



## ORIGINAL ARTICLE

## Assessing Populations Diversity of Small Panel Oak (*Quercus brantii*) in Western Forests of Iran: a Major Effort in Reforestation Programs

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## KEY WORDS

Ecotype;  
Hierarchical clustering;  
Multivariate analysis;  
Persian oak;  
Population structure

## ABSTRACT

Persian oak (*Quercus brantii*) is a critical, economic, and environmental species of Zagros forests in Iran. The effects of climate change and drought have caused a decline in Persian oak populations, leading to a severe reduction in genetic resources for future conservation programs. This study aims to evaluate the diversity and population structure of Persian oak in the western forests of Iran using morphological features. A total of 187 samples were collected from 15 locations in the Ilam province. Twenty phenotypic traits related to leaf, seed, and trunk characteristics were evaluated. Several multivariate statistical analyses were performed. The results revealed significant morphological diversity among the Persian oak ecotypes. Correlation analyses revealed a significant positive correlation between leaf length attribute and distance from leaf base to maximum leaf width (0.55) and maximum width of the leaflet (0.64) traits. The leaf width at 50% attribute with the maximum width of the leaflet and distance from leaf base to maximum leaf width have a positive (0.8 and 0.51 respectively) and significant correlation ( $p \leq 0.05$ ). According to principal component analysis, the components of leaf and seed traits have the most impact on morphological variance. Hierarchical cluster analysis divided the locations into two groups, with some oak locations distributed in two clusters, indicating higher diversity of this species in different locations. Further research is needed to determine the optimal ecotype; however, the oaks in Ghallaje region have characteristics that can increase their ability to resist water scarcity, making them potentially appropriate for reforestation in Ilam province.

## Introduction

Forests are valuable national assets in any country, and their management is crucial to ensure their sustainability for all generations. Nowadays, the conservation of genetic diversity and biological species has become a significant concern with broad public outreach. Each species is unique, and their loss would be

irrecoverable, emphasizing the need for genetic conservation. (Aliyeva *et al.*, 2020; Baghzadeh Daryaii *et al.*, 2020; Mehri *et al.*, 2022). Selecting populations with higher genetic diversity may be appropriate for genetic conservation, as plant populations with higher genetic diversity can better withstand natural disasters

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(Vahdati *et al.*, 2015; Akça *et al.*, 2020). Accurate measurement of genetic diversity is essential for practical studies on ecology and evolution (Sarikhani *et al.*, 2023). Therefore, better management and conservation of forest ecosystems requires awareness of genetic diversity among populations, and appropriate management strategies for the conservation of genetic reserves are necessary, along with a proper understanding of the geographical pattern of genetic diversity. The ultimate goal of such measures is to ensure the survival of populations of a species and to maintain their evolutionary capacity (Hamrick and Godt, 1996; Sayadi *et al.*, 2012a; Ansari and Gharaghani, 2019).

Zagros forests are one of the most important biological, and genetic resources in Iran, with around 508 thousand hectares located in Ilam province. The Persian oak is the most important tree species in this area, accounting for almost 90% of the forest cover in the province. This species is native to Iran, Iraq, Syria, and Turkey and is one of the most important wood species in the Zagros forest (Jazirehi and Rostaghi, 2003). However, these forests are at risk due to various factors, including habitat loss, invasive species, climate change, pests, and diseases (Sayadi *et al.*, 2012b). The decline of Persian oak has been exacerbated by climate change and successive droughts over the past few years, which may lead to a severe decline in native and useful genetic resources for future conservation programs.

In the recent century, there has been a growing concern and an alarming trend observed regarding the magnitude of drought conditions and the diminishing Iranian oak populations in the Ilam province. The increased attention given to this particular phenomenon due to its significance and the escalating nature of the issue. Unfortunately, more than 50% of forests in the Ilam province are in critical condition, indicating the urgency of executive action in this regard (Moradi *et al.*, 2021). Persian oak, is grouped as a vulnerable species according to IUCN regulations (Panahi and Jamzad,

2017). Drought has many socioeconomic effects, including reducing agricultural production, unemployment, reducing capital, malnutrition, and increasing the vulnerability of society in the studied area (Naveen and Cynthia, 2014). The socioeconomic problems associated with climate change primarily affect rural areas (Anthony, 2013). The *Quercus* genus, comprising roughly 600 species, is distributed across the globe. It is native to the Northern Hemisphere and includes deciduous and evergreen species that extend from the cold regions of Asia to the tropics of the United States. Many species of oak grow as ornamental trees around the world (Maya - Garcia, 2020). The Persian oak is predominant in forests of Iran among other oak species. It is a diploid species ( $2n = 2x = 24$ ) that sometimes reaches a height of 20 meters. It has a major presence in Iranian forests and is characterized by a long life while being a wind-pollinating species. Therefore, its range of distribution can spread over wide areas of geographical locations and show high levels of diversity. Oaks have high intra-population genetic diversity and low inter-population differentiation, and hybridization and introgression can occur due to their high frequency of genetic variation between taxa (Aldrich and Cavnder, 2011; Usli and Bakis, 2014).

Leaf morphology is affected by various climatic and ecological factors and can serve as a marker for genetic differentiation within and between populations. Leaf appearance is primarily determined by hereditary characteristics, but environmental factors can also influence the physiological activities of the plant and thus, alter leaf morphology. The study of leaf morphology can provide valuable information about genetic diversity and population structure, which is important for identifying lower species and classifications. Morphological similarity between individuals within and between can indicate genetic exchange through the evolutionary history of plants (Aliyeva *et al.*, 2020). Different environmental conditions and habitat factors such as altitude change

can cause variation in leaf morphology in oak species, and phenotypic traits have been used to develop keys for species and section interpretations within oak (Panahi *et al.*, 2011; Taleshi and Maasoumi-Babarabi, 2013).

Although Persian oak is native to Iran, limited research has been conducted on this species worldwide, and there is a lack of comprehensive information on its current situation. Accordingly, much research has been done on the morphological diversity of the leaves of trees of different oak species. The investigation carried out by Di Pietro and colleagues (2020) leaf morphological characteristics were examined in *Q. pubescens*, which identified groups that did not conform to current taxonomical frameworks and the latest flora and checklists. Aliyeva *et al.* (2020) conducted a study that investigated the genetic diversity of leaf phenotypic traits in different oak species populations and found significant genetic diversity among them, which was largely attributed to leaf area, leaf length-to-width ratio, and leaf shape. These traits also showed a significant correlation with altitude. In a separate investigation carried out in Mexico by Vega-Ortega *et al.* (2021), an inquiry into the genetic diversity of *Q. calophylla*, *Q. rugosa*, and *Q. laurina* was conducted, with the findings indicating a notable genetic variability among the populations of the three aforementioned species. Although traces of an old process of genetic isolation were detected among the populations of these three species, low genetic differentiation was observed, indicating gene flow and a lack of genetic isolation. Zumwalde *et al.* (2021), studied genetic, morphological, and environmental differentiation of different populations of oak species *Q. havardii* Rydb. The results revealed significant differences in genetic, morphological, and environmental features due to large-scale population divergence. Jurksiene *et al.* (2019) also classified *Q. robur* and *Q. petraea* oaks into six groups based on leaf morphological traits. In northern Spain, the genetic diversity of different communities of *Q. subpyrenaica* hybrid oak was studied based on

morphological and ecophysical traits, revealing that hybrid rootstocks have higher diversity than parental rootstocks in terms of characteristics (Himrane *et al.*, 2004). Ebrahimi *et al.* (2017) studied variations between *Q. brantii* populations in the forests of southwestern Iran using leaf morphological traits but found that cluster analysis and principal components analysis (PCA) were unable to isolate populations based on these traits. Borazan and Babac (2002) studied morphological features of the leaf in different oak species and showed that hybridization between the species had historically led to a high degree of diversity in the leaf morphology of these species. Raniers *et al.* (2020) found a positive correlation between leaf cleft and photosynthesis rate in their study on leaf shape diversity, suggesting that variations in leaf shape have functional effects on the adaptation of *Q. lobate* oaks to environmental stress in the Mediterranean climate of California. Understanding the genetic diversity of natural populations and reassessing the genetic structure of their habitat is essential for trees with an allogamous pollination system, such as oaks, especially if the level of gene diversity within populations decreases. This can enhance their capacity for adaptation to internal variables within each forest and can be considered a long-term solution for sustainability. Therefore, there is a need for socio-economic and environmental protection for this valuable species, which covers more than half of the western Zagros forests and can play a major role in restoring deforested areas. In this study, we aimed to evaluate the diversity and population structure of Persian oak (*Q. brantii*) ecotypes in the western forests of Iran using morphological features. Our main goal was to improve the information of phenotypic diversity in the selected geographical locations to aid in selecting appropriate *Q. brantii* populations in reforestation programs.

**Materials and Methods**

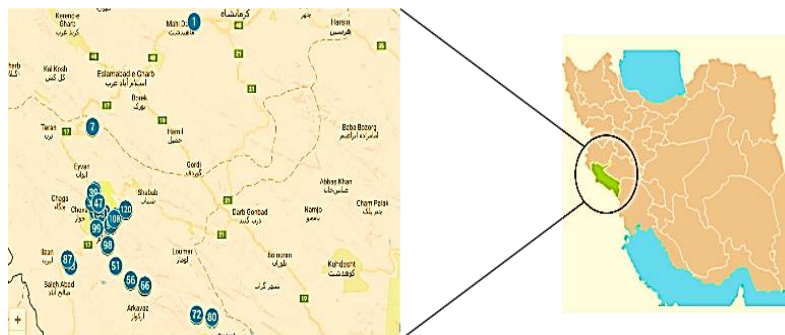
**Plant material**

The study was conducted on Persian oak ecotypes in several locations of the forests of Ilam province to assess their morphology (Fig. 1). A total of 187 oak samples were collected from 15 locations (Fig. 2), with a maximum of 20 samples per location, depending on the tree cover density (Table 1). Samples were taken after complete leaf growth cessation, comprising at least 10 leaves and 20 seeds randomly selected from four

different crowns of each tree. The samples were then transferred to the laboratory for morphological measurements. One tree was randomly selected as the sampling center, and the other trees were systematically sampled at even spaces with distances of 100 to 200 meters to ensure that genetic affinities caused by vegetative propagation were eliminated (Ginwal *et al.*, 2005). The first point was selected randomly, making all sampled points random, and even-spaced distances were observed to ensure habitat coverage and statistical population representation (Mirmohammadi, 2014).



**Fig. 1.** Pictures of the geographical locations of collected ecotypes  
 a: Chagha sabz. b: Mehran Salehabad. c: Eyvan. d: Dareshahr10k. e: Dareshahr27kL. f: Badre



**Fig. 2.** Geographical locations of collected *Q. brantii* ecotypes

**Table 1.** Sampling locations, number of ecotypes sampled in each location, and climatic features of each location

Location (Symbol)	Number of ecotypes in each location	Altitude (approx. Meters)	Longitude (E)	Latitude (N)	Average annual temperature (Celsius)	Average annual rainfall (mm)
Ghallaje (Gh)	15	1900	47.159167	33.965556	13.0	720
Tangeye dalab (TD)	10	1400	47.178889	34.503333	16.7	571
Tangeye dalab-Neck (TN)	15	1700	47.2175	34.099167	16.7	571
Tangeye dalab-Road (TR)	10	1500	47.000278	34.495556	16.7	571
Eyvan (Ey)	16	1400	46.80043	34.46531	17.0	663
Dareshahr10k (D10)	9	1300	47.089722	33.728333	16.9	537.7
Dareshahr20k (D20)	9	1300	47.151944	33.990278	16.9	537.7
Dareshahr27kR (DR)	10	1400	47.321389	33.473056	16.9	537.7
Dareshahr27kL (DL)	7	1400	47.123056	33.476038	16.9	537.7
Badre (Ba)	14	700	47.262222	33.573333	19.0	484
Mehran Salehabad (MS)	9	900	46.32234	34.35238	24.6	225.8
Dareye arghavan (Dg)	14	1600	46.553056	33.776111	16.9	537.7
Chagha sabz (CS)	10	1400	47.035278	33.598333	16.9	537.7
Lomar (Lo)	20	1300	46.775833	33.785278	20.8	447.8
Sarable (Sa)	15	1400	46.627222	34.009444	18.5	520.8

### Agro morphological evaluation

A total of 20 phenotypic traits related to leaf, seed, and trunk characteristics were evaluated (Table 2). Leaf morphological attributes were evaluated visually based on the method of Bruschi *et al.* (2003) using a ruler with millimeter accuracy. Indices of 10 leaves and 20 seeds from each tree were measured and then their average values were considered. Seed length, seed width, and seed volume were measured using a caliper tool and a marked cylinder with a precision of cubic millimeters. To measure breast height and collar diameter, the circumference of the breast and collar of each tree was measured (cm), and then the breast height (1.30 m height of the tree) and collar diameter were calculated. In coppice trees, breast height and collar diameter were calculated using Equation 1.

$$DRC^1 = \sqrt{\sum_{i=1}^n SD_i^2} \quad (1)$$

Where SD represents the collar diameter of each coppice shoot and n is the number of coppice shoots per group of coppice shoots. This relationship was also used to measure breast height in coppice trees (Rogers and Chojnacky, 1999).

### Multivariate statistical analysis

Differences between and within each geographical location were examined in terms of all morphological traits, after the data normality test, analysis of variance (ANOVA) in a completely randomized design with unequal replicates, and multiple comparisons using

non-parametric Spearman correlation approach was used to evaluate the correlation between phenotypic traits. PCA was performed to determine the most diverse phenotypic features among the studied attributes. Bayesian Information Criterion (BIC) was used to select the best model for clustering the samples, followed by model-based clustering. Internal validation clustering

was performed using k-mean partitions comparison analysis based on the Calinski criterion. Finally, hierarchical cluster analysis was carried out using the ward's method and Euclidean distance. The statistical analysis and drawing of the plots were performed by R software 4.0.4 and XLSTAT software (Version 2016.1)

**Table 2.** Evaluated morphological traits and their unit of measurement.

Symbol	Morphological traits (Definition)	Unit of measurement
LL	Leaf length (distance between leaf tip and leaf-leaf connectivity)	Millimeter
LW	Maximum width of the leaflet (width of the leaf at its widest point)	Millimeter
DBW	Distance from leaf base to maximum leaf width (distance between leaf base to the widest point of the leaf)	Millimeter
LP	Petiole length (distance between leaflet-petiole connectivity and petiole-branch connectivity)	Millimeter
LR	Leaf shape (maximum leaf width/leaf length)	-
BW90	Leaf width at 90% of leaf length	Millimeter
BW50	Leaf width at 50% of leaf length	Millimeter
LN	Number of leaf teeth	-
VN	Number of veins	-
LOD	Leaf teeth distance	Millimeter
LOL	Leaf teeth length	Millimeter
LDE	Leaf teeth depth	Millimeter
SL	Seed length	Millimeter
SW	Seed width (seed width at its widest point)	Millimeter
VS	Seed volume	Milliliter
1000SW	Weight of 1000 seeds	Grams
D130	breast height (diameter of tree trunk at a height of 130 cm from the ground)	Centimeter
DC	collar diameter (diameter of the tree trunk where the tree grows at ground level)	Centimeter
TH	Total tree height (tree height from collar to crown tip)	Centimeter
CH	Crown height (height of the tree from the place of growth to the tip of the crown)	Centimeter

## Results

### Comparison of mean data and analysis of variations

The comparison of mean data using Duncan's multiple range test revealed that oaks of the TR location had the highest mean value of LL (98.75 mm), SW (19.53 mm), and DC (101.77 cm). On the other hand, the Ba location had the lowest average values for attributes SL (40.69 mm), SV (6.67 ml), 1000SW (6669.15 g), and DC (41.2 cm). There were specifications regarding the maximum value of average LOL (6.84 mm) and the LOD (10.49 mm) traits. The

ecotypes of TD location had the highest average value of attributes SV (14.11 ml) and 1000SW (16566.95 g). Oaks of the Gh location showed the lowest mean value of attributes LL (77.57 mm), BW50 (34.4mm), LW (40.51 mm), LP (11.47 mm), LOL (4.15 mm), and LOD (8.24 mm) (S1 Table). The results of the one-way analysis of variance (ANOVA) of the morphological traits also indicated significant differences ( $p < 0.005$ )

between *Q. brantii* sampling geographical locations in terms of morphological characteristics (Table 3).

**Table 3.** Results of one-way analysis of variance (ANOVA) of morphological features.

Dependent variable	df	Average squares	F	Sig.
<b>LL</b>	14	466.029	3.147	000.0
<b>LW</b>	14	135.130	2.959	000.0
<b>DBW</b>	14	160.658	3.247	000.0
<b>LR</b>	14	0.092	2.006	0.020
<b>LN</b>	14	72.412	10.021	000.0
<b>VN</b>	14	36.282	5.936	000.0
<b>BW90</b>	14	96.748	7.077	000.0
<b>BW50</b>	14	205.862	4.355	000.0
<b>LP</b>	14	41.653	6.339	000.0
<b>LOD</b>	14	12.312	10.429	000.0
<b>LOL</b>	14	5.250	6.255	000.0
<b>LDE</b>	14	19.840	6.493	000.0
<b>SL</b>	14	198.593	5.552	000.0
<b>SW</b>	14	58.214	14.732	000.0
<b>SV</b>	14	44.626	8.042	000.0
<b>SW1000</b>	14	87649616.27	10.022	000.0
<b>D130</b>	14	2536.644	6.376	000.0
<b>DC</b>	14	3520.259	5.301	000.0
<b>TH</b>	14	81472.636	2.427	0.004
<b>CH</b>	14	53286.950	2.202	0.010

The Plotbox for the Leaf length (LL), Maximum width of the leaflet (LW), and Leaf width at 50% of leaf length (BW50) traits demonstrated more details of how the data spread in mentioned regions (Fig. 3). The difference in LL, LW, and BW50 traits is evident in studied locations. In the TR location, the leaf length ranged from 90 to 110 mm with a spread of

approximately 80 to 115 mm. Half of the data had a length of around 100 mm. Additionally, the highest values and variances for LW and BW50 were observed in the D10 location. On the other hand, the Gh location had the lowest values for LL, LW, and BW50 traits, with the highest diversity. Half of the data of these traits were near the lower values.

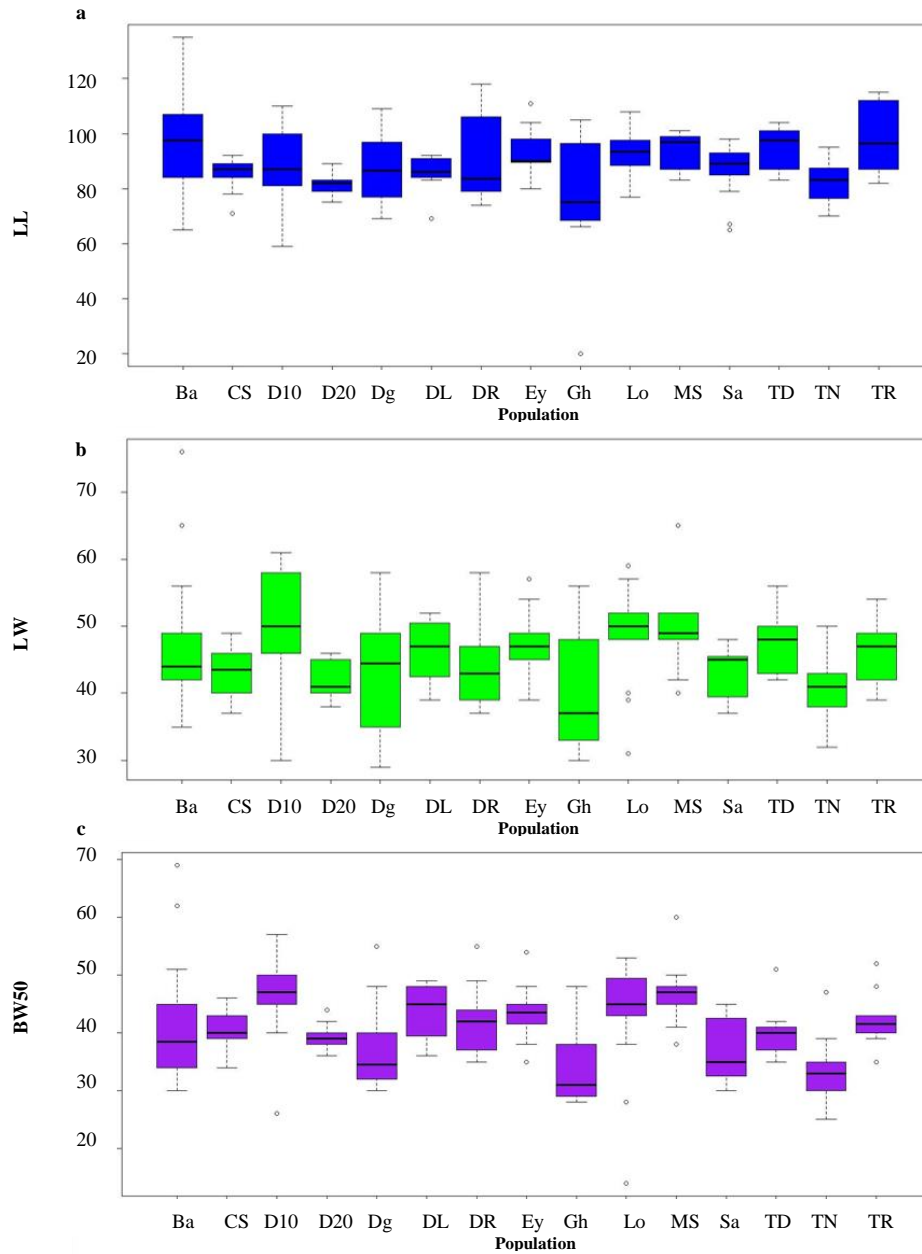


Fig. 3. Boxplot for some morphological traits in studied locations: a) For LL. b) For LW. c) For BW50

**Correlation between morphological features**

The results of the correlation analysis revealed that the LL trait had a significant and positive correlation with DBW (0.55) and LW (0.64) traits at the level of 5%. BW50 trait with LW and DBW have a positive and significant correlation (0.8 and 0.56 respectively) at 5% probability level (S2 Fig.). These positive correlations indicate that genes related to these traits might be located on similar chromosome. Consequently, selecting

an ecotype based on one trait may lead to the selection of another trait in that ecotype, making it important to consider all relevant traits in reforestation. The classification of the 20 evaluated traits resulted in three distinct groups. The first group comprised leaf-related attributes such as LDE, LOD, LOL, BW50, BW90, LW, LL, and DBW. The second group consisted of LR and four tree trunk features associated parameters. The third



group included three characteristics related to leaf and

all seed-size related traits (Fig. 4).

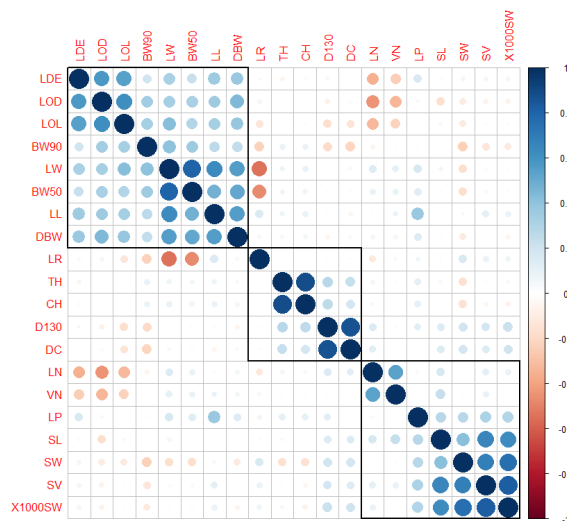


Fig. 4. Spearman correlation coefficients among 20 morphological traits in the studied locations.

**Principal components analysis (PCA) and clustering of**

**ecotypes**

The results of Principal Component Analysis (PCA) indicated that the three main components described 50% of the total data variance. The first component explained 20.9% of the total variance and the traits of maximum leaf width, the distance between leaf teeth, and leaf width explained 50% of leaf length, thereby exhibiting the highest share in this component. The second component comprised 17% of the total data variance and included the 1000-seed weight, seed volume, and seed length, which had the largest share in this component. Therefore, leaf traits played a more important role in the first component. Accordingly, the maximum width of

the leaf and the distance between leaf teeth were more important. In the second component, seed traits were more important since the first and second components explained the groupings more precisely. The components of leaf traits and seed traits played a substantial role in this regard. Meanwhile, traits such as leaf width, leaf teeth spacing, 1000-seed weight, and seed volume were among the important traits of these components, as affected mostly by genetic factors (Fig. 5a). According to the PCA graph, the Oak ecotypes studied can be divided into two separate groups (Fig. 5b).

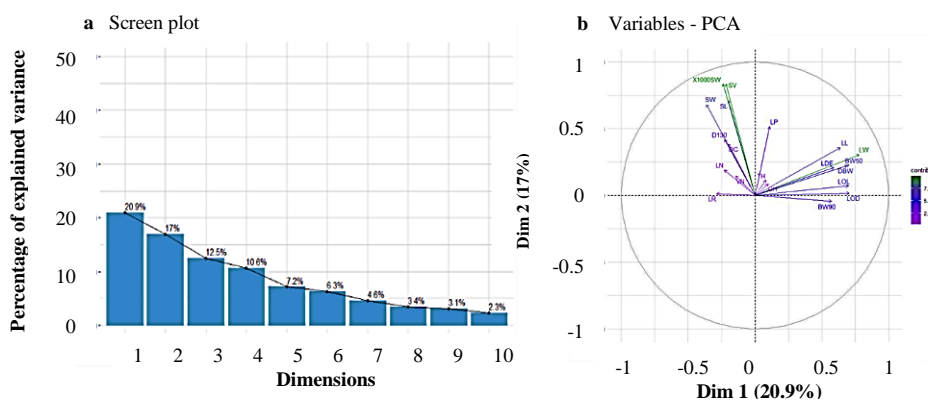


Fig. 5. a) Results of principal component analysis for the morphological traits. b) PCA graphs depicting the distribution of studied oak ecotypes.

Using the Bayesian Information Criterion (BIC), the most suitable model for clustering the studied samples was determined. Fig. 6 illustrates the model-based clustering, as a matter of which the highest BIC value corresponded to two clusters (-9000), indicating the optimal number of clusters and the optimal model for this VVE clustering. As a result, this model revealed the

differences in the size and shape of all clusters, while all clusters had similar directions or orientations in p-dimensional space. According to the k-mean partitions comparison analysis based on the Calinski criterion, K= 2 was selected as the optimal k, and the 187 ecotypes were grouped into two main categories (Fig. 7).

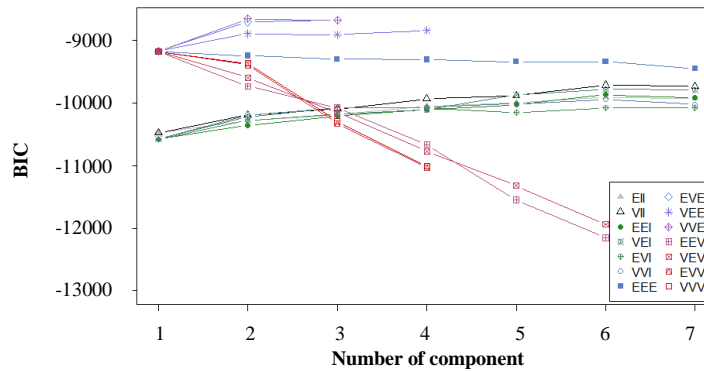


Fig. 6. The Bayesian Information Criterion values for model-based clustering in oak ecotypes

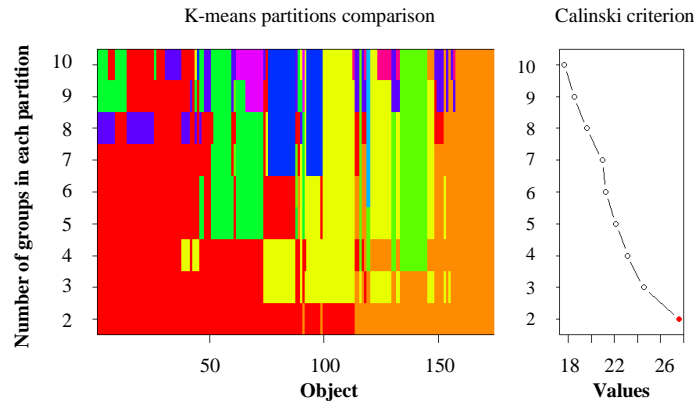


Fig. 7. K- mean analysis to determine the best k for grouping 187 oak ecotypes based on the scatter plot.

The results of cluster analysis using the K-means method and assuming the optimal number of two groups showed that the obtained clusters do not overlap and the

distribution of groups is segregated. Ecotypes 106, 105, 20, 44, and 2 showed a large genetic distance from other ecotypes (Fig. 8).

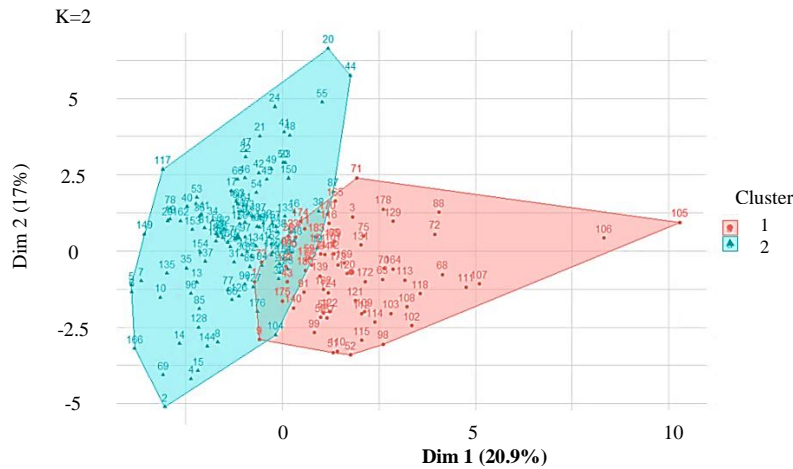


Fig. 8. The distribution of evaluated ecotypes based on k = 2

On the other hand, hierarchical clustering analysis based on Euclidean distance revealed two clusters that were not related to each other in terms of morphological diversity or geographical location (Fig. 9). This suggests that individuals were not separated based on locations and groups included ecotypes from different locations.

Only sampling regional ecotypes of TD, TR, TN, D20, and Sarable locations were in the second group, whereas Ba sampling regional ecotypes were completely in the first group. The remainder were ecotypes of different sampling locations in both groups.

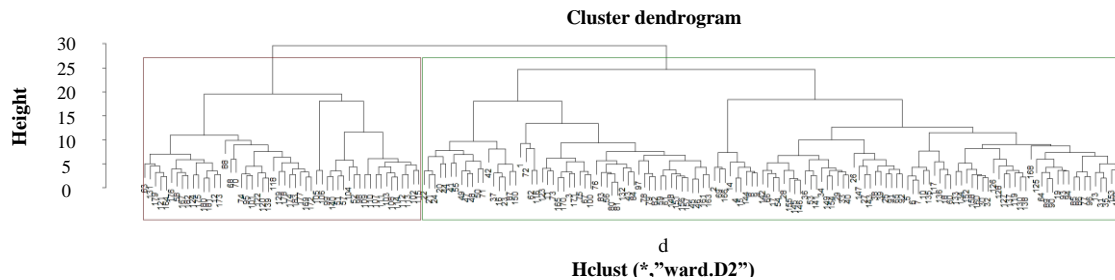


Fig. 9. Ward cluster analysis of studied oak ecotypes based on morphological traits using Euclidean distances.

## Discussion

The morphological traits of leaves and seeds are crucial for the distribution of plant species in different geographical regions and altitudes. These factors cause diversity in morphological characteristics since environmental factors impact plant populations, leading to observed morphological differences in most species. Leaves, as one of the main organs of plants, have high adaptability due to their widespread distribution and ability to react to environmental conditions such as light and temperature (Lusk et al., 2008). Leaf structure maintains a balance between photosynthesis and evapotranspiration, making them particularly important

for carbon sequestration in tree growth (Bruschi et al., 2003). Based on analysis of variance results we observed a significant variation in morphological traits at the level of 5% among and within populations. Similar findings were reported in a study on the diversity of Italian oak populations (*Q. Petrae*) where morphological traits were significantly different at the intra-population and inter-population levels (Bruschi et al., 2003). Many factors are involved in increasing genetic diversity in plant species (Sarikhani et al., 2021). Many factors contribute to the genetic diversity in plant species, including hybridization, speciation, genetic drift

(due to shrinkage of species population), and reproductive strategy, which shape the dynamics of plant species (Conte *et al.*, 2004; Ranker *et al.*, 2004). Oak species are largely incompatible and anemophile in pollination (Chung *et al.*, 2002). Therefore the obligatory allogam is self-incompatible and the spread of pollen grains should essentially happen by the wind to maintain gene flow. According to the crossing of gametes in oak, the current system of the species maintains the genetic diversity within populations and between populations, while gradually enhancing gene flow. The contribution of genetic diversity to plant species reflects their cross-breeding system (Hamrick and Godt, 1996). Genetic diversity is also significantly associated with the distribution distance of pollen. The genetic diversity among oak populations has been confirmed by previous cases of research (Zhanqing *et al.*, 2007; Gonzalez-Rodriguez *et al.*, 2005; Alikhani *et al.*, 2014; Coelho *et al.*, 2006; Coart *et al.*, 2002; Kashani and Dodd, 2002; Ebrahimi *et al.*, 2017; Taleshi *et al.*, 2013; Gailing *et al.*, 2012). The intense gene flow between populations and the typical pollination system are major contributors to the high variance of morphological traits observed in many forest trees, including oaks (Bruschi *et al.*, 2003). Therefore, conservation programs should focus on maintaining the highest possible genetic diversity of the target species in natural ecosystems and its germplasm reservoir. The genetic diversity of forest trees needs to be high to achieve successful planting and enrichment of different populations. Although a great level of diversity was estimated using morphological characteristics of the leaves and seeds, not all populations of *Q. bantii* were covered in the current research. These features can be used as reference measures in conservation programs of genetic resources of this species in the forests of the Middle-Zagros region.

Principal Components Analysis (PCA) showed that leaf traits are more important in the formation of the first component and seed traits in the formation of the second component. Therefore, the attributes LW and LOD were

the most important factors in the morphological variation of populations. The largest LOD was observed in the leaves of oaks in the Ba location, where all samples were grouped, and the lowest mean value was observed in traits related to seeds. In contrast, the ecotypes in the opposite group (i.e., TR and D10) had the highest mean value of leaf-related traits.

Fundamentally, the leaf margin (i.e., leaf teeth spacing) is an important manifestation of oak diversity and differentiation among populations. Research on the morphological diversity of Persian oak leaves in the Zagros woodlands of Fars province, located in Iran, suggested the importance of diversity in the length of leaf blade, the distance between the first tooth and the last tooth on the leaf margin, and the width of leaf blade (Taleshi and Maasoumi Babarabi, 2013). Kremer *et al.* (2002) found leaf length, leaf veins, and cuticle to be reliable descriptors in distinguishing between *Q. petraea* and *Q. robur* species. Di Pietro *et al.* (2020) also obtained similar results while examining the morphological principal component analysis of oak trees in Italy. Morphological traits are effective in clustering oak populations based on environmental conditions and seasonal changes, leading to variations from year to year (Martinik *et al.*, 2014). The use of multiple morphological traits in statistical analysis can provide reliable results at both the intra- and inter-species level (Viscosi and Fortini, 2011; Fortini *et al.*, 2015), contrary to the previously held hypothesis that using too many traits may lead to errors.

The clustering of Persian oak ecotypes in different forest locations of Ilam was studied, and it was found that the first group consisted of TD, TN, TR, D20, and Sa locations, whereas the second group included the ecotypes of Ba location. These were the only locations in which all their ecotypes belonged to the same original cluster, while the remaining oak locations were distributed in two clusters. The dispersion of Persian oak populations from one location to another was also suggested by previous reports, such as the work by

Nocchi *et al.* (2022) on the diversity and genomic structure of oak populations in British Parkland, which involved sequencing the whole genome of 360 oak trees from four regions. According to their findings, there were no clear signs of differentiation among the four sampling regions in 360 samples of *Q. robur* oak. Similar results were also reported by Shabanian *et al.* (2016b) and Taleshi and Maasoumi (2013) in their studies on oak populations. Zeng *et al.* (2011) proposed that subspecies may exist in oak forests, indicating that the genetic diversity found in the current study may not be relevant in terms of species affinity to different subspecies. Pettenkofer *et al.* (2020) also cited the hybrid nature of some samples as reasons for this diversity. The natural occurrence of interbreeding within species populations is a major reason for the significant diversity observed in oak species. Therefore, the consistent genetic diversity between the various ecotypes of oaks in this research can be attributed to hybridization and the conserved, inherited polymorphism in this and other species of oak.

Tree species, such as the oak, generally demonstrate a tendency towards pronounced genetic variability and comparatively limited genetic divergence across various populations. This pattern is likely due to factors such as the long lifespan and durability of trees, high fertility, anemophily pollination, and good seed propagation by animals. Such factors lead to a low-level differentiation among populations, resulting in increased homogeneity of allele frequency among adjacent local populations (Alfonso-Corrado *et al.*, 2014; Wang *et al.*, 2014).

Several studies have suggested that Persian oak seedlings have high compatibility and survival rates in the Zagros forests, making them a suitable choice for reforestation (Alvani Nejad, 2009; Hemati, 1996; Aminpour, 2009). Compared to co-occurring oak species, *Q. brantii* is more adaptable to changes in soil nutrient and water availability (Jazirehi and Rostaghi, 2003). Moreover, various investigations have shown that the decrease in leaf area plays a significant role as an

adaptation mechanism in plants, as it reduces the rate of photosynthesis, thereby enhancing survival in water-constrained conditions (Larcher, 2003; Royer *et al.* 2005; Xu *et al.*, 2008). During our examination, it was observed that the oaks located in the Ghallaje region exhibited the lowest averages in characteristics such as LL, LW, and BW50, all of which are recognized for enhancing resistance to water scarcity. As a result, considering the morphological features exhibited by the *Q. brantii* populations in this area, they may be potentially appropriate for reforestation efforts in Ilam province, with a specific focus on the Mehran Salehabad and Lomar regions, which are subject to low levels of annual rainfall and high levels of annual temperatures. However, further research must be conducted to determine the optimal ecotype.

#### Conflict of interests

The authors have no conflicts of interest to declare

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