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**Research paper**

# Characterization of some wild *Berberis sp.* genotypes distributed in the northeast of Iran

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**Abstract**
The wild barberry shrubs, commonly known as black barberry, are naturally widespread in the north and northeast elevations of Iran. Besides a rich gene bank, these are highly considered concerning food and medicinal purposes. The present research work was undertaken to evaluate the genetic diversity among 15 different barberry genotypes (14 wild and one cultivated genotypes) through morphological, biochemical, and molecular markers. The morphological traits of leaf, thorn, and berry were measured. The biochemical properties of the studied genotypes were also measured at the fruit ripening stage. Furthermore, the genotypes were subjected to simple sequence repeat analysis to ascertain their genetic diversity at the molecular level. The studied accessions were diverse in the case of morphological traits and they were classified into five distinct groups. Moreover, some rare and remarkable morphological characteristics were found in the fruit shape and fruit clusters of some genotypes not reported earlier. Though wide differences were obtained concerning fruit biochemical compounds, the differences didn't have a clear trend. The accessions were characterized based on microsatellite analysis into eight groups in which the closely related genotypes had relatively higher geographical similarities. Access to the genetic diversity of these genotypes may be considered as the backbone of their future breeding programs and the reported data supported that the northeast of Iran may be assumed as a rich source for the diversity of *Berberis* germplasm.

**Keywords:** *Berberis*; breeding; diversity; marker; microsatellites

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## Introduction

Iran is a mountainous land with a high central plateau which is surrounded by mountains in all four geographical directions. Almost 54% of the land area is covered with mountains (Sagheb-Talebi 2014). Owing to its large size and varied ecosystems, Iran is one of the most important countries in the Middle East and Western Asia for the conservation of biological diversity. In the Iranian ecosystems, over 8,000 plant species have been recorded. This includes a large number of wild relatives of commercial plant species, confirming Iran's status as a center of genetic biodiversity (Anonymous 2010).

Barberry (*Berberis* sp.; Berberidaceae), is a compact and spiny shrub that commonly grows 1.5 to 2 meters in height but may rarely reach three meters long (Cadic 1992; Kafi *et al*. 2002). The inner bark of the barberry stem has a specific yellow color. As a medicinal plant, it has been

known and utilized for a long time in Iran and many other world ancient civilizations (Zargari 1990; Rahimi-Madiseh *et al.* 2017). It has been reported that Berberidaceae have approximately 15 genera and 650 species found in temperate regions of the northern hemisphere (Bottini *et al.* 2002). In Iran, some barberry species such as *Berberis vulgaris*, *B. orthobotrys*, *B. khorasanica*, *B. crataegina*, and *B. integerrima* were already reported (Kafi *et al*. 2002; Alemardan et al. 2013). Besides the common seedless barberry (*Berberis vulgaris* C.K. Schn. var. asperma Don), which is the only cultivated species in the eastern parts of Iran (Kafi *et al*. 2002; Rezaei *et al.* 2012), seeded barberries (commonly known as black barberry) are naturally widespread in north and northeast elevations of Iran. The differences between seedless and seeded barberries are represented in Table 1. Furthermore, Iran is the largest producer of barberry in the world and the Khorasan province holds 95% of the world barberry production with about 97% of the cultivated lands (Mokhber Dezfuli *et al.* 2013).

Table 1. The summarized differences between seedless and seeded barberries

| Judgment item | Seedless barberry | Seeded barberry | References |
|---|---|---|---|
| Scientific name | *Berberis vulgaris* C.K. Schn. var. asperma Don | *B. vulgaris* L., *B. orthobotrys* Bienert, *B. crataegina* D.C., *B. integerrima* and *B. khorasanica* Browicz | Balandary and Kafi (2001) |
| Common name | Barberry (Bidaneh and Pofaki in Iran) | Black barberry, Wild barberry | http://flora-iran.com/ |
| Common uses | Food flavor and jam | Mostly used as barberry juice | - |
| Medicinal properties | Seedless barberries are mostly used for food purposes but they used to treat fever, cough, and liver diseases | Fever, cough, liver disease, depression, hyperlipidemia, hyperglycemia, and bleeding | Mokhber Dezfuli *et al.* (2013)<br>Alimardan *et al.* (2013) |
| Growing sites in Iran | 95% in east of Iran (south Khorasan) | 82 sites in 21 different provinces | Balandary (2017) |
| Plant type morphology | Small bushes | Bushes mostly in dense populations | Kafi *et al.* (2002) |
| Berry color | Only red | Mostly purple-black, some deep red and red | http://flora-iran.com/ |
| Number of seeds | No seed | 1-2 and in some cases degenerated seeds | -do- |
| Seed size | No seed | 5-7 mm | -do- |
| Thorn size | Usually less than 3 cm | Sometimes to 5 cm | Talebi (2019) |
| Leaf color | Green | Both green and red | -do- |
| Decorative plants | Not common | Mostly used as ornamentals and developing hedgerows | -do- |

The seeded wild barberries widespread in eastern parts of Iran, are dense bushes, grown as populations in elevations higher than 1000 meters (Tehranifar 2003; Talebi 2019). The fruits of these accessions are mostly collected by native people to prepare barberry juice (known as "Abzereshk" in Iran). The juice seems to slightly reduce blood sugar levels in people with diabetes. The berries of the seedless barberry also are traditionally used to make jams and jellies. The herb also has a long history as a folk remedy for digestive disorders, including constipation, diarrhea, dyspepsia, heartburn, and loss of appetite (Rahimi-Madiseh *et al.* 2017). The phytochemical and pharmacological properties of *Berberis* species were already reviewed by Mokhber Dezfuli *et al.* (2013). As it is obvious in the literature, most of the studies have been focused on the medicinal properties of barberry, but the evaluation of genetic diversity and structure of its populations in northeastern parts of Iran has yet not been extensively investigated.

It is obvious that genetic diversity is the basis of plant selection and it provides the viability of a species or a community through the ability to adapt to different environmental conditions (Bataillon *et*

*al*. 1996). Access to genetic diversity may be considered as the backbone of breeding works and most breeding techniques widely depend on plant genotypes and species biodiversity. In the study of genetic diversity, morphological, biochemical, and molecular markers are used. Breeders usually prefer the use of DNA-based molecular markers to others to ensure the accuracy of variation based on morphological markers. However, the evaluation of morphological traits still is a routine procedure in diversity studies (Singh 2014). Studies on the genus of barberry have shown that morphological variation among barberry species is high (Butini *et al*. 2000). The microsatellite (SSR) markers have advantages such as high polymorphism, homologous inheritance, easy reproducible, abundance and wide distribution, and randomization across large genomic DNA sequences, hence, these markers are used in numerous plant breeding programs (Weising *et al.* 2008; Singh 2014). The DNA markers are more differentiated than morphological and protein markers (Smith *et al.* 1997). Micosatellite markers generally show higher levels of polymorphism and are able to distinguish even very similar strains due to the frequency of alleles at each locus (Nachit *et al.* 2001). Other features of microsatellites are co-dominance expression and the ability to detect heterozygous individuals (Ovesna *et al.* 2002).

Studies on the identification, diversity, and genetic structure of populations in barberry are limited and insufficient (Talebi 2019). In research works already performed by Rezaei *et al.* (2012) and Heidary *et al.* (2009), the genetic diversity of Iranian barberry genotypes was studied using SSR and AFLP markers, respectively. Varas *et al.* (2013) introduced 18 microsatellite markers (SSRs) from *B. microphylla* and their results showed that these new SSR markers are highly polymorphic and useful in genetic studies in any species of *Berberis*.

The study of the barberry species in different parts of the world suggests the existence of a great diversification due to the processes of mutation and recombination as a result of natural crosses among different species. Such conditions have created numerous strains and absolutely new individuals that their botanical relationships are remained unknown (Talebi 2019). Bottini *et al.* (2002) in southern Argentina examined the genetic diversity of 13 barberry species. They evaluated the relationship among diploid and polyploid populations with the AFLP marker. Rob and Durka (2006) studied microsatellites from *Mahonia aquifolium* (another genus of berberidaceae) and showed the usefulness of these markers for detecting the genetic origin of *Mahonia* populations and characterization of native populations.

Due to incidence of close phenotypic similarities in *Berberis* genus and the existence of multiple inter-phenotypic hybrids as well as the existence of many genotypes in the different barberry species, their classification based on morphological traits is difficult (Bottini *et al.* 2007). The present research objective was to characterize the barberry genotypes based on morphological and biochemical characters. The genotypes were also subjected to the SSR analysis for molecular characterization. Furthermore, it was attempted to compare these wild genotypes with a

seedless cultivar which is the only cultivated species collected from barberry orchards located in east of Iran.

## Materials and Methods

### *Plant materials and growing sites*

The wild, seeded barberry genotypes are well-known shrubs in northeast of Iran and 15 different accessions from four populations were selected for the analysis. The seeded genotypes were collected from Khorasan as well as Golestan provinces and the cultivated, seedless variety sample was procured from Birjand, Sothern Khorasan (east of Iran). This seedless variety which is commonly known as "*Berberis vulgaris* C.K. Schn. var. asperma Don" (Figure 1) is the only cultivated seedless cultivar in Iran. The accessions were labeled as, D1-D3: Daregaz 1-3; G1-G6: Golestan 1-6, SH1-5: Shirvan 1-4 and BD: Bidaneh. These names were selected based on the name of barberry growing sites. The last genotype was also named BD because the seedless barberry in Iran is so-called "Bidaneh". The address sites and geographical situation of the studied area are shown in Table 2. These shrubs are distributed as small populations over some north and northeast altitudes of Iran. The selection and sample preparation were undertaken based on obvious morphological differences. In each growing site, the genotypes were sampled at least 200 meters apart from each other. The leaves were collected in spring (April) but the collection of fruits were performed over multi-step visits (during October) due to different ripening times already observed among genotypes grown in different sites. The harvesting time for barberry fruits was determined based on ethno-botanical information obtained from native people who were involved in wild barberry fruit collection.

### *Morphological traits*

The morphological traits were observed and recorded in leaves, thorns, and fruits of the barberry genotypes. A set of 20 leaves, 20 thorns and 20 clusters were randomly selected from each genotype for morphological measurements. Number of leaves per each node, petiole length, thorn size, thorn angle, cluster length, and number of adjoining leaves per cluster were recorded. The berrys' physical dimensions (length, width, ratio of length to width) were measured by a digital slide-caliper, thorn angle by a half circle protractor, and

Table 2. The address and geographical features of barberry (*Berberis sp.*) collection sites

| Genotype name | Genotype code | Collection site address | Latitute (msl) | GPS data |
|---|---|---|---|---|
| Daregaz 1 | D1 | Khorasan Razavi, Ghoochan-Daregaz Road, Zoviab, Dareh Gharehkhan | 1376 | N37 34 47, E58 36 17 |
| Daregaz 2 | D2 | -do- | 1379 | N37 34 47, E58 36 14 |
| Daregaz 3 | D3 | -do- | 1385 | N37 34 47, E58 36 12 |
| Shirvan 1 | SH1 | North Khorasan, Shirvan, between Ghanlogh and Hanameh villages | 1434 | N37 31 48, E58 02 34 |
| Shirvan 2 | SH2 | -do- | 1429 | N37 31 48, E58 02 32 |
| Shirvan 3 | SH3 | -do- | 1505 | N37 32 14, E58 44 66 |
| Shirvan 4 | SH4 | -do- | 1502 | N37 32 13, E58 04 40 |
| Shirvan 5 | SH5 | -do- | 1558 | N37 32 30, E58 05 10 |
| Golestan 1 | G1 | Golestan, Gorgan, Toskestan Road | 1945 | N36 41 13, E54 33 57 |
| Golestan 2 | G2 | Golestan, Gorgan, Shahkooh village | 2077 | N36 34 40, E54 26 34 |
| Golestan 3 | G3 | -do- | 2092 | N36 33 33, E54 26 44 |
| Golestan 4 | G4 | -do- | 2080 | N36 34 37, E54 26 34 |
| Golestan 5 | G5 | Golestan, Ramian, Olang | 1804 | N36 50 45, E55 14 45 |
| Golestan 6 | G6 | Golestan, Azadshahr-Shahrood forked road | 1771 | N36 46 18, E55 18 10 |

| | | | | |
|---|---|---|---|---|
| Bidaneh | BD | South Khorasan, Birjand | 1491 | N32 87 00, E59 21 00 |


Figure 1. Fruit clusters of common seedless barberry (*Berberis vulgaris* C.K. Schn. var. asperma Don), the only cultivated variety in Iran.

the berry weight by electronic balance. The volume of 100 dried berries was recorded by volumetric cylinder without pushing the surface of the fruit mass.

***Biochemical characters***

The fresh berry samples were collected in October. The damaged and smashed fruits were discarded and the residues were divided into two groups. Some were freshly used for analysis and the others were shade dried in room temperature (25 $^{\circ C}$) for future biochemical measurements. The electrical conductivity (EC) of the fruit juice was recorded by an electrical conductivity meter and the pH of the fruit juice was measured with a pH-meter. The soluble solids content was measured using a digital refractometer. The acidity of the fruits was measured by 0.1 N sodium hydroxide. The fruit juice acidity was calculated based on the dominant acid of barberry fruits, *i.e.* malic acid (Farhadi Chitgar *et al.* 2016) and was reported in mg of malic acid per 100 ml of fruit juice (AOAC 1984). The amount of some important carbohydrates *i.e.* total soluble sugars (TSS), glucose, fructose, and sucrose were measured in already shade dried fruits. TSS content was measured by the method of McCrady *et al.* (1950). Glucose was measured according to the Miller method (1959). Handel (1968) method was used to measure sucrose. Fructose was also measured using the method of Ashwell (1957).

***Molecular analysis***

The SSR markers were used for molecular evaluation of the genotypes. The DNA extraction was performed by the CTAB method (Doyle 1987). DNA quantification and quality were determined by 0.8% agarose gel electrophoresis and spectrophotometer, respectively. Seven pairs of SSR primers already reported to show reasonable polymorphism in *Mahonia* (another genus belongs to Berberidaceae) (Rob and Durka 2006) and *Berberis* (Rezaei *et al.* 2012), were selected and applied for PCR reaction (Table 3).

Polymerase chain reaction was performed using a Bio-Rad thermocycler in a volume of 25 µl (Williams *et al*. 1990). Components of the PCR reaction consisted of 3µl genomic DNA, 2 µl 10X PCR buffer, 0.5 µl dNTP 1 mM, 10 µl of each primer (10 µM), 0.2 µl Taq DNA polymerase (5 units per µl), 1.6 µl of 50 mM $MgCl_2$, 14.8 µl of double distilled water, and the final reaction volume was 25 µl. Thermal cycles for the polymerase chain reaction consist of an initial denaturation step at 94 °C for 3 min followed by 35 cycles where each cycle comprised a denaturation at 94 °C for 30 seconds, the annealing phase for 60 second and the extension step at 72 °C for 2 minutes. Primer annealing was performed at the specific temperature of each primer at 57 °C to 63 °C, and the replication was at 72 °C for 90 seconds, and the final replication was continued at 72 °C for 10 minutes. PCR products were subjected to polyacrylamide gel electrophoresis. The band scoring was performed based on presence (1) or absence (0) of bands. Then, the polymorphism and genetic distance in microsatellite sites were measured. Finally, cluster analysis of barberry genotypes based on SSR markers with the UPGMA method was undertaken.

Table 3. The list of SSR primers tested for molecular characterization of barberry accessions (The annealing temperature was optimized from 57 to 63 ºC [as] proposed by Rob and Durka (2006)

| S/N | Primer Name | Primer sequence (3'>5') |
|---|---|---|
| 1 | GA05 | Forward: AGTCATCCCTCCATCATTCG<br>Reverse: TGTGAGAGCTCTGTTGGACTG |
| 2 | GA31 | Forward: TCACAATAGTTTATTTGAGTTTATTTG<br>Reverse: CACTGTCTGGCTCAATTTTGTC |
| 3 | GA33 | Forward: GATCAGGTCAATATCAAAGTTC<br>Reverse: CAGACAAGGAGAGTGCTTGATCC |
| 4 | GA34 | Forward: GGATGAGGGAGGTGTAACAATG<br>Reverse: ACCCATTGGAGCTCTCTCAG |
| 5 | GA04 | Forward: TTGATTTTGAAGCCGAGATG<br>Reverse: TGCATTTTCGACCCATCTAC |
| 6 | CA30 | Forward: TCTCCTCACATGCAACAAAAG<br>Reverse: TCCGCTTTCCACTTACCATC |
| 7 | CA03 | Forward: GGGGTGTGACCGTTTTTATG<br>Reverse: CAATGCCCGAAAGTTACGTC |

***Statistical analysis***

The experiment was carried out as completely randomized design with three replications for the biochemical traits. The morphological traits were also measurde in triplicate but the average for each plot was obtained from 20 observations. Analysis of variance was performed by the GLM procedure in SAS software (SAS Institute Inc. 2003) and the means were compared by the Least Significant Difference (LSD) test with at $p \leq 0.01$

**Results and Discussion**

***Morphological analysis***

Heidary *et al.* (2009) have already reported that morphological markers had lower efficiency than AFLP markers for grouping and systematic studies of the Berberidaceae family. Bottini *et al.* (2002) also stated that the taxonomic treatment of species in the *Berberis* genus based on external morphology is still a matter of debate. But, still these markers were frequently used for characterization of many fruit crops (Lacis *et al.* 2009; Singh, 2014; Tatari et al. 2019). The wild barberry shrub forms dense stands in natural

habitats. The off-shoots produced from root is a natural way for barberry propagation (Balandary and Kafi 2002) which led to establishing spiny and very dense impermeable populations. Hence, the shrubs close to each other are mainly cloned through vegetative propagation during previous years. However, there are also some reports that reproduction in wild barberry is primarily taken place through seeds (high germination rate) and then seeds are distributed by birds and mammals (Talebi 2019). According to the latter points, there would be absolutely morphological differences among populations. In the present study differences in leaves, thorns, and fruits were recorded. A view of bearing shoots collected from each accession representing leaf, thorn, as well as fruits, is depicted in Figure 2.

The diversity in thorn and leaf characteristics

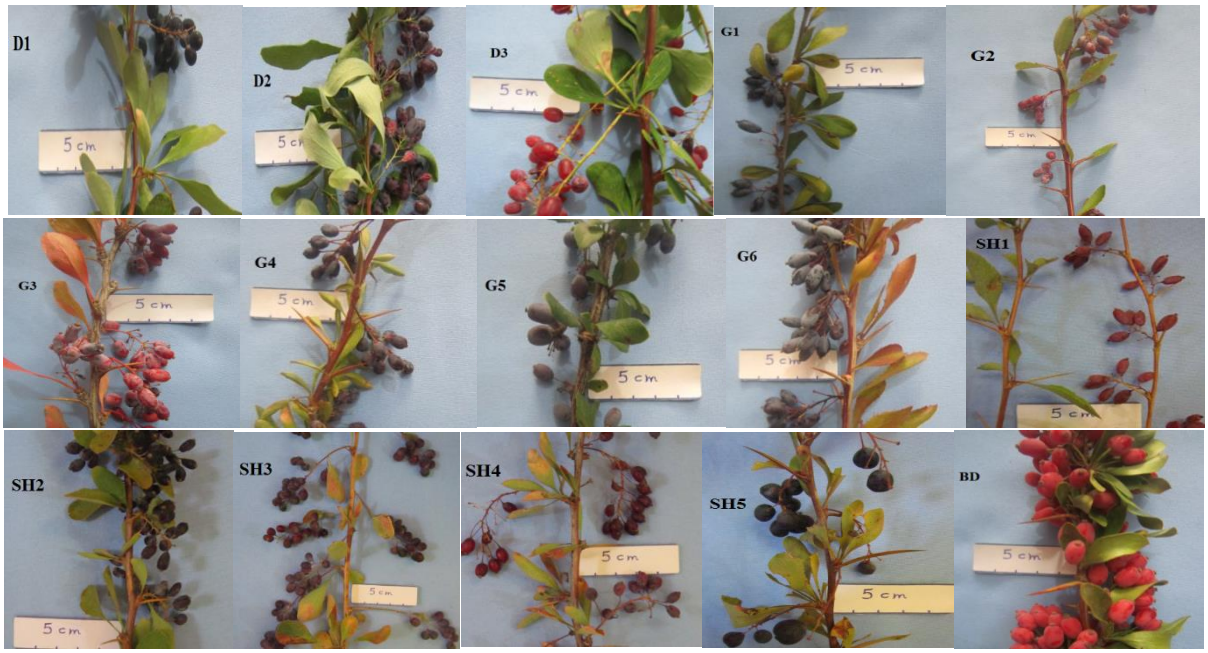


Figure 2. The leaf and fruit cluster characteristics of studied barberry (*Berberis sp*.) accessions (D1-D3: Daregaz 1-3, G1-G6: Golestan 1-6, SH1-5: Shirvan 1-5 and BD: Bidaneh).

is presented in Table 4. The genotypes were different in case of number of leaves produced per node and the highest number of leaves per each node (8.6) was recorded in D3 genotype which was significantly higher than others. The similar diversity was also seen with respect to petiole length (0.3 to 1.39 cm: long petiole in D3 to very short in G5 accession). Among the Golestan accessions, G2 had lower values with respect to number of leaves per node, petiole length, thorn length, thorn angle, cluster length, and number of leaves per cluster. Such diversity may have arisen due to acclimation and adaptation to special climatic conditions. Heidary *et al.* (2009) also demonstrated considerable diversity among *Berberis* populations of Khorasan province (Iran). Bottini *et al.* (2000) and Farhadi Chitgar *et al.* (2016) also observed extensive morphological diversity among barberry genotypes. Another report from Pakistan (Ahmed *et al.* 2013) stated that genotype and growing site are the most

important factors determining growing habit, yield, and nutritional value of barberries.

Table 4. The diversity in leaf and thorn characteristics of barberry (*Berberis sp*.) genotypes in north-east of Iran

| Accessions | | Measured characteristics | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Code | No. of leaves per node | Petiole length (cm) | Thorn length (cm) | Thorn angle (degree) | Cluster length (cm) | No. of leaves per cluster |
| Daregaz 1 | D1 | 5.44c | 1.39a | 1.81ef | 76de | 3.85fg | 10.3de |
| Daregaz 2 | D2 | 8.60a | 1.18bc | 3.96a | 106a | 4.23de | 17.8a |
| Daregaz 3 | D3 | 5.55c | 1.39a | 1.10g | 94bc | 7.22a | 13.7bc |
| Shirvan 1 | SH1 | 4.3ef | 0.94de | 2.13cd | 84d | 5.03gh | 11.4cd |
| Shirvan 2 | SH2 | 5.25cde | 1.42a | 1.54f | 102ab | 3.07gh | 10.7bc |
| Shirvan 3 | SH3 | 4.20f | 0.83ef | 1.19g | 65fg | 7.3a | 14.3b |
| Shirvan 4 | SH4 | 6.13bc | 1.00d | 2.26c | 97bc | 4.55cd | 13.1bc |
| Shirvan 5 | SH5 | 6.92b | 1.32ab | 3.27b | 82d | 5.2b | 10.1bc |
| Golestan 1 | G1 | 4.33def | 0.63g | 0.96g | 63gh | 2.76h | 8.33efg |
| Golestan 2 | G2 | 3.00g | 0.36h | 1.20g | 56h | 2.8h | 6.33g |
| Golestan 3 | G3 | 4.33ef | 0.66fg | 1.83ef | 66fg | 3.3fgh | 8.00g |
| Golestan 4 | G4 | 5.33cd | 1.06cd | 1.66f | 66fg | 6.44bcd | 9.33def |
| Golestan 5 | G5 | 5.33cd | 0.30h | 1.83ef | 76de | 2.9h | 10.33de |
| Golestan 6 | G6 | 4.33def | 0.63g | 1.76ef | 73ef | 5.06bc | 13.33bc |
| Bidaneh | BD | 5.66c | 0.40h | 2.23c | 93c | 3.70ef | 18.00a |

*Means in the same column followed by different letter(s) are significantly different at $p \leq 0.01$using the LSD test.

The differences in berry characteristics may be more important in our studied accessions (Table 5), because the fruit traits are typically more significant breeding objectives in the barberry improvement (Talebi 2019). The longer berries were observed in G series, particularly in G6 with longest berry length (11.97 mm). The berry diameter was also different among accessions and the bold berries (7.2 mm) were found in SH5 fruits. The length to diameter ratio determines the geometric shape of the fruits and according to Table 5 the berries of SH5 accession had the roundest fruits. Also, duo to this special fruit shape, the volume of 100 berries in this accession was found to be higher than others.

The pedicle length varied from 4.33-9.6 mm in these accessions (D1 and BD, respectively). This trait may be helpful during fruit harvesting. It is noteworthy that barberry fruits are harvested by three methods: branch-cutting, impact force, and cluster-picking (Alavi and Mazloumzadeh 2009). In the cluster picking approach, which is essentially used for consuming as fresh fruits, the workers separate the clusters one by one from the branches by hand. This method is time consuming and due to the sharp thorns within the branches, the harvest time is severely increased. The longer pedicle (G5 and BD accessions) and presence of shorter thorns (G1) may lead to facilitate harvesting with ease. So, these characters may be useful for future barberry breeding programs.

As the berry shape and size were different in these genotypes, the 100 berry fresh and dry weights were also statistically different (Table 5). Among these accessions only BD was seedless and it may be expected that its fruit weight must be lower than the seeded ones but it was demonstrated that most of seeded accessions attained lower fruit weight compared to this seedless genotype. The highest fruit fresh weight (28.5 g in 100 berries) was recorded in G5 accession.

The cluster analysis of different *Berberis* accessions based on morphological data is shown in Figure 3. The accessions were grouped in five distinct branches (Euclidean distance of 0.5). In the first group only D1 accession was located. This accession was superior to others with regard to berry diameter and dry berry weight. Similarly, G5 accession was located in another separate branch. The seedless BD genotype was morphologically close to D2 and these were located together in a another group. The characterization of these accessions in five distinct groups revealed diverse morphological differences among accessions in north-east of Iran. This variation may be useful in future breeding programs and isolation of promising lines. Regardless of morphological diversity observed in different barberry accessions, we found two interesting unique characters (Figure 4) in the studied genotypes, which were not already reported in the literature. The first character was grapevine-like, long fruit clusters demonstrated in the SH3 accession. Furthermore, this accession had typical fruit shapes (jug-like fruits) as well. The second trait was long thorn size (about 5 cm) observed in the D2 accession (Figure 4).

Table 5. The differences in berry characteristics of barberry (*Berberis sp*.) genotypes in north-east of Iran

| Accession | | Measured characteristics | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Code | Berry length (mm) | Berry width (mm) | Berry length: width ratio | Fresh weight of 100 berry (g) | Dry weight of 100 berry (g) | Volume of 100 berry (ml) | Pedicle length (mm) | Berry color (mature stage) |
| Daregaz 1 | D1 | 7.35h | 5.63cde | 1.3ef | 16.44g | 5.43de | 125h | 4.33g | Purple |
| Daregaz 2 | D2 | 7.88fg | 5.23ef | 1.49cd | 10.6m | 3.16i | 170d | 5.66def | Purple |
| Daregaz 3 | D3 | 7.57gh | 6.17b | 1.22ef | 21.47d | 6.79c | 150fg | 6.00de | Bright red |
| Shirvan 1 | SH1 | 8.46e | 5.09fg | 1.66b | 14.86i | 4.59fg | 155ef | 4.33g | Red |
| Shirvan 2 | SH2 | 7.85fgh | 5.16ef | 1.52c | 14.47j | 4.46fgh | 160e | 4.33g | Bluish black |
| Shirvan 3 | SH3 | 9.41d | 6.86a | 1.36de | 26.67b | 8.55a | 200b | 4.66fg | Scarlet |
| Shirvan 4 | SH4 | 7.60gh | 5.38def | 1.41cde | 15.94h | 4.95ef | 155ef | 8.66ab | Scarlet |
| Shirvan 5 | SH5 | 8.21ef | 7.23a | 1.13g | 21.03e | 6.58c | 225a | 8.66ab | Bluish black |
| Golestan 1 | G1 | 10.53d | 5.41def | 1.95a | 16.54h | 5.57d | 145g | 6.00de | Black |
| Golestan 2 | G2 | 9.12d | 4.66g | 1.96a | 11.43l | 3.91h | 160e | 5.66def | Brick red |
| Golestan 3 | G3 | 10.00c | 5.84bcd | 1.72b | 13.9k | 5.31de | 149fg | 5.00efg | Red |
| Golestan 4 | G4 | 8.56e | 5.80bcd | 1.47cd | 16.26gh | 4.99def | 149fg | 7.66bc | Purple |
| Golestan 5 | G5 | 11.38b | 6.80a | 1.67b | 28.5a | 7.8b | 199b | 9.00a | Purple |
| Golestan 6 | G6 | 11.97a | 6.02bc | 1.98a | 24.6c | 7.75b | 190c | 6.60cd | Purplish black |
| Bidaneh | BD | 10.46c | 6.00bc | 1.74b | 18.8f | 4.37gh | 169c | 9.60a | Bright red |

*Means in the same column followed by different letter(s) are significantly different at $p \leq 0.01$ using LSD test.

***Biochemical analysis***

The results of the measured biochemical characteristics are presented in Tables 6 and 7. The clear differences were observed among the accessions based on their carbohydrate profile. They were significantly different with respect to TSS. The SH3 was found to have the highest levels of TSS (2.52 mg/g FW). The amount of TSS in SH populations was generally higher than other groups. There were no significant differences among accessions with respect to their sucrose content, but they were different with respect to fructose and glucose content. The BD seedless cultivar was superior in fructose content compared to others. According to Farhadi Cheitgar *et al.* (2014), the amount of reducing sugars in the seedless species was almost twice that of the wild

seeded species. This statement justifies the sweeter fruits of seedless barberry.

There was a large variation in ionic characteristics in the studied accessions (Table 6). The highest and lowest pH values (3.17 and 2.66)

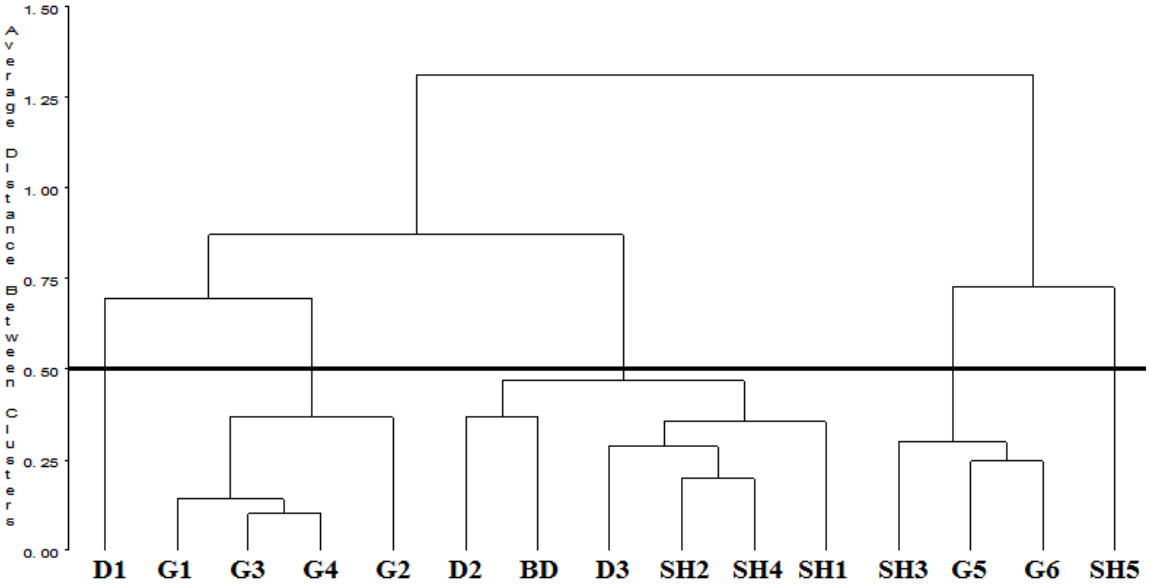


Figure 3. Dendrogram from the cluster analysis of barberry accessions based on morphological traits (D1-D3: Daregaz 1-3, G1-G6: Golestan 1-6, SH1-5: Shirvan 1-5 and BD: Bidaneh).

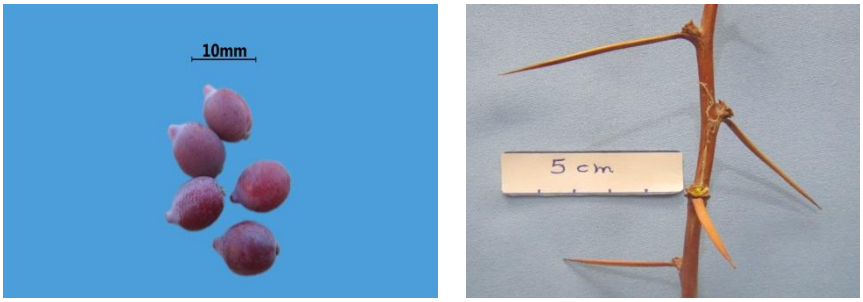


Figure 4. Long fruit cluster (up) and rare jug-like berry shape of SH3 (down-left) and long thorn size in the D2 barberry accession (down-right).

were measured in D3 and G4 accessions. BD had the highest TSS (10.76%) that was significantly higher than all other samples. The fruit juice acidity in SH5 genotype was higher than other genotypes (3.31 compared to lowest acidity 1.38) in fruit juice of D3). Farhadi Cheitgar *et al.* (2014) have already stated that generally wild species have higher acidity and lower pH. D3, which had higher pH than other accessions, also had lower acidity than others. Also, the amount of acidity in different genotypes is mainly related to their growth environment and altitude (Nazari *et al.* 2015).

Table 6. The carbohydrates and ionic characteristics of barberry (*Berberis sp*.) accessions in north-east of Iran

| Measured characteristics | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Accession | | Carbohydrates | | | | Ionic traits | | | |
| Name | Code | Total sugars (mg/ g DW) | Sucrose (mg/g DW) | Fructose (mg/g DW) | Glucose (mg/g DW) | Acidity | TSS (%) | pH | EC (ds/m) |
| Daregaz 1 | D1 | 2.22ef | 0.096a | 0.34h | 1.49e | 1.58g | 7.20h | 3.10b | 5.36a-d |
| Daregaz 2 | D2 | 2.30c-f | 0.102a | 0.43de | 1.57cde | 2.14d | 9.40b | 2.90gh | 5.44abc |
| Daregaz 3 | D3 | 2.35b-e | 0.111a | 0.38fg | 1.63a-d | 1.38h | 8.36c | 3.17a | 4.70c-g |
| Shirvan 1 | SH1 | 2.41abc | 0.106a | 0.4ef | 1.67abc | 1.77f | 7.76e | 3.05d | 5.69ab |
| Shirvan 2 | SH2 | 2.24def | 0.105a | 0.35gh | 1.53de | 2.33c | 7.70ef | 2.88h | 5.29a-e |
| Shirvan 3 | SH3 | 2.52a | 0.118a | 0.43de | 1.70ab | 2.03de | 7.63f | 2.95f | 5.55ab |
| Shirvan 4 | SH4 | 2.3c-f | 0.107a | 0.34h | 1.57cde | 1.89ef | 6.83i | 2.99e | 5.95a |
| Shirvan 5 | SH5 | 2.48ab | 0.114a | 0.44cd | 1.72a | 3.31a | 7.33g | 2.66l | 4.65d-g |
| Golestan 1 | G1 | 2.25c-f | 0.095a | 0.34c | 1.58cde | 1.48gh | 4.60l | 3.07c | 4.44fg |
| Golestan 2 | G2 | 2.20ef | 0.101a | 0.34h | 1.59b-e | 1.36h | 4.63l | 3.10b | 4.04g |
| Golestan 3 | G3 | 2.17f | 0.097a | 0.35gh | 1.57cde | 2.38c | 6.03k | 2.73j | 4.49fg |
| Golestan 4 | G4 | 2.28c-f | 0.106a | 0.51b | 1.62a-d | 3.10b | 8.10d | 2.66l | 5.07b-f |
| Golestan 5 | G5 | 2.32b-f | 0.112a | 0.35h | 1.59b-e | 2.34c | 4.23j | 2.76i | 4.70c-g |
| Golestan 6 | G6 | 2.38a-d | 0.113a | 0.44cd | 1.64a-d | 2.49c | 7.40f | 2.69k | 5.05b-f |
| Bidaneh | BD | 2.35b-e | 0.110a | 0.65a | 1.63a-d | 2.08d | 10.76a | 2.92g | 4.60e-g |

*Means in the same column followed by different letters are significantly different at $p \leq 0.01$ using LSD test; TSS: Total soluble sugars

Earlier, Farhadi Cheitgar *et al.* (2014) reported that TSS was about 18.9% for *B. vulgaris* and about 17.15% for *B. crataegina*. In our study, TSS in different genotypes varied between 4.23 to 10.76%. Meanwhile, the TSS of fruits collected from Golestan province was lower than Shirvan and Daregaz genotypes. It seems that geographical location of different barberry genotypes certainly affects their phytochemical traits, especially TSS. Therefore, the genotypes of Golestan province,

collected from higher altitudes (over 1900 msl), compared to other genotypes had lower TSS. Nazari *et al.* (2015) also stated that with increasing altitude of the growing location of different barberry species, the percentage of TSS in their fruit decreased. Fallahi *et al.* (2010) also indicated that the amount of TSS in barberry species depends on their location and time of collection. Farhadi Cheitgar *et al.* (2013) have already reported that high carbohydrate content in wild genotypes is related to the existence of seed in these genotypes. for total phenols, flavonoids, anthocyanin, tannin, and pectin are shown in Table 7. The highest phenol content was observed in the D1 accession. The BD accession had the lower phenols as compared to other populations. This is a common phenomenon and was already confirmed by other researchers such as Zovko *et al.* (2010), who stated that wild genotypes produce high phenolics compared to the cultivated varieties. The variation in the flavonoid content was also significantly different among genotypes (Table 7). Montora *et al.* (2005) stated that phenols and flavonoids are important secondary metabolites in plants, such as *Berberis*, and they have different roles including antioxidant, anti-microbial, and anti-cancer properties. It has been also stated that the highest levels of phenols is synthesized in barberry fruits while more flavenoids are present in the leaves. Barberry is a rich anthocyanin fruit (Mokhber Dezfuli *et al.* 2013) and among the studied accessions, the amount of anthocyanin in D1 and SH5 was estimated to be more than 600 µg/g DW. It was found that the anthocyanin content is highly correlated with altitude. For example, within the G populations, the anthocyanin was negatively affected by the growing site elevation. A similar trend was also demonstrated by Nazari *et al.* (2015).

There was a considerable difference among populations with regard to the berry pectin content and the BD accession had the lowest pectin level. The D3 was found to have the highest level of pectin even. As the barberry fruit is mostly utilized for jam and jelly preparation (Talebi 2019), this trait in the D3 genotype may be considered in future by food researchers for pectin extraction.

Andola *et al.* (2011) also indicated that wild genotypes had higher carbohydrate content than the cultivated seedless genotypes. The differences in the sugars measured between these genotypes may be attributed to species differences, climatic conditions, and maturity (Table 7).

Analysis of variance revealed that the accessions are significantly different with respect to their phytochemical attributes; hence, the means

***Molecular analysis***

Out of the seven primer pairs tested, six had appropriate polymorphism and revealed putative and scorable bands on the polyacrylamide gel. The summarized results of molecular characterization

Table 7. The differences in phyochemical characteristics of barberry (*Berberis sp*.) accessions in north-east of Iran

| Accession | Phytochemical characteristics |
|---|---|

| Name | Code | Total phenols (mg/g DW) | Flavonoids (mg/g DW) | Anthocyanin (μg/g DW) | Pectin (g/g DW) | Tannin (mg/g DW) |
|---|---|---|---|---|---|---|
| Daregaz 1 | D1 | 169a | 84.0bc | 567f | 3.11efg | 70.33abc |
| Daregaz 2 | D2 | 152fg | 74.0e | 600b | 3.60cde | 57.6def |
| Daregaz 3 | D3 | 163cd | 85.0b | 490i | 4.406a | 78.60a |
| Shirvan 1 | SH1 | 160d | 87.0a | 552g | 4.00abc | 74.30ab |
| Shirvan 2 | SH2 | 152fgh | 83.0bc | 591bcd | 4.36ab | 66.30bcd |
| Shirvan 3 | SH3 | 164bc | 59.0h | 599bc | 3.76cd | 55.00f |
| Shirvan 4 | SH4 | 154f | 82.0c | 590b-e | 4.38ab | 62.00def |
| Shirvan 5 | SH5 | 157e | 62.0g | 619a | 4.05abc | 53.30f |
| Golestan 1 | G1 | 168a | 88.6a | 593bcd | 3.53c-f | 58.00def |
| Golestan 2 | G2 | 150hi | 88.1a | 580e | 2.45h | 62.00c-f |
| Golestan 3 | G3 | 152fg | 88.0a | 525h | 3.73cd | 52.00.6f |
| Golestan 4 | G4 | 165b | 71.0f | 589cde | 3.93bc | 55.60ef |
| Golestan 5 | G5 | 150ghi | 71.9f | 592bcd | 2.71gh | 66.00b-e |
| Golestan 6 | G6 | 161d | 78.7d | 593bcd | 3.4def | 58.30def |
| Bidaneh | BD | 149i | 83.0bc | 584de | 3.01fg | 54.60f |

*Means in the same column followed by different letters are significantly different at $p \leq 0.01$using LSD test.

of barberry accessions based on SSR analysis are presented in Table 8. Out of the responsive primers, CA34 with 40 alleles had the highest and GA31 primer with only five alleles had the lowest number of alleles per each primer. Also, CA34 had the highest Shanon index (0.24) among the tested primers. The efficacy of these primers to evolve polymorphic bands has already been confirmed by Rezaei *et al.* (2012) in some other *Berberis* species. However, they found that GA33 and CA04 as highly discriminative primers. The primers utilized in the present study and the report of Rezaei *et al.* (2012) were already reported by Rob and Dorca (2006) who designed 10 SSR primer pairs to describe *Mahonia* species and among them seven pairs were identified to be efficiently polymorphic. The highest Shannon index was observed for the CA34 primer (0.24) and the lowest for GA05 and GA31 primers (0.11). Rezaei *et al.* (2012) reported that the Shannon index in their studied primers varied from 1.8 to 4.12.

In the present study, a total of 96 polymorphic alleles were identified in 15 barberry accessions with an average number of 16 alleles amplified per primer ranging from 5 to 40 alleles per gene locus. The GA05 primer had the lowest number of different and effective alleles. The highest number of different alleles belonged to the GA04 primer and the highest number of effective alleles belonged to the CA34 primer. According to Roder *et al.*, (1998), the average number of alleles per microsatellite marker indicates the suitability of each gene locus for estimating the gene diversity. Therefore, primers with higher number of alleles are suitable for genetic diversity studies. In the present experiment, the highest and lowest allelic frequencies belonged to the primers GA31 and GA05, with frequencies of 0.4 and 0.21, respectively.

The highest and lowest polymorphism information content (PIC) values were 0.95 and 0.84 in the GA05/GA33 and GA31 primers, respectively. PIC represents gene diversity and shows the discrimination power of a marker by the number of marker alleles and the relative frequency of these alleles in the population under study (Senior *et al.* 1998). The mean PIC for all loci was 0.91 (Table 8). According to Singh (2014), the

markers with the highest PIC are better than other markers to determine the genetic distance of the cultivars and markers with lower PIC values are not well capable of isolating genotypes. Therefore, according to the results, it can be concluded that the GA05/GA33 primers with the highest PIC were able to show the genetic distance among the samples better than other markers and can be used as an indicator in the diagnosis of barberry genetic diversity. According to Bracci *et al.* (2011), the high content of polymorphism information indicates that this marker can be used to distinguish genotypes with close relationship. PIC is an estimate of the differentiation power of each microsatellite considering the relative number and frequency of alleles. But, because of the difference in the frequency of these alleles, they have shown different PICs. The primers GA05, GA33, and GA04 showed the highest PIC and were able to identify different barberry accession. Therefore, they can be useful primers to study the barberry genetic diversity.

Table 8. The results of molecular characterization of barberry (*Berberis sp.*) accessions based on SSR markers

| Measures | SSR primers | | | | | |
|---|---|---|---|---|---|---|
| | GA05 | GA31 | GA33 | CA34 | GA04 | CA30 |
| Number of different alleles | 0.71 | 0.95 | 0.73 | 1.07 | 1.09 | 0.88 |
| Number of effective alleles | 1.10 | 1.10 | 1.14 | 1.27 | 1.25 | 1.25 |
| Shanon index | 0.11 | 0.11 | 0.15 | 0.24 | 0.23 | 0.21 |
| Expected heterozygosity | 0.07 | 0.07 | 0.09 | 0.16 | 0.15 | 0.14 |
| Observed heterozygosity | 0.08 | 0.08 | 0.11 | 0.18 | 0.17 | 0.16 |
| Polymorphism information content (PIC) | 0.95 | 0.84 | 0.95 | 0.89 | 0.94 | 0.90 |
| Polymorphic percentage | 0.37 | 0.17 | 0.46 | 0.22 | 0.17 | 0.23 |
| Allelic frequency | 0.21 | 0.4 | 0.23 | 0.33 | 0.24 | 0.32 |

Cluster analysis of different barberry accessions using Jaccard's similarity matrix and the UPGMA method divided the 15 barberry accessions into eight separate groups (Figure 5). Each of the 1st, 2nd, 3rd, 5th, 6th, 7th, and 8th groups comprised of only one accession (D1, D3, D2, G5, G2, BD, G6). However, eight accessions were included in the 4th group (Sh1, Sh2, Sh3, Sh4, Sh5, G1, G3, G4). Besides aforementioned clustering pattern, there were some significant points with regard to these barberry groups. It was clear that most of the closely related accessions had relatively high geographical similarities. Hence, most of the genotypes grouped in a same cluster were also closer to each other with respect to their growing sites and even some of them were already collected from the same province. For example, all SH1-5 accessions were characterized in the same group. It is noteworthy that, though G6 was also a seeded genotype but it was very dissimilar to other G populations as well as other seeded genotypes with respect to the SSR markers. The data also showed a clear genetic difference of the BD cultivated variety with other accessions. It may be concluded that the BD variety is genetically different with the barberry accessions distributed in north of Iran. Therefore, although the BD accession was morphologically similar to D2 and some of SH accessions, but it was grouped as an individual accession.

## Conclusions

The north and northeast elevations of Iran may be considered as a rich source of the wild *Berberis*

germplasm. The study of the diversity of these genotypes would be beneficial to future breeding programs. In the present study, genetic diversity of 15 barberry accessions was studied using morphological, biochemical, and molecular markers. The accessions were diverse (five distinct groups) with regard to morphological traits measured in their fruits, leaves, and thorns. Moreover, some rare phenotypic traits such as the jug-like fruit shape and cluster length were observed in these accessions, which had not earlier been reported in the literature. The genotypes were also different with respect to their fruit biochemical compositions but it seems that characterization of these genotype based on biochemical traits may not be precise. However, such reported phytochemical diversity would provide essential facts for food researchers. The accessions were divided in to six groups based on SSR markers (seven primer pairs) in which the CA34 showed highest polymorphism. The diversity of barberry accessions observed in northeast of Iran may be considered as rich source

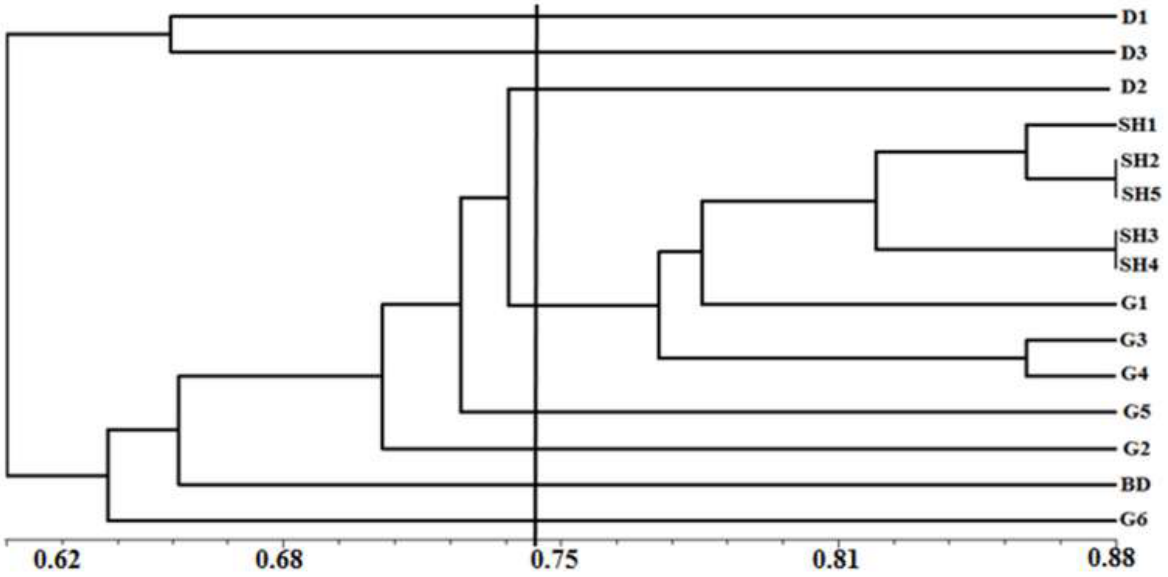


Figure 5. Dendrogram from the cluster analysis of barberry accessions based on SSR markers with the UPGMA method (D1-D3: Daregaz 1-3, G1-G6: Golestan 1-6, SH1-5: Shirvan 1-5 and BD: Bidaneh).

of wild relatives to be utilized in the future breeding programs for the improvement of cultivated *Berberis* species.

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**Conflict of interest**

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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## ارزیابی خصوصیات برخی از ژنوتیپ‌های زرشک وحشی (*Berberis* sp.) در شمال شرق ایران

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**چکیده**

درختچه‌های زرشک وحشی که به طور معمول، به نام «زرشک سیاه» شناخته می‌شوند، به‌طور طبیعی در ارتفاعات شمال و شمال شرق ایران پراکنش دارند. این توده‌ها، علاوه بر یک بانک ژن غنی، از نظر اهداف غذایی و دارویی بسیار مورد توجه می‌باشند. پژوهش حاضر به منظور بررسی تنوع ژنتیکی ۱۵ ژنوتیپ مختلف زرشک (۱۴ ژنوتیپ وحشی و یک ژنوتیپ زراعی) از طریق نشانگرهای مورفولوژیکی، بیوشیمیایی و مولکولی انجام شد. صفات مورفولوژیکی برگ، خار و میوه اندازه‌گیری شد. خواص بیوشیمیایی ژنوتیپ‌های مورد مطالعه نیز در مرحله رسیدن میوه اندازه‌گیری شد. علاوه بر این، ژنوتیپ‌ها با نشانگرهای ریزماهواره تجزیه و تحلیل شدند تا تنوع ژنتیکی آن‌ها در سطح مولکولی نیز مشخص شود. توده‌های مورد مطالعه از نظر صفات مورفولوژیکی متنوع بودند و در پنج گروه مجزا طبقه‌بندی شدند. علاوه بر این، برخی از ویژگی‌های مورفولوژیکی کمیاب و قابل توجه در شکل میوه و خوشه‌های میوه در تعدادی از ژنوتیپ‌ها یافت شد که قبلاً گزارش نشده بود. اگرچه تفاوت‌های زیادی در مورد ترکیبات بیوشیمیایی میوه به دست آمد، ولی این تفاوت‌ها روند مشخصی نداشتند. این توده‌ها، بر اساس نشانگرهای ریزماهواره در هشت گروه قرار گرفتند که در آن‌ها ژنوتیپ‌های نزدیک به هم شباهت‌های جغرافیایی نسبتاً بیشتری داشتند. دسترسی به تنوع ژنتیکی این ژنوتیپ‌ها را می‌توان به عنوان ستون فقرات برنامه‌های اصلاحی آتی آن‌ها در نظر گرفت و داده‌های گزارش شده در این پژوهش حاکی از آن است که شمال شرق ایران می‌تواند یک منبع غنی برای تنوع ژرم پلاسم زرشک در نظر گرفته شود.

**واژه‌های کلیدی:** به‌نژادی؛ تنوع؛ ریزماهواره؛ زرشک؛ نشانگر