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ORIGINAL ARTICLE

Morphological characterization of *Prunus incana* Pall. by multivariate analysis

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Abstract Iran is one of the most important growing centers for wild and domesticated species and varieties of *Prunus* subgenus *Cerasus* plants. Due to the suitable adaptation of wild species to the environmental and edaphic conditions of this country, they can be used as rootstock for sweet and sour cherry cultivars as well as in breeding programs for rootstock improvement, and a program has been initiated for this purpose. As a first step towards exploring the genetic diversity of *P. incana* Pall., morphological traits were used to evaluate the variation within its population dispersed in East and West Azerbaijan and Kordestan Provinces of Iran. In this study, 32 accessions of *P. incana* and 3 accessions from related species (*Prunus avium* L., *P. cerasus* L., and *P. mahaleb* L.) were used. Seventeen quantitative and two qualitative traits (vegetative and reproductive) were analyzed, and significant differences among accessions were found for most traits. Results of simple correlation analysis showed significant positive or negative correlations among some important traits such as tree height, leaf area, and leaf blade length and width. Factor analysis showed that leaf area, leaf blade length and width, petiole length, fruit and stone weight, fruit length and diameter, stone volume, and plant height constructed the main factors. Cluster analysis clearly discriminated *P. incana* accessions from other *Prunus* species and also differentiated *P. incana* accessions according to their geographic growing sites. Scatter plot analysis using two main factors also strongly confirmed the cluster analysis results.

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Introduction

Prunus incana Pall. belongs to the genus *Prunus* subgenus *Cerasus* in the family Rosaceae (Rehder 1940; Ingram 1948). According to the division of subgenus *Cerasus* by Ingram (1948), *P. incana* is included in the *Microcerasus* section of this genus. *P. incana* is native to northwest temperate regions of Iran where local people usually consume its fruits. This species is a wild deciduous shrub with tart small fruits and may have potential as a dwarfing rootstock for cherries. This plant is well adapted to diverse conditions of its growing regions, providing an extensive germplasm resource for domestication and improvement. It is usually found on dry calcareous and rocky mountain slopes at 1,400–2,100 m elevation, and is well adapted to severe winter (temperatures to about −20 °C) and dry, hot summer conditions.

Morphological characterization continues to be the first step for germplasm description and classification, and the statistical method of factor analysis is a useful tool for screening the accessions of a collection (Badenes et al. 2000). The genetic variation within a cultivated germplasm is generally low, and this restricts *Prunus* production to specific areas and conditions (Scorza et al. 1985). Zhang et al. (2008) studied the morphological traits of 44 tomentosa cherry (*Prunus tomentosa* Thunb.) accessions from 10 ecogeographical regions of China. They observed high morphological variation among populations, where the highest variations were in fruit weight, fruit width, and leaf width. Also using morphological traits, Rodrigues et al. (2008) studied sweet and sour cherry varieties in a

Table 1 Plant materials used in this study and their collecting locations

No.	Accession	Species	Collection area (province-region)	Longitude	Latitude	Altitude (m)
1	Inc-Ta1	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,698
2	Inc-Ta2	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,704
3	Inc-Ta3	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,703
4	Inc-Ta4	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,701
5	Inc-Ta5	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,711
6	Inc-Ta6	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,720
7	Inc-Ta7	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,731
8	Inc-Ta8	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,754
9	Inc-Ta9	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,757
10	Inc-Ta10	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,758
11	Inc-Ta11	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,769
12	Inc-Ta12	*P. incana*	East Azerbaijan-Tabriz	38°05	46°19	1,796
13	Inc-Kh1	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,340
14	Inc-Kh2	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,379
15	Inc-Kh3	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,407
16	Inc-Kh4	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,404
17	Inc-Kh5	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,400
18	Inc-Kh6	*P. incana*	West Azerbaijan-Khoy	38°28	44°46	1,371
19	Inc-Gosh1	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,717
20	Inc-Gosh2	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,756
21	Inc-Gosh3	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,931
22	Inc-Gosh4	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,947
23	Inc-Gosh5	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,962
24	Inc-Gosh6	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,979
25	Inc-Gosh7	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	2,012
26	Inc-Gosh8	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,900
27	Inc-Gosh9	*P. incana*	West Azerbaijan-Salmas	38°00	44°56	1,904
28	Inc-Sa1	*P. incana*	Kordestan-Sanandaj	35°17	47°08	2,039
29	Inc-Sa2	*P. incana*	Kordestan-Sanandaj	35°17	47°08	2,069
30	Inc-Sa3	*P. incana*	Kordestan-Sanandaj	35°17	47°08	2,088
31	Inc-Sa4	*P. incana*	Kordestan-Sanandaj	35°17	47°08	2,069
32	Inc-Sa5	*P. incana*	Kordestan-Sanandaj	35°17	47°08	2,141
33	Mahaleb	*P. mahaleb*	Tehran-Karaj	35°46	50°55	1,253
34	Sweet	*P. avium*	Tehran-Karaj	35°46	50°55	1,253
35	Sour	*P. cerasus*	Tehran-Lavasan	35°49	51°38	1,675

germplasm bank in Portugal. Their results showed that crown, trunk, leaf, flower, fruit, and seed characters could be useful for classification of sweet and sour cherry genotypes.

In several breeding programs, aiming to develop better-adapted rootstocks, introduction of genes from related *Prunus* species through interspecific hybridization has been utilized (Webster 1996; Martínez-Gómez et al. 2005). Nevertheless, some commonly used rootstocks have undesired traits; For example, dwarfing cherry rootstocks that provide precocious bearing, i.e., Gisela 5 and Maxma 14, are susceptible to crown rot by *Phytophthora* spp. (Exadaktylou and Thomidis 2005). Success in plant breeding requires a large gene pool from which to select. Thus, there is still a need to evaluate the genetic diversity of plants in order to develop new rootstocks and/or cultivars.

Fruit trees of small size are easier and cheaper to manage, are more precocious, and often bear fruits of higher quality than traditional, large trees. In addition, dwarf trees would also allow use of plastic roofs as well as net covering of trees (Balmer 1998; Webster 1996). Most of the dwarfing rootstocks for sweet cherry trees are hybrids. Among the dwarfing rootstocks, the GiSelA series are the best known dwarfing rootstocks for sweet cherry trees. These rootstocks are hybrids of *P. avium* L. and *P. cerasus* L. with

Fig. 1 *Prunus incana* tree, leaf, and fruit

P. fruticosa Pall. or *P. canescens* Boiss. (Webster and Schmidt 1996). However, the same rootstocks showed incompatibility with some of the important sweet cheery cultivars (Sitarek 2006).

In Iran, most sweet cherries are grown on mahaleb seedlings, and sour cherries are mainly nongrafted seedings. Therefore, improved rootstocks are needed for commercial production of cherries in Iran, and wild species are worth considering as potential sources of new rootstocks.

A breeding program on *Prunus* subgenus *Cerasus* in Iran started recently to develop new rootstocks for cherries. In the present study, as a first step towards exploring the genetic and horticultural potential of *P. incana*, morphological traits are used to evaluate the variation within its population, dispersed in East and West Azerbaijan and Kordestan Provinces of Iran. Moreover, evaluation of the genetic relationships between *P. incana* and the cultivated *Prunus* species has been performed, as this may also be useful for breeding and improving cherry rootstocks.

Materials and methods

Plant material

A total of 35 accessions including 32 accessions of *P. incana* (Table 1) and 3 accessions of cultivated *Prunus* species, including one each of *Prunus avium*, *P. cerasus*, and *P. mahaleb*, were used as materials for this study. The *P. incana* accessions (Fig. 1) were collected from four locations in three different provinces in Iran. The accessions of *P. avium*, *P. cerasus*, and *P. mahaleb* were collected from Lavasan and Karaj of Tehran Province (Fig. 2).

Morphological characters

Seventeen quantitative and two qualitative traits (Table 2) were recorded during 2 years in this study. Some characters were evaluated at their natural stands, while others were measured in the laboratory. From each genotype, 10 adult leaves and fruits were sampled and the length characters measured using a digital caliper with sensitivity of ±0.01 mm. Weight of fruit was measured using analytical balance with sensitivity of ±0.01 g. Leaf area (LA) was determined using an area measurement system (Delta-T, England). The content of total soluble solids (TSS) was determined using juice samples of fruit pulp with a hand refractometer at room temperature. Stone volume (SV) was determined using the formula $4/3\pi r^3$, where $r =$ (seed length + seed diameter)/4 (Rodrigues et al. 2008). Height of plant was determined by coding (1 m > height = 1, 1 m < height < 2 m = 3 and 2 m < height = 5); fruit skin color was coded as 1–7, representing yellow, orange-red, vermilion on yellow ground color, vermilion, wine red, mahogany, and black, respectively.

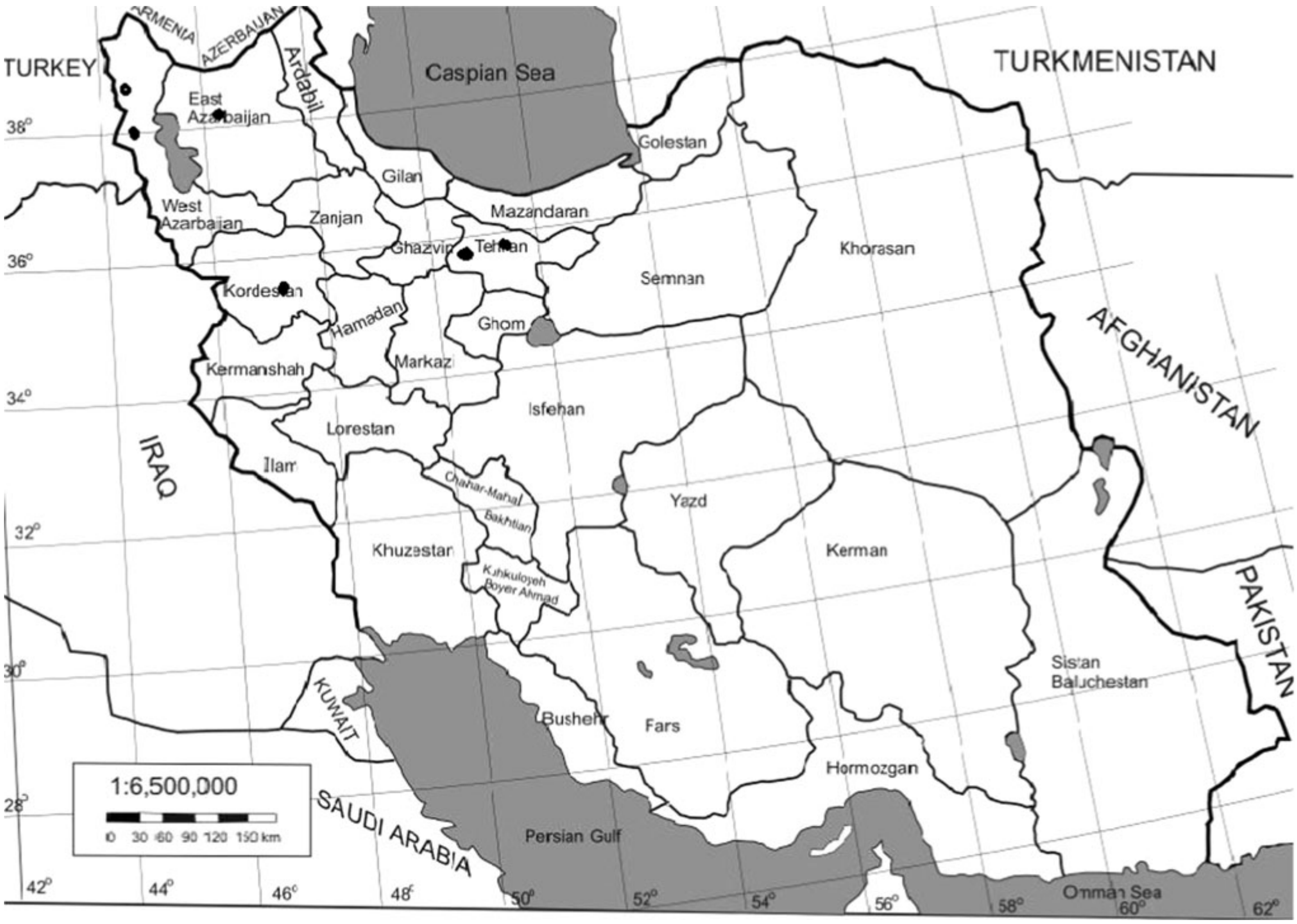

Fig. 2 Geographical locations of collecting sites of *Prunus incana* accessions and the three related accessions (*P. avium*, *P. cerasus*, and *P. mahaleb*) used in this study

Data analysis

Quantitative data were analyzed using SPSS software for Windows to perform analysis of variance, comparison of means, and coefficient of variation (CV). Simple correlations and factor analysis were also carried out using SPSS software. Cluster analysis and scatter plot analysis were carried out using PAST software (Hammer et al. 2001). Factor analysis was done by the Varimax factor rotation technique. A dendrogram of genetic similarities among accessions was compiled using the Euclidean method.

Results

Results showed that the range of height of Marmareh was 0.5–2 m, with an upright growth habit. Newly developed shoots are white in color and have pubescence, but older shoots are without pubescence and have gray color. Leaf is of small size (1–5 cm length, 0.7–1.8 cm width), with oval shape and serrated edges. The lower surface of the leaf has higher pubescence than the upper surface, which together with the small leaf size can help to improve drought tolerance. This species has red to dark fruit with large stone and thin flesh with a small peduncle. Compared with domesticated species (sweet and sour cherries), *Prunus incana* is in lower state for vegetative and fruit characteristics.

Simple correlations

The correlations between pairs of morphological traits are presented in Table 3. It was found that leaf area positively correlated with traits such as fruit weight ($r = 0.98$), leaf blade length and width (0.94), and petiole and fruit length (0.86). Fruit weight was also highly correlated with stone weight (0.90).

Factor analysis

Factor analysis determined the main factors for reducing the number of effective traits for discrimination of accessions.

Table 2 Quantitative and qualitative traits measured in *Prunus incana*: range of variability, mean, and coefficient of variation

No.	Trait	Abbreviation	Unit	Min.	Max.	Mean	CV (%)
1	Leaf area	LA	mm^2	84.82	607.52	192.44	53.77
2	Leaf blade length	LBL	cm	1.44	4.48	2.51	27.88
3	Leaf blade width	LBW	cm	0.72	1.88	1.2	24.79
4	Petiole length	PL	cm	0.16	0.9	0.5	38.59
5	Leaf serration	LS	per cm	5.6	10	7.57	15.69
6	Fruit weight	FW	g	0.17	0.62	0.36	32.45
7	Stone weight	SW	g	0.03	0.24	0.1	42.79
8	Total soluble solids	TSS	%	13	29	–	–
9	Stone length	SL	mm	4.76	9.14	6.8	16.48
10	Stone diameter	SD	mm	3.27	5.94	4.85	13.15
11	Fruit length	FL	mm	6.26	10.26	8.31	13.26
12	Fruit diameter	FD	mm	5.28	9.21	7.93	12.57
13	Stone length/diameter	SL/SD	Ratio	0.99	1.6	1.4	10.28
14	Fruit length/diameter	FL/FD	Ratio	0.9	1.24	1.05	7.33
15	Stone volume	SV	mm^3	0.03	0.21	0.1	50.46
16	Blade length/blade width	BL/BW	Ratio	1.09	3.3	2.12	25.6
17	Petiole length/blade length	PL/BL	Ratio	0.05	0.41	0.21	49.79
18	Fruit skin color	FSC	code	1	7	–	–
19	Plant height	HP	code	1	5	–	–

For each trait, factor loading of more than 0.64 was considered as significant (Table 4).

According to factor analysis, 12 of the vegetative traits accounted for 45.63 % of the variance as the first main factor, while the other eight traits distributed within the three next factors, and these four factors determined 80.62 % of the total variance.

In the first factor, traits including leaf area, leaf blade length and width, petiole length, fruit and stone weight, stone diameter, fruit length and diameter, stone volume, and plant height with positive impacts indicating 45.63 % of variance. The second factor with 15.30 % of total variance included the traits of stone length, stone length/diameter ratio, and petiole length/blade length ratio with significant positive effects, and the trait of leaf blade length/width ratio with negative effect.

The third factor with 13.28 % of the total variance included the two traits of total soluble solids (with negative effect) and fruit length/diameter ratio (with positive effect). The remaining effective characters were leaf serration and fruit skin color with positive effects determining the fourth factor.

Cluster analysis

Cluster analysis (Fig. 3) clearly discriminated *P. incana* accessions from other *Prunus* species, producing two major clusters and *P. incana* accessions distributed within two main groups.

The first major cluster is divided into two subgroups: subgroup I consisted of *Prunus avium* and *P. cerasus*, and subgroup II contained *P. mahaleb*. The second major cluster, which contained *P. incana* accessions, is divided into two subgroups. Subgroup I accessions are mainly from East and West Azerbaijan Provinces, and subgroup II accessions are from West Azerbaijan and Kordestan Provinces. Cluster analysis indicated lower genetic variation within *P. incana* accessions that belonged to the same geographic sites.

Scatter plot

The two-dimensional scatter plot (Fig. 4) shows the distribution of accessions according to factor 1 and factor 2. Starting from the negative to the positive values of factor 1, the accessions of *Prunus incana* and related *Prunus* species showed a general increase in leaf, fruit, and stone size, and plant height. Starting from the negative to the positive value of factor 2, the accessions increased in stone size and petiole/blade length ratio, and decreased in leaf blade length/width ratio. The scatter plot results support the results of cluster analysis. *P. incana* accessions clearly separated in the scatter plot from other related *Prunus* accessions, but they were not thoroughly discriminated from each other.

Discussion

The present study focused on the morphological diversity among 35 accessions of *Prunus incana*. The results clearly

Table 3 Correlation coefficients among 19 characteristics in *Prunus incana* and related *Cerasus* subgenus accessions

		1 LA	2 LBL	3 LBW	4 PL	5 LS	6 FW	7 SW	8 TSS	9 SL	10 SD
1	LA	–									
2	LBL	0.94**	–								
3	LBW	0.94**	0.93**	–							
4	PL	0.86**	0.86**	0.94**	–						
5	LS	−0.22	−0.26	−0.21	−0.29	–					
6	FW	0.98**	0.87**	0.92**	0.84**	−0.17	–				
7	SW	0.83**	0.71**	0.82**	0.80**	−0.18	0.90**	–			
8	TSS	−0.21	−0.23	−0.29	−0.39*	−0.1	−0.18	−0.2	–		
9	SL	0.39*	0.27	0.42*	0.47**	−0.1	0.52**	0.69**	0.04	–	
10	SD	0.57**	0.58**	0.66**	0.72**	−0.07	0.61**	0.72**	−0.19	0.73**	
11	FL	0.86**	0.73**	0.82**	0.75**	−0.23	0.93**	0.91**	−0.05	0.70**	0.62**
12	FD	0.80**	0.65**	0.70**	0.59**	−0.17	0.88**	0.86**	0.06	0.65**	0.51**
13	SL/SD	−0.32	−0.40*	−0.31	−0.24	0.13	−0.25	−0.2	0.15	0.37*	−0.12
14	FL/FD	0.19	0.25	0.40*	0.54**	−0.13	0.17	0.18	−0.44**	0.15	0.41*
15	SV	0.54**	0.48**	0.56**	0.59**	−0.17	0.62**	0.78**	−0.06	0.85**	0.75**
16	BL/BW	−0.2	−0.03	−0.36*	−0.36*	−0.12	−0.31	−0.42*	0.02	−0.55**	−0.42*
17	PL/BL	0.09	−0.04	0.19	0.44**	−0.12	0.18	0.39*	−0.22	0.55**	0.41*
18	FSC	0.31	0.34	0.38*	0.3	0.11	0.29	0.19	−0.09	0.10	0.21
19	HP	0.73**	0.73**	0.83**	0.82**	−0.22	0.74**	0.65**	−0.26	0.33	0.53**

		11 FL	12 FD	13 SL/SD	14 FL/FD	15 SV	16 BL/BW	17 PL/BL	18 FSC	19 HP
1	LA									
2	LBL									
3	LBW									
4	PL									
5	LS									
6	FW									
7	SW									
8	TSS									
9	SL									
10	SD									
11	FL	–								
12	FD	0.95**	–							
13	SL/SD	−0.08	−0.09	–						
14	FL/FD	0.11	−0.18	0	–					
15	SV	0.72**	0.68**	0.01	0.1	–				
16	BL/BW	−0.43*	−0.32	−0.26	−0.37*	−0.35*	–			
17	PL/BL	0.31	0.16	0.3	0.46**	0.40*	−0.65**	–		
18	FSC	0.19	0.14	−0.17	0.19	0.13	−0.15	−0.05	–	
19	HP	0.64**	0.53**	−0.18	0.45**	0.33	−0.38*	0.32	0.43*	–

Abbreviations of traits according to Table 2

* Indicates significant difference at 0.05

** Indicates significant difference at 0.01

point out that the diversity of accessions from different geographic regions was greater than that of the accessions from a particular region. Several researchers have reported the morphological variation between some *Prunus* subgenus *Cerasus* genotypes such as for sweet cherry (*P. avium*), sour cherry (*P. cerasus*), mahaleb (*P. mahaleb*), and

Table 4 Eigenvalues and cumulative variance for four major factors obtained from factor analysis and traits within each factor for *Prunus incana* and related *Cerasus* subgenus accessions (*P. avium*, *P. cerasus*, and *P. mahaleb*)

No.	Factor	1	2	3	4
	Eigenvalue	8.67	2.91	2.52	1.22
	Variance cumulative (%)	45.63	60.93	74.2	80.62
	Traits	Factor loading			
1	LA	0.95**	−0.11	0.17	0.03
2	LBL	0.89**	−0.27	0.26	−0.01
3	LBW	0.9**	−0.02	0.37	0.08
4	PL	0.81**	0.11	0.53	−0.07
5	LS	−0.24	0.13	−0.13	0.81**
6	FW	0.97**	0.03	0.1	0.06
7	SW	0.9**	0.27	0.09	−0.04
8	TSS	−0.08	0.05	−0.67**	−0.08
9	SL	0.55	0.77**	−0.11	−0.1
10	SD	0.66**	0.4	0.28	0.02
11	FL	0.93**	0.26	−0.04	−0.04
12	FD	0.91**	0.19	−0.29	−0.01
13	SL/SD	−0.3	0.65**	−0.18	0.02
14	FL/FD	0.07	0.23	0.88**	−0.04
15	SV	0.7**	0.48	−0.08	−0.12
16	BL/BW	−0.24	−0.73**	−0.23	−0.25
17	PL/BL	0.11	0.77**	0.42	−0.17
18	FSC	0.3	−0.14	0.25	0.64**
19	HP	0.7	0.03	0.49	0.11

Abbreviations of traits according to Table 2

** Significant factor loadings (considered for values above 0.64)

tomentosa cherry (*P. tomentosa*) (Perez-Sanchez et al. 2008; Khadivi-Khub et al. 2008; Ganjimoghadam and Khalighi 2007; Raconjac et al. 2010; Zhang et al. 2008).

A highly significant correlation was observed between fruit diameter and fruit weight in the present study. This result is in agreement with the finding of previous reports (Theiler-Hedricth 1990; Demirsoy and Demirsoy 2004). A highly significant negative correlation was observed between the traits of fruit length-to-diameter ratio and total soluble solids content. This result explains that the increase in fruit length to fruit diameter ratio coincides with a decrease in total soluble solids content. This might be due to the ripening stage of the fruit, as with the progress of fruit ripening the diameter increases.

In this study, for *P. incana* accessions, diverse skin fruit colors were observed, from yellow in accessions from Salmas Region to black in accessions from Sanandaj Region. The accessions from Tabriz Region had the smallest plant height in comparison with other regions. The accessions from Khoy Region had the highest leaf area; also, these accessions had red-colored fruits.

Shahi-Gharahlar et al. (2010) observed variation in leaf pubescence in *Prunus* subgenus *Cerasus*, and *P. incana* showed high pubescence on the leaf upper and lower surface, suggesting that it might be a highly suitable candidate for breeding with the objective of developing rootstocks with resistance to drought. Taiz and Zeiger (2002) suggested that leaves of plants having a gray-white appearance because of densely packed hairs reflect a large amount of light.

Factor analysis showed that parameters of the leaf, stone, and fruit contributed within the first main factor, accounting for 45.63 % of total variance. Zhang et al. (2008) observed that, among morphological traits, fruit width, leaf length, stem length, branch type, fruit color, and fruit shape were the most useful to assess accessions of tomentosa cherries. In addition, in this study, leaf area, fruit weight, petiole length to blade length ratio, fruit length/diameter ratio, and leaf serration were the most useful traits to evaluate *P. incana* accessions.

Cluster analysis clearly separated *P. incana* accessions from the other *Prunus* species and could separate *P. incana* accessions according to their geographic sites. Shahi-Gharahlar et al. (2010) reported that dendrogram obtained from morphological traits clearly separated the *Cerasus* subgenus genotypes. In addition, Perez-Sanchez et al. (2008)

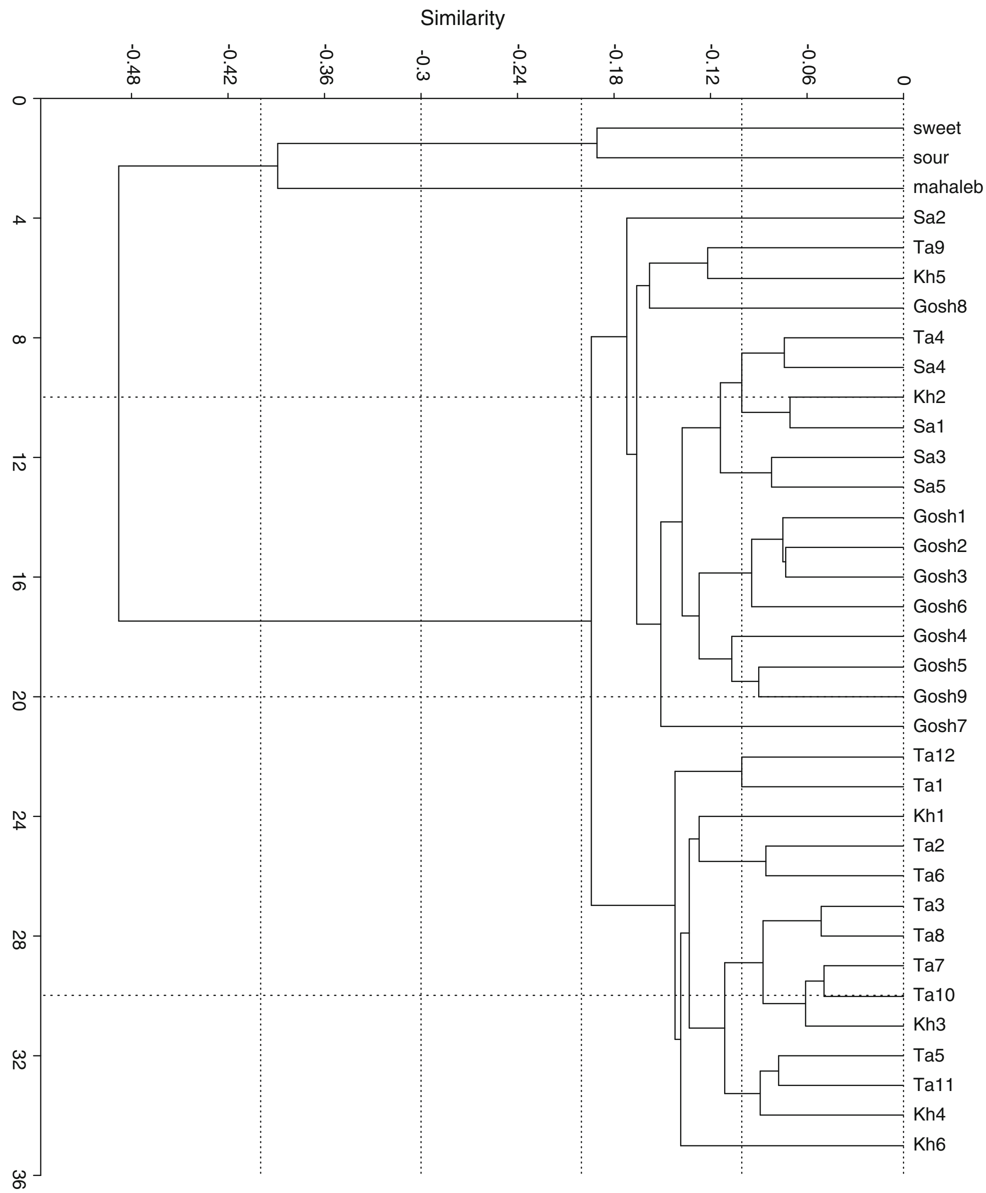

Fig. 3 Dendrogram of 32 *Prunus incana* accessions and the 3 related species accessions (*P. avium*, *P. cerasus*, and *P. mahaleb*) based on morphological traits

suggested that dendrogram gained from morphological characteristics clearly showed the relationships among the cultivars of sweet, sour, and duke cherries.

Scatter plot could not clearly separate the *Prunus incana* accessions from each other, and they were mostly distributed along the factor 2 axis. This means that seed length, seed length/diameter, blade length/width, and petiole length/blade length were effective for separating the *P. incana* accessions on scatter plot. Some *P. incana* accessions were more separated from the others on scatter plot. These separated accessions had special characteristics. An example is accession Kh1, which had larger leaf size and plant height compared with others. Also, accession Kh5 had the minimum stone size, and Gosh3 had the maximum of this trait.

Most of the dwarfing rootstocks for sweet cherry trees are interspecies hybrids with some graft incompatibility reports and susceptibility to disease as well as soil condition; therefore, development of new rootstocks is essential for sweet cherry production. For this purpose, identification of indigenous germplasm could help in terms of their use in breeding programs for rootstock improvement. Thus, due to small trees, *P. incana* could be a good candidate as dwarfing rootstock for sweet cherries.

In conclusion, accessions of *Prunus incana* included in this study were diverse, and variation existed between populations. This provides a good germplasm for breeding targets for *Prunus* species, especially for rootstocks with good adaptation to climatic and edaphic conditions of Iran.

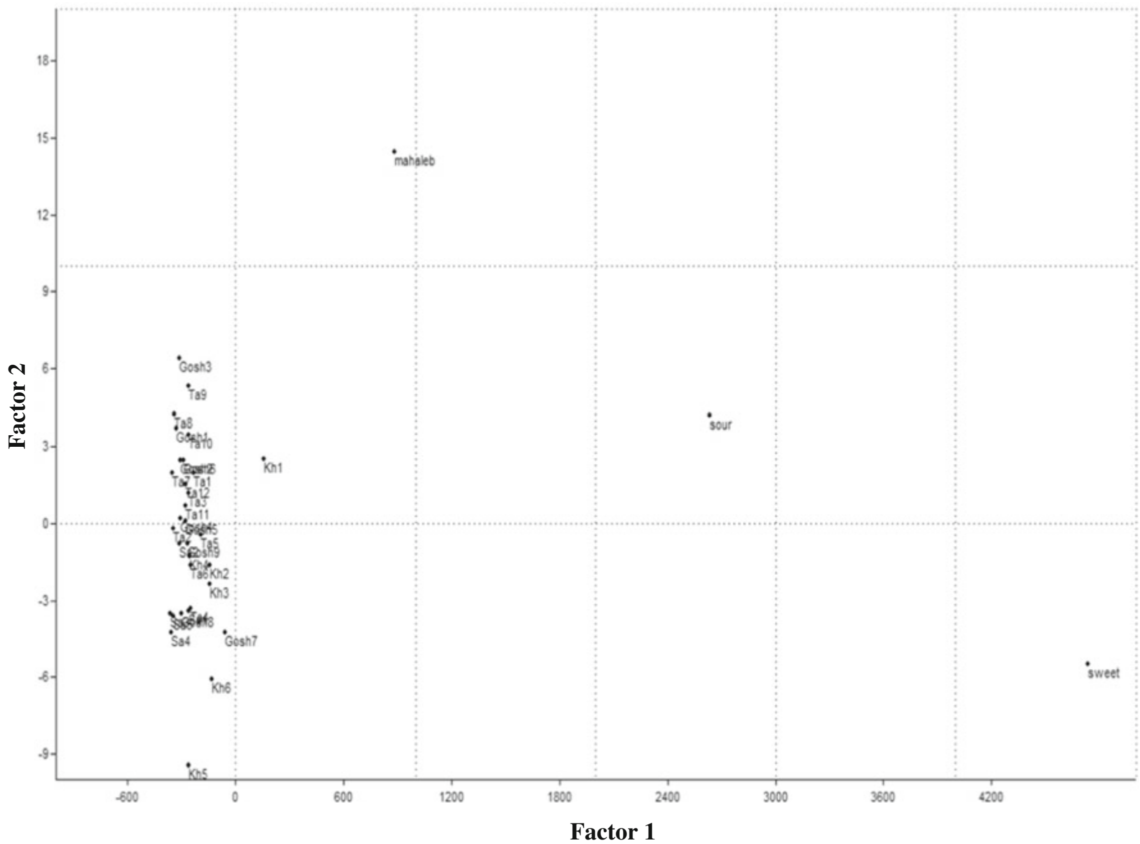

Fig. 4 Scatter plot of accessions according to the first two main factors for 32 accessions of *Prunus incana* and the 3 related *Cerasus* subgenus accessions (*P. avium*, *P. cerasus*, and *P. mahaleb*)

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