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LEAF MORPHOLOGY IN QUERCUS ROBUR L. GENETIC RESOURCES ACROSS ROMANIA

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Abstract: *We studied the leaf morphology of pedunculate oak (Quercus robur L.) in seven natural stands. Fourteen leaf descriptors were used. For most of the morphological traits the differences between stands were non-significant. For petiole ratio, petiole length and lobe depth ratio were significant and for basal shape were highly significant. By using a Principal Component Analysis (PCA) no groups were distinguished. The values of leaf descriptors are quite similar to those reported in Western Europe.*

Key words: *Q. robur, genetic resources, leaf morphology, ANOVA, PCA.*

1. Introduction

The genus *Quercus L.* is characterized by high polymorphism [19] and consists of about 400 to 450 species (deciduous and evergreen trees and shrubs) spread from temperate to semiarid areas across the northern hemisphere [20]. In Europe, there are 24 oak species [16]. Among them, one of the most important species from the economic aspect is *Q. robur.*

The pedunculate oak was taxonomically classified in many ways. It was included in subfamily *Quercoidae,* subgenus *Lepido-balanus* [8] or in subgenus *Quercus,* section *Quercus s.l.* (sensu lato), group *Quercus s.s.* (sensu stricto) [20]. It is one of the most economically important forest species in Romania.

It is also valued for its longevity and beauty and as a host for a wide range of organisms [18].

Its Romanian distribution range is very fragmented and intensely marked by human's activities, as a result of highly deforestation, especially in the plains for increasing the farming areas. As a consequence of its fragmented range the vitality and the biological features might suffer in the future [22].

So far, no detailed observations on the leaf morphology of the pedunculate oak were carried out in Romania. By contrast, many stands of pedunculate oak were investigated regarding the leaf morphology in other parts of Europe [2], [3], [5], [13], [14], [18], [21].

The aim of this study is to determine the morphological leaf variability within this species and to identify intraspecific varieties

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or forms. It is well known that *Q. robur* has one of the highest variability, especially in size and shape of the leaves [16].

2. Materials and Methods

2.1. Plant Material

The individuals of pedunculate oak were selected from seven stands distributed throughout a broad ecological range from Romania (Figure 1). The geographical coordinates of these stands are shown in Table 1.

These stands have been included on the list of Forest Genetic Resources in Romania.

Geographical coordinates Table 1

Stand	Geographical coordinates	
	Longitude	Latitude
Cenuşa	27°13'40"E	47°3'9"N
Dacia	29°32'15"E	45°19'53"N
Letea	29°32'15"E	45°19'53"N
Noroieni	22°55'2"E	47°50'19"N
Păunoaia	25°59'8"E	44°43'37"N
Reşca	24°26'6"E	44°9'21"N
VânjuMare	22°50'59"E	44°25'11"N

Only adult trees were selected with a minimum distance of 50 m between them to avoid relatedness as much as possible.

From each stand approximately 50 oaks were sampled. The collecting position of branches was standardized.

From every branch, fully developed leaves were collected from the sun-exposed upper crown [4]. Afterwards, three undamaged and healthy leaves were chosen from every tree to form the herbarium collection which will be the object for the analysis.

2.2. Morphological Analysis

The protocol of leaf evaluation followed the procedure described in a study in Western Europe [17]. The following variables were assessed: five dimensional traits - *lamina length* (LL), *petiole length* (PL), *lobe width* (LW), *sinus width* (SW), *length of lamina at largest width* (WP), two counted variables - *number of lobes* (NL) and *number of intercalary veins* (NV), two observed variables - *basal shape of the lamina* (BS), scored as an index varying from 1 to 9

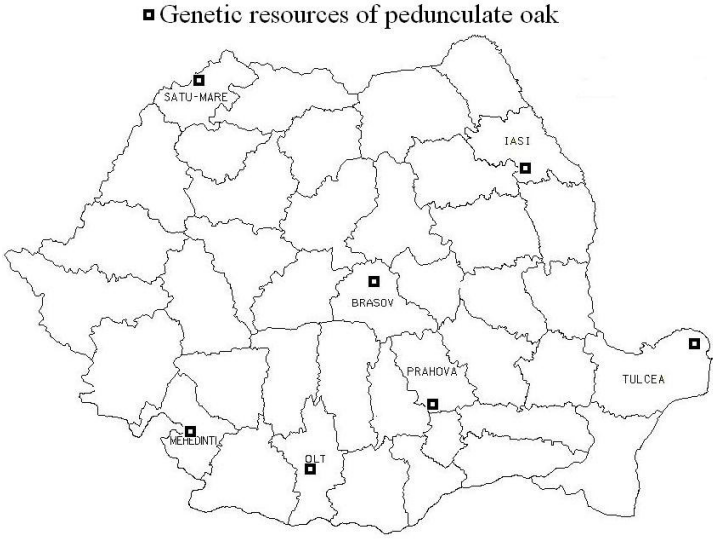

Fig. 1. *Locations of the sampled stands*

and *abaxial laminar pubescence* (PU), evaluated according to Kissling's grading system from 1 (no pubescence) to 6 (dense hairness) [15] with an optical microscope (x8) and five transformed variables: *lamina shape or obversity* (OB): OB = 100 x WP/LL, *petiole ratio* (PR): PR = 100 x PL/(LL+PL), *lobe depth ratio* (LDR): LDR = 100 x (LW-SW/LW), *percentage venation* (PV): PV = 100 x NV/NL and *lobe width ratio* (LWR): LWR = 100 x LW/LL.

WinFolia software [24] was used to assess the measured variables. The data were then centralized in an Excel file for further analyses.

2.3. Statistical Analysis

Data were further analyzed with STATISTICA software, version 8.0 [23]. Arithmetical mean values, variances, standard deviations and coefficients of variation (CV%) were calculated for all variables.

Comparisons between the stands were computed with analysis of variance (ANOVA) and Principal Component Analysis (PCA). Multivariate analyses are used in order to find which leaf descriptors discriminate best between stands and within them.

The correlations between variables and the discriminant functions (ID) proposed for pedunculate oak and sessile oak by Kremer et al. [17] and Dupouey & Badeau [10] were also tested.

3. Results and Discussion

3.1. Values of Leaf Descriptors

For every stand, the mean values and coefficients of variation of variables, except for the calculated ones are shown in Table 2. The values for the coefficients of variation indicate that the populations are homogeneous (C.V.% < 35-40%) [12].

Means and coefficients of variation for each descriptor Table 2

Variable	Stand	Cenuşa	Dacia	Letea	Noroieni	Păunoaia	Reşca	Vânju Mare
PU	Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	C.V.%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BS	Mean	8.2	8.7	8.4	8.4	7.7	8.1	8.4
	C.V.%	9.5	3.7	11.0	6.8	17.0	7.4	6.1
NL	Mean	8.9	10.0	9.9	9.8	10.5	10.1	10.4
	C.V.%	16.8	14.5	17.0	16.0	16.0	15.0	13.1
NV	Mean	3.3	4.6	5.1	4.0	3.3	4.4	5.2
	C.V.%	34.6	24.2	22.3	32.2	34.4	24.7	25.7
LL [mm]	Mean	116.0	106.4	108.1	108.3	126.6	133.3	114.0
	C.V.%	14.3	13.7	14.1	13.8	15.0	12.3	15.2
PL [mm]	Mean	7.2	6.8	7.4	6.2	5.7	6.1	5.3
	C.V.%	43.2	31.9	27.8	28.2	35.4	25.9	29.0
LW [mm]	Mean	39.4	35.6	35.9	35.9	38.5	44.5	36.0
	C.V.%	16.3	15.7	16.4	13.9	18.3	15.4	14.5
SW [mm]	Mean	16.5	13.3	11.8	12.6	14.6	16.2	13.3
	C.V.%	27.8	24.4	26.5	26.2	29.7	25.1	26.8
WP [mm]	Mean	66.3	64.2	63.9	63.6	75.4	76.2	71.5
	C.V.%	17.3	17.2	17.7	16.1	17.2	17.1	18.5

However, there are two exceptions: PL in Cenuşa stand and for PU in all stands. The results of the leaf descriptors are very similar to those reported in dendrology books (e.g. PL = 5-7 mm, LL = 6-20 cm, NL = 8.9-10.5) [22] and other morphological studies done elsewhere [17]. There were neither stellate nor fasciculate hairs (the mean values for PU descriptor for all stands were 1) observed on the abaxial surface of the lamina [9], [21]. Our data indicate that all sampled trees belong to *Q. robur* var. *glabra* (Gord.) Schwz.

The average value of the BS variable (8.3) indicates a typical auriculate basal shape of the lamina.

This is in agreement with other studies [21] concerning the leaf morphology of pedunculate oak, where such well developed auricles have been also observed. The number of lobes (NL) and the number of intercalary veins (NV) have similar average values (9.9, respectively 4.3) with those reported in other studies done elsewhere in Europe [10], [11], [21]. For the NL, the lowest and highest values of the coefficient of variation (13.1%, respectively 17%) are close to those reported in Bulgaria (12%, respectively 16.7%) [7].

The mean values of the measured variables (LL, PL, LW, SW and WP) are alike those found in France [1], [3], [10]. It turned out that the smaller leaves are found in Dacia, Letea and Noroieni stands and the largest ones originate from Reşca stand.

3.2. Correlations between Variables

Table 3 gives the correlations between variables. As showed before [11], [21], dimensional characters are positive correlated. Especially the variables LW and WP are significantly correlated with LL. The variable PV was positive correlated with NV and negative correlated with NL [11].

Correlation between variables Table 3

Variable	LL	PV
BS	–0.14	0.15
NL	0.33	–0.42
NV	0.06	0.88
LL	x	–0.09
PL	0.06	–0.02
LW	0.80	–0.07
SW	0.32	–0.33
WP	0.82	–0.12
OB	–0.06	–0.06
PR	–0.37	0.04
LDR	0.14	0.31
PV	–0.09	x
LWR	–0.19	0.03

3.3. Testing ID Functions

Kremer's et al. [17] and Dupouey & Badeau's discriminant (ID) functions [10] developed for discriminating between pedunculate oak and sessile oak were tested for all 349 pedunculate oaks taken into account in this study.

The first discriminant function [17] gave negative values for 12 trees (3.43%) and only for 9 oaks (2.57%) in case of the second [10] function (Table 4).

Testing discriminant functions Table 4

Tree		Results	
No.	Stand	ID Kremer	ID Dupouey
41	Cenuşa	–1624.6	–2254.7
30	Cenuşa	–553.1	–686.3
7	Cenuşa	–441.4	–360.3
24	Cenuşa	–410.4	–451.0
72	Dacia	–396.1	–299.7
62	Dacia	–288.1	–22.7
47	Cenuşa	–246.3	–319.3
28	Cenuşa	–186.0	–18.0
25	Cenuşa	–167.4	–81.3
214	Paunoaia	–125.0	19.7
108	Letea	–17.0	296.7
231	Paunoaia	–0.2	54.7

The discriminant function proposed by Kremer et al. is 357-97*PL+205*NV and Dupouey & Badeau's is 407*NV-130*PL+357. Both of them are taken into account the PL and NV variables, which are used in dendrological literature to distinguish pedunculate oak from sessile oak.

It is known that pedunculate oak has more intercalary veins than sesile oak [17] and a shorter petiole [22]. It has been established that these functions gave positive ID values for pedunculate oak exemplars and negative values for sessile oak individuals.

3.4. Analysis of Variance (ANOVA)

By using the multivariate analysis from STATISTICA software package [23] it was found that the "Population" effect is statistically significant (acoording to Wilks test F value was 6.3 at $p = 0.0$). It can be seen that Levene's test for homogeneity of variances showed significant differences ($p < 0.05$) among the seven stands in 4 cases: BS, followed by PR, PL and LDR, which have almost the same F values (Table 5). This means that in the case of these four variables differences among stands could be observed. The variable PU was not included in the analysis because its variances for all stands were zero. The p value for NV variable (0.819), correlated with the mean values for this descriptor for every stand, indicates that is almost impossible to distinguish these populations, if we are taken into account only this aspect.

3.5. Principal Component Analysis (PCA)

Multivariate techniques are particularly useful in taxonomy because they allow comparing samples of trees taken from different places [6]. Figure 2 shows the PCA graph of all seven populations. The first two principal components (factors) explain about 40% of the total variation. It is clear from the figure that the pedunculate oak individuals tend to form only one morphological group.

Only a few individuals appear to be separated from the group. Some of them (e.g. 41, 7, 72, 47, 62, 24 or 30) are the ones that showed negative ID values for the two discriminant functions (see 3.3). However, there are individuals that showed negative values for the discriminant functions (e.g. 28 or 25), which means that they are not *Q. robur*, but they still remain in the group.

Levene's test for homogenity of variances Table 5

Variable	MS Effect	MS Error	*F*	*p*
BS	2.12	0.31	**6.823**	**0.000**
PR	2.71	1.06	**2.568**	**0.019**
PL	4.52	1.85	**2.452**	**0.025**
LDR	74.18	32.31	**2.296**	**0.035**
SW	10.70	5.36	1.999	0.065
LWR	7.16	3.99	1.794	0.099
LW	22.54	14.28	1.579	0.152
LL	128.85	94.80	1.359	0.230
PV	74.02	62.10	1.192	0.310
NL	0.81	0.76	1.072	0.379
OB	12.68	11.90	1.066	0.383
WP	31.97	54.90	0.582	0.744
NV	0.24	0.49	0.486	0.819

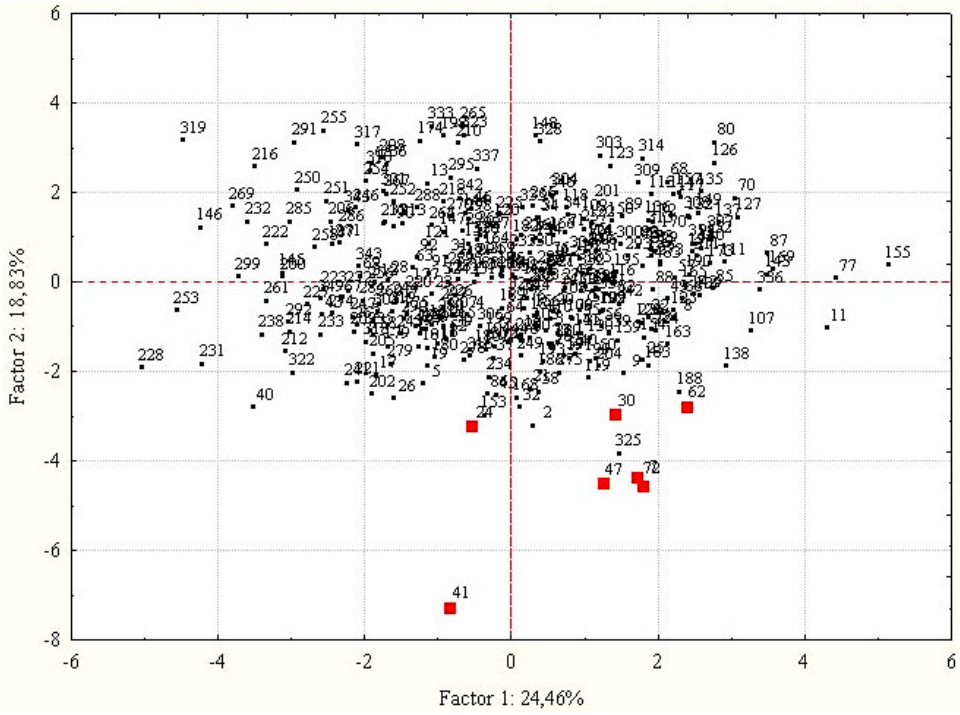

Fig. 2. *PCA diagram*

4. Conclusions

Even though the leaves were sampled from seven stands from Romania, growing under different ecological conditions, scattered on a broad geographical area, a relatively small variation was revealed by the fourteen descriptors we used.

The differences between the sampled stands were for most of the leaf descriptors non-significant ($p > 0.05$). However, for three variables the differentiation among stands were significant ($0.01 < p < 0.05$). Moreover, for the basal shape (BS) showed highly significant differences among the stands (p value was 0.00).

As a result of this study we can conclude that the values of the leaf descriptors were very similar to those reported in different morphological studies which followed almost the same methodology done in Western and Central Europe.

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