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Variation in leaf morphology of *Quercus pontica* natural populations in Turkey

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Abstract

Quercus pontica K. Koch is a relict plant species which is classified as vulnerable (VU) in Turkey. Within the scope of this study it needed to be investigated are there variations in leaf morphological characteristics within and among *Q. pontica* populations. Variation in leaf traits in relation to seven different natural provinces was identified. In total, 99 individuals were selected, and 30 leaves were collected from each shrub to determine leaf variations in *Q. pontica* populations. Mean values for petiole length (1.07 cm), leaf length (17.13 cm), lamina length (16.13 cm), leaf width (8.10 cm), leaf area (93.76 cm^2), leaf vein angle (54.22 degree), total number of primary veins (38.72), total number of teeth (40.73), petiole ratio (6.34%), relative length of lamina at largest width (50.50%), and percentage of venation (98.65%) were determined in all populations. The results of the analysis of variance showed significant differences ($p < 0.01$) among individuals within populations for all leaf characteristics. Variations among populations were detected by cluster analysis and principal components analysis. According to the analysis results, the 11 populations were grouped in three categories. Using correlation analysis, statistically significant relationships between leaf traits were determined. In this study, we detected no significant relationship between environmental factors (altitude, aspect, annual temperature and annual precipitation) and leaf traits.

Keywords: leaf morphology, *Quercus pontica*, relict, variation

Introduction

Quercus L. is the largest genus of the *Fagaceae* family and includes tree species and shrubs growing in many temperate forests in the Northern Hemisphere (Krüssmann 1986). In Turkey, oak forests are the most widely distributed forest types, covering approximately 5.9 million hectares (26.34%), including high forest and coppice (Anonymous 2015). Turkey has the richest oak forests of Central Europe in terms of species variety (Hedge and Yaltırık 1982).

Quercus L. shows a high diversity in leaf shape and size (Krüssmann 1986), and its leaf characteristics are important in the classification and valuable of delimiting. Most oak species can easily be identified by their leaf morphology, which may also be a good indicator of putative hybridization (Borazan and Babaç 2003). In various oak species, differentiation in foliar characteristics occurs among populations, among trees within populations, and among branches within a tree (Blue and Jensen 1988, Bruschi et al. 2000). Leaves, which are the main photosynthetic organs, play an important role in plant growth and survival (Xu et al. 2008). The differences in the morphological characteristics of leaves are probably related to the anatomical characteristics of the vessel elements because of the strong correlation between physiological and morphological leaf characteristics (Eguchi et al. 2004, Bayramzadeh et al. 2008, Güney et.al 2016, Bayraktar et al. 2018). The arrangement, size, shape, and anatomy of leaves differed greatly among plants growing in different environments (Bruschi et al. 2003, Kusi and Karsai 2020, Yücedağ et al. 2021).

Quercus pontica K. Koch is an Euxine element and relict species; it varies little in its characteristics and is quite distinct from other Turkish oaks. Its leaves are large, regularly serrate, and have numerous prominent parallel veins (Hedge and Yaltırık 1982, Yaltırık 1984). It is a deciduous tall shrub, 3–5 m high, of white Oak. *Q. pontica* is not important economically but is considered as the most primitive species of oaks found, making it important from phylogenetic and systematic viewpoints. This oak species exists as individual tree or in small groups, in particular in mixed forests of beech, oriental spruce, and rhododendron in Artvin, Rize, and Trabzon, Turkey. It is used as an ornamental tree in parks and gardens, particularly in botanical gardens (Anşin and Özkan 2006, Terzioğlu et al. 2012, Öztürk 2013). However, it is also naturally distributed in south-western Georgia and included in the “Red List” of Georgia (Patarkalashvili 2017). Although there are numerous studies about leaf morphological variations and genetic characteristics of *Quercus* (Jurkšienė and Baliuckas 2014, Ebrahimi et al. 2017, Gailing et al. 2018, Kusi and Karsai 2020, Yücedağ et al. 2021), leaf variation in *Q. pontica* has not been investigated. Although studies were carried out on the traits of seed (Aksu and Tilki 2015), wood (Serdar and Mazlum 2014, Özgenç 2020), vegetation (Ermakov et al. 2020) and silviculture (Yıldırım et al. 2018) of this species, no studies on leaf morphology have been found.

In this context, the aim of the present study is to investigate variations in leaf morphological characteristics within and among *Q. pontica* populations in natural distribution areas in Turkey. Also this species according to is in the vulnerable (VU) category (Ekim et al. 2000). Thus, to help sustain biological diversity and support future studies, more studies should be devoted to further reveal the general characteristics of this species.

Material and methods

Plant material

The populations sampled are distributed in the provinces of Trabzon, Rize, and Artvin in the Eastern Black Sea area of Turkey (Figure 1).

All leaf materials were collected from 11 populations in total. The geographical coordinates, altitude, aspect, number of individuals for each region, annual temperature, and annual precipitation are shown in Table 1. Climatic data for all populations were obtained in the Trabzon Agency of Meteorology (TSMS 2019).

Leaf samples from seven provinces were collected from a total of 99 specimens in July 2019. All leaf samples were obtained hand-picked when leaf growth had stopped. The distance among separate investigated trees was approximately 50 m to minimize positional variability within the tree (Blue and Jensen 1988, Franjić et al. 2006, Toader et al. 2009). Adult specimens were about 16–45 years old and 3–12 cm in diameter at breast height (DBH), naturally distributed. Leaves were collected in the middle of the upper crown with four different aspects of the shrub (Kremer et al. 2002). These leaves were first pressed, dried, and scanned. Leaf images were scanned by using a ruler. Leaf traits were measured using ImageJ analysis software package (Schneider et al. 2012) that was calibrated by the ruler for accuracy (Mhamdi et al. 2013, Güney et al. 2016, Batos et al. 2017). This software package has been used in various research studies examining leaf variations (Bayramzadeh et al. 2008). Measurements were made on a total of 2,970 leaves, with 30 leaves from each specimen.

Figure 1. Distribution of the investigated *Q. pontica* populations used for leaf material collection in Turkey

Assessment of leaf morphological characteristics

Thirty leaves from each of the 99 specimens were measured in each population, determining 11 leaf traits as described in Ebrahimi et al. (2017), Ponton et al. (2004), and Kremer et al. (2002). The following characteristics were measured: 1) lamina length, 2) petiole length, 3) total leaf length, 4) lamina width, 5) leaf vein angle, 6) leaf area, 7) total number of teeth, 8) total number of primary veins, 9) petiole ratio, 10) relative length of lamina at largest width, and 11) percentage of venation (Table 2 and Figure 2).

Table 1. *Q. pontica* populations with province, coordinates, altitude, aspect groups, and climatic details

Population No	Province	Label	Total number of specimens	Coordinates x	Coordinates y	Altitude	Aspect Groups	Average Annual Temperature (°C)	Annual Precipitation (mm)
1	Trabzon-Hayrat	TH1*	11	618263	4515205	1711	N	6.9	2089.1
2		TH2	5	618729	4514342	1873	SE	6.1	2176.6
3	Rize-Cimil	RC	10	645177	4511547	1795	NE	6.7	1008.0
4	Rize-Çamlıhemşin	RÇ1	10	668178	4536984	1815	SE	10.8	1118.1
5		RÇ2	10	667796	4537811	1626	NE	12.2	967.4
6	Artvin-Arhavi	AA1	10	694754	4569606	1490	SW	7.3	2612.0
7		AA2	10	695910	4565345	1686	NE	6.4	2717.9
8	Artvin-Karagöl	AK1	5	737279	4586873	1653	SW	3.6	808.6
9		AK2	8	736958	4586915	1475	NE	4.5	712.5
10	Artvin-Camili	AC	5	737357	4591124	1627	NE	3.8	794.5
11	Artvin-Murgul	AM	15	710806	4570806	1482	NE	4.5	716.2

* 1 and 2 defines different altitudes of populations.

Table 2. List of measured or calculated descriptors. Abbreviation numbers are defined based on Figure 2 A, B in parentheses

Abbreviations	Descriptors
LL:	Lamina length (cm) (1–2)
PL:	Petiole length (cm) (2–3)
TLL:	Total leaf length (LL+PL, cm) (1–3)
WL:	Width of lamina (cm) (4–5)
VA:	Leaf vein angle (°) (1–6–7)
LA:	Leaf area (cm^2)
NT:	Total number of teeth
NV:	Total number of primary veins
PR:	Petiole ratio, (PL/TLL*100) (%)
LWRL:	Relative length of lamina at largest width, (WL/LL*100) (%)
PV:	Percentage of venation, (NV/NT*100) (%)

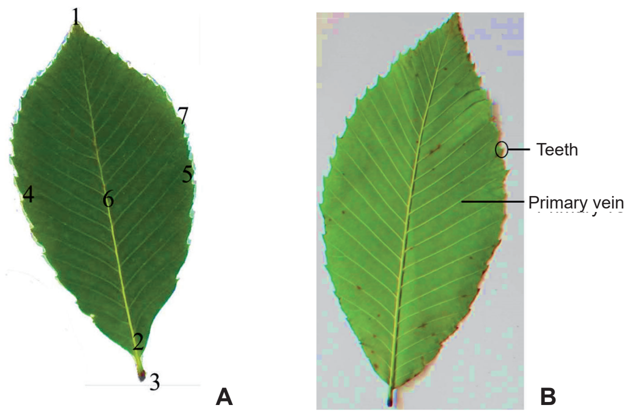

Figure 2. Analyzed morphological leaf traits of *Q. pontica*: A) upper surface of leaf, B) lower surface of leaf

Statistical analysis

All analyses of leaf morphological characteristic were performed using analysis of variance (ANOVA) in SPSS 20.0 software package (IBM 2020). To test the normality of the data, normality test was used, and there was no need to conversion because of the data showed normal distribution. Variance analysis (one-way ANOVA) was used to determine variations among and within population. Means and standard deviations were calculated separately for populations. Duncan's test was performed to determine the groups that were found in terms of populations for all leaf traits. Cluster analysis using the Euclidian 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. Principal components analysis (PCA) was carried out using the PC-ORD™ 6.0 software package (McCune and Mefford 2016) to determine variations in leaf samples.

Results

Petiole length (PL), total leaf length (TLL), lamina length (LL), lamina width (WL), leaf area (LA), leaf vein angle (VA), total number of primary veins (NV), total number of teeth (NT), petiole ratio (PR), relative length of lamina at largest width (LWRL), and percentage of venation (PV) were measured using leaves from natural populations. The average values and standard deviations are presented, along with the results of analysis of variance and Duncan´s test (Table 3).

Table 3. Results of analysis of variance and Duncan's test for all *Q. pontica* leaf traits

Province	Label	PL* (cm)	TLL (cm)	LL (cm)	WL (cm)	LA (cm^2)	VA (Degree)	NV	NT	PR (%)	LWRL (%)	PV (%)
Trabzon-Hayrat	TH1	1.03 ±0.26 bc***	16.65±3.09 cd	15.63±2.98 bcd	7.65±1.62 ab	84.91±31.71 bcd	52.69±6.68 c	39.61±5.12 d	41.50±7.31 c	6.28±1.50 bc	49.16±6.47 b	97.18±14.40 c
	TH2	0.91±0.34 a	16.23±2.75 bc	15.32±2.51 bcd	8.08±1.68 b	88.04±33.09 cde	48.08±6.68 a	36.69±4.60 b	49.19±9.29 f	5.53±1.51 a	52.60±6.16 ef	76.56±13.74 a
Rize-Cimil	RC	1.07±0.27 c	16.81±2.89 cd	15.75±2.79 cd	7.33±1.47 a	82.52±30.26 abc	55.10±8.07 de	35.65±5.30 b	31.77±8.67 a	6.39±1.57 bcd	46.65±5.93 a	121.46±44.39 f
Rize-Çamlıhemşin	RÇ1	1.17±0.33 d	18.80±3.21 f	17.63±3.01 e	8.73±1.73 c	111.22±38.62 f	56.76±7.16 f	42.54±6.29 f	42.43±8.03 cd	6.20±1.33 b	49.44±4.50 bc	102.34±18.53 de
	RÇ2	1.19±0.25 d	18.06±3.03 e	17.26±7.14 e	7.97±1.54 b	93.74±32.52 e	51.01±6.78 b	33.25±4.76 a	32.76±6.14 a	6.62±1.22 def	47.14±5.45 a	103.56±19.06 e
Artvin-Arhavi	AA1	1.09±0.35 c	16.15±3.41 bc	15.06±3.18 abc	7.88±1.92 b	86.12±37.85 bcde	54.31±6.86 d	38.09±5.64 c	38.81±6.73 b	6.73±1.59 ef	52.49±7.59 e	98.71±5.48 cd
	AA2	0.94±0.24 a	16.96±2.96 d	16.02±2.88 d	8.07±1.50 b	91.76±31.51 de	56.08±7.03 ef	36.60±4.73 b	43.73±13.11 d	5.65±1.49 a	50.61±5.14 cd	88.13±17.43 b
Artvin-Karagöl	AK1	1.31±0.51 d	19.86±3.75 g	18.55±3.46 f	9.65±2.05 d	132.99±56.21 h	54.99±6.10 de	42.45±6.08 f	43.21±7.73 cd	6.55±2.06 cde	52.19±7.07 e	99.29±9.74 cd
	AK2	1.09±0.29 c	19.25±5.21 fg	18.41±6.43 f	9.34±2.52 d	125.66±70.12 g	56.73±7.13 f	41.50±6.35 ef	47.17±11.97 e	5.80±1.49 a	51.61±6.37 de	90.44±12.12 b
Artvin-Camili	AC	0.99±0.31 b	15.84±3.90 ab	14.84±3.71 ab	7.37±1.85 a	76.88±36.94 a	50.75±7.59 b	39.94±7.54 d	41.67±9.39 c	6.36±1.51 bcd	50.08±6.35 bc	97.42±11.64 c
Artvin-Murgul	AM	1.05±0.30 bc	15.45±3,50 a	14.45±3.40 a	7.84±3.84 b	80.00±35.46 ab	55.47±7.01 def	40.56±5.47 de	42.18±8.01 cd	6.91±1.81 f	53.85±9.88 f	97.71±12.80 c
Avg.		1.07	17.13	16.13	8.10	93.76	54.22	38.72	40.73	6.34	50.50	98.65
Anova	F	27.410	45.059	32.169	24.927	49.555	33.835	77.861	89.613	22.497	35.533	75.643
	P	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

* Abbreviations of the traits can be seen in Table 2. ** $p < 0.01$ (there is a statistical difference). *** Duncan's groups for each trait.

As seen in Table 3, there were statistically significant differences ($p < 0.01$) among the populations for all leaf traits measured. The highest and lowest leaf and lamina lengths were observed in AK1 (Artvin-Karagöl) and AM (Artvin-Murgul), respectively. The population of TH2 (Trabzon-Hayrat) had the lowest values in terms of petiole length, leaf vein angle, petiole ratio, and percentage of venation, while the population of RC (Rize-Cimil) had the lowest values in terms of lamina width, number of teeth, and relative length of lamina at largest width, and the population of AC (Artvin-Camili) showed the lowest leaf area value. The lowest number of primary veins was found for the RC2 (Rize-Cimil) population.

Regarding petiole length, lamina width, and leaf area, the highest values were found for the populations of AK1. The population of RÇ1 (Rize-Çamlıhemşin) had the highest values in terms of leaf vein angle and number of primary veins, while the population of TH2 had the highest values in terms of number of teeth; petiole ratio and relative length of lamina at the largest width were the highest for the populations of AM. The population of RC had the highest value in terms of percentage of venation.

Groups formed by the populations for each leaf traits were determined by Duncan's test. Based on the results of the Duncan test, there were five groups in terms of PL and WL, seven groups in terms of NV, NT and PV, eight groups in terms of LL, VA, PR, and LWRL, 10 groups in terms of TLL, and 11 groups in terms of LA. Regarding the variance analysis for all populations, there was a 99% meaningful result in terms of petiole length, leaf length, lamina length, width of lamina, leaf area, leaf vein angle, total number of primary veins, total number of teeth, petiole ration, relative length of lamina at the largest width, and percentage of venation. The results of Pearson's correlation analysis for the measured 11 leaf traits s are presented in Table 4.

According to the results of the correlation analysis, there were statistically significant positive and negative correlations for most of the characteristics. There was positive and significant ($p < 0.01$) correlation between TLL and LL ($r = 0.991$), WL (0.889) and LA (0.911). There were positive and significant ($p < 0.01$) correlations between LL and WL ($r = 0.853$) and LA ($r = 0.889$), and also between WL and LA ($r = 0.959$) and NV ($r = 0.637$). In addition, there were negative and significant ($p < 0.05$) correlations between NT and PR, LWRL and PV, and also between NT and PV.

Hierarchical clustering analysis was performed to determine how the populations can be graphically grouped according to the measured leaf parameters (Figure 3). Subsequently, discriminant analysis was applied to determine the significance of these groups. The results show that the formation of three different groups was statistically significant.

The results of the cluster analysis using the Euclidian method for all leaf traits showed that AK1 and AK2 populations were in the same group, the population from RC formed one group, while the other populations could be grouped in a different group. To find the most important traits for discriminate the accessions, principal component analysis was applied on all 11 leaf traits. We therefore obtained three varimax-rotated principal component analyses (Table 5). The analysis of eigenvectors provides informa-

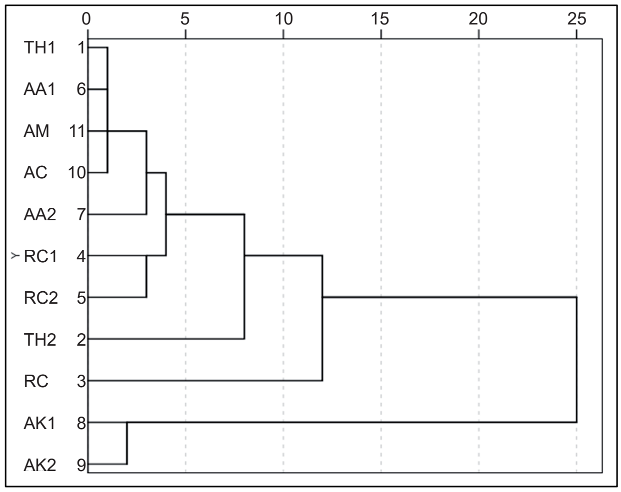

Figure 3. A dendrogram obtained by means of hierarchical cluster analysis related to all leaf traits

Table 4. Pearson's correlations among *Q. pontica* leaf traits

Variable	PL	TTL	LL	WL	LA	VA	NV	NT	PR	LWRL	PV
PL***	1	.044	.034	.274	.220	.500	.080	-.111	.594	.501	.133
TLL		1	.991**	.889**	.911**	.438	.505	.093	-.136	-.168	.078
LL			1	.853**	.889**	.453	.447	.030	-.108	-.231	.117
WL				1	.959**	.471	.637*	.418	-.147	.301	-.221
LA					1	.394	.546	.314	-.162	.160	-.138
VA						1	.434	-.064	.170	.072	.348
NV							1	.540	-.084	.324	-.128
NT								1	-.623*	.686*	-.876**
PR									1	.012	.629*
LWRL										1	-.615*
PV											1

* Significance level (P) < 0.05 statistically difference (2-tailed). ** Significance level (P) < 0.01 statistically difference (2-tailed).
*** Abbreviations of the traits can be seen in Table 2.

tion about the traits responsible for the separations along with the first three principal components.

Separate percentages of variation attributable to the first five components by decreasing order are 41.85%, 26.74% and 18.02%, 18.02%. PC1 had 41.85% of the total variation. TLL, LL, WL, LA and NV contributed negatively to PC1. PC2 had 26.747% of the total variation and was positively associated with petiole ratio (PR) and percentage of venation (PV), whereas total number of teeth number (NT) was negatively associated. PC2 is responsible for the separation of TH2 and AK2 from other accessions. PC3 had 18.027% of the total variation and was negatively associated with petiole length (PL) and relative length of lamina at largest width (LWRL). The results of principal component analysis and cluster analysis were comparable (Figure 4).

The results of the principal component analysis show that the AK1 and AK2 populations separated from the other populations. Additionally, three main groups could be distinguished via principal component analysis.

Table 5. Eigenvalues, cumulative variance, and factor loadings of morphological traits to PC axes

	PC1	PC2	PC3
Eigenvalues	4.603	2.941	1.982
Variance (%)	41.846	26.740	18.018
Cumulative variance (%)	41.846	68.585	86.603
Leaf traits			
PL*	–0.221	0.302	–0.860
TLL	–0.890	0.300	0.309
LL	–0.857	0.353	0.335
WL	–0.984	0.011	–0.041
LA	–0.948	0.086	0.078
VA	–0.533	0.451	–0.334
NV	–0.721	–0.129	–0.146
NT	–0.454	–0.871	–0.102
PR	0.211	0.666	–0.574
LWRL	–0.255	–0.584	–0.744
PV	0.206	0.929	0.035

* Abbreviations of the traits can be seen in Table 2.

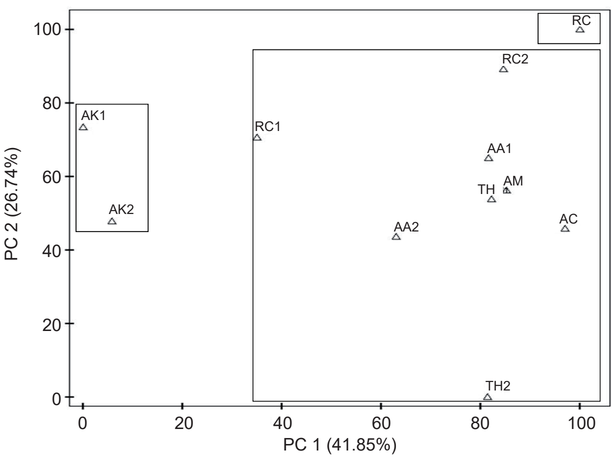

Figure 4. Principal component analysis ordination plot of the analyzed populations

Discussion

The results of analysis of variance and Duncan´s test demonstrated that the measured morphological traits of *Q. pontica* leaves were very variable. Within the scope of this study, variations in leaf traits could be determined by measuring PL, TLL, LL, WL, LA, VA, NV, NT, PR, LWRL, and PV in the natural distribution area of *Q. pontica*. The average values of these parameters were 1.07 cm, 17.13 cm, 16.13 cm, 8.10 cm, 93.76 cm^2, 54.22 degree, 38.72, 40.73, 6.34%, 50.50%, and 98.65%, respectively. Highest values for petiole length (1.31 cm), leaf length (19.86 cm), lamina length (18.55 cm), leaf width (9.65 cm), and leaf area (132.99 cm^2) were determined in the AK1 populations (see Table 3). Even though this study is based on a low number of individuals from a narrow geographical zone, all analyses showed some among-populations differentiation. All measured leaf traits changed significantly across populations. Based on leaf traits the populations can be divided into three major groups (see Figure 3 and 4).

Investigating leaf size variation is a relatively quick method to identify the population structure of less studied forest tree species and can be useful in designing more detailed population genetics studies. Similarly, Shiran et al. (2011) used leaf form along with molecular markers to investigate the population structure of Persian oak (*Q. brantii*). Bruschi et al. (2000), measured several morphological leaf parameters of sessile oak (*Q. petraea*) and pubescent oak (*Q. pubescens*) populations and found differences among populations. Yücedağ and Gailing (2013), determining leaf traits of *Q. petraea* and pedunculate oak (*Q. robur*) natural populations showed significant (99%) correlations (positive and negative) with lamina length. Similar to these works, there were statistically significant differences regarding leaf length, lamina length, width of lamina, leaf vein angle, total number of teeth, petiole ratio, and relative length of lamina at largest width in the present study. In another study, leaf traits analysis showed high degrees of variation because of hybridization between four taxa in Bolu populations (*Q. pubescens, Q. virgiliana, Q. petraea*, and *Q. robur*), while *Q. cerris* was clearly separated from other populations (Borazan and Babaç 2003).

Leaf morphology variation showed significant differences among and within populations. Also, PR, PV, LL, and TLL had the greatest impact on ordination (see Table 5); similar results have been observed by Ebrahimi et al. (2017). According to Taleshi and Maasoumi-Babarabi (2013), components accounted for 70% of the cumulative variance of leaf blade length, which agrees with our study findings (86.6%). Ertan (2007), in his study on sweet chestnut (*Castanea sativa*) found no significant correlation between different petiole lengths. This finding has shown a harmony with results obtained in our study.

Most traits do not vary independently of each other, and particular combinations of morphological traits are more correlated than it would be expected randomly (Kudoh et al. 2001). The correlation analysis carried out within the study confirmed the expected correlation between some of the studied characters (lamina length/width of lamina), previously stated by Batos et al. (2017). Lamina length was strongly and positively correlated with total leaf length (r = 0.991; see Table 4).

Leaf size, shape, leaf specific area, nitrogen content, and teeth number are related to mean annual temperature, precipitation, and elevation (Royer et al. 2005, Peppe et al. 2011, Read et al. 2014, Temel 2018), but other parameters excluding the teeth number of leaves which were not measured in the present study, and there was no significant relationship between the teeth number of leaves and ecological factors. Yücedağ et al (2021) determined that most leaf traits of Kasnak oak (*Q. vulcanica*) (an endemic species) showed significant positive and negative correlations with lamina length but no correlation with stomata density, altitude and precipitation effectiveness index in either comparison. In this study, we detected no significant relationship between environmental factors (altitude, aspect, annual temperature and annual precipitation) and leaf traits, which might be due to the fact that the leaf samples were obtained from the same region (Eastern Black Sea region). The climate types at population locations display mostly humid characteristics. The restricted group of *Q. pontica*, a relict and Colchic endemic species and located only in the eastern region of Turkey, is geographically closer to Georgia. The species has varying little in its characters and quite distinct among all the other Turkish oaks on account of the very large, regularly serrate leaves with numerous prominent parallel veins (Hedge and Yaltirik 1982). On the other hand, Olsson (1975) recommend that all leaf materials should be collected at the same height and aspect on each tree, which was done in our study.

Conclusions

Determination of variation within and among populations of *Q. pontica* was carried out based on the measurement of morphological leaf traits. Consequently, for *Q. pontica* with an optimal distribution between 800–2.100 m (Hedge and Yaltirik 1982) for natural populations, there are variations within and among populations. The small habitat range of endemic species often leads to a high risk of extinction (Rodrigues et al. 2006, Mhamdi et al. 2013). Therefore, determination of morphological and genetic characterization of *Q. pontica*, an endemic and relict plant in natural forest resources, is crucial for the implementation of *in situ* and *ex situ* conservation activities. We observed obvious differences between the populations of Artvin-Karagöl province, AK1 and AK2, and other populations. In this sense, biodiversity preservation and the continuity of variation are important for the conservation of genetic resources.

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