



## Morphometric Leaf Variation in Oaks (*Quercus* L.) of Azerbaijan

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### Abstract

The aim of this study was the assessment of interpopulation and intrapopulation of the oak leaf morphology and variability for new sources of genetic diversity and relationships between different population origins. Therefore, we aimed to demonstrate segregation among *Quercus* species in Azerbaijan and to present morphological variations within and among the species. Variations within and among populations of species were detected by cluster analysis (CA) and principal component analysis (PCA). Our results showed that the leaf characters presented a high differentiation of studied oak species in PCA at the population level. The relationships between *Q. robur* subsp. *pedunculiflora* (K. Koch) Menitsky, *Q. petraea* subsp. *iberica*, *Q. macranthera* subsp. *macranthera* showed complex groups in CA. We obtained discrimination among the *Quercus* species from Azerbaijan based on leaf characters, which is quite useful for those herbarium specimens without acorns and in other systematic observations.

**Keywords:** Oaks, leaf morphology, Cluster analysis, PCA.

## Azərbaycan Meşələrində (*Quercus* L.) Yarpağın Morfometrik Çeşitliliği

### Öz

Bu çalışmanın amacı, yeni genetik çeşitlilik kaynaklarını ve popülasyonların farklı kökenleri arasındaki ilişkileri belirlemek için, meşe yapraklarının morfolojisinin interpopülasyon ve intrapopülasyon çeşitliliğini değerlendirmektir. Bu nedenle, Azerbaycan'daki *Quercus* türleri arasındaki ayrımı göstermeyi ve türler içinde ve arasında morfolojik varyasyonları sunmayı amaçladık. Azerbaycan'ın farklı bölgelerinden 5 tür, 7 popülasyon ve 91 ağaçtan yaprak örnekleri toplanmıştır. Tür popülasyonları içindeki ve arasındaki varyasyonlar, cluster analysis (CA) ve principal component analysis (PCA) ile tespit edilmiştir. PCA'da sonuçlarımız, yaprak karakterlerinin popülasyon düzeyinde meşe türlerinde yüksek bir farklılaşma sergilediğini göstermiştir. *Q. robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Q. petraea* subsp. *iberica* ve *Q. macranthera* subsp. *macranthera* CA'da kompleks gruplar gösterdi. Azerbaycan'daki *Quercus* türleri arasında, meşe palamudu içermeyen herbaryum örnekleri ve diğer sistematik gözlemler için çok faydalı olan yaprakların özelliklerine dayanan bir ayrım elde ettik.

**Anahtar Kelimeler:** Meşe, yaprak morfolojisi, Cluster analizi, PCA.

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## 1. Introduction

*Quercus* (oak) has the highest diversity among temperate trees with more than 500 species distributed worldwide (Ardi et al., 2012). Oak is a woody, long-lived, and wind-pollinated species. This genus is native to the northern hemisphere and includes deciduous and evergreen species spreading from cold latitudes to tropical Asia and America (Ardi et al., 2012). Hybridization and hybrid zones are common among oaks. The major reason for the phenotypic diversity of oaks is the high frequency of hybridization among species (Borazan and Babach, 2003; Aykut et al., 2017, Jensen, 1990). Therefore, they spread through wide geographic regions and they show high levels of variation (Kremer and Petit, 1993). Since Darwinian time, botanists have used oaks as a model for studying evolutionary processes and speciation. Oaks have special characteristics such as high levels of phenotypic plasticity, interspecific gene flow, and genetic variation, which significantly contributed to the genesis of several hundreds of species, subspecies, and ecotypes (Neophytou et al., 2010). These characteristics influence the biological advantage of the genus *Quercus*. However, these attributes also pose difficulties in the classification of this genus, estimating genetic differentiation among species and genetic architecture of populations.

It is well known that the arrangement, size, shape, and anatomy of leaves differ greatly in plants growing in different environments (Bruschi et al., 2003). Polymorphism of individuals in leaf morphology is induced by the interaction between the genetic structure and environmental effects. At the same time, it improves species adaptation to various environmental conditions (Castro-Díez et al., 2000; Bayramzadeh et al., 2012). Long generations, inadequate seed production relative to demand, the impossibility of storing seeds for long periods, and hardly vegetative propagation, pose difficulties in forestry and *Quercus* improvement programs (Valladares et al., 2006). Protection and conservation of high-value forest genetic resources require information on the patterns of genetic variation among and within populations (Aldrich and Cavender, 2011, Boratynski et al., 2008).

The study of genetic differentiation of leaf morphology 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 (Batos et al., 2017).

*Quercus*, which is one of the most important woody genera of the Northern hemisphere, is considered as one of the main forest tree genus in Azerbaijan (Menitsky, 2005). The oak tree has a special symbolic, ecological, and economical value in Azerbaijan. Studies of Azerbaijani oaks have been carried out in a traditional way until now (Bandin and Prilipko 1964, Menitsky, 2005, Qurbanov 2004, Asgarov, 2010, Mammadov 2016.). But we used modern equipment, methodology, and analysis in our study. It is a part of a larger study on the ecological, morphological, and molecular characterization of these five species in Azerbaijan. Morphometric analyses are generally used to demonstrate discrimination among the operational taxonomic units (OTUs) (Uslu and Bakish, 2014). Therefore, the aim of the study is to study the morphological features of oak species in Azerbaijan and investigate differences interspecific and intraspecific.

## 2. Materials and Methods

### 2.1. Plant materials

A total of 91 tree specimens (*Q. castaneifolia* C.A.Mey., *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Quercus petraea* subsp. *iberica*, *Quercus macranthera* subsp. *macranthera* and *Q. ilex* L.) were collected from 7 sites around Azerbaijan (Fig. 1). Chestnut-leaved oak (*Q. castaneifolia* C.A.Mey.) leaf samples were collected from Hirkan National Park (HNP)-Lankaran-Astara, Lankaran plain (LP) and Mardakan arboretum (MA). Georgian oak (*Quercus petraea* subsp. *iberica*) leaf samples were collected from Ismaili and Baku. The study areas of pedunculate oak (*Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky) were Baku (Botanical garden), Absheron (Mardakan arboretum), and Ganja. Caucasian oak (*Quercus macranthera* subsp. *macranthera*) leaf samples belong to Goygol National Park. Finally, holm oak (*Q. ilex* L.) leaf samples were gathered from Baku (Botanical garden and Officers' Park) and Absheron (Mardakan arboretum). The same sampling design and methods were applied to each population. Ten mature trees of a 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 crown 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 (Jensen, 1990., Bruschi et al., 2003, Viscosi and Cardini, 2011).



**Figure 1.** Oak samples collected areas: Baku, Absheron, Lankaran, Astara, Goygol, Ganja, and Ismayilli.

## 2.2. Morphometric analysis

The morphological study of the oak leaf included 6 characters, 10 leaves per tree, on 91 trees in 14 populations, which makes a total of 910 leaves (10 trees per population) (Bruschi et al. 2003, Jensen, 1990; Sokal and Rohlf, 1995). The morphological characters utilized in this study are as follows: LA-(cm<sup>2</sup>) - 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

Two statistical tests namely KMO (Kaiser-Meyer-Olkin) and Bartlett were used for correctly performance of PCA. The most important data on population and individual variability were described by results of descriptive statistics. Species was treated, as a fixed variable; trees were considered as a random factor nested within species because trees were representative of each population (Aliyeva et al, 2020). We computed a dissimilarity matrix using Euclidean distance coefficients (Dunn and Everitt 1982; Abbot et al. 1985) for the Cluster Analysis. A dendrogram was, therefore, produced using the unweighted pair group arithmetic averages method (UPGMA).

## 3. Results and Discussion

All studied leaf morphological traits varied considerably among sampling sites (Figure 2.). For the aim of discrimination of taxa for each single character representation, minimum, and maximum average values have been tabulated (Table 2). It separated taxa into three distinct groups clearly. Three plots, at the population level, are presented in Table 3. Components in the PCA plot of populations revealed 86.97% of the total variations, approximately. PCA based on the populations' data gives a clear-cut discrimination of all five taxa from the remaining taxa. PRIN1 is significant because it explains 39.29% of the total variations (Table 3). R and LL were evaluated at maximum value in the current PRIN. LL and R have been among the best discriminative characters for *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Quercus petraea* subsp. *Iberica* and *Quercus macranthera* subsp. *macranthera*. The second indicator element (PRIN2) explained 32.49% of the total variation (Table 3). Significant traits in this PRIN were LA, LW, and F for: *Quercus robur* subsp. *pedunculiflora* (K.Koch)



Menitsky, *Quercus petraea* subsp. *Iberica*, and *Q. castaneifolia* C.A.Mey. Although many characters revealed that *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky and *Quercus petraea* subsp. *Iberica*, have been grouped together, some characters such as LW and R have discriminated *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Quercus petraea* subsp. *Iberica* and *Quercus macranthera* subsp. *macranthera* clearly. The third indicator element (PRIN3) contains 15.9% of the total variation. LL, LW, and P traits were the most important traits in these PRIN. These traits have been the best discriminative characters for *Q. castaneifolia* C.A.Mey.

Interestingly, *Q. ilex* L. did not produce a similar group with other studied species. Results of CA (Figure 3) and PCA (Tables 2,3) have also supported these complexes. This was most probably caused by the size depending property of leaf characters since most of the characters were based on measurements. Our results are consistent with other research (Stace, 1989; Menitsky, 2005; Aldrich, 2011).



**Figure 2.** The morphological differences of oak species in interspecific and intraspecific level in Azerbaijan: a) *Quercus petraea* subsp. *Iberica* b) *Quercus castaneifolia* C.A. Mey. c) *Quercus macranthera* subsp. *macranthera* d) *Quercus robur* subsp. *pedunculiflora* (K. Koch) e) *Quercus ilex* L.

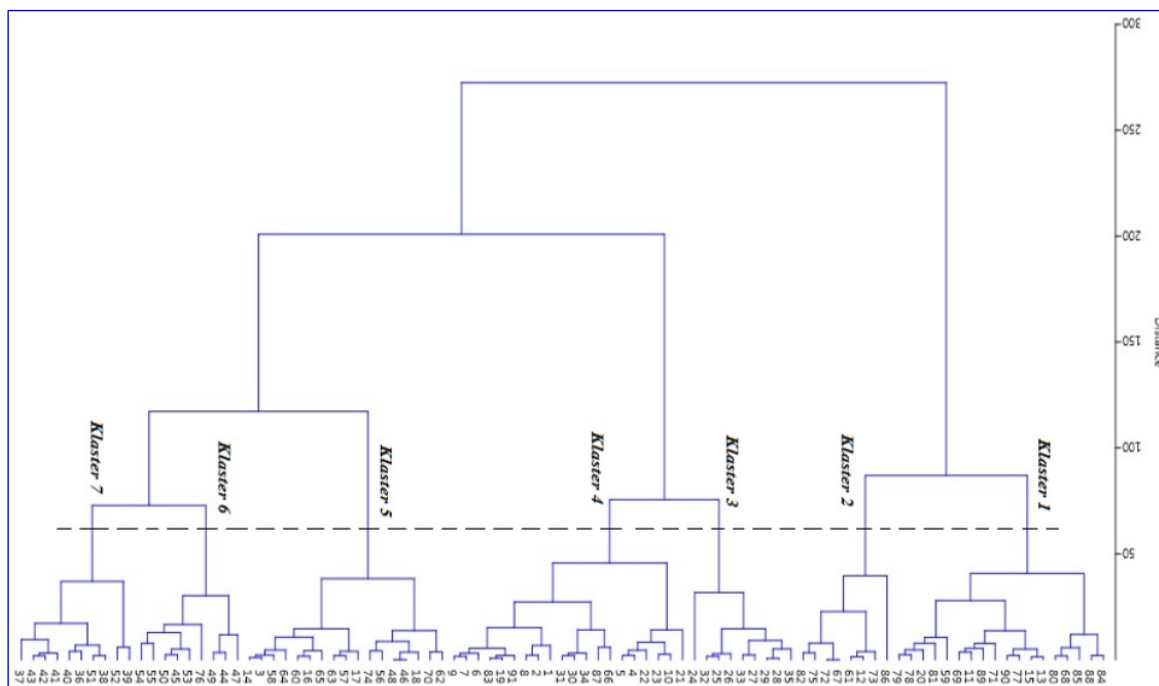
**Table 2.** Comparison of minimum-maximum averages of leaf characters for the studied taxa.

Characters\ Taxa	<i>Q. castaneifolia</i> C.A. Mey	<i>Q. robur</i> subsp. <i>pedunculiflora</i> (K.Koch) Menitsky	<i>Q. macranthera</i> subsp. <i>macranthera</i>	<i>Q. petraea</i> subsp. <i>Iberica</i>	<i>Q. ilex</i> L.
LA (cm <sup>2</sup> )	46.96-93.62	61.13-95.69	48.27-125.40	48.08-94.03	23.97-51.24
LL (cm)	13.68-21.16	14.62-23.84	18.97-23.84	9.00-27.61	3.07- 4.52
LW (cm)	5.59-9.03	7.98-10.72	7.95-16.75	5.53-10.08	1.83-4.59
LP (cm)	95.87-146.86	125.44-170.71	144.97-172.13	92.56-171.76	95.87-16.44
R	1.69-27.06	1.58-2.73	2.00-2.95	1.67-3.87	2.76-4.15
F	0.04-60.11	0.03-0.09	0.02-0.05	0.01-0.08	0.01-0.03

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

**Table 3.** Results of the analysis of components for each studied trait (Aliyeva et al., 2020).

Morphological characters	PCA1	PCA2	PCA3
Leaf area	0.13	0.60	0.27
Leaf length	0.29	-0.05	0.54
Leaf width	-0.01	0.50	0.35
Perimeter	0.09	-0.201	0.57
Ratio	0.94	-0.01	-0.27
Factor	-0.06	0.58	-0.33
Variation percentage	39.29	32.49	15.19
Total variation	39.29	71.78	86.97

**Figure 3.** Dendrogram of populations generated by Cluster Analysis using the UPGMA method.

1-10 *Q. ilex* L. (Absheron), 11-20 *Quercus petraea* subsp. *Iberica* (İsmayilli), 21-25 *Q. ilex* L. (Baku 1), 26-35 *Q. ilex* L. (Baku 2), 36-45 *Q. castaneifolia* C.A.Mey (Hirkan), 46-55 *Q. castaneifolia* C.A.Mey (Lankaran), 56-63 *Q. castaneifolia* C.A. Mey (Absheron), 64-66 *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky (Absheron), 67-76 *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky (Ganja), 77-86 *Quercus macranthera* subsp. *macranthera* (Goygol), 87-91 *Quercus robur* subsp. *pedunculiflora* (K.Koch) Menitsky (Baku)

On the other hand, to determine considerable differences in the six biomorphological-quantitative signs among studied oak specimens and to examine the morphological relationships among the populations sampled, Ward and UPGMA methods were used and a cluster analysis of Euclidean genetic distance was conducted. The hierarchical clustered based on morphological characters for *Quercus* populations and the distance index are shown in Figure 3 revealing that populations are separated into five different species. The studied oak phenotypes were grouped into seven groups in the dendrogram as a result of the cluster analysis of biomorphological signs of oak leaves. In the CA, three main groups were obtained by a phenon line at a 6.32 dissimilarity level (Figure 3). According to the diagram, it is clear that the first cluster was a group of 18 oak samples, the second cluster consists of 8, the third cluster 9, the fourth cluster 20, the fifth cluster 17, the sixth cluster 9 and the seventh cluster consists of 10 samples. The result of cluster analysis allows predicting the hybridization among samples by determining genetic distances between genotypes and achieving success in this area.

Cluster analysis was used to determine the genetic diversity and genetic distance based on biomorphological quantitative characters among the studied oak samples, and the results on researched oaks were grouped into dendrograms. A comparison of taxa ranges within the characters (Table 3) had shown that most of them were separated at least into three groups.

According to the dendrogram, the first cluster was a group of 18 oak samples. These samples were from *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky (Ganja, Baku), *Quercus petraea* subsp. *Iberica* (Gabala), and *Quercus macranthera* subsp. *macranthera* (Goygol). All of them belong to *Quercus* section, beside the sample number 59. The samples number 84, 88, 85, 68, 80, 13, 15, 77, 90, 71, 89, 11, 69, 59, 81, 20, 78 and 79 are in the current cluster. The current group incorporates 18.9% of genotypes. The second cluster classified only 8.8% - eight of the oak samples. They are from *Quercus macranthera* subsp. *macranthera* (Goygol), *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky (Ganja), and *Quercus petraea* subsp. *Iberica* (Gabala). This cluster classifies genotypes numbered 86, 73, 12, 61, 67, 72, 75 and 82. The grouping of 9 genotypes in the third cluster demonstrates that current oak samples are closer to each other in terms of genetic distance. They are genotypes number 35, 28, 29, 27, 33, 26, 25, 32 and 24. These samples account for 9.89% of the investigated oaks and all of them belong to *Ilex* section, *Q. ilex* L. from 2 populations. Twenty genotypes belong to the fourth cluster, it contains 21.98% of studied oaks. They are genotypes number 21, 10, 23, 22, 4, 5, 66, 87, 34, 30, 31, 1, 2, 8, 91, 19, 83, 6, 7 and 9. The nearest genetic distance within the current cluster is between genotypes number 10, 23, 4, 5, 30, 31, 2, 8, 19, 83, and 7 and 9. This cluster combine samples from *Ilex* and *Quercus* section, but most of the samples belong to the *Ilex* section. The fifth cluster consists of 17 genotypes and they organize 18.68% of studying samples. There are two subgroups in the fifth cluster. There are genotypes number 62, 70, 18, 46, 48, 56 and 74 in first subgroup, and genotypes number 17, 57, 63, 65, 16, 60, 64, 58, 3 and 14 in the second subgroup. Genotypes number 47, 44, 49, 76, 53, 45, 50, 55 and 54 are belong to the sixth cluster. Samples 44 with 49 and 45 with 50 are the closest genotypes in this cluster. Beside genotype number 76, all samples in this cluster belong to the *Cerris* section, *Q. castaneifolia* C.A. Mey species from different populations. Finally, the seventh cluster was a group of genotypes number 39, 52, 38, 51, 36, 40, 41, 42, 43 and 37. The two genotypes contained in this cluster, that is, genotypes 39 and 52, are in a subgroup and these two samples differ in their morphological distance in the current group. According to the study, we can conclude that *Quercus macranthera* subsp. *macranthera*, *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky, and *Quercus petraea* subsp. *Iberica* distributed in the flora of Azerbaijan, are closer to each other for morphological distance. Leaves are particularly significant as the availability of fruits depends on the seasons (Stace 1989; Jensen et al. 1990). The biennial maturation of fruits as characteristic to *Quercus* (Hedge and Yaltrık 1982; Borzan and Babach, 2003) also makes studies based on fruit morphology difficult. This indicated that the chosen characters were also informative solely. In this study, we have conducted the most comprehensive morphometric analysis of leaves belonging to the Azerbaijani oaks to date. On the other hand, high morphological diversity was recorded as a result of the study of morphological differences of five oak species. These results provide us with ample opportunities to use current materials for future breeding and other genetic programs as appropriate parental forms.

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