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Morphological and Molecular Differentiation in Populations of Persian Oak (*Quercus Brantii* Lindl.) in Southwestern Iran

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PERSIAN oak (*Quercus brantii* Lindl.) is a common woody species in the Zagros forests of Iran. The current study evaluates the variation among the *Q. brantii* populations in the southwestern forests of Iran using amplified fragment length polymorphism (AFLP) and leaf morphology. A total of 135 trees were sampled from 27 populations representing nine regions in the provinces of Khuzestan (GOL, EMA, BAB, MOG, ALG), Chaharmahal and Bakhtiari (MON), Lorestan (KHA), Kohgiluyeh and Boyer-Ahmad (DIS) and Fars (BAA). Twelve morphological leaf traits were analyzed using PCA and Ward's clustering methods. The results of ANOVA showed significant differences between populations, but the PCA graph and clustering analysis could not separate the populations on the basis of leaf characteristics. An analysis of molecular variance indicated that most genetic variation was contained within populations (95%); differences between populations accounted for only 1% and 4% was attributed to variation between regions. The five regions of Khuzestan have similar genetic structures. This was observed for the regions in Fars, Lorestan and Chaharmahal and Bakhtiari. Region DIS of Kohgiluyeh and Boyer-Ahmad had a separate genetic structure. The Dendrogram of *Quercus brantii* populations based on AFLP marker and Cluster analysis of populations using Ward's method based on morphological data don't confirm each other and in both, populations mix together. The current study revealed the some morphological and molecular differences in some populations of Persian oak in Iran. Population genetic information can provide critical insights into range expansion and evolutionary potential to adapt to environmental changes.

Keywords: AFLP, Molecular, Morphological, *Quercus brantii*.

Introduction

The genus *Quercus* L. (Fagaceae) is a diversified group of temperate trees with about 500 species distributed worldwide (Mehrnia et al., 2012). Zagros oak forests are the largest in Iran with coverage of about 40% (Sagheb-Talebi et al., 2004). These forests have a sub-Mediterranean semiarid temperate climate with dominant species being *Quercus brantii* Lindl. and other deciduous, broad-leaf trees such as *Pistacia atlantica* Desf., *Pistacia khinjuk* Stocks., *Pyrus* spp., *Amygdalus* spp., *Celtis* spp. and *Acer monspessulanum* L. With more than 50% coverage of the western forests of Iran, *Q. brantii* is the most important tree species in the region (Heydari et al., 2013).

Its genetic variation and high levels of phenotypic plasticity contribute to the success of the genus *Quercus*; however these characteristics pose difficulties when estimating the genetic architecture of populations (Kashani & Dodd, 2002). Genetic variation is essential for a species' long-term survival and adaptation under a changing environment. As species encounter new stresses (climate change and pollution) and management measures are required to conserve their gene pool (Crăciunesc et al., 2011).

Plant taxonomists believe that the leaves of some oak species under environmental change and habitat factors such as elevation change or altitudinal gradients show different morphological forms; therefore, several dichotomous keys based on morphological characteristics have been developed to describe species and sections within Quercus (Panahi et al., 2011; Mehrnia et al., 2012 and Taleshi & Maasoumi-Babarabi, 2013), but the

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use of these characteristics alone is not sufficient because of the influence of environmental conditions. For this reason, molecular markers are commonly used to evaluate genetic variation in plant communities. DNA-based markers have numerous benefits over morphological markers because they lack the influence of environment (Duran et al., 2009).

Evaluation of genetic diversity is necessary for germplasm conversion and optimized forest management. Because oaks are important components of temperate forests, they have been the subject of many studies using different morphological and molecular markers (González-Rodríguez & Oyama, 2005; Birchenko, 2008; Ballian et al., 2010; Crăciunesc et al., 2011; Lind & Gailing, 2013; Yücedağ1 & Gailing, 2013; Bakiş & Babaç, 2014 and Lee et al., 2014).

Amplified fragment length polymorphism (AFLP) technique is a highly repeatable method that produces a large number of markers, which makes it suitable for genetic population studies (Vos et al., 1995). AFLP produces a notably higher amount of markers in comparison with microsatellite markers (Mariette et al., 2002). It has been shown that the vast majority of diversity present occurs by within-population variation rather than between populations or between species (Coart et al., 2002; Kashani & Dodd, 2002 and Coelho et al., 2006).

Shiran et al. (2011) used morphological and AFLP markers and reported a high rate of variation within the Iranian populations of *Q. brantii*. This study was performed only in one province (Chaharmahal va Bakhtiari) of Iran. Issues such as genetic structure and the impact of environmental factors on morphological traits of populations have not been investigated. Zolfaghari et al. (2009) evaluated genetic diversity in the Persian oak using simple sequence repeat (SSR) and found that middle altitude populations have higher genetic diversity than those at lower and higher altitudes. Using internal transcribed spacer (ITS) markers, Mehrnia et al. (2012) grouped the all populations of oaks in the Zagros Mountains into eight species. Other studies on oaks from other parts of Iran used both molecular and morphological markers (Taleshi & Maasoumi-Babarabi, 2013 and Alikhani et al., 2014).

Historically, oaks have been an important source of fuel, fodder and building materials. They are also sources of tannin and dye. Oak bark and leaves are used for tanning leather. The fruit of this tree is used in a decoction or as a powder to treat acute diarrhea, inflammation and stomachache (Shikov et al., 2014) in traditional medicine.

The present study evaluates the genetic and morphological variation of *Q. brantii* populations in the provinces of Lorestan, Khuzestan, Chaharmahal and Bakhtiari, Kohgiluyeh and Boyer-Ahmad and Fars using AFLP markers technique and leaf morphology. This study answer to some important questions; (1) If there morphological and molecular differences between and among populations of Quercus brantii? (2) If the morphological and molecular results confirm each other? (3) In ordination of populations, morphological marker is better or molecular marker? (4) How is that the genetic variation within populations, among populations and among regions? (5) How is that the genetic structure of populations? (6) If the environmental conditions affect the morphological traits?

Materials and Methods

Plant materials

This study was performed on the materials from 27 populations collected from nine regions in southwestern Iran (Table 1 and Fig. 1). The regions were located in the provinces of Lorestan (KHA), Chaharmahal and Bakhtiari (MON), Khuzestan (ALG, GOL, MOG, BAB, EMA), Kohgiluyeh and Boyer-Ahmad (DIS) and Fars (BAA). The geographical coordinates, altitude, aspect, annual precipitation average and annual temperature average for each region are shown in Table 1. The collection of acorns was carried out at low, middle and high altitudes at each site during the autumn of 2014. Each population was represented by at least five individual trees at least 50 m apart. All step of this study carried out 2014-2015, in laboratory and herbarium of department of biology, science and research branch, Islamic Azad University, Tehran, Iran.

DNA extraction

Because the mature leaves of oaks contain high amounts of tannin and phenolic compounds, immature fresh leaves of seedlings were used as the DNA source. Fully mature acorns of *Q. brantii* were collected in the

TABLE 1. Geographical and climatic details of 27 populations of *Quercus brantii* collected for this study. Abbreviations: Pop., population; Reg., Region; Prov., Province; Abr., Abbreviation; Alt., Altitude; Lat., Latitude; Long., Longitude; Asp., Aspect; APA, Annual Precipitation Average (mm); ATA, Annual Temperature Average (°C). Climatic Data were obtained from the Iran Agency of Meteorology (IRIMO) (http://www. irimo.ir).

Pop.	Reg.	Prov.	Abr.	Alt.	Lat.	Long.	Asp.	APA	ATA
1	Golzar	Khuzestan	GOL	850	3254.3 ˊ12ˊˊ	4940.1ˊ43ˊˊ	South	35	21
2				1350	329.3ˊ11ˊˊ	4946.2ˊ42ˊˊ	West		
3				1600	3216.4ˊ9ˊˊ	4936.5ˊ43ˊ ˊˊ	East		
4	Emamzadeh-Abdollah	Khuzestan	EMA	1630	3146.1 ˊ24ˊˊ	5054.1 ˊ9ˊ ˊˊ	East	132	27
5				1801	3138.6 ˊ23ˊˊ	5046.6 ˊ9ˊˊ	West		
6				2086	312.1 ˊ23ˊˊ	5021.9 ˊ9ˊ ˊˊ	West		
7	Baloot-Boland	Khuzestan	BAB	910	3126.4ˊ45ˊˊ	5047.1ˊ8ˊˊ	South	34	19
8				1540	3151.1ˊ41ˊˊ	5025.7ˊ18ˊˊ	South		
9				2030	3143.5ˊ39ˊˊ	5026.6ˊ23ˊ ˊˊ	North		
10	Mongasht	Khuzestan	MOG	950	3154.9ˊ47ˊˊ	499.3ˊ59ˊ ˊˊ	East	35	21
11				1285	3130.1ˊ46ˊˊ	4937.0ˊ59ˊˊ	West		
12				1621	3115.7ˊ46ˊˊ	5023.7ˊ0ˊˊ	West		
13	Alvar-Garm-siri	Khuzestan	ALG	650	3224.7ˊ50ˊˊ	4834.5ˊ22ˊˊ	East	20	26
14				800	3245.4ˊ49ˊˊ	482.8ˊ16ˊˊ	West		
15				1000	328.2ˊ53ˊˊ	4853.0ˊ15ˊˊ	East		
16	Dishmook	Kohgiluyeh and Boyer-Ahmad	DIS	700	3129.1ˊ14ˊˊ	500.2ˊ18ˊˊ	East	372	25
17				1435	318.5ˊ17ˊˊ	5051.8ˊ16ˊˊ	West		
18				2000	3120.8ˊ16ˊˊ	5012.1ˊ22ˊˊ	East		
19	Monj	Chaharmahal and Bakhtiari	MON	1550	3127.1ˊ30ˊˊ	5055.5ˊ38ˊˊ	South	35	16
20				1830	3145.9ˊ29ˊ ˊˊ	503.8ˊ365ˊˊ	West		
21				2245	3110.3ˊ30ˊˊ	5029.0ˊ35ˊˊ	West		
22	Khorram-Abad	Lorestan	KHA	1162	3318.1ˊ26ˊˊ	485.3ˊ12ˊˊ	South	30	17
23				1420	332ˊ20.5ˊˊ	489ˊ25.18ˊˊ	East		
24				1874	334ˊ17.29ˊˊ	481ˊ26.15ˊˊ	East		
25	Barm-Arjan	Fars	BAA	1249	2946.6ˊ33ˊˊ	5119.5ˊ51ˊˊ	North	13	22
26				1655	2928.7ˊ29ˊˊ	5122.4ˊ58ˊˊ	North		
27				2101	2919.3ˊ32ˊˊ	5131.8ˊ57ˊˊ	West		

Fig. 1. Map of Iran indicating locality of collected plant material. Each point represents the region in which three populations are collected (further details in Table1). Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar- Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

autumn and then planted in pots. After 3-4 week the leaves appeared. Young fresh leaves were collected from each seedling and stored in plastic bags filled with silica gel pearls until DNA extraction. The total DNA was extracted from the silica-gel dried leaf samples using the modified protocol of Doyle & Doyle (1990) and DNeasy Plant Mini Kits (Germany) following manufacturer protocols. The quality of the extracted DNA was checked on 1% agarose gel. High-quality DNA samples were stored at -20°C until required.

PCR-AFLP analysis

For the AFLP, the Scalone & Albach (2012) method was used with the following modifications. The total DNA was simultaneously digested with 0.3 µl *Eco*RI and 0.1 µl *Mse*I (10 U/µl; Thermoscientific; Lithuania) and ligated with T4 DNA ligase (5 U/µl; Thermoscientific; Lithuania) in a reaction buffer (10 mM tris-HCl (pH 7.5), 10 mM Mg acetate, 50 mM K acetate, 1 µl 0.5 mol NaCl and 0.25 µl bovine serum albumin (BSA; 2 mg/ml; Thermoscientific; Lithuania) and 0.091 µl T4 DNA ligase) for 3h at 37°C. Amplification was carried out in a standard polymerase chain reaction (PCR) cocktail containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 mM of Taq DNA polymerase, PCR buffer, 1% Tween 20 (Ampliqon; Denmark), 0.125 µl 10 pmol/µl preselective primers E01 and Mo2, 2.25 µl DEPC H_2O, 1 µl DMSO and 3 µl diluted product (1:3) of the digestion-ligation reaction. For the primers, the *Eco*RI primer sequence was identical to the adapter sequence, but the *Mse*I primer had an extra cytosine (C) as a selective nucleotide. Two adaptors, one for the 0.5 µl 5 pmol/µl *Eco*RI ends and one for the 0.5 µl 5 pmol/µl *Mse*I ends to avoid the reconstruction of the restriction sites. Following incubation, enzymes were inactivated by at 65°C for 10 min. Subsequently, the ligation product was diluted at 1:3 for primary amplification. Primary The PCR reaction was performed in a thermocycler (Labcycler; Sensquest; Germany) for 20 cycles using the following cycling parameters: 20 s at 94°C, 30 s at 56°C, 2 min at 72°C and 30 min at 60°C. The primary amplification product was then diluted at 1:10 for selective amplification selective amplification which involved the use of 4 different primer pair combinations. Selective amplification was carried out in a

standard PCR reaction cocktail containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 mM of Taq DNA polymerase, PCR buffer, 1% Tween 20, 0.18 µl 10 pmol/µl *Eco*RI primer and 0.25 µl 10 pmol/µl *Mse*I primer, 2.07 µl DEPC H_2O and 5 µl diluted product of the preselective amplification. The four primer combinations for the selective PCR were E38-Hex combined with M50; E31-Pet and M54; E45-Fam and E40-Ned with M57 (Table 2). The PCR program had two cycles: 10 cycles of 30 s at 94°C, 30 s at 65°C and 2 min at 72°C, followed by 25 cycles of 30 s at 94°C, 30s at 56°C and 2 min at 72°C. Selective amplification products were resolved on 6% polyacrylamide native gel. The gels were stained with Gelstar (FMC Bioproducts; USA) following manufacturer protocol and documented with a Kodak DC 120 digital camera.

TABLE 2 . Name and sequences of primer pairs that was used in selective-PCR reactions.

Reverse primer sequences (non- labeled)	Reverse primer	Forward primer sequences (labeled)	Forward primer	Row
5'-GATGAGTCCTGAGTAACAT-3'	M50	5'HEX-GACTGCGTACCAATTCACT-3'	E38	1
5'-GATGAGTCCTGAGTAACCT-3'	M54	5'PET-GACTGCGTACCAATTCAAA-3'	E31	2
5'-GATGAGTCCTGAGTAACGG-3'	M57	5'FAM-GACTGCGTACCAATTCATG-3'	E45	3
'5-'GATGAGTCCTGAGTAACGG-3	M57	5'NED-GACTGCGTACCAATTCAGC-3'	E40	4

Statistical data analysis

The bands on the gel obtained using Gelstar and scored as present (1) or absent (0) in the readable region of the bands from 50-500 bp in length using Gene Marker (v. 1.95). Each peak having a signal intensity of more than 1000 was selected and checked for presence in each sample. The genetic diversity and structure of populations was calculated using GenAlex (v. 6.501) and Structure (v. 2.3.4), respectively. Some of this work was done in SPSS (v. 22) and Adobe Illustrator CS6.

Morphological data

Five leaves from each of the 135 trees were selected randomly and measured for biometrical verification of the 27 populations using 12 leaf characteristics (Table 3 and Fig. 2). All measurements were linear and taken with a digital caliper except the number of rays in stellate trichomes on leaf adaxial side and number of rays in stellate trichomes on leaf abaxial side of the leaf, which were measured using a light microscope.

TABLE 3. Measured traits of leaves (Abbreviation letters are defined based on Fig. 2).

Measured traits of Leaf	Abbreviation
Leaf blade length	LBL
Leaf blade width (at widest point)	LBW
Leaf petiole length	LPL
Total leaf length (leaf blade length + leaf petiole length)	TLL
Total number of tooth	TNT
Number of intercalary vein on right side	NVR
Number of intercalary vein on left side	NVL
Nervure length in the center of blade	NLC
Interval between central nervure in apical leaf	INA
Leaf blade shape (leaf blade length/ leaf blade width (at widest point))	LBS
Number of rays in stellate trichomes on leaf adaxial side	RAD
Number of rays in stellate trichomes on leaf abaxial side	RAB

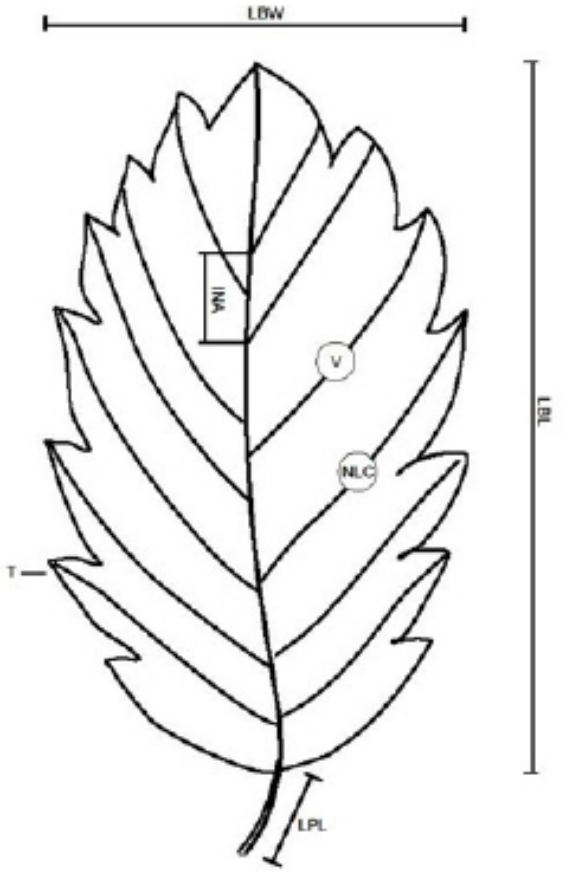

Fig. 2. Definition of oak leaf traits used in this study. Abbreviations: T, Teeth; V, Vein; LBW, leaf blade width; LBL, leaf blade length; LPL, leaf petiole length; INA, interval between central nervure in apical leaf; NLC, nervure length in the center of blade.

Statistical analysis

Analysis of variance (ANOVA) was used with 5% error and multiple comparisons were done using Duncan's test. Principal component analysis (PCA) was used to determine the most differentiating characteristics of the leaves. Cluster analysis using Ward's method was performed to examine leaf variation within and between populations. Pearson's correlation was carried out to test for correlations between leaf morphological characteristics, climate and geographical data. Data analysis was performed using SPSS (v. 22).

Results and Discussion

Analysis of molecular variance (AMOVA) in GenAlex showed that 95% of the total variation could be attributed to differences between individuals within the same population; 1% of the overall variation could be attributed to differences between populations and 4% between regions (Table 4). The greatest genetic variation was within populations, which agree with the findings of Coart et al. (2002), Kashani & Dodd (2002), Mariette et al. (2002), Coelho et al. (2006), Shiran et al. (2011) and Alikhani et al. (2014). But, there are drawbacks in interpreting results. Five individuals representing each population is very small sample size which for sure affects the results. It doesn't allow to capture all genetic diversity maybe present in populations and hence results in less differentiation among populations.

A general pattern in temperate tree species is high genetic diversity and low genetic differentiation between populations. This pattern has been explained to be the result of long generation times, woody life forms, out-crossing mating system, high fecundity, mechanism of pollination (anemophily), long age and dispersal of seeds by animals. These are responsible for the low levels of differentiation between populations, which

TABLE 4. Analysis of molecular variance (AMOVA) of genetic variation in *Quercus brantii* populations. Abbreviation: Df, Degrees of freedom; SS, Sum of squares; MS, Mean squares; Est.Var., Estandard variation; %, Percent of genetic variation.

Source of variation	df	SS	MS	Est.Var.	%
Among Regions	8	55.426	6.928	0.189	4%
Among Populations	26	127.244	4.894	0.048	1%
Within Populations	100	515.600	5.156	5.040	95%
Total	134	698.270		5.345	100%

improves the homogeneity of the allele frequency between adjacent local populations (González-Rodríguez & Oyama, 2005; Coelho et al., 2006; Shiran et al., 2011; QIN et al., 2012; Zhang et al., 2013; Alfonso-Corrado et al., 2014; Porth & El-Kassaby, 2014; Valencia-Cuevas et al., 2014 and Wang et al., 2014).

The dendrogram of AFLP data and indicates that populations mixed and were not separate from each other (Fig. 3 and Table 5). Shiran et al. (2011) showed that, at the morphological level, macromorphological traits significantly differentiate between populations. The clustering pattern from AFLP and morphological analysis was complex in detail, but some general features were evident. The AFLP tree of individuals from different populations were mixed, and this case may correlate with shared old genes by individuals or gene exchange between individuals (González-Rodríguez & Oyama, 2005 and Shiran et al., 2011).

The oak populations may retain high genetic variation because they are long-lived and wind-pollinated plants with large geographic ranges for which a higher number of diverse or more heterozygous individuals may survive over time. They do not generally show separate and specified genetic foundations (Kittelson et al., 2009). Moreover, gene flow is a key factor in species evolution that affects the hybridization and local adaptation (Gerber et al., 2014). Factors such as forest fragmentation, rapid climate change and interference of humans effect genetic diversity of the population in forest trees that are long-lived (Birchenko, 2008).

Figure 4 shows the results of evaluation of genetic structure and the two gene pools available in the populations are denoted in red and green. Calculation of the percentage of alleles in different regions (Table 6) indicated that five regions have the same genetic pool (GOL, EMA, BAB, MOG and ALG). This was also observed between regions in Fars (BAA), Lorestan (KHA) and Chaharmahal and Bakhtiari (MON). The DIS region of Kohgiluyeh and Boyer-Ahmad had a specific and separate genetic structure.

The pattern of diversity and population genetic structure are determined by the interaction of different factors related to life history traits and ecological features of species, evolutionary history, and natural disturbances. The balance achieved by a population among these factors determines its special genetic structure (Valencia-Cuevas et al., 2014). Genetic structure can be associated with geographical region, climate conditions (Neophytou et al., 2010), hybridization (Curtu et al., 2014), breaking of primary reproductive

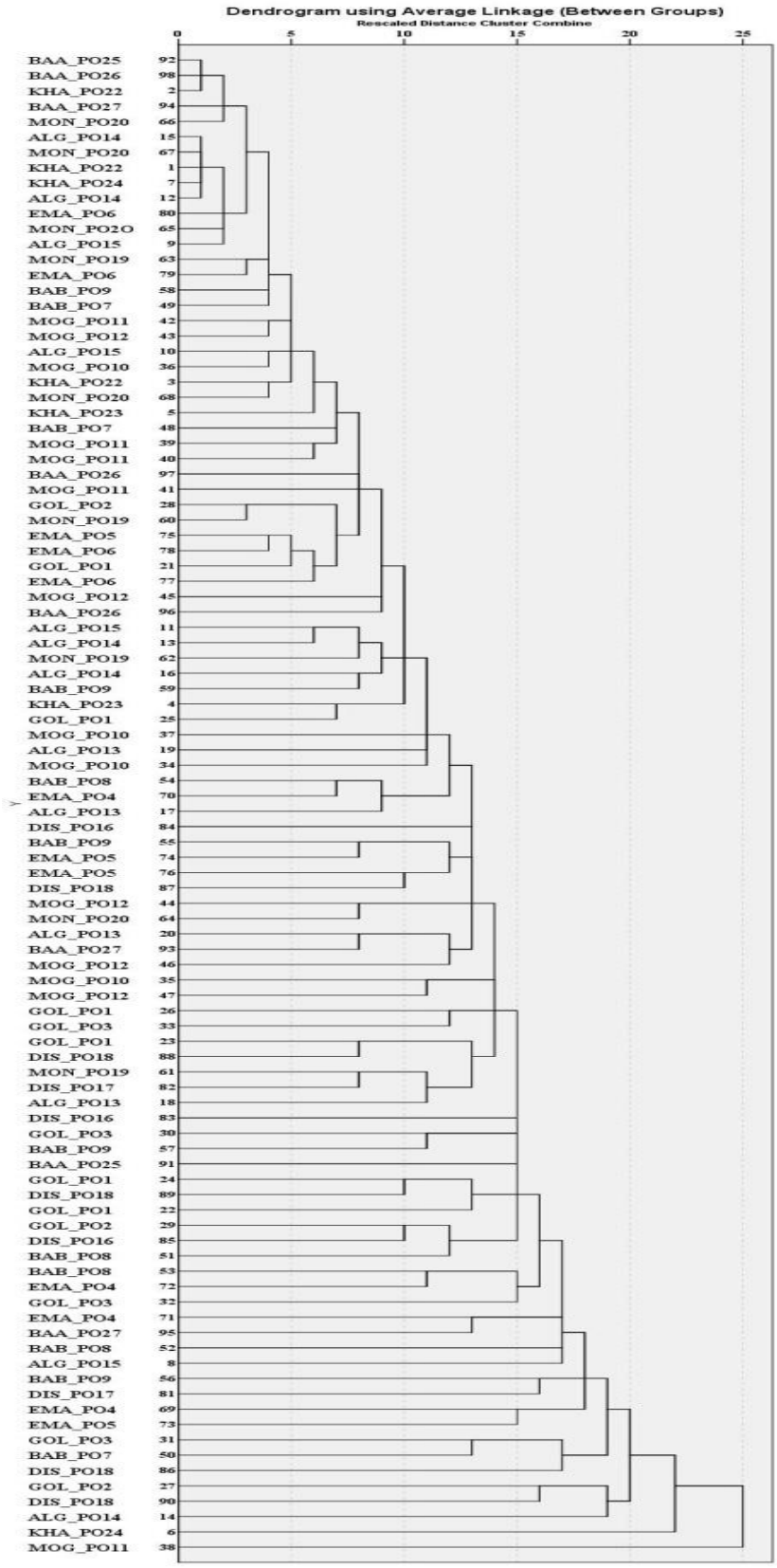

Fig. 3. Dendrogram of Quercus brantii populations based on AFLP marker.

TABLE 5. Explanation of letters and numbers in figure 3. Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar- Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

Region	GOL	EMA	BAB	MOG	ALG	DIS	MON	KHA	BAA
Population	PO1-PO27								
Individual	Numbers (1-135): individuals of each population								

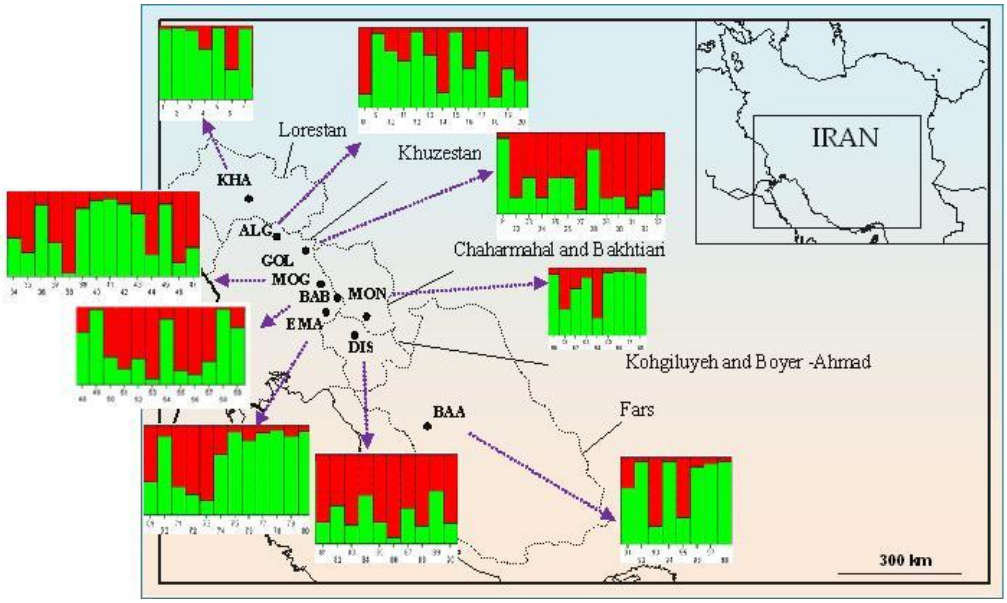

Fig. 4. Correlation between genetic structures of *Querus brantii* populations in studied regions. Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

TABLE 6. Mean % of allele in *Quercus brantii* populations in studied regions. The descriptive statistics are presented in terms of the Mean ± SD (Standard deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test.

Region	(%) Red allele	(%) Green allele
Golzar (GOL)	69.38 ± 25.70 cd	30.62 ± 25.70 ab
Emamzadeh-Abdollah (EMA)	34.08 ± 29.51 ab	65.92 ± 29.51 cd
Baloot-Boland (BAB)	58.08 ± 27.97 bcd	41.92 ± 27.97 abc
Mongasht (MOG)	43.29 ± 32.46 ab	56.71 ± 32.46 cd
Alvar-Garmsiri (ALG)	45.92 ± 29.02 abc	54.08 ± 29.02 bcd
Dishmook (DIS)	74.00 ± 13.70 d	26.00 ± 13.70 a
Monj (MON)	26.78 ± 25.51 a	73.22 ± 25.51 d
Khorram-Abad (KHA)	20.71 ± 21.83 a	79.29 ± 21.83 d
Barm-Arjan (BAA)	31.63 ± 28.76 a	68.38 ± 28.76 d

barriers (Valencia-Cuevas et al., 2014), altitude, historical movements, evolution, phenology and seed dispersal over a large area by animals (Ohsawa et al., 2007; Zhang et al., 2013 and Wang et al., 2014).

A one-way analysis of variance (ANOVA) of all leaf characteristics showed that the populations were significantly different at the 5% level (Table 7). Measures carried out are summarized as three varimax-rotated principal components in Table 8. Principal component analysis (PCA) showed that the variation explained by the first component was mostly accounted for by variables related to TLL (total leaf length) and LBL (leaf blade length) and comprised 37% of the total variation. These traits had the greatest impact on ordination. The second component explained 18% of the total variation and was mostly due to the variation in NVR (number of intercalary veins on the right side) and NVL (number of intercalary veins on the left side). Morphological characters on the third principal component explained 15% of the total variation. In total, these three factors explained 70% of the total variance in the data; thus, the traits of total leaf length, leaf blade length, number of intercalary veins on the right side and number of intercalary veins on the left side had the greatest impact on ordination.

The principal component analysis graph of the first two components from principal component analysis could not separate the populations on the basis of leaf characteristics (Fig. 5). One of the first steps was to determine and identify population variation using morphological markers. Among the morphological traits, the leaves and fruit have special importance. In the current study, despite significant differences between populations for leaf morphological traits, the populations could not be separated and the results of the morphological traits data differed from the AFLP markers. Mehrnia et al. (2012) found that leaf morphological traits were not important for separation of the *Quercus* species. The current study and Taleshi & Maasoumi-Babarabi (2013) showed that the principal component analysis graph and cluster analysis were unable to separate populations on the basis of leaf characteristics but that leaf blade length was an important trait in ordination.

The results of cluster analysis by Ward's method using of total leaf characteristics showed that all populations fell into major clades A and B and except a few (8-9,10-11 and 17-18) populations that could not be separated. One point in this cluster was for the population at high altitudes in clade B (Fig. 6). Shiran et al. (2011) reported that the dendrogram obtained from AFLP markers showed a general pattern that was quite different from the pattern obtained by morphological analysis.

Table 9 shows the correlation between leaf morphological characteristics, geographical conditions and climatic factors. The results indicate that geographical conditions influence a number of leaf characteristics, but that temperature and precipitation effect more leaf characteristics.

Conclusion

Despite the fact that this investigation was carried out in part of the Zagros forest, analysis revealed the some morphological and molecular differentiations in some populations of Persian oaks in Iran. The pattern of diversity differs depending on the kind of marker and the analysis performed. Further molecular investigations are required in this case to test for historical evolutionary forces that shape species population structure.

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TABLE 7. The results of Duncan's mean comparison test for leaf morphological characters in different regions of *Quercus brantii.* The descriptive statistics are presented in terms of the Mean ± SD (Standard deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test. Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

Region	LBL	LBW	LPL	TLL
GOL	8.34±1.35bcd	4.66±1.10abc	1.54±0.30ab	9.88±1.44bc
EMA	8.26±1.68bcd	4.82±1.04bc	1.57±0.39ab	9.82±1.86bc
BAB	8.44±1N73bcd	5.21±1.42c	1.60±0.43ab	10.04±2.04c
MOG	7.61±1.70ab	4.82±1.32bc	1.38±0.49a	8.99±1.83ab
ALG	7.28±1.54a	4.05±0.83a	1.50±0.40ab	8.78±1.75a
DIS	9.14±1.67d	4.69±1.23abc	1.93±0.58c	11.06±2.09d
MON	8.22±1.33bc	4.73±1.10abc	1.60±0.53ab	9.83±1.61bc
KHA	8.53±1.42cd	4.36±0.93ab	1.65±0.38b	10.18±1.66cd
BAA	7.11±1.51a	4.57±1.48abc	1.55±0.45ab	8.67±1.81a
Region	**TNT**	**NVR**	**NVL**	**NLC**
GOL	21.90±6.34bc	11.03±2.62bcd	11.40±2.62bc	3.03±0.67abc
EMA	20.80±5.14abc	10.37±1.97ab	10.47±2.13ab	3.12±0.72bc
BAB	23.83±5.65c	11.97±1.90cd	11.87±1.98c	3.31±0.96c
MOG	21.10±7.38abc	10.53±2.73ab	10.60±2.63abc	3.02±0.69abc
ALG	18.77±6.67ab	9.50±2.01a	9.40±2.28a	2.64±0.45a
DIS	20.50±4.67abc	12.03±2.13d	11.53±1.85bc	3.09±0.80bc
MON	19.67±5.25ab	11.57±2.32bcd	11.07±2.13bc	3.04±0.78abc
KHA	21.90±5.75bc	10.43±2.61ab	10.47±2.39ab	2.74±0.66ab
BAA	18.03±4.86a	10.73±1.84abc	10.83±2.04bc	2.83±0.80ab
Region	**INA**	**RAD**	**RAB**	**LBS**
GOL	1.02±0.18ab	5.77±1.04d	5.83±0.99cd	1.84±.300bc
EMA	0.99±0.26a	5.40±1.07cd	6.07±0.98d	1.73±.182ab
BAB	0.89±0.30a	5.73±1.34d	6.03±1.45d	1.66±.255ab
MOG	1.06±0.88ab	5.37±0.10bcd	5.83±1.02cd	1.62±.293a
ALG	0.88±0.31a	5.37±1.16bcd	5.67±1.06bcd	1.84±.417bc
DIS	1.33±1.47b	4.77±1.14ab	5.63±1.10bcd	2.01±.370c
MON	0.86±0.27a	4.37±1.03a	5.20±1.19ab	1.78±.341ab
KHA	0.92±0.33a	5.07±0.83bc	4.93±0.91a	2.01±.379c
BAA	0.70±0.22a	4.83±1.02abc	5.23±0.94abc	1.61±.299a

TABLE 8. Principal component analysis carried out on morphological traits: Factor loadings and % of exploited variability.

Characters	Component			
	1	2	3	4
LBL	0.931	0.240	-0.015	0.014
LBW	0.678	0.104	0.697	0.003
LPL	0.692	-0.035	-0.005	-0.213
TLL	0.969	0.199	-0.015	-0.040
TNT	0.025	0.824	-0.090	0.059
NVR	0.136	0.913	0.017	-0.098
NVL	0.108	0.935	0.027	-0.022
NLC	0.689	0.033	0.588	0.021
INA	0.355	-0.288	0.037	-0.071
RAD	-0.077	-0.042	0.085	0.821
RAB	-0.083	0.022	-0.063	0.817
LBS	0.131	0.112	-0.957	-0.017

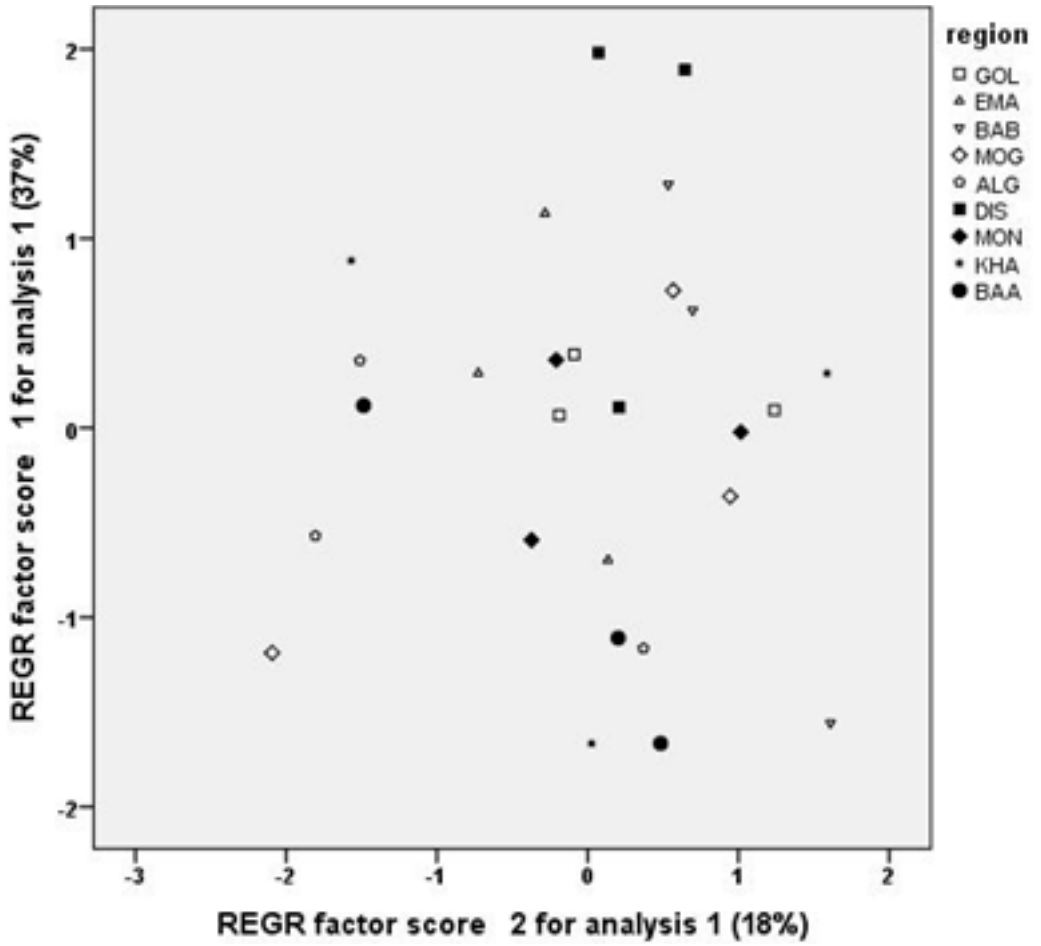

Fig. 5. The scatter plot of the populations on the basis of first two component of PCA. Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

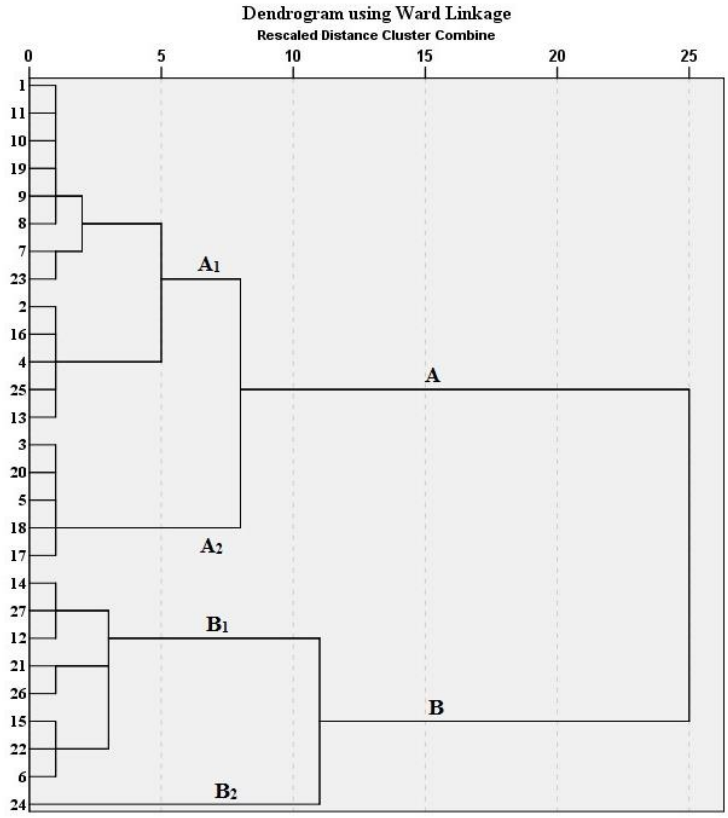

Fig. 6. Cluster analysis of *Quercus brantii* population using Ward's method based on morphological data..

TABLE 9. Correlation between leaf morphological characters, geographical conditions and climatic factors. Pearson's correlation coefficient is indicated with level of significance (P ≤ 0.05 and P ≤ 0.01). Minus sign (-) shows the negative and plus sign (+) shows the positive correlation between factors. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Characters	Altitude	Latitude	Longitude	Aspect	Temperature	Precipitation
LBL	0.063	0.089	-0.089	0.057	*-0.142	**0.308
LBW	0.024	*-0.121	*0.121	0.013	-0.097	0.104
LPL	*0.149	-0.020	0.020	0.051	-0.064	*0.132
TLL	0.090	0.072	-0.072	0.062	*-0.138	**0.297
TNT	*-0.122	0.092	-0.092	**-0.178	*-0.130	*0.154
NVR	0.040	-0.119	0.119	**-0.197	**-0.204	*0.126
NVL	0.019	-0.097	0.097	**-0.216	**-0.175	0.098
NLC	0.025	-0.087	0.087	0.037	-0.070	*0.151
INA	-0.039	0.056	-0.056	0.048	0.039	**0.200
RAD	*-0.146	*0.154	*-0.154	-0.078	0.103	0.035
RAB	-0.039	-0.001	0.001	-0.006	**0.157	*0.132
LBS	0.001	**0.250	**-0.250	0.027	-0.045	**0.171

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