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A MULTIVARIATE APPROACH TO DIFFERENTIATE THREE ROMANIAN OAK SPECIES: A CASE STUDY

C.M. ENESCU[1] N. ŞOFLETEA[1] A.L. CURTU[1]

Abstract: *The main objective of this study was to highlight the morphological leaf descriptors that differentiate three oak species, namely pedunculate oak, sessile oak and pubescent oak, by using multivariate analysis (PCA and MANOVA). Fifty oak individuals were sampled for every species and nine morphological traits were assessed. The results of the statistical analysis confirmed the great discriminating power of certain descriptors. Abaxial laminar pubescence and basal shape of the lamina were the variables that separate pedunculate oak individuals from the pair pubescent oak-sessile oak. In conclusion, in order to achieve a better separation other morphological traits should be taken into consideration.*

Key words: *PCA, MANOVA, pubescent oak, pedunculate oak, sessile oak.*

1. Introduction

Among the multivariate statistical analysis used for oak species (genus *Quercus* L., *Fagaceae*) differentiation and for evaluating their great morphological variability, Principal Component Analysis (PCA) is one of the most used method [1-2], [4-6], [9], [12]. PCA is a multi-factorial method which purpose is to build new synthetic variables that are combinations of the original ones, unrelated one to each other and called principal components [8]. The number of principal components (factors) is equal to the number of original variables. It is well known that the first two principal components explain the biggest part of the variability from the data set, reason for which they are usually used for designing the diagrams. In other words, PCA is able to produce a lower-dimensional picture, easier to interpret by the researcher.

Similarly, Multivariate Analysis of Variance (MANOVA) is also used for highlighting the variables that determine significant differences between the analyzed objects (i.e. between the three oak species, in our study).

2. Objectives

Firstly, the objective of this study was to test the ability of PCA to differentiate three Romanian oak species, namely *Quercus pubescens* Willd. – pubescent or downy oak, *Q. robur* L. – pedunculate oak and *Q. petraea* (Matt.) Liebl. – sessile oak by using leaf morphological descriptors. Secondly, by using MANOVA and PCA we wanted to highlight the variables that have the biggest discriminating power

[1] Dept. of Silviculture, *Transilvania* University of Braşov.

between the same oak species in order to use them in field determinations based only on leaf morphology.

3. Materials and Methods

Leaf morphological assessment followed the methodology used in other similar studies [3], [7], [11], but without taking into consideration the five transformed variables. Thus, five leaves from every oak tree were chosen and nine leaf descriptors were assessed: five dimensional characters – *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) and two observed variables – *abaxial laminar pubescence* (PU), evaluated according to the grading system from 1 (no pubescence) to 6 (dense hairness) with a binocular (x30) and *basal shape of the lamina* (BS), scored as an index varying from 1 to 9.

The dimensional descriptors were evaluated by the aid of WinFOLIA software, after the leaves were dried and scanned.

Fifty trees for each oak species were sampled from three stands located in different geographical regions. So, 50 pubescent oaks were sampled from Vlădila stand (Olt county, south of Romania), 50 sessile oaks from Ronişoara (Maramureş county, north of Romania) and fifty pedunculate oaks from Podul Iloaiei (Iaşi county, eastern Romania).

During sampling, species determination on field was made according to the recent Romanian dendrology manual [10], by taking into consideration the trunk bark (aspect and thickness), twig (glabrous or pubescence), leaf (pubescence, basal shape, lamina length, petiole length) and fruit morphological traits (cupula peduncle length, cup dimensions, acorn dimensions).

PCA was carried out on a correlation matrix by the aid of STATISTICA software, version 8. The correlation matrix was used because the assessed variables were on different scales. So, by using the correlation matrix the data is standardised.

MANOVA was performed also by STATISTICA software, by un-checking the *Sigma-restricted* option from *Parameterization* field and selecting the type III (orthogonal) of sums of squares.

4. Results and Discussions

By using all the nine variables two main groups resulted in PCA diagram (Figure 1). The first one, on the left part of the graph, included mainly pedunculate oak individuals. On the other hand, the second one, from the right part of the diagram, was composed by pubescent oak and sessile oak individuals.

The overlapping of *Q. petraea* and *Q. pubescens* individuals can be explained by their morphological leaf similarities more pronounced compared to *Q. robur*.

In addition, by taking into consideration the contribution of the variables to the first two principal components (Figure 2 and Table 1), the presence of the pubescent oak – sessile oak mixed group on the right part of the graph was determined by the highest values of certain variables (abaxial laminar pubescence and petiole length) compared to those found for pedunculate oak.

Thus, the leaf petiole is longer and the lamina is hairy in the case of pubescent oak and sessile oak, compared to pedunculate oak. Similarly, the oaks with high values for basal shape of the lamina (almost ear-like basal shape) and lamina length appeared on the left part of the diagram. This case is typically for pedunculate oak (*Q. robur*).

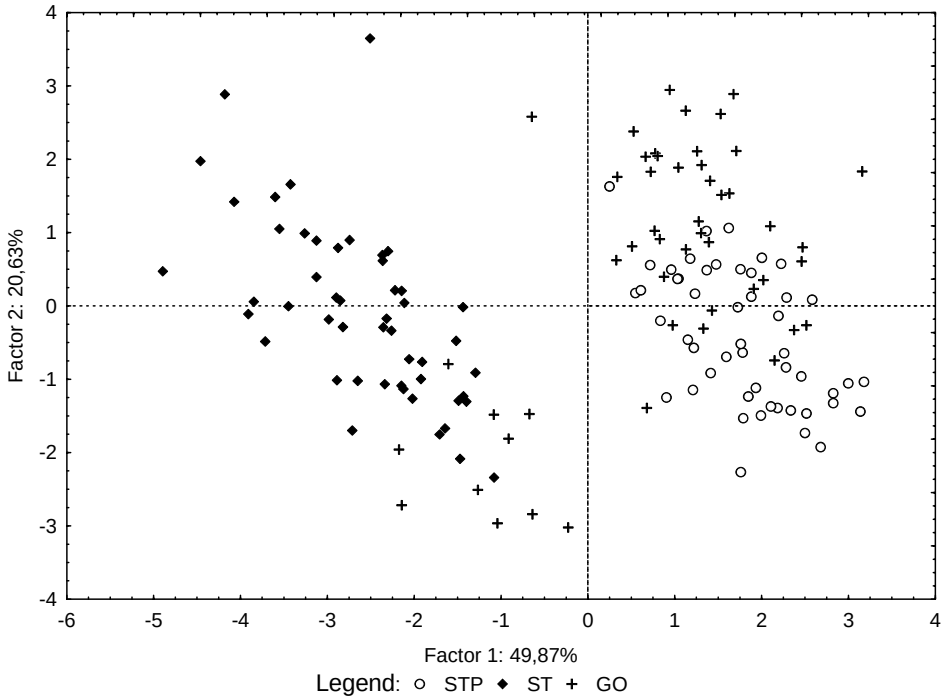

Fig.1. *PCA diagram (nine variables)*

Briefly, we can conclude that the dimensional leaf descriptors, in combination with the basal shape of the lamina and the intensity of the abaxial laminar pubescence are able to separate *Q. robur* from the other two oak species.

Table 1

Contributions of variables to the first two principal components

Variable	Principal Component	
	1	2
PU	**0.18**	0.01
BS	**0.17**	0.04
NL	0.03	0.15
NV	0.03	0.22
LL	**0.15**	0.08
PL	0.10	0.14
LW	0.13	0.06
SW	0.05	0.25
WP	**0.16**	0.05

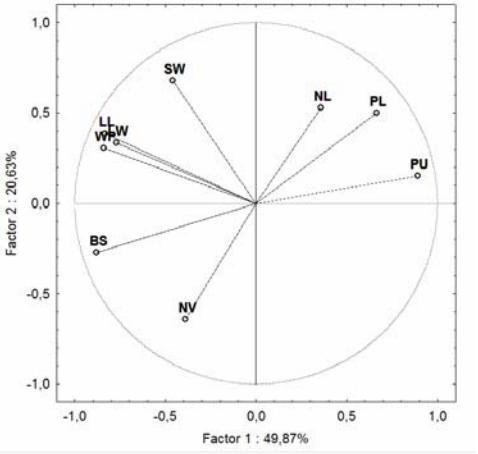

Fig.2. *Projection of the variables on the factor-plane (factor 1 x factor 2)*

Similar interpretations like those presented above could be provided by the values of the Pearson's correlation coefficients. For example, the positive and strong correlation between abaxial laminar

pubescence and petiole length (0.64; Table 2) is typical for all three oak species. While the lamina of *Q. robur* has no hairs and the petiole is short, in the case of *Q. pubescens* and *Q. petraea* the lamina is hairy and the petiole is longer. Moreover, a positive and strong correlation was observed between the dimensional traits, except for petiole length which was negatively correlated with the other dimensional characters (Table 2). In other words, as the length of lamina is increasing, the length of petiole is decreasing. This case is typically for pedunculate oak, the petiole ratio having lower values compared to the other two species.

Correlations of variables Table 2

Var.	PU	BS	NL	NV	LL	PL	LW	SW	WP
PU	1.00	-0.87	0.36	-0.37	-0.62	**0.64**	-0.54	-0.36	-0.66
BS		1.00	-0.43	0.42	0.55	-0.72	0.54	0.28	0.59
NL			1.00	-0.22	-0.04	0.38	-0.24	0.07	-0.01
NV				1.00	0.18	-0.44	0.11	-0.32	0.23
LL					1.00	**-0.30**	**0.81**	**0.47**	**0.89**
PL						1.00	**-0.25**	**-0.04**	**-0.41**
LW							1.00	0.49	0.65
SW								1.00	0.47
WP									1.00

Afterwards, in order to achieve the second objective of this study, we performed a MANOVA, by using Levene's test for homogeneity of variables, with the nine variables as dependent variables and species as main effect. The first four variables with the highest *F* values and lowest *p* values (i.e. PU, BS, NV and WP; Table 3) were retained for performing another PCA. Three, namely PU, BS and WP, out of these four variables had the highest contributions to the first factor in PCA.

In this case, even if the first two factors accounted for approximately 86% of the total variance, the overlapping of the sessile oak and pubescent oak individuals was also observed (Figure 3).

Levene's Test for Homogeneity of Variances Table 3

Variable	MS Effect	MS Error	F	p
PU	12.3080	0.25247	**48.75033**	**0.000000**
BS	21.0695	0.74401	**28.31884**	**0.000000**
NL	0.7991	0.76531	1.04421	0.354565
NV	9.9033	0.93896	**10.54711**	**0.000052**
LL	309.7933	64.33809	4.81508	0.009429
PL	58.1433	8.93838	6.50490	0.001964
LW	47.1408	9.12060	5.16860	0.006772
SW	16.9941	5.31327	3.19843	0.043683
WP	233.5708	24.52386	**9.52423**	**0.000129**

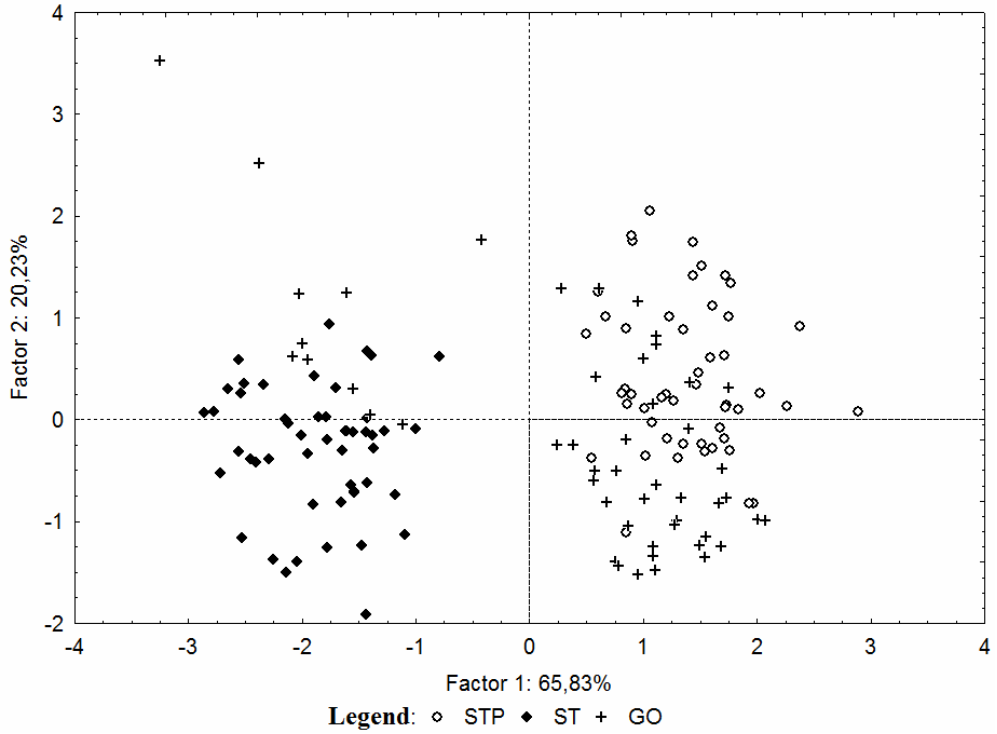

Fig.3. *PCA graph by using only 4 variables (PU, BS, NV and WP)*

5. Conclusions

The results of this multivariate survey are in accordance with the morphological description from the literature [10]. More precise, the group of variables PU-BS-LL-NV-WP were able to differentiate pedunculate oak individuals from the pair pubescent oak – sessile oak. Among them, laminar abaxial pubescence and basal shape of lamina were the variables which determined significant differences between the three oak species both in PCA and MANOVA.

No significant differences were observed for the two PCAs, the case with the nine variables and the case with only four. For this dataset, it can be explained by the lower discriminating power of the other five. In order to achieve a better separation between *Q. petraea* and *Q. pubescens* other morphological descriptors should be taken into consideration, for example the types of the hairs from the abaxial part of the leaf or the twig hairiness.

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