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Genetic diversity (RAPD) in natural Lithuanian populations of common ash (*Fraxinus excelsior* L.)

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Randomly amplified polymorphic DNA (RAPD) analysis was used to examine the genetic diversity and structure of ten natural Lithuanian common ash (*Fraxinus excelsior* L.) populations. A total of 99 trees were studied using six arbitrarily selected primers. Seventy-six RAPD bands ranging in size from 0.45 to 2.8 kbp were scored for analysis. Differences in the level of DNA polymorphism among the populations were established. The percentage of the polymorphic DNA fragment in the ten populations studied varied from 57.9 to 92.1%. The estimates of Shannon's phenotypic diversity index ranged from 0.312 for the Pakruojis population to 0.532 for the Šilutë population, averaging to 0.44. Most of the genetic variations were found among individuals within the populations, while the interpopulation variance component accounted for 8.3% ($P = 0.001$). The low interpopulation differentiation implies an extensive gene flow among them. Genetic differences among the populations appeared due to the frequency differences of amplified fragments rather than to the absence or presence of particular RAPD bands. Both the principal component analysis and UPGMA cluster analysis of genetic distance values revealed a varying degree of separation among the populations with a considerable overlap among them; however, the clusters did not precisely reflect the geographic position of the populations. A medium-to-strong negative correlation between population genetic diversity characteristics and the longitude and climate continentality index of population locality was found to indicate existence of a clinal trend and a higher genetic diversity in more maritime West Lithuania.

Key words: genetic diversity, RAPD markers, *Fraxinus excelsior*, population structure

INTRODUCTION

Common ash (*Fraxinus excelsior* L., Oleaceae) is a temperate tree species that exists as small discontinuous populations in European mixed deciduous forests. The populations of common ash are mostly natural and occupy about 2.6% of the territory covered by forests in Lithuania [1]. In spite of a rather wide spread of this species, a high mortality of trees was observed in natural populations [2, 3]. The damage has no geographic pattern; more serious damage has been monitored in 20–40-year-old stands [4]. Species composition and the percentage of ash in stands do not appear to be influential factors. The severe damage of ash stands can be considered as ash dieback. Most European countries also consider *Fraxinus* to be threatened at the population level [5]. The capacity of forest tree species to persist in spatially and temporarily heterogeneous environments is dependent on their adaptive potential, which is determined by the intraspecies genetic diversity at the inter- and intrapopulation levels [6]. Eriksson et al. [7] strongly argued that the present-day genetic structure is transient and that maximum fitness is not achieved in nature for multiple reasons such as too rapid changes of the environment, genetic drift, gene flow among populations and other evolutionary forces. Therefore the existing adaptedness and genetic structure should not be regarded as the objective for gene conservation but as the starting material. Comprehensive studies of common ash populations were performed using nuclear and chloroplast microsatellite markers [8–12]. Two Lithuanian common ash populations were included in the latest studies [11, 12]. In the recent study, Nowakowska et al. [13] made an attempt to measure genetic varia-

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bility within and among the Lithuanian populations of common ash in comparison with Polish populations, using RAPDs. Three Lithuanian populations were analysed in this study.

The random amplified polymorphic DNA (RAPD) technique, first described by Williams et al. [14], despite some limitations provides a useful approach for evaluating population's genetic differentiation, particularly in the species that are poorly known genetically [15, 16]. The problem of low reproducibility in early RAPD analyses can now be overcome through improved laboratory techniques and band scoring procedures [16, 17]. Another limitation of RAPD markers is their dominant nature [14]. This peculiarity of RAPDs impedes direct estimations of allele frequency and can bias calculations of population differentiation [18]. This problem can be overcome by use of appropriate statistical methods, *e.g.*, analysis of molecular variance (AMOVA) [19], which is not influenced by the dominance problem [20, 22].

Lithuanian common ash populations are facing dramatic fluctuations with an unknown impact on the genetic diversity and structure. Information on their genetic variation and population structure is urgently needed for developing the national gene conservation and restoration program for common ash. This work was aimed to study the positioning of variation of RAPD markers among different regions of provenance, distribution of variation among and within the populations, and a relation between genetic and geographical distances among the populations.

MATERIALS AND METHODS

Plant material. Ten Lithuanian common ash populations from four out of five regions of provenance were included in this study (Table 1). Two and more populations represented each of the four provenance regions sampled. The regions of provenance for *Fraxinus excelsior* in Lithuania were established in 2001, based on the climatic, edaphic and growth parameters of ash stands that are typically registered during routine forest inventory. Forest enterprises were used as administration units and their boundaries superpose with ones delineated for provenance regions [23]. The principles of provenance region establishment were similar to those used in most European countries. Ten individuals, with one exception, represented each population. Trees were selected so that the distance between them was no less than 40 metres, thus preventing a close relatedness. We studied 99 individual trees in total. Wood increment cores were used for exact age estimation.

DNA extraction and amplification. Genomic DNA was extracted from 100 mg of fresh leaf tissue with the aid of the Genomic DNA purification Kit (Fermentas). We performed PCR amplification, electrophoresis and visualization of RAPD markers according to the conditions described earlier [24]. RAPD bands were scored as either present (1) or absent (0) in each DNA sample, and a matrix of RAPD phenotypes was assembled. Only data from clear, intensely staining unambiguous bands were used for analysis. The reproducibility of an amplified fragment pattern was tested in at least two independent experiments.

Data analysis. Genetic distance (GD) among individual trees in the study populations was estimated according to the Nei and Li formula [25]: $GD = 1–[2N_{xy} / (N_x + N_y)]$, where N_x is the number of bands in individual X, N_y is the number of bands in individual Y, and N_{xy} is the number of RAPD bands present in both X and Y. The GD value was calculated using the TREECON for Windows [26].

The number of polymorphic loci, measures of heterozygosity and the Shannon index of phenotypic diversity (I), genetic distances among populations [27] were computed with PopGene3.2 [28] assuming all loci to be dominant and in Hardy–Weinberg equilibrium. AMOVA [19] was used to

Table 1. **Sampling locations and the number of individuals analysed in Lithuanian common ash (*Fraxinus excelsior*) populations. Population name corresponds to the respective forest enterprise name (see Fig. 1). The abbreviations for population names given in this table will be used further, in all tables and figures**

No	Population	Provenance region	n	Mean age, yr.	Latitude N	Longitude E	Altitude, m asl
1.	Telšiai (Tels)	1	10	71	56°04′19″	22°27′23″	138
2.	Šilute (Sil)	1	10	91	55°14′47″	21°42′29″	16
3.	Šakiai (Sak)	4	10	.	55°01′28″	23°04′33″	59
4.	Kaišiadorys (Kai)	4	10	47	54°53′29″	24°21′30″	89
5.	Marijampolë (Mar)	4	10	74	54°26′18″	23°27′29″	119
6.	Këdainiai (Ked)	4	10	91	55°11′22″	23°59′20″	67
7.	Pakruojis (Pak)	2	10	73	56°16′31″	24°02′28″	39
8.	Kupiškis (Kup)	2	10	54	55°51′20″	25°10′34″	97
9.	Nemenèine (Nem)	3	10	71	54°59′03″	25°29′41″	163
10.	Ignalina (Ign)	3	9	81	55°16′29″	26°33′33″	137

n, number of plants studied per population.

estimate variance components for RAPD phenotypes, partitioning the variation into intrapopulational, interpopulational, and interregional values. Variance components were tested statistically by nonparametric permutational procedures using 999 permutations. Analyses were performed using the GenAlEx V5 software [29]. Estimates of gene flow (Nm) among populations were calculated according to Wright [30].

The dendrogram of UPGMA cluster analysis based on genetic distance values [27] was generated in order to present relationships among the populations. The relationships among individuals and populations can be summarised by principal component analysis (PCA). The PCA method in factor analysis was applied to analyse the data from RAPD fingerprints using the FACTOR procedure [31]. Only polymorphic fragments were used for data evaluation. The STEPDISC SAS procedure was applied to select the loci that contributed most to the differentiation of populations. The lowest significance level used for the selection of single RAPD was 0.01.

The Pearson correlation and its *p* value were calculated to relate the genetic characteristics of the populations to the geographic and climatic characteristics of the population origin (CORR SAS procedure). The climate continentality index of the population origin was calculated using Chromov's method [32].

RESULTS

Six informative primers (summarized in Table 2) of the 13 tested generated 76 unambiguous polymorphic amplification products in the range of 450–2800 bp. The total number of bands scored per primer ranged from 11 (Roth 170-03) to 14 (primers A6, Roth 170-02), with an average of 12.7 bands per primer. The applied primers revealed a different number of RAPD loci in the populations analyzed (Table 3).

The average population diversity using the Shannon information index (*I*) was 0.440 and ranged from 0.312 for the Pakruojis population to 0.532 for the Ðilutë population (Table 4). Populations with the highest *I* values also exhibited the highest percentage of polymorphic loci for a single population, which ranged from 57.9% (Pakruojis population) to 92.1% (Ðilutë and Telðiai populations; Table 3). No population-specific RAPD bands were found. The largest average genetic distance between individuals was detected in the Telðiai population (0.307) and the smal-

Table 2. **Codes and sequences of primers used for analysis, total number of bands counted and DNA fragment size**

Primer	Sequence (5'→ 3')	Total number of bands	Fragment size range (bp)
A6	GACCCGTCCC	14	450–2800
A7	GAAACGGGTG	13	550–2250
Roth 170-02	CAGGGTCGAA	14	480–2000
Roth 170-03	ACGGTGCCTG	11	500–2500
Roth 170-04	CGCATTCCGC	12	490–2500
Roth 170-09	TGCAGCACCG	12	500–2550

Table 3. **Number and percentage of polymorphic DNA bands detected by the analysis of selected six primers for ten Lithuanian common ash populations**

Primer	Tels	Sil	Sak	Kai	Mar	Ked	Pak	Kup	Nem	Ign
A6*	14	13	11	8	14	11	10	13	5	11
%	100	92.9	78.6	57.1	100	78.6	71.4	92.9	35.7	78.6
A7	13	13	13	13	13	10	9	11	11	13
%	100	100	100	100	100	76.9	69.2	84.6	84.6	100
Roth 170-02	14	14	14	12	10	13	8	14	11	9
%	100	100	100	85.7	71.4	92.7	57.1	100	78.6	64.3
Roth 170-03	10	8	7	6	8	10	6	9	7	8
%	90.9	72.7	63.6	54.6	72.7	90.9	54.6	81.8	63.6	72.7
Roth 170-04	9	12	12	7	9	6	4	9	6	5
%	75	100	100	58,3	75	50	33,3	75	50	41,7
Roth 170-09	10	10	11	7	9	9	7	9	10	8
%	83.3	83.3	91.7	58.3	75	75	58.3	75	83.3	66.7
Total	70	70	68	53	63	59	44	65	50	54
Mean %	92.1	92.1	89.5	69.7	82.9	77.6	57.9	85.5	65.8	71.1

* A6, A7 et al. – codes of the primer; % – percentage of polymorphic loci in a common ash population.

Table 4. **Patterns of genetic diversity for ten Lithuanian common ash populations**

Population	Observed number of alleles, N_a	Mean heterozygosity	Shannon index	GD_{xy}*
Tels	1.921	0.359	0.526	0.307
Sil	1.921	0.365	0.532	0.248
Sak	1.895	0.358	0.522	0.235
Kai	1.697	0.254	0.378	0.217
Mar	1.829	0.313	0.461	0.259
Ked	1.776	0.277	0.411	0.271
Pak	1.579	0.210	0.312	0.183
Kup	1.855	0.343	0.500	0.294
Nem	1.658	0.245	0.361	0.187
Ign	1.711	0.273	0.401	0.205
Mean	1.784 ± 0.113	0.3 ± 0.053	0.440 ± 0.075	0.241 ± 0.041

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

lest (0.183) in the Pakruojis population (Table 4). All the trees studied had unique RAPD phenotypes.

The mean heterozygosity within the populations of *F. excelsior* varied from 0.210 to 0.365 within the ten populations. The Šilutë population showed the highest level of mean estimated heterozygosity (0.365) over 76 polymorphic loci, whereas the Pakruojis population showed the lowest one (Table 4).

In PCA, only 5.8% of the total variation was described by the first three principal components (Fig. 1) when only the loci that were significant for population differentiation were analysed (13 of 76 loci). The same procedure, but with all polymorphic RAPD loci analysed, resulted in 15.1% of variation (data not shown). The low variation described by principal components indicates that many uncorrelated loci contributed to the interpopulation differentiation with a low individual contribution. This analysis revealed that populations can be characterised by varying degrees of separation, but there is a considerable overlap among them (Fig. 1). Nei's [27] genetic distance values between pairs of populations ranged from 0.021 between Kupiðkis and Telðiai to 0.209 between Ignalina and Këdainiai (Table 5). The mean value of genetic distance was 0.115 (SD = 0.046). A dendrogram based on UPGMA cluster analysis of genetic distance values shows that clusters did not precisely reflect the geographic position of populations, except the Marijampolë–Ðakiai–Ðilute cluster (Fig. 2). The dendrogram also showed that populations that originated from the same region of provenance were distributed over different clusters and homogenous groupings could not be recognized. PCA, cluster analysis and Nei's genetic distance estimates showed that the most genetically distant populations are Ignalina and Këdainiai. Despite an almost complete absence of correlation between the interpopulation genetic distance, geographic distance (–0.12) and the change of

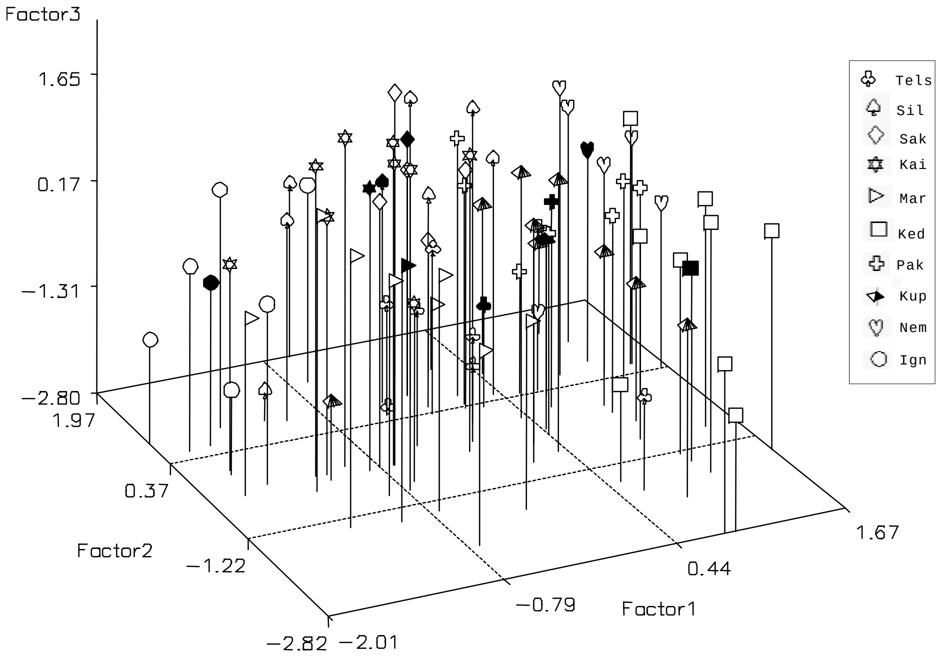

Fig. 1. Ordination of the tree estimate based on three principal components. Only the RAPD loci significant for population discrimination ($A6_{450}$, $A6_{550}$, $A6_{900}$, $A6_{1700}$, $A7_{850}$, $R170\text{-}02_{1200}$, $R170\text{-}03_{600}$, $R170\text{-}04_{490}$, $R170\text{-}04_{700}$, $R170\text{-}04_{900}$, $R170\text{-}04_{2500}$, $R170\text{-}09_{650}$, $R170\text{-}09_{750}$) were analysed. Trees belonging to the same population have the same designations. Designations with black background indicate the population mean estimate

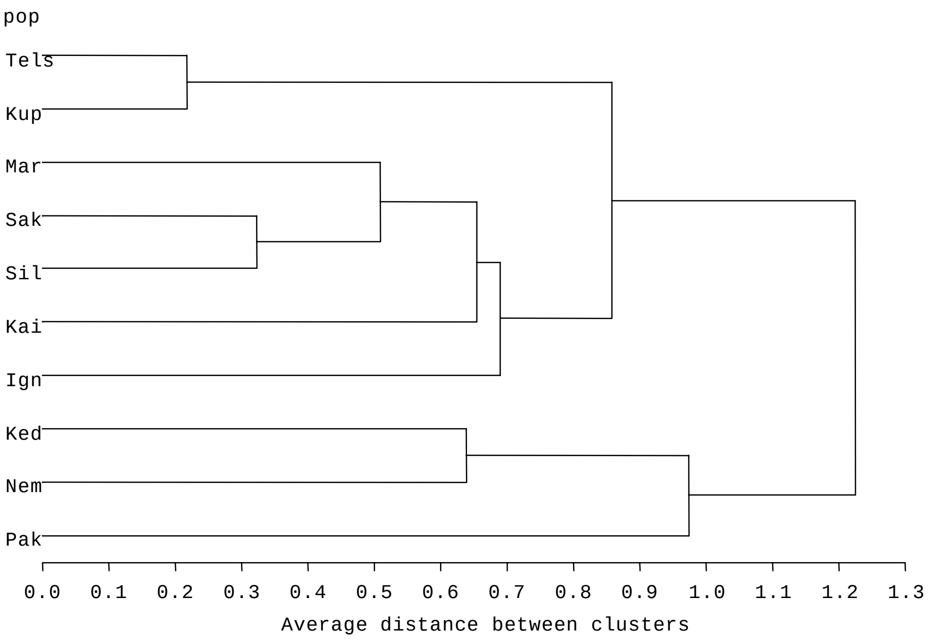

Fig. 2. Population clustering based on Nei's genetic distance using the UPGMA method

the climate continentality index of population origin (–0.16), the correlation of population genetic diversity characteristics with longitudinal data and the climate continentality index of population origin was from medium negative to strong (Table 6). The correlations for all characteristics gave negative values, except the correlation for mean genetic distance among individual plants in population. AMOVA revealed that 8.3% of the total genetic variance was due to the interpopulation differences and 91.7% was due to an individual variation within the populations. The average number of migrants per generation (N_em) among populations was 2.53. No differentiation among the provenance regions was observed.

DISCUSSION

RAPD markers have been used to evaluate the levels of genetic diversity within and among ten Lithuanian populations of *F. excelsior* from four regions of provenance. The used six primers were highly informative and revealed an average of 12.7 bands per primer. The common ash populations in the present study exhibited different levels of genetic diversity; the percentage of polymorphic loci in these popula-

Table 5. **Nei's [27] genetic distance among ten common ash populations studied**

Pop ID	Tels	Ked	Nem	Kup	Pak	Mar	Ign	Kai	Sak
Tels									
Ked	0.084								
Nem	0.087	0.098							
Kup	0.021	0.072	0.092						
Pak	0.132	0.165	0.137	0.117					
Mar	0.061	0.13	0.143	0.067	0.158				
Ign	0.102	0.209	0.190	0.133	0.174	0.077			
Kai	0.119	0.191	0.169	0.099	0.198	0.094	0.107		
Sak	0.089	0.159	0.114	0.082	0.146	0.076	0.097	0.077	
Sil	0.08	0.175	0.16	0.073	0.181	0.063	0.076	0.085	0.028

Table 6. **Pearson's correlation between genetic and geographic characteristics of common ash populations. The significance of estimate is given in italics below the correlation value**

Characteristics	Observed number of alleles, N_a	Mean heterozygosity	Shannon index	GD_{xy}	Percentage of polymorphic loci
Latitude	–0.09	–0.03	–0.04	0.15	–0.09
	0.812	*0.933*	*0.907*	*0.674*	*0.812*
Longitude	–0.63	–0.58	–0.59	–0.43	–0.63
	0.051	*0.078*	*0.073*	*0.212*	*0.051*
Altitude	–0.14	–0.13	–0.13	0.02	–0.14
	0.699	*0.710*	*0.711*	*0.949*	*0.699*
Continentality	–0.67	–0.64	–0.65	–0.55	–0.67
	0.035	*0.045*	*0.044*	*0.097*	*0.035*

tions ranged from 57.89 % to 92.11%. According to previous studies, high genetic diversity is usual in wind-pollinated trees [33, 34]. Mean heterozygosity also differs substantially among the common ash populations studied (varies from 0.210 to 0.365). Currently, the view prevails that the level of genetic diversity indicates the adaptive potential of a species to environmental changes and a higher heterozygosity means a higher fitness [8, 35, 36]. The pronounced and significant negative correlations among genetic diversity characteristics and the longitudinal or climate continentality index in the locality of population origin gives an indication of the possible clinal trend (Table 6). The higher genetic diversity in West Lithuania possibly reflects the north-eastward migration route of the species.

Our results indicate that the majority of genetic diversity (91.7%) is contained within populations, but there is also a highly significant (P = 0.001) differentiation among populations (8.3%), which can be considered as appreciable [22, 37]. This level of population differentiation was lower than the one estimated by Nowakowska et al. [13] in Polish (G_{ST} = 0.198) and Lithuanian (G_{ST} = 0.122) populations of common ash. The genetic differences among the Lithuanian populations of common ash established in our study appeared largely due to amplified fragment frequency differences rather than because of a complete absence or presence of a particular band. The majority of RAPD bands is common for most populations. Some RAPD loci were not found in particular populations. No population-specific loci were obtained, although 13 loci were qualified as important for population differentiation (Fig. 1). The low interpopulation differentiation in trees in comparison to other plant groups usually implies an extensive gene flow among tree populations. Wind-pollinated tree species typically have interpopulation differentiation levels less than 10%, which transforms to more than two successfully established migrants per population in each generation [33, 34]. Thus, the number of migrants ($N_e m$ = 2.53) among populations of *F. exccelsior* estimated in the present study is in accordance with the values presented for other wind pollinated trees.

Contrasting patterns of diversity in western and eastern European populations of *F. excelsior* were established in the study on the basis of nuclear microsatellite markers, where two Lithuanian populations (Kaišiadorys and Pakruojis) were included [12, 38]. Lithuanian ash populations were attributed to the regions with a high allelic and genetic diversity within populations, but a very low differentiation among populations (F_{st} = 0.01 for the two Lithuanian populations studied). The discrepancy between these data and our results obtained on the basis of RAPD markers partially could be explained by the different molecular basis of RAPD and SSR markers [39, 40]. Discrepancies between RAPD and SSR data obtained on the basis of the same material are described elsewhere [40, 41].

The population structure and ratio of intra- and interpopulation variations are influenced by the pattern of species distribution, its role and stage in a forest ecosystem, pollen and seed vectors, particular biological features, etc. In tree species with large and continuous ranges, the interpopulation differentiation is often below 3% [34]. The present scattered distribution and specific ecological requirements indicate that common ash populations are probably more differentiated than those of wind-pollinated species with continuous distribution [5]. The habitat fragmentation may affect the population dynamics by modifying gene flow patterns. Gene flow through pollen and seed dispersal is a key determinant in the establishment of genetic structure [38]. According to Heuertz et al. [10], a mean seed dispersal distance established in a Romanian population of *F. excelsior* was less than 14 m and the moderate pollen flow was in a range of 70–140 m. In a study of a French common ash population [42] these numbers were a few times higher (*e.g.*, mean seed dispersal distance was about 140 m). With regard to the present studies, limited seed dispersal or pollen transfer also might have contributed to the genetic differentiation among Lithuanian populations of common ash.

Geographic distances among the populations studied vary from 41 to 308 km. Within this range, no significant correlation (r = – 0.12, P = 0.418) was detected between the genetic and geographic distances among populations in our study. The UPGMA dendrogram in the present study showed that some populations from different regions of provenance were located in the same cluster (Fig. 2). For example, the Telšiai and Kupiškis populations were grouped together despite the geographical distance of 170 km between them. On the contrary, two geographically close populations those of Kaiðiadorys and Këdainiai, were distributed into separate clusters and showed a rather large genetic distance. An AMOVA analysis and PCA also did not show any regional divergence among the populations. The lack of such correlation could be partially explained by a possible adaptive RAPD eco-geographical differentiation associated with habitat fragmentation [43] or by severe bottlenecks in some populations in the past. This would have resulted in a new allelic composition of the populations, independently on their geographic location [44].

Our study was an attempt to establish the genetic structure of Lithuanian common ash populations with the aid of RAPD markers. High levels of polymorphism and the existence of population differentiation of *F. excelsior* found in the present work showed that RAPD markers are a suitable tool for genetic diversity studies in the common ash and can be useful for accumulation and management of genetic-breeding resources of this species. The unique RAPD phenotype found for each tree indicates that RAPD markers can be used for identification of clones in seed orchards and clone archives. Although no population-specific RAPD bands were found, the amplified fragment frequency differences among populations, which were qualified as most important for their differentiation, allow identification of populations for control of the origin/provenance of forest reproductive material.

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References

1. Lithuanian Statistical Yearbook of Forestry. State Forest Survey Service. Kaunas, 2004: 47–8.
2. Ozolinèius R, Stakënas V. Miðkininkystë 1998; 1(41): 59–71.
3. Skuodienë L, Grybauskas K, Palionis V, Maslinskas R. Miðkininkystë 2003; 2 (54): 86–96.
4. Juodvalkis A, Vasiliauskas A. Vagos 2002; 56(9): 17–22.
5. Pliura A. In: Report of the Third Meeting, Noble Hardwoods Network. Ed. J. Turok, J. Jensen, C. Palmberg-Lerche. European long-term gene conservation strategies: Ash (*Fraxinus* ssp). International Plant Genetic Resources Institute. Rome, 1999: 8–20.
6. Müller-Starck G. Forest Genet 1995; 2: 121–4.
7. Eriksson G, Namkoong G, Roberds J. Forest Ecol Manag 1993; 62: 15–37.
8. Heuertz M, Hausman J-F, Tsvetkov I et al. Mol Ecol 2001; 10: 1615–23.
9. Morand ME, Brachet S, Rossignol P et al. Mol Ecol 2002; 11: 377–85.
10. Heuertz M, Vekemans X, Hausman J-F et al. Mol Ecol 2003; 12: 2483–95.
11. Heuertz MS, Fineschi M, Anzidei R et al. Mol Ecol 2004; 13: 3437–52.
12. Heuertz M, Hausman J-F, Hardy OJ et al. Evolution 2004; 58(5): 976–88.
13. Nowakowska J, Jablonowski S, Mockeliunaite R, Bieniek J. Balt Forest 2004; 10(1): 57–64.
14. Williams GK, Kubelik AR, Livak J et al. Nucleic Acids Res 1990; 18: 6531–5.
15. Silva EP, Russo CAM. Hydrobiologia 2000; 420: 119–35.
16. Nybom H. Mol Ecol 2004; 13: 1143–55.
17. Volis S, Yakubov B, Shulgina I et al. Biol J Linn Soc 2001; 74: 289–303.
18. Lynch M, Milligan BG. Mol Ecol 1994; 3: 91–9.
19. Excoffier L, Smouse PE, Quattro JM. Genetics 1992; 131: 479–91.
20. Huff DR, Peakall R, Smouse PE. Theor Appl Genet 1993; 86: 927–34.
21. Bussell JD. Mol Ecol 1999; 8: 775–89.
22. Dîaz V, Mu¹iz LM, Ferrer E. Mol Ecol 2001; 10: 2593–603.
23. Lithuanian forest genetic resources and their conservation. Ministry of Environment of the Republic of Lithuania. Vilnius, 2003: 42–4.
24. Þvingila D, Verbylaitë R, Abraitis R et al. Balt Forest 2002; 8: 2–7.
25. Nei M, Li W. Proc Natl Acad Sci USA 1979; 76: 5269–73.
26. Van de Peer Y, De Wachter R. Comput Applic Biosci 1994; 10: 569–70.
27. Nei M. Genetics 1978; 89: 583–90.
28. Yeh FC, Yang RC, Boyle TBJ et al. POPGENE 3.2, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, 1999.
29. Peakall R, Smouse PE. GenAlEx: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, 2001. http://www.anu.edu.au/BoZo/GenAlEx/
30. Wright S. Ann Eugen 1951; 15: 323–54.
31. SAS Institute Inc. 1999: SAS/STAT® User's Guide, Version 8, Cary, NC, USA.
32. Khromov SP. Meteorology and Climatology. Leningrad, Hidrometeoizdat, 1968 (in Russian).
33. Hamrick JL, Godt MJW, Sherman-Broyles SL. New Forests 1992; 6: 95–124.
34. Slavov GT, DiFazio SP, Strauss SH. In: Gene Flow Workshop. Eds. A. Snow, C. Mallory-Smith, N. Ellstrand, J. Holt, H. Quemada. The Ohio State University, 2002: 113–26.
35. David P. Heredity 1998; 80: 531–37.
36. Hansson B, Westerberg L. Mol Ecol 2002; 11: 2467–74.
37. Wright S. In: Evolution and Genetic Populations. Ed. S. Wright. University of Chicago Press, Chicago, 1978; 4: 242–322.
38. Heuertz M. Population genetic structure in common ash: a focus on southeastern European genetic resources. PhD diss., Free University of Brussels, Belgium, and Public Research Centre – Gabriel Lippmann, Luxembourg, 2003.
39. Tautz D, Trick M, Dover GA. Nature 1986; 322: 652–6.
40. Mengoni A, Gori A, Bazziclupo M. Plant Breeding 2000; 119: 311–17.
41. Pejic I, Ajmone-Marsan P, Morgante M et al. Theor Appl Genet 1998; 97: 1248–51.
42. Morand ME, Gerber S, Frascaria-Lacoste N. In: International Conference Dygen 'Dynamics and Conserva-

tion of Genetic Diversity in Forest Ecosystems'. Eds. A. Kremer, O. Savolainen, J. Turok. Strasbourg, 2002: 34.
43. Owuor ED, Fahima T, Beiles A et al. Mol Ecol 1997; 6: 1177–87.
44. Gaudeul M, Taberlet P, Till-Bottraud I. Mol Ecol 2000; 9: 1625–37.

D. Þvingila, R. Verbylaitë, V. Baliuckas, A Pliûra, S. Kuusienë

GENETINËS ÁVAIROVËS TYRIMAS LIETUVOS PAPRASTOJO UOSIO (*Fraxinus excelsior* L.) POPULIACIJOSE RAPD METODU

Santrauka

Atsitiktinai pagausintos polimorfinës DNR (RAPD) metodu tirta 10-ies Lietuvos paprastojo uosio populiacijø genetinë ávairovë ir struktûra. Panaudojant ðeðis oligonukleotidinius pradmenis tirti 99 medþiai. Genetinës ávairovës analizei tarp populiacijø ir populiacijø viduje pasirinkti 76 DNR fragmentai, kuriø dydis – nuo 0,45 iki 2,8 kbp. Tirtose populiacijose nustatyti DNR polimorfizmo skirtumai. Polimorfiniai lokusai jose sudarë nuo 57,9 iki 92,1%. Maþiausias Shannon fenotipinës ávairovës indeksas nustatytas Pakruojo populiacijoje (0,312), o didþiausias – Ðilutës populiacijoje (0,532). Populiacijø diferenciacija pagal RAPD lokusus siekë 8,3% (P = 0,001). Genø migracijos daþnis ($N_e m$) buvo lygus 2,53. Nustatyta tam tikra koreliacija tarp genetinës ávairovës rodikliø (polimorfiniø lokusø procento, vidutinio heterozigotiðkumo, Shannon indekso, stebimo aleliø skaièiaus) ir populiacijø augavieèiø kai kuriø geografiniø parametrø (kontinentalumo indekso, ilgumos). Priklausomybës tarp populiacijø genetinio ir geografinio atstumo nerasta.