



## Variability of Leaf Characteristics in Seedlings of Pubescent Oak of Kırıkkale, Turkey

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study and managed the literature searches. Author HBO made the measurements of the field. Author CY performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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### ABSTRACT

In the present study, the variability of leaf characteristics in seedlings of six populations of pubescent oak was studied at Kırıkkale, Turkey. For this purpose, 3 years old pubescent oak (*Quercus pubescens* Willd) seedlings were used and about 30 leaves of completely developed and matured were taken from each population for collecting data. Data were analyzed by using univariate, Pearson's correlation coefficient and Hierarchical cluster analysis. A high variability was revealed by petiole length, total leaf length and width. There is no differentiation among populations in terms of number of intercalary veins and number of lobes. Groups of Duncan test conformed to those of hierarchical cluster analysis. For example, the northern populations were in another group while the southern populations replaced in one homogeneity group. Future studies on all Turkish populations of Pubescent oak will help to find centers of genetic diversity and populations with specific adaptations to drought and frost.

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## 1. INTRODUCTION

As the primary photosynthetic organs, leaves have an important role in the survival and growth of a plant and also provide evidence for plant taxonomy [1-2]. Considering leaves as the main photosynthetic organs of trees, their structure is important from the aspect of biomass production [3]. For example, while studying poplar clones, Orlović et al. [4] found the positive correlation between leaf anatomy and organic matter production. Thus, parameters like this should be taken into consideration in the selection and breeding of tree species [3].

Leaves are organs sensitive to environmental changes in the process of evolution and may exhibit phenotypic plasticity as a response to abiotic stress. Significant variations occurred in many parameters due to the effects of the environment and/or allometry. There were broadly consistent trends for leaf morphological variations along the gradients. In instance, the leaf size became smaller with a short supply of resources [5]. Some leaf traits, such as the leaf area index, also can reflect the status of the whole plant [6].

Recent studies in plant traits have found that some relationships between specific leaf traits are globally repeated despite large variations in the values of the traits across individual species with very diverse phylogenetic, biogeographical and environmental affinities [1,7]. Leaf physiognomy can be regarded as an excellent tool for ecological studies [8]. There are so many types of leaves in nature, from blades to needles. Differences in leaf size can significantly alter whole-lamina- and whole-leaf-integrated chemical and structural characteristics [9]. Theoretical studies suggest that, for a common biomass investment in major veins, palmate- and parallel-veined leaves are hydraulically and mechanically more efficient than pinnate-veined leaves due to a more uniform distribution of major veins in parallel/palmate-veined leaves [10]. So, the minor veins play a more important part in leaf support and will be more sensitive to environmental variations. [11].

Trees adjust the functional balance among organs and tissues responsible for water acquisition (fine roots), transport (sapwood) and transpiration (leaves) [12]. Pubescent oak maintains high transpiration rates despite the

incidence of drought [13], partly due to the ability to extract water from deep soil layers and groundwater [14]. Pubescent oak has higher predawn leaf water potentials, which is indicative of a better access to soil moisture [15-16]. Consequently, Pubescent oak seems to be capable of withstanding and surviving extreme drought events [17-18]. In addition, oak seedlings, on the other hand, are usually subjected to the severities of summer drought before they develop large root systems [19].

Pubescent oak (*Quercus pubescens* Willd.) is a middle-sized (15-20 m, rarely 25 m tall) deciduous or semi deciduous tree. Its alternate leaves are mostly ovate-oblongate, (3)5-10 cm long, bear a short petiole (5 to 20 mm), with 5-6 more or less deep lobes. Its leaves are densely pubescent [20-22]. It has a long petiole, but its leaf blade is elliptic-obovate, the basal region is cordate and the apex is obtuse [23]. The species has a wide distribution range, occupying almost all of central and southern Europe from western Spain to Ukraine and Anatolia with some isolated populations in the Caucasian area. It grows widely in the Central Anatolian region of Turkey and is available up to 1700 m. Its wood is mainly used as firewood. Pubescent oaks are among the most frequent hosts of all the economically important truffles [24-26]. It is a xerophilous species and typically grows on dry, lime-rich soils in the sub-Mediterranean region, which is characterized by hot dry summers and mild dry winters [17,27].

So far, leaf variability of adult pubescent oaks has been investigated using traditional morphological analyses by many authors [23,28-32]. However, there is not a scientific study on morphological characteristics of leaves of its seedlings. In the present study, the variability of leaf characteristics of seedlings of six pubescent oak populations at Kırıkkale, Turkey was investigated.

## 2. MATERIALS AND METHODS

Three years old pubescent oak seedlings of six different populations in Kırıkkale-Turkey (Fig. 1; Table 1) were used as material. Containerized seedlings were in temporary nursery within the boundaries of Kırıkkale (700 m asl.). The soil of containers included approximately 50% coarse clay and 50% forest soil. Average annual precipitation of this area is about 383.4 mm with

an average temperature of 12.6°C based on 90 years (1926-2016) climate records [33].

Seedlings were irrigated based on the operational regime for the nursery, but not fertilized. First weeding was done when the seedlings were seventy days old. The measurements were started when leaves had been completely developed and matured in September of 2016. For measurements 30 leaves were used for each population. The following characteristics per population were evaluated: Petiole length (cm), total leaf length and leaf width (cm), number of intercalary veins and number of lobes.

The mean values for each of the six populations based on the 13 characters were calculated. Univariate was used to compare means of populations. The means were separated by using the adjusted Duncan mean comparison test. Correlation between pairs of the measured

characteristics for each component was evaluated by using Pearson's correlation coefficient.

For multivariate analyses, variables measured at different scales were standardized by means of Z scores of SPSS to equally contribute to the analysis. Hierarchical cluster analyses, completely based on numerical analysis, are aiming to group objects based on their similarity through different steps to determine consecutive clusters and the distance values (or similarities) of the units to be included in these clusters. Nearest neighbor was used as cluster method. Morphological differences among populations were visualized with both an unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on hierarchical cluster analyses based on Euclidean distances. All statistical analysis was performed by using SPSS program [34].



**Fig. 1. Location of the studied Pubescent oak populations**

**Table 1. Concise information for the studied populations**

Population	Latitude (°)	Longitude (°)	Altitude (m)	Exposure
Yağbasan	40°05'55"	33°37'40"	1092	E
Talipoğlu	40°07'59"	33°37'45"	1065	E
Kösedurak	40°04'41"	33°42'49"	1155	NE
Selamlı	40°05'34"	33°45'43"	1145	NW
Sulakyurt	40°10'14"	33°42'06"	950	N
Sarıklı	40°10'10"	33°46'49"	864	NE

### 3. RESULTS AND DISCUSSION

Mean values of leaves of 3 years old seedlings, F ratios and significance levels are given in Table 2. The analysis of variance revealed significant differences between populations at the 0.001 probability level for all morphological characters with the exception of number of intercalary veins and number of lobes. Population Yağbasan and Talipoğlu showed the lowest performance for the majority of the characters. The mean values of petiole length, total leaf length, leaf width, number of intercalary veins and number of lobes for pubescent oak seedlings were 1.02 cm, 7.79 cm, 4.47 cm, 12.27 and 17.03, respectively. The mean values of the other characters apart from number of lobes showed similarity with those reported for adult pubescent oaks by Bruschi et al. [29]. The mean number of lobes in the present study was found higher than the mean values indicated by Dupouey and Badeau, [28], Bruschi et al. [29] and Franjic et al. [31]. The average petiole length of the present study was within the ranges reported by Pasta et al. [25] and Franjic et al. [31]. The mean number of intercalary veins found by Dupouey and Badeau [28] was less than that of the current study.

Pearson coefficient correlation between pairs of the characteristics were evaluated (Table 3). There were found only positive relations ( $p \leq 0.05$ )

between the studied characteristics. Accordingly, petiole length has a positive correlation with total leaf length and width at 0.01 level, and number of intercalary veins at 0.05 level. Total leaf length correlated with leaf width at 0.01 level and number of intercalary veins at 0.05 level. On the other hands, number of lobes has a correlation with only number of intercalary veins at 0.01 level. Gailing [35] and Enescu et al. [36] found positive correlations between the pairs of morphological characters of *Quercus robur* L. leaves.

In order to visualize the degree of similarity among populations, a cluster analysis (UPGMA) based on a hierarchical cluster analysis on the basis of all measured characters was conducted (Fig. 2). According to the hierarchical cluster analysis, three main groups could be distinguished at the 20.0 distance unit. The first group included Sulakyurt, Sarıkızlı, Kösedurak and Selamlı populations, the second group consisted of Talipoğlu population, and Yağbasan population individually formed a minor group. A geographic pattern was observed with congruent results obtained by UPGMA cluster analysis. Furthermore, groups of Duncan test conformed with those of hierarchical cluster analysis. For example, the northern populations were in another group while the southern populations replaced in one homogeny group.

**Table 2. Mean, F ratio, significance levels and Duncan test results of characteristics**

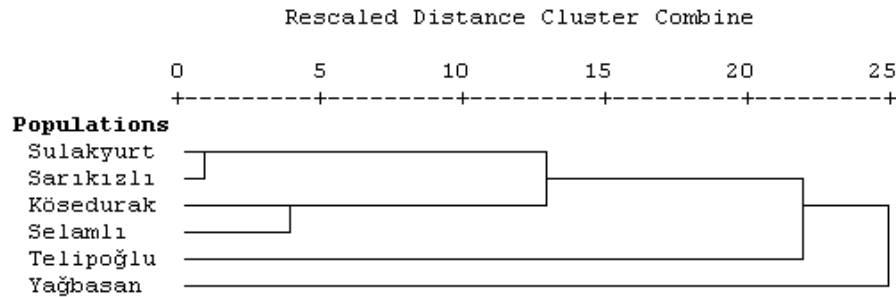
Population	Petiole length (cm)	Total leaf length (cm)	Leaf width (cm)	Number of intercalary veins	Number of lobes
Yağbasan	0.84a*	7.05a	3.80a	12.33	17.43
Talipoğlu	0.85a	7.02a	4.03ab	11.73	16.77
Kösedurak	1.02b	7.59b	4.30ab	12.50	16.90
Selamlı	1.03b	7.76b	4.49b	12.23	16.97
Sulakyurt	1.21c	8.73c	5.13c	12.50	17.13
Sarıklı	1.17c	8.59c	5.05c	12.43	17.00
Mean	1.02	7.79	4.47	12.27	17.03
F ratio	54.90	18.01	9.44	1.09	0.22
P	0.000	0.000	0.000	0.365	0.955

\* The difference between means showed same letter was not significance ( $p \leq 0.05$ )

**Table 3. Pearson coefficient of correlation between pairs of characteristics**

	Petiole length	Total leaf length	Leaf width	Number of intercalary veins	Number of lobes
Petiole length	1.000				
Total leaf length	0.506**	1.000			
Leaf width	0.389**	0.649**	1.000		
Number of intercalary veins	0.157*	0.186*	0.102 <sup>ns</sup>	1.000	
Number of lobes	0.057 <sup>ns</sup>	0.109 <sup>ns</sup>	0.104 <sup>ns</sup>	0.486**	1.000

\* Correlation significant at 0.05 level (2-tailed); \*\* Correlation significant at 0.01 level (2-tailed); NS: Non-significant



**Fig. 2. Cluster diagram (UPMGA) based on Euclidean distances among populations**

#### 4. CONCLUSION

Although the leaves were sampled from six populations from Kırıkkale, scattered on a restrained geographical area, a high variability was revealed by the studied three characters (petiole length, total leaf length and width). There is no differentiation among populations in terms of number of intercalary veins and number of lobes. Future studies on all Turkish populations of Pubescent oak will help to find centers of genetic diversity and populations with specific adaptations to drought and frost.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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