

Comparison of DNA polymorphism in seedlings of *Pinus sylvestris* L. from different populations by RAPD markers

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We used random amplified polymorphic DNA (RAPD) to examine genetic differences among three populations of *Pinus sylvestris* L. (Labanoras, Druskininkai (Veisiejai) and Telšiai) situated in ecologically different environments. The influence of original habitats was excluded by growing seedlings in experimental plots at the Botanical Garden of Vilnius University. There were identified 63 RAPD loci in 90 plants. All individuals had specific RAPD phenotypes. Allele frequency data were used to calculate within-population ($H_s = 0.203 \pm 0.03$) and within-species ($H_T = 0.249 \pm 0.04$) genetic diversity. The level of average heterozygosity was highest in Labanoras population (0.23 ± 0.03) and the lowest in Telšiai population (0.18 ± 0.02). The UPGMA dendrogram based on the genetic distance and PCO analysis gave similar results and showed that Druskininkai and Telšiai populations were most genetically distant. A possible relationship between the level of genetic diversity and the properties of the populations studied is discussed.

Key words: *Pinus sylvestris* L., comparison of populations, RAPD, genetic diversity, mean heterozygosity

INTRODUCTION

Among- and within-population genetic diversity is a material for the breeding of forest trees and conservation of valuable genes. It is essential to analyze the genetic structure of the most important forest tree populations in order to make an effective use of available woodland resources and to foresee the strategy of preserving their genetic diversity [1].

Scots pine (*Pinus sylvestris* L.) is the most important forest tree species in Lithuania. It occupies up to 37.2% of the total forest area in the country [2]. Studies of the genetics of *P. sylvestris* have been started in Lithuania in 1956 [2, 3]. Since then, the research work involves analysis of the phenotypical structures of pine populations, the efficiency of population transfer, the use of geographical variability by means of population hybridization, the sampling of valuable genotypes, inter-specific hybridization, accumulation of genetic resources, etc [2]. Differences among populations of *P. sylvestris* were used as the basis for delineation of seed zones in the country [4]. The small territory of Lithuania is significantly different in ecological conditions which may have an impact on genetic structure of the local plant popu-

lations. The perennial plant *P. sylvestris* is a suitable model for such studies. The local populations of *P. sylvestris* used in this work represent the regions of Lithuania with well expressed ecological differences and are favorable for investigators of *P. sylvestris* populations. So, a significant many-year information has been accumulated concerning biological peculiarities of the local *P. sylvestris* populations from Telšiai, Druskininkai and Labanoras forests. They show a rather different branching pattern, sporification time, biological productivity, adaptation to new environments, etc. [5, 6]. Despite these peculiarities, the genetic variability within populations of Scots pine is known to be high [2, 7].

The former studies of Lithuanian *P. sylvestris* populations were based mainly on the morphological and phenological traits. As an alternative to these traditionally measured features, DNA markers offer the advantages of being unaffected by the environment or the developmental stage of the plant. Besides, they are very polymorphic and numerous. As tools in forest tree improvement, DNA markers are most useful for estimating genetic diversity in breeding populations and for germplasm identification [8]. One type of molecular markers that have received much attention in studies of genetic diversity in natural populations is randomly amplified polymorphic DNA

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(RAPD) [9]. They have been applied to many plant species, including pines [10, 11]. Most of RAPD markers are likely neutral, although it has been demonstrated that part of these loci are adaptive [10, 12].

The purpose of the present study was to assess RAPD diversity within and among three seedling groups derived from three Lithuanian populations of *P. sylvestris*.

MATERIALS AND METHODS

Plant material. Seeds from 20 trees of each population (Telšiai, Druskininkai (Veisiejai) and Labanoras) were collected and sown in experimental plots of Botanical Garden of Vilnius University (Table 1). Genomic DNA from the needles of one-year-old seedlings was extracted using the CTAB method of Doyle and Doyle [13], and 30 seedlings per population were analyzed.

RAPD analysis. PCR was performed as described earlier [14]. Arbitrary decamer primers were purchased from Carl Roth (Germany). Amplification products were separated by electrophoresis on 1.5% agarose gels using 1 × Tris-borate-EDTA buffer and detected by staining with ethidium bromide (Serva, Hei-

delberg, Germany). The GeneRuler™ DNA Ladder Mix (Fermentas, Lithuania) was used for sizing DNA amplification products.

Only clear and reproducible DNA bands were used for subsequent statistical analyses. The presence (1) or absence (0) of the fragment in each sample was assessed and the data were assembled into a binary matrix. DNA bands of equal mobility were treated as identical. Genetic distance among individuals (GD_{xy}) was calculated in accordance with the Nei and Li formula [15]: $GD_{xy} = 1 - 2 N_{xy} / (N_x + N_y)$, where N_{xy} – is the number of DNA bands present in both x and y, N_x is the number of DNA bands inherent in the plant x, and N_y is the number of DNA bands inherent in the plant y. GD_{xy} was used to construct a UPGMA dendrogram using TREECON for Windows [16]. The genetic parameters of the populations, such as the number of polymorphic loci (P), the average observable number of alleles per locus (n_p), the effective number of alleles per locus (n_e) [17], the Nei [18] gene diversity (h), Shannon's information index of phenotypic diversity (I) [19], within-population diversity (H_s), within-species diversity (H_p) and Nei's Gst were computed with PopGene v.1.31 [20].

Analysis of the principal coordinates (PCO) and distribution of DNA fragments in pine populations was done using GenAIEx (*Genetic Analysis in Excel*) software [21].

RESULTS AND DISCUSSION

Seven arbitrary decamer primers yielding reproducible and unambiguous fragments were chosen

Table 1. The study populations of *Pinus sylvestris* L.

Population name	No. of analysed seedlings	Latitude (°N)	Longitude (°E)
Labanoras	30	54°02'	23°42'
Druskininkai	30	55°14'	25°44'
Telšiai	30	55°57'	22°29'

Table 2. Primers used in RAPD analyses, numbers and sizes of generated bands

Primer	Primer sequence 5'→3'	No. RAPD loci	Number of polymorphic DNA fragments				DNA band size range (bp)
			Labanoras	Druskininkai	Telšiai	Total	
270-1	GTCTCGTCGG	13	8	7	6	9	480-1880
270-2	GGCCTACTCG	7	4	3	2	4	490-1600
270-3	GTGTAGGGCG	9	6	5	5	6	320-1600
270-4	CGGGTTCGATC	8	4	3	4	4	800-2000
270-5	GCCCTCTTCG	9	6	4	6	7	500-1500
270-6	CAGGGGCATC	7	4	3	2	4	470-1140
170-1	CATCCCGAAC	10	6	10	8	10	480-1880
Total number of DNA bands		63	38	35	33	44	
Total number of polymorphic DNA bands (%)			60.3	55.5	52.4	69.8	

Table 3. Summary of genetic variation statistics for all 63 loci in populations of *Pinus sylvestris* L.

Populations	Observed number of alleles, n_a	Nei's gene diversity, h	Effective number of alleles, n_e	Shannon's information index, I	GD_{xy}^*
Labanoras	1.603	0.225	1.402	0.330	0.150
Druskininkai	1.539	0.206	1.364	0.303	0.116
Telšiai	1.539	0.176	1.294	0.267	0.091

* – GD_{xy} , mean genetic distance among individual plants in population.

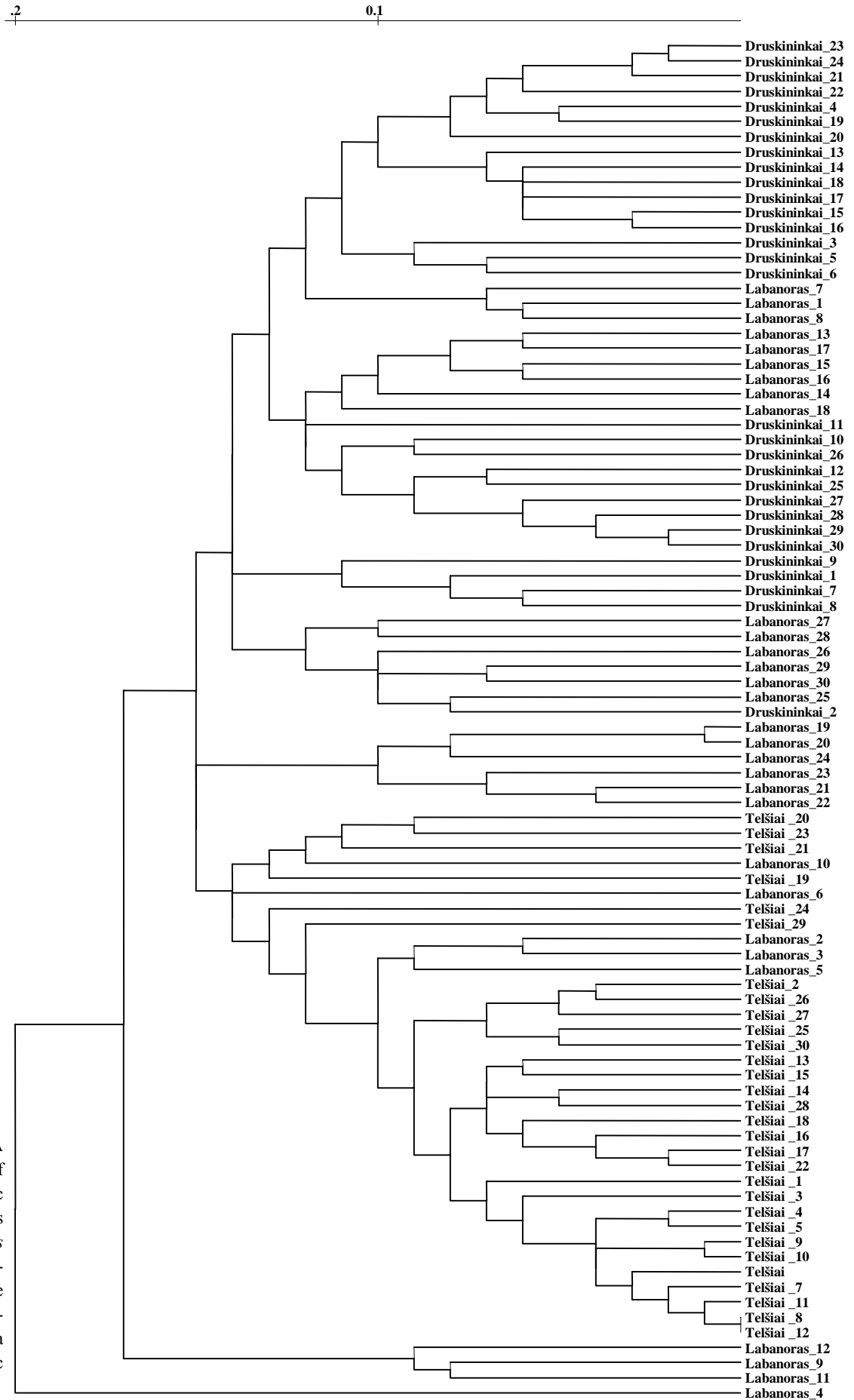


Fig. 1. UPGMA dendrogram of putative genetic relatedness among 90 *Pinus sylvestris* individuals from three populations generated from a matrix of genetic distance values

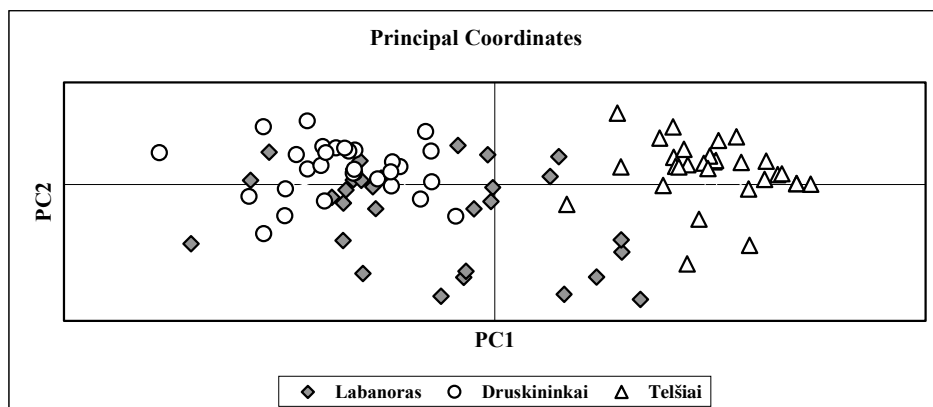


Fig. 2. Two-dimensional representation of principal coordinate analysis of *Pinus sylvestris* populations based on RAPD genetic distances. Percentage explained variability: PC1 – 15.64%, PC2 – 8.46%

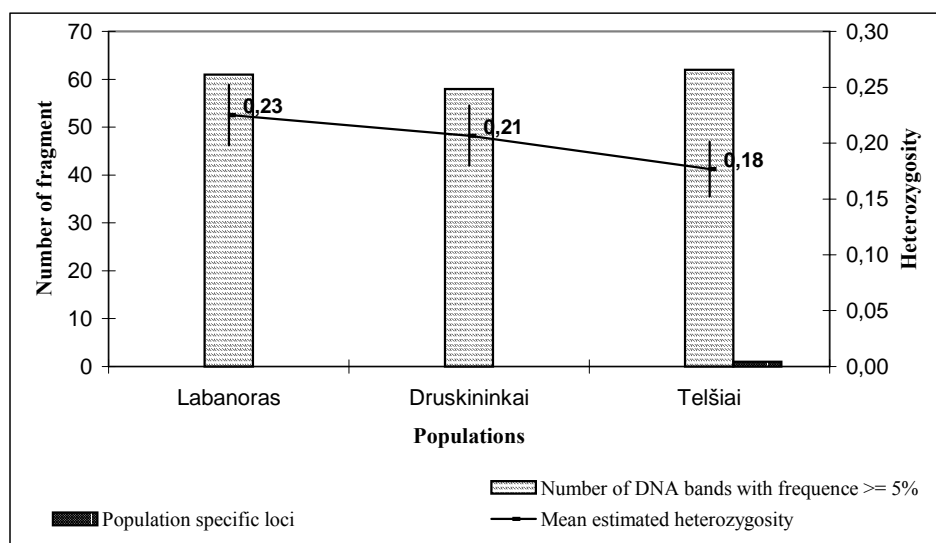


Fig. 3. The level of average expected heterozygosity in the study populations of *Pinus sylvestris*

for RAPD analysis in three populations of *P. sylvestris*. These primers revealed 63 loci (Table 2). The percentages of monomorphic and polymorphic bands in the 90 genotypes studied were 30.2% and 69.8%, respectively. The size of scored DNA fragments ranged from 320 bp to 2000 bp. The highest number of RAPD loci (13) was identified using the primer 270-1 and the least (7) with 270-2. The mean number of bands identified per primer was 9. The most polymorphic population Labanoras possessed 60.3% of polymorphic loci, while the Telšiai population showed 52.4% of loci. 44 bands were polymorphic in all populations. There was identified one locus (270 – 5₅₀₀) possibly specific of Telšiai population.

We also measured the genetic distances among individuals in each population and a genetic distance among the populations. The mean genetic distance among individual seedlings ranged from 0.091 for Telšiai to 0.150 for Labanoras population (Table 3). Genetic relationships among the individual plants of *P. sylvestris* were assessed by means of

cluster analysis (UPGMA) (Fig. 1). This dendrogram is based on the genetic distance matrix of 4005 possible pairs of 90 individuals. The dendrogram shows that individuals from Druskininkai and Telšiai populations were clustered in two remote and rather compact groups. Individual seedlings derived from Labanoras population were distributed among a number of small groups. Four genotypes of this population are located outside the main clusters of the plants.

The principal coordinate analysis supported the results of UPGMA analysis (Fig. 2). It demonstrated that Labanoras population was most genetically heterogeneous. Both the dendrogram and PCO showed that seedlings from Labanoras population possessed the highest genetic relation to Druskininkai population. Nei's genetic distance value between

these two populations was 0.062 (data not shown). The largest genetic distance (0.105) was established between Telšiai and Druskininkai populations. From the ecological point of view, Telšiai population is located in the area of maritime climate. The other two populations are situated in the zone of a more continental climate. It has been established earlier that pollen release periods of some populations from different parts of country are not overlapping and therefore these populations cannot exchange pollen directly [3]. Such divergence in timing of pollen dispersal could cause the genetic differentiation between them.

Nei's gene diversity for each population is presented in Table 3. The Labanoras population showed the highest diversity value (0.225) while the Telšiai population had the lowest value (0.176). The estimates of the genetic parameters of the other populations (n_s , n_e , I) showed a similar pattern of distribution between these populations: the highest values in Labanoras and the lowest in Telšiai population. The differences in genetic diversity may be as-

sociated with biological properties of the Scots pine populations studied. In the former studies [22] it has been demonstrated that seedlings of the population from the most continental conditions (Labanoras) exceeded by growth and biological productivity traits the seedlings from maritime (Telšiai) and southern medium continental (Veisiejai) populations. The Telšiai population showed also the most negative reaction to new climatic conditions in population transfer experiments. Besides, the Labanoras population demonstrated the best performance in earlier forest studies [2]. Although not in all cases [23], a positive correlation between genetic diversity and fitness is often observed [24, 25]. For example, Dvorak et al. [25] have reported that provenances of *Pinus maximinoi* selected in trials for good volume production were generally the most genetically diverse by biochemical and molecular marker assessment. Moreover, the observed heterozygosity patterns in 25 populations of *P. maximinoi* detected in the RAPD analysis correlated reasonably well with provenance performance [25]. There could be many reasons of such a correlation. For example, mating between related individuals increases the proportion of homozygous loci in offspring, therefore increasing the probability of recessive deleterious alleles occurring in a homozygous state and resulting in a lower fitness [26]. In our study, the highest level of average expected heterozygosity (0.23 ± 0.03 , SE) was detected in Labanoras population and the lowest (0.18 ± 0.02 , SE) in Telšiai population (Fig. 3). The higher heterozygosity is expected to result in somatic, reproductive and adaptive heterosis [27].

The mean within-population diversity (H_d) and within-species diversity (H_s) established in studied population were 0.203 ± 0.03 and 0.249 ± 0.04 , respectively. These results are similar to those obtained by Nowakowska [28], where $H_T = 0.262$. The other genetic characteristics of the populations tested in Nowakowska's study also showed a good agreement with our results (for example, in five groups of 29 Polish *P. sylvestris* provenances the number of observed alleles ranged from 1.58 to 1.76, the Shannon index from 0.25 to 0.38, while in our work n_a ranged from 1.390 to 1.603 and I from 0.267 to 0.330).

Our study included only three populations that can be considered as model populations in Lithuanian *P. sylvestris* studies, because they have been used in many former forestry and tree genetics studies and so are able to display the genetic diversity of this species in the country. To our knowledge, it is the first attempt to examine a population structure by RAPD loci in the Lithuanian *P. sylvestris* populations. It demonstrates some differences in DNA polymorphism among the populations studied and supplements the genetic researches of this most important Lithuanian forest tree species with new molecular data.

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**DNR POLIMORFIZMAS PAPRASTOSIOS PUŠIES
(*PINUS SYLVESTRIS* L.) SKIRTINGOS KILMĖS
SĖJINUKUOSE**

S a n t r a u k a

RAPD (Random Amplified Polymorphic DNA) metodu ištirtas DNR polimorfizmas paprastosios pušies (*Pinus sylvestris* L.) sėjinukuose, išaugintuose iš sėklų, surinktų Labanoro, Druski-

ninkų (Veisiejų) ir Telšių populiacijose. Nustatyti 63 RAPD lokusai, iš kurių 69,8% buvo polimorfiški. Pagal alelių dažnius apskaičiuotas genetinės įvairovės lygis populiacijos ($H_s = 0,203 \pm 0,03$) ir rūšies ($H_T = 0,249 \pm 0,04$) viduje. Didžiausias vidutinis heterozigotiškumas nustatytas Labanoro kilmės sėjinukuose ($0,23 \pm 0,03$), o mažiausias – Telšių kilmės sėjinukuose ($0,18 \pm 0,02$). UPGMA (Unweighted-pair group method with arithmetic means) giminingumo medis bei gautų duomenų principinių koordinatų analizė rodo, kad labiausiai genetiškai nutolusios Druskininkų ir Telšių populiacijos. Straipsnyje aptariama galima heterozigotiškumo įtaka tirtų paprastosios pušies populiacijų biologinėms savybėms.