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The Estimation of Genetic Diversity within and between Lithuanian Populations of Norway Spruce (*Picea abies* (L.) Karst.) by using RAPD

RAMUNĖ AREŠKEVIČIENĖ[1], DONATAS ŽVINGILA[1,3], RIMVYDAS GABRILAVIČIUS[2] AND SIGUTĖ KUUSIENĖ[1]

[1]Lithuanian Forest Research Institute, Laboratory of Molecular Genetics and Biotechnology, Liepu 1, Girionys, LT-53101 Kaunas district, Lithuania

[2]Lithuanian Forest Research Institute, Department of Tree Genetics and Breeding, Liepu 1, Girionys, LT-53101 Kaunas district, Lithuania

[3]Vilnius University, Departament of Botany and Genetics, M. K. Ciurlionio 21, LT-03001 Vilnius, Lithuania

** - author for correspondence: ramuneareskeviciene@yahoo.com, phone +37065144443*

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Abstract

The genetic diversity and genetic differentiation of eight Lithuanian populations of Norway spruce (*Picea abies* (L.) Karst.) were studied using random amplified polymorphic DNA method. Seven random oligonucleotide primers produced 74 scorable fragments. Differences in the level of DNA polymorphism among populations were established. Out of the 74 amplified loci scored, 69 (93.2%) were polymorphic among all studied populations. The highest RAPD polymorphism (79.73%) and observed number of alleles (1.8) was obtained for Alytus population, but the highest effective number of alleles (1.453), Shanon's index (0.391) and Nei's gene diversity (0.262) were found for Telsiai population. According to Nei's genetic distances the most geneticaly close are Dubrava and Rokiskis populations, whereas the most distant - Rokiskis and Plunge. The results showed the high intra-populational (90.35%) and comparatively low inter-populational (9.65%) genetic variation. The average number of migrants per generation (Nm) among populations was 4.7.

Key words: RAPD, *Picea abies*, population, genetic diversity

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is one of the most important species of European trees, both from the economic and ecological point of view. Accordingly, it is a traditional object of forest genetics and breeding research. In Lithuania spruce forests cover about 22.8% of the total forest area (Lithuanian Statistical Yearbook of Forestry 2003). Lithuanian spruce forests are situated in the Baltico-Nordic domain of the natural range of *P. abies* (Schmidt-Vogt 1986, Collignon and Favre 2000). In 1994 Lithuania entered the EUFORGEN programme aiming at ensuring the effective conservation and sustainable utilization of forest genetic resources in Europe (Koski *et al.* 1997). Since national database on Norway spruce genetic resources was created (Danusevičius and Gabrilavičius 1995, Gabrilavičius and Danusevičius 1996), national catalogue of the plant genetic resources with a section on the forest genetic resources was published (Pliura *et al.* 1997) and regions for transfer of Norway spruce seeds were re-approved (Danusevičius *et al*. 1999). Much valuable information on Lithuanian *P. abies* genetic structure was derived from morphological, eco-climatic, karyotype, isozyme and other analysis (Gabrilavičius and Danusevičius 2003).

In recent years there has been increasing interest in the use of DNA-based markers for a variety of applications in population genetics, conservation and

tree improvement. Williams *et al.* (1990) experimentally demonstrated that short primers of arbitrary nucleotide sequence can be used to reproducibly amplify segments of genomic DNA from a wide variety of species. By random amplified polymorphic DNA (RAPD) was in general use for various forest trees species: pine (Plomion *et al*. 1995, Thomas *et al.* 1999, Boscherini *et al.* 1994, Hurme and Savolainen 1999, Žvingila *et al*. 2002, Szyp-Borowska and Staniulyte 2003), aspen (Yeh *et al.* 1995), oak (Yakovlev and Kleinschmidt 2002, González-Rodríguez *et al*. 2004), birch (Howland *et al.* 1995, Zeng *et al.* 2003) and other. In the spruce research RAPD was used to study provenances and populations genetic differentiation (Collignon and Favre 2000, Kraj 2002), genetic mapping (Binelli and Bucci 1994, Bucci *et al*. 1997, Troggio *et al.* 2001), for clone verification and phenotypic selection (Scheepers *et al.* 1997), for genetic validation and characterization of closely related species (Nkongolo *et al*. 2003) and in the study of genetic stability of embryogenic lines (Nkongolo *et al.* 1998). However the overwhelming majority of studies concerning the genetic structure of *P. abies* are based on morphological and allozyme markers (Tigerstedt 1973, Lagercrantz and Ryman 1990, Goncharenko *et al*. 1995, Krutovskii and Bergmann 1995, Lewandowski *et al*. 1997, Gabrilavičius and Danusevičius 2003, Gončarenko *et al.* 2005).

The general aim of the present study was to analyse the genetic diversity within and between eight Lithuanian populations of Norway spruce using RAPD markers.

Materials and methods

Plant material and DNA extraction. The plant material was collected in the experimental plantation of the Lithuanian Forest Research Institute, established in Kazlu Ruda experimental and training forest enterprise in 1983 (Gabrilavičius and Danusevičius 2003). Eight Lithuanian Norway spruce populations were included in the present study (Fig. 1, Table 1). From 16 to 21 individuals of different families represented each population – a total of 152 trees.

Genomic DNA was extracted from needles using a modified CTAB method (Doyle and Doyle 1990). Additionally RNase A (Fermentas) was used to eliminate RNA. The DNA was checked by subjecting it to 1.0% agarose gel electrophoresis and concentration was estimated by spectrophotometer (Eppendorf) at 260 nm.

RAPD analysis. Seven decamer random primers (Table 2) were selected for RAPD analysis according to the primary study of Norway spruce (Staniulytė *et al.* 2004). The main criterion for the selection

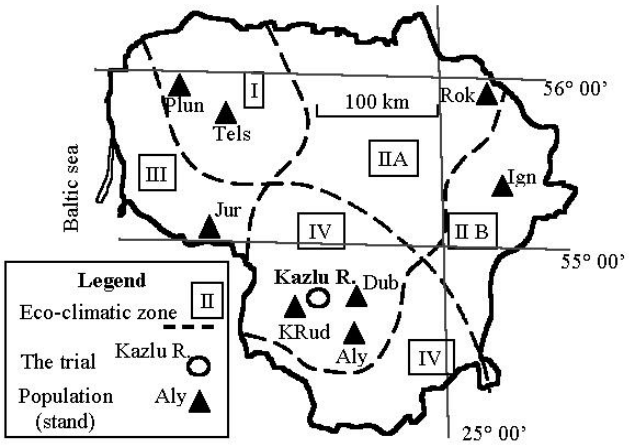

Figure 1. Origin of studied Lithuanian Norway spruce populations located in Kazlu Ruda trial. The abbreviated population names are given according to Table 1

Table 1. Sampling locations and the number of individuals analysed in the studied Lithuanian Norway spruce (*Picea abies* (L.) Karst.) populations

No	Population	Individuals analysed	Latitude, N	Longitude, E	Altitude, m
1	Plunge (Plun)	20	55º52´	21º50´	130
2	Telsiai (Tels)	16	55º50´	22º15´	190
3	Rokiskis (Rok)	20	55º55´	25º35´	140
4	Ignalina (Ign)	17	55º20´	26º10´	230
5	Jurbarkas (Jur)	18	55º05´	22º15´	40
6	Kazlu Ruda (KRud)	20	55º43´	23º33´	100
7	Alytus (Aly)	20	54º30´	24º05´	120
8	Dubrava (Dub)	21	54º50´	24º00´	65

was production of clear polymorphic loci (as polymorphic are considered bands that occurs in some of investigated individuals). DNA bands reproducible in at least two independent experiments were scored for the analysis.

RAPD reactions were carried out in a volume of 25 µl containing 1.0 U of termostable *Taq* polymerase (Fermentas), 0.4 µM single primer, 0.2 mM each dATP, dCTP, dGTP and dTTP, 3.0 mM $MgCl_2$, 2.5 µl Taq buffer and approximately 150 ng of DNA. Amplifications were performed in a DNA thermocycler *TGradient* (Biometra) using a period of 4 min of initial denaturation at 94˚C, followed by 45 consecutive cycles of 1 min of denaturation at 94˚C, 1 min of annealing at 35˚C, 2 min of extension at 72˚C, and a final extension step of 5 min at 72˚C (Žvingila *et al.* 2002). Each reaction was performed at least twice.

The amplification products were separated by

electrophoresis on 1.5% agarose gel in Tris-borate-EDTA (TBE) buffer and stained by ethidium bromide. DNA bands were observed under UV light and photographed using BioDocAnalyse system (Biometra).

Data analysis. Only clear and polymorphic DNA bands were used for data analysis. The bands were scored as present (1) or absent (0) and a binary data matrix was constructed. DNA fragments of identical size amplified with the same primer were considered to be the same DNA marker. The PopGene3.2 software (Yeh *et al*. 1999) was used to estimate values, which describe genetic diversity: number of polymorphic band (as polymorphic are considered bands that occurs in some of investigated individuals), observed number of alleles (n_a), effective number of alleles (Kimura and Crow 1964), Nei's (1973) gene diversity (h), Shannon's information index (I) (Lewontin 1972) and genetic distances among populations (Nei 1978). A dendrogram of the populations was constructed which has been based on the unweighed pair group method (UPGMA) analysis of Nei's (1978) genetic distances. Population differentiation as G_{ST} was calculated using McDermott and McDonald (1993) formula: $G_{ST} = (h_t - h_s) / h_t$, where h_s – intra-populational variation for polymorphic bands and h_t – total variation for polymorphic bands. According to G_{ST} values, estimates of gene flow (Nm) among populations were calculated using formula $Nm = 0.5 (1 - G_{ST}) / G_{ST}$.

Results

In the present study eight Lithuanian populations of *P. abies* were analysed by RAPD. The seven informative primers were used to examine the level of polymorphism (Table 2). On average 10.6 scorable bands were generated per primer. Primer Roth 370-10 gave the largest (12), whiles Roth 370-04 the smallest (9) number of bands. The size of the amplification products ranged from 480 bp to 3000 bp. Out of the 74 amplified loci scored, 69 (93.2%) were polymorphic among all studied populations. The pattern of DNA polymorphism established with primer Roth 370-10 is shown in Figure 2.

Different primers revealed various number of polymorphic RAPD fragments in studied populations (Table 2). One genotype specific 530 bp band was detected with primer Roth 370-10 for one individual from Ignalina population.

The values of genetic diversity indices for tested Norway spruce populations are given in Table 3. The highest n_a was observed for Alytus population (1.797) and the least – for Jurbarkas (1.716), showing the lowest Shannon's index (0.342) and Nei's gene diversity (0.22) as well. The highest n_e (1.453),

Table 2. Primers used for RAPD analysis, total number of scored fragments and their size range

Primer	Sequence (5'→3')	Total number of bands	Fragment size range (bp)
Roth 170-05	5' GCACCGAACG 3'	10	620 -2150
Roth 170-08	5' CTGTACCCCC 3'	11	850 - 2500
Roth 170-10	5' CAGACACGGC 3'	11	760 - 2500
Roth 370-01	5' TCCCTGTGCC 3'	11	780 - 1800
Roth 370-02	5' GCTCTCCGTG 3'	10	500 - 3000
Roth 370-04	5' GTATGCCGCG 3'	9	480 - 1350
Roth 370-10	5' CTGTCCGGTC 3'	12	510 - 2600

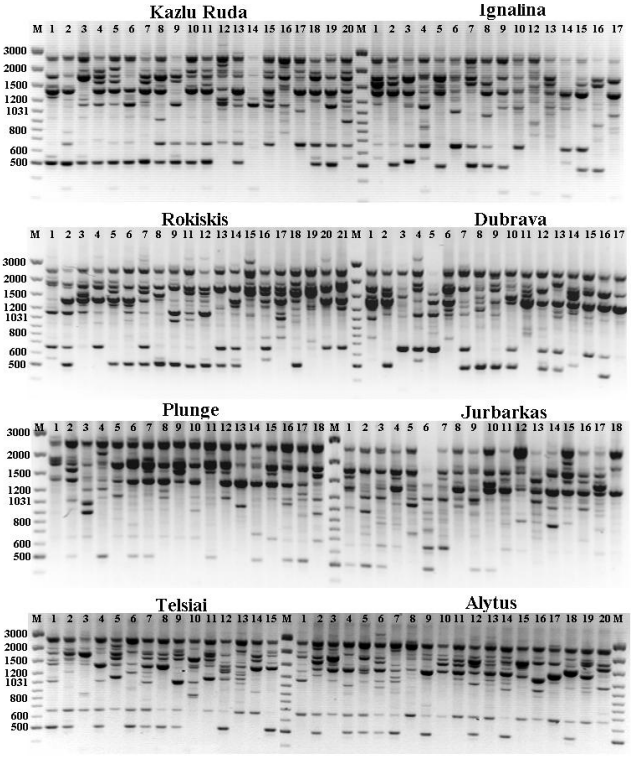

Figure 2. RAPD agarose gel electrophoresis profiles of eight Lithuanian populations of Norway spruce using primer Roth 370-10. Lines marked by numbers (1, 2, 3…) represent the individuals that belong to populations listed in table 1. M – DNA size marker is given in base pairs (bp)

Shanon's index (0.391) and Nei's gene diversity (0.262) were found for Telsiai population. The lowest effective number of alleles and Nei's gene diversity (1.37 and 0.23 respectively) were found in the population of Plunge.

Nei's (1978) genetic distance (Table 4) among studied Norway spruce populations was calculated and varied from 0.009 to 0.061. Accordingly the most genetically close were Dubrava and Rokiskis populations, whereas the most distant - Rokiskis and Plun-

Table 3. Genetic diversity indices for eight Norway spruce Lithuanian populations. n_a – observed number of alleles, n_e – effective number of alleles, h - Nei's (1987) gene diversity, I - Shannon's information index

Population	Number of polymorphic loci	Percentage of polymorphic loci	n_a	n_e	h	I
Plunge	58	78.38	1.784	1.371	0.230	0.354
Telsiai	56	75.68	1.757	1.453	0.262	0.391
Rokiskis	56	75.68	1.757	1.416	0.248	0.375
Ignalina	53	71.62	1.716	1.388	0.232	0.352
Jurbarkas	53	71.62	1.716	1.374	0.224	0.342
Kazlu Ruda	57	77.03	1.770	1.413	0.244	0.370
Alytus	59	79.73	1.797	1.438	0.256	0.386
Dubrava	55	74.32	1.743	1.436	0.255	0.382
Mean	55.9	75.51	1.755 ±0.029	1.411 ±0.031	0.244 ±0.014	0.369 ±0.018

Table 4. Nei's (1978) genetic distance among eight studied Norway spruce populations. The abbreviated population names are given according to Table 1

Plun							
Tels	0.042						
Rok	0.061	0.022					
Ign	0.054	0.024	0.027				
Jur	0.024	0.032	0.046	0.032			
KRud	0.039	0.024	0.023	0.031	0.036		
Aly	0.032	0.014	0.031	0.032	0.032	0.026	
Dub	0.052	0.021	0.009	0.033	0.037	0.017	0.023
	Plun	Tels	Rok	Ign	Jur	KRud	Aly

ge. Dendrogram based on UPGMA cluster analysis of genetic distance values (Fig. 3) grouped all populations in two clusters. One cluster contained two populations (Plunge and Jurbarkas), the other cluster contained six populations from different parts of Lithuania. The clusters did not precisely reflect the geographic position of populations.

To quantify genetic differentiation of populations,

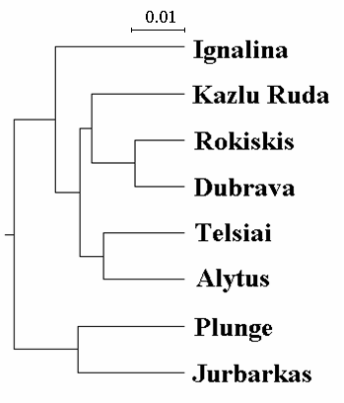

Figure 3. The dendrogram for eight Lithuanian populations of Norway spruce obtained employing the UPGMA method

G_{ST} was calculated. The results showed that population differentiation amount 0.0965. Most of the total genetic variation (90.35%) was found between individuals within populations, while 9.65% of the genetic variation was because of among population differences. The average number of migrants per generation among populations was 4.7.

Discussion and conclusions

Woody plant species, among which trees, are generally long-lived organisms presenting a high level of adaptation to different types of environment and a very high level of genetic variability. The mean percentage of polymorphism per population estimated in our study was 75.51%. Populations were similarly polymorphic for the whole set of scored bands (Table 3). No bands were fixed exclusively in a single population, except one individual specific band in Ignalina population. Earlier studies of isozyme polymorphism carried out in two distant Lithuanian populations (Jurbarkas and Ignalina) of Norway spruce revealed that 74% of the loci studied were polymorphic. Furthermore, a large variation in morphological traits was observed in natural stands of Norway spruce in Lithuania. A number of distinct expressions of shape of cone scales, cone color, branching type, bark patterns were found (summarized in Gabrilavičius and Danusevičius, 2003). Thus our results obtained on the basis of molecular data support the former evidence of a large genetic variation within natural populations of *P. abies*. The great genetic variability of spruce as other wind pollinated woody plants is influenced by a large number of factors: ancient origin and immigration history, natural selection, an extensive gene flow caused by pollen dispersal, genetic drift due to small population size, and human activities (Hamrick *et al*. 1992, Collignon and Favre 2000).

The G_{ST} indicating how much of the genetic diversity is among populations reached in our study the value 0.0965. The value of genetic differentiation of the same order was established using RAPD analysis among Polish (6.4%) and French (4.2%) populations of Norway spruce (Collignon and Favre 2000, Kraj 2002). Former allozyme studies also showed low, but significant differentiation among populations of *P. abies*. For example Langercrantz and Ryman (1990) estimated that the genetic differentiation of 70 populations of spruce distributed over whole European range reached 5%. Giannini *et al*. (1991) studied nine Italian populations and found the G_{ST} value of 4.4%. Genetic differentiation calculated among 20 Swiss populations of spruce was 4.3% (Müller-Starck 1995). A

bit lower differentiation in allozyme loci among populations from north-eastern and southern Poland was observed (2.8%) by Lewandowski and Burczyk **(**2002). Isoenzyme polymorphism of fifteen Norway spruce populations of the international IUFRO provenance-testing programme of 1964/1968 showed vaguely major genetic differentiation at 19 coding gene loci. The overall genetic diversity between the provenances was 6.7%. According to Kannenberg and Gross (1999) the genetic variation within the populations increased in going from the southwest to the north or northeast (most forest tree species are characterized by having high rates of gene flow among their populations). On average, woody species have more than 90% of their genetic diversity within their populations and less than 10% among their populations. This implies high rates of gene flow and corresponds to more than 2 successfully established migrants per population in each generation. The estimate of Nm for widely spread woody plants can reach more than 7 migrants per generation (Hamrick *et al* 1992).Thus our results confirm this tendency.

The estimates of genetic distance between eight studied populations mainly were geographically independent. They showed larger genetic distance over short geographic distances, against low genetic distance between geographically distant populations. For example, the genetic distance obtained between Dubrava and Alytus populations, located only about 80 km apart, was 0.023, whereas the GD between Dubrava and Rokiskis populations separated by more than 190 km, was only 0.009 (Table 4). The dendrogram drawn on the basis of genetic distances using UPGMA method demonstrates the possible relationships between studied Lithuanian populations (Figure 3). One of two clusters of the dendrogram includes only Plunge and Jurbarkas populations from the western part of Lithuania. These populations are from different seed zones but geographically close. The second cluster is very heterogenic and involves populations from different parts of Lithuania. Ignalina population is more genetically distant and forms separate branch from other populations (Kazlu Ruda, Rokiskis, Dubrava, Telsiai and Alytus), of which 60% are from the central part of Lithuania (Kazlu Ruda, Dubrava and Alytus). The incorporation of two geographicaly distant populations Dubrava and Rokiskis into one subcluster presents the most problematic feature of this dendrogram (Figure 3). Close genetic relationship between these two spatially remote populations is difficult to explain. In spite of the fact that RAPD markers usually are considered as neutral characters, various authors point out that some of these markers are at least partially adaptive and are influenced by natural diversifying selection (Latta and Mitton 1997, Owuor *et al.* 1999, Fahima *et al.* 1999, Semagn *et al*. 2000). In such situation the general structure of clusters can be influenced by local ecological factors. Similar ecological conditions of population's habitats could cause the convergence of noncoding DNA sequences. On the other hand, the evolutionary history of populations (for example severe bottlenecks occurred in the past) can strongly influence the genetic structure of population (Gaudeul *et al*. 2000).

Our research gave new information on the genetic structure of Lithuanian Norway spruce populations by using RAPD markers. It demonstrated the ability of this technique to detect the genetic differentiation among Lithuanian populations. It also showed that genetic divergence among Lithuanian populations of *P. abies* is largely based on RAPD markers frequency differences rather than upon complete absence or presence of particular bands. The lack of population specific RAPD markers indicates that the management of Lithuanian spruce genetic resources at the populational level should be based on the integrated employment of morphological and molecular markers.

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ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ В ПОПУЛЯЦИЯХ ЕЛИ ОБЫКНОВЕННОЙ (*PICEA ABIES* (L.) KARST.) ЛИТВЫ МЕТОДОМ RAPD

Р. Арешкевичене, Д. Жвингила, Р. Габрилавичюс, С. Куусене

Резюме

Генетическое разнообразие и дифференциация восьми литовских популяций ели обыкновенной (*Picea abies* (L.) Karst.) изучалось методом RAPD. С помощъю семи олигонуклеотидных праймеров был изучен ДНК полиморфизм 74 локусов. Установлены различия в полиморфизме ДНК между популяциями. Самое большое число полиморфных фрагментов ДНК (79,7%) установлено в популяции Алитус. Однако наибольшими значениями числа эффективных аллелей (1,453), индекса фенотипического разнообразия Шенонўа (0,391) и генного разнообразия Нея (0,262) отличалась популяция Тельшяй. Соответственно значениям генетических дистанций Нея наиболее генетически близкими являются популяции Дубрава и Рокишкис, а наболее отдаленными – популяции Рокишкис и Плунге. Генетическая дифференциация между изученными популяциями ели обыкновенной составила 9,65%. Интенсивность генного потока (Nm) между популяциями, определённая при помощи показателя генетической дифференциации (G_{ST}), составила в среднем 4,7 мигрантов на поколение.

Ключевые слова: RAPD, *Picea abies*, популяция, генетическое разнообразие