	8	0.60	0.77	1.75	0.29

[a] Observed alleles, [b] observed heterozygosity, [c] expected heterozygosity , [d] Shannon index, [e] Polymorphic information content.

Table 4. Genetic parameters of the two populations.

Population	Parameters						
	N [a]	Na [b]	Ne [c]	I [d]	He [e]	PIC [f]	P% [g]
Iranian	13	1.82±0.08	1.28±0.03	0.35±0.02	0.19±0.2	0.28	91.07%
Foreign	7	1.36±0.13	1.31±0.43	0.3±0.03	0.19±0.02	0.25	67.86%

[a] Number of individuals, [b] number of observed alleles, [c] expected alleles, per locus, [d] Shannon index, [e] expected heterozygosity, [f] Polymorphic information content, and [g] Percentage of polymorphism.

Table 5. Analysis of molecular variance (AMOVA) among and within populations**.**

Source of variation	*Df*[a]	Sum of squares	Variance components	Percentage of variation
Among population	1	14.43	0.68	8
Whitin population	18	148.37	8.24	92
Total	19	162.68	8.92	100

[a] Degrees of freedom.

dendrogram. The first group included four red fleshed apples: Haji Qermez (1), Qazvin-1(6) (Figure 1), Qazvin-2 (7), and Qazvin-3 (8). The second group was composed of four apples: Gousht Qermez (2), Shafiei (9), Golden Delicious (12), and Gala (19). There were four cultivars in the third group: B.9 (5), Shafi Abadi (20), Jonathan (11), and Granny Smith (13). The forth group consisted of two Iranian cultivars: Golab-e Kohanz (10) and Golab-e Sahneh (18). Two foreign cultivars including Fuji (15) and Red Delicious (16) constituted the fifth group. There were three Iranian cultivars, namely, Shahroud-10 (3) (Figure 1), Soltani Shabestar (14), and Heydar Zadeh (17) cultivars in the sixth group. Finally, the last group included only one cultivar, Arous Gousht Qermez (4).

According to the obtained similarity matrix (Table 6), the genetic distance varied between 1.00 for Qazvin's genotypes and

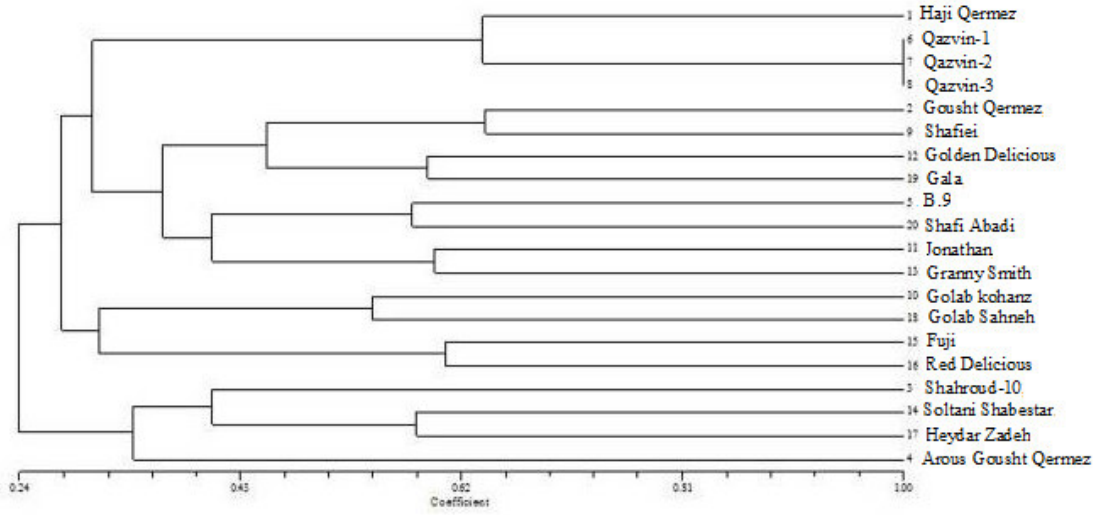

Figure 3. Phylogenetic dendrogram of 20 apple genotypes constructed using data from seven polymorphic SSR markers. The phenogram was produced using the UPGMA method of Dice between varieties.

Table 6. Genetic distance for 20 genotypes using SSR markers based on Dice similarity cophenetic.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1																			
2	0.09	1																		
3	0.40	0.28	1																	
4	0.26	0.08	0.31	1																
5	0.18	0.52	0.15	0.32	1															
6	0.70	0.28	0.50	0.26	0.35	1														
7	0.70	0.28	0.50	0.26	0.35	1	1													
8	0.60	0.38	0.10	0.17	0.27	0.90	0.50	1												
9	0.10	0.17	0.30	0.17	0.45	0.20	0.20	0.20	1											
10	0.21	0.30	0.12	0.27	0.38	0.42	0.42	0.31	0.31	1										
11	0.10	0.38	0.30	0.34	0.45	0.40	0.40	0.30	0.40	0.62	1									
12	0.30	0.66	0.50	0.26	0.63	0.40	0.40	0.30	0.60	0.31	0.50	1								
13	0.15	0.46	0.38	0.33	0.52	0.38	0.38	0.28	0.66	0.40	0.56	0.66	1							
14	0.27	0.26	0.61	0.18	0.41	0.45	0.45	0.36	0.46	0.47	0.51	0.46	0.52	1						
15	0.10	0.38	0.10	0.34	0.45	0.30	0.30	0.30	0.30	0.31	0.60	0.40	0.33	0.36	1					
16	0.10	0.47	0.30	0.17	0.27	0.20	0.20	0.20	0.80	0.31	0.80	0.30	0.23	0.36	0.60	1				
17	0.19	0.17	0.15	0.40	0.26	0.27	0.27	0.48	0.27	0.28	0.36	0.27	0.31	0.66	0.27	0.45	1			
18	0.31	0.20	0.31	0.18	0.28	0.42	0.42	0.31	0.31	0.65	0.31	0.21	0.40	0.38	0.21	0.81	0.17	1		
19	0.30	0.28	0.10	0.26	0.27	0.20	0.20	0.40	0.50	0.21	0.80	0.40	0.47	0.27	0.20	0.80	0.36	0.52	1	
20	0.26	0.80	0.52	0.30	0.61	0.62	0. 30	0.64	0.43	0.43	0.34	0. 52	0.45	0.56	0.34	0.43	0.40	0.35	0.26	1

0.08 for two red fleshed apples, i.e. Gousht Qermez (2) and Arous Gousht Qermez (4).

DISCUSSION

In our study, the average number of alleles per locus (8) (Table 3, Figure 2) was considerably more than previous studies (Hokanson *et al.,* 1998; Silfverberg–Dilworth *et al.,* 2006; Gharaghani *et al.,* 2009; Farrokhi *et al.,* 2011). The average of Shannon Index was 1.75 across the loci (Zhang *et al.,* 2007; Gharaghani *et al.,* 2009). The Shannon Index showed a positive correlation with the n_e and the H_e (Gharaghani *et al.,* 2009) (Table 3). Thus, microsatellite markers are useful for assessment of genetic diversity in apple (Hokanson *et al.,* 1998; Gianfranceschi *et al.,* 1998; Goulão and Oliveira, 2001; Liebhard *et al.,* 2002; Silfverberg –Dilworth *et al.,* 2006; Mac an Tsaoir *et al.,* 2006; Zhang *et al.,* 2007; Zhang *et al,* 2009; Gharaghani *et al.,* 2009; Farrokhi *et al.,* 2011; Mingyang and Fengwang, 2012).

The bands ranged from 100 to 400 bps in length for all genotypes (Figure 2) (Celton *et al.,* 2009; Espley *et al.,* 2009). The two SSR primers, MdBHLH3 (Figure 1) and MdMYB12, showed polymorphism but did not have any linkage with the red color of the flesh; because of presenting these SSRs in all genotypes (Chagné *et al.,* 2007; Espley *et al.,* 2009). A minisatellite region in MYB10 R6 promoter is strongly associated with red fleshed apples (Espley *et al.,* 2009), but this allele is neither sufficient nor required for this trait in all genotypes (van Nocker *et al.,* 2012).

The obtained dendrogram (Figure 3) showed 18 genotypes from 20 individuals. The observed bands for Qazvin's genotypes (Qazvin-1 (6), Qazvin-2 (7) and Qazvin-3 (8)) had an overall homology. These Qazvin's genotypes (Figure 1) had a very close relationship with Haji Qermez (1), another member of this group. All individuals in this group had pink flesh, seeds, and skin (Figure 1). Having small-size and globose-shape fruit with three (Table 7), four or, on rare occasions, five seeds are their other characteristics. Qazvin's genotypes were own-rooted in the orchard and had anthocyanin in other parts of the plant such as trunk, branches, and flowers. Anthocyanin content in shoot cortexes shows a canonical correlation with the freezing tolerance of apple trees (Leonchenko, 1988).

According to the dendrogram and genetic distance table (Figure 3, Table 6), the red fleshed apples were classified into different groups, suggesting a high degree of variation among them. Red fleshed apple is a natural form of *Malus sieversii* native to central Asia (Harris *et al.,* 2002; van Nocker *et al.,* 2012). Hence, Iran, due to its location in the center of apple genetic diversity, i.e. Central Asia (Janick *et al.,* 1996), can be considered as an important center of diversity of red fleshed apples (Harris *et al.,* 2002; Gharaghani *et al.,* 2009; Richards *et al.,* 2009; Farrokhi *et al.,* 2011).

Golab-e Kohanz (10) and Golab-e Sahneh (18), in a distinct group, are common with red flesh-apples in some properties such as early ripening (Table 7); but their taste is mild and flavor ('Golab ' is an Iranian name, composited of Gol= Flower and Ab= Water, means fragrant), while red-flesh apples are tasty and somewhat sour.

In the fifth group, there were Fuji and Red Delicious genotypes. The parentage of Fuji apple is Ralls Janet and Red Delicious (Nafro, 1930); and, as revealed in the UPGMA dendrogram, Fuji is shown to be more closely related to Red Delicious. As expected, Gala and Golden Delicious were in the same group, an expected close relationship because Gala is a hybrid of crossing between Golden Delicious and the less-known variety of Kidd's Orange Red, developed in New Zealand (Kidd, 1930). The phylogenetic dendrogram (Figure 3) seems to be consistent with the pedigree of apple cultivars, rather than geographical regions, because Golab apples were collected from Kermanshah and Karaj

Table 7. Morphological traits of the individuals in this study.

Cultivars / Fruit traits	Fruit shape	Fruit size	Color of flesh	Time of harvesting
Haji Qermez [a]	Gobose	Small to medium	Red	Early
Gousht Qermez [a]	Globose	Small to medium	Green	Mid-season
Shahroud-10 [a]	Flat	Medium	Red	Mid
Arous gusht Qermez [a]	Globose	Medium	Yellow	Mid
B.9 [a]	Flat	Small to medium	Red	Mid
Qazvin-1 [a]	Flat	Small	Pink	Early
Qazvin-2 [a]	Flat	Small	Pink	Early
Qazvin-3 [a]	Flat	Small	Pink	Early
Shafiei	Globose	Medium to large	Cream	Mid-season
Golab-e Kohanz	Conical	Medium	Green	Early
Jonathan	Ellipsoid	Medium	Cream	Mid-season
Golden Delicious	Conical	Small to medium	Cream	Late
Granny Smith	Ellipsoid	Medium	Yellow	Late
Soltani Shabest	Ellipsoid	Medium	Green	Mid-season
Fuji	Ellipsoid	Medium	Green	Late
Red Delicious	Conical	Large	Yellow	Late
Heydar Zadeh	Ellipsoid	Medium to large	Cream	Mid-season
Golab-e Sahneh	Ellipsoid	Medium	Green	Early
Gala	Ellipsoid	Medium to large	Yellow	Mid
Shafi Abadi	Ellipsoid	Medium to large	Cream	Mid

[a] Red fleshed apple cultivar.

(Table 1), two different provinces of Iran (Gharaghani *et al.,* 2009).

These results along with similarity matrix can be used as an adequate tool for selection from among Iranian red fleshed apples for further apple breeding programs. This selection is possible because the highest distance was observed between Gusht-e Qermez (2) and Arous Gousht Qermez (4), while the lowest distance was seen between Qazvin's varieties (Table 4), all of which are classified as red fleshed apples. Since apples do not propagate easily from cutting and propagate more from seed (Juniper, 1999), the suitable material for crossing and releasing new cultivars are available in Iran. In New Zealand, a number of varieties have been developed by crossing between red fleshed apples originated from Kazakhstan (Espley *et al.,* 2009) and transgenic apples (Chagne *et al.,* 2009; Espley *et al.,* 2009).

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بررسی مقدماتی تنوع ژنتیکی سیب‌های گوشت قرمز ایرانی با استفاده از نشانگرهای ریزماهواره

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چکیده

سیب‌های گوشت قرمز حاوی مقادیر بالایی از آنتوسیانین هستند. ایران به علت واقع شدن در آسیای مرکزی، دارای تنوع قابل توجهی در این ارقام سیب می‌باشد. در این مطالعه، به منظور بررسی تنوع ژنتیکی موجود در بین ارقام سیب گوشت قرمز، ۲۰ ژنوتیپ سیب شامل هشت سیب گوشت قرمز ایرانی و ۱۲ رقم تجاری ایرانی و خارجی انتخاب شد. با استفاده از ۱۱ نشانگر ریزماهواره (SSRs) تنوع ژنتیکی و همبستگی رنگ قرمز در ارقام گوشت قرمز و گوشت سفید بررسی شد. از میان نشانگرهای استفاده شده، هفت نشانگر چندشکلی بالایی نشان دادند. در مجموع، ۵۶ آلل با دامنه ۳ تا ۱۱ و میانگین ۸ برای هر پرایمر شناسایی شد. تجزیه خوشه ای بر اساس الگوریتمUPGMA و ضریب تشابه Dice با استفاده از نرم افزارNTSYS-PC ver. 2.02 انجام شد. بر اساس دندروگرام بدست آمده، ژنوتیپ های مورد مطالعه به هفت گروه طبقه بندی شدند. هتروزیگوسیتی و شاخص شانون برای پرایمرها با استفاده از نرم افزارPOPGEN ver. 1.32 و برای دوگروه ایرانی و خارجی با استفاده از نرم افزارGene Alex ver. 6.3 برآورد شد. این نتایج برای حفاظت از این منابع ژنتیکی و انتخاب آنها برای برنامه های اصلاحی سیب در آینده مفید خواهد بود.