

Leaf morphological variation of *Quercus brantii* Lindl. along an altitudinal gradient in Zagros forests of Fars Province, Iran

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ABSTRACT

Quercus brantii Lindl. species is known as Persian oak, West oak and Zagros oak. This species is one of the most important woody species of the Zagros forests. In this study three Persian oak populations were selected with three ranges of altitude in several regions. Then, in each of these sites, 10 trees were selected. In each tree, 10 leaves were collected. The leaves of each tree were mixed and then 5 leaves were selected randomly and 20 traits were measured. Comparison of means by using Duncan's test showed that 10 traits of 20 leaf characteristics had the lowest value in second population but there is no significant difference between first and third populations. In conclusion we can say that the second population had the highest difference of leaf morphological characteristics compared to other populations. PCA showed that the traits of leaf blade length, interval between apical and basal tooth, angle of leaf blade base, leaf blade width, interval between basal pair of tooth and blade width in 0.1 length of blade from leaf base had the greatest impact in classification. On the basis of cluster analysis three populations were grouped in five categories. This issue indicated that in each of the second and third population, the leaf traits were more similar than the first population and this population could have genetic diversity of within population. Totally, trees of the first population had the most Euclidean distance from each other that this issue might indicate genetic diversity of within population.

Key words: Leaf Morphology, Altitude, Zagros Forests, Persia Oak, Fars Province

INTRODUCTION

Fagaceae family is one of the dicotyledonous angiosperms and contains 9 genera in the worldwide. This family has three genera in Iran consist of beech (*Fagus spp.*), oak (*Quercus spp.*) and chestnut (*Castanea spp.*) that oak genus is distinguished from the other two genera because of the bowl, fruit with no gap and only one seed per fruit. The diverse oak species in the north and west of Iran (the Caspian Sea and Zagros Mountains) comprise widespread and valuable forests [14]. *Quercus brantii* Lindl. species is known as Persian oak, West oak and Zagros oak. *Quercus brantii* species is native of temperate regions of Asia and western Asia, including Iran, Iraq, Syria, and Turkey [22] and it's the boundary of Irano- Turanian vegetation region [16]. This species is one of the most important woody species of the Zagros forests [22]. Persian oak is a big tree with height of 20 m and a big spherical crown and it has generally ovoid leaves with serrated margins. The Fruit of *Q. brantii* is elongated and oval in velvety white bowl and conical [16]. This species usually appears in pure stand and altitudinal distribution of 900 to 2400 meters above sea level that it is indicated ecological flexibility of this species [9]. In classification of Iranian forests, Zagros forests with area about 5 million hectares is the largest forest site that they aren't commercial forests with relation to

production of wood, but they have unique importance in terms of soil and water conservation, production of byproducts and environmental values. These forests are starting from Piranshahr in Western Azerbaijan and extend along Zagros Mountains to Firoozabad in Fars province. Area of these forests are estimated about 5 million hectares with canopy cover greater than 5 percent [13, 2]. The investigation of morphological traits has been one of the most ancient classifications of plants. The morphological markers are the same as morphological traits which they are one of the first evidence that plants have been classified accordingly. Some of them, such as leaves, branches, bud, or traits like viability, resistance to cold and pests and properties of wood are considered in the early age [21, 2, 10]. Plant taxonomy experts believe that the leaves of some oak species under environmental changes show different morphological forms [14]. More physical, mechanical and anatomical properties of tree species affected by habitat factors such as elevation changes [7]. Study of the altitude role and its impact on the diversity of forest tree species according to the terms of topography is indicated their importance [12]. Morphological characters are typically controlled by a single locus and can be used as a genetic marker [6]. Several studies were performed on the morphological evaluation of plant organs especially about leaves and fruits. Asadi et al (2004) used morphological traits such as leaves and branches to distinguish between different poplar clones of *P.alba*, *P. deltoids*, *P. euphratica* and *P. nigra*. Espahbodi et al. (2006) studied the genetic diversity of *Sorbus torminalis* species by using leaves and fruits morphology Li et al. (2006) studied morphological and physiological responses of *Quercus aquifolioides* leaves with altitude changes. Sardans et al. (2006) surveyed the morphological characteristics of *Quercus ilex subsp. Ballota* leaves and they found that variability of leaves morphology were reply to fertilization and environmental changes in competitive position.. Kaffash et al. (2008) studied the morphological characteristics of *Quercus infectoria* leaves and this species was described by using morphological parameters. Yousefzadeh et al. (2008) studied the level of *Parrotia persica* population's diversity on the base of leaves morphological traits. Boratynski et al. (2008) investigated leaf morphological differences between adult and young trees of *Q. robur* and *Q. petraea*. Xu et al. (2008) studied the effect of habitat on leaf morphology of *Quercus acutissima* and they concluded that the leaves of these plants responded differently to changes in habitat. Alimohammadi et al. (2009) evaluated the use of leaf morphology characteristics in detection of genetic variation in *P. nigra* trees stands. Sattarian et al. (2011) studied on the leaf morphological variation among natural populations of *Quercus castaneifolia* and *Q. macranthera* and also identification of intermediate phenotypes in the Caspian forests. Saeedi and Azadfar (2011) studied about morphological variation of leaves in three species of poplar clones and they concluded that the morphological characters were useful technique for determination of inter and intra specific variation. This study was carried out to evaluate morphological variation of *Q. Brantii* population along an altitudinal gradient in Zagros Forests, Iran

MATERIALS AND METHODS

Materials

Studied area is in Bovan village in Noorabad-e-Mmasani city of Fars province. Studied area is a part of Zagros forests and its distance from Noorabad-e-Mamasani is 50 Km. In this area tree and shrub species change in relation to altitude and climatic condition and they have specific habitat. One of the most important species is *Quercus brantii*. In the lowest elevation *Q. brantii* and *Pistacia atlantica* species and in highest elevation *Amygdalus orientalis* and individual trees of *Juniperus polycarpus* species are observable [4].

Methods

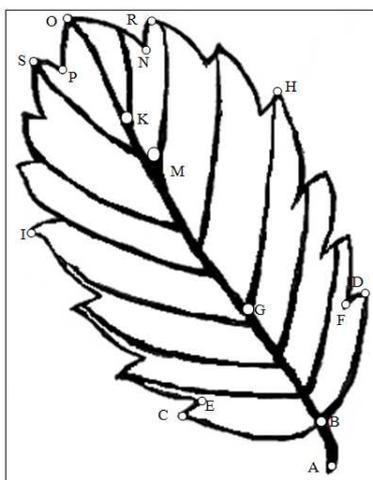
In this study, three Persian oak populations were selected with three ranges of altitude in several regions (Table 1). Then, in each of these sites with respect to the aspect (the trees were chosen in southern aspect), 10 trees were selected with different characteristics and at least 100 m from each other [21]. In each tree and altitude, 10 leaves were collected from exterior and northern of tree crown in the first half of December. The leaves of each tree were mixed and then 5 leaves were selected randomly [1]) and 20 traits were measured that these characters were shown in the table2. To reduce the calculation amount while maintaining the accuracy of the experiment, the mean of mentioned leaf characteristics was calculated for each tree individual Data normality was examined by Kolmogorov-Smirnov normality test. Because the all data were normal, Multivariate Analysis of Variance (MANOVA) was used with 5% error and multiple comparisons were done using Duncan's test. Using multivariate statistical method of Principal Component Analysis (PCA) was determined the most important factors of leaves to investigate the genetic diversity. The cluster analysis by using Ward's method was performed and investigated leaves variation within and between populations. Data analysis was performed using SPSS ver. 15 and PCORD ver. 4 software.

Table 1: Characteristic of studied area

Area	population	altitude	longitude	latitude	Zone
Noorabad-e- mamasani	1 (A1-A10)	1000 m	559644	3327326	39
	2 (A11-A20)	1200 m	562347	3327795	39
	3 (A21-A30)	1400 m	568397	3317237	39

Table 2: Measured characteristic of leaves (Abbreviation letters are defined based on Fig. 1)

measured traits of Leaf	Abbreviation letters
leaf thickness	Li
leaf blade length	Bo
leaf Blade width (at widest point)	lh
leaf Petiole length	Ab
the distance between the widest point and the leaf base	Bh
total number of tooth	N
interval between apical pair of tooth	Sr
interval between apical pair of sinuses	Pn
interval between basal pair of tooth	Cd
interval between basal pair of sinuses	Ef
interval between apical and basal tooth	Rc
nervure length in the center of blade	Gh
interval between central nervure in apical leaf	Km
blade width in 0.1 length of blade from leaf base	W11
blade width in 0.9 length of blade from leaf base	W12
Angle of the base of leaf blade	An
relative petiole length (leaf petiole length: leaf blade length)	Ltl
leaf blade shape (leaf blade length: leaf blade width)	Lf
leaf basal shape (blade width in 0.1 length of blade from leaf base: leaf blade width)	Bf
leaf apical shape (blade width in 0.9 length of blade from leaf base: leaf blade width)	Lpf

**Fig1. Definition of oak leaf characteristics used in this study (The figure should be consulted in conjunction with table1)**

RESULTS AND DISCUSSION

The results of multivariate analysis of variance (MANOVA) based on the all leaves characteristics showed that populations were significantly different at 5% level. Except for traits consist of interval between apical pair of tooth, interval between apical pair of sinuses, blade width in 0.9 length of blade from leaf base, angle of leaf blade base, leaf blade shape and leaf apical shape, were found significant differences between other characters in populations. Comparison of means by using Duncan's test showed that leaf blade length, leaf blade width, leaf petiole length, total number of tooth, interval between basal pair of tooth, interval between basal pair of sinuses, Interval between apical and basal tooth, Blade width in 0.1 length of blade from leaf base, relative petiole length and leaf basal shape had the lowest value in the second population but there is no significant difference between the first and third populations. Also, traits of nervure length in the center of blade and interval between central nervure in apical leaf had the least value in the third population but there is no significant difference between the first and second populations. The leaf thickness was the lowest in the first population but there is no significant difference between the second and third populations. Principal component analysis (PCA) showed that in the formation of the first component, traits of leaf blade length, interval between apical and basal tooth and angle of leaf blade base and in the formation of the secondary component traits of leaf blade length, leaf blade width, interval between basal pair of tooth, interval between apical and basal tooth and blade width in 0.1 length of blade from leaf base showed more important than other traits. According to the table 3, the first two components accounted for 70% of the cumulative variance. Thus, the traits of leaf blade length, Interval between apical and basal tooth, angle of leaf blade base, leaf blade width, interval between basal pair of tooth and blade width in 0.1 length of blade from leaf base had the greatest impact in the classification. Also, in the formation of the first and secondary components traits of leaf thickness, relative petiole length, leaf blade shape, leaf basal shape and leaf apical shape had the least important. The results of cluster analysis that was performed by using of total leaf characteristics showed that according to the

variance of the clusters formation, the three populations were divided into five groups. In the first group, trees number A1, A8, A2 and A7 of the first population, trees number 12A, 14A, and 18A of the second population and tree number A30 of the third population were placed. In the second group were seem trees number A9 and A10 of the first population and trees number A11, A13, A16 and A17 of the second population. The third group was included of trees A3, A4 and A5 of the first population and trees A25, A26, A28 and A29 of the third population. Forth group consist of tree A6 of the first population, trees A15 and A19 of the second population and tree A24 of the third population. In the fifth group were placed trees A21, A22, A27 and A30 of the third population. The PCA graph based on first two components derived from principal components analysis couldn't separate individually each population on the base of leaf characteristics. Although in the first population trees 3 and 9 had similar habitat conditions but from but they had the greatest Euclidean distance from each other.

Table 2: The results of Duncan's mean comparison test (Means with the same letter are not significantly different at p=0.05)

Studied leaf characteristics	Mean		
	Population 1	Population 2	Population 3
leaf thickness	0.2314 ^b	0.2832 ^a	0.2822 ^a
leaf blade length	74.6356 ^a	58.6220 ^b	77.2172 ^a
leaf Blade width (at widest point)	46.3440 ^a	36.1652 ^b	52.1964 ^a
leaf Petiole length	15.0340 ^a	6.2268 ^b	12.6720 ^a
the distance between the widest point and the leaf base	43.0236 ^a	35.0354 ^b	40.4960 ^{ab}
total number of tooth	11.8200 ^a	7.7400 ^b	9.9400 ^a
interval between apical pair of tooth	7.8344 ^a	8.7700 ^a	9.0892 ^a
interval between apical pair of sinuses	5.7144 ^a	5.4100 ^a	5.8972 ^a
interval between basal pair of tooth	40.5012 ^a	33.0364 ^b	47.0044 ^a
interval between basal pair of sinuses	38.2292 ^a	30.1860 ^b	43.6036 ^a
interval between apical and basal tooth	63.5912 ^a	43.5500 ^b	63.9260 ^a
nervure length in the center of blade	26.0132 ^a	22.1908 ^a	30.7832 ^b
interval between central nervure in apical leaf	9.0132 ^a	9.7184 ^a	12.7144 ^b
blade width in 0.1 length of blade from leaf base	36.7372 ^a	21.6492 ^b	33.0984 ^a
blade width in 0.9 length of blade from leaf base	18.6604 ^{ab}	16.5736 ^b	20.8272 ^a
Angle of the base of leaf blade	138.6800 ^a	141.9400 ^a	163.5800 ^a
relative petiole length (leaf petiole length: leaf blade length)	0.1996 ^a	0.1096 ^b	0.1609 ^a
leaf blade shape (leaf blade length: leaf blade width)	1.6329 ^a	1.6766 ^a	1.5026 ^a
leaf basal shape (blade width in 0.1 length of blade from leaf base: leaf blade width)	0.7127 ^a	0.5894 ^b	0.9622 ^a
leaf apical shape (blade width in 0.9 length of blade from leaf base: leaf blade width)	0.4129 ^a	0.4404 ^a	0.4038 ^a

Table 3: Hidden roots of studied leaf characteristic in the first six axes of PCA

Studied leaf characteristic	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6
leaf thickness	0.0001	0.0002	0.0024	0.0040	0.0013	0.0029
leaf blade length	0.1564	0.4676	0.3093	0.4937	0.3049	0.2277
leaf blade width (at widest point)	0.0641	0.3369	0.2816	0.1853	0.0897	0.0184
leaf Petiole length	0.0553	0.1286	0.0679	0.2669	0.5560	0.6151
the distance between the widest point and the leaf base	0.0734	0.1898	0.1370	0.3111	0.4842	0.4852
total number of tooth	0.0043	0.0374	0.2038	0.2277	0.0036	0.1696
interval between apical pair of tooth	0.0005	0.0139	0.1649	0.0387	0.2220	0.2108
interval between apical pair of sinuses	0.0024	0.0148	0.1081	0.0187	0.1211	0.1560
interval between basal pair of tooth	0.0740	0.3069	0.4314	0.1296	0.1883	0.0391
interval between basal pair of sinuses	0.0650	0.2976	0.3586	0.0683	0.1730	0.0131
interval between apical and basal tooth	0.1059	0.5840	0.5305	0.2347	0.1818	0.1691
nervure length in the center of blade	0.0383	0.1748	0.2292	0.0998	0.0640	0.1716
interval between central nervure in apical leaf	0.0145	0.0618	0.1801	0.1935	0.0432	0.0436
blade width in 0.1 length of blade from leaf base	0.0314	0.3094	0.1654	0.5989	0.2051	0.1333
blade width in 0.9 length of blade from leaf base	0.0038	0.1020	0.1077	0.1195	0.3790	0.3956
Angle of the base of leaf blade	0.9692	0.2343	0.0297	0.0515	0.0330	0.0171
relative petiole length (leaf petiole length: leaf blade length)	0.0003	0.0007	0.0006	0.0045	0.0066	0.0080
leaf blade shape (leaf blade length: leaf blade width)	0.0013	0.0017	0.0172	0.0201	0.0102	0.0100
leaf basal shape (blade width in 0.1 length of blade from leaf base: leaf blade width)	0.0003	0.0020	0.0010	0.0102	0.0030	0.0002
leaf apical shape (blade width in 0.9 length of blade from leaf base: leaf blade width)	0.0005	0.0007	0.0004	0.0031	0.0071	0.0092

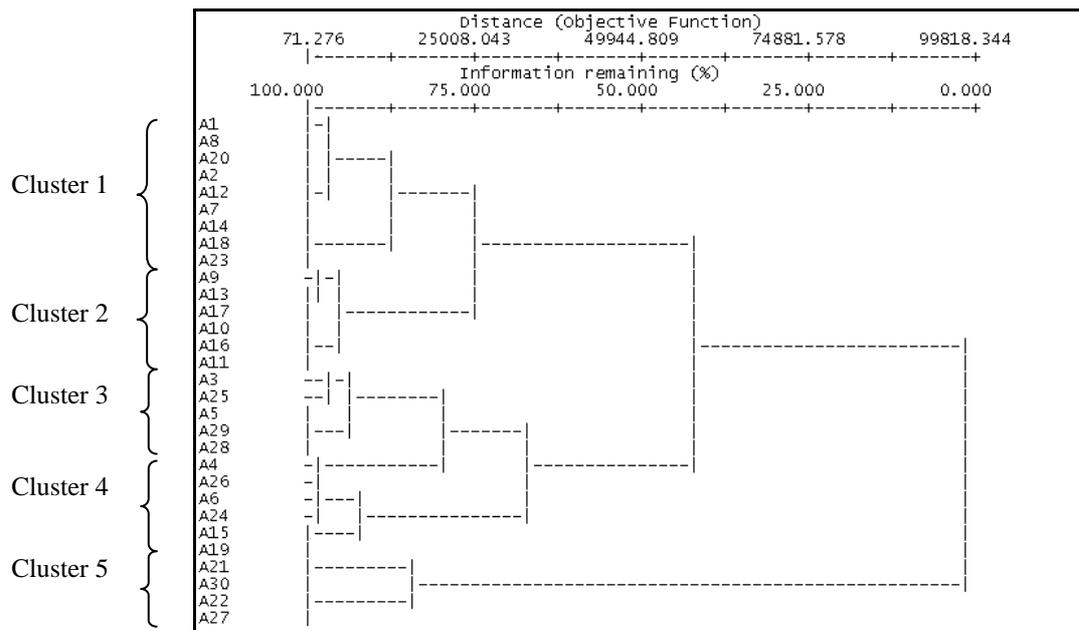


Fig 2: Cluster analysis of *Quercus brantii* population using Ward's method

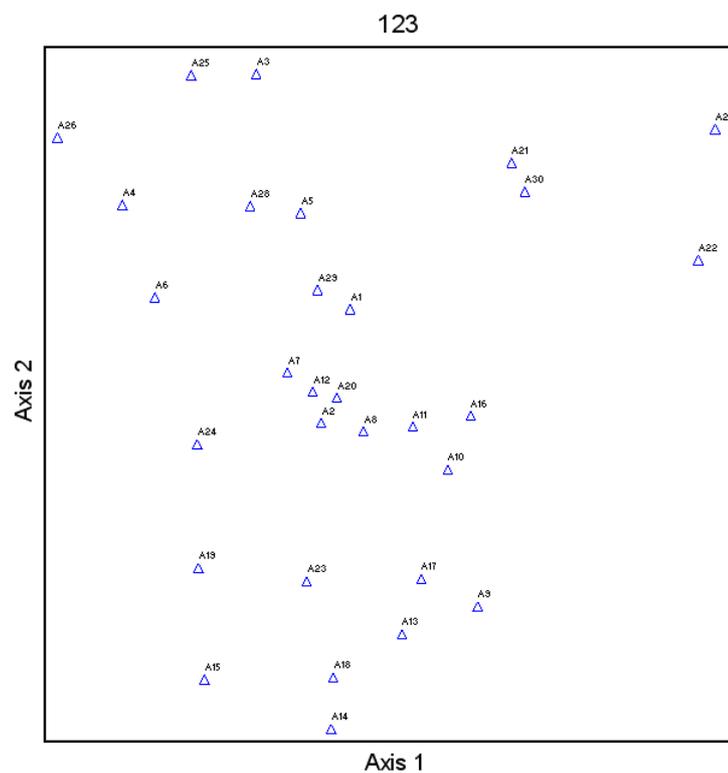


Fig 3: The scatter plot of the tree individuals on the basis of first two component of PCA (A1-A10: Population 1, A11-A20: Population 2 and A21-A30: Population 3)

CONCLUSION

One of the first steps in determining and identifying of the intra and inter specific variation is using of morphological markers. Among the morphological traits, the leaves have special importance due to growth and regeneration of trees on the basis of photosynthesis and carbon sequestration [21]. Comparison of means by using Duncan's test showed that 10 traits of 20 leaf characteristics had the lowest value in second population but there is no significant difference between first and third populations. In conclusion we can say that the second population had the highest difference of leaf morphological characteristics compared to other populations. This issue could indicate that the trees of second population were genetically different than the first and third populations, which genetic studies are needed to prove.

Also, on the other hand more trees of second population were coppice; it is possible that differences in leaf characteristics were probably in relation to this issue. PCA showed that the traits of leaf blade length, interval between apical and basal tooth, angle of leaf blade base, leaf blade width, interval between basal pair of tooth and blade width in 0.1 length of blade from leaf base had the greatest impact in classification. Also in the formation of the first and secondary components the traits of leaf thickness, relative petiole length, leaf blade shape, leaf basal shape and leaf apical shape had the least important. In this regard, Yousefzadeh *et al.* (2008) reported that leaf width and angle of the base of the leaf blade of *Parrotia persica* were important in the classification. Also, Espahbodi *et al.* (2006) showed that the traits of leaf blade width and the Leaf apical shape of *Sorbus torminalis* were more effective in formation of PCA component. On the basis of cluster analysis three populations were grouped in five categories. Most of the trees of second population were placed in the second group next to each other (4 trees). On the other hand, in fifth group was observed only trees of the third population. In other groups the trees of different population were put together. This issue indicated that in each of the second and third population, the leaf traits were more similar than the first population and this population could have genetic diversity of within population. Although, in the first population trees 3 and 9 had similar habitat conditions but they had the greatest Euclidean distance from each other. Totally, trees of the first population had the most Euclidean distance from each other that this issue might indicate genetic diversity of within population.

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