

# Evaluation of Morphological Diversity of Different Ecotypes of *Amygdalus scoparia* Spach: A Medicinal Plant Resistant to Hard Environmental Conditions

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## ABSTRACT

*Amygdalus scoparia* Spach is a potentially multi-purpose wild almond species and a perennial medicinal plant which belonging to Rosaceae family where grows naturally in arid and semi-arid areas of Iran. In the current investigation, genetic variation of five ecotypes of *A. scoparia* from Hormozgan and Fars provinces, including Bekhun, Homag, Sekhuran, Sarvestan and Fasa, was evaluated using morphological markers. 10 important characteristics such as sub-branch length, number of secondary branches on the sub-branch, number of flowers on the sub-branch, dry weight of sub-branch and percentage of dry weight of sub-branch were investigated. The results of variance analysis showed that significant differences ( $p \leq 0.01, 0.05$ ) were found among the studied *A. scoparia* natural ecotypes for studied traits. The ecotypes of Homag, Sekhuran and Bekhun had the highest values of the important breeding traits including the sub-branch length, the number of secondary branches on the sub-branch, the number of flowers on the sub-branch, the dry weight of sub-branch and the percentage of dry weight of sub-branch. The results showed that ecotypes had a wide variation in terms of all studied morphological attributes. The correlation coefficients between evaluated traits showed significant positive and negative correlations between some important traits. PCA analysis results illustrated that studied characteristics divided in three groups which justified 64.01% of the total variance. Hierarchical cluster analysis identified three major clusters with several sub-clusters. The results of this investigation highlighted the efficiency of translation initiation codon polymorphism for genetic characterization and accurate authentication of *A. scoparia* ecotypes as well as detecting and tagging morphologically important traits in this species that would be helpful for implementation of effective conservation strategies and even broaden current genetic diversity.

## INTRODUCTION

Climate change resulting from human activities is occurring so rapidly that many plant species will not be able to adapt with it. In fact, it is predicted that by the end of this century, we will lose a large portion of global biodiversity [1]. In recent years, climate change along with the impact of other factors such as human intervention and industrial development has caused changes and also tensions in the natural ecosystems of wild medicinal plants

species [2]. Wild medicinal plants ecosystems in arid and semi-arid regions, which have become what they are today over consecutive years, have a decisive role in maintaining the balance of ecosystems, and the exploitation of these resources should be based on a careful study of habitat characteristics [3].

One of the medicinal plants species that is cultivated to protect water and soil in arid and semi-arid regions with hard environmental conditions is

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*Amygdalus scoparia* Spach, which botanically belongs to Rosaceae family [4]. From the phenotypic point of view, this plant is a shrub up to 6 meters tall, which is branched from the base and has many branches (with non-angled branches). This shrub, as the most advanced flowering shrub before spring, with large and white flowers, offers beautiful and pleasant landscapes for every tourist and nature lover. *A. scoparia* can be found along with other wild *A.* species in the Irano-Touranian zones [5].

This shrub is resistant to adverse environmental conditions (drought, salinity, low soil fertility, wind, and high and low temperatures) in most climates of Iran [6]. *A. scoparia* can grow on loose conglomerates, limestone cliffs, loose volcanic rocks, crevices in rock slopes, and in clay and sandy soils. It most commonly can be found at high altitudes (1200 up to 2700 m). This species is appreciated mostly due to its economic importance (the usage of its products and by-products in the food, pharmaceutical and chemical industries) and its use as a natural resource as well as in controlling soil erosion control [7].

The medicinal properties of *A. scoparia* such as gum and fruit have been confirmed and thus, they can be applied in food, sanitary, cosmetic, industrial and pharmaceutical industries [8]. In addition, its kernels are used as nuts and dried fruits by the Iranian people [9]. Furthermore, its kernel oil has significantly higher unsaturated to saturated fatty acids ratio, total tocopherols and phenolics contents and calculates oxidizability value than those of olive oil [10]. The gum of *A. scoparia* is consumed as a functional ingredient for nutritional and pharmaceutical purposes: appetite stimulant, crushing bladder stones and food emulsions [11]. Food production and security are largely influenced by conservation of agrobiodiversity and genetic resources. On-farm conservation through international initiatives can protect genetic diversity of local varieties [12]. According to the Convention on Biology Diversity, the conservation and sustainable utility of plant gene pool effect food and agriculture security [13].

Morphological feature is necessary to evaluate the genotypes recorded in gene banks for knowledge of their important traits such as culture-related characteristics [14]. An important step for efficient use of crop germplasm is morphological description

of plant materials with desired traits. Comprehension of the components restricting crop success is crucial, as well as finding solutions for accessing genetic diversity inside and outside the species is important [15]. To detect similarity indices among species, genotypes and varieties, cluster and principal component analyses are adopted as the main multivariate statistical methods [16].

Iran is rich in genetic resources of many medicinal and aromatic plants species. Identifying wild and native varieties in each area is the first step in protecting genetic resources. Hence, the aim of the current study was to use morphological characteristics to understand the phenotypic diversity of *A. scoparia* naturally grown in Fars and Hormozgan provinces in Iran.

## MATERIALS AND METHODS

### Plant Materials

In order to study the distribution site and harvesting of different ecological areas of *A. scoparia* in the southern regions of Iran, five different natural habitats, including three different altitude areas in Hormozgan province, including Bekhun, Homag and Sekhuran areas and two habitats in Fars province including Sarvestan and Fasa habitats were selected and the desired sampling was performed in these five habitats. Habitat information for each area, including latitude and longitude and altitude, was determined using a Global Positioning System (GPS) (Garmin eTrex 30x GPS). Climatic data related to the last 18 years of each habitat including average annual temperature, minimum and maximum temperature as well as average annual rainfall were collected from meteorological stations in the region (Table 1).

In order to study the morphological traits of *A. scoparia*, 10 genotypes from each habitat and 4 perennial branches from each genotype in different directions were cut from the stem and transferred to the laboratory for morphological studies. To reduce the error and to prevent the collection of clones belonging to a tree and ultimately increasing accuracy, a proper distance of 200m between the trees in each area was considered.

**Table 1** Some characteristics of the collected *A. scoparia* natural habitats

Collection places	Altitude (m.a.s.l*)	Longitude (E)	Latitude (N)	Mean annual temp. (°C)	Maximal temp. (°C)	Minimal temp. (°C)	Rainfall (mm/year)
Bekhun	1500	56° 10'	28° 10'	+25.4	+46.6	-3.8	206.5
Sekhuran	785	56° 48'	27° 37'	+28.4	+47.8	+4.6	158.8
Homag	1285	56° 27'	27° 54'	+25.1	+45.6	-1.8	162.2
Fasa	1790	53° 64'	28° 94'	+19.7	+39.8	-4.8	247.8
Sarvestan	1850	53° 12'	29° 18'	+18.4	+40.5	-5.0	270

\*meter above sea level

### The Characters Recorded

A total of 10 quantitative morphological characteristics (Table 2) were used for phenotypic evaluations. The following parameters were measured:

Sub-branch length, base diameter of sub-branch, internode distance, number of secondary branches on the sub-branch, number of flowers on the sub-branch, distance between the base and the first branch, diameter of the first sub-branch, fresh weight of sub-branch, dry weight of sub-branch and percentage of dry weight of sub-branch. The quantitative characters such as diameter of the first sub-branch and internode distance were measured by a digital caliper. An electronic balance with 0.01 g precision was used for measuring the weight of fresh and dry of sub-branch.

### Statistical Analysis

The average values were used for data analysis and conclusions. One-way analysis of variance (ANOVA) was used to determine significant differences between genotypes in terms of the traits measured by SAS software version 9.4 in the form of Nested design. The parameters including minimum, maximum, mean, standard deviation (SD), and coefficient of variation (CV) were calculated. Data obtained from measured characteristics were entered SAS for analysis of variance. The comparison of characteristics was meaned by Duncan's multi-domain test at 1% probability level. To determine the relationship between characteristics, Pearson simple correlation and grouping of ecotypes by cluster analysis (based on Ward method) were used by SPSS software version 16.

Principal component analysis (PCA) was applied to determine the relationship between genotypes using SPSS software. In each main and independent factor, factor coefficients of 0.55 and above were considered significant. Euclidean distance coefficient and Ward's method were used to perform cluster analysis by SPSS software.

## RESULTS

### Diversity Index and Trait Description

Most of the characters measured showed meaningful differences among the genotypes selected of which seven characters had CVs greater than 20.00%. Distance between the base and the first branch showed the highest CV (71.07%) and followed by Number of flowers on the sub-branch (53.15%). In contrast, three characters had lower CVs ( $CV \leq 20.00\%$ ) among all the genotypes that included percentage of dry weight of sub-branch, diameter of sub-branch and Sub-branch length.

According to the analysis of variance, the results of data analysis of morphological characteristics of different *A. scoparia* bases in different ecotypes showed a statistically significant difference among most of the evaluated characteristics in different ecotypes, which was observed at a probability level of one percent or five percent. Accordingly, among the measured appearance and morphological characteristics, five characteristics including, Sub-branch length, Internode distance, Number of secondary branches on the sub-branch, Number of flowers on the sub-branch and Diameter of the first sub-branch showed a statistical difference of 1%. In simpler terms, except Number of flowers on the sub-branch trait, in different ecotypes, these vegetative characteristics of *A. scoparia* demonstrated a significant difference at the statistical level of one percent (Table 3).

Also, five other traits among the measured morphological characteristics such as Diameter of sub-branch, Distance between the base and the first branch, Fresh weight of sub-branch, Dry weight of

sub-branch and Percentage of dry weight of sub-branch illustrated significant at 5% probability level. Comparison of the mean of morphological characteristics of *A. scoparia* branches in different.

**Table 2** Descriptive statistics for morphological traits utilized in the studied *A. scoparia* genotypes

No.	Character	Abbreviation	Unit	Min.	Max.	Mean	SD	CV (%)
1	Sub-branch length	SBL	cm	67.02	117.33	89.55	14.61	16.31
2	Diameter of sub-branch	DSB	mm	6.77	12.57	8.97	1.17	13.00
3	Internode distance	ID	mm	9.7	32.7	18.06	4.87	26.99
4	Number of secondary branches on the sub-branch	NSBS	-	4	19.67	11.27	3.72	33.03
5	Number of flowers on the sub-branch	NFS	-	19.67	258.33	100.5	53.41	53.15
6	Distance between the base and the first branch	DBF	cm	1	29.02	6.97	4.95	71.07
7	Diameter of the first sub-branch	DFS	mm	1.25	7.58	4.96	1.18	23.74
8	Fresh weight of sub-branch	FWS	gr	74.67	281.33	140.02	48.08	34.34
9	Dry weight of sub-branch	DWS	gr	32	188.67	81.97	31.06	37.90
10	Percentage of dry weight of sub-branch	PDW	-	43.53	82.24	59.29	5.90	9.96

**Table 3** Analysis of variance of the measured traits in different studied *A. scoparia* ecotypes

S.O.V.	df	Mean of squares									
		SBL	DSB	ID	NSBS	NFS	DBF	DFS	FWS	DWS	PDW
Ecotype	4	1391.2 **	3.50 *	123.11 **	53.06 **	16734.15 **	33.72 *	6.14 **	6609.72 *	2214.04 *	75.45 *
Error	45	108.67	1.17	14.94	10.37	1619.23	23.72	0.96	1929.29	853.93	31.23

\*, \*\*, ns: significant at 5% and 1% probability levels and non-significant, respectively

**Table 4** Mean comparisons of the ecotypes of *A. scoparia* for morphological traits.

No.	Character	Bekhun	Sekhuran	Homag	Fasa	Sarvestan
1	Sub-branch length	103.85 a	93.39 b	94.61 ab	82.57 c	73.34 c
2	Diameter of sub-branch	8.49 b	8.25 b	9.21 ab	9.17 ab	9.71 a
3	Internode distance	20.65 a	20.31 a	20.88 a	13.89 b	14.58 b
4	Number of secondary branches on the sub-branch	12.93 ab	9.26 c	14.43 a	10.36 bc	9.36 c
5	Number of flowers on the sub-branch	158.20 a	127.18 a	75.73 b	59.23 b	82.17 b
6	Distance between the base and the first branch	9.37 a	6.55 ab	7.74 ab	4.86 b	6.29 a
7	Diameter of the first sub-branch	4.47 b	4.88 b	4.30 b	4.86 b	6.29 a
8	Fresh weight of sub-branch	121.2 b	120.87 b	171.33 a	121.97 b	164.80 a
9	Dry weight of sub-branch	71.93 b	73.23 b	102.13 a	69.00 b	93.53 ab
10	Percentage of dry weight of sub-branch	59.34 ab	63.59 a	59.72 ab	56.74 b	57.05 b

Different letters represent statistically different values for  $p \leq 0.01$  (Duncan's test).

**Table 5** Simple correlations among morphological variables utilized in the studied *A. scoparia* genotypes

Trait	SBL	DSB	ID	NSBS	NFS	DBF	DFS	FWS	DWS	PDW
SBL	1	-	-	-	-	-	-	-	-	-
DSB	-0.11	1	-	-	-	-	-	-	-	-
ID	0.48 **	-0.15	1	-	-	-	-	-	-	-
NSBS	0.28	0.01	0.35 *	1	-	-	-	-	-	-
NFS	0.29 *	-0.14	0.28	0.06	1	-	-	-	-	-
DBF	0.35 *	-0.19	0.08	0.22	-0.01	1	-	-	-	-
DFS	-0.23	0.55 **	-0.22	-0.41 **	-0.16	-0.18	1	-	-	-
FWS	-0.01	0.68 **	0.06	0.03	-0.01	-0.09	0.32 *	1	-	-
DWS	0.06	0.63 **	0.11	0.01	0.04	-0.08	0.30 *	0.96 **	1	-
PDW	0.14	-0.11	0.19	-0.07	0.23	-0.05	0.03	-0.02	0.21	1

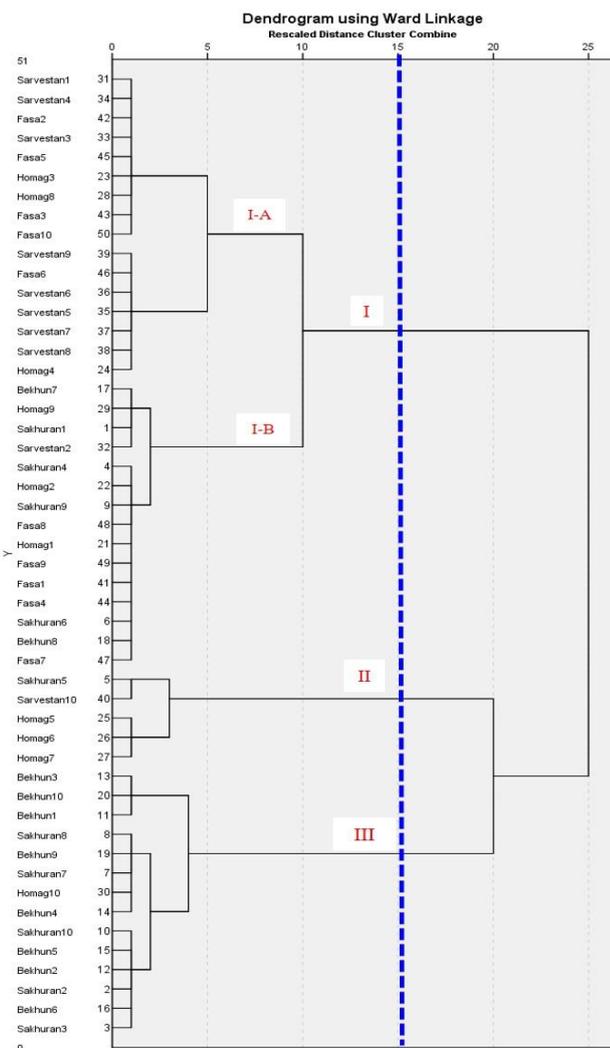
\*\* Correlation is significant at the 0.01 level.

\* Correlation is significant at the 0.05 level.

**Table 6** Eigenvalues of the principal component axes from the PCA of morphological characters in the studied *A. scoparia* genotypes

Character	Component		
	1	2	3
Sub-branch length	0.84 *	-0.14	-0.21
Diameter of sub-branch	0.95 *	0.04	0.02
Internode distance	0.92 *	0.01	0.19
Number of secondary branches on the sub-branch	0.02	0.63 *	0.44
Number of flowers on the sub-branch	0.05	0.77 *	-0.05
Distance between the base and the first branch	-0.11	0.59 *	-0.12
Diameter of the first sub-branch	0.49	-0.56 *	-0.10
Fresh weight of sub-branch	0.06	0.52	0.55 *
Dry weight of sub-branch	-0.06	0.09	0.70 *
Percentage of dry weight of sub-branch	0.02	-0.21	0.75 *
Eigenvalue	2.89	2.18	1.32
% of Variance	28.92	21.85	13.23
Cumulative %	28.92	50.77	64.01

Eigenvalues  $\geq 0.55$  are significant.

**Fig. 1** Ward cluster analysis of the studied *A. scoparia* genotypes based on morphological traits using Euclidean distances

Regions using Duncan's multiple range test indicated that *A. scoparia* trees were in three groups in terms of Sub-branch length. The first group is Bekhun and Homag habitats and there was no statistically significant difference between these two habitats. The second group included the habitats of Homag and Sekhuran, and there was no statistically significant difference between the two habitats.

The third group included the habitats of Fasa and Sarvestan, and there was no statistically significant difference between the two habitats. Accordingly, the longest Sub-branch length (103.85 cm) was related to the genotypes of Bekhun habitat and the shortest branch length (73.34 cm) was related to the genotypes of Sarvestan habitat (Table 4).

Comparison of the mean of morphological characteristics of *A. scoparia* branches in different natural habitats using Duncan's multiple range test showed that *A. scoparia* trees were divided into two groups in terms of Diameter of sub-branch. The first group was Sarvestan, Homag and Fasa habitats and the second group included the habitats of Homag, Fasa, Bekhun and Sekhuran, and there was no statistically significant difference between these four habitats. Accordingly, the highest Diameter of sub-branch (9.71 mm) was related to the trees of Sarvestan region and the lowest diameter of the Diameter of sub-branch (8.25 mm) was related to the trees of Sekhuran region (Table 4).

Comparison of the mean of morphological characteristics of *A. scoparia* branches in different regions using Duncan's multiple range test illustrated that *A. scoparia* trees were in three groups in terms of Number of secondary branches on the sub-branch. The first group was Homag and Bekhun habitats. The second group was the habitats of Bekhun and Fasa and the last group included habitats of Fasa, Sarvestan and Sekhuran and there was no statistically significant difference between these three habitats. Accordingly, the highest number of secondary branches on the sub-branch (14.43) was related to the genotypes of Homag region and the lowest number of secondary branches on the sub-branch (9.26) was related to the genotypes of Sekhuran region (Table 4).

Comparison of the mean of morphological traits of *A. scoparia* in different natural habitats using Duncan's multiple range test demonstrated that *A. scoparia* genotypes were in two separated groups in terms of Number of flowers on the sub-branch. The first group was the habitats of Bekhun and Sekhuran and the second group insisted on the habitats of Sarvestan, Homag and Fasa and there was no statistically significant difference between these three habitats. Accordingly, the highest number of flowers on the sub-branch (156.20) belongs to the trees of Bekhun natural habitat and the lowest number of flowers on the sub-branch (59.23) belonged to the genotypes of Fasa region (Table 4).

Comparison of the mean of morphological characteristics of *A. scoparia* genotypes in different regions using Duncan's multiple range test indicated that *A. scoparia* genotypes were in two groups in terms of Percentage of dry weight of sub-branch. The first group was Sekhuran, Homag and Bekhun habitats and the second group consisted of the habitats of Homag, Bekhun, Sarvestan and Fasa, and there was no statistically significant difference between these four habitats. Accordingly, the highest percentage of dry weight of sub-branch (63.59%) is related to the genotypes of Sekhuran ecotype and the lowest percentage of dry weight of sub-branch (56.74%) is related to the genotypes of Fasa ecotype (Table 4).

### Correlations between Traits

Significant correlations were observed among some variables (Table 5). Sub-branch length showed positive and meaningful correlations with internode distance ( $r = 0.48$ ), number of flowers on the sub-

branch ( $r = 0.29$ ) and distance between the base and the first branch ( $r = 0.35$ ). Diameter of the first sub-branch showed significant and positive correlations with diameter of sub-branch ( $r = 0.55$ ), while that character showed negative and significant correlation with number of secondary branches on the sub-branch ( $r = -0.41$ ). Dry weight of sub-branch had significant and positive correlations with diameter of sub-branch ( $r = 0.63$ ), diameter of the first sub-branch ( $r = 0.30$ ) and fresh weight of sub-branch ( $r = 0.96$ ).

### Principal Component Analysis (PCA)

The PCA showed 3 independent components that could explain 64.01% of total variance (Table 6). The first factor with 28.92% included parameters such as sub-branch length, diameter of sub-branch and internode distance. The second factor with 21.85% consisted of parameters including number of secondary branches on the sub-branch, number of flowers on the sub-branch, distance between the base and the first branch and diameter of the first sub-branch. The third factor with 13.23% included fresh weight of sub-branch, dry weight of sub-branch and percentage of dry weight of sub-branch (Table 6).

### Cluster Analysis

Ward dendrogram created according to the data obtained revealed the variation among genotypes and showed three major clusters (Fig. 1). The first cluster formed two sub-clusters; Sub-cluster I-A consisted of 16 genotypes and sub-cluster I-B included 15 genotypes. The second cluster contained 5 genotypes, while the rest genotypes were placed into the third cluster. Fasa and Sarvestan areas, where belonged to Fars province, were placed into the first group. The genotypes of these ecotypes with common characteristics such as Diameter of sub-branch, Diameter of the first sub-branch and Percentage of dry weight of sub-branch were the most similar among the studied ecotypes. Homag, Sekhuran and Bekhun, where belonged to Hormozgan province, formed the other groups. The common characteristics of the genotypes of these ecotypes of the second cluster were Distance between the base and the first branch and Number of secondary branches on the sub-branch. Finally, Dry weight of sub-branch, Number of flowers on the sub-branch and Sub-branch length were the common traits of the genotypes of the third group.

## DISCUSSION

Estimation of the existing genetic diversity is one of the primary fundamental steps and an important component of each plant resource management, which pave the way for better utilization of the elite plant materials and their subsequent applications in crop improvement. This is the first report on the comprehensive evaluation of the genetic relationships in the plant. Five *A. scoparia* ecotypes that were investigated in the current study have a wide geographic distribution and are considered as hardy and low-expectation tree species bearing edible fruit with significant nutritional and medicinal properties. The significant differences in the morphological data may be caused by various habitat conditions of the studied ecotypes. These valuable traits were considered by plant scientists towards this genus for land validation and fruit production especially in the regions that most of the other fruit crops cannot be cultivated commercially [17].

Knowledge of the level of genetic diversity and accurate estimation of it in plant germplasms and determination of genetic relationships between breeding materials is the basis of many breeding programs. Acquiring information about the genetic distance between individuals or ecotypes and being aware of the relationships of the species in the breeding program, the possibility of organizing hereditary reserves and effective sampling of genotypes and better use of diversity in the program provides correction [18]. Distance between the base and the first branch showed the highest CV (71.07%) and Percentage of dry weight of sub-branch illustrated the lowest CV (9.96%). The range of 7.74-89.95% as CV has been reported in a study on *A. scoparia* germplasm [19]. Moradi *et al.* (2020) observed the highest CVs for suture opening of shell (289.09%), leaf upper surface color (79.83%), and nut shape (75.75%), while they reported that four characters such as nut width (11.75%) and nut thickness (11.77%) had the lowest CVs in a set of genotypes belonging to *A. scoparia* [20]. For almond breeding programs, the knowledge of relationships that exist between the quantitative characteristics, which must be improved, is of significant interest as the choice of one trait can favor the appearance or disappearance of others affecting the efficiency of the program [21]. Furthermore, the presence of significant correlations

between characteristics and their estimation would expedite an advanced procedure of indirect selection aimed at improving a character by selecting it over another. This improvement is significant for recognizing and enhancing those characteristics, which are difficult to identify during the segregation of the offspring or for conducting an early selection procedure in cases where a character is not manifested until the sexual maturity of the tree [22]. The presence of a high value of phenotypical correlations between the characteristics can be attributed, at least in part, to the pleiotropic effects or linkages that exist among the genes that influence the different characteristics [23].

Examination of the results of comparing the mean of morphological features related to natural populations of *A. scoparia* showed the maximum sub-branch length and number of flowers on the sub-branch were observed in Bekhun ecotype. On the other hand, the highest number of secondary branches on the sub-branch was obtained in Homag ecotype. Due to the fact that the formation of flowers and more fruit in this species depends on more branches, so genotypes of Bekhun and Homag can be selected as candidates to continue the breeding process [24]. Morphological characterization is essential to evaluate the genotypes recorded in gene banks for knowledge of their important traits such as culture-related traits. For efficient utilization of crop germplasm, morphological description of plant materials with desired characters is an important step. Knowledge of the components restricting crop success is crucial, as well as finding solutions for accessing genetic diversity inside and outside the species is necessary [25].

In addition, significant positive and negative correlations were detected between different pairs of morphological traits. Besides the internode distance, number of flowers on the sub-branch and distance between the base and the first branch attributes also had significant positive correlations with sub-branch length. Our results corroborate the prior reports indicating high correlation between these traits [19,20]. The branches contain leaves that are the site of photosynthesis. Plant leaves are the main specialized tissue for capturing sunlight energy and converting it to photosynthetic products. Therefore, the availability of a high level of carbohydrates in the plant and increase the ratio of C to N will

provide desirable conditions for the growth of reproductive organs and subsequently will lead to the improvement in the size and weight of fruit [17]. Information about the correlation between morphological traits would be of great importance in the breeding programs, as this information enables the breeders to predict one trait from another one and subsequently will reduce the period required for selection which is highly advantageous especially in the long-lived woody species plants.

The PCA showed that the variables with higher scores in the PC1 and PC2 were related to flower production and finally fruit yield. Previously, PCA had been used to establish genetic relationships among accessions and genotypes, to study correlations among morphological traits and to evaluate germplasm of different almond related species [19,20, 26,27].

In general, based on the results of this study, there is a wide genetic diversity among the natural populations of *A. scoparia* species in terms of morphological characteristics, which indicates the value of these reserves and the need for more attention to conservation. Selection requires diversity, and as genetic diversity increases in a community, the ability to select superior genotypes increases. Knowledge of the various aspects of morphology helps us to determine the strategies of exploitation, improvement and domestication. Finally, at present, based on the results of genetic diversity of these ecotypes using morphological markers, the superior natural populations of *A. scoparia*, including Homag, Sekhuran and Bekhun are suitable in breeding projects in order to domesticate and create good cultivars for the needs of different aspects.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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