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Original article

Diversity in the nut and kernel characteristics of seven populations of *Prunus scoparia* from the central and southern Zagros regions of Iran by comparison with three other almond species

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Summary

Introduction – **Several Iranian almond species in the wild are valuable plant genetic resources which can be used in breeding almond cultivars and rootstocks. Among them, *Prunus scoparia* (Spach) C.K. Schneider, holds an outstanding position that needed to be clarified.** *Materials and methods* – **To evaluate the variability of the nut and kernel characteristics, six populations of *P. scoparia* were sampled from Shiraz, Marvdasht, Firouz Abad, Eqlid, Nour Abad and Mian Jangal Fasa in the Fars province, and one population was sampled from the Lordegan region in Chaharmahal and Bakhtiari province. Three populations of other species including *P. dulcis* Mill., *P. eburnea* Spach. and *P. elaeagnifolia* Spach. were also studied. Twelve quantitative and four qualitative traits of the nuts and kernels were evaluated according to the almond descriptor.** *Results and discussion* – **Considerable variations were observed with statistical significance in the nut and kernel traits within and between the populations. Among *P. scoparia* populations, the Shiraz was superior to other ones with respect to most of the nut attributes. However, among the species studied here, the common almond (*P. dulcis*) had the largest nuts. Interesting and useful correlations were noticed between the measured nut characteristics, among which the highest correlation (r = 0.94) was observed between nut length and nut weight. By using cluster analysis, the accessions could be placed in two main clusters of wild and domesticated populations. The main cluster of wild populations was divided into four sub-clusters based on species and geographic proximity.** *Conclusion* – **In general, this study demonstrated that there is a considerable variation in the nut and kernel characteristics of the wild almond species in Iran, especially with respect to *P. scoparia*, offering some potential in future breeding programs.**

Keywords
Iran, almond, *Prunus scoparia*, genetic diversity, morphological traits, cluster analysis

Significance of this study

What is already known on this subject?

- Recently, wild almond species have received a great deal of attention, especially from fruit breeders, mainly because of their capability in being used as rootstocks or scion cultivars in breeding programs. With more than 20 wild almond species, Iran is within the center of diversity for almond and Iran's almond genetic resources is regarded as one of the most diverse and valuable germplasm for almond improvement.

What are the new findings?

- A wide range of variations in the nut and kernel traits were observed within and between populations of the *Prunus scoparia* (Spach) C.K. Schneider, the mostly distributed wild almond species in Iran. In this regard and in comparison to tree other wild and domesticated almond species investigated in this study, thin but sealed shells, high kernel percentage and light-colored kernels are important characteristics that *P. scoparia* could share for almond breeding programs. Interesting and useful correlations which were observed between the measured characteristics of the nut and kernel could be utilized in breeding programs for indirect selection. Nut and kernel characteristics are not only useful for the selection of desirable clones but also can be considered for the assessment of genetic diversity.

What is the expected impact on horticulture?

- Climate change is an increasing challenge affecting agriculture at a global scale, especially in arid and semi-arid climatic conditions. This challenge will undoubtedly lead to a growing demand for the development of new cultivars and rootstocks, adapted to these changing conditions such as lower amounts of rainfall and higher temperatures. Therefore, the *P. scoparia* as one of the wild relatives of common almond could be used as a valuable source of new traits and genes for breeding new adapted almond scion and rootstocks cultivars.

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Résumé

Diversité des caractéristiques des fruits et des graines de sept populations de *Prunus scoparia* des régions centrales et méridionales du Zagros par comparaison avec trois autres espèces d'amandier.

Introduction – **Plusieurs espèces iraniennes d'amandier présentes dans la nature représentent de précieuses ressources génétiques végétales pouvant être utilisées en sélection de cultivars et de porte-greffes d'amandier. Parmi elles, *Prunus scoparia* (Spach) C.K. Schneider est dans une position exceptionnelle qui a besoin d'être clarifiée.** *Matériel et méthodes* – **Pour évaluer la variabilité des caractéristiques des fruits secs (noix) et des graines (amandes), six populations de *P. scoparia* ont été échantillonnées à Shiraz, Marvdasht, Firouz Abad, Eqlid, Nour Abad et Mian Jangal Fasa dans la province de Fars, et une population dans la région de Lordegan, province de Chaharmahal et Bakhtiari. Trois populations d'autres espèces, dont *P. dulcis* Mill., *P. eburnea* Spach. et *P. elaeagnifolia* Spach. ont également été étudiées. Douze caractères quantitatifs et quatre caractères qualitatifs des noix et des amandes ont été évalués selon le descripteur de l'amandier.** *Résultats et discussion* – **Des variations considérables statistiquement significatives ont été observées dans et entre les populations étudiées. Parmi les populations de *P. scoparia*, celle de Shiraz était supérieure aux autres pour ce qui concerne la plupart des attributs des noix. Cependant, parmi les espèces étudiées, l'amande commune (*P. dulcis*) a présenté les plus grosses noix. Des corrélations intéressantes et utiles ont été obtenues entre les caractéristiques mesurées sur les noix, parmi lesquelles la corrélation la plus élevée (r = 0,94) entre la longueur et le poids des noix. En utilisant l'analyse en clusters, les génotypes ont pu être placés dans deux groupes principaux de populations sauvages et domestiques. Le groupe principal de populations sauvages a été divisé en quatre sous-groupes en fonction de l'espèce et de la proximité géographique.** *Conclusion* – **D'une manière générale, cette étude a démontré qu'il existe une variation considérable parmi les caractéristiques des noix et des amandes des espèces d'amandier sauvage en Iran, et particulièrement en ce qui concerne *P. scoparia*, ce qui offre un certain potentiel pour les futurs programmes de sélection.**

Mots-clés
Iran, amandier, *Prunus scoparia*, diversité génétique, caractère morphologique, analyse en clusters

Introduction

Climate change is an increasing challenge affecting agriculture at a global scale, especially in arid and semi-arid climatic conditions (Gharaghani *et al*., 2017). This challenge will undoubtedly lead to a growing demand for the development of new cultivars and rootstocks, and even new crops adapted to these changing conditions such as lower amounts of rainfall and higher temperatures. Therefore, the wild relatives of domesticated crops would be increasingly looked upon as a source of new plant genetic material for breeding new adapted cultivars (Martinez-Gomez *et al*., 2005; Gharaghani and Eshghi, 2015).

Recently, wild almond species have received a great deal of attention, especially from fruit breeders, mainly because of their agricultural value and the assessment of their capability in being used as rootstocks or scion cultivars in breeding programs, through the contribution of useful traits such as early crop maturity, sealed nut, late blooming, drought, and tolerance to lime and salinity (Socias I Company, 1992; Sorkheh *et al*., 2009).

Nearly 20 wild almond species have been reported in Iran (Khatamsaz, 1992), indicating that Iran is within the center of diversity for almond (Ladizinsky, 1999). Iran's almond germplasm is regarded as one of the most diverse and valuable genetic resources for almond improvement (Gharaghani *et al*., 2017; Sepahvand *et al*., 2015). They are found at various altitudes between 600 and 2,700 m above sea level, in different environments, *e.g.*, alpine areas, foothills and semi-arid areas. They are the dominating plant species of the mountainous sub-region and may also occur in the plain sub-regions of ecological zones in the Zagros of Iran and the Irano-Touranian ecological zone where areas usually receive over 100 mm rainfall (Sabeti, 1994). The Fars province is among the largest provinces of Iran which covers parts of central Zagros and almost the entire southern Zagros region. The species *Prunus scoparia, P. elaeagnifolia*, and *P. eburnea* are not only among the widely distributed wild almond species in Iran (Gharaghani *et al*., 2017; Sorkheh *et al*., 2009) but also are the dominating wild almond species in the Fars province. These wild almond species have been used directly as rootstocks for almonds (Sorkheh *et al*., 2009), and *P. elaeagnifolia* also has been used as a rootstock for the plum (Gholami *et al*., 2010) in Iran, usually under non-irrigated conditions.

Among these three species, *P. scoparia* is a potentially multi-purpose wild almond species in Iran which has the capability of becoming a crop of choice in arid and semi-arid areas. The species *P. scoparia* can adapt to adverse climatic conditions, and the kernel and gum of this species could be used in many industries, such as pharmaceuticals, chemical and the food industry, which makes it not only a good choice for reforestation but also a good candidate for selection as a new multipurpose nut crop (Gharaghani and Eshghi, 2015). *Prunus scoparia* is a potential source of vegetable oil with relatively high oxidative stability for human nutrition, higher unsaturated to saturated fatty acids ratio, oxidisability value, and phenolic contents comparable to those of olive oil (Farhoosh and Tavakoli, 2008). Comparing the results obtained by Farhoosh and Tavakoli (2008) and Roncero *et al.* (2016) with regard to its chemical composition, *P. scoparia* oil is very similar to virgin almond oil which is characterized by its low content of saturated fatty acids and the predominance of mono-unsaturated fatty acids, especially oleic acid. Furthermore, *P. scoparia* and almond oils contain fat-soluble bioactive compounds and antioxidants that make them oils with nutritional and cosmetic properties (Farhoosh and Tavakoli, 2008; Roncero *et al*., 2016). Kernel oil and zedu gum (also called Persian gum) are exuded from the bark or branches of *P. scoparia*, and even the shoots, bark and leaves of this species have been used in Iranian traditional folk medicine to treat many diseases such as cancer, kidney stone, heart disease, diabetes, spasm, toothache, bronchitis, asthma and cough (Zargari, 1989–1992). Zedu gum is also being used as a suspending or emulsifying agent in cosmetic, food and textile industries, as well as in isolating the surface of boats (Rahimi *et al.*, 2013). The activated carbon produced from *P. scoparia* shells (endocarp) is also proposed to be of use as an alternative low-cost adsorbent for the adsorption of lead – Pb (II) (Mohammadi *et al.*, 2010). Debittered, salted and roasted nuts of this species are used as edible nuts, while they can also be applied in the confectionaries of some regions in Iran (Gharaghani and Eshghi, 2015).

Modern objectives in plant breeding may be achieved by the evaluation of desirable traits amongst genetic resources so as to combine them in one cultivar. Recent studies have used morphological, protein and molecular markers for the genetic characterization of Iran's wild almond species, and they have illustrated a wide range of genetic diversity in these species regarding the many traits of interest (Sorkheh *et al.*, 2009; Zeinalabedini *et al.*, 2008; Rahemi *et al.*, 2012). Morphological characteristics must be recorded for the selection of parents, as they are a first choice that can be used for describing and classifying the genetic resources. Statistical methods including multivariate analysis can also be used as useful tools for screening the germplasm accessions. Additionally, high and constant correlations between morphological characteristics could be useful for indirect selection in breeding programs (Karimi *et al.*, 2009).

While a few studies have been conducted on the assessment of genetic diversity and relationships among Iranian wild almond species with regard to morphological analyses (Zeinalabedini *et al.*, 2008), we still do not have comprehensive information about the ecological behavior, phenotypic and genetic diversity of the mostly distributed wild almond species in Iran, especially in the southern Zagros region. Accordingly, the aim of the present study (as a subproject of more comprehensive studies on wild almond species in the central and southern Zagros region) is to investigate the diversity, relationships and correlation between nut and kernel attributes in seven populations of *P. scoparia,* collected from the Fars and Charmahal and Bakhtiari provinces. We also sought to compare the nut characteristics of *P. scoparia* with other almond species including two wild species (*P. eburnea* and *P. elaeagnifolia*) and common almond (*P. dulcis* Mill.) from the same region.

Materials and methods

Almond species and collecting regions

The populations of almond species studied here included *P. scoparia* and *P. eburnea* in section Spartioides Spach., as well as *P. elaeagnifolia* and *P. dulcis* (which included commercial cultivars) in section Euamygdalus Spach. (Kester and Gradziel, 1996). Field trips were organized in 2013–2014 to collect plant materials. Nuts were harvested at the same maturity stage. They were collected from the central and southern Zagros regions including different sites in the Fars province and one site in the Charmahal and Bakhtiari province (Figure 1). Six populations of *P. scoparia* were collected from different regions in the Fars province including Shiraz, Marvdasht, Firouz Abad, Eqlid, Nour Abad and Mian Jangal Fasa, as well as one population from Lordegan (Kareh Bas and Gerd Bisheh area) region in the Charmahal and Bakhtiari province. In addition to *P. scoparia*, this research included a population of *P. elaeagnifolia* (including accessions from both provinces), one population of *P. eburnea* (including accessions only from Fars province), as well as a population of *P. dulcis* (including three cultivars sampled from the agricultural research station located in the Charmahal and Bakhtiari province) (Table 1; Figure 3). In each population, up to 10 genotypes (10 different trees) were selected from their natural habitats, while the nut samples were collected and transferred to the laboratory after recording their geographic position data.

Evaluation of nut and kernel attributes

Nut and kernel characterization was performed based on almond descriptors developed by the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) (Gulcan, 1985). In this study, 16 phenotypic characteristics of the nut and kernel were evaluated, comprising of 12 quantitative traits including nut weight, nut length, nut width and nut thickness, shell thickness, kernel weight, kernel length, kernel width and kernel thickness, kernel percentage (ratio between kernel weight and in-shell nut weight), percentage of empty nut, rate of twin kernel (%), as well as 4 qualitative traits including nut shape, markings on the outer shell, shell color intensity and kernel color (Tables 2 and 3). Measurements were performed on 20 nuts per replicate (3 replications) in each accession (total of 60 nuts per accession) and the mean values were used to assess the range of morphological variation within and among the populations and species.

Variables such as nut and kernel length, width and thickness were measured by using a digital caliper. Nut and kernel weight were measured by using an electronic balance with 0.001 g precision. Qualitative traits were determined by using a coding method according to the almond descriptor with minor modifications as follows: nut shape (1. Round, 2. Ovate, 3. Oblong, 4. Cordate, 5. Extremely narrow), markings on the outer shell (0. Without pores, 3. Scattered pores, 5. Medium pores, 7. Dense pores, 9. Scribed), nut and kernel color (1. Extremely light, 2. Very light, 3. Light, 4. Slightly light, 5. Medium dark, 6. Slightly dark, 7. Dark).

Statistical analyses

The experiment was carried out based on completely randomized design with three replications. Data analysis was based on an analysis of variance procedure with mean values tested for significant differences at $P < 0.05$ by using Duncan's multiple range test in SAS, version 9.1. The correlation analysis between variables was performed by using the Pearson's correlation coefficient with the SPSS statistics software version 16 (SPSS Inc., Chicago, IL, USA). Cluster analyses were performed on the basis of all measured traits by using the Ward's method and the graph representing the classification as a dendrogram of dissimilitude with standardized Euclidean distances representing the closest accessions in homogeneous groups.

Results and discussion

Variations in the nut and kernel traits

Results showed that there were large differences between the almond species studied in this research, and there were significant differences between populations of *Prunus scoparia* in all of the characteristics of the nut and kernel being measured (Tables 2 and 3). In these regards, considering the mean values, not surprisingly, the largest nut (3.79 g) and kernel (1.24 g) were observed in the common almond (*P. dulcis*), while *P. eburnea* had the smallest nut (0.33 g) and kernel (0.11 g). These results are in line with those reported by Sorkheh *et al.* (2009) who reported average nut weights of

FIGURE 1. A map of Iran representing the sampling regions (colored provinces; Fars, Chaharmahal and Bakhtiari).

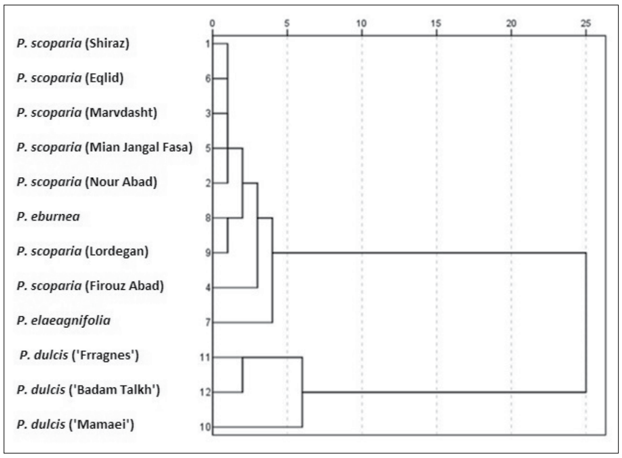

FIGURE 3. Dendrogram based on all measured nut and kernel characteristics by using Ward clustering method depicting the relationship of studied almond populations.

5.1 and 0.6 g, and average kernel weights of 1.4 and 0.4 g for *P. dulcis* and *P. eburnea*, respectively, although the mean values reported by this research are smaller. Among *P. scoparia* populations, the largest nut (0.76 g) and kernel (0.25 g) were recorded in the Lordegan population, while the smallest nut (0.46 g) and kernel (0.15 g) were seen in the Marvdasht population. These values are smaller than those reported by Sorkheh *et al.* (2009) for the Lodegan/Karebas population of *P. scoparia* which is found in common between the two studies (1.6 and 0.7 g for nut and kernel weight in Kareh bas population, respectively). Results of the current study for different populations of *P. scoparia* are in accordance with findings reported by Khadivi-Khub and Anjam (2014) but the ranges and mean values for measured traits are slightly less than those reported by these researchers. For example, they reported 12.70–25.50 mm for the range and 17.4 mm for the average value of nut length in four populations of *P. scoparia* from different parts of Iran (compared to 11.66–20.40 mm for the range and 15.07 mm for the mean value in our study). These differences could be mainly because of the wider geographic regions and climatic zones covered by those researchers who performed sampling, compared to our study that covered more sampling sites but from a more limited geographic region (mainly the warm and dry climatic zones of the southern Zagros). Another reason behind these differences could arise from the variation in climatic conditions, especially in rainfall through the different years in which these studies were carried out. The variation in annual precipitation is very common in the arid and semi-arid climate of Iran. The variation of rainfall in Shiraz, for example, in which one of the almond populations were sampled, have ranged between 120–670 mm throughout the last 40 years.

In breeding programs, where high nut weight is desired, the small nut size of *P. scoparia* is undesirable (Gradziel and Kester, 1998), although some accessions studied herein have shown a relatively high nut weight (such as the accessions of Shiraz 2 and 4, Lordegan 1 and 2, Eqlid 7) which could be considered in almond breeding programs and for the local cultivation of this species for nut production and oil extraction. It should be mentioned that collection is now mainly from the natural habitats of this species. Therefore, the Shiraz population has been considered as the most diverse population of *P. scoparia,* and could be searched more comprehensively as a source for the selection of superior genotypes to be used for future breeding and selection programs.

The thickest (2.71 mm) and thinnest (0.97 mm) nut shell were recorded in *P. dulcis* and *P. scoparia* respectively. Among *P. scoparia* populations, the Shiraz population had the thickest nut shell (1.21 mm), and the thinnest nut shell (0.99 mm) was seen in both Marvdasht and Eqlid populations (Table 2). The range and mean value recorded for shell thickness in the current study for *P. scoparia* (0.47–1.63 and 1.09 mm, respectively) are much higher than in those reported by Khadivi-Khub and Anjam (2014) for this species (0.20–0.87 and 0.55 mm, respectively). These differences could be mainly explained by the differences in climatic condition of the sampling areas, and to some extent, the number of accessions in each study. The well-sealed shell is common in *P. scoparia* and is reported to be more resistant to fungus and insect infestation (Gradziel and Martinez-Gomez, 2002; Ledbetter and Shonnard, 1992). This offers new opportunities in breeding already not readily available in domesticated almond genetic resources. The presence of a relatively high variation in shell thickness among populations of *P. scoparia* offers the opportunity of selecting thin-shell nuts, what is important for the local production of this species as a nut crop.

Nut shapes varied from ovate (code 2) to extremely narrow (code 5) among accessions of the four species (Table 2; Figure 3). Ovate and oblong (code 3) were the most common nut shapes in *P. scoparia* populations. The difference in nut shapes is probably due to the genetic variations caused by heterogamy and species variations, and also environmental influence which are contrary to Talhouk *et al.* (2000), who believed that almond quantitative traits are much less under the influence of environmental conditions.

Various types of shell marks were recorded in the species studied here in (except for *P. scoparia* which had nuts with no marks). This can be considered as an important and distinct criterion in classifying wild almond species (Khatamsaz, 1992).

Prunus eburnea and *P. duclia* represented the darkest and lightest nut color, respectively. Among *P. scoparia* populations, the color intensity of nuts varied and ranged from extremely light (1) to dark (7) and by considering the mean values, the Mian Jangal Fasa and Lordegan populations had

Table 1. List and geographic information of studied almond species, populations and genotypes collected from different areas of Central and Southern Zagros, Iran.

Populations	Genotypes	Longitude E	Latitude N	Altitude (m)	*Mean annual precipitations (mm)
Prunus scoparia (Shiraz)	1	52 34.558	29 37.082	1,535	297.76
	2	52 35.822	29 44.275	1,820	297.76
	3	52 35.784	29 44.287	1,816	297.76
	4	52 35.811	29 44.269	1,819	297.76
	5	52 34.601	29 37.103	1,569	297.76
	6	52 34.449	29 37.465	1,565	297.76
	7	52 33.704	29 37.946	1,549	297.76
	8	52 34.586	29 39.490	1,670	297.76
	9	52 32.642	29 40.263	1,728	297.76
	10	52 35.789	29 44.353	1,821	297.76
P. scoparia (Nour Abad)	1	51 53.00	29 38.00	1,925	420.65
	2	51 37.194	30 4.238	1,191	420.65
	3	51 20.875	30 4.695	1,179	420.65
	4	51 39.730	29 48.513	934	420.65
	5	51 32.377	30 1.167	1,086	420.65
	6	51 23.931	30 0.745	1,285	420.65
	7	51 39.823	29 48.605	946	420.65
	8	51 21.011	30 6.513	1,183	420.65
	9	51 31.694	30 1.252	1,067	420.65
	10	51 58 42.7	30 01 08.4	1,592	420.65
P. scoparia (Marvdasht)	1	52 54.983	30 3.162	1,728	283.64
	2	52 54.800	30 6.249	1,730	283.64
	3	53 00.807	30 6.800	1,812	283.64
	4	53 12.117	30 5.742	1,837	283.64
	5	53 10.524	30 1.617	1,828	283.64
	6	53 12.643	29 59.116	1,803	283.64
	7	53 14.080	29 57.712	1,765	283.64
	8	53 6.493	29 48.754	1,667	283.64
	9	53 6.535	29 48.816	1,663	283.64
	10	53 8.662	29 47.443	1,640	283.64
P. scoparia (Firouz Abad)	1	52 38.222	29 09.564	1,845	323.43
	2	52 32.509	29 8.712	1,725	323.43
	3	52 34.486	28 58.157	1,503	323.43
	4	52 22.587	28 54.317	1,578	323.43
	5	52 32.400	28 55.816	1,445	323.43
	6	52 38.719	29 5.885	1,917	323.43
	7	52 23.202	28 53.222	1,524	323.43
	8	52 38.310	29 3.808	1,732	323.43
	9	52 32.801	29 9.124	1,763	323.43
	10	52 41.274	28 13.319	1,275	323.43
P. scoparia (Mian Jangal Fasa)	1	52 46.117	29 26.327	1,481	261.97
	2	52 50.130	29 19.653	1,526	261.97
	3	–	–	2,187	261.97
	4	53 24.351	29 9.542	1,729	261.97
	5	53 23.894	29 9.939	1,754	261.97
	6	53 26.033	29 7.617	1,720	261.97
	7	53 26.054	29 7.632	1,716	261.97
	8	53 22.759	29 10.821	1,815	261.97
	9	53 19.277	29 12.351	1,825	261.97
P. scoparia (Eqlid)	1	52 40.003	30 15.126	1,701	323.54
	2	52 38.310	30 16.346	1,742	323.54
	3	52 36.405	30 18.062	1,800	323.54
	4	52 35.080	30 22.196	2,321	323.54
	5	52 23.051	30 19.324	1,843	323.54
	6	52 23.764	30 19.060	1,750	323.54
	7	52 24.085	30 18.382	1,715	323.54

Table 1. Continued.

Populations	Genotypes	Longitude E	Latitude N	Altitude (m)	*Mean annual precipitations (mm)
P. scoparia (Lordegan)	1	51 11.609	31 33.619	1,752	551.46
	2	51 13.020	31 34.354	1,962	551.46
	3	–	–	1,948	551.46
	4	–	–	1,963	551.46
P. elaegnifolia	1	52 35.806	29 44.098	1,801	297.76
	2	52 35.746	29 44.158	1,804	297.76
	3	–	–	2,128	551.46
	4	–	–	2,570	190.47
P. eburnea	1	51 32.320	30 1.169	1,084	420.65
	2	52 22.173	30 19.865	1,912	323.54
	3	53 24.368	29 09.592	1,732	261.97
	4	–	–	2,280	190.47
	5	52 24.585	30 18.376	1,711	323.54
	6	52 23.745	30 19.046	1,746	323.54
P. dulcis	'Mamaei'	–	–	1,910	340.95
	'Ferragnes'	–	–	1,910	340.95
	'Badam talkh'	–	–	1,910	340.95

* Average of 10 years obtained from closest meteorology stations to the sampling sites of each population or accession.

Table 2. Diversity of nut characteristics in populations of wild and domesticated almond species collected from different areas of Central and Southern Zagros, Iran.

Populations (No. of accessions)	Index	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Shell thickness (mm)	Nut weight (g)	Nut shape (1-5)	Marking of outer shell (0–9)	Shell color intensity (1–7)
Prunus scoparia Shiraz (10)	Range	11.66-20.40	8.20-13.30	6.29-8.96	0.85-1.54	0.28-1.02	2-3	0.0-0.0	1-6
	Mean*	15.20^{c}	10.15^{c}	7.77^{c}	1.21^{a}	0.58^{b}	2.90^{b}	0.0^{a}	5.0^{e}
P. scoparia Nour Abad (10)	Range	12-70-17.44	8.40-10.10	6.32-8.04	0.47-1.50	0.35-0.95	2-5	0.0-0	3-7
	Mean	14.77^{d}	9.29^{f}	7.44^{f}	1.07^{e}	0.51^{e}	3.20^{a}	0.0^{a}	5.0^{e}
P. scoparia Marvdasht (10)	Range	13.40-16.30	8.40-11.50	6.30-8.22	0.81-1.15	0.35-0.61	2-3	0.0-0.0	2-7
	Mean	14.64^{e}	9.68^{e}	7.44^{f}	0.99^{f}	0.46^{f}	2.90^{b}	0.0^{a}	5.4^{d}
P. scoparia Firouz Abad (10)	Range	12.50-16.05	8.50-11.70	6.75-8.48	0.88-1.52	0.32-0.72	2-3	0.0-0.0	3-7
	Mean	14.60^{e}	10.03^{d}	7.70^{d}	1.09^{d}	0.51^{e}	2.90^{b}	0.0^{a}	5.7^{b}
P. scoparia Mian Jangal Fasa (9)	Range	12.90-15.80	8.90-12.50	7.41-8.88	0.92-1.63	0.41-0.80	2-3	0.0-0.0	5-7
	Mean	14.37^{f}	10.26^{b}	7.95^{b}	1.13^{c}	0.56^{c}	2.77^{d}	0.0^{a}	6.2^{a}
P. scoparia Eqlid (7)	Range	12.40-19.30	9.20-11.40	6.81-8.08	0.82-1.09	0.39-0.76	2-3	0.0-0.0	5-6
	Mean	15.35^{b}	10.29^{b}	7.65^{e}	0.99^{f}	0.54^{d}	2.85^{c}	0.0^{a}	5.6^{c}
P. scoparia Lordegan (4)	Max	15.50-17.80	9.50-12.60	7.78-9.82	1.02-1.39	0.52-0.92	2-3	0.0-0.0	2-5
	Mean	16.57^{a}	11.35^{a}	8.43^{a}	1.15^{b}	0.76^{a}	2.50^{e}	0.0^{a}	3.5^{f}
	C.V.	0.18	0.25	0.20	0.39	0.17	0.08	0.00	0.04
Prunus species (No. of accessions)									
P. scoparia (60)	Range	11-66-20.40	8.20-13.30	6.29-9.82	0.47-1.63	0.28-1.02	2-5	0.0-0.0	1-7
	Mean**	15.07^{C}	10.15^{C}	7.76^{C}	1.09^{C}	0.56^{C}	2.85^{B}	0.0^{D}	5.19^{B}
P. elaeagnifolia (4)	Range	14-30-19.70	9.40-12.20	7.37-8.74	1.55-1.87	0.48-1.07	2-3	3.0-9.0	2-4
	Mean	16.80^{B}	10.45^{B}	7.95^{B}	1.69^{B}	0.81^{B}	2.75^{C}	6.0^{A}	2.75^{C}
P. eburnea (5)	Range	10.20-12.90	7.90-9.50	6.85-7.59	0.77-1.17	0.29-0.46	2-4	0.0-9.0	6-7
	Mean	11.58^{D}	8.63^{D}	7.19^{D}	0.97^{D}	0.33^{D}	2.5^{D}	1.5^{C}	6.83^{A}
P. dulcis (3)	Range	34.80-37.70	18.80-21.80	14.00-17.20	2.42-3.02	3.07-4.79	3-4	7.0-7.0	1-3
	Mean	36.13^{A}	20.70^{A}	15.34^{A}	2.71^{A}	3.79^{A}	3.33^{A}	5.7^{B}	1.66^{D}
	C.V.	0.15	0.46	0.14	0.18	0.01	0.05	0.04	0.04

* and **: Mean comparisons of *Prunus scoparia* populations (small letters) and four species (capital letters) respectively, values with same letters in the columns do not have significant differences based on Duncan multiple range test at $P < 0.05$.

Table 3. Diversity of Kernel characteristics in populations of wild and domesticated almond species collected from different areas of Southern Zagros, Iran.

Populations (No. of accessions)	Index	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Kernel weight (g)	Kernel percentage (%)	Empty kernels (%)	Twin kernels (%)	Kernel color (1–7)
Prunus scoparia	Range	9.75-16.33	6.00-9.43	4.17-5.28	0.11-0.32	28.39-39.28	0.00-1.66	0.00-1.66	1-4
Shiraz (10)	Mean*	12.48^{c}	7.52^{c}	4.75^{c}	0.199^{b}	35.41^{b}	0.33^{b}	0.16^{a}	2.80^{e}
P. scoparia	Range	11.13-14.28	6.48-8.06	4.01-5.36	0.14-0.21	32.75-45.71	0.00-5.0	0.00-0.00	1-5
Nour Abad (10)	Mean	12.40^{d}	7.21^{e}	4.82^{b}	0.179^{c}	38.43^{a}	0.50^{b}	0.00^{a}	3.50^{b}
P. scoparia	Range	10.15-12.62	5.62-7.58	3.59-5.45	0.10-0.20	427.02-1.30	0.00-0.00	0.00-0.00	1-5
Marvdasht (10)	Mean	11.58^{g}	6.86^{f}	4.50^{e}	0.157^{g}	33.47^{d}	0.00^{b}	0.00^{a}	2.70^{f}
P. scoparia	Range	10.40-13.15	6.79-8.33	4.10-5.36	0.12-0.22	25.00-40.00	0.00-23.30	0.00-3.31	2-5
Firouz Abad (10)	Mean	11.89^{e}	7.41^{d}	4.55^{d}	0.166^{f}	32.80^{e}	2.49^{a}	0.33^{a}	3.60^{a}
P. scoparia	Range	10.33-13.25	6.47-8.92	4.44-5.60	0.13-0.22	25.00-33.33	0.00-1.66	0.00-1.66	1-5
Mian Jangal Fasa (9)	Mean	11.72^{f}	7.49^{c}	4.80^{b}	0.173^{e}	31.28^{g}	0.37^{b}	0.37^{a}	3.00^{d}
P. scoparia	Range	10.48-15.75	7.07-8.50	4.34-4.74	0.13-0.26	25.49-35.29	0.00-3.33	0.00-1.66	1-5
Eqlid (7)	Mean	12.70^{b}	7.74^{b}	4.57^{d}	0.175^{d}	32.46^{f}	0.47^{b}	0.23^{a}	3.14^{c}
P. scoparia	Range	12.00-14.10	6.50-8.90	4.90-6.53	0.19-0.34	27.77-36.95	0.00-0.00	0.00-0.00	1-5
Lordegan (4)	Mean	13.24^{a}	7.92^{a}	5.33^{a}	0.255^{a}	33.88^{c}	0.00^{b}	0.00^{a}	2.50^{g}
	C.V.	0.20	0.43	0.41	0.12	0.003	60.54	26.46	0.27
Prunus species (No. of accessions)									
P. scoparia (total, 60)	Range	9.75-16.33	5.62-9.43	3.59-6.53	0.10-0.34	25-00-45.71	0.00-23.30	0.00-3.31	1-5
	Mean**	12.28^{C}	7.45^{B}	4.76^{B}	0.187^{B}	33.96^{A}	0.59^{A}	0.15^{B}	2.93^{B}
P. elaeagnifolia (4)	Range	11.22-15.73	5.97-7.77	3.57-4.75	0.10-0.24	14.01-29.85	0.00-0.00	0.00-10.00	1-3
	Mean	13.36^{B}	6.74^{C}	4.24^{D}	0.172^{C}	21.88^{D}	0.00^{B}	3.75^{B}	2.00^{D}
P. eburnea (5)	Range	8.58-10.40	5.55-8.25	4.40-5.01	0.09-0.12	26.08-41.37	0.00-0.00	0.00-0.00	2-5
	Mean	9.66^{D}	6.28^{D}	4.68^{C}	0.113^{D}	33.47^{B}	0.00^{B}	0.00^{B}	3.66^{A}
P. dulcis (3)	Range	24-59-26.13	11.18-12.93	6.15-10.74	0.82-1.75	26.71-36.53	0.00-0.00	26.71-36.53	1-4
	Mean	25.52^{A}	12.32^{A}	8.12^{A}	1.243^{A}	32.03^{C}	0.00^{B}	32.03^{A}	3.00^{C}
	C.V.	0.16	0.29	0.41	0.29	0.01	0.41	28.72	0.05

* and **: Mean comparisons of *Prunus scoparia* populations (small letters) and 4 species (capital letters) respectively, values with same letters in the columns do not have significant differences based on Duncan multiple range test at $P < 0.05$.

the darkest and lightest nut colors respectively. In *P. elaeagnifolia,* most of the genotypes had nuts with light color (Table 2; Figure 3). Seed coats of light color and smooth surface are preferred in almonds (Khadivi-Khub and Anjam, 2014). Among the wild almond species studied herein, the *P. elaeagnifolia* had the lightest shell color, what can be considered as a good source for the improvement of this trait in almond breeding programs.

Kernel yield percentage varied from 14.01–45.71% among the four species and ranged between 25.00–45.71% in the *P. scoparia* populations (Table 3). Considering the mean values, the highest (33.96%) and lowest (21.89%) mean values of kernel percentage were recorded in *P. scoparia* and *P. elaeagnifolia,* respectively. Among *P. scoparia* populations, the highest (38.44%) and the lowest (31.29%) mean kernel percentages belonged to the Nour Abad and Mian Jangal Fasa populations, respectively. The average kernel weight and percentage of kernel are important factors in determining the success of the marketable yield (Khadivi-Khub and Anjam, 2014). The highest kernel weight was recorded in the common almond, but the highest kernel percentage belonged to *P. scoparia* which could be considered in almond breeding programs for the development of cultivars with higher ratios of kernel/nut.

A few populations of the *P. scoparia* showed some degrees of empty nuts, while some others and the populations of *P. elaeagnifolia, P. duclia* and *P. eburnea* species were recorded as populations with 0.0% empty nuts (Table 3).

Variations in kernel size, percentage of kernel yield and the occurrence of empty nuts can be due to variations in humidity and the rainfall occurring in the natural habitats of these species. In fact, when the almond tree is subjected to drought stress, it will start to use the kernel moisture which then results in the shrinking of the kernel and the decrease in nut size (Yadollahi and Rahemi, 2005). Kester *et al.* (1977) observed a highly significant effect of the environment on the occurrence of empty nuts, while Sánchez-Pérez *et al.* (2007) indicated a diminutive annual variation in this particular trait.

The highest (32.03%) and lowest (0.00%) rates of occurrence for twin kernels were observed in *P. dulcis* and *P. eburnea*, respectively. In *P. dulcis*, the highest rate of twin kernels (68.04%) was recorded in the local cultivar 'Mamaei' (Table 3). The influence of the environment on the production of twin kernels is also well-known (Kester and Asay, 1975). The occurrence of double pistils is markedly increased when the trees are exposed to high temperatures throughout the period of flower differentiation (Beppu *et al.*, 2001). Arteaga and Socias I Company (2002) reported that the development

TABLE 4. Correlation coefficients (Pearson) of nut and kernel quantitative characteristics in studied wild and domesticated almond species.

	Nut length	Nut width	Nut thickness	Shell thickness	Nut weight	Kernel length	Kernel width	Kernel thickness	Kernel weight	Kernel percentage	Empty kernels	Twin kernels	Altitude	Mean precipitation
Nut length		0.03	0.75**	0.82**	0.94**	0.93**	0.85**	0.70**	0.21**	-0.17**	-0.04	0.62**	0.18**	0.08
Nut width			0.03	0.01	0.02	0.04	0.07	0.00	0.00	-0.04	-0.01	0.00	0.25**	0.08
Nut thickness				0.71**	0.81**	0.69**	0.67**	0.75**	0.18**	-0.05	-0.02	0.60**	0.20**	0.06
Shell thickness					0.84**	0.75**	0.72**	0.59**	0.20**	-0.38**	0.02	0.60**	0.23**	0.01
Nut weight						0.87**	0.84**	0.83**	0.23**	-0.12	-0.04	0.76**	0.19**	0.05
Kernel length							0.82**	0.66**	-0.11	-0.13*	-0.05	0.55**	0.14*	0.10
Kernel width								0.71**	0.18**	-0.06	-0.02	0.51**	0.15*	0.11
Kernel thickness									0.17**	0.23**	-0.05	0.78**	0.03	0.17*
Kernel weight										0.02	-0.03	0.21**	0.14*	0.06
Kernel percentage											0.00	0.01	-0.44**	0.22**
Empty kernels												-0.02	-0.11	-0.12
Twin kernels													0.11	-0.03
Altitude														-0.04
Mean precipitation														

* and **: Significance at $P < 0.05$ and $P < 0.01$, respectively.

of twin kernels was a quantitative trait, comprising a complex value of inheritance and heritability that is difficult to estimate, mainly due to environmental effects.

Prunus eburnea and *P. elaeagnifolia* were recorded herein as species with the darkest and lightest kernel color, respectively. Among *P. scoparia* populations, the Firouz Abad and Lordegan populations had the darkest and lightest kernel colors, respectively (Table 3). Since a light kernel color is a characteristic of important preference in modern almond breeding programs, the very light kernel color in *P. scoparia* and *P. elaeagnifolia* species can be considered for these purposes.

The general analysis of all measured traits illustrates a high morphological diversity of accessions belonging to the *P. scoparia* in Iran. Natural hybridization, cross-incompatibility, propagation by seed, gene flow and human selection may have contributed to this variation (Sefc *et al.*, 2000). In accordance with our findings, some other authors (Socias I Company *et al.*, 2008; Yadollahi and Rahemi, 2005; Sorkheh *et al.*, 2010; Zeinalabedini *et al.*, 2012) have shown that the morphological evaluation is an efficient tool for characterizing almond genetic resources and distinguishing the various species. Reports have also certified the usefulness of some physical traits in nuts, compared to the chemical parameters, to confirm authenticity of pistachio nuts, kernels and by-products depending on the cultivar and its origin (Tsantili *et al.*, 2010; Rabadán *et al.*, 2017). Our findings are in general agreement with those of such previous studies. On the other hand, many of the almond nut and kernel characteristics are controlled genetically (Arteaga and Socias I Company, 2002). However, other factors such as crop load, tree vigor, environment and the soil moisture in which plants are grown may also have important effects on these attributes, and should be considered while describing and interpreting the observed variations (Imani, 2000; Sánchez-Pérez *et al.*, 2007).

The almond commercial quality refers to all aspects related to the external appearance of the product, including size, shape, surface texture, kernel color, absence of twin kernels, and ultimately, the success in making kernels marketable (Khadivi-Khub and Anjam, 2014). In this regard, some characteristics of *P. scoparia* for almond breeding programs are important to add: a thin but sealed shell, high kernel percentage and a light color of the kernel. The bitter taste of kernel and small nut size are undesirable characteristics of wild almond species that limit the full utilization of this species in breeding programs. Nonetheless, genetic studies have revealed that many of these undesirable traits are controlled by a limited number of genes which could easily be eliminated in the breeding process (Martínez-Gómez *et al.*, 2007). On the other hand, sweet seed genotypes have been reported among the bitter in some of these species (Emam *et al.*, 2013).

Correlation between traits

The simple correlation coefficient analysis revealed significant positive/negative correlations between many characteristics (Table 4). Not surprisingly, nut weight correlated positively and highly with almost all of the dimensions pertaining to the nut and kernel (ranging from r = 0.76 to 0.94), and with the percentage of twin kernels (r = 0.62). A positive, significant, but relatively low correlation was also observed between nut weight and kernel weight (r = 0.23). These results are in accordance with those of previous studies in wild and domesticated almond species (Sorkheh *et al.*, 2010; Zeinalabedini *et al.*, 2012). Shell thickness was neg-

TABLE 5. Correlations (Spearman's ranked order) of qualitative characteristics in studied wild and domesticated almond species.

	Nut shape	Marking of outer shell	Shell color intensity	Kernel color	Altitude	Mean precipitation
Nut shape		-0.08	-0.13*	-0.02	-0.30**	-0.17*
Marking of outer shell			-0.40**	-0.16*	0.36**	-0.07
Shell color intensity				0.19**	-0.19**	-0.40**
Kernel color					0.31**	0.17*
Altitude						-0.03
Mean rainfall						

* and **: Significance at $P < 0.05$ and $P < 0.01$, respectively.

atively correlated with kernel percentage (r = -0.38) which is in agreement with previous results obtained in wild and domesticated almond species (Khadivi-Khub and Anjam, 2014; Sánchez-Pérez *et al.*, 2007). This result indicates that cultivars or genotypes with softer shells have a tendency to bear relatively larger kernels. Twin kernels also correlated positively with almost all of the nut and kernel quantitative traits. The occurrence of twin kernels is an undesirable trait, and its occurrence is markedly increased when the trees experience high temperatures throughout the period of flower differentiation (Beppu *et al.*, 2001). Among the qualitative attributes, there was a significant negative correlation (r = -0.40) between markings on the outer shell and shell color intensity (Table 5).

Many of the nut and kernel attributes measured in this experiment showed a significant and positive correlation with altitude, but some of them (nut shape, shell color intensity, kernel color, kernel thickness and kernel percentage) correlated with the average annual precipitation. This is contrary to the results reported by Sorkheh *et al.* (2009) indicating that characteristics of the fruit did not appear to associate with neither rainfall nor elevation. This finding partially agrees with results obtained by Orsanic *et al.* (2009) who studied the influence of altitude on fruit size of *Sorbus torminalis* (L.) Crantz, thereby concluding that the fruit size increases because of higher altitude. The same authors ascribe this effect to the different climatic and soil conditions existing in the three monitored altitudes. Surprisingly, altitude and the average rainfall did not correlate with each other (Table 4). The different results obtained in this study regarding the correlation between nut and kernel traits with the altitude and average annual precipitation could arise from the different methods of data collection for these two environmental parameters. The real data of altitudes for sampled plant materials have been used, while the same average annual precipitation data (from the closest meteorological station) were considered for all plant accessions sampled from each region (see Table 1).

The most important characteristics of the majority of plants are the quantitative traits that are largely influenced by the environment and, therefore, have low heritability. Accordingly, the response to direct selection for these characteristics may be unpredictable unless there is a good control of environmental variation (Hatami Maleki *et al.*, 2011).

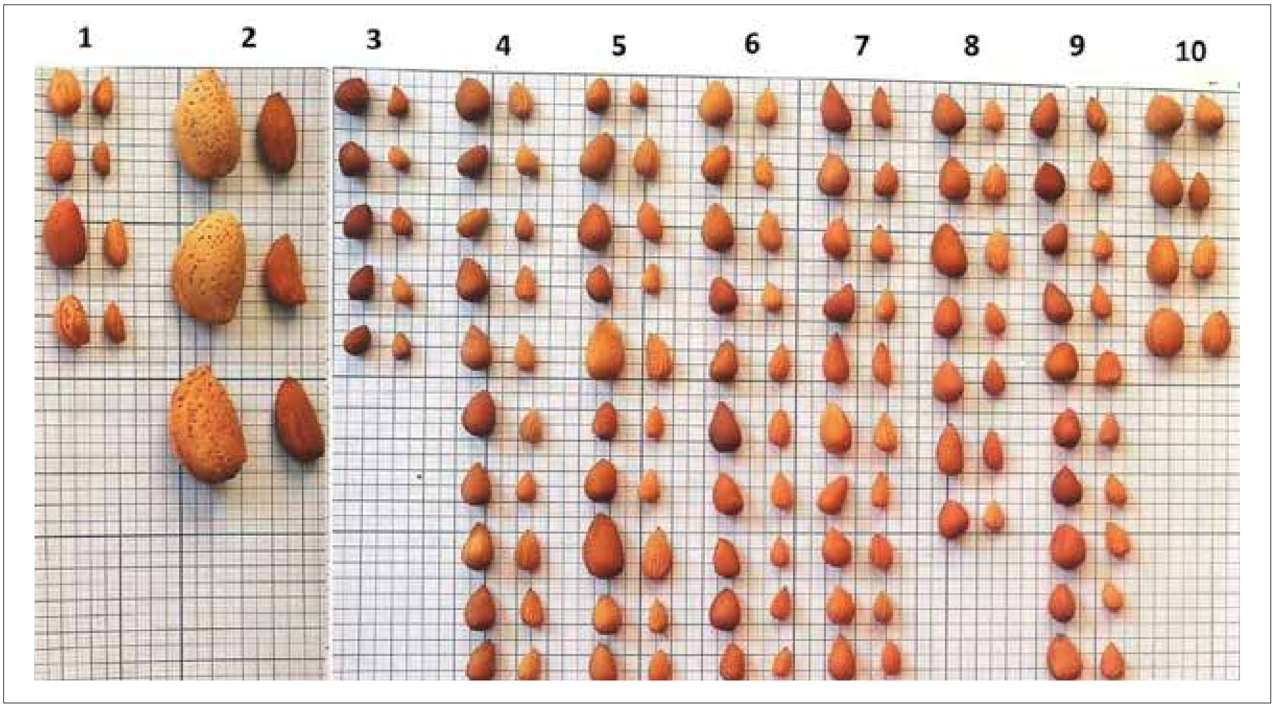

FIGURE 2. Nuts (left) and kernels (right) of all studied almond genotypes of 10 populations assigned to four species collected from different areas of Central and Southern Zagros, Iran. (1. *Prunus elaeagnifolia*; 2. *P. dulcis*; 3. *P. eburnea*; 4–10: Firouz Abad, Shiraz, Marvdasht, Nour Abad, Eqlid, Mian Jangal Fasa and Lordegan populations of *Prunus scoparia,* respectively). Note that the order of the genotypes within populations is not similar to that in Table 1. Size of the squares in scaled paper: 5 × 5 mm.

The success of any breeding program is highly dependent on the extent of diversity and knowledge of the behavior of desirable traits through crosses (Sánchez-Pérez *et al*., 2007). The existence of correlation between traits helps the selection of important traits indirectly, and this can facilitate and accelerate the breeding programs (Vargas *et al*., 2001). A close relationship between traits could also facilitate or hinder gene introgression since strong selections for a desirable trait could favor the presence of another desirable trait from the same population. In addition, established relationships between some traits can help breeders set goals for parental-partner selection and breeding (Dicenta and Garcia, 1992).

Cluster analysis

The cluster analysis clarified some of the relationships among the studied populations of *P. scoparia* as well as three other almond species. In general, the species were classified into two major groups including common (cultivated) almond and wild almonds. Wild almonds were divided into four subgroups (mainly based on the species and, to some extent, according to the geographic distribution). Three cultivars of common almond (*P. dulcis*) were positioned in two different subgroups (Figure 2). Five out of six populations of *P. scoparia* from Fars Province including Shiraz, Eqlid, Mian Jangal Fasa, Marvdasht and Nour Abad did not separate clearly and were located very closely to each other, but surprisingly the Firouz Abad population of this species stood alone, with considerable distance from others due to some unique characteristics, such as a relatively small nut length, a relatively thin shell, small nut weight, relatively small kernel thickness, medium kernel percentage, highest prevalence of the empty nut, relatively low rate of twin kernels, smooth nut shell, relatively dark nut color and relatively light kernel color. The only population of *P. scoparia* which has been sampled very distantly from Fars populations was collected from the Charmahal and Bakhtiari province (Lordegan), and only population of *P. eburnea* were positioned very close to each other. However, these populations are from two different species, but these two species belong to the same section (Spartioides Spach.) (Kester and Gradziel, 1996), and the studied genotypes of both population share traits such as a medium nut thickness, small shell thickness, medium kernel thickness, medium kernel percentage, zero percentage of empty nut and twin kernels, light nut color and similar nut shape (Figure 3). Among the three cultivars of the common almond (*P. dulcis*), large nut weight, high twin kernels and an extremely light kernel color separated 'Mamaei' cultivar from the other two ('Ferragnes' and 'Badam Talkh').

Hierarchical cluster analysis could be properly utilized in assessing the similarity or dissimilarity among individuals and for clarification of relationships among them (Costa *et al*., 2011). Our results showed that nut and kernel traits are suitable for characterization of almond germplasm and that they could appropriately separate the almond species from each other. The traits could also relatively separate the distinct and distant populations of the *P. scoparia* into distinct morphological groups, which is in agreement with previous results obtained by Khadivi-Khub and Anjam (2014) in cluster analysis of *P. scoparia* populations from four distinct regions in Iran. In accordance with our findings, Kelleher *et al.* (2005) showed that morphological evaluations prove to be efficient for the characterization of oak germplasm and for the distinction of species.

Conclusion

The overall analysis of all traits illustrates a wide range of variations in the nut and kernel traits that occur within and between populations of the wild almond species under study, especially in *Prunus scoparia,* which may have important implications for the management of genetic resources and future breeding programs. In this regard, having thin but sealed shells, high kernel percentage and light-colored kernels are important characteristics that *P. scoparia* could share for almond breeding programs. The Shiraz population proved to be the most diverse population of *P. scoparia,* which could be used as a source for further selections of superior genotypes. In breeding programs, where the heavy nut weight is a desirable trait, the small nut size of *P. scoparia* is undesirable, although some of the studied accessions showed a relatively heavy nut weight which could be considered in almond breeding programs and could be used for the local cultivation of this species for nut production and oil extraction. Such samples are mainly collected from natural habitats where this species exists. Interesting and useful correlations were observed between the measured characteristics of the nut and kernel, which could be utilized in breeding programs for indirect selection. By using cluster analysis, genotypes were classified into two main clusters of wild and domesticated populations. The wild populations were grouped inside the main cluster based on species and geographic proximity. These results suggest that the characteristics of the nut and kernel are not only useful for the selection of desirable clones but also can be considered for the assessment of genetic diversity. The morphological characterization of almond accessions, as established in this study, deserves future research by using molecular marker analysis.

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