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

Sh. Faramarzi, A. Yadollahi, and B. M. Soltani

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; Kakhonen 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...
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; Silverberg-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
Genetic Diversity Among Iranian Red Fleshed Apples

Table 1. Apple genotypes used in this study.

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

The genotypes that are red-fleshed apple cultivars.

Collected from Horticultural Research Station at Kamal-Abad (Karaj, Iran).

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 mM of each primer, 0.2 mM of dNTP, 0.5 unit of Taq polymerase, 2mM MgCl₂, 10 mM of Tris- HCl (pH 8.5), and 19.1 µl of distilled H₂O; (Taq polymerase, MgCl₂ 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 (nₐ), effective alleles (nₑ), expected heterozygosity (Hₑ) (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 2. SSR primers sequence used in this study.

<table>
<thead>
<tr>
<th>Primer name*</th>
<th>Sequence</th>
<th>Tm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MdMYB17</td>
<td>F 5'-TGTCCTCTCTCTAGCTATTGCATAAT-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-AAGACTCAAAACTAGTCTGAAT-3</td>
<td></td>
</tr>
<tr>
<td>MdBHLH3</td>
<td>F 5'-CAACTCCCCCTATTCTTCTTCTTC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-CACCTGACCTCTCTCTCTCACTC-3</td>
<td></td>
</tr>
<tr>
<td>MdMYB12</td>
<td>F 5'-CTCGGCAATCGTAAAGCTA-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-TATGAAACGTGAAACCCTTACCCCTA-3</td>
<td></td>
</tr>
<tr>
<td>NZmsPa18</td>
<td>F 5'-GGCACAAGCACAAGGAAACA-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTTGAGCGCAGTCCATTCTCTTAT-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa113</td>
<td>F 5'-TCGATGAAACAGCCTGAAAG-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTAGGGGGACGGAAGAACAAAA-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa136</td>
<td>F 5'-CCTCAACAAAATATAAGACTCTCTC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTTCTCCACTCTCTCTCCGTACATT-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa145</td>
<td>F 5'-AAACCCAAACACAGAC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GCTTCTGTCGATCTGATG-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa151</td>
<td>F 5'-GATTTCCTCAGTAATCCTGCC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTTAAAAACACTCAAGCTCTGC-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa192</td>
<td>F 5'-GTTTCCTGTTTCACTGATT-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTGCCACATTTTCACCATA-3</td>
<td></td>
</tr>
<tr>
<td>NzmsPa1213rd</td>
<td>F 5'-CAACCTTTCCTCATTCTAC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTTCCTTTATGTACCGGCTT-3</td>
<td></td>
</tr>
<tr>
<td>NZmsPa1443rd</td>
<td>F 5'-AAAAACTCTCCTACCTCC-3</td>
<td>60-56</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTTTATCCTGCAAATCTGAGACC-3</td>
<td></td>
</tr>
</tbody>
</table>

*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.

Touchdown PCR by reducing 1°C for 4 steps.

RESULTS

SSR Polymorphism

Seven out of the 11 primers used to measure genetic diversity of the 20 genotypes showed polymorphism. Three SSRsMdMYB17, 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).

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.
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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 heterozygosities (H_o), expected heterozygosities (H_e), and Shannon index (I).

<table>
<thead>
<tr>
<th>Locus</th>
<th>N_a</th>
<th>H_o</th>
<th>H_e</th>
<th>I</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MdBHLH3</td>
<td>11</td>
<td>0.55</td>
<td>0.92</td>
<td>2.35</td>
<td>0.25</td>
</tr>
<tr>
<td>MdMYB12</td>
<td>5</td>
<td>0.25</td>
<td>0.54</td>
<td>1.03</td>
<td>0.23</td>
</tr>
<tr>
<td>NZmsPa18</td>
<td>8</td>
<td>0.90</td>
<td>0.85</td>
<td>1.92</td>
<td>0.35</td>
</tr>
<tr>
<td>NZmsPa113</td>
<td>11</td>
<td>0.75</td>
<td>0.88</td>
<td>2.15</td>
<td>0.33</td>
</tr>
<tr>
<td>NZmsPa136</td>
<td>8</td>
<td>0.30</td>
<td>0.83</td>
<td>1.84</td>
<td>0.26</td>
</tr>
<tr>
<td>NZmsPa145</td>
<td>3</td>
<td>0.45</td>
<td>0.52</td>
<td>0.88</td>
<td>0.26</td>
</tr>
<tr>
<td>NZmsPa1213</td>
<td>10</td>
<td>1.00</td>
<td>0.87</td>
<td>2.07</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean</td>
<td>8</td>
<td>0.60</td>
<td>0.77</td>
<td>1.75</td>
<td>0.29</td>
</tr>
</tbody>
</table>

* Observed alleles, ‡ observed heterozygosity, † expected heterozygosity, ‡ Shannon index, ‡ Polymorphic information content.
Table 4. Genetic parameters of the two populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Iranian</td>
<td>N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Na&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>1.82±0.08</td>
<td>1.28±0.03</td>
</tr>
<tr>
<td>Foreign</td>
<td>7</td>
<td>1.36±0.13</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among population</td>
<td>1</td>
<td>14.43</td>
<td>0.68</td>
<td>8</td>
</tr>
<tr>
<td>Within population</td>
<td>18</td>
<td>148.37</td>
<td>8.24</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>162.68</td>
<td>8.92</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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...
### 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   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 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| 0.60| 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| 0.20| 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; Goulao 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, compositied of Gol= Flower and A= 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.

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

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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