Multivariate analysis of leaf shape of the common oak (Quercus robur L.) in the Gajno provenance test (Croatia)

Franjić, Josip; Gračan, Joso; Kajba, Davorin; Škvorc, Željko; Dalbelo-Bašić, Bojana

Source / Izvornik: **Glasnik za šumske pokuse: Annales Experimentis Silvarum Culturae Provehendis, 2000, 37, 469 - 479**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:108:051628

J. Franjić, et al.: Multivariate analysis of leaf shape of the common oak (*Quercus robur* L.) in the "Gajno" provenance test (Croatia). Glas. šum. pokuse 37: 469–479, Zagreb, 2000.

UDK 630*165+232.1 (Quercus robur L.)

MULTIVARIATE ANALYSIS OF LEAF SHAPE OF THE COMMON OAK (*QUERCUS ROBUR* L.) IN THE "GAJNO" PROVENANCE TEST (CROATIA)

JOSIP FRANJIĆ*, JOSO GRAČAN**, DAVORIN KAJBA*,
ŽELJKO ŠKVORC*, BOJANA DALBELO-BAŠIĆ*

*Faculty of Forestry, University of Zagreb, Svetošimunska 25,
HR-10000 Zagreb, Croatia
** Forest Research Institute, HR-10450 Jastrebarsko, Croatia

In this work, the results of morphological research of the common oak (*Quercus robur*) leaf shape in the provenance test in the locality of Gajno (Jastrebarsko, Croatia) are given. The provenance test was established in autumn 1987 with annual seedlings. The test included in total 16 provenances (15 from Croatia and one from Vojvodina) belonging to various bioclimatic regions. The plot was arranged in the form of a randomised block system with 100 plants per each repetition. The leaf sample for analysis was picked in July 1998, and 200 leaves of each provenance (50 per repetition) were analysed. On each leaf five traits (leaf blade length, leaf blade width, petiole length, number of lobes on the leaf blade left side and number of lobes on the leaf blade right side) were measured. Regularities in the variability of particular traits under identical stand conditions corresponding to genetic differences were determined. All measured parameters were submitted to the description and regression analysis, the variance analysis and the multivariate analysis from which the differences between the studied provenances can be clearly seen. The results of this research will be very useful in the artificial regeneration of common oak forests, which is nowadays becoming more and more important and in which the selection of adequate regeneration material is of major importance, since the microsite conditions are fundamental to adequate decision making. Besides this, the quantity of acorns per area unit would be considerably reduced.

Key words: *Quercus robur*, leaf variability, provenance test

INTRODUCTION

The common oak forests in Croatia are generally regenerated naturally. However, for reasons of a poor and irregular acorn yield in the last few decades, artificial regeneration with acorns and seedlings has been applied in addition to natural regeneration. In this process, very often seedlings which do not belong to the domicile population are used. To avoid mistakes in the common oak stand through artificial regeneration by using inadequate seedling material, intensive research of the local population genetic variability has been made in the last decade (cf. Gračan et al. 1991; Stojković 1991; Franjić 1993, 1994, 1994a, 1996, 1996a; Gračan 1993, 1993a; Gračan & Perić 1993; Krstinić et al. 1996). Since the common oak is an endangered species, it is absolutely necessary to preserve the gene pool of this species. To do this, knowledge of the genetic differentiation at the individual and population level is extremely important. Data on the genetic differentiation of local populations can generally be obtained by researching the phenotypic and genetic variability. On the basis of the phenotypic variability of local populations, conclusions can be made as to the variability type, which can be of a continuous or a discontinuous character (cf. Krstinić et al. 1996).

Continuous variability is typical of compact populations and is generally expressed as a clinal variability. Through research of phenotypic variability, the clinal variability of morphological leaf traits in Croatia has been determined (cf. Franjić 1994), although discontinuous populations were involved, but their compactness has been affected anthropogenically by the large felling of forests in the Sava River region where the research was carried out.

Discontinuous variability suggests the existence of morphological and physiological races, known by the name of geographical variability. This type of variability is the consequence of changed macroecological conditions in the area where a species grows, as well as of the spatial isolation of local populations. Through earlier morphometric research of discontinuous populations belonging to various climatic regions, a very strong resemblance of populations with similar microsite characteristics was determined (Franjić 1993, 1994, 1996, 1996a, 1996b; Franjić et al. 1999).

In his research of the common oak populations in the Sava River and the Drava River regions in Croatia, Franjić (1993a, 1994, 1996, 1996a) arrived at the conclusion that each population has approximately the same number of genotypes, and the only difference occurs in the frequency of particular genotypes in the respective population. Either by ignorance or because the common oak variability and adaptability were not taken into account sufficiently, acorns were very often transported and planted in unsuitable sites and in this way the regeneration or refilling of the common oak stands was made. Today we are in a situation where the common oak is endangered and where considerable funds are earmarked for the regeneration of its forests and the preservation of its gene pool. But in spite of that, control of its regeneration is still inadequate. The problem is that the consequences cannot be seen immediately and that very often they coincide with other causes (such as acid rain, heavy defoliation and the like), that makes identification of the real cause of common oak withering rather difficult.

For all these reasons, it is necessary to determine experimentally and as soon as possible the genetic differentiation of local populations (provenances) and this by (generative or vegetative) progeny testing under the same conditions. Such research is important for all exploitable species, and in particular for the common oak as the most important exploitable tree species in Croatia.

The purpose of this research is to obtain a clearer picture of the genetic structure of each researched provenance. On the basis of such research, for artificial regeneration it will be possible to use a considerably smaller number of plants per

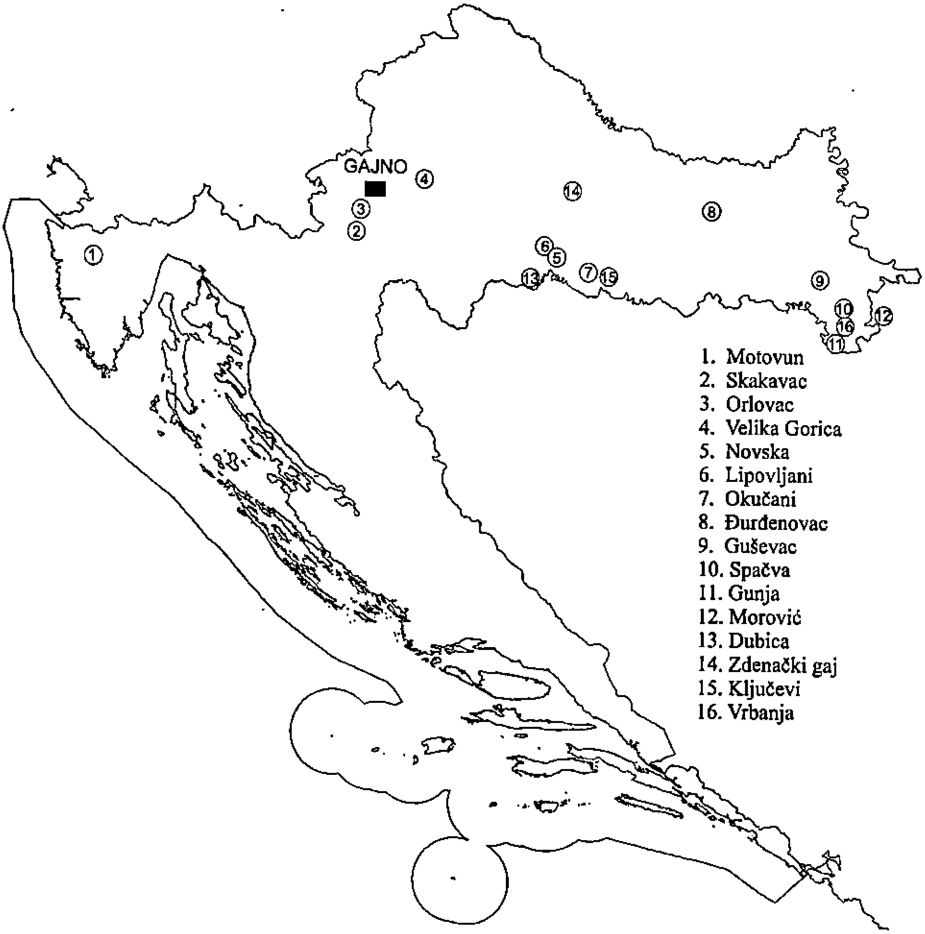

Figure 1. Distribution of 16 provenances of common oak (*Quercus robur* L.) in field trialy "Gajno" (Croatia)

area unit, thus reducing substantially the costs of artificial regeneration of the common oak forests.

MATERIAL AND METHODS

Acorns necessary for the establishment of provenance tests were harvested in common oak seed stands in autumn 1985 (cf. Gradečki & Poštenjak 1987; Gračan et al. 1991). The provenance test "Gajno" was established in autumn 1987 and in spring 1988. In the provenance test, there are in total 16 provenances (15 from Croatia and 1 from Vojvodina) belonging to various bioclimatic regions (cf. Fig.1). The plot was arranged in the form of a randomised block system with 100 plants in each repetition. The soil in the "Gajno" plot was marshy-amphigley-mineral-clay soil over clay deposits of the Okičnica and Gonjeva streams, at an elevation of 115 m (cf. Mayer 1989; Gračan et al. 1991). The leaf sample for analysis was picked in

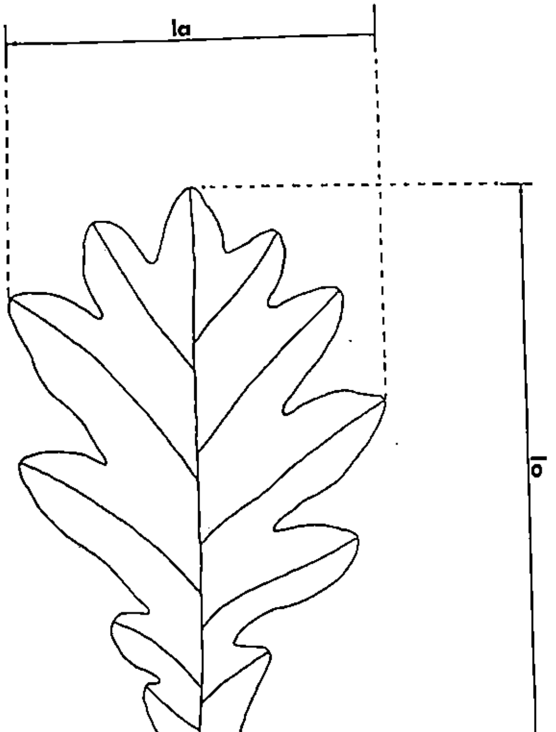

Figure 2. Representation of the measured leaf parameters (la - leaf blade width; lo - leaf blade lenght; lp - petiole lenght)

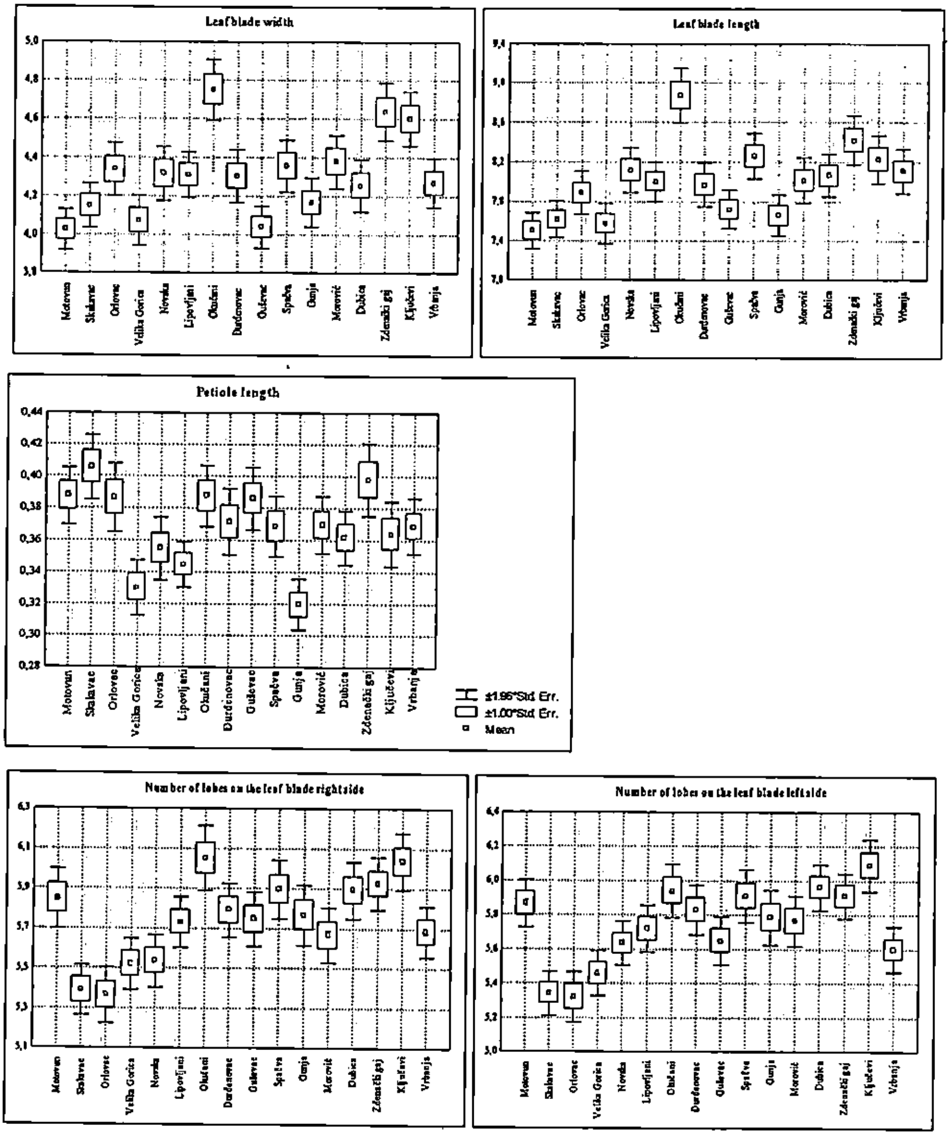

Figure 3. 95 % confidence interval (Box & Whisker Plot) of means by provenances for all five traits

July 1998 and put in the herbarium. Measuring was made immediately after herbarising and included 200 healthy and fully developed leaves of each provenance (50 per repetition). On each leaf five traits were measured (leaf blade length, leaf blade width, petiole length, number of lobes on the left side and number of lobes on the right side of leaf blade; cf. Fig. 2). Measuring was made with an accuracy of 1 mm.

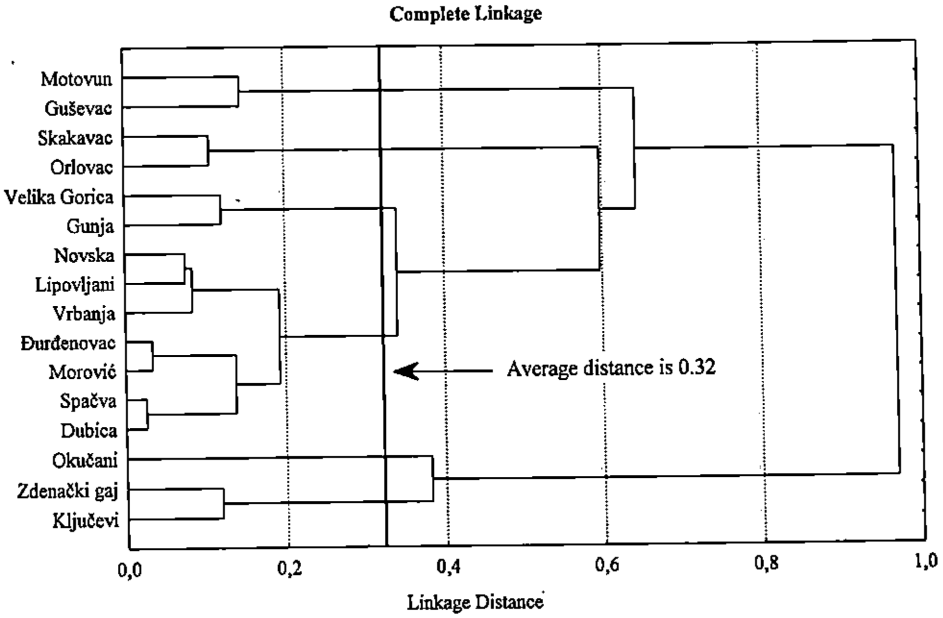

Figure 4. Dendrogram obtained by the Complete Linkage method

The measured traits were analysed in detail using descriptive statistics. The variance univariant analysis (ANOVA) for particular leaf traits (cf. Fig. 3), the variance multivariate analysis (MANOVA) and the multivariate cluster analysis were made. For this, algorithms for Ward's Complete Linkage method were used, namely Unweighted Pair Group Average (UPGMA), Weighted Pair Group Average (WPGMA), Unweighted Pair Group Centroid (WPGMC), and Weighted Pair Group Centroid (WPGMC), (cf. McClare & Dietrich 1988; Sharma 1996; Johnson 1998). All methods show a similar form and regularity of provenance linkage, but, since in this particular case the "Complete Linkage" method has the highest cophenetic correlation coefficient, this method was used for interpretation purposes (cf. Fig. 4). All used statistical data processing methods were done in the program package Statistics for Windows 4.5 (cf. StatSoft™ 1994).

ANALYSIS OF THE RESEARCH RESULTS AND DISCUSSION

The analysed provenances belong to various bioclimatic regions (areas) which differ between them very much by their macroecological characteristics (cf. Gračan et al. 1999). The common oak is a species very sensitive to changes in its site, so during evolution this species has undergone a fine adaptation to microsite conditions that has resulted in its differentiation into a series of ecotypes (cf. Trinajstić 1974, 1988; Franjić 1993, 1993a, 1994, 1996, 1996a, 1996b; Trinajstić & Krstinić 1993). For the territory of Croatia, the common oak differs very clearly, both morphologically and ecologically, from other oak species, because it occupies floodplain and, for other oaks, very selective sites. The only problem is introgression, where one or more nonselective traits of another congenial oak species (such as the sessile oak or bitter oak) can enter the common oak genom, which can cause great difficulties in the determination of the common oak (cf. Franjić 1993, 1993a, 1994).

From the results of the descriptive analysis, it can be seen that the researched provenances link between them into certain groups, and in these the form and the regularity of linkages do not depend on the macroecological belonging of the provenances (belonging to a certain area), (cf. Gračan 1996; Gračan et al. 1999). However, as stated in the introduction, this linkage certainly depends on the microsite conditions (cf. Franjić 1993, 1994, 1996, 1996a; Franjić et al 1999). Moreover, through the descriptive analysis at a very high level, for all researched properties and for all researched provenances, the right hand (+) asymmetry and the positive (+) flatness have been determined, which corresponds to the research made by Franjić (1996, 1996a, 1996b) on adult plants. The arithmetical average absolute values for all researched traits and all provenances in certain parts of the range in Croatia show a tendency of an increase or a decrease in the east→west direction that can be connected with clinal variability. Such a tendency is disturbed, however, by some provenances, which suggests ecotypical differences under the influence of microsites.

The multivariate analysis (MANOVA) confirmed the existence of significant differences in the researched traits between the provenances. Differences in traits for particular provenances can also be seen in the descriptive statistics graphs. For reasons of limited space, we shall present here the 95% confidence interval (Box & Whisker Plot) of the means by provenances for all five traits (cf. Fig. 3).

Considering that the statistically significant difference of provenances was obtained, various cluster analysis methods were used. All methods show a similar form and regularity of provenance linkage, and since in this particular case the "Complete Linkage" method has the highest cophenetic correlation coefficient, this was used for interpretation purposes (cf. Fig. 4).

In Fig. 4, six clusters which differ at a very high level can be clearly seen. The number of clusters was determined on the basis of the average distance which is 0.32 (cf. Fig. 4; Mahalanobis 1936), and the provenances are linked into clusters in the following way:

1. To the first cluster belong the provenances Motovun and Guševac
2. To the second cluster belong the provenances Skakavac and Orlovac
3. To the third cluster belong the provenances Velika Gorica and Gunja
4. To the fourth cluster belong the provenances Novska, Lipovljani, Vrbanja, Đurđenovac, Morović, Spačva and Dubica
5. To the fifth cluster belongs the provenance Okučani

6. To the sixth cluster belong the provenances Zdenački gaj and Ključevi.

From the mentioned clusters, it can be seen that very rarely do the provenances belonging to the same area link together (cf. Fig. 1 and 4), which, in practice, is the basis for seed (acorn) distribution. Such a situation is absolutely logical and expected, since, as said before, similar results have already been obtained (cf. Franjić 1993, 1994, 1996, 1996a, 1999) where it was emphasised that for seed distribution the most important factor is to determine the compatibility of microsites and not the geographical belonging. Thus, for instance, there is a linkage between:

- Motovun (Istria) and Guševac (Lower Posavina)
- Velika Gorica (the Upper Sava Region) and Gunja (Lower Posavina)
- Novska and Lipovljani (Central Posavina), Vrbanja and Spačva (Lower Posavina); Đurđenovac (Podravina), Morović (Srijem) and Dubica (Upper Posavina).

Although almost no regional links exist here, the soil and phytosociological belonging are basic properties linking these provenances into certain entities (cf. Gradečki & Poštenjak 1987; Gračan et al. 1991; Gračan & Perić 1993).

The provenances are very rarely linked according to their geographical belonging, such as:

- Skakavac and Orlovac (Upper Posavina)
- Zdenački gaj and Ključevi (Central Posavina), to which, at a somewhat higher level, the Okučani provenance belonging to Central Posavina is also linked.

All this can be connected to the accidental selection of provenances during the establishment of the tests, as well as to the coincidence of the soil type and the phytosociological belonging. By statistical analysis, it was again determined on this material, similar to earlier research (cf. Franjić 1993, 1994, 1996, 1996a, 1999), that in each common oak population in Croatia there is an approximately identical number of genotypes, and that the proportion of genotypes in the respective population depends on the microecological properties of the site.

CONCLUSION

The common oak forests in Croatia are generally regenerated naturally, but for reasons of a poor and irregular acorn yield during the last few decades, in addition to natural regeneration, artificial regeneration with acorns and seedlings has also been applied. By doing this, very often the seedling material which does not belong to the domicile population has been used. To avoid mistakes in the artificial regeneration of the common oak stands by using inadequate seedling material, intensive research of the genetic variability of local populations has been done in Croatia in the last decade.

Thus, in autumn 1987 and in spring 1988, the provenance test "Gajno" was established with a total of 16 provenances (15 from Croatia and 1 from Vojvodina) belonging to various bioclimatic regions (cf. Fig. 1; Gračan et al. 1991, 1999; Gračan 1996). In this test, a leaf sample for morphometrical analysis was harvested. In total, 200 fully developed and healthy leaves of each provenance were analysed and on each leaf five traits (leaf blade length, leaf blade width, petiole length, number of lobes on the leaf blade left side and number of lobes on the leaf blade right side cf. Fig. 2) were measured. The purpose of this research was to obtain a clearer picture of the genetic structure of each researched provenance.

All measured parameters were analysed. From the results of the analysis it can be seen that the researched provenances link between themselves into certain groups (cf. Fig. 3 and 4), and the form and the regularity of this linkage of provenances do not depend on their macroecological belonging (area belonging) but (cf. Gračan 1996; Gračan et. al. 1999), as pointed out in the introduction, the linkage of provenances undoubtedly depends on the microsite conditions (cf. Franjić 1993, 1994, 1996, 1996a; Franjić et al. 1999). From the given results (cf. Fig. 1 and 4), it can be seen that the linkage of provenances belonging to the same area, which in practice is the basis for seed (acorn) distribution, is very rare. Such a situation is absolutely logical and expected, since earlier research has given similar results (cf. Franjić 1993, 1994, 1996, 1996a; Franjić et al. 1999). Attention was then brought to the fact that of greatest importance in seed distribution is to determine the compatibility of microsites, not the geographical belonging.

By statistical analysis it was determined that on this material, similar to earlier research (cf. Franjić 1993, 1994, 1996, 1996a; Franjić et al. 1999), in each common oak population in Croatia there is approximately the same number of genotypes, and that the proportion of genotypes in the respective population depends on the microecological properties of the site.

REFERENCES

Franjić, J., 1993: Veličina žira kao pokazatelj individualne varijabilnosti hrasta lužnjaka (*Quercus robur* L.). Glas. šum. pokuse, Posebno izd. 4: 195-205.

Franjić, J., 1993: Morfometrijska analiza lista i ploda hrasta lužnjaka (*Quercus robur* L.) u Hrvatskoj. Magistarski rad - PMF. Zagreb.

Franjić, J., 1994: Morphometric leaf analysis as an indicator of common oak (*Quercus robur* L.) variability in Croatia. Ann. Forest. 19(1): 1-32.

Franjić, J., 1994a: Dužina i širina plojke lista kao pokazatelji varijabilnosti hrasta lužnjaka (*Quer-cus robur* L.). Simpozij Pevalek - Flora i vegetacija Hrvatske, 25-34. Koprivnica-Zagreb.

Franjić, J., 1996: Multivarijatna analiza posavskih i podravskih populacija hrasta lužnjaka (*Quercus robur* L., *Fagaceae*) u Hrvatskoj. Doktorska disertacija PMF. Zagreb.

Franjić, J., 1996a: Multivariety analysis of leaf properties in the common oak (*Quercus robur* L., *Fagaceae*) populations of Posavina and Podravina in Croatia. Ann. Forest. 21(2): 23-60.

Franjić, J., 1996a: Morfometrijska analiza varijabilnosti lista posavskih i podravskih populacija hrasta lužnjaka (*Quercus robur* L., *Fagaceae*) u Hrvatskoj. Glasn. šum. pokuse 32: 153-214.

Franjić, J., B. Dalbelo-Bašić, Ž. Škvorc, 1999: Acorn form variability in the common oak (*Quercus robur* L.) in Croatia. Sauteria 10: (in press).

Gračan, J., 1993: Preliminary results of common oak (*Quercus robur* L.) provenance experiments in Croatia. Ann. Sci. For. 50(1): 215-221.

Gračan, J., 1996: Masovna selekcija. In: D. Klepac: Hrast lužnjak (*Quercus robur* L.) u Hrvatskoj. Hrvatska akademija znanosti i umjetnosti, Centar za znanstveni rad Vinkovci, J.P. Hrvatske šume. Vinkovci - Zagreb.

Gračan, J., N. Komlenović, P. Rastovski, 1991: Pokus provenijencija hrasta lužnjaka (*Quercus robur* L.). Šum. list 115(6-9): 245-260.

Gračan, J.,I. Trinajstić, Ž. Orešković, Z. Perić, J. Franjić, 1995: Growth of common oak (*Quercus robur* L.) provenances in Croatia. Rad. Šum. Inst. Jastreb. 30(2): 109-123.

Gračan, J., A. Krstinić, S. Matić, Đ. Rauš, Z. Seletković, 1999: Šumski sjemenski rajoni (jedinice) u Hrvatskoj. Rad. Šum. Inst. Jastreb. 34(1): 55-93.

Johnson, D. E., 1998: Applied Multivariate Methods for Data Analysts, Duxbury Press.

Krstinić, A., Trinajstić, I., Gračan, J., Franjić, J., Kajba, D., Britvec, M., 1996: Genetska izdiferen-ciranost lokalnih populacija hrasta lužnjaka (*Quercus robur* L.) u Hrvatskoj. In: Matić, S.,J. Gračan (ed.): Skrb za hrvatske šume od 1846. do 1996. Zaštita šuma i pridobivanje drva 2: 159-168. Hrvatsko šumarsko društvo. Zagreb.

Mahalanobis, P. C., 1936: On the generalized distance in statistics. Proc. Nat. Inst. Sci. India 2: 49-55.

Mayer, B., 1989: Pedološka karta područja nizinskih šuma Pokupskog bazena (kompilacija) 1:50000 (disertacija). Šumarski institut Jastrebarsko.

McClare, J. T., F. M., Dietrich, 1988: Statistics, Fourth Ed., Dellen Publishing Company, San Fransisco.

Sharma, S., 1996: Applied multivariate techniques. John Wiley & Sons. New York.

StatSoft™, 1994: Statistics for Windows, Volume I: General Conventions & Statistics I.

Stojković, M., 1991: Varijabilnost i nasljednost listanja hrasta lužnjaka (*Quercus robur* L.). Glas. šum. pokuse 27: 227-259.

Trinajstić, I., 1974: *Quercus* L. In: I. Trinajstić (ed.) Analitička flora Jugoslavije 1(3): 460-481.

Trinajstić, I., 1988: Taksonomska problematika hrasta lužnjaka *Quercus robur* L. u flori Jugoslavije. Glas. šum. pokuse 24: 101-116.

Trinajstić, I., A. Krstinić, 1993: Varijabilnost hrasta lužnjaka (*Quercus robur* L.). Glas. šum. pokuse, Posebno izd. 4: 35-44.

MULTIVARIJATNA ANALIZA OBLIKA LISTA HRASTA LUŽNJAKA (*QUERCUS ROBUR* L.) U TESTU PROVENIJENCIJA GAJNO (HRVATSKA)

U radu će biti prikazani rezultati morfometrijskih istraživanja oblika lista hrasta lužnjaka (*Quercus robur*) u testu provenijencija na lokalitetu Gajno. Test provenijencija osnovan je u jesen 1987. godine s jednogodišnjim sadnicama. Testom je obuhvaćeno ukupno 16 provenijencija (15 iz Hrvatske i 1 iz Vojvodine), koje pripadaju različitim bioklimatskim područjima. Pokusna je ploha postavljena u obliku nasumce izabranoga blok-sustava sa 100 biljaka po svakome ponavljanju. Uzorak lišća za analizu ubran je u srpnju 1998. godine, a analizirano je po 200 listova svake provenijencije (50 po ponavljanju). Na svakom listu mjereno je 5 svojstava (duljina i širina plojke, duljina peteljke, broj režnjeva s lijeve i s desne strane plojke). Istraživanjima se nastoji utvrditi zakonitost varijabilnosti pojedinih svojstava u identičnim stanišnim uvjetima, koji bi odgovarali genetskim razlikama. Svi izmjereni parametri podvrgnuti su deskriptivnoj, neparametrijskoj i multivarijatnoj analizi iz koje se mogu jasno uočiti sličnosti i razlike među istraživanim provenijencijama. Rezultati će ovih istraživanja biti veoma korisni pri umjetnoj obnovi šuma hrasta lužnjaka, koja danas sve više dobiva na značenju. Prilivno je važan izbor odgovarajućega materijala za obnovu jer su mikrostanišni uvjeti od izuzetnoga značenja pri toj odluci. Prethodnim je istraživanjima utvrđeno da svaka populacija ima po prilici isti broj genotipova, a razlika se javlja jedino u preživljavanju pojedinih genotipova. Rezultatima ovih istraživanja dobit će se jasnija slika genetske strukture svake istraživane provenijencije, a pri umjetnoj će se obnovi moći ići s manjim brojem jedinki po jedinici površine, čime će se znatno smanjiti troškovi umjetne obnove lužnjakovih šuma.

Ključne riječi: *Quercus robur*, varijabilnost lista, test provenijencija