

The morphological and genetical characterisation of a native Norway spruce (*Picea abies* (L.) Karst.) population in the area of Pokljuka mire

Morfološke in genetske značilnosti naravne populacije smreke (*Picea abies* (L.) Karst.) na območju poključke barja

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Abstract. Morphometrical and genetical characteristics of two morphologically different sub-populations of spruce trees grown in a frost area of the mire Šijec (altitude 1170 m) on the Pokljuka plateau were studied. The tree height & diameter, needle length & needle volume of 70, approximately 120 to 200 years old trees, were measured. The genetic structure of the two spruce sub-populations were estimated by isozyme analysis on starch gel at 17 polymorphic gene loci. Soil conditions on the research plots examined with a gauge sound and then mapped and investigated in details by lab analyses of soil samples from representative soil profiles. The analysed spruce trees grown on the more productive site belonging to the association *Rhytidiadelpho lorei* - *Piceetum* in edge of the mire with mainly automorphic soils were in average 3,8 times taller and thicker than the trees from the extreme site belonging to the association *Sphagno girgensohnii* - *Piceetum*. The needle length of these trees was 25,7 % longer and their needle volume 68 % bigger than of the trees from the mire plot on the hydromorphic peat soils. The comparison of the genetic structures has shown distinct differences in frequencies of some alleles and genotypes between the analysed spruce sub-populations.

Key words: *Picea abies* (L.) Karst., sub-population, soil conditions, morphological variability, genetic structure, Julian Alps, Slovenia

Izvleček. Na Poključki planoti smo na mraziščnem področju barja Šijec (na n. v. 1170 m) proučevali morfološke in genetske značilnosti dveh različnih smrekovih subpopulacij. Okoli 120 do 200 let starim smrekam smo izmerili drevesne višine, premere debel v prsni višini ter dolžine in volumne iglic. Genetsko strukturo obeh subpopulacij smo proučili z elektroforetsko analizo 17 polimorfnih izoencimskih genskih lokusov v škrobem gelu. Analizirane smreke z roba barja, ki rastejo na rastišču asociacije *Rhytidiadelpho lorei* - *Piceetum*, na katerem prevladujejo trdinska tla in je rodovitnejše, so imele v povprečju 3,8 krat večjo drevesno višino in premer ter za 25,7 % daljše iglice in za 68 % večjo prostornino iglic od smrek barjanske ploskve, ki poraščajo ekstremno rastišče asociacije *Sphagno girgensohnii* - *Piceetum*

na hidromorfnih šotnih tleh. Primerjava genetskih struktur je pokazala izrazite razlike v frekvencah nakaterih alelov in genotipov med analiziranimi smrekovima subpopulacijama.

Ključne besede: *Picea abies* (L.) Karst., subpopulacija, talne razmere, morfološka variabilnost, genetska struktura, Julijske Alpe, Slovenija

Introduction

Forests cover about 20 km² of the Pokljuka plateau is calcareous and mixed moraines. Natural forests of beech (*Fagus sylvatica* L.) and fir (*Abies alba* Mil.) and secondary forests of Norway spruce (*Picea abies* (L.) Karst.) predominate, both with a high wood productivity. At the altitude of about 1200 m a.s.l. primary and supposedly autochthonous Norway spruce forests are still present and form two plant associations.

The spruce plant association *Rhytidiadelpho lorei* - *Piceetum* (ZUPANČIČ 1981 emend.) covers automorphic soils developed on moraines, spruce growth of these stands is relatively good. The spruce association *Sphagno girgensohnii* - *Piceetum* (W. KUOCH 1954 corr. ZUPANČIČ 1982) var. geogr. *Carex brizoides* (ZUPANČIČ 1982 corr.) grows on hydromorphic soils of mires. This spruce-mire plant community on peat soils lives in extremely poor and severe site conditions, spruces there grow slowly and dwarfly.

The aim of this study was to evaluate the influence of different site conditions on spruce growth characteristics and to determine the genetic structure of this species in two primary Norway spruce associations.

Materials and methods

Two research plots were established in the natural growing sites of spruce in the frosty area on the Pokljuka plateau. The research plot "Mire" lies in the south-eastern part of the mire Šijec in the bushy and gappy spruce forest (association *Sphagno girgensohnii*-*Piceetum*) between dwarf-pine community (*Sphagno-Pinetum mughi*) and high spruce forest on the mire's edge. The research plot "Edge" is located inside a one-hectare large permanent plot "Šijec" of the Slovenian Forestry Institute where sites of the spruce association *Rhytidiadelpho lorei*-*Piceetum* predominate (Figure 1). The plots are located at 1170 m altitude, the distance between them is 500 m.



Figure 1: Norway spruce population of the mire's Šijec area on the Pokljuka plateau (left) with locations of the research plots "Mire" (in the middle) and "Edge" (right). Photo: G. Božič

Trees from every plot are considered as a sub-population from the whole spruce population of the mire's Šijec area. On each plot soil conditions were studied and 35 vital randomly chosen dominant spruce trees were dendrometrically analysed and their genetic structure determined.

Soil conditions and morphologic properties of soil in the research plots (see also BOŽIČ & URBANČIČ 2001) were examined with a semicircular sound, which reaches down to 110 cm deep. According to soil heterogeneity of plots the locations for representative soil profiles were chosen. After the description of profiles soil samples were taken and analysed in the lab. For each soil sample pH in de-ionised water (H_2O) and in 0.01 M calcium chloride ($CaCl_2$), content of: carbonates, organic carbon, organic matter, total nitrogen and C/N ratio were determined. Contents of exchangeable cations (K^+ , Ca^{2+} , Mg^{2+} , Al^{3+} , Fe^{3+} , Mn^{2+} and H^+), cation exchange capacities, base saturation and texture classes were determined for samples from mineral part of soil.

The morphological characteristics of the sub-populations were determined by analysis of morphometric measurements of needle lengths and volumes and by dendrometric analyses of heights and diameters at the height of 1,3 m of dominant, approximately 120 to 200 years old spruce trees. Studied samples were taken from the 2 years old shoots exposed to sun in the southern upper third of the tree crown of 70 randomly chosen trees. Each tree sample consisted of 100 randomly chosen needles. All samples were taken during the second week in November and stored in the laboratory at $-20^{\circ}C$ until further use. Morphometric analysis of fully developed *Picea abies* needles were done by using a computer – aided image system (Optimas 5.0 programme) in the laboratory of Applied Botany and Physiology, Agronomy Department of Biotechnical Faculty, University of Ljubljana, under the supervision of MSc. T. Sinkovič. For calculation of needle volume (V) the equation of RIEDERER *et. al.* (1988) was used: $V \text{ (mm}^3\text{)} = 0,208 \times (\text{projected needle area})^{1,353}$. The age of trees was estimated by measurements of tree rings from the cores taken at 0,4 m using a dendrochronological table LINTAB with 1/100 mm accuracy (BOŽIČ & LEVANIČ 1998).

To determine the genetic structure of the sampled trees, endosperms from six seeds per tree were analysed. In the mire's spruce sub-population there was no fructification during analysis, so dormant winter buds were used for analysis. The genetic variability of the two spruce sub-populations was analysed by means of isozyme gene markers using starch electrophoresis as the separation method. Enzyme extraction from buds and seeds (endosperm) was performed according to RHODES (1977). Electrophoresis conditions, staining, and genotyping followed KONNERT & MAURER (1995). The following isozyme gene loci were investigated: *Aco-A*, *Gdh-A*, *Got-A*, *Got-B*, *Got-C*, *Idh-A*, *Idh-B*, *Lap-B*, *Mdh-A*, *Mdh-B*, *Mdh-C*, *Mnr-A*, *Mnr-C*, *Pgi-A*, *Pgi-B*, *Pgm-A*, *Skdh-A*, *6-Pgdh-A*, *6-Pgdh-B*, *6-Pgdh-C*. The results of isozyme electrophoretic analyses were evaluated by relative allele and genotype frequencies calculated on different gene loci (recalculated from BOŽIČ 1997). The genetic structure was described for all loci for which polymorphisms were found in at least one spruce sub-population and compared with respect to the average actual heterozygosity H_a calculated as the arithmetic mean of single locus values (NEI 1973). The genetic differentiation between the sub-populations was studied with chi-square tests of homogeneity among allele frequencies for particular gene loci at the level $\alpha = 0,05$ and estimated with allelic and genotypic genetic distances (d_p) proposed by GREGORIUS (1974).

Results and discussion

On the bog site plot "Mire" all 35 studied spruce trees grow on hydromorphic organic soils which have the peat horizon lying over wet, softy and gelatinous lake sediments (gyttia). Thickness of the peat layer is from about 60 cm to over one meter. The reaction of its peat is very acidic

(measured values of $\text{pH}(\text{CaCl}_2)$ are between 2,88 and 3,18). Soil is classified as ombrotrophic form of the middle deep to deep subtype of peat acrohistosol and according to FAO (1989) soil classification it belongs to soil unit of Fibric Histosols. On this site spruces have extremely bad growth conditions. The ages (after BOŽIČ & LEVANIČ 1998) of spruces on the plot "Mire" at the height of 0,4 m varied between 65 and 142 (in average 95) years, at breast height they had diameters from 6 to 19 (in average 12) centimetres and tree heights from 4 to 13 (in average 8) metres. Analysed needles had lengths from 7,2 to 14,3 (in average 10,5) millimeters and volumes from 0,8 to 3,9 (in average 1,9) mm^3 .

On the plot "Edge" soils have developed on mixed moraine lying over lake chalk. Mixed moraine is composed by unconsolidated material of limestone, dolomite, marl, cherts, shales and sandstones. On this parent material heterogenous, mostly dystric soils have developed. They are covered with mainly acidophilic vegetation. Soil sounding discovered that 14 sampled trees grow on dystric cambisols, 16 on podzols and 5 on gleysols. Properties of these FAO (1989) soil units were examined closely by three representative soil profiles. Podzols and dystric cambisols have in upper half meter depth very acid reaction (pH 3,02–4,37) and very low base saturation levels (BS 3,15 %–34,17 %). Gleysols are under the influence of groundwater and lake chalk and are less dystric (pH 3,57–4,9; BS 43,9 %–53,8 %). These soils are rather deep, have suitable loamy texture and moisture regime and are rather fertile for spruce. The age of studied spruces at the height of 0,4 m in the plot "Edge" varied between 87 and 147 (in average 116) years, they had diameters at breast height from 31 to 60 (in average 46) centimetres and tree heights from 27 to 36 (in average 31) metres. Analysed needles had lengths from 9,9 to 17,5 (in average 13,2) millimeters and volumes from 1,0 to 6,3 (in average 3,2) mm^3 (modified after BOŽIČ 2000 and BOŽIČ & LEVANIČ 1998).

Also according to (BOŽIČ & LEVANIČ 1998) true ages of selected trees on the plot "Mire" were in average about 40 to 50 years greater and on the plot "Edge" from 30 to 35 years greater as in the sampling height 0,4 m. The age of the oldest trees is about 200 years, which means that trees in the two research plots started to grow at least 50 years before the first huge clearcuts and artificial regeneration with planting was started on the Pokljuka plateau in the years 1848–1859 (SMOLEJ 1984).

Between two sampled groups of trees on the research plots "Mire" and "Edge" remarkable differences in allele and genotype frequencies were noted. The most distinct differences in frequency of alleles and genotypes was observed at locus *Mnr-A* for alleles *Mnr-A*₂, *Mnr-A*₃, and genotypes *Mnr-A*₂₂ and *Mnr-A*₂₄. The homozygote type was more common in sampled trees from the plot "Edge" while the heterozygote type was more common in sampled trees from the plot "Mire". The heterozygote types *Mnr-A*₂₄ and *Mnr-A*₃₄ reached much higher values in sampled group of trees on the mire site than on the edge site. The reverse situation was occurred at homozygote type *Mnr-A*₂₂ which was much more frequent in group of trees "Edge" than in "Mire" group of trees (45,7 % vs. 11,4 %, respectively). In average, a higher heterozygosity (H_a) on the plot »Mire« than on the plot »Edge« was observed. If the most differentiated locus in heterozygosity *Mnr-A* is taken in consideration, trees sampled on the »Mire« had much higher heterozygosity as sampled group of trees on the »Edge« plot (77,1 % and 40,0 %, respectively). Results of the contingency χ^2 tests for homogeneity of genetic structure between sub-populations has shown that allele frequencies significantly differed at the level $\alpha = 0,05$ in two gene loci (chi-square = 8,13; 3 d.f. for *Lap-B*) and (chi-square = 5,27; 1 d.f. for *Mnr-A*). At this two gene loci, also the genetic distances between »Edge« and »Mire« group of trees were very high with allelic distances of 17,2 % for *Lap-B* and 21 % for *Mnr-A* and genotypic distances of 25,8 % for *Lap-B* and 37,2 % for *Mnr-A*. Remarkable differences of allele and genotype frequencies were also noted for loci *Aco-A*, *Got-C*, *Lap-B*, *Mnr-A*

and 6-*Pgdh-C*. Only for gene locus *Gdh-A*, the genetic structures of the two sub-populations were identical and the genetic distances equal with zero. The single-locus values mean value of Gregorius allelic and genotypic genetic distance (5,9 % and 10,7 %, respectively), further confirms that the genetic differences between the sampled spruce sub-populations on the "Mire" and "Edge" plots are comparatively high. For the population genetic structure of Norway spruce a relative high levels of differentiation between the sub-populations were observed.

Genetic differentiation between the sub-populations from true different forest site conditions may be a consequence of different selection processes to which spruce in this location has been exposed. As spruces from the mire site with organic (peat) soils were 3,8 times lower and thinner as spruces in similar age from the site with mineral soils, is there a great opportunity for selection to act. Since temperature and oxygen availability in the soil are closely associated with soil moisture, further experiments are required to understand the environmental factors which are most directly associated with the selection factors which acting on the forest sites with mainly automorphic (out of underground water reach) and mineral or hydromorphic (strong influence of underground water) peat soils.

The very high allelic and genotypic distances observed in loci *Lap-B* and *Mnr-A* indicated that specific site conditions could have an impact to these two loci. The response to site conditions could affect the observed locus directly, or any other loci tightly linked to them, as well as any coadapted gene complexes that they mark (HAMRICK 1989). As the isoenzyme variants (allozymes) may be both, selectively equivalent (i.e. neutral) and adaptive, depending on the respective environmental conditions under which the particular population is living (BERGMANN 1991), present results would allow to hypothesise that decrease in frequency of allele *MNR-A₂* from 65,7 % observed in forest site with mainly automorphic and mineral soils to 44,3 % on mire site, may have an adaptive origin and could be connected with increased level of heterozygosity of individuals existing in environmental site conditions with hydromorphic soils because only heterozygotic individuals can be better adapted to extreme site conditions.

Although the obtained results are incomplete, also because of possible sampling mistakes on account of the large size of analysed samples, and do not allow us to infer any certain conclusions on the possible adaptive role of the enzyme systems under analysis, they verify the existence of morphological and genetical differentiation between spruce sub-populations within single population associated with variation of environmental conditions resulting from differences in site conditions.

Conclusions

The research plots were established on Pokljuka in sites within the area of natural distribution of Norway spruce. On both plots dominant from about 120 to 200 years old spruces were chosen. The oldest trees were growing at least 50 years before the first huge clearcuts and artificial regeneration with spruce seedlings on Pokljuka plateau in the middle of 19th century began so the analysed spruce sub-populations in the Šijec area can be regarded as autochthonous.

Striking differences in soil and other site conditions between the research plots have been reflected in the spruce's growth. Spruces from the plot "Edge" were in average 3,8 times taller and thicker as trees from the plot "Mire". Needle length and needle volume of spruces from the site with better growing conditions were 26 % longer and 68 % bigger than of the trees from the peat bog. Different site conditions resulting from soil conditions were attributed particularly to the tree growth and its morphological attributes.

Environmental conditions resulting from differences in site conditions could have an impact to the genetic structures of sub-populations growing in such habitats. The obtained results would also allow to hypothesise an adaptive response of the allele *Mnr-A*₂ to different environmental conditions resulting from differences in site conditions.

Aknoweledgeement

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Povzetek

Raziskovalni ploskvi sta bili osnovani v mraziščnem delu Pokljuke, v okolici visokega barja Šijec, v dveh smrekovih subpopulacijah, na njenih naravnih rastiščih. Na vsaki ploskvi smo za raziskavo izbrali 35 vitalnih, nadraslih oziroma soraslih smrek različnih starosti (od 120 do 200 let). Najstarejša drevesa so rastle vsaj 50 let pred prvimi velikopovršinskimi goloseki in sadnjami smrekovih sadik sredi 19 stoletja, zato menimo, da so analizirane smreke še avtohtone.

Ploskev »Mire«, ki jo porašča asociacija barjanskega smrekovja (*Sphagno - Piceetum* s. lat. R. KUOCH 1954 emend. ZUPANČIČ 1981), leži na šotnem barju. Njena srednje globoka do globoka šotna tla po FAO (1989) razvrstitvi spadajo v talno enoto fibrični histosoli. Na njej vladajo za smreko skrajno neugodne rastiščne razmere.

Ploskev »Edge« smo osnovali na robu barja. Njeno rastišče porašča rastlinska združba smreke in smrečnega resnika (*Rhytidiadelpho lorei - Piceetum* (M. WRABER 1953 n.nud.; ZUPANČIČ (1976), 1981 em. 1999). Na njem so se na mešani moreni, ki prekriva jezersko kredo, razvili podzoli, distrična rjava tla in hipogleji. Po mednarodni FAO (1989) klasifikaciji jih uvrščamo v talne enote: podzoli, distrični kambisoli in glejsoli. Ta tla so distrična (imajo zelo kisle reakcije in zelo nizke stopnje nasičenosti z izmenljivimi bazami) in so vsaj pol metra globoka. Imajo ugodno ilovnato teksturo in vodni režim, tako da so za smreko dokaj rodovitna.

Analizirane smreke z roba barja so bile v povprečju 3,8- krat višje in debelejšje ter so imele v povprečju za 25,7 % daljše in za 68,4 % volumensko večje iglice od smrek s šotnega barja. Te razlike v rasti in v morfoloških lastnostih smrek so po našem prepričanju odraz velikih razlik v talnih in drugih rastiščnih razmerah med ploskvama.

Te razlike v okoljskih razmerah pa bi lahko vplivale tudi na genetski strukturi analiziranih smrekovih subpopuacij. Izidi naših analiz genetske variabilnosti subpopulacij smreke z metodo izoencimske gelske elektroforeze, ki je zajemala uporabo škrobnega gela in ekstrakcijo izoencimov iz popkov in endosperma semen, podpirajo to domnevo. Razlika v heterozigotnosti med subpopulacijama na genskem lokusu *Mnr-A* nakazuje možni učinek preživetvene selekcije.

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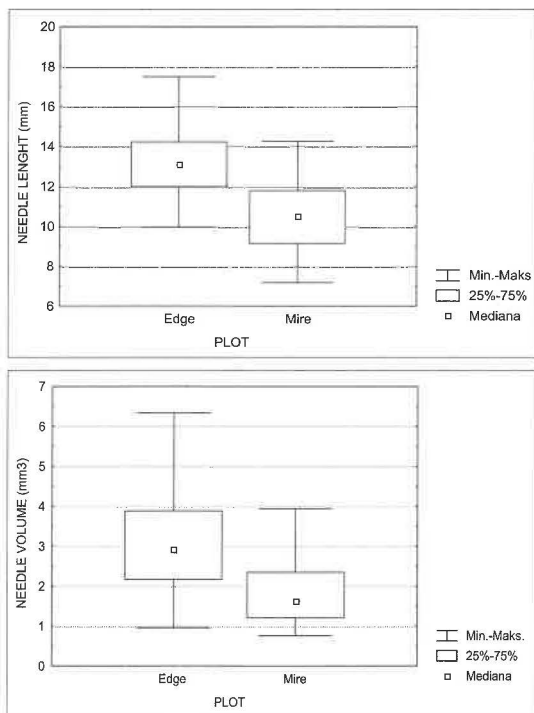
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Appendices

Appendix 1: The tree age on the height of 0,4 m, diameter at 1,3 m, tree height, needle length and needle volume of sampled spruce trees on the research plots "Mire" and "Edge"

Parameter		Plot "Mire"	Plot "Edge"
Age	Min. (years)	65	87
	Max. (years)	142	147
	Mean (years)	95	116
Diameter	Min. (cm)	6	31
	Max. (cm)	19	60
	Mean (cm)	12	46
Height	Min. (m)	4	27
	Max. (m)	13	36
	Mean (m)	8	31
Needle length	Min. (mm)	7,2	9,9
	Max. (mm)	14,3	17,5
	Mean (mm)	10,5	13,2
Needle volume	Min. (mm ³)	0,8	1,0
	Max. (mm ³)	3,9	6,3
	Mean (mm ³)	1,9	3,2

Appendix 2: The distribution of needle length and needle volume values of sampled spruce needles per each research plot



Appendix 3: Genotype frequencies at the 20 analysed enzyme gene loci of sampled spruce trees on the research plots "Mire" and "Edge"

Locus	Genotype	Plot "Mire"	Plot "Edge"	Locus	Genotype	Plot "Mire"	Plot "Edge"
ACO - A	11	0,086	0,086	MDH - C	24	0,086	0,000
	12	0,200	0,343		44	0,914	1,000
	22	0,714	0,571	MNR - A	12	0,057	0,000
GDH - A	12	0,029	0,029		22	0,114	0,457
	22	0,971	0,971		24	0,600	0,400
					34	0,114	0,000
GOT - A	12	0,000	0,029		44	0,114	0,143
	22	1,000	0,971	MNR - C	12	0,057	0,000
GOT - B	12	0,000	0,029		22	0,943	1,000
	22	1,000	0,971	PGI - A	22	1,000	1,000
GOT - C	22	0,171	0,086	PGI - B	22	0,000	0,086
	24	0,514	0,429		23	0,600	0,514
	25	0,029	0,029		33	0,400	0,400
	44	0,257	0,429	PGM - A	22	0,829	0,857
	45	0,029	0,029		23	0,143	0,114
IDH - A	23	0,029	0,114		33	0,029	0,029
	33	0,971	0,886	SKDH - A	13	0,029	0,057
IDH - B	22	1,000	1,000		22	0,143	0,086
					33	0,829	0,800
LAP - B	11	0,000	0,029		35	0,000	0,029
	13	0,000	0,029		36	0,029	0,029
	14	0,029	0,086	6PGDH - A	12	0,000	0,029
	33	0,000	0,029		22	0,971	0,971
	34	0,114	0,200		23	0,029	0,000
	36	0,000	0,029	6PGDH - B	22	0,400	0,486
	44	0,571	0,429		25	0,486	0,343
	46	0,229	0,143		35	0,000	0,029
	47	0,029	0,000		55	0,114	0,143
	66	0,029	0,029	6PGDH - C	15	0,000	0,029
MDH - A	22	1,000	1,000		22	0,457	0,314
					25	0,257	0,400
MDH - B	12	0,029	0,000		26	0,029	0,000
	22	0,914	0,971		55	0,257	0,257
	23	0,057	0,029				