Zbornik gozdarstva in lesarstva, 46, 1995, s. 75 - 96

GDK 176.1 Quercus robur (L.) : 181.6 / .7 : 165.3 : (497.12)

MORPHOLOGICAL AND GENETIC VARIABILITY OF PEDUNCULATE OAK (*QUERCUS ROBUR* L.) POPULATIONS IN SLOVENIA

Franc BATIČ *, Tomaž SINKOVIČ **, Branka JAVORNIK ***

Abstract

The article presents a taxonomic study carried out in three pedunculate oak populations in Slovenia by leaf morphometry and by DNA analysis with RAPD markers. About one hundred leaves were sampled from five trees from three seminatural pedunculate oak stands in the Sub-pannonian vegetation region of Slovenia. Samples were collected from the well lit upper part of the tree crowns, and only leaves from short twigs were analysed. Leaf samples were herbarised, and measured and assessed in the laboratory. Leaf dimension (lamina length and width, petiole length, number of lobe pairs) were measured and characters like type of leaf pubescence and leaf lamina base type were assessed. Analyse of leaf morphometry showed that all sampled trees belonged to the *Quercus robur* L. species. Nevertheless, on some trees, leaves also showed characters typical of sessile oak (*Quercus petraea* /Matt./ Leibl.), especially the leaf lamina base type and number of lobe pairs. Other parameters analysed were within the variation of pedunculate oak. More populations of both oak species should be analysed, especially when growing together, in order to obtain reliable data for taxonomic studies of oak hybrids and population genetic diversity by means of classic leaf and acorn morphometry and molecular markers.

Key words: pedunculate oak, morphology, multivariate analysis, RAPD markers, genetic polymorphism, natural hybridisation

MORFOLOŠKA IN GENETSKA VARIABILNOST POPULACIJ DOBA (*QUERCUS ROBUR* L.) V SLOVENIJI

Izvleček

Delo predstavlja taksonomske raziskave pri treh populacijah doba in vključuje morfometrijske meritve listov in DNK analizo z uporabo RAPD markerjev. Analizirali smo do 100 listov iz petih označenih dreves v subpanonskem vegetacijskem območju. Nabrali smo jih dobro osvetljene zgornje tretjine krošnje samo s kratkih poganjkov. Liste smo herbarizirali, merili in ovrednotili v laboratoriju. Ovrednoteni parametri listov so obsegali dolžino in širino listne ploskve, dolžino listnega peclja, število listnih krp, tip listnega dna in gostoto ter tip dlačic na listih. Raziskave morfometrijskih podatkov listov so pokazale, da raziskovana drevesa pripadajo dobu (*Quercus robur* L.). Posamezna drevesa na različnih ploskvah kažejo nekatere morfološke lastnosti gradna (*Quercus petraea* /Matt./Liebl.), posebej tip listnega dna in število listnih krp. Ostali morfološki parametri so v okviru variacijske širine doba. Analizirati bi morali večjo populacijo dreves predvsem v mešanih sestojih, kjer omenjeni vrsti rasteta skupaj. Podatki iz večje populacije bi bolj zanesljivo prikazali taksonomske probleme hibridov pri hrastih s primerjavo klasičnih morfoloških parametrov in molekularnih markerjev.

Ključne besede: dob, morfologija, multivariantna analiza, RAPD markerji, genetska variabilnost, naravna hibridizacija

---

* Prof., dr., dipl. biol., Argonomy dept., Biotehnical faculty, University in Ljubljana, Jamnikarjeva 101, 61000 Ljubljana, Slovenia

** Assist., mag., dipl. biol.,Argonomy dept., Biotehnical faculty, University in Ljubljana, Jamnikarjeva 101, 61000 Ljubljana, Slovenia

*** Prof., dr., dipl. ing., food tech.l., Argonomy dept., Biotehnical faculty, University in Ljubljana, Jamnikarjeva 101, 61000 Ljubljana, Slovenia

TABLE OF CONTENTS

1 INTRODUCTION

Lowland forests of oaks are the most changed forest ecosystems in Slovenia, as elsewhere in Europe. Due to agricultural activity and urbanisation, they have been transformed into cultural steppes and urban areas. Only a few remnants of them remain, and even they suffer from various disturbances like a changed water table, input of fertilisers, air pollution impact, cuttings etc. A new kind of forest decline has also seriously affected oak forests throughout Europe. The situation is not improving but getting increasingly drastic. In addition to the above mentioned human activities, increasing drought and inappropriate land management are the major reasons for the decline of oak. In this connection, there is growing interest in better knowledge of oak species and populations, especially the most important oak species, the pedunculate oak (*Quercus robur* L.) and the sessile oak (*Q. petraea* (Matt.) Leibl.), which are of interest to foresters because of their valuable timber, as well as for their landscape value.

Taxonomic problems of oaks are related to their great morphological variability and have attracted a large number of botanists, foresters, dendrologists, florists in the past time (Cousens 1962, 1963, 1965, Carlisle & Brown 1965, Wigston 1974, 1975, Olsson 1975, Rushton 1978, etc.). Up to the present time, taxonomic problems with pedunculate and sessile oak have not been fully solved. The great polymorphism of pedunculate oak is probable the result of hybridisation of different species in the genus *Quercus* L. (Martinis et. al. 1987; Trinajstić 1988), due to incomplete reproductive barriers between oak species, or is a result of adaptation to environment. In Slovenia, six indigenous oak species appear: *Q. ilex* L., *Q. crenata* Lam., (*Q. pseudosuber* Santi.), *Q. cerris* L., *Q. pubescens* Willd. (*Q. lanuginosa* Thuill.), *Q. petraea* (Matt.) Liebl., (*Q. sessiliflora* Salisb., *Q. sesisilis* Ehrh.) *Q. robur (Q. pedunculata* Ehrh.) and one questionable taxon *Q. virgiliana* Ten. (*Q. pubescens* L. subsp. *dalechampii* Hay., *Q. croatica* Posp.); (Schwarz, 1964; Martinčič and Sušnik 1969, 1984).

According to the literature (Schwarz 1936, Trinajstić 1974; Garcke 1972; Rothmahler 1991; Tutin 1964), all oak species are interfertile. In the differentiation of the lower taxons of the genus *Quercus,* the morphology of the leaves has the most important role. The second important taxonomic character is the morphology of the acorns and the peduncule length (Schwarz 1936, 1964, Jones 1968, Wigston 1975, Rushton 1978, Franjić 1993). Great polymorphism of oak leaves has led to the description of many forms and varieties that were not justified according to taxonomic nomenclature (Trinajstić 1988). This also happened in former Yugoslavia where Erdeši (1985) described 84 forms of pedunculate oak, some of them growing sympatrically under the same ecologic conditions. Only recently some more realistic morphometric studies of pedunculate oak have been carried out (Franjić 1993, Trinajstić & Krstinić 1993) in order to resolve taxonomic problems of oak hybrids, forms and varieties. Some of more recent morphological studies on hybridization between *Q. robur* and *Q. petraea*, like Aas (1990) in Germany and Dupouney & Badeau (1993) in France reached different conclusions revealing that both species were clearly separated showing some morphological intermediate individuals. As Dupouney & Badeau pointed out, the main discrepancy of results estimating morphological data and giving evidence of hybrids or separated species was in the choice of variables and different approaches used for morphological data analysis. Today, the distinction of species in the *Quercus* complex is still a matter of debate, with prevalent opinion that many morphological intermediate forms are due to hybridization and introgression between pure parent species. Several species have been reported as potentially inbreeding in Europe, especially *Q. robur* and *Q. petraea*. Rushton (1993) concluded that before any radical revisions of the genus was attempted a wider application of possible techniques for the study of hybrids should be applied in order to clarify the true extent of gene flow between *Quercus* species.

The taxonomy of oaks in Slovenia has not been studied well. Even on the species level, all cited species are neither confirmed nor herbarised. The situation at the subspecies level has not been studied at all, although forestry practice requires such data for establishing seed orchards and resolving the problems of oak decline. The origin of planting material should be known and the homogeneity of preserved populations of pedunculate and sessile oak should be studied in order to enable proper forest management. The study of leaf and acorn morphometry carried by Smole & Batič (1992) has confirmed a high degree of variation of measured morphological parameters, probably due to hybridisation among all sympatric oak species. The presented paper is a continuation of that study. Additionally, the application of molecular markers was started (RAPD, Random Amplified Polymorphic DNA) in order to assess genetic variability in populations of oak species and to contribute to the studies of morphological data on the hybridization between oak species. The use of molecular markers in oaks has been limited to the use of rRNA markers which are often considered as a good species specific marker. Petit et al. (1993) used length variant frequencies of the rDNA unit. They were not able to distinguish pedunculate and sessile oak variants and their calculated gene diversity of both species was extremely high (0.829). Chloroplast DNA (cpDNA) polymorphism has also been studied in oaks, showing that the same cpDNA genotype is shared by different oak species of the same geographic region (Petit et al. 1993).

RAPD markers have been successfully used due to their simplicity, low cost and reasonable discriminatory power in many different genetic studies of plants, including tree species. Our approach has been to screen populations of different oak species with RAPD markers in order to evaluate genetic diversity in oak species and populations and to find out whether this type of markers could result in species specific marker. In this work the first results of three pedunculate oak populations in Slovenia are represented.

2 MATERIALS AND METHODS

Five permanent research plots of pedunculate and five of sessile oak have been established for various studies by the Slovenian Forestry Institute. The plots were chosen in forests where the two oak species represented an important or even the prevailing part of the woodstock. The preliminary taxonomic study of pedunculate oak from three research plots, Cigonca, Dobova and Hraščica is presented in the article. All three plots are located in the lowlands, in the Sub-pannonian vegetation region of Slovenia. All plots are on natural sites of pedunculate oak, but the past planting of some foreign provenience cannot be excluded. The distance to the nearest locality of other oak species, mostly sessile oak, is probably not large enough to exclude cross pollination, especially in the case of wind pollination.

The taxonomic studies include the morphology of leaves and acorns. A DNA analysis on the base of RAPD markers was used to differentiate oak species in order to compare the results with the morphological data. Five numbered trees on each of the research plots were investigated. For the morphology, up to 100 leaves per tree were sampled from the upper third of a well lit crown. Only leaves from short twigs were herbarised. Leaves were observed and measured under a stereo microscope (50x magnification). The type and density and occurrence of leaf pubescence were observed and assessed on leaf laminas, veins and leaf margins (Smole & Batič 1992). In addition to pubescence, the morphological observations and measurements of leaves included the following parameters: the length of lamina, the width of lamina, the type of leaf lamina base, the length of petiole and the number of lobe pairs. The leaves were measured with a vernier ruler (up to 1mm), later a computer attached digitalizer was used to overcome mistakes in ruler measurements. Multivariate statistical methods were used for the interpretation of morphological data results, box plots, cluster analysis, ANOVA, correlations etc. (Aas, 1993).

Variables measured and used for estimating the morphological homogeneity of pedunculate oak populations are:

Variable 1 = petiole length (cm)
Variable 2 = leaf petiole percentage (petiole percentage to total lamina length)
Variable 3 = leaf lamina base types (cordate (auric.)-1, cuneate-2, asymmetrical
Variable 4 = leaf lobe pair number
Variable 5 = leaf index (lamina length/lamina width)
Variable 6 = the pubescence of upper lamina surface (type, density)
Variable 7 = the pubescence of veins on upper lamina (type, density)
Variable 8 = the pubescence of margins on upper lamina (type, density)
Variable 9 = the pubescence of lower lamina surface (type, density)
Variable 10 = the pubescence of veins on lower lamina (type, density)
Variable 11 = the pubescence of margins on lower lamina (type, density)
Variable 12 = lamina length (cm)
Variable 13 = lamina width (cm)
Variable 14 = number of lobes on the left side
Variable 15 = number of lobes on the right side

RAPD markers: Approximately 30 leaves from the same tree were taken and stored in a deep freeze for the extraction of DNA. Total genomic DNA was extracted by the adapted method of Saghai-Maroof (1984). The amplification of arbitrary DNA fragments using random 10-mer primer (Operon Technologies) was performed according to the optimised procedure as described by Javornik & Kump (1992) using Taq polymerase from Fermentas MBI (Lithuania).
Seven different primers were used: OPA-01, OPA-20, OPB-01, OPB-04, OPB-08, OPB-11 and OPB-12.

Data were scored for the presence/absence of RAPD markers and evaluated by the NTSYS-pc program. A simple matching coefficient of similarity (SM=m/n, m is the number of matches and n is total sample size) was used to generate matrices of similarity. Genetic diversity at the population level was estimated by $H_e=1-\Sigma pi^2$, where pi is the frequency of allele i in the population.

3 RESULTS

Only the data of leaf morphometry and DNA analysis are presented. Data for leaf morphometry are presented in Fig. 1 to Fig. 7, sampling sites (plots) are denoted by a- Cigonca, b- Dobova and c- Hrašica.

In Fig.1, the box plot diagram of leaf petiole length is presented to estimate possible oak hybrids between *Q. robur* and *Q. petraea*. In plot Cigonca, 600 leaves were measured. The median value of the petiole length was around 0.5 cm. The longest petiole was measured in leaves collected from trees numbered 186 and 264. From Dobova plot, 332 leaves were measured. The mean value of petiole length was 0.7 cm, and the longest petioles were measured in leaves from tree no. 440. The mean values of the leaf petiole length from the Hrašica plot was 0.5 cm, with 500 leaves having been measured. The longest petioles were measured in leaves from tree no. 102.

The leaf petiole percentage is presented in Fig. 2. The mean value for the leaves from Cigonca is around 6 %, similar for leaves from Dobova, and more scattered for Hrašica, from 6 to 2%.

The frequency distribution of oak leaf lamina base types is presented in Fig. 3. On the Cigonca plot only cordate-auriculate (1) and asymmetrical (3) types , which are typical for pedunculate oak were established, the leaves from Dobova were also similar but on the Hrašica plot several leaves, from trees no. 276, 211 and 303, also had cuneate (2) leaf lamina base type, without auricula, which is a typical morphological character of sessile oak.

Leaf lobe pair number is presented in Fig. 4. Most of the leaves from all three plots (Cigonca, Dobova, Hrašica) have 3 or 4 pairs of leaf lobes, which are typical of pedunculate oak, but for some trees from the Hrašica, where the number of leaf lobe pairs exceeds 4, typical of sessile oak. In any case, the number of such leaves varies from tree to tree.

The leaf lamina index, expressed as a ratio between leaf length and leaf width, is presented in Fig. 5. Median values of that index are very similar for all trees from Dobova, being around 1.6, and much more scattered on the other two plots. The median value for the leaf index for leaves from Cigonca lies between 1.4 and 1.7, and for Hrašica between 1.6 and 2.0.

Leaf pubescence type is presented in Fig. 6. Only simple leaf hair types were established, and with few exceptions at Hrašica, pubescence was mostly established on the lower leaf surface. Pubescence was observed and assessed separately on leaf upper and lower surfaces (UPP.S - hairs on upper surface, UPP.V- hairs on veins of upper surface, UPP.M - hairs on the margins of upper surface; LW- lower leaf surface). Stellate hair types, typical of sessile oak, were not observed. The correlation between leaf width and length, presented in Fig. 7. as a regression line, is similar in all three oak populations.

In the application of RAPD markers and testing of their suitability in studies of genetic variation in oaks we used seven different primers which generated 34 polymorphic RAPD bands in all analysed trees. In Fig. 8. RAPD markers amplified by primer OPA-20 in trees from locations Cigonca and Hrašica are shown. Only well amplified fragments were scored for the presence/absence in each sample and these makers were used in the calculation of genetic diversity within populations (He) and for genetic distances, with the simple matching coefficient of similarity (SM) being used. The estimated genetic diversity within populations was 0.31 for Dobova, 0.27 for Cigonca and 0.27 for Hrašica. These figures are within the reported average genetic diversity of 0.27 reported for *Q. robur* from the isozyme data (Kremer & Petit, 1993), despite a limited number of trees used in this work. Similarity between trees in tree populations was estimated by SM and ranged from 0.412 for the most distant D284-C244 trees and for D284-H302 ones to 0.824 for the most closely related H211 and H302 trees from Hrašica. The range of SM coefficients within populations (Dobova: 0.529-0.735; Cigonca: 0.559-0.794; Hrašica: 0.588-0.824) did not indicate lower variability of trees within populations. On the basis of morphological data, trees no. C124 from Cigonca and no. D134 from Dobova, were identified as theoretical species type trees (TST) according to Cousens (1963), but SM of these two trees was only 0.676, showing a low level of similarity.

4 DISCUSSION

Using morphological data to establish oak hybrids requires that attention be paid to variation within tree species and attempts made to standardise methods of scoring and data analysis (Trinajstić, 1988, Rushton, 1993). Replicate samples of leaves from the same tree, combined with population variation and use of multivariate computer data management, are needed for the estimation of morphological data in oak populations. Typical morphological characteristics for evaluating oak hybrids are: pubescence type and density, the length of petiole and peduncle (fruit stalk), the type of leaf lamina base, the dimensions of the lamina, the number of lobes, their depth and regularity, leaf venation pattern and the morphology of acorns (Schwarz 1936, Jones 1968, Cousens 1962, 1963, 1965, Carlisle & Brown 1965, Wigston 1975, Franjić 1993, etc.). Pedunculate oak leaves are very often hairless or with a few simple hairs especially on the veins and leaf margins. The hairs are more developed on the lower surfaces of leaves, so it is important to score the hairs on the lower surface of leaves, especially on the leaf lamina. In statistical analyse, all such morphological data were used for dividing the populations of pedunculate oaks in search of possible hybrids with sessile oak (*Q. petraea* /Matt./ Liebl). According to Flora Europaea (Tutin et al., 1964) the petiole length is up to 5 mm in pedunculate oak and between 18 and 25 mm in sessile oak (Aas, G., 1993). The occurrence of hairs on the lower surfaces on veins and margins, together with petiole length and the number of lobes and leaf base type, seem to be good parameters in separating oak populations and have an important role in morphological differentiation.

The results obtained by this study show that all analysed trees from the three quite distinct pedunculate oak populations can be ranked as *Quercus robur* species type. The morphological data also indicate some evidence of hybridisation with *Quercus petraea*, especially the trees from Hrašica in the Prekmurje district, showing frequent occurrence of the cuneate leaf lamina base type without auricula and according to this character, are classified as hybrids according to that caracter. The same population has also leaves with a higher lobe pair number, typical of sessile oak. Other leaf characters like petiole length and pubescence type are of *Q. robur* type. The sampled trees from Cigonca and Dobova are more typical pedunculate oaks according to the morphological data analysis. Some trees from Cigonca, trees no. C124 and C183, and two trees from Dobova (D134, D284) can even be classified as theoretical species type (TST) according to Cousens (1965) yet unfortunately, the data do not match with the DNA analysis of the same trees. Trees from Hrašica are a from TST trees in morphological characters, DNA analysis, however, shows more similarity, in spite of the different morphology.

According to leaf index values, petiole length and percentage and pubescence type, all analysed trees are within the variation of pedunculate oak, with a slight indication of hybridisation, which is mainly demonstrated by lobe pair number and leaf lamina base type. Consequently, the introgression of sessile oak cannot be excluded, only that many more samples from different localities in Slovenia should be analysed. The presented results of the application of RAPD markers are the first attempt to use this type of molecular markers in oak. This preliminary study has shown high level of polymorphism revealed by RAPD markers within the pedunculate oak despite the limited number of analysed trees and a relatively low number of primers. The estimated values of genetic diversity within populations surprisingly match the reported values for pedunculate oak but the values on genetic similarities would require further analysis for comparison with morphological data. However, RAPD markers seem to be applicable for the study of genetic diversity in oaks; further work will focus on the analysis of a higher number of trees within the populations of species and also the analysis of other oak species.

5 REFERENCES

AAS, G. 1993. Taxonomic impact of morphological variation in *Quercus robur* and *Q. petraea*: a contribution to the hybrid controversy. Ann Sci For, 50, Suppl 1, 107s-113s.

AAS, G. 1990. Kreuzbarkeit und Unterscheidung von Stiel- und Traubeiche. All. Forstzeitschrift 9-10:219-221.

CARLISLE, A. & BROWN, A.H.F. 1965. The assessment of the taxonomic status of mixed oak (*Quercus* spp.) populations. Watsonia 6(2):120-127.

COUSENS, J.E. 1962. Notes on the status of the sessile and pedunculate oaks in Scotland and their identification. Sco. For. 16:170-179.

COUSENS, J.E. 1963. Variation of some diagnostic characters of the sessile and pedunculate oaks and their hybrids in Scotland. Watsonia 5(5):273-286.

COUSENS, J.E. 1965. The status of the pedunculate and sessile oaks in Britain. Watsonia 6(3):161-176.

DUPOUEY, J.L. & BADEAU, V. 1993. Morphological variability of oaks (Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd.) in northeastern France: preliminary results. Ann Sci For 50, Suppl 1, 35s-40s.

ERDEŠI, J. 1985. Iconography of common oak in Yugoslavia. University of Beograd, Bulletin of the Faculty of Forestry, Belgrade, (64), 109-140.

FRANJIĆ, J. 1993. Acorn as the indicator of individual variability of the common oak (*Quercus robur* L.). Glasnik, p. 9.

Garcke, A. 1972. *Quercus* L. In: Illustrierte Flora, 23. Auflage, 429-430.

JAVORNIK, B. & KUMP, B. (1992). Random amplified polymorphic DNA (RAPD) markers in buckwheat. Fagopyrum 13:35-39.

JONES, E. E. 1968. The taxonomy of the British species of *Quercus*. Proc. bot. Soc. Br. Isl. 7:183-184.

MARTINČIČ, A., F. SUŠNIK. 1969. *Quercus*. In: Mala flora Slovenije, p.213-214.

MARTINČIČ, A., F. SUŠNIK. 1984. *Quercus*. In: Mala flora Slovenije.

MARTINIS, Z., Ž. LOVAŠEN-EBERHARD AND M. TUĐA. 1987. Trihomografske i palinomorfološke karakteristike hrasta lužnjaka (*Q. robur* L.) u odnosu na druge hrastove u Jugoslaviji. Glasn. Šum. Pok., Pos. Izd. 3:347-355.

OLSSON, U., 1975. A morphological analysis of phenotypes in a population of *Quercus* (*Fagaceae*) in Sweden. Bot. Notiser. 128:55-68.

PETIT, R. J., KREMER, A., WAGNER, D.B. 1993. Geographic structure of chloroplast DNA polymorphism in Europaean oaks. Theor Appl Genet. 87:122-128.

ROTHMAHLER, W. 1991. *Quercus* L. In: Exkursionsflora, 3. Band, 8. Aufl. Berlin, 66-67.

RUSHTON, B.S. 1978. *Quercus robur* L. and *Quercus petraea* (Matt.) Leibl.: a multivariate approach to the hybrid problem, 1. Data aquisition, analysis and interpretation. Watsonia 12:81-101.

RUSHTON, B.S. 1993. Natural hybridization within the genus *Quercus* L. Ann. Sci. For. 50:73-90.

SAGHAI-MAROOF, M.A., SOLIMAN, K.M., JORGENSEN, R.A., ALLARAD, R.W. 1984. Ribosomal DNA spacer length polymorphysim in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci 81:8114-8018.

SCHWARZ, O. 1936. Monographie der Eichen Europas und der Mittelmeergebietes, Feddes Report. Sonderbuch D(5):72-173.

SCHWARZ, O. 1964. *QUERCUS* L. IN: TUTIN, T. G. et al.: Flora Europaea (1), Cambridge University Press:61-64.

SMOLE I. & BATIČ, F. 1992. The importance of morphological characteristics for identification of oak species. Zbornik gozdarstva in lesarstva (Ljubljana) 39:133-172.

TRINAJSTIĆ, I. 1974. *Quercus* L. In: Trinajstić, I. (ed). Analitička flora Jugoslavije 1 (3):460-487.

TRINAJSTIĆ, I. 1988. Taxonomy of pedunculate oak - *Quercus robur* L. in the flora of Yugoslavia. Glas. šum. pokuse, (24):101-116, Zagreb.

TRINAJSTIČ, I. & KRSTINIĆ, A. 1993. Variabilnost hrasta lužnjaka (*Quercus robur* L.). Glas. Šum. Pokuse, pos. izd. 4: 185-194. Sveučilište u Zagrebu, Šumarski Fakultet, Zagreb, Hrvatska.

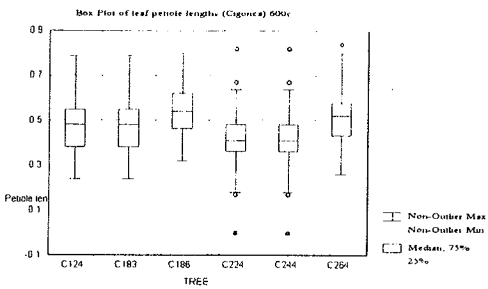
WIGSTON, D. L. 1974. Cytology and genetics of oaks. In: The British oak (Morris, M.G. & Perring, F.H., eds.): 27-50. The Botanical Society of the British Isles, E.W. Classey LTD., Faringdon, UK.

WIGSTON, D. L. 1975. The distribution of *Quercus robur* L., *Quercus petraea* (Matt.) Leibl. and their hybrids in south-western England. 1. The assessment of the taxonomic status of populations from leaf characters. Watsonia 10:345-369.

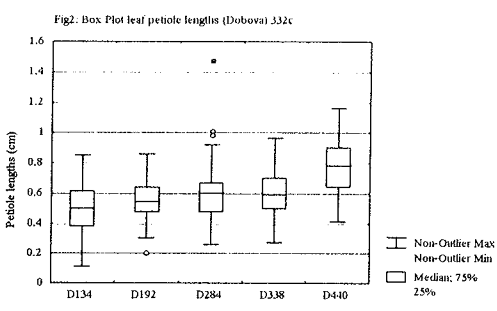
6 APPENDICES

Fig. 1: Petiole lengths (cm) of pedunculate oak leaves (Variable 1)

a) Cigonca



b) Dobova



c) Hrašica

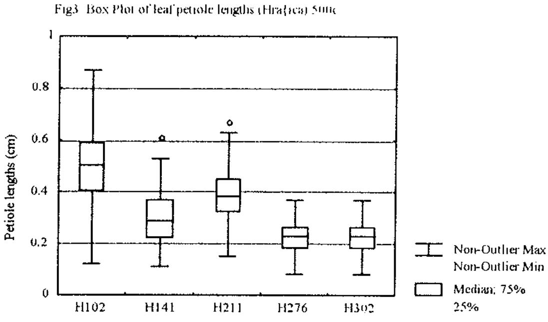
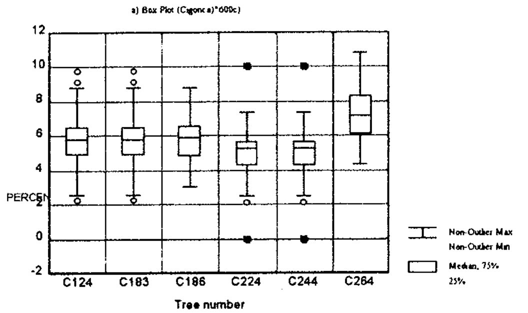
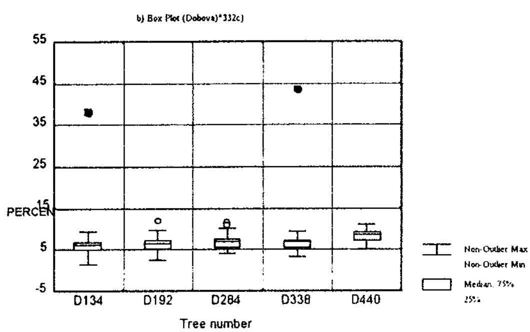


Fig. 2: Leaf petiole percentage (Variable 2)

a) Cigonca



b) Dobova



c Hrašica

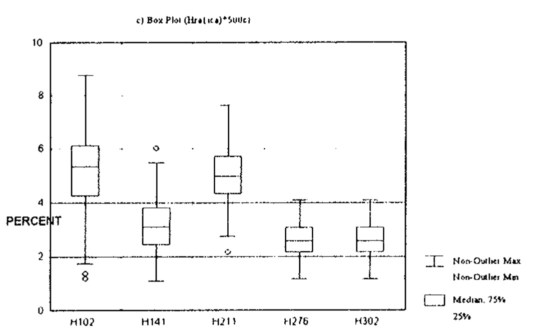
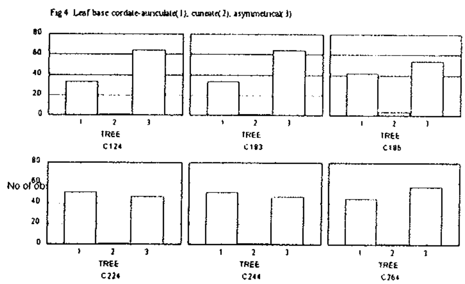
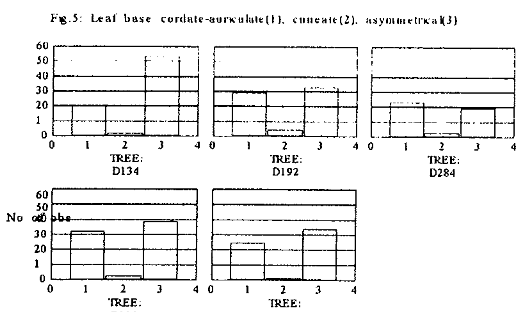


Fig. 3: Leaf lamina base types: 1-cordate (auriculate), 2-cuneate, 3-asymmetrical (Variable 3)

a) Cigonca



b) Dobova



c) Hrašica

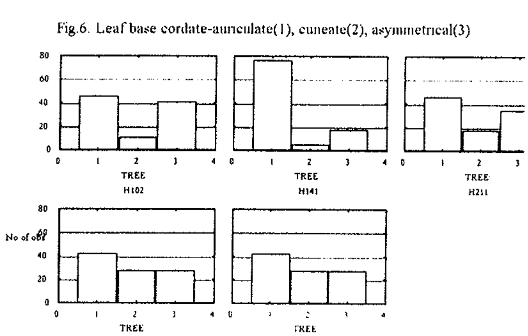
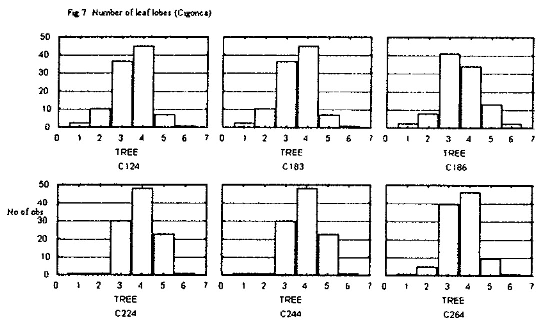
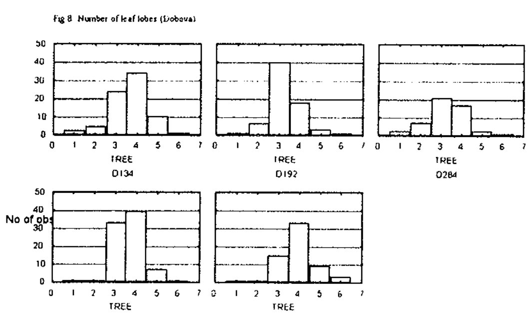


Fig. 4: Leaf lobe pair number of pedunculate oak (Variable 4)

a) Cigonca



b) Dobova



c) Hrašica

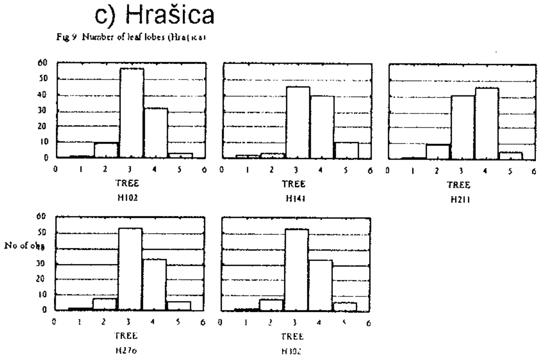
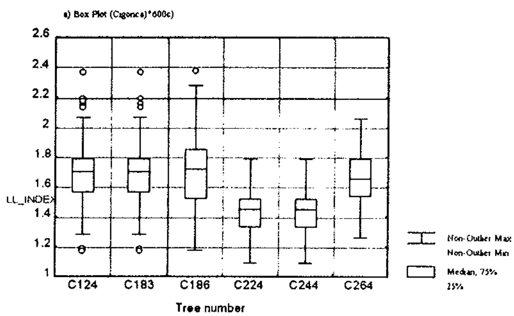
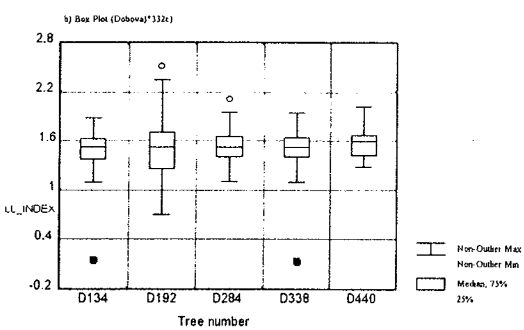


Fig. 5: Leaf lamina index (lamina length/lamina width) of pedunculate oak Variable 5)

a) Cigonca



b) Dobova



c) Hrašica

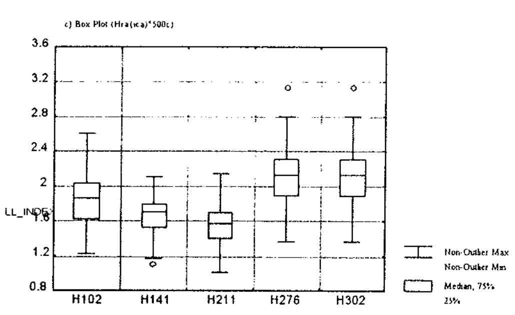
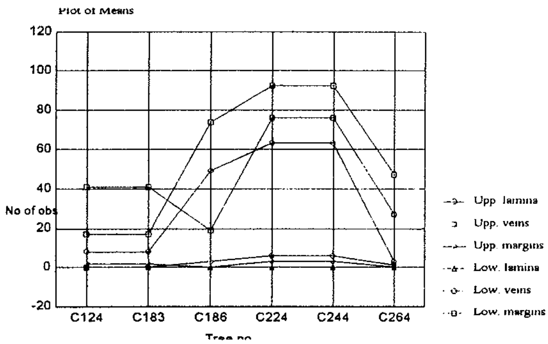
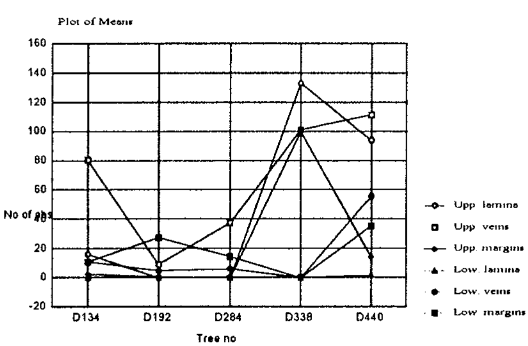


Fig. 6: Leaf pubescence of the pedunculate oak (Variable 6-11)

a) Cigonca



b) Dobova



c) Hrašica

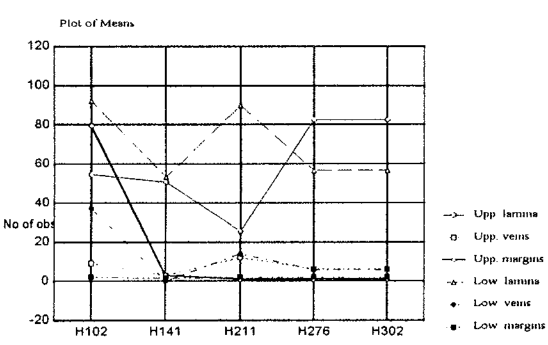
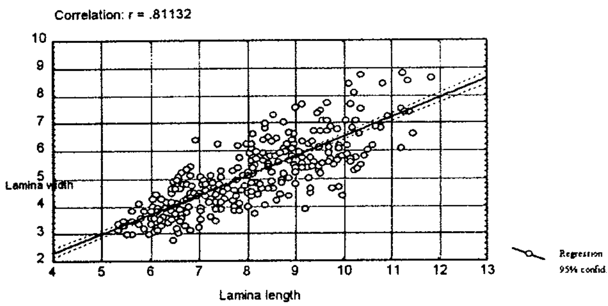
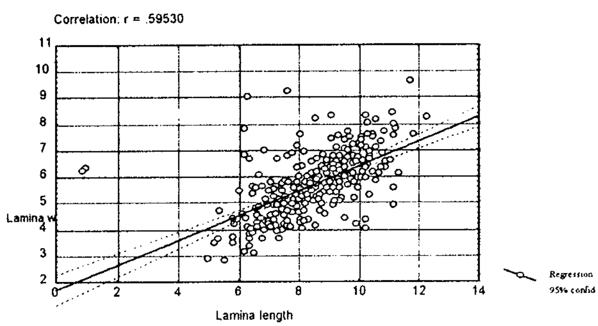


Fig. 7. Correlation between leaf lamina width and length of pedunculate oak

a) Cigonca



b) Dobova



c) Hrašica

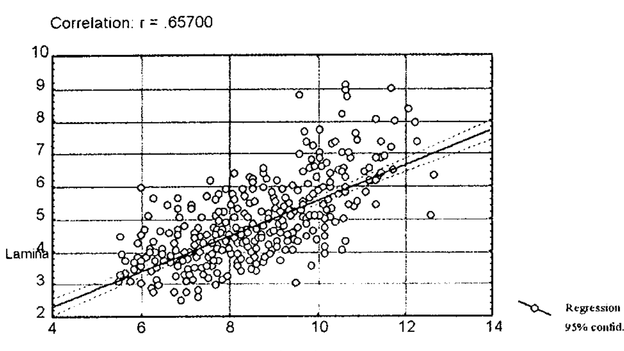


Fig. 8: RAPD markers generated from three trees from Cigonca (1,2,3) and five trees from Hrašica (4,5,6,7,8) of pedunculate oak using primer OPA 20. Line M is a DNA size marker. Arrow indicate the scored fragments.