Evaluation of Genetic Diversity in Selected *Fraxinus excelsior* L. Trees and Their Offspring using RAPD and Microsatellite Methods

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Abstract

The Random Amplified Polymorphic DNA (RAPD) technique was used to assess the genetic polymorphism of *Fraxinus excelsior* L. in three families (K-59, T-3, and N-5) obtained by mature embryo culture *in vitro*. Analysis of 15 offspring showed polymorphic DNA bands with 12 oligonucleotide primers. A total of 139 RAPD bands were scored, of which 92% were polymorphic. These results confirm that the studied offspring exhibited high genetic variation between three families of common ash. We have revealed nine primers, each of which, when used separately can identify all studied genotypes.

The simple sequence repeat (microsatellites) method was established together with the necessary basic laboratory techniques as developed by an Austrian lab. An assessment of genetic diversity of selected *Fraxinus excelsior* trees and their offspring from Lithuania served as the case study. Results demonstrated that in contrast to RAPD, many more alleles at each locus were present, and some offspring of individual trees grouped together after clustering.

Key words: *Fraxinus excelsior*, microsatellites, RAPD, offspring, maternal tree, genetic diversity

Introduction

Common ash (*Fraxinus excelsior* L., Oleaceae) is one of the more important hardwood forestry species in many European countries, often forming small discontinuous stands in European mixed deciduous forests. The populations of common ash are mostly natural and occupy about 1.8% of the territory covered by forests in Lithuania (Lithuanian Statistical Yearbook of Forestry 2010). Massive dieback of *F. excelsior* has been observed not only in Lithuania, but in other European countries as well (Pliūra 1999, Skuodienė 2005, Bakys et al. 2008). Forest trees are exposed to many stress factors, such as pollution, climate change, and habitat fragmentation, and assuring the potential for adaptation must play an increasingly important role in the plant species‘ response. Jump and Penuelas (2005) stated that in fragmented landscapes, rapid climate change has the potential to overwhelm the capacity for adaptation in many plant populations, and to dramatically alter the genetic composition. As genetic diversity is the basis of the evolutionary potential of species to respond to environmental changes, the maintenance of this diversity has become the main aim for conservation of forest genetic resources programmes (Palmberg-Lerche 2001, Toro and Caballero 2005).

Williams et al. (1990) first described the randomly amplified polymorphic DNA (RAPD) technique, and is used frequently, as it is easily available in order to acquire information on the genetic diversity of a species. The RAPD analysis was used to examine the genetic diversity and structure of ten natural Lithuanian common ash (*F. excelsior*) populations (Žvingila et al. 2005). The RAPD markers were also used to recognize two closely related European ash species, *F. excelsior* and *F. oxyphylla* and their putative hybrids (Jeandroz et al. 1996). The genetic diversity of common ash in five Polish and three Lithuanian populations was evaluated based on RAPD analysis by Nowakowska et al. (2004).

Microsatellites are highly polymorphic DNA markers with discrete loci and codominant alleles. Microsatellite markers have been developed in *F. excelsior* initially by Lefort et al. (1999). Comprehensive studies of common ash Bulgarian and French populations

were performed using the nuclear microsatellite markers (Heuertz et al. 2001, Morand et al. 2002). Genetic variability of 31 Italian common ash populations was evaluated using another similar set of six microsatellite markers (Ferrazzini et al. 2006). Heuertz et al. (2004) studied genetic diversity between Western and Southeastern European common ash populations using the nuclear microsatellites. The genetic diversity of 14 populations of *F. excelsior* in southern Germany was analysed using nuclear and chloroplast microsatellites (Hebel et al. 2006).

The aim of the present investigation was to evaluate the level of genetic diversity in Lithuanian common ash offspring using RAPD markers, and to assess the genetic diversity of Lithuanian common ash offspring and maternal trees with the use of four polymorphic microsatellite loci.

Materials and methods

Plant material. The offspring of three different Lithuanian common ash families [Kėdainiai-59 (K-59), Nemenčinė-5 (N-5), Telšiai-3 (T-3)] were analysed by using RAPD markers. The biological material for genetic analysis was collected from plantlets grown in isolated mature embryo culture at the early developmental stage (first true leave) (Mockeliunaite and Kuusiene 2004). The original seeds had been selected for sampling in November 2001 (previously: Lithuanian forest genetic resources, seed and plant service, now: Forest service). The 83 samples of one *F. excelsior* Lithuanian population (Kėdainiai, with three different half-sib families: K-58, K-59, and K-60) were used for the microsatellite analysis. The seeds were collected in the same way as for RAPD, and material for this DNA experiment was taken from leaves of seminal (maternal) trees in June 2004.

Genomic DNA isolation. The DNA of the offspring of three different Lithuanian common ash populations for RAPD analysis was extracted using the genomic DNA purification Kit (*Fermentas,* Lithuania), applying the protocol recommended by the supplier of the kit. The DNA was extracted from 15 plantlets of three families which were propagated in isolated embryo culture. Then 80 samples of offspring DNA were extracted from the embryos of three mother trees, and the DNA of the mother trees was extracted from the leaves. The DNA of embryos (from the seeds) was extracted according to an *Arabidopsis thaliana* genomic DNA extraction method in the Lithuanian laboratory as follows: each embryo from seed was ground in an Eppendorf tube with a tightly fitting plastic pestle, before addition of 400 µl extraction buffer (EB) and vortexing, then centrifugation in a microcentrifuge for 1 min at 14,000 rpm. The supernatant (300 µl) was transferred to a new tube, 300 µl of isopropanol was added and the mix was incubated for 2 min at room temperature, then spun in a microcentrifuge for 10 min at 14,000 rpm. Finally, the supernatant was removed, the pellet washed with 70% (ice cold) ethyl alcohol, spun for 1 min at full speed, after which the supernatant was again removed, the pellet air-dried, and re-suspended in 100 µl water. The extracted DNA was carried to the Austrian laboratory in an ethanol-precipitated state and was dissolved in TE buffer (10 mM Tris, 0.1 mM EDTA) upon arrival.

PCR amplification. A total of 18 different RAPD primers were chosen for the three Lithuanian ash populations (Table 1). The Polymerase Chain Reaction (PCR) amplification was carried out in 25 µl of a mix consisting of: 2 µl of extracted DNA, 2.5 µl 10x reaction buffer as supplied with the polymerase, 13.8 µl ddH_2O, 3 µl $MgCl_2$, 2.5 µl dNTP mix (2mM; obtained from *Fermentas*, Lithuania), 0.2 µl Taq Polymerase (*Fermentas,* Lithuania), and 1 µl primer (sources see Table 1). Amplifications were performed in a DNA thermocycler *TGradient* (Biometra, Germany) as follows: 4 min of initial denaturation at 94°C, followed by 45 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 fi-

Table 1. Number of total and polymorphic DNA bands obtained with different RAPD primers used to assess genetic diversity in *Fraxinus excelsior* offspring

RAPD primer	Total bands[1]	Polymorphic bands[2]	Size range of DNA fragment (bp)	Genotype-specific bands[3]
Roth 170-01	17	16	630-2830	2
Roth 170-02	12	12	500-2800	1
Roth 170-03	Uninformative	-	-	-
Roth 170-04	Uninformative	-	-	-
Roth 170-05	Uninformative	-	-	-
Roth 170-06	1	1	3450	0
Roth 170-09	10	8	820-2800	2
Roth 170-10	5	4	1120-2800	0
Roth 370-01	9	8	830-2500	0
Roth 370-02	9	8	500-1750	0
Roth 370-03	Uninformative	-	-	-
Roth 370-04	12	10	1050-3100	1
Roth 370-05	18	17	400-3000	1
Roth 370-06	12	11	750-3000	0
Roth 370-07	Uninformative	-	-	-
Roth 370-08	16	15	500-3200	0
Roth 370-09	Uninformative	-	-	-
Roth 370-10	18	18	560-3100	0

[1] – total number of scorable bands detected
[2] – total number of bands detected as polymorphic in at least one genotype
[3] – number of possible genotype-specific bands, generated with corresponding primer

nal extension step after the cycles of 5 min at 72°C (Žvingila et al. 2002). Every PCR reaction was repeated at least twice.

The PCR conditions for the other set of 83 samples for microsatellite analysis were conducted as in the protocol of the host laboratory. For mother tree and offspring parentage analysis, five highly polymorphic nuclear microsatellite markers (FEMSALT4, 11, 16, 19, and M2-30) were selected initially (Lefort et al. 1999). Those loci had shown the best results in the host laboratory in Vienna, Austria. The PCR products were tested using electrophoresis through 2.0% agarose gels. Gels were stained with ethidium bromide and visualized using ultra-violet light. Polyacrylamide electrophoresis on large (40 x 20 cm) denaturing gels was used for separating microsatellite alleles. Staining of these large polyacrylamide gels was conducted by using either SYBR-Gold or silver nitrate. Comparison to 10 bp and 100 bp marker ladders (Invitrogen, Canada) determined the exact sizes of the microsatellite fragments. The SYBR-Gold staining was preferred to silver staining for time saving and easier gel removal, so most data were obtained with SYBR-Gold staining. All samples were analysed with four labelled primer pairs (FEMSALT 4, 11, 16, and M2-30) using a dedicated sequencing automat (Beckman CEQ 8000, UK), using standard procedures in the host laboratory (Hecker and Roux 1996).

Data analysis. The amplified RAPD fragments of each sample were scored independently, and only for reproducible and clear bands. For each sample, the presence or absence of DNA fragments were recorded as 1 or 0, respectively, and treated as discrete characters. A dendrogram was generated using the TREECON for Windows software (Van de Peer and De Wachter 1994). The data of the microsatellite analysis was processed with the software GenAlEx 6 (Peakall and Smouse 2006) and the Pop-Gene v. 1.31 Software (Yeh et al. 1999).

Results

The genetic variability of common ash (*F. excelsior* L.) was assessed using RAPD molecular markers. Of the 18 studied primers, six produced no amplification at all, while the other remaining primers amplified polymorphic products (Table 1). The 12 informative RAPD primers produced very different numbers of DNA bands, and the size of amplified products ranged from 400 to 3450 bp. The highest number of bands resulted from amplification with the primers Roth 370-05 and Roth 370-10, while the primer Roth 170-06 produced only one band. The pattern of DNA polymorphism established with primer Roth 170-01 was shown in Figure 1.

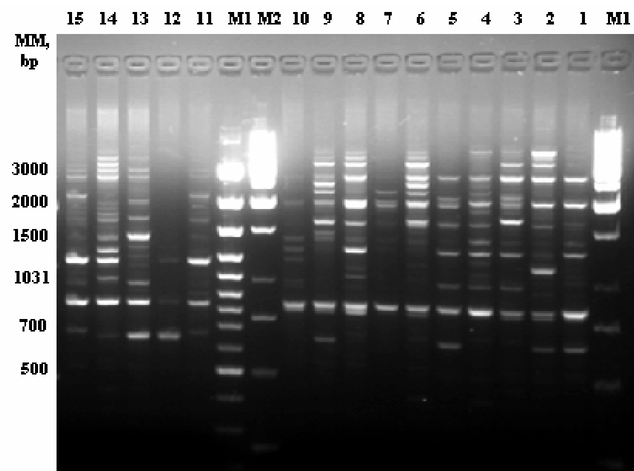


Figure 1. RAPD agarose gel electrophoresis profiles of 15 common ash offspring using primer Roth 170-01. Lines 1 to 15 represent the offspring listed in table 2. Size markers (M1 and M2) were given in base pairs (bp). The arrow indicated genotype-specific RAPD bands

A closer examination of the RAPD gel patterns showed a number of amplification products that were genotype-specific and could also identify individual offspring in this set. Genotype-specific bands were revealed by the primers Roth 170-01, Roth 170-02, Roth 170-09, Roth 370-04, and Roth 370-05 (Table 1). The primer Roth 170-01 identified a genotype-specific 990 bp band for offspring N-5/3 and another at 1030 bp for offspring N-5/5. Two genotype-specific RAPD bands were amplified for genotypes K-59/26 and K-59/7 (primer Roth 170-09). One genotype-specific band for K-59/4 was identified with primer Roth 170-02. Two genotype-specific bands with the same primer (Roth 170-09) were identified in the investigation of genotypes K-59/26 and K-59/7. One genotype specific band was amplified with the primers Roth 370-04 and Roth 370-05 for offspring K-59/6 and K-59/4, respectively.

The relationship between analysed offspring was shown in the dendrogram (Fig. 2). The offspring of families N-5 and K-59 appear in two separate clusters. One offspring of family T-3 clusters apart from its family cluster.

The PCR products were obtained from most of the samples of microsatellites. A small group of samples which had been extracted from tissue culture embryos consistently showed problems after amplification. After some initial technical difficulties, we managed to pour and run polyacrylamide gels adequate for electrophoresis, staining, and scoring. We did not succeed, however, in analysing all samples with the polyacrylamide gels. Therefore, we turned to the sequencer for the rest of the samples. Data were presented as a sim-

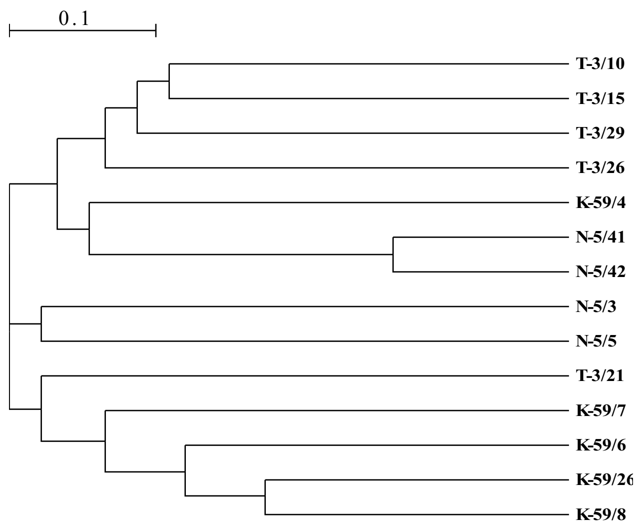


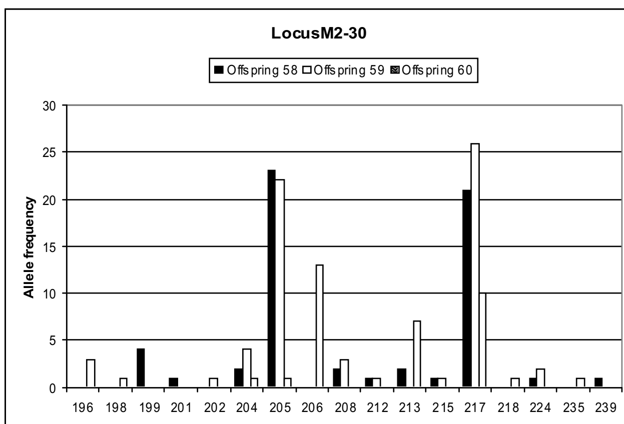
Figure 2. Dendrogram of three Lithuanian families of plantlets (K-59, T-3, and N-5) of common ash RAPD fragments obtained using UPGMA method

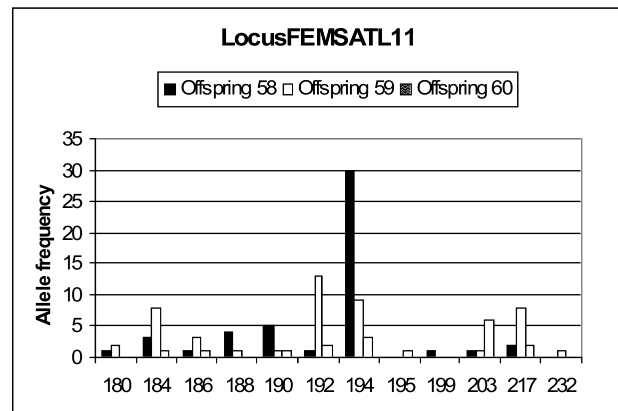
ple allele frequency graph (Fig. 3), in order to check Mendelian inheritance of the alleles from mother to offspring. As the result, the K-59 offspring matched the maternal tree at the highest rate of 64.8% (Table 2).

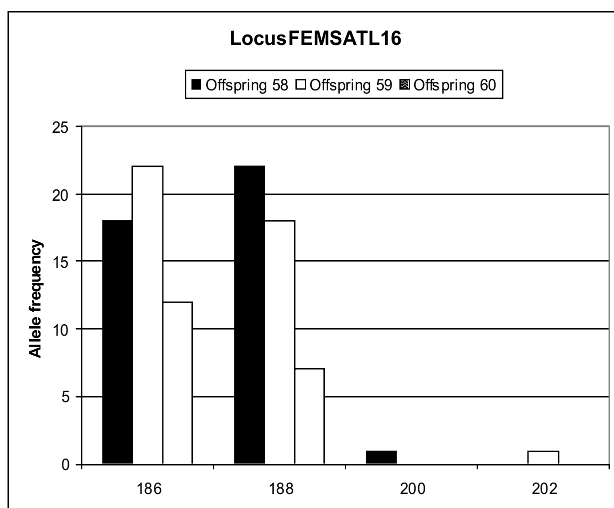
Table 2. Percentage of offspring microsatellite alleles of common ash which matched with the mother trees

	K-58-offspring	K-59-offspring	K-60-offspring
K-58-mothertree	**62.2±0.03%**	48.4±0.02%	21.3±0.03%
K-59-mothertree	57.7±0.03%	**64.8±0.03%**	31.2±0.03%
K-60-mothertree	34.4±0.02%	36.0±0.02%	**42.0±0.05%**

The dendrogram of offspring using microsatellites showed separate clusters of analysed families' similarly to RAPD experiment, but with more exceptions (Fig. 4). The maternal trees of the three families made up the different clusters with one or more offspring.







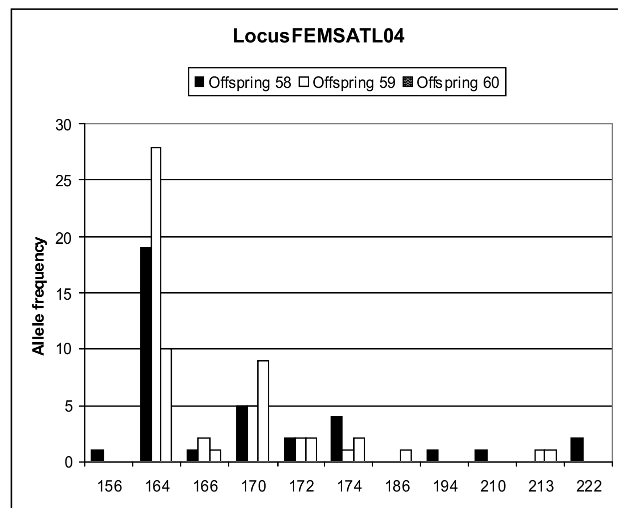


Figure 3. Allele distributions at four selected microsatellite loci encountered for three offspring families of common ash

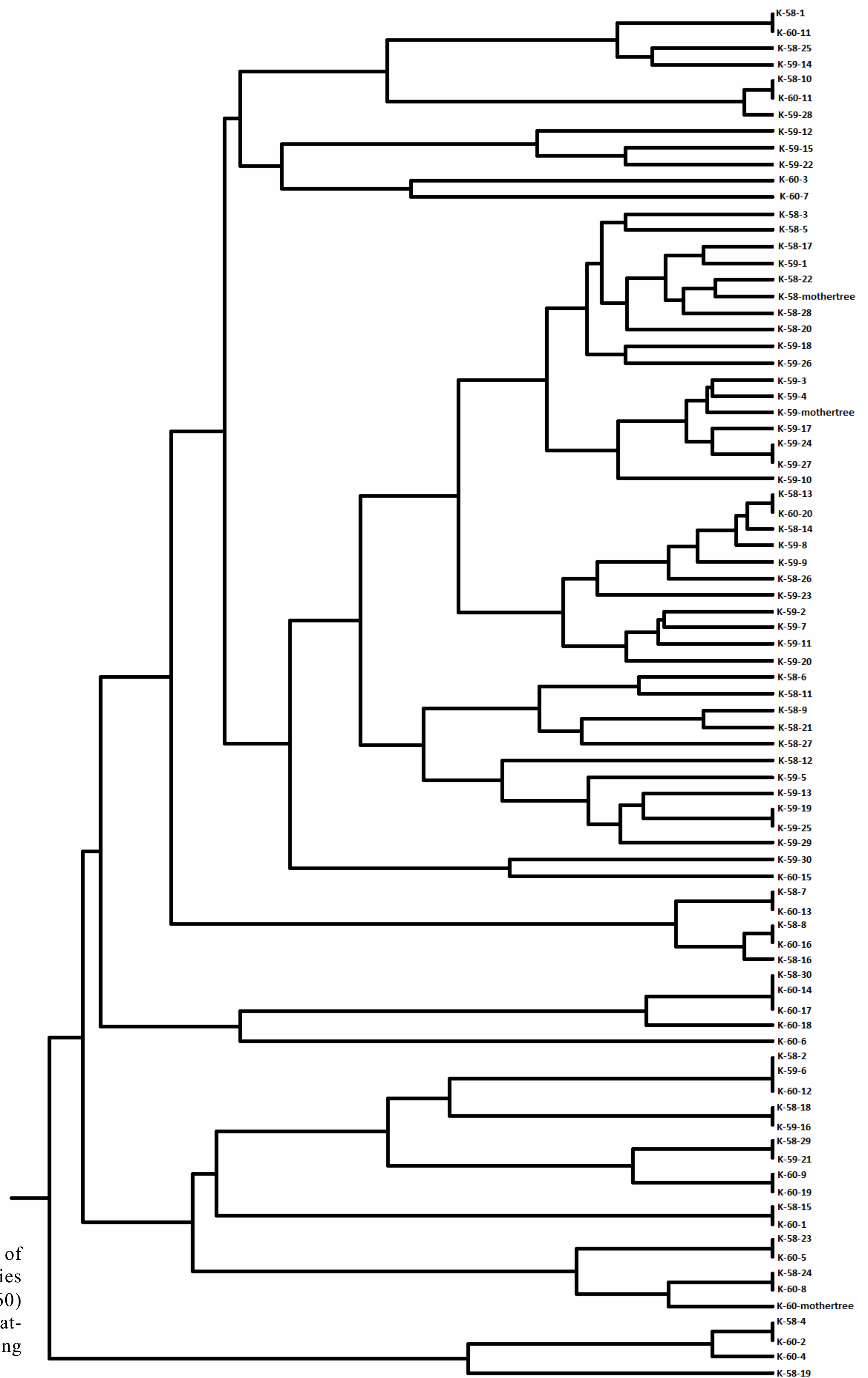


Figure 4. Dendrogram of three Lithuanian families (K-58, K-59, and K-60) for common ash microsatellite loci obtained using UPGMA method

Discussion and conclusions

The RAPD method was used to assess genetic diversity in common ash (*F. excelsior* L.) individuals of three maternal trees from different populations of Lithuania. Plantlets from mature embryo culture *in vitro* were chosen randomly. The RAPD technique was chosen for its simplicity and availability (Williams et al. 1990) and was very effective in identifying genetic differences between common ash offspring. All chosen informative primers showed polymorphic bands in the studied genotypes. A total of 139 RAPD bands were scored, of which 92% were polymorphic. This percentage of polymorphic DNA fragments was almost the same as the one estimated by Žvingila et al. (2005) in the ten Lithuanian common ash populations (92.1%). Nowakowska et al. (2004) showed that 97% of the loci were polymorphic in Polish common ash populations and 95.2% in Lithuanian populations. Our results confirmed that the analysed genotypes exhibited high genetic variation between three families of common ash. These results showed that the chosen RAPD technique was effective in revealing a high level of DNA polymorphism in common ash offspring.

The dendrogram showed the genetic relationship between common ash offspring in simplified form (Fig. 4). Some seeds or plantlets from certain families clustered away from the family. Similar observations were reported for genetic diversity of common ash and Norway spruce populations (Areškevičienė et al. 2005, Žvingila et al. 2005) The clustering patterns of the populations was independent of the geographic distribution, and showed cases of larger genetic diversity over short geographic distances, as well as cases of low genetic distance between geographically distant populations. Many tree species have been found to harbour high genetic diversity within populations, but low differentiation among populations (Hamrick et al. 1992, Heuertz et al. 2001, Morand et al. 2002). Some biological characteristics of tree species such as long generation time and predominant wind pollination in temperate areas (Hamrick and Godt 1989) can explain this phenomenon. The fragmented nature of *F. excelsior* stands may add to this phenomenon in Lithuania. Single offspring not clustering with the bulk of the family may result from long-distance pollinations.

We have identified genotype-specific DNA bands. Five of the primers (Roth 170-01, Roth 170-02, Roth 170-09, Roth 370-04, and Roth 370-05) showed such genotype-specific DNA bands, resulting in a total of seven genotype-specific DNA fragments. The assumption can be made that these fragments were genotype-specific, but it was equally likely that these represented rare variants that may appear again in a wider set of common ash offspring.

Depending on the microsatellite locus, different patterns were obtained for the three different offspring families. For example, offspring Kėdainiai 60 (which had the smallest number of samples) was clearly different when locus FEMSATL 04 or M2-30 was considered. The other two families can be distinguished by the patterns in FEMSATL 11.

The microsatellite primers were also used for evaluation of genetic structure within and among Bulgarian populations of common ash (Heuertz et al. 2001), in western and eastern European populations of common ash (Heuertz et al. 2004), e.g. showing a general heterozygote deficiency in French common ash populations (Morand et al. 2002).

The fixation index, F_{ST}, was expressed as the proportion of genetic diversity as a result of allele frequency differences among populations (in contrast to F_{IS}, differentiation within a population). The genetic variation observed in Northeastern and Southeastern common ash populations in Europe by microsatellite DNA markers (F_{ST} = 0.090 and F_{ST} = 0.088, respectively, Heuertz et al. 2001, Heuertz et al. 2004) was higher than in our samples (F_{ST} = 0.125). This could be explained by the fact that we worked with one population consisting of three subpopulations (the three families). This was in line with the common finding that in forest trees, within-stand genetic diversity was often higher than among-population diversity (Smith and Devey 1994). The fragmented nature and small size of the populations of common ash may also contribute to this result.

The RAPD method was very efficient for detecting genetic differentiation of offspring, given its low requirements for equipment, handling, and skills. Microsatellite markers were more precise for searching parents of an individual, if clean amplification patterns can be obtained. All microsatellite loci were shown to be polymorphic in our samples from Lithuania, and we will take this as a basis for future investigations.

Acknowledgements

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References

Areškevičienė, R., Žvingila, D., Gabrilavičius, R. and Kuusienė, S. 2005. The estimation of genetic diversity within and between Lithuanian populations of Norway spruce (*Picea abies* (L) Karst.) by using RAPD. *Baltic Forestry* 11 (2): 2-8.

Bakys, R., Vasaitis, R., Barklund, P., Thomsen, I.M. and Stenlid, J. 2008. Occurrence and pathogenecity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. *European Journal of Forest Research* 128: 51-60.

Ferrazzini, D., Monteleone, I. and Belletti, P. 2006. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior* L.). *Annals of Forest Science* 64: 159-168.

Hamrick, J.L. and Godt, M.J.W. 1989. Allozyme diversity in plant species. In: Brown ADH, Clegg MT, Kahler AL and Weir BS, editors. Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Inc., Sunderland, MA, USA, 43–63 p.

Hamrick, J.L., Godt, M.J.W. and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.

Hebel, I., Haas, R. and Dounavi, A. 2006. Genetic variation of common ash (*Fraxinus excelsior* L.) populations from provenance regions in southern Germany by using nuclear and chloroplast microsatellites. *Silvae Genetica* 55 (1): 38-44.

Hecker, K.H. and Roux, K.H. 1996. High and low annealing temperatures increase both specifity and yield in touchdown and stepdown PCR. *Biotecgniques* 20 (3): 478-485.

Heuertz, M., Hausman, J.-F., Hardy, O.J., Vendramin, G.G., Frascaria-Lascote, N. and Vekemans, X. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and eastern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* 58 (5): 976-988.

Heuertz, M., Hausman, J.F., Tsvetkov, I., Frascaria-Lacoste, N. and Vekemans, X. 2001. Assessment of genetic structure within and among Bulgarian populations of the common ash (*Fraxinus excelsior* L.). *Molecular Ecology* 10 (7): 1615-1623.

Jeandroz, S., Frascaria-Lacoste, N. and Bousquet, J. 1996. Molecular recognition of the closely related *Fraxinus excelsior* and *F. oxyphylla* (Olaceae) by RAPD markers. *Forest Genetics* 3 (4): 237-242.

Jump, A. S. and Penuelas, J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* 8 (9): 1010-1020.

Lefort, F., Brachet, S., Frascaria-Lacoste, N., Edwards, K.J. and Douglas, G.C. 1999. Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae). *Molecular Ecology* 8 (6): 1088-1090.

Lietuvos miškų ūkio statistika. 2010. (Lithuanian Statistical Yearbook of Forestry 2010). Kaunas, 14 p. (in Lithuanian).

Mockeliunaite, R. and Kuusiene, S. 2004. Organogenesis of *Fraxinus excelsior* L. by isolated mature embryo culture. *Acta Universitatis Latviensis, Biology* 676: 197-200.

Morand, M. E., Brachet, S., Rossignol, P., Dufour, J. and Frascaria-Lacoste, N. 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. *Molecular Ecology* 11 (3): 377-385.

Nowakowska, J., Jablonowski, S., Mockelūnaitė, R. and Bieniek, J. 2004. Genetic variability within and among Polish and Lithuanian populations of common ash (*Fraxinus excelsior* L.) based on RAPD analysis. *Baltic Forestry* 10 (1): 57-64.

Palmberg-Lerche, C. 2001. Conservation of forest biological diversity and forest genetic resources, in: Palmerg-Lerche P., Iversen P.A., Sigaud P. (Eds.), Forest Genetic Resources 29, Food and Agriculture Organization of the Untied Nations, Rome, 70 pp.

Peakall, R. and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6 (1): 288-295.

Pliūra, A. 1999. European long-term gene conservation strategies: Ash (*Fraxinus* spp.). In: Turok, J., Jensen, J., Palmeberg-Lerche, C., Rusanen, M., Russell, K., de Vries, S. and Lipman, E. (Eds.), Noble hardwoods network – Report of the third meeting, 13-16 June 1998, Sagadi, Estonia, International Plant Genetic Resources Institute, Rome: 8-20 pp.

Skuodienė, L. 2005. Medžių stresas ir jo fiziologinė indikacija (Tree stress and its physiological indication). Monografija. ARX Baltica spaudos namai, Kaunas, 84 pp. (in Lithuanian).

Smith, D. and Devey, M. 1994. Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genome* 37 (6): 977-983.

Toro, M.A. and Caballero, A. 2005. Characterization a conservation of genetic diversity in subdivided populations. *Philosophical Transactions of the Royal Society Biological Sciences* 360 (1459): 1367-1378.

Van de Peer, Y. and De Wachter, R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in the Biosciences* 10: 569-570.

Williams, G.K., Kubelik, A.R., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18 (22): 6531-6535.

Yeh, F.C., Yang, R. and Boyle, T. 1999. POPGENE v.1.31, Microsoft Window – based Freeware for Population Genetic Analysis.

Žvingila, D., Verbylaitė, R., Abraitis, R., Kuusienė, S. and Ozolinčius, R. 2002. Assessment of genetic diversity in plus tree clones of *Pinus sylvestris* L. using RAPD markers. *Baltic Forestry* 8 (2): 2-7.

Žvingila, D., Verbylaitė, R., Baliuckas, V. Pliūra, A. and Kuusienė, S. 2005. Genetic diversity (RAPD) in natural Lithuanian populations of common ash (*Fraxinus excelsior* L.). *Biologija* 3: 46-53.

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ОЦЕНКА ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ ОТОБРАННЫХ ДЕРЕВЬЕВ *FRAXINUS EXCELSIOR* L. И ИХ ПОТОМСТВА ИСПОЛЬЗУЯ МЕТОДЫ RAPD И МИКРОСАТЕЛЛИТОВ

Р. Лукшене, Б. Хэинзе и С. Куусене

Резюме

Для установления генетического полиморфизма в трёх семействах *Fraxinus excelsior* L. (К-59, Т-3 и Н-5) была использована техника RAPD, используя культуру зрелых эмбрионов *in vitro*. Анализ 15 отпрысков показал полиморфизм ДНК с 12-ти праймерах. Всего было обнаружено 139 RAPD фрагментов, 92 % из которых были полиморфными. Эти результаты подтверждают, что исследованные отпрыски обладают высокой генетической вариацией в трёх семействах ясеня европейского. Было обнаружено девять праймеров, при использовании которых в отдельности позволяя идентифицировать все исследованные генотипы.

Для установки некоторых основных техник работы с микросателитами (простого повторения порядка) и для их применения был использован метод, который был разработан в Австрии для оценки генетического разнообразия отпрысков *Fraxinus excelsior* и материнских деревьев из Литвы. Работа была проведена в 2006 году в Генетическом Отделе Федерального Учреждения при Центре Исследований Леса, в Вене, Австрия. По сравнению с методом RAPD, метод микросателлитов выявил значительно большее количество аллелей в каждом локусе. Потомство отдельных деревьев лучше группировалось в отдельные кластеры.

Ключевые слова: *Fraxinus excelsior*, микросателлиты, RAPD, материнские деревья, потомки, генетическое разнообразие