

**GENETIC CHARACTERISATION OF GENOTYPES OF AUSTRIAN PINE
(*Pinus nigra Arnold*) POPULATIONS USING PROTEIN MARKERS**

Aleksandar LUČIĆ¹, Ana NIKOLIĆ², Snežana MLADENOVIĆ DRINIĆ²,
Vasilije ISAJEV³ and Vera LAVADINOVIĆ¹

¹Institute of Forestry, Belgrade

²Maize Research Institute, Zemun Polje, Belgrade

³Faculty of Forestry, Belgrade

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The occurrence of Austrian pine over large areas attacked by erosion processes or on completely degraded and denuded areas on which it produces excellent results, classifies it among the most important economic species in forestry. The aim of present research is to study intra- and inter-population genetic variation in Austrian pine by the application of biochemical markers. A better knowledge of the genetic potential of Austrian pine populations will enhance the production of seed and planting material, and in this way also the success of afforestation and the establishment of Austrian pine specific-purpose plantations. The polymorphism of protein markers was determined based on the selected genotypes originating from six populations (seed stands), which actually included all Austrian pine populations in Serbia. Based on the derived electrophoregram, qualitative and quantitative differences (number and

Corresponding author: Aleksandar Lučić, Institute of Forestry, Kneza Višeslava 3, 11000 Belgrade, Serbia, Phone:011-3553355, e-mail:aleksandar.lucic@gmail.com

pattern) in protein fractions were identified and the seed protein profile was constructed for each tree, as well as for each of the six study populations. This showed the “conservativeness“ of some polypeptides, i.e. the presence in all analysed genotypes. On the other hand, some protein fractions were variable at the population level, and some were variable depending on the population. Based on the electrophoregram, the coefficient of genetic similarity/distance was calculated by comparing the band numbers and patterns. Cluster analysis dendrograms were also constructed. The degree of genetic variation among the provenances was higher than the variation within the provenances. There was also a high genetic variation in seed proteins within the analysed populations.

Key words: Austrian pine, proteins, polymorphism, seed

INTRODUCTION

For more than a century, Austrian pine has been in the focus of forestry scientific and professional public. The interest in this species is primarily the result of its characteristics: high genetic potential and genetic variation, as well as the taxonomic complexity and plasticity of the species. Its protective and reclamation function on the most severe terrains is the consequence of its extraordinarily low site demands. Austrian pine thriving over large areas attacked by erosion, or on completely degraded and denuded areas, where it produces excellent results, classifies it among the most important economic species in forestry. All the above Austrian pine characteristics, along with its wide native range and disjunct distribution, led to Austrian pine intensive introduction to the sites outside its native range, which resulted in a great number of subspecies and transition forms. The result of this natural variation is a high genetic potential which provides the base and the potential for the successful breeding of the species.

Taking into account the advancement of the concepts of nursery production, i.e. the tendency of abandoning the planting material production at the level of the species (TUČOVIĆ *et al.*, 1996, ISAJEV *et al.*, 1998, MATARUGA *et al.*, 1998), and in the aim of the most efficient and fast realisation of the tasks, it is necessary to enhance the study of the genetic potential of Austrian pine provenances, because its directed implementation can improve the production of seed and planting material, and in this way also the success of afforestation and establishment of specific-purpose plantations of this species.

Consistent with the above problem which is assigned to modern forestry, the objective of the present study is to research and identify the genotypes of Austrian pine in Serbia, as one of the most important commercial species, irreplaceable in afforestation.

MATERIAL AND METHOD

The Official List of seed stands in Serbia includes six Austrian pine seed stands, which are presented in Table 1.

Table 1: Main data on seed stands (Source – Official List of seed stands in 2005)

No.	Registration number	Name of seed stand	User or owner
I	S 01.03.02.08	MU "Divan-Breze", 27a.	FE "Šumarstvo" Raška
II	S 01.03.02.14	MU "Divan-Lokva", 21a	FE "Šumarstvo" Raška
III	S 01.03.02.15	MU "Crni Vrh Ljeskovac", 69 c.	FE "Prijeopolje" Prijeopolje
IV	S 01.03.02.07	MU "Šargan", 22 b.	FE "Užice" Užice
V	S 01.03.02.03	MU "Goč-Gvozdac", 92b.	Faculty of Forestry –Belgrade - Goč
VI	S 01.03.02.04	MU "Studenica-Polumir", 17c, 26a.	FE "Stolovi" Kraljevo

Thanks to the occasional abundant yield of Austrian pine seed stands in Serbia, in October and November 2005, it was possible to collect the seeds at all six sites and in this way to embrace all Austrian pine seed stands.

The proteins were isolated from seeds of 55 trees of six populations, according to WANG *et al.*, 1994, and separated by PAGE according to LEAMMLI, 1970. The qualitative and quantitative differences (number and pattern) in protein fractions were detected based on the electrophoregrams. Coefficients of similarity were calculated after JACCARD (1908) and SOKAL and MICHENER (SM) (1958). Cluster analyses were carried out on the matrix of genetic similarities using the unweighted pair-group method using arithmetic averages (UPGMA) clustering algorithm. The dendrograms were constructed using NTSYS-PC software (ROHLF, 2000).

RESULTS AND DISCUSSION

The polymorphism of protein markers intra and inter Austrian pine populations was identified based on the selected genotypes within each provenance. The analysis of the derived electrophoregrams shows clearly that the analysed samples had a specific protein pattern.

In order to determine the genetic similarity/distance among the study genotypes, cluster analysis was performed based on the analysis of protein markers within Austrian pine populations.

The similarity matrices after Jaccard and Sokal and Michener produced dendrograms with the identical pattern of genotypes within population I, and

genotypes within population II, i.e. VI, whereas the values of genetic similarity were in different ranges. The dendrogram analysis of genotypes in population II distinguishes two subclusters and one line (Fig. 1). One subcluster consists of genotypes II-1, II-7 and II-8, and the other subcluster is formed of genotypes II-2, II-4, II-3, II-5 and II-6. It should be noted that the grouping within subclusters occurs at small genetic distances. The line which consists of genotypes II-9 and II-10 is loosely linked to the above clusters. Based on protein image, genotypes II-2 and II-4, as well as II-9 and II-10, are genetically identical.

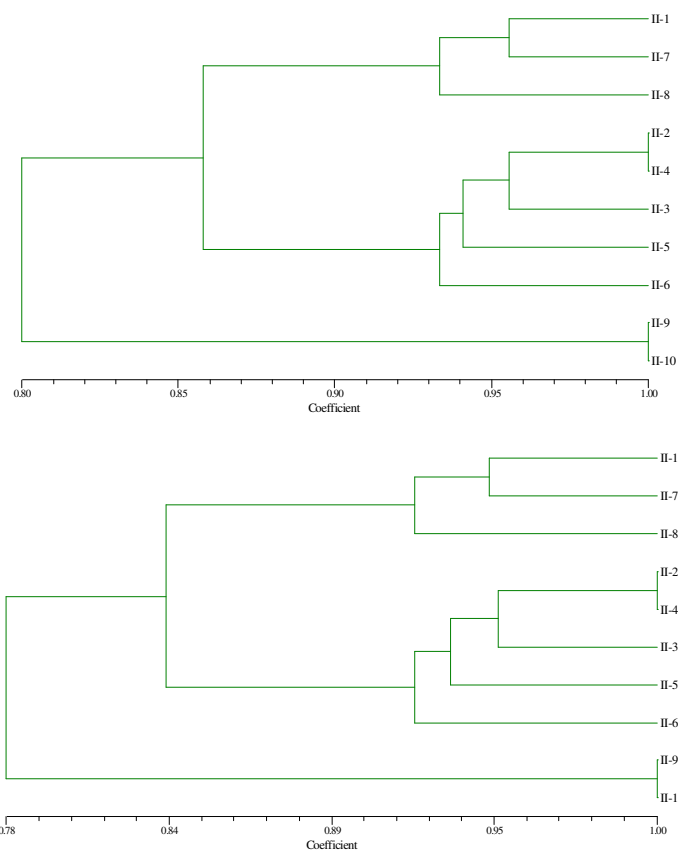


Figure 1. Dendrogram of population II genotypes after Sokal and Michener (a) and after Jaccard (b)

It is characteristic for the populations III, IV and V, that the similarity matrices after Jaccard and Sokal and Michener did not produce dendrograms with the identical patterns of genotypes, and the values of genetic similarity were also in different ranges. The dendrogram analysis for population IV shows two subclusters

and one loosely linked line, which consists of IV-3 genotype (Fig. 2). One subcluster consists of genotypes IV-1, IV-4, IV-9, IV-10 and IV-5, while the other subcluster consists of genotypes IV-2, IV-7, IV-6 and IV-8, which have different patterns depending on the applied method. Based on the analysis of protein markers, it is not possible to separate the genotypes IV-4 and IV-9.

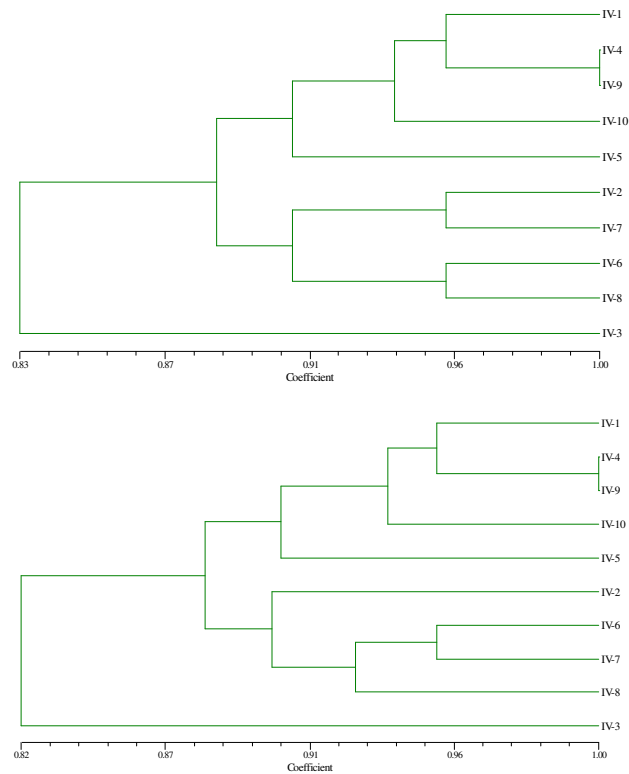


Figure 2. Dendrogram of population IV genotypes after Sokal and Michener (a) and after Jaccard (b)

Population V is characterised by the fact that it has a significantly different pattern of genotypes, depending on the applied method. The common characteristic of both methods is the loose link of genotypes V-2 and V-6 to other genotypes (Fig. 3).

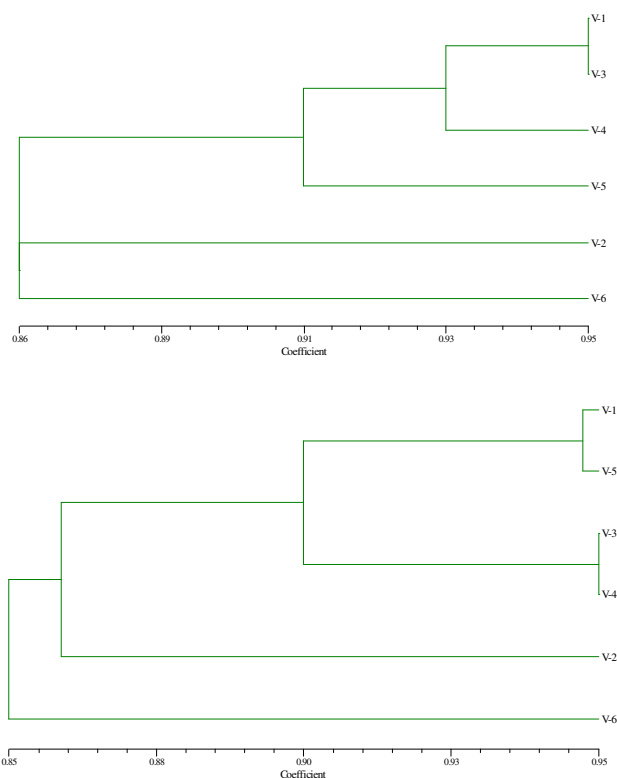


Figure 3. Cluster analysis dendrogram of population V after Sokal and Michener and after Jaccard

The values of intrapopulation diversity in protein markers were different among the six Austrian pine populations under study. The similarity coefficients of seed proteins of the above populations showed a high variation, which ranged from 0.64 to 0.95.

The analysis of polypeptide pattern, individual for each genotype, based on the cluster analysis (Fig. 4) shows, with minor deviations, that the genotypes clustered in two groups, based on both similarity coefficients. The larger group consists of the genotypes in five provenances (I, II, III, V and VI) and the other group consists of the genotypes in provenance IV. The genotype pattern in groups and subgroups differs depending on the applied coefficient. Based on Jaccard coefficients, the genotypes in provenance III are grouped together, making one subgroup, which also includes genotype V-5. The genotypes in provenance I, II and VI are grouped in smaller subgroups within group I, while the genotypes in provenance V are grouped without any rule. Based on Sokal and Michener coefficients, genotypes in provenance IV form a special group. Within the first

group, there are several differences in genotype patterns in subgroups according to Jaccard's dendrogram. Thus, genotype I-8, according to Sokal and Michener, is grouped together with another three genotypes in provenance I, whereas according to Jaccard, this genotype is grouped together with genotype II-7. Three genotypes in provenance V are also grouped together with the genotypes in provenance III. Based on the above dendrograms, at the population level, the genotypes are grouped within individual provenances. This confirms the minor differences in site conditions and the differences among genotypes, and emphasises the significance of the provenances.

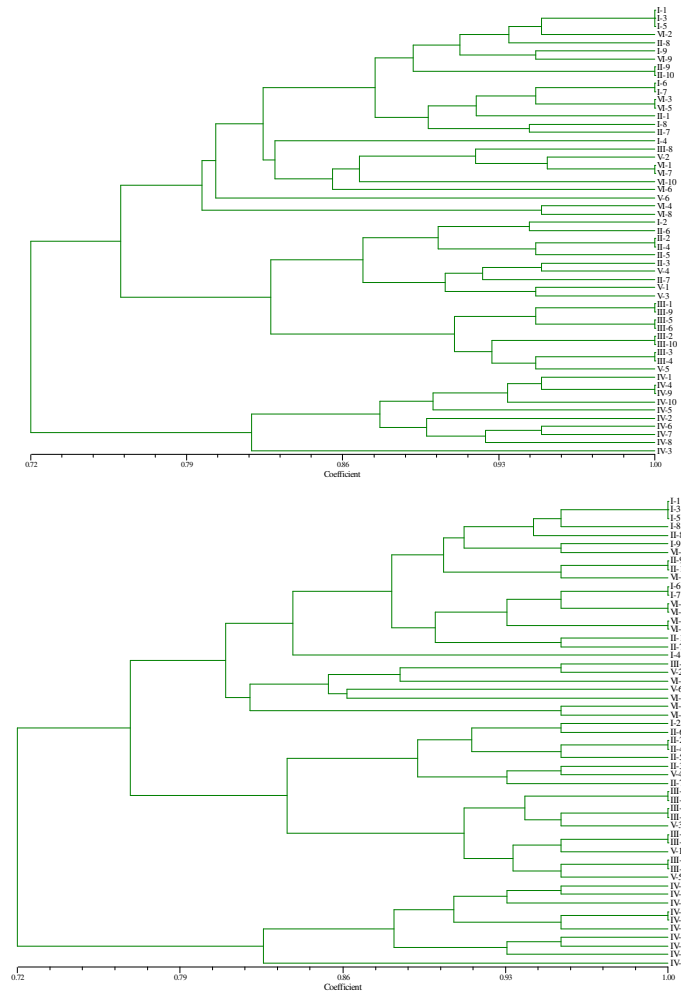


Figure 4. Dendrogram of genotypes of six Austrian pine populations after Sokal and Michener (a) and after Jaccard (b)

The results of the analysis of protein complex show that seed samples from different provenances had different protein images. Two seeds were taken from each of 55 trees in six provenances and grouped by localities, after which seed proteins were isolated from the formed sample and analysed by electrophoresis. The differences in number, pattern and intensity of protein fractions were determined. Total number of protein fractions in gel was 22, of which 11 were polymorphic (50%). Seed protein profile was established based on the electrophoregram (Fig. 5) for each of the six study provenances and the genetic similarity coefficients among the study provenances were calculated.

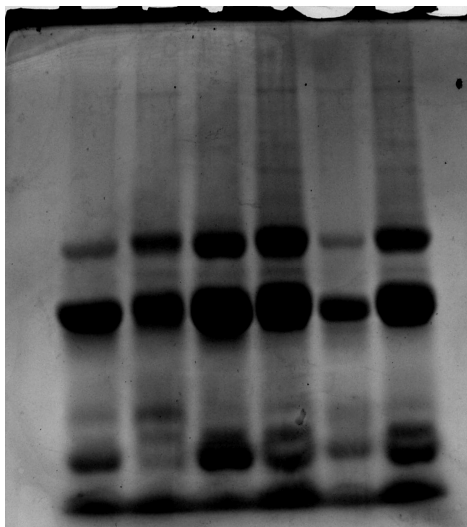


Figure 5. Electrophoregram of seed proteins of group samples

To determine the genetic similarity/distance among the study genotypes, cluster analysis was performed based on the analysis of protein markers among Austrian pine populations (Fig. 6).

Similarity matrices after Jaccard and Sokal and Michener generated the dendrograms with the identical pattern of genotypes, while the values of genetic similarity were in different ranges. Cluster analyses of the populations were characterised by a high genetic diversity among the study populations expressed by the dendrogram form. The comparative analysis of the generated dendrogram shows clearly that, populations I and VI, as well as III and V, were at the smallest genetic distances. Population II was more loosely linked to populations I and VI than to III and V. An interesting characteristic of both dendrograms is the character of the link of population IV to other populations. Austrian pine on Mt. Šargan had

an extremely loose link to other populations, which was the result of a great genetic distance compared to other populations. Similarity matrices according to Jaccard and Sokal and Michener were compared by Mantel test. A high degree of correlation was established between pairs of similarity matrices ($r=0.997$).

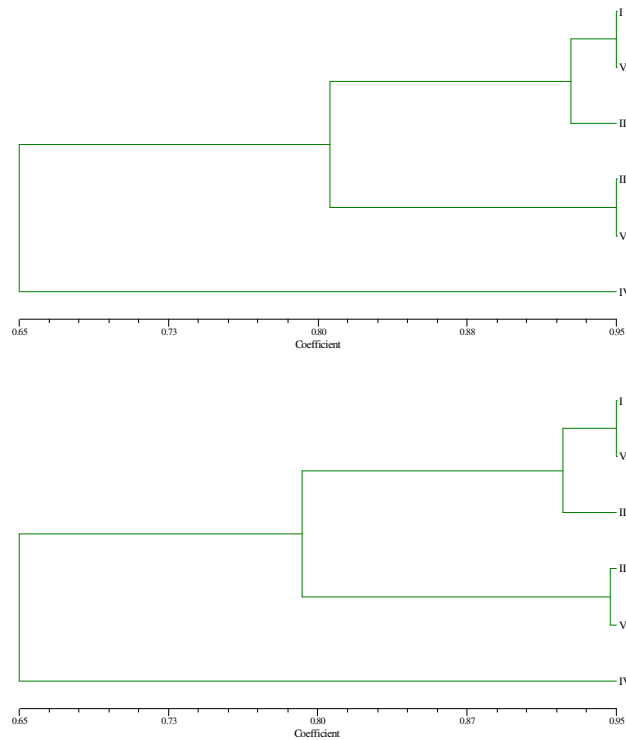


Figure 6. Cluster analysis dendrogram of populations after Sokal and Michener (a) and after Jaccard (b)

Seed proteins are relatively frequently applied as genetic markers in the study of forest tree genomes, but there are relatively few studies on Austrian pine (NIKOLIĆ *et al.* 1983; HAJDUCH *et al.* 2001, MATARUGA 2006; ISAJEV 2007; LUČIĆ *et al.* 2008). The polymorphism of Austrian pine seed proteins was researched aiming at the assessment of genetic variation intra- and inter- provenances. There was a higher degree of genetic variation among provenances than within provenances. Also there was a high genetic variation in seed proteins within provenances. These results are compatible with the results reported by FORREST (1994) and MATARUGA (2006), which point to the absence of site effects on the protein complex, as well as with the results which show the variation among the analysed populations which are spatially isolated, BAHRMAN (1994). In general,

conifers are characterised by a very high level of genetic variation, HAMRICK (1979), and the potential factors are a) longevity, free pollination with high fertility, and b) divergent selection for macro-micro geographic adaptation. According to SCALTSOYIANNES *et al.* (1994), *Pinus nigra* is characterised by a high total variation because of the high intrapopulation genetic variation, which points to a high genetic variation in local populations and the possibility that the same alleles are distributed throughout the entire range of this species.

CONCLUSION

Based on the study results, it can be concluded that the analysis of seed proteins shows a specific protein pattern of all analysed genotypes. This showed the "conservativeness" of some polypeptides, i.e. the presence in all analysed genotypes. On the other hand, some protein fractions were variable at the population level, and some were variable depending on the population.

Based on the cluster analysis, the provenance IV, MU "Šargan" 22b, FE "Užice" - Užice was clearly separated from other provenances, and provenances III and V were grouped together at smaller distances. Population IV had the smallest genetic similarity among the analysed genotypes, showing the greatest distance from populations I, II and V, and a slightly greater genetic similarity with populations III and VI.

The values of intrapopulation diversity in the applied markers are much higher than it was recorded among the study six Austrian pine seed stands. From the aspect of commercial forest seed production, the study results are significant for future activities on defining Austrian pine provenance regions in Serbia, because they point to the fact that the genetic distances among the analysed seed stands are such that they can affect the future trade of seed material in the sense of its use in specific-purpose nursery production.

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**GENETIČKA KARAKTERIZACIJA GENOTIPOVA POPULACIJA
CRNOG BORA (*PINUS NIGRA ARNOLD*) UPOTREBOM PROTEINSKIH
MARKERA**

Aleksandar LUČIĆ¹, Ana NIKOLIĆ², Snežana MLADENović DRINIĆ²,
Vasilije ISAJEV³ i Vera LAVADINOVIĆ¹

¹Institut za šumarstvo, Beograd

²Institut za kukuruz, Zemun Polje, Beograd

³Šumarski fakultet, Beograd

I z v o d

Javljanje crnog bora na velikim površinama zahvaćenim erozionim procesima, ili potpuno degradiranim, ogoljenim površinama na kojima daje odlične rezultate, svrstavaju ga u najvažnije ekonomske vrste korišćene u šumarstvu. Cilj ovih istraživanja je bilo ispitivanje unutar i među populacijske genetičke varijabilnost crnog bora primenom biohemijskih markera. Bolje upoznavanje genetskog potencijala populacija crnog bora omogućuje poboljšanje proizvodnje semena i sadnog materijala, a time i uspeh prilikom pošumljavanja i podizanja namenskih kultura ovom vrstom. Polimorfizam proteinskih markera utvrđen je na osnovu odabranih genotipova poreklom iz šest populacija (semenskih sastojina) čime su obuhvaćene sve populacije crnog bora u Srbiji. Na osnovu dobijenih elektroforegrama utvrđene su kvalitativne i kvantitativne razlike (broj i raspored) proteinskih frakcija i određen je proteinski profil semena za svako stablo kao i za svaku od 6 ispitivanih populacija ponaosob. Tom prilikom je zapažena „konzervativnost“ pojedinih polipeptida, tj. prisustvo u svim analiziranim genotipovima. S druge strane utvrđene su proteinske frakcije koje su varijabilne na nivou populacija, kao i one čija varijabilnost varira u zavisnosti od populacija. Na osnovu dobijenih elektroforegrama je izračunat koeficijent genetičke sličnosti, odnosno udaljenosti, upoređivanjem broja i rasporeda traka i urađeni su dendogrami klaster analize. Utvrđen je veći stepen genetskog variranja između provenijencija nego unutar njih. Takođe je utvrđena velika genetska varijabilnost proteina semena unutar analiziranih populacija.

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