

Picea abies (L.) Karst. in Slovenia – genetic investigations

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Abstract: Autochthonous Norway spruce (*Picea abies* (L.) Karst.) populations from Slovenia and Croatia were investigated by means of isozyme gene markers and compared with high elevation central European spruce populations. The degree of differentiation among populations was measured with genetic distances proposed by GREGORIUS (1974) for 14 polymorphic gene loci. The allelic distances varied from 2.2 % to 7.1 % with Slovenian and Croatian populations. Between Slovenian and German spruce populations distance values from 3.1 % to 6.9 % were found while between Slovenian and French populations distance values between 3.3 % and 11.9 % were calculated. The results of hierarchical clustering into group show clear genetic differences between high-montane spruce stands from the French Alps (Western Alps) and populations from the Bavarian and Slovenian parts of the Alps (Eastern Alps), as well as spruce stands from the north-western Dinaric mountains, all the latter of which are genetically similar.

Key words: *Picea abies* (L.) Karst., isozymes, genetic differentiation, Alps, Dinaric mountains

Izvleček: S pomočjo izoencimskih genskih označevalcev smo proučili avtohtone populacije smreke (*Picea abies* (L.) Karst. v Sloveniji in na Hrvaškem in jih primerjali z visokogorskimi srednjeevropskimi smrekovimi populacijami. Sopno genske diferenciacije med njimi smo izmerili z genskimi razdaljami, ki smo jih izračunali po GREGORIUS (1974) za 14 polimorfnih genskih lokusov. Alelne genske razdalje med populacijami (d_0), se nahajajo med 2,2 % in 7,1 % v Sloveniji in na Hrvaškem, v primerjavi slovenskih z nemškimi populacijami med 3,1 % do 6,9 %, v primerjavi slovenskih s francoskimi populacijami pa od 3,3 % do 11,9 %. Rezultati hierarhične združevanja v skupine nakazujejo jasno gensko diferenciacijo med sestoji smreke z območja francoskih Alp (zahodne Alp) ter populacijami smreke z bavarskega in slovenskega predela Alp (vzhodne Alpe) kakor tudi sestojev smreke iz severozahodnega predela Dinariskega gorstva, ki so si med seboj gensko bolj podobni.

Ključne besede: *Picea abies* (L.) Karst., izoencimi, genska diferenciacija, Alpe, Dinaridi

1. Introduction

The Republic of Slovenia measures 2.027 million ha. The north and north-western parts are mountainous, forming part of the Alps and some old magmatic mountains, the south-western part borders the Adriatic Sea, the east borders the Pannonian plain and the southern part is the far end of the Dinaric mountains. Forests cover 56 % of the area and it could be said that they are a symbol of Slovenia. Norway spruce (*Picea abies* (L.) Karst) is a common and

economically significant tree species in Slovenia. It grows on 83.36 % of forest land and constitutes 32.5 % of the total growing stock (Božič *et al.*, 2001; according to the Annual Report of Slovenian Forest Service, 1998). Today, it grows naturally only on ecologically suitable sites, i.e. on locally colder and edaphically more humid sites (ZUPANČIČ, 1980). Spruce usually accompanies beech and silver fir, but can also grow as a dominant species in high montane and subalpine regions from the Julian Alps to the Pohorje mountain range. In

the Dinarics, it occurs in groups on extreme cold sites, otherwise its distribution is as individual trees. In Slovenia, 18 phytocenological spruce associations are known, 5 of them are potential natural, 6 conditionally potential natural and 7 are secondary associations on beech forest sites (ZUPANČIČ, 1999).

The aim of this study was to analyse the genetic differentiation of spruce populations in their natural distribution range in the south-eastern Alps and the north-western Dinaric mountains and to evaluate genetic differences from corresponding populations in the central European alpine region.

2. Materials and methods

Twenty six spruce populations from potential natural and conditionally potential natural sites (ZUPANČIČ, 1999) in Slovenia and Croatia were included in the study. In each population, 50 to 70 randomly chosen 100 to 200 year old dominants were sampled. The genetic structures of individual trees were determined by means of isozyme gene markers. Isozymes were extracted from dormant buds and separated by starch gel electrophoresis following the methodology given in KONNERT and MAURER (1995) with slight modifications. The following 14 isozyme gene loci were analysed: *Gdh-A*, *Got-A*, *Got-B*, *Got-C*, *Idh-A*, *Idh-B*, *Lap-B*, *Mdh-B*, *Mdh-C*, *Pgi-B*, *Pgm-A*, *Skdh-A*, *6-Pgdh-B*, *6-Pgdh-C*. The allelic structures of the 22 Slovenian (P1 – P22) and 4 Croatian (P23 – P26) populations were calculated from diploid genotypes of individual trees and then compared with the allelic structures of 6 spruce populations from the Bavarian Alps – Germany (Koenigssee, Oberammergau) (P 27 – P 32) and 7 populations from the French Alps (Boreon, Nevache, Sainte Foy Tarentaise) (P 33 – P 39). The genetic data of – German and French populations originated from the EU-funded project »Biodiversity of Alpine Forest Ecosystems, FAIR-PL96-1949«.

The genetic differences between stands were quantified by allelic genetic distances (d_0) after Gregorius (1974), calculated with the GSED-programme (GILLET 1998). The matrix of the average genetic distances between popu-

lations was interpreted with the aid of hierarchical cluster analysis after Ward, and by the method of un-weighted pair-group arithmetic average (UPGMA). The hierarchical cluster analysis was done by the programme STATISTICA.

3. Results and discussion

The genetic differences between the studied spruce populations are relatively small for the gene pool studied. The average genetic distances d_0 (GREGORIUS, 1974) varies in Slovenia between 2.1 % and 6.5 %, in Croatia between 3.4 % and 4.5 %, in the Bavarian Alps from 1.9 % – 5.1 % and in the French Alps from 3.1 % – 9.1 %. Comparison of spruce populations within the Slovenia and Croatia region showed only a slight increase of genetic distances – (from 3.0 % to 7.1 %). Similar values were observed between Slovenian and Bavarian populations (from 3.1 % to 6.5 %) while much greater differences were found in the comparison with French populations (3.2 % to 11.9 %).

The dendrogram of the cluster analysis performed by the Ward method showed clustering of populations into three relatively well recognisable groups. The first group includes the French stands from the regions Boreon (33, 34), Nevache (35, 36) and Sainte Foy Tarantaise (37, 38, 39). Next to them are the German stands from the Oberammergau region (27, 28, 29) and some alpine stands from Slovenia (03, 04, 09). The first group is further subdivided into two subgroups: the first contains nearly all the French stands (except population 39), and the other the German and Slovenian stands of alpine origin, which, within the subgroup, are genetically more similar among themselves. Populations 33 in 34 (Boreon – France) differ most in the first group.

The second group contains stands from the dinaric region in Slovenia (19, 20, 22) and Croatia (23, 24, 25, 26) and some alpine Slovenian stands (5, 7, 10, 15), which are genetically more similar among themselves. In the third group are concentrated mainly stands from the alpine region of Slovenia (1, 2, 6, 8, 11, 12, 13, 14) and Germany (30, 31, 32). The

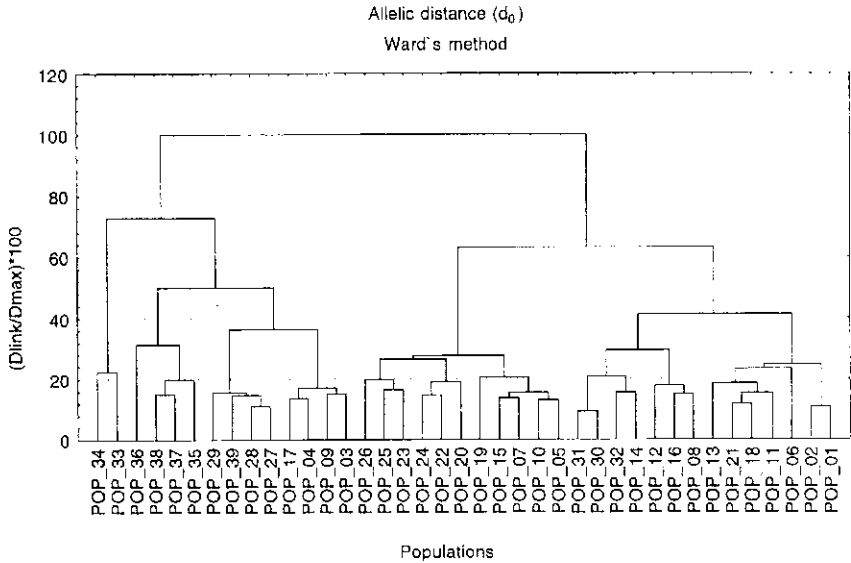


Figure 1: Results of hierarchical cluster analysis by the Ward method based on the Gregorius (1974) genetic distances between 39 sampled spruce populations; P1 – P15 Slovenia / Alps, P16 – P22 Slovenia / Dinaric mountains, P23 – P26 Croatia / Dinaric mountains, P27 – P32 Germany / Alps, P33 – P39 France / Alps

Slika 1: Prikaz rezultatov hierarhičnega združevanja v skupine z metodo po Wardu, izvedene na osnovi povprečnih genetskih razdalj po Gregoriusu (1974), med 39 vzorčenimi populacijami smreke: P1 – P15 Slovenija / Alpe, P16 – P22 Slovenija / Dinaridi, P23 – P26 Hrvaška / Dinaridi, P27 – P32 Nemčija / Alpe, P33 – P39 Francija / Alpe

similarity between groups is lower than within the groups. The second and third groups are genetically much more similar but are clearly differentiated from the first group.

Cluster analysis performed by the method of un-weighted pair-group arithmetic average (UPGMA) showed similar results as the Ward method.

It can be seen from the second dendrogram that two French populations (33, 34) from the Boreon region represent independent units and are most distinct from all the other analysed stands, which cluster into two genetically well differentiated groups. The first group includes only stands from the French Alps (35, 36, 37, 38), while the second, larger group includes all stands from Slovenia (1–22), Croatia (23–26), Germany (27–32) and also the French population 39.

The results of hierarchical clustering into groups show clear genetic differences between the highmontane spruce stands from the French Alps (Western Alps) and the populations from the Bavarian and Slovenian parts of the Alps (Eastern Alps), as well as spruce stands from the north-western Dinaric mountains, all the latter of which are genetically similar.

Direct comparison of data from this study to other investigations of Norway spruce in the Alps is difficult because of similar but not identical methods used in various studies. Indirect comparisons of the average gene pool differentiation (δ) between spruce populations from Slovenia and Croatia (Božič, 2002) and the central alpine region (Italy / GIANNINI *et al.*, 1991, MORGANTE, VENDRAMIN, 1991, Austria / GEBUREK, 1999 and Switzerland / MÜLLER-

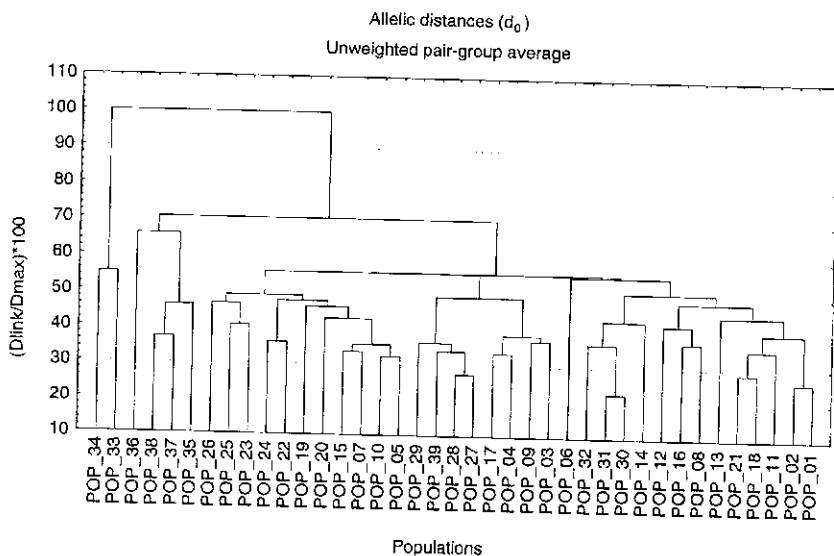


Figure 2: Results of hierarchical cluster analysis by the UPGMA method based on the Gregorius (1974) genetic distances between 39 sampled spruce populations; P1 – P15 Slovenia / Alps, P16 – P22 Slovenia / Dinaric mountains, P23 – P26 Croatia / Dinaric mountains, P27 – P32 Germany / Alps, P33 – P39 France / Alps

Slika 2: Prikaz rezultatov hierarhičnega združevanja v skupine z UPGMA metodo, izvedene na osnovi povprečnih genskih razdalj po Gregoriusu (1974), med 39 vzorčenimi populacijami smreke; P1 – P15 Slovenija / Alpe, P16 – P22 Slovenija / Dinaridi, P23 – P26 Hrvaška / Dinaridi, P27 – P32 Nemčija / Alpe, P33 – P39 Francija / Alpe

STARCK, 1995) shows that genetic differences between populations are more marked between Italian (5.0 %) and Swiss (4.3 %) populations than between those in Austria (2.6 %) and our sampled regions (3.2 %). Based on the limited comparability among the studies, a careful conclusion would be that Slovenian and Croatian spruce populations are genetically very similar to central alpine populations that have not been affected by other southern refugial populations.

4. Conclusions

Spruce populations from the natural distribution range in the south-eastern Alps and north-western Dinaric mountains showed specific genetic differences in comparison with high-montane spruce stands in the French Alps. The obtained results indicate that spruce popula-

tions from Slovenia, Croatia and the Bavarian Alps had a much more similar evolutionary history than populations from the Western Alps, especially the French Alps.

5. Povzetek

Namen raziskave je spoznavanje gozdnih genskih virov smreke (*Picea abies* (L.) Karst.) v Sloveniji. Želeli smo analizirati genetsko diferenciranost smreke iz naravnega območja razširjenosti v predelu jugovzhodnih Alp in severnozahodnih Dinaridov in ugotoviti stopnjo genetske diferenciranosti (sorodnosti) smreke v Sloveniji v primerjavi z ustreznimi populacijami smreke v širšem srednjeevropskem alpskem prostoru.

Raziskovalne objekte smo izbrali v subalpinskem in zgornjem montanskem pasu, na

rastiščih, ki jih smreka gradi po svoji naravni razširjenosti. Material za analizo smo nabrali v 26 smrekovih sestojih iz alpskega (Pohorje, Kamniško Savinjske Alpe, Karavanke, Pokljuka, Jelovica) in dinarskega fitogeografskega območja v Sloveniji (Trnovski gozd, Snežnik, Goteniški Snežnik) in na Hrvaškem (Gorski Kotar, Velika Kapela, Velebit). V raziskave je bilo vključenih od 50 do 70 zrelih dreves s posamezne ploskve. Vzorec sestavljajo naključna vitalna, nadrasla oziroma sorasla drevesa različne starosti (večinoma od 100 do 200 let). Z vsakega od poskusnih dreves smo v zimskem obdobju odvzeli vejo s spežimi popki, ki smo jih uporabili kot izhodiščni material za pripravo encimskih izvlečkov. Genetsko strukturo posameznih smrekovih dreves smo analizirali s pomočjo izoenzimskih genskih označevalcev. Uporabili smo metodo škrobne gelske elektroforeze. Elektroforezo, barvanje gelov in odčitavanje elektroforegramov smo izvajali po standardnih metodoloških postopkih za analizo smrekovih vzorcev (KONNERT & MAURER, 1995) z manjšimi modifikacijami. Analizirali smo naslednjih 14 genskih lokusov: *Gdh-A*, *Got-A*, *Got-B*, *Got-C*, *Idh-A*, *Idh-B*, *Lap-B*, *Mdh-B*, *Mdh-C*, *Pgi-B*, *Pgm-A*, *Skdh-A*, *6-Pgdh-B*, *6-Pgdh-C*.

Populacije smreke iz Slovenije (P1 – P22) in Hrvaške (P23 – P26), smo primerjali z ustreznimi populacijami iz alpskih območij v Nemčiji (Oberammergau, Koenigssee) (P27 – P32) in Franciji (Boreon, Nevache, Sainte Foy Tarentaise) (P33 – P39), ki so jih že uporabili pri drugih raziskavah (v okviru projekta Biodiverziteta alpskih gozdnih ekosistemov, FAIR-PL96-1949) in so zaradi enake uporabljene metodologije in zaradi enakih kombinacij analiziranih lokusov pri analizi neposredno primerljive z našimi. Razlike v genetskih strukturah posameznih populacij smreke smo kvantificirali s pomočjo genetske razdalje (d_0) po GREGORIUS (1974). Matriko povprečnih alelnih genetskih

razdalj (d_0) med posameznimi populacijami, smo nato interpretirali s pomočjo hierarhičnega združevanja v skupine. Za interpretacijo smo uporabili Wardovo metodo, ki temelji na minimalni razliki variance znotraj posameznih skupin ali klastrov (sl. 1) in metodo neponderirane aritmetične sredine (sl. 2).

Rezultati primerjave povprečnih alelnih genetskih razdalj med posameznimi populacijami, ki smo jih izračunali za 14 lokusni genski sklad so pokazali, da je genetska diferenciacija med sestoji znotraj primerjanih območij v Sloveniji od 2.1 – 6.5 %, na Hrvaškem od 3.4 – 4.5 %, v Bavarskih Alpah od 1.9 – 5.1 %, v francoskih populacijah pa od 3.1 – 9.1 %. Nadaljnja primerjava slovenskih in hrvaških populacij nakazuje le na manjše povečanje genetskih razdalj (od 3.0 % do 7.1 %). Podobno odstopanje opazimo tudi pri primerjavi slovenskih populacij z nemškimi (od 3.1 % do 6.5 %). Še najbolj opazno odstopanje je pri primerjavi slovenskih populacij s francoskimi sestoji (od 3.2 % do 10.2 %). Slovensko hrvaške populacije smreke se manj razlikujejo od nemških (3.1 – 6.9 %) kot pa od francoskih smrekovih populacij (3.3 – 11.9 %).

Rezultati hierarhične združevanja v skupine nakazujejo sklep o jasni genetsko pogojeni diferenciaciji naravnih visokogorskih sestojev smreke z območja francoskih Alp (območje zahodnih Alp) od populacij smreke z bavarskega in slovenskega obrobla Alp (vzhodne Alpe) kakor tudi sestojev smreke iz severozahodnega predela Dinarske verige, ki so si med seboj genetsko bolj podobni. To dejstvo nakazuje možnost podobne razvojne zgodovine naših smrekovih populacij v primerjavi s populacijami smreke iz bavarskih Alp ter možnost različne razvojne zgodovine le teh v primerjavi s smreko na zahodnem predelu Alp, še zlasti iz francoskih Alp.

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