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VARIATION ANALYSIS OF LEAF MORPHOLOGICAL TRAITS IN SOME OAK SPECIES (*Quercus* spp.), AZERBAIJAN REPUBLIC

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Abstract. Variation in leaf morphology is an important indicator of how plants respond to different environmental conditions. We analyzed the variation in the leaf morphological traits of oak species and its relationships with environmental variables across the distribution of this species to evaluate population differentiation using multidimensional statistical methods. Study species were *Q. castaneifolia* C.A. Mey*., Q. pedunculiflora* C.Koch*., Q. iberica* Stev*., Q. macranthera* Fisch*.&* C.A.Mey. ex Hohen. Plants were collected in diverse forest types between -28 and 2200 m in elevation in Azerbaijan. We measured and analyzed the differentiation in morphological of 910 leaves from 91 trees from 8 populations multivariate analyses. Interpopulation variability was confirmed by the results of the analysis of variance (ANOVA). A statistically significant ($P<0.01$) effect of population was obtained for the most of the studied morphological characters. The leaf area (A), the ratio of leaf length to width (R) and shape factor (F) had the greatest effect on population differentiation and they were significantly correlated with elevation.

1.   Introduction

The genus *Quercus* (oak) is one of the most diversified groups of temperate trees with more than 600 species distributed worldwide. This genus is native to the northern hemisphere, and includes deciduous and evergreen species extending from cold latitudes to tropical Asia and America (Aldrich & Cavender**,** 2011; Fortini *et al*., 2009). Oaks are considered as oneof the main forest tree species in Azerbaijan (Menitsky, 2005; Mammadov, 2016; Asgarov, 2016).

Oaks are woody, long-lived and wind-pollinated species. Therefore, they can spread across wide geographic regions and so they show high levels of variation. Since breeding barriers among oak species are extremely weak, oaks living in mixed populations show hybridization behavior either in the same or in different sections. A probable high frequency of genetic exchange between the involved taxa, hybridization and introgression, incomplete sorting of polymorphisms during relatively rapid and recent speciation processes are factors that cause the neutral genetic variation among oak species (Aldrich & Cavender, 2011) Oaks generally have high levels of within-population genetic variation and low differentiation among population (Kremer & Petit 1993; Borzan & Babaç, 2003; Uslu & Bakış, 2014). Individual variability in leaf morphology is induced by the interactions between the genetic structure and the effects

of the environment. Changes in the interaction between these two factors may result in evolution of morphological traits such as shape. At the same time, it makes the potential of a species to adapt to changing environmental conditions (Castro-Dıez *et al*., 2000; Bayramzadeh, 2012).

Oaks (*Quercus* spp.) are of global ecological and economic importance and have great symbolic value in many countries. They have provided a major source of timber and coppice wood for centuries and continue to provide a significant proportion of temperate hardwood production. Other useful products derived from oaks include fuelwood, charcoal, medicinal products, dyes, cork and bark used in tanning (Oldfield & Eastwood, 2007). Acorns have provided food in times of famine and continue to provide animal fodder. The genus *Quercus* is a keystone taxon from both ecological and economic perspectives, forming an important source of food and habitat for wildlife, and wood and paper products for humans. Many species of oak are grown ornamentally around the world (Aldrich & Cavender 2011; Maya-Garcia *et al.,* 2020)

The study of leaf morphology from the aspect of genetic differentiation provides useful information on population and intrapopulation variability and can be the basis for the determination of species and lower categories as well as intraspecific or interspecific hybrids. The similarity between individuals of the same or different populations or between distant and separate populations can point to their historical connections and common descent. Morphological determination is a good basis for further studies of this kind and it is often combined with chemotaxonomic, cytological and molecular analyses (Ardi *et al*., 2012; Aykut *et al.,* 2017; Lind-Riehl, 2014).

Oak forests make up about a quarter of the total forest area of our republic (Aliyev *et al*., 2008; Asgarov, 2016). Six oak species (*Q. castaneifolia, Q. pedunculiflora., Q. iberica., Q. macrhantera*, *Q. araxina*,*Q.pubecens*) are growing in Azerbaijan in natural flora according to the latest literature. In addition to these species, six new species (*Q. ilex, Q.erucifolia, Q.hypochrisa,Q.crispata, Q.robur, Q.infectoria)*were introduced. In general, there are 12 oak species in Azerbaijan (Gurbanov, 2004). The oak plant has been widely used in folk medicine as a medicinal plant since ancient times. Oak forests can be used as an extensive raw material base at the time when the development of the pharmaceutical industry in our country is planned. The using of oak acorns as fodder in a cattle breeding and poultry has a positive effect on the quality of milk, meat and eggs.

The conservation of genetic diversity on earth and the conservation of all biological species are some of the most important problems of our time. Each species is unique, not a single lost species can be restored in the absence of its last representative and the accompanying genetic information. It is necessary to protect the high genetic resources and biodiversity of oak forests, conduct research in this area and make appropriate requests and recommendations. The above reflect the relevance of studying oaks, the main biomass of the Azerbaijan flora.

It is a part of a larger study on the ecological, morphological, and molecular characterization of these five species in Azerbaijan. The main goals of the study were: 1) to collect comparative morphological and genetic data of some species of the *Quercus* genus in the country 2) study of genetic diversity based on polymorphism and biomorphological quantitative traits of leaves 3) to test the possibility of morphological differentiation within and among the oak speices (*Q. castaneifolia, Q. pedunculiflora., Q. iberica., Q. macrhantera*, *Q.ilex*) and to evaluate learned morphological characters and genotypes using multidimensional statistical methods 4) If the environmental conditions affect the morphologial traits?

2. Materials and Methods

2.1. Plant materials. 91 tree specimens (*Q. castaneifolia* C.A. Mey.*, Q. pedunculiflora* C.Koch.*, Q. iberica* Stev.*, Q. macrhantera* Fisch. & C.A.Mey. exHohen*, Q. ilex* L.) werechosen (Bruschi *et al.*, 2003) from 8 sites of oak trees around Azerbaijan in 2017. Chestnut-leaved oak (*Q. castaneifolia*) leaf samples were collected from Hirkan National Park (HNP), Lankaran, Lankaran plain (LP) and Mardakan arboretum (MA). Georgian oak (*Q.iberica* Stev.) leaf samples were collected from Ismaillan Garden, Baku. The study areas of pedunculate oak (*Q. pedunculiflora*) were Baku (Botanical garden), Absheron (Mardakan arboretum) and Ganja. Caucasian oak (*Q. macranthera*) leaf samples belong to Goygol National Park. Finally**,** holm oak (Q.*ilex*L.) leaf samples were taken from Baku (Botanical garden and Officers' Park) and Absheron (Mardakan arboretum). The geography and ecology of these areas are given in Table 1. We collected leaf samples based on Vincenzo and Andrea, 2011 and Jensen, 1990 protocols. The same sampling design and methods were applied for each population. 10 mature trees of small area (0.5–1 ha) of homogeneous open oak forest were selected. 8–10 m tall trees were chosen and four outermost branches (light subsample) and four innermost branches (shade subsample) of each tree crowns were randomly selected. To avoid seasonal and positional variations, samples were collected from different branches at approximately the same height and location, where leaf growth had stopped. Branches were collected from the four cardinal compass directions. The leaves' ages were practically the same, although there is a small variation in budburst among and within trees. In experimental design, only branch position considered (Jensen, 1990: Vincenzo & Andrea, 2011). The most important factor within-plant variation is inner vs outer position of branch regardless of compass direction or height.

Table 1. Geographic location and climate conditions of the sampled oak populations.
Pa annual precipitation (millimeters), *T* mean annual temperature (°C) (Mammadov *et al.,* 2010)

Locality	Geographic coordinates	Altitude (ma.s.l.)	Pa (mm)	T (°C)
Baku	40"23'N 49"51'E	-28	216	14.2
Absheron	40"33'N 49"30'E	8	180-300	14-15
Ismaıllı	40"35'N 47"45'E	500-800	500-1000	14-14.5
Gabala	41"25'N 47"23'E	900	800-850	10-12
Ganja	40"40'N 46"21'E	400-450	200-300	13.1
Goygol	40"37'N 46"34'E	1000-2200	500-900	13.5
Lankaran	39"24'N 48"58'E	-28-200	1280	14.1

2.2 Morphometric analysis. The morphological study of the oak leaf included 10 leaf samples per tree, on 91 trees in 7 populations, which makes a total of 910 leaves (10 trees per population) (Jensen, 1990; Bruschi *et al*., 2003; Vincenzo & Andrea, 2011). Generally, 6 morphometric parameters were analyzed. The morphological characters utilized in this study are as follows:

LA-(cm^2) - leaf area
LL (cm) - leaf length
LW (cm) - leaf width
LP (cm) - leaf perimeter
R-Ratio (R=LL/LW)
F-Leaf shape factor (LW/LP)

Morphological traits were measured by CI-202 LESER AREA METER (USA) on ten leaves stripped of the petiole for each subsample. For each character, mean values of each population were calculated.

2.3. Statistical analysis. Compilation and mathematical analysis of the variation sequence according to the morphological parameters of the leaves, centralize and preparation of data for further analysis were carried out using Excel software. Variability degree of parameters was determined by variation analysis (ANOVA) and Principle Component Analysis (PCA) based on RCBD (Randomized Complete Block Design).

3.   Results and Discussion

According to the results of the leaf morphological assessment, the variability of most of the studied characters was very high, both between the sampled leaves within individual trees and between trees in a population or among populations.

In this study, samples from 7 populations over Azerbaijan belonging to the *Quercus* genus were studied by Principal Component Analysis (PCA). The results of the Analysis of Variance (ANOVA) of genotypes for all studied characters are shown in Table 2. The ANOVA tests showed differences in all leaf morphological among the populations. The value of the variance coefficient of all morphological traits, which we get using RCBD method, represents the high reliability of the variance analysis. In turn, this result is the beginning of the performance of other statistical analysis of the studied parameters. Thus, if there is not statistical significance of any trait during ANOVA, it means that discontinue statistical analysis for this trait.

Leaf area. The result of the variation analysis of LA, showed a significant difference between genotypes (Table 2). The variation coefficient of 34.01 for LA indicates a large variety of this character among studied genotypes. ANOVA showed a statistically significant differentiation of 5% for LA. LCD=4.55% (leaf significant differentiation). Correlation analysis was used to test linear connection between biomorphological traits.As shown in Table 3 there was a high positive dependence with the LA (r = 0.33), LW (r =0.684) and F (r = 0.46). Among the studied oak populations, maximum, minimum and middle values of LA were determined for *Q. macranthera* (125.26 cm^2, Goygol), *Q. ilex* (Absheron) 23.97 cm^2 and *Q. iberica* (Gabala) 57.62 cm^2. During the study, *Q. macranthera* (Goygol) 10 (127. 26 cm^2)*, Q. pedunculiflora* (Ganja) 7 (95.69 cm^2), *Q. pedunculiflora* (Absheron) 1 (92.56 cm^2), *Q. iberica* (Ismayilli) 2 (94.02 cm^2), *Q. castaneifolia* (Absheron) 6 (93.62 cm^2), *Q. castaneifolia* (Lankaran) (92.24 cm^2) were estimated as the highest samples according to the LA character. The most common value

(median) of the current character for all populations was 56.19 cm^2.

Leaf length. ANOVA test revealed that high diversity of LL among all studied oak samples. The CV was 9.28, it indicates the validity of ANOVA (Table 2). The least genetic diversity among studied characters was for LL. The study found positive correlation between LL, LW (r = 0.2.70 and LP (r = 0.652), but there were negative significant dependence between LL and F (r = -0.43) (Table 3). The variation coefficient for LL was 10.78, and it had the lowest value compared to other parameters (Table 4). The maximum, minimum and medium values of LL were 23.84 cm, 4.52 cm, 19.71 cm, respectively, but the average value was 19.63 cm. When comparing populations, it was determined that *Q. pedunculiflora* (Ganja) 4 (23.84 cm), *Q. iberica* (Ismayilli) 1 (23.31 cm), *Q. castaneifolia* (Absheron) 5 (22.46 cm), *Q ilex* (Absheron) 6 (4.52 cm), *Q. acranthera* (Goygol) 7 (22.93 cm) - samples were the longest leaves. The study of morphological features such as leaf length and width helps to identify important patterns in the population (Jensen, 1990).

Leaf width. The results of variation analysis, CV=5.98 for LW, it confirmed the degree of statistically significant ANOVA for this character. The least significant difference (LSD5%) belong to LW, 1.28 (Table 2). However, the 21.06 value of the variation coefficient does not indicate the high phenotypic variation of leaf width in the oak samples. According to the results of linear connection, it is clear that there was 1% of positive correlation between LW and LA, LL and LP, but it was 5% between LW and F (Table 3).*Q. pedunculiflora* (Baku) 1 (16.75 cm), *Q. pedunculiflora* (Ganja) 10 (11.32 cm), *Q. iberica* (Gabala) 7 (10.72 cm) samples had the maximum value of LW. The lowest value of this character was recorded in *Q. ilex* (Baku) 1 (4.54 cm) and 4 (4.59 cm) samples. In addition,, the average values were obtained in the genotypes *Q. ilex* (Officers' Garden) 8 (5.55 cm) and *Q. castaneifolia* (Hirkan) 1(5.59 cm). The average and the medium values of LW were 7.81 cm and 7.54 cm for all populations.

Table 2. Results of variation analysis based on RCDP method for the studied characters

Leaf characters	Repeat	Genotype	Error	LSD %5	C.V %
df	1	90	90	-	**-**
Leaf area (cm^2)	4.98[*]	352.88[**]	1.63	4.55	**10.38**
Leaf length (cm)	0.06[n.s]	2.586[**]	0.805	2.16	**9.28**
Leaf width (cm)	0.049[n.s]	1.587[**]	0.758	1.28	**5.98**
Leaf perimeter (cm)	2.65[**]	3.25[**]	0.725	2.89	**4.96**
Ratio	0.182[*]	189.2[**]	1.28	4.89	**3.89**
Factor	0.497[**]	98.89[**]	2.09	1.89	**11.28**

**It shows significance at the 0.01 level (2-tailed).
* It shows significance at the 0.05 level (2-tailed).

Leaf perimeter. Variation analysis based on the RCBD method has proved the existence of a high variation of perimeter among all oak samples included in the experiment. The LSD was 2.89 for current character. Obtaining 4.96 in the ANOVA analysis for LP indicates statistical significance of the RCBD method. Correlation analysis showed a 1% positive statistical significant probability dependence among the perimeter, width and length of the leaf. However, a negative statistical significant correlation was found between perimeter and ratio. There is no correlation among any

studied quantitative characters, as well R and P. 5% statistical significant probability dependence between R and P (Table 3). The highest values of LP were in *Q. castaneifolia* (Lankaran) 4 (187.78 cm), *Q. macranthera* (Goygol) 10, 6, 9 (173.34 cm, 172.13 cm, 171.25) genotypes. Moreover, the lowest value of LP was in *Q. ilex* (Baku) 6, 2 and 7 samples (95.87 cm, 96.82 cm and 97.82 cm, respectively). The medium value was found to be 144.97 cm for this character. It should be noted that although the ANOVA explains the existence of differences between the oak genotypes studied on the perimeter trait, a value of 14.59 of the phenotypic variation coefficient indicates the mean diversity on the current trait.

Table 3. The correlation of the studied quantitative traits

Leaf characters	Area	Length	Width	Perimeter	Ratio	Factor
Leaf area (cm^2)	1					
Leaf length (cm)	0.333**	1				
Leaf width (cm)	0.684**	0.269**	1			
Perimeter (cm)	0.127	0.652**	0.297**	1		
Ratio	-0.089	-0.154	-0.187	-0.259*	1	
Factor	0.46**	-0.432**	0.242*	-0.66**	0.137	**1**

Table 4. Values of statistical parameters for the studied characters

	Area	Length	Width	Perimeter	Ratio	Factor
Min	23.96	13.68	4.54	95.87	1.576	0.01
Max	125.39	23.84	16.75	187.78	27.01	0.12
Average rate	57.62	19.63	7.81	140.69	3.19	0.042
Standard error	±2.05	±0.22	±0.17	±2.15	±0.37	±0.01
Variation	384.05	4.48	2.71	421.35	12.33	0.01
Standard discriminate	19.59	2.12	1.65	20.53	3.51	0.02
Median	56.19	19.71	7.54	144.97	2.68	0.04
CV	34.01	10.78	21.06	14.59	110.03	53.12

Ratio (R=LL/LW). A ratio may represent a comparison based on homologous attributes, if care has been taken to evaluate ratios based on measurements between landmarks. But, even if the two components of the ratio (e.g., length and width) are derived from identification of homologous pairs of landmarks, the ratio carries no information about the positional relationships among these landmarks, e.g., whether there has been an orthogonal displacement or rotation of one line with respect to the other (by definition, width is orthogonal to length; but width defined by reference to a pair of landmarks need not be orthogonal to the longer axis of the object). This is exactly the type of information needed for comparing two shapes (Jensen, 1990).

The result of the ANOVA based on the RCBD design explained existence of major difference of R among all oak samples. High genetic diversity among 6

quantitative characters was noted for this character. Linear correlation among quantitative characters did not show any correlation between the studied characters - R and P. However, there was negative statistical correlation between R and P (α= 5%) (Table 3). The highest genetic diversity among the studied 6 quantitative characters was noted for R. This result is totally confirmed by the value of variation coefficient (CV=110.03) (Table 4). The maximum, minimum and average values of R were 27.01, 1.58 and 3.19, and they were found in *Q. ilex* (Absheron) 4, *Q. macrhantera* (Goygol) 7, *Q.iberica* (Baku) 9 genotypes, respectively.

Leaf Shape Factor (LW/LP). Variation analysis based on RCBD experimental design suggests a high diversity of F among all studded oak genotypes. The RCBD design coefficient of variance for this character (CV=11.28) reflects the novelty of the current experiment. At the same time, LSD 5% for F was 1.89. During the study of interpersonal linear dependence, the presence of positive correlation between F and LA was found 1% and 5% between F and LW. However, there was 1% negative dependence probably between F and LL, F and P (Table 3). The average value for F in the 91 studied samples was 0.04 ($\pm$0,002), and the median value was 0.039. The maximum and minimum values were computed 0.12 and 0.01, respectively. The highest values of F among studied oak genotypes were registered in *Q. castaneifolia* (Hirkan) 7 and *Q. castaneifolia* (Hirkan) 10 according to 0.122 and 0.113, at the same time in *Q.castaneifolia* (Lankaran) 4 and *Q.pedunculiflora* 4 (Ganja) respectively 0.1 and 0.09 . Among the studied genotypes, the highest genetic diversity was found for R with F. Thus, the value of the coefficient of variation for this criterion was high (53.12).

In this study, the relative importance of two sets of driving variables (i.e., environmental and geographical) on the morphology of leaves was analyzed. It has been documented that the leaf morphological variability of species along elevational gradients is related to environmental factors identified a strong relationship between morphological features and environmental factors (mainly elevation). The high phenotypic diversity observed in the studied biomorphological traits (Table 4) is due to the direct association of the genotypes and the environment. It has a particular importance for species. Genetic information of the population provides adaptation of the species to changing environmental conditions for wide distribution, it also provides evolutionary potential (Ardi *et al.,* 2012). The reason of the high variety of morphological features observed in most forest trees are special reproductive system - typical pollination and intensive inter-population gene flow (Bruschi *et al.*, 2003). 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, and mechanism of pollination (anemophily), long age and dispersal of seeds by animals. These are responsible for the low levels of differentiation between populations, which improves the homogeneity of the allele frequency between adjacent local populations (Zhang *et al*., 2013; Alfonso-Corrado *et al.,* 2014; Porth & El-Kassaby, 2014; ValenciaCuevas *et al.,* 2014 and Wang *et al*., 2014).

The traits of an organism (its phenotype) can be very different from each other in the same genotype, but in different developmental conditions. The reveal of genes and genotypic effects in the body depends on environmental conditions (Guliyev & Aliyeva, 2002). This form of variability, which is not related to genotype is called modification. The limits of the modification variability under different conditions for different traits are called its reaction norm. The trait is not inheritance in itself, but a certain phenotypic

ability of an organism (its genotype) formed as a result of interaction with developmental conditions is inheritance (Rajora, 2019; Putenikhin, 2013). In other words, the organism's reaction norm to the external environment is inherited. When development condition is more monotonous, modification variability will be the less noticeable, and the variation row will be wider (Guliyev & Aliyeva, 2002).

Thus, high genetic diversity was recorded as a result of the study of genetic diversity of 91 oak samples based on quantitative indicators of biomorphological characteristics. They are formed by different environmental factors and have an adaptive character.

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