



Diversity of Nut and Seedling Characteristics and Its Relationship to Habitat Climate in some Almond Species

Sama Rahimi Devin¹, Ali Gharaghani^{*1,2}, Saeid Eshghi¹

¹ Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

² Drought Research center, College of Agriculture, Shiraz University, Shiraz, Iran

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ABSTRACT

This study carried out to evaluate variations and relationships of nut size, seedling morphological attributes and habitat climatic parameters in of *Prunus scoparia* (Spach) C. K. Schneid populations in comparison to three other almond species. A total of 10 populations including seven populations of *P. scoparia* and three of other almond species including *P. elaeagnifolia* Spach., *P. eburnea* Spach., and *P. dulcis* Mill. were raised from seeds in greenhouse condition. Results showed noticeable variations in the studied seedlings characteristics within and among the populations. Among *P. scoparia*, Nourabad, Shiraz and Lordegan populations were superior to other populations in most of the measured traits. Useful correlations were observed between nut and seedling traits, among them, the highest correlation ($r = 0.89$) was between nut weight and leaf width. Altitude and annual precipitation correlated positively with some of the nut and seedling characteristics (ranging from $r = 0.13$ to $r = 0.33$). Based on principal components analysis (PCA), the first three components explained 76.2 % of the total variation of morphological characteristics. Using cluster analysis as well as a bi-plot of PCA, the genotypes classified into two main clusters of wild and domesticated populations. The main cluster of the wild populations divided into three sub-clusters based on their botanical categorization, whilst *P. scoparia* populations clustered based on geographic proximity and climate similarity. Results may have important implications for managing these genetic resources as well as their use in future breeding programs especially for the development of new rootstocks for almond and stone fruits.

Introduction

Iran located in the mid-latitude belt of arid and semi-arid regions of the earth and more than 60% of the country is covered by such regions (Vahdati *et al.*, 2019b). Accordingly, the use of plants resistant to such climate is of paramount importance (Imani *et al.*, 2021; Ansari *et al.*, 2019; Modarres & Silva, 2007; Vahdati *et al.*, 2014). Iran is highly rich in wild almond species and based on the latest survey, 20 wild species and six inter-specific hybrids have been reported in Iran (Khatamsaz, 1992). Thus, local cultivars and landraces, as well as

related wild species, provide an extensive gene pool for desirable traits including tree and nut characteristics as well as resistance to biotic and abiotic stresses, which can be utilized in fruit trees breeding programs (Gharaghani *et al.*, 2017; Vahdati *et al.*, 2019a). Some of the wild almonds can survive water shortage due to some anatomical characteristics such as defoliation of leaves during the hot seasons and higher ability of roots to store and absorb soil moisture (Madam *et al.*, 2011; Zokaee-Khosroshahi, 2014). These species are among

*Corresponding author: Email address: agharaghani@shirazu.ac.ir

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the rare trees that naturally grow in shallow, rocky and eroded soils (Ali *et al.*, 2015).

Prunus scoparia, *P. elaeagnifolia*, and *P. eburnea* are among the widely distributed wild almond species in Iran (Sorkheh *et al.*, 2009). *P. scoparia* is a less thorny shrub up to 6 m tall and its local name is Badame kuhi (Gharaghani *et al.*, 2017). This is an important multi-purpose wild almond species in Iran and has the potential of being used as a dwarf rootstock for almond (Gharaghani and Eshghi, 2015). It is a very suitable candidate for soil stabilization and soil erosion prevention in arid and semi-arid areas (Mozaffarian, 2005). Tolerance to drought and infertile soils, evergreen and dense canopy, beautiful flowers and the relatively long flowering period has made it a very suitable candidate for landscape in arid and semi-arid areas especially for green belts of cities in mountainous and non-fertile soils (Gharaghani & Eshghi, 2015). *P. elaeagnifolia* is indigenous to Iran and is called locally Badame Kermani or Barg Senjed (Gharaghani *et al.*, 2017). Its shrub reaches up to 3–4 m tall. *P. elaeagnifolia* has been used as a rootstock for the plum in Iran, usually under non-irrigated conditions (Gholami *et al.*, 2010). *P. eburnea* is also endemic to Iran and is locally called Badame Ajee or Khakestary Badam. Its thorny shrub reaches up to 1.5 m tall (Gharaghani *et al.*, 2017).

These species can also be used for afforestation, especially in arid and semi-arid zones, where water resources are limited, as a way to protect and develop vegetative cover (Mardani, 2006). For example, stands of almond of the Irano-Turanian zone are found in Badamak forests near Firuzabad in Fars province and also near Mohammadabad Maskun in Kerman province and in Badameshk forests in South Khorasan province of Iran (Talebi *et al.*, 2013).

The commercial use of wild almond species as rootstocks in Iran dates back to about 300 years (Madani *et al.* 2006). A large area of almond cultivars grafted on

wild rootstocks has been reported in the Fars, Kerman, Boushehr and Hormozgan provinces of Iran (Gharaghani *et al.*, 2017). Alberghina (1978) stated that nectarines can be grafted on wild almond trees as rootstocks.

Nowadays, the tree architectural analysis makes up a basic framework in pomological research (Lauri & Laurens, 2005). The height of the first lateral from the ground is vital for mechanized harvesting and the expansion of leaves is also associated with stronger aerial growth. Breeders and growers have selected the wild almond species as a rootstock probably due to their ability in controlling tree size in order to gain balance between reproductive and vegetative growth (Khadivi-Khub *et al.*, 2016). Tree growth reduction by using genetically dwarf rootstocks is a key component for high density, mechanized orchard systems (Cousins, 2005) and promotion of earlier flowering (Fallahi *et al.*, 2001). Several independent genetic traits determine the tree size, including internode length, branch location (basitonic, mesotonic and acrotonic), and growth rate (Faust, 1989). The diameter of the trunk is also vital in some tree species regarding the production of resin for industrial purposes (Lombardero *et al.*, 2006).

Genetic diversity plays a fundamental role in the breeding of fruit crops (Danesh *et al.*, 2015; Vahdati *et al.*, 2015; Azimi *et al.*, 2016; Pourkhaloe *et al.*, 2017; Sharifani *et al.*, 2017; Saddoud Debbabi *et al.*, 2021; Sarikhani *et al.*, 2021). Thus, recognizing and measuring such diversity is of great importance. Local cultivars and landraces in addition to the related wild species make up a useful source of genetic diversity and an excellent potential for crop improvement. Thus, identifying the promising genotypes is crucial in breeding programs (Khadivi-Khub, 2014). The plant morphology analysis is a common method in the identification and characterization of the germplasm. The characterization of morphology combined with multivariate statistical methods such as principal

component analysis (PCA) and cluster analysis are valuable tools for screening genotypes (Sorkheh *et al.* 2009; Ansari & Gharaghani, 2019). These statistical techniques together provide comprehensive information which contributes to the genetic diversity of plants (Mohammadi & Prasanna, 2003). Moreover, high and constant correlations between morphological characteristics may be valuable for indirect selection in breeding programs (Karimi *et al.*, 2009). The detection of the possible relationship between geographical/ or climatic parameters of plant's original habitats and plants morphological/ or physiological characteristics will yield useful information that could be used to identify the most suitable species, genotypes, or cultivars for cultivation in a particular area as well as the most appropriate areas for the production of the crop of choice (Ghasemi Soloklui *et al.*, 2017).

Thus the aim of the present study is to evaluate the diversity, relationships, and correlation of nut and seedling morphological attributes in seven populations of *P. scoparia*, collected from the Central and Southern Zagros region in Fars and Charmahal-o Bakhtyari provinces. We also sought to compare nut and seedling characteristics of *P. scoparia* with other almond species including two wild species (*P. eburnea* and *P. elaeagnifolia*), common almond (*P. dulcis* Mill.) and also GF677 (*Prunus dulcis* × *Prunus persica*) from the same region. Finally, the possible correlations between environmental parameters of plant's natural habitats and plant characteristics of collected plant materials were investigated.

Materials and Methods

Almond species and collection regions

The studied almond species consisted of *P. scoparia* and *P. eburnea* in section Spartioides Spach., as well as *P. elaeagnifolia*, and *P. dulcis* in section Euamygdalus Spach (Kester & Gradziel, 1996), alongside with GF677, a peach almond inter-specific hybrid. Some field trips were taken in 2013-2014 in order to gather plant materials (nuts) from Southern and Central Zagros regions of Iran consisting of different sites in Fars province and Charmahal-o-Bakhtiari province. Six populations of *P. scoparia* were gathered from different regions of Fars province including Mian Jangal Fasa, Firuzabad, Nourabad, Shiraz, Marvdasht, and Eqlid, as well as one population from Lordegan (Kareh Bas and Gerdbisheh) region in Charmahal-o Bakhtiari province. Along with *P. scoparia*, a population of *P. eburnea* (consisting of accessions only from Fars province), one population of *P. elaeagnifolia* (consisting of accessions from both provinces), a population of *P. dulcis* (including three cultivars of 'Mamaei', 'Ferragnes', 'Badam talkh' sampled from Agricultural Research Station located in Charmahal-o Bakhtiari province as well as GF677 (*Prunus dulcis* × *Prunus persica*) sampled from Neyriz in Fars province) were included (Fig. 1 and Table 1). For each region, the geographic and climatic data (approximately a period of 10 to 20 years, upon the availability of the locations from which accessions were collected) including altitude, minimum and maximum temperatures, mean daily temperature, average wind speed and average rainfall were obtained from meteorological stations in Iran (Table 1).



Fig. 1. Approximate distribution of almond species and populations habitats in Iran that used in this study (the green colored provinces). The original map is obtained from d-map (https://d-maps.com/carte.php?num_car=5496&lang=en) and modified (colored) using paint software of Microsoft Windows

Table 1. List of the studied plant materials and the geographical and climatic information of their collection sites.

Population	Genotype	E	N	Altitude (m)	Annual precipitation (mm)	Minimum temp (0°C)	Maximum temp (0°C)	Mean daily temp (0°C)	Mean wind Speed (m/s)						
<i>P. scoparia</i> (Shiraz)	1	52° 34.558"	29° 37.082	1535	297.76	4.99	30.72	18.78	1.73						
	2	52° 35.822	29° 44.275	1820											
	3	52° 35.784	29° 44.287	1816											
	4	52° 35.811	29° 44.269	1819											
	5	52° 34.601	29° 37.103	1569											
	6	52° 34.449	29° 37.465	1565											
	7	52° 33.704	29° 37.946	1549											
	8	52° 34.586	29° 39.490	1670											
	9	52° 32.642	29° 40.263	1728											
	10	52° 35.789	29° 44.353	1821											
	11	52° 35.785	29° 44.351	1818											
<i>P. scoparia</i> (Nourabad)	3	51° 20.875	30° 4.695	1179	420.65	7.93	34.81	23.48	1.46						
	4	51° 39.730	29° 48.513	934											
	5	51° 32.377	30° 1.167	1086											
	6	51° 23.931	30° 0.745	1285											
	7	51° 39.823	29° 48.605	946											
	8	51° 21.011	30° 6.513	1183											
	9	51° 31.694	30° 1.252	1067											
	10	51° 58.42.7	30° 01.08.4	1592											
	<i>P. scoparia</i> (Marvdasht)	1	52° 54.983	30° 3.162						1728	283.64	3.11	30.16	19.22	1.38
		2	52° 54.800	30° 6.249						1730					
3		53° 00.807	30° 6.800	1812											
4		53° 12.117	30° 5.742	1837											
5		53° 10.524	30° 1.617	1828											
6		53° 12.643	29° 59.116	1803											

	7	53 14.080	29 57.712	1765					
	8	53 6.493	29 48.754	1667					
	9	53 6.535	29 48.816	1663					
	10	53 8.662	29 47.443	1640					
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<i>P. scoparia</i> (Firouzabad)	2	52 32.509	29 8.712	1725					
	3	52 34.486	28 58.157	1503					
	5	52 32.400	28 55.816	1445					
	6	52 38.719	29 5.885	1917					
	8	52 38.310	29 3.808	1732					
	9	52 32.801	29 9.124	1763	323.43	9.65	31.18	21.96	2.49
	10	52 41.274	28 13.319	1275					
	11	----	----	1578					
<hr/>									
<i>P. scoparia</i> (Mian Jangal Fasa)	1	52 46.117	29 26.327	1481					
	2	52 50.130	29 19.653	1526					
	3	--	--	2187					
	4	53 24.351	29 9.542	1729					
	5	53 23.894	29 9.939	1754					
	6	53 26.033	29 7.617	1720					
	7	53 26.054	29 7.632	1716	261.97	6.01	32.50	19.74	1.78
	9	53 22.759	29 10.821	1815					
	10	53 19.277	29 12.351	1825					
	<hr/>								
<i>P. scoparia</i> (Eqlid)	3	52 40.003	30 15.126	1701					
	4	52 38.310	30 16.346	1742					
	5	52 36.405	30 18.062	1800					
	6	52 35.080	30 22.196	2321					
	7	52 23.051	30 19.324	1843	323.54	0.05	24.28	13.16	2.73
	8	52 23.764	30 19.060	1750					
<hr/>									
<i>P. scoparia</i> (Lordegan)	1	51 11.609	31 33.619	1752					
	2	51 13.020	31 34.354	1962					
	3	--	--	1948	551.46	2.15	28.88	15.44	1.33
	4	--	--	1963					
<hr/>									
<i>P. elaeagnifolia</i>	1	52 35.806	29 44.098	1801					
	2	52 35.746	29 44.158	1804	297.76	4.99	30.72	18.78	1.73
	3	--	--	2128	551.46	2.15	28.88	15.44	1.33
	4	--	--	2570	190.47	7.70	30.04	20.89	2.34
<hr/>									
<i>P. eburnea</i>	2	52 22.173	30 19.865	1912	323.54	0.05	24.28	13.16	2.73
	4	--	--	2280	190.47	7.70	30.04	20.89	2.34
	5	52 24.585	30 18.376	1711	323.54	0.05	24.28	13.16	2.73
	7	52 22.130	30 19.854	1902	323.54	0.05	24.28	13.16	2.73
	8	53 24.101	29 9.071	1763	261.97	6.01	32.50	19.74	1.78
<hr/>									
<i>P. dulcis</i>	Mamaei	---	---						
	Ferragnes	---	---	1910	340.95	-5.12	25.21	11.13	2.15
	Badam talk	---	---						
	GF667	---	---	2280	190.47	7.70	30.04	20.89	2.34

Nut and seedling trait assessment

A total number of 60 nuts per accession selected randomly from the collected nut batches of each accession.

1. A group of 30 nuts devoted for nut parameter measurements including nut weight and kernel percentage using an electronic balance with 0.001 g precision.

2. A group of 30 nuts used to grow the seedlings. The nuts scarified mechanically and then soaked in water for 24 h (Fig. 2). The seeds were mixed with

perlite and stratified at $4 \pm 1^\circ\text{C}$ for 45 days. After stratification, the single seeds were transferred to 5 kg pots filled with a mixture of fine sand, leaf mould and soil (1:1:1, volume ratio). The pots were then transferred to the greenhouse with an average temperature of $26 \pm 3^\circ\text{C}$ under natural photoperiod for the whole period of the experiment. After the growth of one-year-old seedling, for each accession, six healthy seedlings were randomly selected (as replications) for seedling morphological characterization (Fig. 2). Recorded observations of seedlings were: seedling height, stem diameter, number of lateral branches, length of lateral

branches, height of the first lateral branch, internode length, leaf area, leaf length, leaf width, leaf dry matter and leaf ash percentage. Variables such as seedling height, height of the first lateral branch, internodes and length of lateral branches, leaf length and width were measured using a proper ruler. The stem diameter was measured using a digital caliper. Leaf dry weight (g)

was measured after drying samples for 72 h at 70°C, using an electronic balance with 0.001 g precision. Leaf ash weight was also measured after subjection samples to 500 °C for 4 h in an electric oven. Leaf area (mm²) was determined using a leaf area meter (Delta-T devices, Ltd., Cambridge, UK).



Fig.2. Nut samples soaked in water to be used in raising seedlings (down) and raised seedling after one year of growth in greenhouse condition (top).

Statistical analyses

The experiment performed in a completely randomized design with six replications. The results were expressed as means \pm standard error. The diversity index calculated based on the quotient of the norm deviation over the characteristic average using this formula: Diversity Index= (Std. Deviation / Mean) \times 100 (Rahemi *et al.*, 2011). The correlation between variables, including seeds and seedling attributes as well as habitat climatic parameters, was performed by Pearson's correlation coefficient using SPSS statistics software version 16 (SPSS Inc., Chicago, IL, USA). Clusters analysis was conducted on the basis of all the measured characteristics according to Ward's method

using Minitab software, version 16 (Minitab, Inc., 1998). The graph showing classification is a dendrogram of dissimilitude with standardized Euclidean distances which indicates the closest accessions in homogeneous groups (Alinia Ahandani *et al.*, 2014). Principal components analysis (PCA) was standardized to similar dimensions with the biplot procedure using Minitab software, version 16 (Minitab, Inc., 1998). This method also applied in previous studies (Azimi *et al.*, 2018; Hassanzadeh Khankahdani *et al.*, 2019). Cluster analysis conducted on standardized component coordinates to individuate grouping of characteristics and genotypes (Bianco *et al.*, 2013).

Results

Variations of nut and seedling characteristics

The analysis of variance for all traits are presented in Table 2. A very high level of variations was recorded for almost all of the measured traits among the studied genotypes of different species (Table 3). Nut weight and kernel percentage varied from 0.27 to 4.80 g and 9.89 to 45.71 % among all genotypes assigned to four species, respectively and ranged between 0.27-1.03 g and 25.00-45.71 % among *P. scoparia* populations, respectively. The highest (3.93 g) and lowest (0.33 g) average value of nut weight was recorded in *P. dulcis* and *P. eburnea*, respectively, whilst, the highest (33.49 %) and lowest (21.88 %) mean value of kernel percentage were measured in *P. scoparia* and *P. elaeagnifolia*, respectively. Among *P. scoparia* species, the largest mean values for nut weight and kernel percentage (0.76 g and 38.24 %) belonged to Nourabad and Lordegan populations, respectively.

Results indicated that the tallest seedlings belonged to the common almond, *P. dulcis* (57.47 cm), while *P. eburnea* had the smallest seedlings (27.01 cm). Among wild almond species the mean value of seedling height in *P. scoparia* (34.56 cm) was slightly higher than that of *P. elaeagnifolia* (27.69 cm) and *P. eburnea* (27.01 cm) populations.

Stem diameter varied from 1.96 to 9.88 mm among all genotypes of four species and from 1.96 to 7.69 mm among all accessions of *P. scoparia*. The thickest and thinnest stems were recorded in *P. dulcis* and *P. scoparia*, respectively. Among *P. scoparia* populations, Lordegan had the thickest stem (4.73 mm), whereas the thinnest stem (3.65 mm) belonged to Marvdasht.

Results showed that the height of the first lateral varied from 0 to 510 mm among all genotypes of four species and from 0 to 190 mm among all accessions of *P. scoparia*. The greatest height of the first lateral (510 mm) was recorded in *P. dulcis*. Among *P. scoparia* populations, Nourabad was recorded as population having the highest first lateral (190 mm).

Internode length varied from 0.92 to 13.13 mm among four species and ranged between 3.57-13.13 mm among *P. scoparia* populations. Considering the mean values, the highest (8.41 mm) and lowest (5.16 mm) internode length were recorded in *P. dulcis* and *P. eburnea*, respectively. Among *P. scoparia* populations, the highest and the lowest internode length belonged to Lordegan and Marvdasht, respectively.

The largest number (20 branches) and length (50.4 cm) of lateral branches were recorded in *P. eburnea* and *P. scoparia*, respectively. Among *P. scoparia* species, the largest mean values for number and length of lateral branches belonged to Shiraz population (15 branches and 50.40 cm, respectively).

Table 2. Analysis of variance of studied traits in almond genotypes assigned to *Prunus scoparia* populations (upper rows) and four almond species (lower rows).

Sources of variations	Degree of freedom	Mean-square							
		Seed weight (g)	Kernel Percentage (%)	Seeding height (cm)	Height of first lateral (mm)	Stem diameter (mm)	Inter node length (mm)	Number of laterals	Length of laterals (cm)
<i>P. scoparia</i> populations	6	0.297**	286.962**	2200.239**	730.999**	6.843**	72.895*	123.635**	1073.780**
Error	341	0.022	13.424	154.696	288.500	0.708	1.833	10.041	59.003
Almond species	3	88.099**	1453.280**	5125.741**	215440.90**	91.417**	57.031**	276.607**	1233.458**
Error	422	.052	24.421	182.095	1839.412	.817	2.880	12.089	68.190

ns and ** represent non significance and significance at the 1% level respectively.

Table 2 (Continued). Analysis of variance of studied traits in almond genotypes assigned to *Prunus scoparia* populations (upper rows) and four almond species (lower rows).

Sources of variations	Degree of freedom	Mean-square				
		Leaf length (mm)	Leaf width (mm)	leaf area (mm ²)	Leaf dry matter (%)	Leaf ash (%)
<i>P. scoparia</i> populations	6	115.607**	16.539**	3950.029**	1269.690**	15.679**
Error	341	8.857	.595	113.149	38.672	4.251
Almond species	3	805.854**	1857.042**	4644352.784*	1594.221**	10.161 ^{ns}
Error	422	57.308	2.546	6710.321	242.170	4.156

ns and ** represent non significance and significance at the 1% level respectively.

Table 3. Diversity of nut and seedling characteristics in populations of wild and domesticated almond species evaluated in this study.

Population (No. of accession)	Index	Seed weight (g)	Kernel Percentage (%)	Seeding height (cm)	Height of first lateral (mm)	Stem diameter (mm)	Inter node length (mm)	Number of laterals	Length of laterals (cm)
<i>P. scoparia</i> Shiraz (11)	Range	0.27-1.03	28.39-39.28	17.60-71.50	0.00-32.00	3.38-7.69	4.34-12.50	1.00-15.00	7.8-50.4
	Mean*	0.55 ^b ±0.03	35.60 ^b ±0.43	36.42 ^b ±1.31	7.94 ^b ±1.03	4.61 ^a ±0.10	8.07 ^b ±0.20	8.80 ^a ±0.42	18.56 ^b ±0.94
<i>P. scoparia</i> Nourabad (8)	Range	0.34-0.58	32.75-45.71	8.10-77.50	0.00-190.00	1.96-5.75	5.55-12.5	0.00-15.00	0.00-30.16
	Mean	0.47 ^{cd} ±0.009	38.24 ^a ±0.57	37.42 ^b ±0.38	18.77 ^a ±4.49	4.05 ^b ±0.13	7.55 ^b ±0.23	7.06 ^{bc} ±0.56	16.21 ^b ±1.36
<i>P. scoparia</i> Marvdasht (10)	Range	0.35-0.61	27.02-41.3	11.50-63.10	0.00-63.00	2.09-5.56	3.57-8.33	0.00-11.00	0.00-35.65
	Mean	0.46 ^d ±0.01	33.47 ^c ±0.51	25.04 ^c ±1.37	11.96 ^{ab} ±1.86	3.65 ^c ±0.09	5.69 ^d ±0.15	5.06 ^{de} ±0.34	10.45 ^c ±0.74
<i>P. scoparia</i> Firouzabad (8)	Range	0.43-0.72	25.00-40.00	15.70-77.50	0.00-50.00	2.20-6.90	5.44-11.11	0.00-13.00	0.00-38.16
	Mean	0.53 ^{bcd} ±0.01	33.04 ^c ±0.67	31.92 ^b ±1.83	8.87 ^b ±1.92	4.02 ^b ±0.14	8.07 ^b ±0.19	6.16 ^{cd} ±0.43	15.09 ^b ±1.11
<i>P. scoparia</i> Mian Jangal Fasa (10)	Range	0.41-0.90	25-33.33	17.00-60.80	0.00-65.00	7.41-8.88	0.92-1.63	0.41-0.80	2.00-3.00
	Mean	0.57 ^b ±0.01	31.03 ^d ±0.31	34.30 ^b ±1.31	14.90 ^{ab} ±1.92	4.38 ^{ab} ±0.10	6.56 ^c ±0.11	8.15 ^{ab} ±0.41	16.79 ^b ±0.86
<i>P. scoparia</i> Eqlid (7)	Range	0.38-0.76	25.49-35.29	19.01-77.90	0.00-55.00	2.60-5.53	4.61-9.70	1.00-12.00	7.66-38.65
	Mean	0.54 ^{bc} ±0.01	32.46 ^{cd} ±0.47	35.78 ^b ±2.00	14.33 ^{ab} ±1.99	4.10 ^b ±0.11	6.53 ^c ±0.18	5.97 ^{cd} ±0.45	17.98 ^b ±1.13
<i>P. scoparia</i> Lordegan (4)	Range	0.51-0.93	27.77-36.95	29.40-82.90	0.00-75.00	3.37-5.89	7.1-13.13	1.00-8.00	12.25-42.00
	Mean	0.76 ^a ±0.03	33.88 ^c ±0.76	51.31 ^a ±3.02	12.33 ^{ab} ±3.69	4.73 ^a ±0.16	9.84 ^a ±0.30	4.41 ^c ±0.73	26.11 ^a ±1.71
CV		27.30	10.80	36.02	35.27	20.07	18.58	46.60	45.75
<i>P. scoparia</i> Total (60)	Range	0.27-1.03	25.00-45.71	8.10-82.90	0.00-190.00	1.96-7.69	3.57-13.13	0.00-15.00	0.00-50.40
	Mean*	0.54 ^C ±0.008	33.49 ^A ±0.31	34.56 ^B ±0.73	12.53 ^C ±0.92	4.19 ^B ±0.04	7.26 ^B ±0.09	6.79 ^B ±0.18	16.50 ^A ±0.44
<i>P. elaeagnifolia</i> (4)	Range	0.48-1.07	14.01-29.85	5.70-56.50	0.00-150.00	2.16-6.53	4.34-9.52	0.00-8.00	0.00-40.80
	Mean	0.81 ^B ±0.05	21.88 ^C ±1.17	27.69 ^C ±2.87	59.04 ^B ±10.50	4.24 ^B ±0.19	6.50 ^B ±0.25	2.75 ^C ±0.46	10.35 ^{BC} ±2.18
<i>P. eburnea</i> (5)	Range	0.28-0.46	26.08-41.37	9.70-49.80	15.00-174.00	1.98-5.91	2.94-11.11	1.00-20.00	2.20-15.25
	Mean	0.33 ^D ±0.00	34.44 ^A ±0.92	27.01 ^C ±1.59	60.33 ^B ±7.49	4.37 ^B ±0.19	5.16 ^C ±0.28	9.66 ^A ±0.81	6.49 ^C ±0.69
<i>P. dulcis</i> (4)	Range	3.07-4.8	9.89-36.53	30.30-80.00	0.00-510.00	6.68-9.88	6.06-11.76	0.00-13.00	0.00-27.20
	Mean	3.93 ^A ±0.14	26.50 ^B ±2.13	57.47 ^A ±2.73	173.60 ^A ±32.00	7.68 ^A ±0.12	8.41 ^A ±0.28	3.87 ^C ±0.64	11.87 ^B ±1.61
CV		31.05	15.03	38.66	15.54	20.52	23.71	52.66	54.41

* 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 $P < 0.05$.

The range of leaf length varied from 3.79 to 75 mm, leaf width ranged between 1.3 - 31 mm and variations of the leaf area were from 1.75 to 1601.2 mm² among all accessions of four species. Notably, a high level of variations recorded among *P. scoparia* populations for leaf size. Considering the mean values for leaf size again not surprisingly, the largest leaf area belonged to

P. dulcis (804.7 mm²), while *P. scoparia* had the smallest leaf area (19.72 mm²). Among *P. scoparia* species, the largest mean values for leaf size (23.55 mm, 5.12 mm and 46.66 mm² for leaf length, width and area, respectively) were recorded in the Lordegan population, while the smallest mean values (13.42 and 12.81 mm² for leaf length and leaf area, respectively) belonged to

Marvdasht population. Totally, the studied *P. scoparia* germplasm had low leaf area which can indicate better adaptation to drought conditions.

Leaf dry matter and leaf ash varied from 21.95 to 82.45 % and 0.68 to 17.54 % among all studied accessions of four species, respectively. The range of leaf dry matter and leaf ash among *P. scoparia* populations were between 19.79-82.45 % and 0.68-17.54%, respectively. Considering the mean values, the highest (41.43 %) and lowest (31.11 %) mean values for

leaf dry matter belonged to *P. scoparia* and *P. elaeagnifolia*, respectively, while the highest (4.33 %) and lowest (3.22 %) mean values for leaf ash belonged to *P. dulcis* and *P. elaeagnifolia*, respectively. Within *P. scoparia*, the Mian Jangal Fasa and Lordegan populations had the highest (49.71 %) and Lowest (36.73 %) mean values of dry matter, while, Nourabad and Lordegan had the highest (4.87 %) and lowest (3.15 %) mean values of leaf ash.

Table 3. (Continued). Diversity of nut and seedling characteristics in populations of wild and domesticated almond species evaluated in this study.

Population (No. of accession)	Index	Leaf length (mm)	Leaf width (mm)	leaf area (mm ²)	Leaf dry matter (%)	Leaf ash (%)
<i>P. scoparia</i> Shiraz (11)	Range	9.30-23.20	1.40-4.50	1.75-40.35	23.86-50	1.13-10.52
	Mean*	15.80 ^b ±0.38	3.13 ^{cd} ±0.09	17.55 ^c ±1.13	37.46 ^c ±0.33	4.39 ^a ±0.23
<i>P. scoparia</i> Nourabad (8)	Range	9.80-23.60	2.60-6.70	7.00-75.55	31.81-46.83	1.04-15.21
	Mean	15.90 ^b ±0.49	3.69 ^b ±0.11	22.38 ^b ±1.76	38.78 ^c ±0.51	4.87 ^a ±0.42
<i>P. scoparia</i> Marvdasht (10)	Range	9.00-18.8	1.90-5.40	2.19-40.25	31.94-56.36	1.81-10.76
	Mean	13.42 ^c ±0.29	2.99 ^{cd} ±0.08	12.81 ^d ±0.85	44.22 ^b ±0.30	4.48 ^a ±0.19
<i>P. scoparia</i> Firouzabad (8)	Range	10.20-20.30	1.70-4.50	3.85-29.79	21.95-49.18	1.51-17.54
	Mean	15.16 ^b ±0.34	2.96 ^d ±0.08	13.32 ^{cd} ±0.96	36.78 ^c ±0.98	4.26 ^{ab} ±0.23
<i>P. scoparia</i> Mian Jangal Fasa (10)	Range	11.80-22.40	1.50-5.10	6.10-47.14	29.16-82.45	0.68-7.54
	Mean	16.42 ^b ±0.31	3.32 ^c ±0.10	22.83 ^b ±1.30	49.71 ^a ±1.18	3.43 ^{bc} ±0.18
<i>P. scoparia</i> Eqlid (7)	Range	8.40-20.20	1.30-4.30	3.83-35.68	36.11-51.31	1.16-14.70
	Mean	15.15 ^b ±0.40	3.29 ^{cd} ±0.08	17.46 ^c ±1.17	42.90 ^b ±0.48	4.46 ^a ±0.48
<i>P. scoparia</i> Lordegan (4)	Range	13.20-30.40	2.40-7.00	7.71-92.70	28.87-48.38	1.38-5.37
	Mean	23.55 ^a ±0.90	5.12 ^a ±0.25	46.66 ^a ±4.85	36.73 ^c ±0.52	3.15 ^c ±0.17
CV		18.08	22.83	53.71	10.94	6.05
<i>P. scoparia</i> Total (60)	Range	8.40-30.40	1.30-7.00	1.75-92.70	21.95-82.45	0.68-17.54
	Mean**	15.88 ^B ±0.19	3.35 ^C ±0.05	19.72 ^C ±0.71	41.43 ^A ±0.24	4.21 ^{AB} ±0.09
<i>P. elaeagnifolia</i> (4)	Range	17.10-31.00	5.70-12.60	41.85-219.15	19.79-38.85	2.06-5.74
	Mean	24.21 ^A ±0.85	8.75 ^B ±0.39	102.06 ^B ±8.85	31.11 ^C ±0.63	3.22 ^B ±0.11
<i>P. eburnea</i> (5)	Range	10.80-22.00	2.60-5.40	8.25-41.76	29.41-45.90	1.28-11.32
	Mean	14.79 ^B ±0.34	3.95 ^C ±0.12	20.42 ^C ±1.77	37.52 ^B ±0.51	3.62 ^{AB} ±0.36
<i>P. dulcis</i> (4)	Range	3.79-75.00	11.30-31.00	364.5-1601.2	21.69-79.86	1.62-8.17
	Mean	22.19 ^A ±5.85	18.45 ^A ±1.09	804.7 ^A ±70.2	38.02 ^{AB} ±12.13	4.33 ^A ±0.20
CV		45.48	34.98	11.92	37.88	48.80

* 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 $P < 0.05$.

Leaf area, the height of first lateral and nut weight considered as the traits having the highest diversity with the variation coefficient of 289.35, 209.67 and 111.69

%, respectively. The smallest diversity index (21.28 %) was belonged to kernel percentage (Table 4).

Table 4. Mean, standard error, max, min, standard deviation and diversity index of nut and seedling characteristics of wild almond species and populations evaluated in this study.

Traits	Mean	Std. error	Max	Min	Std. dev.	Diversity index
Nut weight (g)	0.74	0.04	4.80	0.27	0.82	111.69
Kernel percentage	32.51	0.34	45.71	1.65	6.92	21.28
Seedling height (cm)	34.94	0.71	82.90	5.70	14.73	42.17
Height of first lateral (mm)	27.59	2.80	510.00	0.00	57.85	209.67
Stem diameter (mm)	4.41	0.059	9.88	1.96	1.21	27.38
Internode length (mm)	7.14	0.09	13.13	2.94	1.81	25.29
The number of laterals	6.61	0.18	20.00	0.00	3.74	56.54
The length of laterals (cm)	15.20	0.42	50.40	0.00	8.74	57.53
Leaf length (mm)	16.63	0.38	75.00	3.79	7.91	47.57
Leaf width (mm)	4.55	0.19	31.00	1.30	3.95	86.90
Leaf area (mm ²)	68.64	9.62	1601.22	1.75	198.61	289.35
Leaf dry matter (%)	41.07	0.08	323.14	19.80	15.87	38.63
Leaf ash (%)	4.12	0.10	17.54	0.69	2.05	49.66

$$\text{Diversity Index} = (\text{Std. Deviation} / \text{Mean}) \times 100$$

Correlation among traits

Simple correlation coefficient analysis revealed significant positive/negative correlations between many pairs of the measured characteristics (Table 5). Nut weight was positively and highly correlated with almost all of the seedling traits (ranging from $r = 0.17$ for leaf dry matter to $r = 0.89$ for leaf width). These are while, kernel percentage negatively correlated with almost all of the growth parameters in raised seedlings (ranging from $r = -0.10$ for seedling height to $r = -0.53$ for leaf length).

Seedling height positively and highly correlated with many traits (ranging from $r = 0.25$ for the height of the first lateral to $r = 0.63$ for the length of the laterals). Seedling height was also correlated to leaf area ($r = 0.36$). There was a positive correlation ($r = 0.47$) between internode length and the seedling height. Results also indicated that the lateral branch's length was also associated with the internode length of the stem ($r = 0.40$).

Stem diameter also correlates positively, but not strongly, with number ($r = 0.17$) and length of the lateral branches ($r = 0.21$) and it appears that large-diameter trees will support more branches. Based on the results, stem diameter associates positively with most of the measured characteristics.

Correlation among seedling morphology and habitat geo-climatic parameters

Simple correlation coefficient analysis revealed significant positive/negative correlations between some of plant morphological characteristics and habitat geographical and climatic parameters (Table 5). Considering the environmental parameters, the number and length of the lateral branches correlated negatively with the altitude. The enhancement of temperature correlates negatively with the seedling height, leaf area and stem diameter ($r = -0.15$, $r = -0.39$ and $r = -0.29$, respectively). It is interesting to know that altitude (at

which the plant materials were sampled) had a significant and positive correlation (but not very strong) with the height of the first lateral, stem diameter, leaf size and nut weight (ranging from $r = 0.18$ to $r = 0.29$).

Precipitation showed correlations with seedling height, internode length, lengths of lateral branches and kernel percentage ($r = 0.21$, $r = 0.33$, $r = 0.21$ and $r = 0.14$, respectively).

Table 5. Correlation coefficients (Pearson) of seedling characteristics in wild almond species

	Seed weight	Kernel Percentage	Seedling height	Height of first lateral	Stem diameter	Inter node length	Number of laterals	Lengths of laterals	Leaf length	Leaf width	Leaf area	Leaf dry matter	Leaf ash
Kernel Percentage	-0.36*												
Seedling height	0.39*	-0.10											
Height of first lateral	0.54**	-0.17*	0.25*										
Stem diameter	0.66**	-0.23*	0.54**	0.42**									
Inter node length	0.21*	0.06	0.47**	0.04	0.28*								
Number of laterals	-0.21*	0.09	0.054	-0.14	0.17*	-0.04							
Lengths of laterals	-0.04	-0.03	0.63**	-0.11	0.21*	0.40**	0.01						
Leaf length	0.29*	-0.53**	0.26*	-0.13	0.22*	0.12	0.03	0.18*					
Leaf width	0.89**	-0.34*	0.33*	0.57**	0.60**	0.17*	-0.25*	-0.10	0.30*				
Leaf area	0.86**	-0.24*	0.36*	0.69**	0.62**	0.18*	-0.20*	-0.08	0.07	0.87**			
Leaf dry matter	0.17*	0.04	0.03	-0.11	0.08	-0.90	-0.04	-0.05	-0.11	0.01	0.12		
Leaf ash	0.02	0.06	-0.09	-0.07	-0.09	-0.04	-0.12	0.06	0.03	-0.07	0.01	0.08	
Altitude	0.28**	-0.42**	-0.05	0.19*	0.18**	-0.08	-0.13*	-0.12	0.26*	0.29**	0.23**	-0.07	0.43**
Mean precipitation	-0.01	0.14*	0.21*	0.002	-0.01	0.33**	-0.13*	0.21**	0.03	0.06	0.01	-0.11	-0.03
Mean daily temp	0.35**	0.16*	-0.15*	0.36**	-0.29**	0.05	0.09	0.01	0.12	0.37**	0.39**	-0.02	-0.11
Maximum temp	0.27**	0.18*	-0.07	0.28**	-0.18**	0.10	0.10	0.07	0.08	0.27**	0.30**	-0.06	-0.04
Minimum temp	0.41**	0.08	-0.14*	0.42**	-0.30**	0.10	0.14*	0.08	0.21*	0.43**	0.48**	0.02	-0.11
Mean wind speed	0.11	-0.15*	0.01	0.13*	0.09	-0.06	0.07	-0.06	-0.02	0.08	0.11	0.07	-0.03

* and **: Significant differences at 5% and 1% probability levels, respectively.

Cluster analysis

According to the cluster analysis, the species were categorized into two main groups, including common almonds and wild almonds (Fig. 3). The division of all the wild species into subgroups correlated with their botanical classification as follow: the only population of *P. elaeagnifolia* species was located in the first subgroup. Four out of seven populations of *P. scoparia* including Lordegan, Nourabad, Shiraz and Firuzabad were positioned in the second subgroup. The only population of *P. eburnea* species and three populations

of *P. scoparia* including Eqlid, Marvdasht and Mian Jangal Fasa were placed together in the third subgroup. *P. eburnea* resembles *P. scoparia* in terms of many traits such as kernel percentage, leaf area, leaf dry weight, leaf ash percentage, leaf width and length, and stem diameter.

Three cultivars of the common almond (*P. dulcis*) including 'Ferragnes', 'Mamaei' and 'Badam talk' (bitter almond) and GF677 were positioned in two

different subgroups within the main cluster of domesticated plant materials.

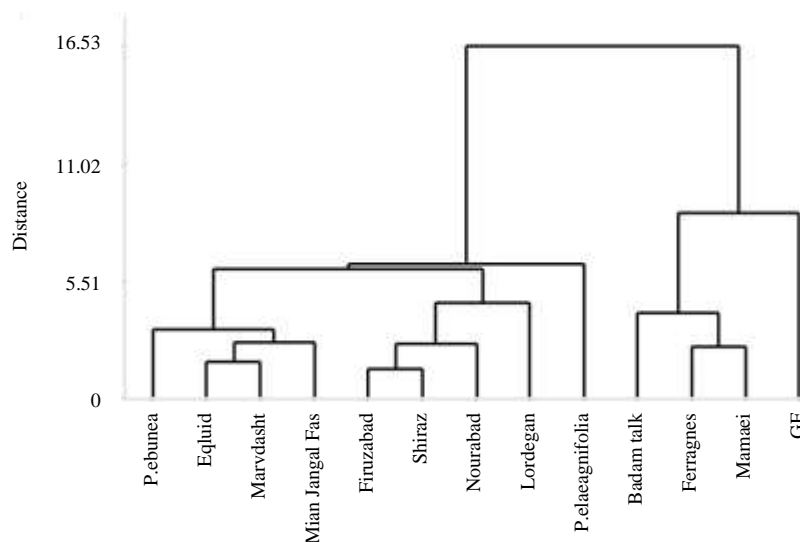


Fig. 3. Dendrogram using Ward (1963) method for clustering almond populations based on their nut and seedling characteristics.

Principal component analysis and Biplot

According to the PCA, five components explained 88.80 % of the total variance (Table 6). The first component contributed 42.80 % of the variation where nut weight, seedling height, the height of the first lateral, stem diameter, the number of laterals, leaf width, leaf area and leaf dry matter had the highest loadings. The second component accounted for 19.80 % of the total variation and featured kernel percentage, leaf length, leaf ash matter while the third component accounted for 13.50 % of the variation featuring a length of laterals and internode length. Additionally, a biplot was prepared according to the PC1 and PC2 that reflected

the relationship among the genotypes in terms of phenotypic resemblance and morphological traits. Accessions were distributed in four quadrants. Marvdasht, Nourabad, Mian Jangal Fasa, Firuzabad, Eqlid and Shiraz populations of *P. scoparia* as well as *P. eburnea*, were grouped together. Lordegan population of *P. scoparia* and *P. elaeagnifolia* were found in the middle portion of the biplot. It is interesting to notice that ‘Ferragnes’, ‘Mamaei’ and ‘Badam talk’ were grouped together and GF667 was located alone (Fig. 4).

Table 6. Eigenvectors of the five principal component axes from PCA analysis of morphological variables in wild almond species.

Trait	Component				
	1	2	3	4	5
Seed weight (g)	0.316	0.148	-0.065	-0.242	-0.027
Kernel percentage	-0.111	-0.432	-0.051	-0.198	0.243
Seedling height (cm)	0.250	0.192	-0.334	0.029	0.204
Height of the first lateral (mm)	0.293	-0.107	-0.026	-0.017	0.277
Stem diameter (mm)	0.320	0.127	-0.114	-0.081	0.165
Internode length (mm)	0.147	-0.030	-0.505	0.094	0.041
The number of laterals	-0.229	0.166	0.090	0.042	0.547

The average length of laterals (cm)	-0.113	0.139	-0.437	0.338	0.082
Leaf length (mm)	-0.017	0.486	-0.080	0.029	-0.231
Leaf width (mm)	0.319	0.078	-0.035	-0.242	-0.150
Leaf area (mm ²)	0.331	0.023	-0.078	-0.134	-0.167
Leaf dry matter (%)	0.159	-0.092	0.078	-0.506	-0.178
Leaf ash (%)	-0.065	0.415	-0.032	-0.203	0.235
Eigenvalue	8.12	3.76	2.57	1.46	0.93
Variance (%)	42.80	19.80	13.50	7.70	4.90
Cumulative (%)	42.80	62.60	76.10	83.80	88.80

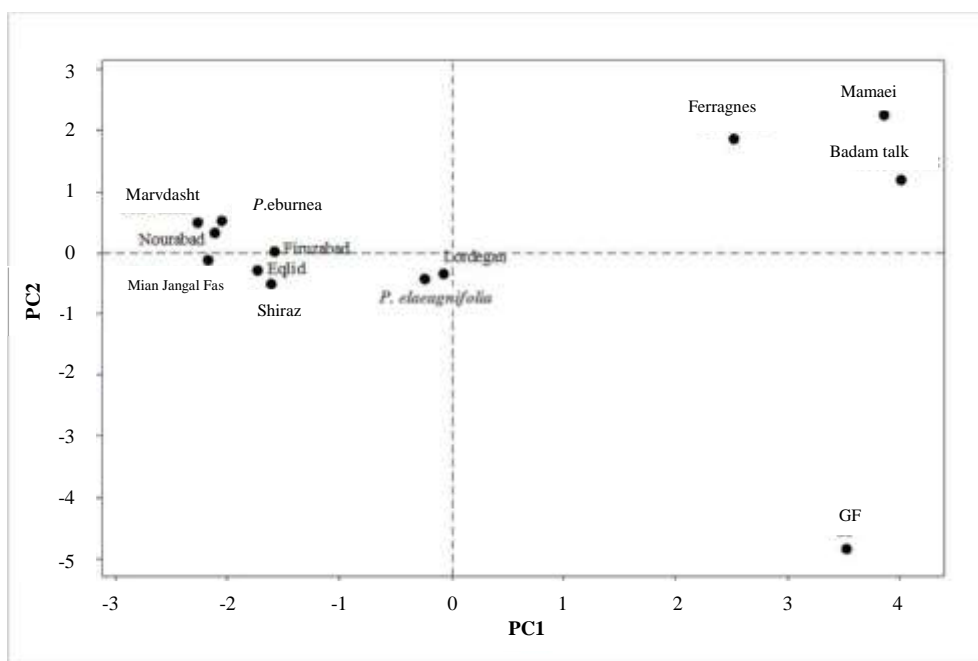


Fig. 4. The biplot diagram of the first and second principal components (explaining 42.80 % and 19.80 % of total variance, respectively), as a result of principal components analysis of almond populations based on their nut and seedling characteristics.

Discussion

Variations of nut and seedling characteristics

High nut weight ought to be considered in breeding programs aiming to nut production and oil extraction (Rahimi Dvin et al., 2017), and it is also believed that corresponded to the vegetative growth of the seedling raised from the nut (Finch-Savage & Bassel, 2015). One of the most interesting points of current study was that the kernel percentage of wild almonds (*P. scoparia* and *P. eburnea*) was even higher than that of the domesticated one.

Among wild almond species the mean value of seedling height in *P. scoparia* (34.56 cm) was slightly higher than that of *P. elaeagnifolia* (27.69 cm) and *P. eburnea* (27.01 cm) populations which all corresponded with the findings of Madam et al. (2011) and Mousavi et al. (2011). Low tree height should be considered in breeding programs as a useful trait for the development of dwarfing rootstocks (Vahdati et al., 2008; Vahdati et al., 2014). Khadivi-Khub and Anjam, (2016) also recommended the *P. scoparia* as a potential species to be used as a dwarfing rootstock for almonds.

The diameter of the stem is vital regarding resin and gum production. The formation of resin-producing ducts by most resin-producing plants to defend themselves has been reported by Lombardero *et al.* (2006). One of the reasons why wild almond trees are resistant to pests and diseases is believed to be associated with their gum production property (Daryaei *et al.*, 2012; Lombardero *et al.*, 2006). Also, the increase of stem and shoot thickness enhances hydraulic conductivity in various plant species (Cochard, 1992). Thick branches are able to allow water and necessary nutrients to the fruit, thus leads to higher growth and fruit quality (Genard & Bruchou, 1992).

The greatest height of the first lateral (510 mm) was recorded in *P. dulcis*. Among *P. scoparia* populations, Nourabad was recorded as population having the highest first lateral (190 mm). This characteristic is notably suitable in mechanized harvesting. It has been reported that the *Pistacia atlantica* sub. *mutica* ('Baneh') tree owing to having a trunk with no lateral branches is a proper rootstock for the pistachio orchard where mechanized harvesting needs trees of 2 or 3 meters high (Daryaei *et al.*, 2012). It seems that the higher the first lateral is from the ground, the more suitable it will be for the rootstock as well as for mechanized harvesting. It has been observed that almond trees have mostly crotonic shapes (Costes *et al.*, 2014) but *P. scoparia* seems to lie in basitonic group because of its particular and dwarf tree morphology. The acrotonic versus basitonic branching position means long shoots are located in the top and basal part of the trunk, respectively (Costes *et al.*, 2006). Moreover, with dwarf and basitonic canopy structures, *P. scoparia* could be a promising source of more compact growth habits and early crop maturity for the further breeding programs (Sorkkeh *et al.*, 2009; Ansari & Gharaghani, 2019).

P. scoparia normally lose its leaves in early summer and its green shoots continue to do photosynthesis supplying carbohydrates for the shoot and root growth

and development in the rest of the growing season (compensating the leaves absence). Since the source of gibberellins and cytokinins are in the roots (Arteca, 2013), transportation of these substances to shoots enhance more internode elongation in *P. scoparia* (instead of new leaf formation) in comparison with *P. eburnea* and *P. elaeagnifolia* (Madam *et al.*, 2011).

Considering the leaf size not surprisingly, the largest leaf area belonged to *P. dulcis* (804.7 mm²), while *P. scoparia* had the smallest leaf area (19.72 mm²). Among *P. scoparia* species, the largest leaf size (46.66) were recorded in the Lordegan population, while the smallest values (12.81 mm²) belonged to Marvdasht population. Totally, the studied *P. scoparia* germplasm had low leaf area which can indicate better adaptation to drought conditions. This result corresponded with the study of Sorkkeh *et al.* (2009) who stated that reduced leaf area is an early response of adaptation to drought conditions. The population of Lordegan grown from the seeds collected from higher altitudes bore larger leaves due to the higher amount of yearly precipitation and cooler climate. As the *P. scoparia* leaves drop in early summer, these species can better match as rootstock since the green shoots keep on photosynthesis, it is probably a mechanism for resistance in drought conditions. Generally, the *P. scoparia* population bore small leaves which were considered as a probable adaptation to the drought conditions (Khadivi-Khub *et al.*, 2016).

The highest (41.43 %) and lowest (31.11 %) leaf dry matter belonged to *P. scoparia* and *P. elaeagnifolia*, respectively, while the highest (4.33 %) and lowest (3.22 %) leaf ash belonged to *P. dulcis* and *P. elaeagnifolia*, respectively. It is interesting to note that *P. scoparia* has higher dry matter than domestic almonds do, although it has a smaller leaf size. The biomass production by plants is indicated by Fresh and dry weights (Barzegargolchini *et al.*, 2017). Interestingly, the accumulation of ash or mineral content is the cheapest and the easiest way in order to predict

the yield and the plant adaptation to drought (Cabrera-Bosquet *et al.*, 2009). As it was reported, the plants which had more dry matter had more yield under salt stress (Wright *et al.*, 1997). It has also been reported that the leaf ash content positively correlates with the yield (Voltas *et al.*, 1998; Monneveux *et al.*, 2004). Additionally, it has been reported that leaf life span is correlates with dry matter content (Wilson *et al.*, 1999).

Leaf area, the height of first lateral and nut weight were considered as the traits having the highest diversity with the variation coefficient of 289.35, 209.67 and 111.69 %, respectively. The smallest diversity index (21.28 %) was belonged to kernel percentage (Table 4). Environmental factors such as water stress could affect leaf area and nutweight (Xie *et al.*, 2001). Studying the fruit characteristics of some Iranian wild almond species, Rahemi *et al.* (2011) reported a high variation coefficient for the nut weight which is in line with the results of the current study. High variation coefficient among the accessions could be owing to species variation and cross-pollination which had resulted in heterozygosity and high genetic variation in almonds during their development and evolution (Kester & Gradziel, 1996). High morphological variation has also been observed in wild almonds gathered from different regions of Iran by Zeinalabedini *et al* (2008). Since almond is an outbreeding tree and spontaneous hybridization occurs among species, high morphological diversity is observed among almond species in Iran. The differences in seedling characteristics among accessions were found to be connected with the evolution of the natural habitat of their ancestors because populations gathered from higher lands had larger leaf sizes, stem diameter, nut weight and height of the first lateral than those situated in lower lands. These results were in line with those reported by Khadivi-Khub and Anjam (2014) in the same almond species.

Correlation among traits

Simple correlation coefficient analysis revealed significant positive/negative correlations between many pairs of the measured characteristics (Table 5). Nut weight was positively and highly correlated with almost all of the seedling traits, these are while, kernel percentage negatively correlated with almost all of the growth parameters in raised seedlings. This is not surprising, as nut weight has a negative correlation with kernel percentage showing that the selection of nut weight would not be effective to enhance the kernel percentage. This result was in agreement with the findings of Thakur *et al.* (2005).

Seedling height positively and highly correlated with many traits. Seedling height was also correlated to leaf area since large trees can enjoy higher leaf photosynthetic capacity and they can receive more light than the smaller ones (Liu *et al.*, 2010). There was a positive correlation ($r = 0.47$) between internode length and the seedling height which is similar to that reported by Baninasab and Rahemi (2007) concerning *P. webbii*. Dwarfism is a vital trait that is very important in developing high-density orchards and simplifies orchard practices. Internode length was found to be positively associated with the papaya tree height (Lim & Siti Hawa, 2007). These results indicated that the lateral branch's length was also associated with the internode length of the stem ($r = 0.40$).

The leaves of trees with long-internode, provides more optimal distribution of light within the canopy, which results in higher content of carbohydrate than those of the short-internode trees (Wang & Faust, 1987). Decreased content of carbohydrate in short internode shoots could be related to lower mean daily exposure to sunshine (DeJong & Doyle, 1984).

Stem diameter also correlated positively, but not strongly, with number ($r = 0.17$) and length of the lateral branches ($r = 0.21$) and it appears that large-diameter trees will support more branches. Thus, higher yield

could be attained from trees with more branches (Vallesteros & Carandang, 2015). Based on the results, stem diameter associates positively with most of the measured characteristics, confirming that larger stems suggest a more optimal physiological condition of the tree and show better and more transmission of nutrients through the trunk. Although this is not exactly in agreement with the results in walnut grafting (Ebrahimi A and Vahdati, 2006; Rezaee *et al.*, 2008), however this correlation may be considered as a proper relationship in order to improve strong rootstocks appropriate for arid or semi-arid lands where fast and vigorous growth is needed at the beginning of the seasonal life cycle to induce and preserve suitable strength in scion and also for gaining a proper size for budding and/or grafting in nurseries as soon as possible (Nikoumanesh *et al.*, 2011). The negative relationship between the height of the first lateral and the number of lateral branches is a vital correlation for the improvement of grafting onto rootstocks.

Correlation among seedling morphology and habitat geo-climatic parameters

Simple correlation coefficient analysis revealed significant positive/negative correlations between some of plant morphological characteristics and habitat geographical and climatic parameters (Table 5). Considering the environmental parameters, the number and length of the lateral branches correlated negatively with the altitude, whilst stem diameter correlated positively with altitude. At higher altitudes, trees take the form of bushes by decreasing the number and the length of the branches so as to be able to resist harsh winds (Orsanic *et al.*, 2009).

The enhancement of temperature correlates negatively with the seedling height, leaf area and stem diameter, showing that access to water is limited as the average temperature increase, as a result, this process causes water stress which results in photosynthesis and

cambium activity decrease. This leads to tree growth reduction which conforms with the result of Kaźmierczak and Zawieja (2014).

Interestingly, altitude (at which the plant materials were sampled) had a significant and positive correlation (but not very strong) with the height of the first lateral, stem diameter, leaf size and nut weight; this was in agreement with the findings of Orsanic *et al.* (2009) who reported that the fruit size of *Sorbus torminalis* increases as the altitude rises and also Roozban *et al.* (2005) who reported various nut quality in pistachios grown in different altitudes. This effect is due to the different climatic conditions experienced at different altitudes.

Precipitation showed correlations with seedling height, internode length, lengths of lateral branches and kernel percentage, because water stress restricts photosynthesis and thus, growth (Niklas, 2007). Considering this correlation, it is clear why Lordegan population of *P. scoparia* (receiving the highest precipitation) was superior to other populations of this species in vegetative attributes.

This information could be used to identify the most suitable species/genotypes for cultivation in a particular area, as well as the most proper areas for the production of wild almonds which is common in Iran.

Cluster analysis

According to the cluster analysis, the species categorized into two main groups, including common almonds and wild almonds (Fig. 3). The division of all the almond species was in accordance with their botanical categorization since *P. scoparia* and *P. eburnea* belong to the section *Spartioides* Spach., while, *P. elaeagnifolia*, and *P. dulcis* are located in the section *Euamygdalus* Spach., within *Rosaceae* family (Kester & Gradziel, 1996). Considering the populations of *P. scoparia*, it seems they clustered to some extent based on their geographic proximity, as Marvdasht and Eqlid

population in cluster I and Shiraz, Firouzabad and Nourabad populations in cluster II, not only are located closely to each other, but also experience quite similar climatic conditions. In this regard Location of Lordegan population of *P. scoparia* in cluster II also could be explained by similar climatic conditions.

The results of the cluster analysis using seedling characteristics are in general agreement with our preceding research considering nut and kernel characteristics (Rahimi Dvin *et al.*, 2017). Genotypes did not entirely cluster according to their geographical origins, indicating a wide diversity among genotypes obtained from the same place. Cross-incompatibility, natural hybridization, propagation by seed, gene flow and plant material exchange between the sampled areas may have involved this variation (Sefc *et al.*, 2000). Since almond is an ancient nut crop in Iran and has a short juvenile period (Kester & Gradziel, 1996), its traditional propagation and distribution were by seeds. Thus, during millennia, owing to communication throughout the country, almond germplasm may have been under exchange among various regions of Iran indicating the possibility of interbreeding among populations. This could explain the overlapping groupings of some populations from various places (for example, the close relationship of Eqlid, Marvdasht, Mian Jangal Fasa populations within *P. scoparia* species). Similar results have been reported in *Corylus colurna*, in which, genotypes coming from the same location were classified in separate clusters, indicating a wide diversity among genotypes coming from the same locale (Srivastava *et al.*, 2010). Similar to our findings, Talhouk *et al.* (2000) showed that morphological assessment is an effective tool for identifying almond germplasm and species differentiation. The total analysis of all traits elucidates a wide diversity that may have important implications for the management of the genetic resources.

Principal component analysis and Biplot

The goal of the principal component analysis is to set numbers of major factors so as to decrease the number of effective parameters to discriminate genotypes. According to the PCA, five components explained 88.80 % of the total variance (Table 6). Results of the biplot according to the PC1 and PC2 supported the findings of the cluster analysis with genotypes distributed into two groups (Fig. 3 and 4). Advancing from the negative to positive values of PC1 (left to right) in biplot, the examined genotypes indicated a gradual increase in the values of seedling height (cm), length of lateral branches (cm) and kernel percentage. Proceeding from the negative to the positive values of PC2 (bottom to upwards), the examined genotypes indicated some reduction in the values of nut weight and leaf area.

The relative agreement between the results of PCA and cluster analysis showed that morphological analysis may present reliable information on the variability in almond germplasm. The dendrogram of cluster analysis and the biplot of PCA confirmed the high variation among genotypes, indicating that the studied germplasm was a good candidate gene pool for breeding programs. In the principal component analysis, nut weight, seedling height, the height of the first lateral, stem diameter, number of laterals, leaf width, leaf area and leaf dry matter had the highest loadings, as a result, they are useful traits for almond germplasm characterization.

PCA has formerly been used to set up genetic associations among cultivars and genotypes of the various *Prunus* species (Sorkheh *et al.*, 2009). Zeinalabedini *et al.* (2008) studied nut, kernel and leaf traits in *P. scoparia*, *P. elaeagnifolia*, *P. hauskenchtii*, and *P. lycioides* and reported that these traits showed significant differences among the species and indicated the possibility of the use of them in distinguishing *Prunus* germplasm which was in line with the results of the current study.

Conclusions

The comprehensive analysis of all measured characteristics elucidates a wide range of variations in the studied nut and seedling characteristics within and among the populations of almond species, particularly in *P. scoparia*. This may have important implications for managing genetic resources as well as their use in future breeding programs especially for the development of new rootstocks for almond and stone fruits. Traits such as the height of the first lateral, nut weight, kernel percentage, dwarf seedling stature and small leaf size are desirable features and could be taken into account in almond breeding programs as well as in the local cultivation of this species for nut production and oil extraction.

Interesting and useful associations were observed among the assessed nut and seedling traits which could be considered in seed materials collection for the production of seedling rootstocks as well as in the breeding programs for indirect selection. Shiraz population as the most diverse population of *P. scoparia* could be used as a source for further selections of superior genotype for rootstock purposes or even plant development for forestry and landscape purposes. Molecular screening of these plant materials is highly recommended in illustrating marker/trait associations to be utilized in marker-assisted breeding.

Using cluster analysis, genotypes were classified into two main clusters of wild and domestic populations, and within the main cluster based on their botanical and geographical proximity. Moreover, principal component analysis (PCA) made it possible to set up similar groups of genotypes as well as to study associations among morphological characteristics.

Further analysis of graft compatibility with commercial almond and stone fruit cultivars will be required before utilization of these plant materials as rootstocks. Since that the stem diameter of the most seedling population is lower than 5 mm, to speed up the

process of grafting, it is recommended to study the possibility of using scions material from in vitro or from mother plant submitted to repeated pruning in such manner that shoot will grow very thin and suitable for being grafted on wild seedling populations.

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Conflict of interest

The authors declare no conflict of interest and confirm that all authors contributed significantly to the work and agree with the contents of the manuscript and its submission to the journal.

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