Genetic Variability within and among Polish and Lithuanian Populations of Common Ash (*Fraxinus excelsior* L.) Based on RAPD Analysis

JUSTYNA NOWAKOWSKA[1], STANISŁAW JABŁONOWSKI[2], RINGAILĖ MOCKELIŪNAITĖ[3], JOLANTA BIENIEK[1]

[1]Forest Research Institute, Dept. of Genetics and Forest Tree Physiology, Bitwy Warszawskiej 1920 R-3, PL-00 973 Warsaw, Poland

[2]Warsaw Agricultural University (SGGW), Faculty of Agricultural Economics, Nowoursynowska 166, PL-02 787 Warsaw, Poland

[3]Lithuanian Forest Research Institute, Laboratory of Genetics and Biotechnology, Girionys, Liepu 1, LT-4312 Kaunas district., Lithuania

Nowakowska J., Jabłonowski S., Mockeliūnaitė R., 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.

This study characterizes the genetic diversity of common ash (*Fraxinus excelsior* L.) in Polish and Lithuanian provenances using random amplified polymorphic DNA (RAPD) markers. A total of 183 ash trees were genotyped with twelve Operon primers. In Polish samples, the average and effective numbers of alleles per 97.0 % polymorphic loci were n_a=1.970 and n_e=1.301, respectively, whereas 95.2 % polymorphic loci and n_a=1.952 and n_e=1.337 characterized Lithuanian ash samples. The high genetic variability was detected within Polish and Lithuanian populations (H_S=0.166 and H_S=0.195, respectively) with G_{ST}=0.198 for Polish and G_{ST}=0.122 for Lithuanian stands. These coefficients were lower than the coefficients of population diversity H_T=0.207 in Poland and H_T=0.222 in Lithuania. We found that four ash populations from the Northern, Southern and Northwest of Poland were clustered apart from one population from the Northeast of the country. These results are discussed in comparison with genetic variation data from social broad leaves species such as oak and beech.

Key words: *Fraxinus excelsior* L., genetic diversity, within and among population variability, RAPD

Introduction

Common ash (*Fraxinus excelsior* L., Oleaceae) is a temperate tree species occurring in mixed deciduous forests throughout Europe. In Poland, *F. excelsior* occurs throughout the country, especially in lowlands forest, preferring fertile moist soils rich in humus, but it can also be found on dry and poor sites, in the mountains below 1000 m altitude (Fober 1995, Boratynska 1995, Dolatowski 1995). Ash ecotype is various, in floodplain forests it occurs very often with black alder and elms. In fertile mixed broadleaved forests, ash is often associated with oak, hornbeam and maple (Thill 1970, Claessens *et al.* 1994). A great economic importance of common ash was the main cause of its mass cutting in the past. Natural latifolious forests with a considerable percentage of this species are very rare nowadays (Denisiuk 1995).

The capacity of Forest tree species to survive in changing environmental conditions largely depends on their adaptive potential determined by genetic diversity. Since the conference in Rio de Janeiro in 1992, many research programs deal with gene reserve conservation and concentrate on genetic diversity determination (Kremer 1994, Barbault 1997). In forest tree species, known for their longevity and adaptation potential to changing environmental conditions, the genetic differentiation assessment is crucial for all programs of *in-situ* and *ex-situ* gene resource conservation (Primack 1998). The genome organization of forest trees is complex and stores the highest genetic diversity among the plant kingdom (Arbrez 1994, Hamrick *et al.* 1992). This fact is probably due to restricted range of distribution of tree species during the last glacial period 18 000 BC in a few refugia in Southern Europe (Kremer 1994, Petit *et al.* 1997). Post-glacial recolonization of the European continent increased within-population diversity rather than among population differentiation (Kremer 1994, Kremer *et al.* 2002).

Many approaches to the diversity conservation, the exploration of plant genetic resources and the design of plant improvement programmes require a

detailed knowledge of the amount and distribution of genetic diversity within species.

Previous studies based on analyses of chloroplast and nuclear DNA markers showed some genetic variation in European common ash populations (Heuertz 2003). A study of 36 different common ash provenances with five nuclear microsatellite loci revealed different genetic patterns between Western and Southeastern populations. Polish and Lithuanian *F. excelsior* provenances appeared to share the Western pattern of genetic variation observed in Ireland, England, France, Germany, Switzerland, Hungary and Slovakia (Heuertz 2003). Based on genetic variation of four allozyme loci, six main genotypes were characterized in seven populations of common ash in Poland (Krzakowa and Przybyl 2002).

The present investigation attempts to evaluate the level of genetic diversity in Polish and Lithuanian ash provenances with the use of RAPD markers. All analyses were focused on the determination of within and among population diversity coefficients and on the construction of the genetic distance dendrogram based on UPGMA (unweighted pair-group method of arithmetic averages) clustering method.

Materials and methods

Sample collection. Samples (buds) were harvested by shooting from 183 adult trees of ten forest districts in Poland and three forest districts in Lithuania (Table 1, Fig. 1) and stored at –75°C before the DNA extraction.

Genomic DNA isolation. After collection, 100 mg of frozen buds were ground to a fine powder in liquid nitrogen and DNA was then extracted according to the QIAGEN procedure using DNeasy 250 Plant Mini Kit. The quality of the DNA was checked by electrophoresis on a 1 % agarose gel containing ethidium bromide (0.5 mg/ml) in 1x TBE (45 mM Tris-borate, pH 8.3, 1 mM EDTA). Gel DocTM 2000 Imaging System (Bio-Rad) quantified the amount of DNA in each sample.

Table 1. Provenance description of studied common ash stands

Provenance location	Latitude / Longitude	Seed micro-zones*	Number of sampled trees	Mean age (years)
Bartoszyce	54°20´N / 20°55´E	201 (PL)	8	113
Choszczno	53°10´40"N / 15°18´09"E	151 (PL)	15	93
Elblag	54°10´N / 19°24´E	103 (PL)	10	83
Jamy / Bialochowo	53°32´59"N / 15°18´09"E	356 (PL)	11	85
Jamy / Chelmno	53°20´59"N / 18°23´52"E	356 (PL)	13	111
Jawor	50°58´58"N / 16°12´23"E	752 (PL)	15	128
Nowogard	53°38´27"N / 15°05´27"E	151 (PL)	14	113
Szczecinek	53°44´35"N / 16°42´28"E	155 (PL)	25	104
Tulowice	50°40´41"N / 17°29´36"E	554 (PL)	20	133
Zloty Potok	50°47´07"N / 19°29´17"E	655 (PL)	22	113
Kedainiai	55°11´22"N / 24°00´01"E	4 (LT)	10	91
Nemencine	54°59´03"N / 25°29´43"E	3 (LT)	10	71
Pakruojis	56°16´20"N / 24°02´56"E	2 (LT)	10	73

* According to Polish (PL) seed micro-zones and Lithuanian (LT) provenance regions

Figure 1. Geographic localization Polish and Lithuanian *Fraxinus excelsior* provenances *i.e.* in Poland: Bartoszyce, Elblag, Szczecinek, Choszczno, Zloty Potok and in Lithuania: Kedainiai, Nemencine and Pakruojis

DNA amplification. Preliminarily, all trees were examined with OPA-05, OPA-06, OPA-08, OPA-09, OPA-11, OPA-16, OPB-08, OPB-13, OPC-04, OPD-12, OPE-08, OPE-12, OPE-17, OPF-07, OPG-09, OPG-10, OPG-12, OPJ-01 and OPJ-08 (OPERON Technologies) RAPD primers. The different primers used for RAPD reactions were chosen in order to generate clear and scorable bands of DNA in Polish and Lithuanian ash samples and were established empirically after preliminary screening. Finally, the following primers (OPERON Technologies) were chosen for the Polish common ash PCR amplification: OPA-08, OPA-09, OPA-11, OPB-08, OPE-08, and OPG-12. Lithuanian ash DNA was amplified with OPA-06, OPB-05, OPB-13, OPD-12, OPE-12 and OPF-07 primers. The PCR amplification was carried out in 25 µl of mix containing: 100 ng of DNA in TE buffer (pH 7.0); 1x reaction buffer; 3.5 mM $MgCl_2$; 1x Q solution; 200 µM dNTPs; 0.6 µM of primer; 0.8 units of Taq Polymerase (QIAGEN Master Kit). After an initial denaturation step at 94°C for 4 min, 45 cycles of 1 min at 94°C, 2 min at 35°C and 2 min at 72°C were performed in T Personal thermocycler (Biometra). The final elongation step consisted in 5-min incubation at 72°C followed by 1 min at 25°C. Every PCR reaction was repeated at least three times. All PCR products were analysed by migration on 1,8 % agarose gel in 1x TBE

buffer under 70V to be further analysed in Gel Doc™ 2000 Imaging System (Bio-Rad).

RAPD product analysis. The amplified RAPD fragments of each sample were scored independently only for reproducible and clear bands. For each sample, the presence and absence of different DNA fragments were recorded as 1 or 0, respectively, and treated as discrete characters. Pair-wise comparison of banding patters was evaluated using BIO-PROFIL Bio-Gene Windows Application V99.05 (Vilber Lourmat) program. The data were analysed to generate similarity coefficients with the Pop-Gene v. 1.32 software (Yeh and Boyle 1997) using UPGMA (unweighted pair-group method of arithmetic averages) and then presented as dendrograms of genetic distances (Nei and Li 1979, Nei 1987). The Chi-square test was carried out with the Pop-Gene software in order to evaluate the frequencies of gene homogeneity across provenances.

Results

RAPD analysis. Data on the genetic diversity in 5 Polish and 3 Lithuanian population samples of common ash are presented in Table 2 and Table 3. In total, 183 sampled trees examined with the Operon primers listed above gave successful DNA amplification in 106 samples. In Jamy/Bialochowo, Jamy/Chelmno, Jawor, Nowogard and Tulowice provenances we obtained very weak banding pattern (2-6 bands per tree) probably related to a very bad health condition of collected buds from these stands. Successful DNA amplification was achieved for other ash provenances (Bartoszyce, Choszczno, Elblag, Szczecinek, Zloty Potok, Kedainiai, Nemencine and Pakruojis, Table 2 and Table 3) probably because of better quality of extracted DNA. In these populations, RAPD primers yielded 369 reproducible fragments reflecting putative loci. The size of the fragments ranged from 244 bp to 4300 bp, whereas the percentage of polymorphic loci ranged from 45.3 % (Bartoszyce provenance) to 74.4 % (Szczecinek provenance, Table 2). The Chi-square test revealed 77 % of homogeneity among amplified loci from Polish ash samples, which means that 23 % of analysed loci are substantially different ($p<0.05$). For Lithuanian loci, Chi-square test revealed 92 % of homogeneous fragments, so that 8 % of loci are significantly different ($p<0.05$).

Table 2: Genetic diversity analysis in *Fraxinus excelsior* Polish provenances

Provenance	Percentage of polymorphic loci	n_a	n_e	I	h
Bartoszyce	45.3	1.453	1.221	0.216	0.138
Choszczno	65.5	1.655	1.249	0.264	0.164
Elblag	55.2	1.551	1.244	0.246	0.156
Szczecinek	74.4	1.744	1.309	0.320	0.201
Zloty Potok	69.5	1.694	1.256	0.275	0.169
All Polish provenances:	97.0	1.970	1.301	0.342	**H_T=0.207 H_S=0.166 G_{ST}=0.198**

The degree of polymorphism were assessed by calculation of: n_a – average number of alleles, n_e – effective number of alleles, **I** –Shannon index (Lewontin 1972), **h** – within population Nei's genetic diversity, H_S – average diversity within populations, H_T - genetic differentiation within all provenances, and G_{ST} – relative differentiation between populations (Nei 1987).

Table 3. Genetic diversity analysis in *Fraxinus excelsior* Lithuanian provenances

Provenance	Percentage of polymorphic loci	n_a	n_e	I	h
Kedainiai	66.9	1.669	1.316	0.307	0.197
Nemencine	73.5	1.735	1.339	0.338	0.216
Pakruojis	62.0	1.621	1.276	0.270	0.172
All Lithuanian provenances:	95.2	1.952	1.337	0.356	**H_T=0.222 H_S=0.195 G_{ST}=0.122**

Explanation as in Table 2

The mean observed number of alleles varied from n_a=1.453 (Bartoszyce provenance) to n_a=1.744 (Szczecinek provenance) with the overall mean of n_a=1.970 for Polish and n_a=1.952 for Lithuanian stands. The mean expected number of alleles varied from n_e=1.221 (Bartoszyce population) to n_e=1.339 (Nemencine population) with the mean of n_e=1.301 for all Polish and n_e=1.337 for all Lithuanian populations (Table 2 and Table 3). The lowest Shannon index was found for the Bartoszyce population (I=0.216) and the highest for Nemencine provenance (I=0.338, Table 2 and Table 3).

Genetic variation among populations was significant for $p<0.05$. The lowest diversity h=0.138 was observed for Bartoszyce provenance and the highest h=0.216 was found in Nemencine provenance. In Poland, the average *F. excelsior* intra-population diversity was estimated as H_S=0.166 and the genetic differentiation within all provenances as H_T=0.207. The latest one was higher than the overall gene diversity G_{ST} = 0.198. Lithuanian provenances showed the following values: H_S=0.195, H_T=0.222 and G_{ST} = 0.122.

Strong geographic differentiation was apparent from the UPGMA analysis of genetic distances (Fig. 2 and 3). Two major clusters of populations charac-

Genetic variation between and within populations. Genetic relationships based on calculations of Nei's genetic distance and variation are presented as population averages in Table 2 and Table 3.

terized Polish populations: one cluster joined provenances from the Northern and Southern parts of Poland (Elblag and Zloty Potok); another cluster grouped the Northeastern provenances (Szczecinek and Choszczno). The genetic distance of D_N=0.087 separated Bartoszyce population from both groups (Fig. 2). We cannot observe clustering of geographically close populations, as the groups of provenances separated by low genetic distances were more than 300 km apart (*e.g.* Elblag, Złoty Potok, Szczecinek and Choszczno, Fig. 1 and Fig. 2).

Figure 2. Dendrogram of genetic distances according to the UPGMA clustering analysis (Nei 1987) calculated for 5 Polish *Fraxinus excelsior* provenances

The dendrogram built by genetic distances for Lithuanian populations of common ash suggests that Nemencine and Pakruojas provenances are much more genetically related than the Kedainiai one (Fig. 3).

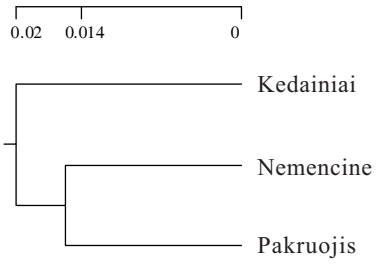

Figure 3. Dendrogram of genetic distances according to UPGMA clustering analysis (Nei 1987) calculated for 3 Lithuanian *Fraxinus excelsior* provenances

Discussion

This study evaluates the genetic relationship between Polish and Lithuanian native common ash provenances.

The level of population differentiation tends to be relatively high in the studied Polish and Lithuanian ash provenances (G_{ST}=0.198 and G_{ST}=0.122, respectively, Table 3) and suggests fragmentation of common ash provenances in these countries in the past. According to Hamrick (1992) mean G_{ST} and H_T found in Angiosperms are 0.110 and 0.287, respectively.

The within-population variation is large (H_T=0.207 and H_T=0.222 for Polish and Lithuanian provenances respectively), probably as a result of the out breeding mating system of *Fraxinus excelsior*. Genetic diversity level found in populations of other deciduous out breeding species such as *Quercus petraea* (H_T=0.233, Le Corre *et al.* 1997) and *Fagus sylvatica* (H_T=0.291, Troggio *et al.* 1996) are also comparable to our data for *Fraxinus excelsior*. The H_T values are slightly lower than genetic variation found in conifer species *e.g.* Scots pine in Poland (H_T=0.262, Nowakowska 2003), Scots pine in Lithuania (H_T=0.252, vingila *et al.* 2002), Norway spruce in France (H_T=0.296, Collignon *et al.* 2002) and Norway spruce in Poland (H_T=0.298, Nowakowska *et al.*, submitted). Genetic diversity for all common ash Polish and Lithuanian provenances (H_T=0.207 and H_T=0.222, respectively) were higher than the intra-population differentiation coefficient (G_{ST}=0.198 and G_{ST}=0.122, Table 3) in agreement with data found in other forest tree species (Heuertz *et al.* 2001).

Nei diversity coefficients (h) estimated from RAPD analysis for Polish Zloty Potok and Choszczno provenances were significantly less different (h=0.169 and h=0.164, respectively, Table 2) than the Wright fixation index (F_{ST}=-0.176 and F_{ST}=-0.800) respectively obtained from the peroxidase allozyme study for the same provenances (Krzakowa and Przybyl 2002). Nevertheless, the negative F_{ST} values were due to the excess of heterozygotes observed among the peroxidase allozyme variation (Krzakowa and Przybyl 2002). In general, RAPD markers detect higher polymorphism because they cover a large part of genome and are not restricted to the coding sequences (Skov 1998). RAPD has been frequently used for the assessment of the genetic population structure in many tree species *e.g.* *Quercus*, *Populus*, *Pinus*, *Picea*, *Ulmus, Citrus*, *Eucalyptus*, *Malus*, *Prunus*, *Olea* and *Fraxinus* (Heinze *et al.* 1996, Jeandroz *et al.* 1996, Barret *et al.* 1997, DeVerno and Mosseler 1997, Hick *et al.* 1998, Coleman 2000, Belaj *et al.* 2002, Ratkiewicz and Borkowska 2002, Nkongolo *et al.* 2002, Collignon *et al.* 2002, Žvingila *et al.* 2002, Rajora *et al.* 2003).

The genetic variation by microsatellite DNA markers observed in Northeastern and Southeastern common ash populations in Europe (F_{ST}=0.090 and F_{ST}=0.088, respectively), indicates the high conserva-

tion level of ash resources in these regions (Heuertz *et al.* 2001, Heuertz 2003). Other deciduous species examined with microsatellites present very various genetic differentiation level, *e.g.* F_{ST}=0.178 (elm), F_{ST}=0.100 (chestnut), F_{ST}=0.018-0.071 (walnut), G_{ST}=0.024 (oak) and G_{ST}=0.054 (beech) (Comps *et al.* 1990, Zanetto *et al.* 1994, Pliūra and Heuertz 2003, Heuertz 2003 and references cited within).

Higher levels of G_{ST} values from our RAPD data comparing to another markers may be explained either by genetic drift in fragmented ash populations or by the influence of some diversifying action of selection on some RAPD loci. Comparing genetic diversity assessed with two nuclear markers in *Pinus flexilis* James, Latta and Mitton (1997) found that F_{ST} values obtained for RAPD markers were much more higher (F_{ST}>0.100) than F_{ST}=0.016 from allozyme study, suggesting a selective pressure on the RAPD studied loci. During evolution, some alleles can drift to high frequency of heterogeneity in one population giving high F_{ST} estimates, while at other loci alleles may drift to high frequency in two populations simultaneously giving low F_{ST} values (Latta and Mitton 1997). The Polish and Lithuanian common ash investigated populations are geographically distant at least 100 km, and we can exclude any pollen flow between them. So we can presume that some strong selection forces or genetic drift probably influenced evolution of our populations in the past, such as fragmentation by deforestation. Forest management practices as well as natural climate disturbances can significantly impact the genetic variability in forest populations. High levels of genetic diversity are generally considered to be essential for facilitating the adaptive responses required to adjust species survival to any change (Mosseler *et al.* 2003).

The UPGMA analysis of genetic distances revealed relationships between Polish and Lithuanian ash tree provenances (Fig.2 and Fig. 3). Two groups of provenances, Elblag and Zloty Potok, as well as Szczecinek and Choszczno, were separated from each other by the mean distance of D_N=0.036 (Fig. 2). Only Bartoszyce population (Northeast of Poland, Fig. 1) was separated from the other provenances by the highest genetic distance (D_N=0.087, Fig. 2). Besides this clustering, there was no association between genetic distance values and geographical distances between sites (data not shown), as Elblag and Zloty Potok provenances are quite distant geographically, as well as Szczecinek and Choszczno provenances (Fig. 1). A similar founding was observed in a common beech differentiation study (Gallois *et al.* 1998) and in the Polish Scots pine RAPD variation (Nowakowska 2003).

Our RAPD data are concordant with the chloroplast microsatellite cpDNA analysis of Polish *F. excelsior* populations: Choszczno, Jawor, Jamy/Chelmno, Jamy/Bialochowo, Nowogard and Szczecinek (Heuertz 2003). In Poland, there is a transition zone between two distinct ash cpDNA haplotypes. Common ash populations from the North-Western Poland (Szczecinek, Choszczno) seem to be originated from a Northern Carpathian refuge, whereas other ash provenances (Jawor, Jamy/Chelmno and Jamy/Bialochowo) are originated from the Alps (Heuertz 2003). This result is supported by the UPGMA dendrogram built from Nei's genetic distances for Szczecinek and Choszczno provenances clustered together (Fig. 2).

Lithuanian provenances were much less related genetically, the distance D_N=0.014 separated Nemencine and Pakruojas populations, both distant from Kedainiai by D_N=0.020 (Fig. 3). There was no correlation between this founding and geographic location of Lithuanian provenances (not shown).

Many symptoms of serious ash dieback have been observed throughout Poland since 1990, independently of class age of common ash stands (Grzywacz 1995). Especially young 1-5 year-old ash trees are subjected to necrosis lesions and brown discoloration of shoot apices resembling to the bark necrosis disease. There was found 31 common ash trees associated with disease changes of apical shoots (Przybyl 2002a). Over 26 species of fungi were identified to be associated with a decay of the thin roots of *F. excelsior* plants in Poland. As we have not achieved a positive DNA amplification in plant material from five Polish *F. excelsior* stands, Jamy/Bialochowo, Jamy/Chelmno, Jawor, Nowogard and Tulowice provenances, we presumed that it was mainly due to the bad health state of harvested buds. At Jamy Forest District the symptoms of apical shoot disease and death of twigs were studied in details by Przybyl (2002b). As it was demonstrated in the case of oak and elm decline in Europe, many abiotic (drought, frost, pesticides and salt pollution of soils) and biotic factors (insects, fungi) are responsible for weakening of ash tree condition, and fungi development is probably the second cause of dieback (Karolewski 1995, Przybyl 2002b).

The development of fast and repeatable DNA analysis may help assess the genetic structure of Polish ash populations in order to find the resistant genotype of this species in the future as it was demonstrated in the case of plant breeding material subjected to some stress factors, like pathogen or insect diseases (Leibenguth and Shoghi 1998). In spring wheat cultivars (*Triticum aestivum* L.), Sun *et al.* (2003) found 3 RAPD markers significantly associated with *Fusarium* resistant genotypes. The molecular differentiation study among the California red oak (*Quercus* section Lobatae) populations infected by *Phytophtora ramo-*

rum suggested that the pathogen tolerance or susceptibility were rather due to some ecological factors than to genetic diversity level (Dodd and Kashani 2003). The disease and insect resistance depend on allelic richness, and RAPD markers due to their dominant nature may underestimate the real genetic basis of the resistance (Sun *et al.* 2003). In forest tree species, the mechanism of resistance is probably more complex because of higher genome size and higher genetic diversity level. In our study, it was difficult to compare the health state of analysed ash populations with the degree of their genetic variability.

The present distribution of genetic diversity in many forest tree species was strongly affected by the last ice age and the postglacial re-colonization period in Europe (Petit *et al.* 1997, Kremer *et al.* 2002, Palmé *et al.* 2003). Additionally, the presence of the ash trees in the landscape of Poland and Lithuania largely depends on present and on going social-economic changes, *e.g.* privatization, increased demand for wood, deforestation, grazing and development of technical infrastructure (Falinski and Pawlaczyk 1995, Pliura and Heuertz 2003). During history, the surfaces of natural forest stands have often declined significantly due to the high anthropogenic pressure. Our further investigations will be focused on genetic diversity assessment using chloroplastic DNA markers, *e.g.* PCR-RFLP of variable introns or microsatellites (Lefort *et al.* 1999, Raquin *et al.* 2002, Heuertz 2003) in order to better highlight the history of genetic structure of Polish and Lithuanian common ash provenances.

Acknowledgments

This study was financed by Polish National Fund for Environmental Protection and Water Management (Grant 12-U-30). The authors thank Dr. Jan Kowalczyk (FRI Warsaw, Poland) for collecting the plant material and mgr. Rafal Hoser for technical assistance. We are very grateful to Prof. Nathalie Frascaria-Lacoste (ENGREF Orsay, France) for providing valuable remarks about the manuscript.

References

Arbrez, M. 1994. Fondement et organisation des réseaux de conservation de ressources génétiques forestičres. Genetics selection evolution, 26: 301-314.

Barbault, R. 1997. Ecologie générale. In: Structure et fonctionnement de la biosphčre. Paris, Masson Eds., p. 288.

Barret, C., Lefort, F., Douglas, G.C. 1997. Genetic characterization of oak seedlings, epicormic, crown and micropropagated shoots from mature trees by RAPD and microsatellite PCR. Scientia Horticulturae, 70: 319-330.

Belaj, A., Satovic, Z., Rallo, L.,Trujillo, I. 2002. Genetic diversity and relationships in olive (*Olea europea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor. Appl. Genet., 105: 638-644.

Boratynska, K. 1995. Systematics and geographical distribution (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 19-54.

Claessens, H., Thibaut, A., Lecomte, H., Delecourt, R., Rondeux, J., Thill, A. 1994. Le frźne en Condroz – station et productivités potentielles. Faculté des Sciences Agronomiques de Gembloux, Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture Eds., Bruxelles, Belgium.

Coleman, M. 2000. Application of RAPDs to the critical taxonomy of the English endemic elm *Ulmus plotii* Druce. Botanic Journal of the Linnean Society, 133: 241-262.

Collignon, A.M., Van de Sype, H., Favre, J.M. 2002. Geographical variation in random amplified polymorphic DNA and quantitative traits in Norway spruce. Can. J. For. Res., 32: 266-282.

Comps, B., Thiébaut, B., Paule, L., Merzeau, D., Letouzey, J. 1990. Allozymic variability in beechwood (*Fagus sylvatica* L.) over Central Europe: spatial differentiation among and within populations. Heredity 65: 407-417.

Denisiuk, Z. 1995. The European ash (*Fraxinus excelsior*) in the system of nature conservation in Poland (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 481-519.

DeVerno, L.L. and Mosseler, A. 1997. Genetic variation in red pine (*Pinus resinosa*) revealed by RAPD and RAPD-RFLP analysis. *Can. J. For. Res.* **27**: 1316-1320.

Dodd, R.S. and Kashani, N. 2003. Molecular differentiation and diversity among the California red oaks (Fagaceae ; *Quercus* section Lobatae). Theor. Appl. Genet., 107: 884-892.

Dolatowski, P. 1995. Ash as a parc and landscape tree (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 337-353.

Falinski, J.B. and Pawlaczyk, P. 1995. Outline of ecology (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe (in Polish) Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 217-305.

Finkeldey, R. and Murillo, O. 1999. Contribution of subpopulations to total gene diversity. Theor. Appl. Genet., 98: 664-668.

Fober, H. 1995. Mineral nutrition (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – Fraxinus *excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 137-147.

Gallois, A., Audran, J.C., Burrus, M. 1998. Assessment of genetic relationships and population discrimination among

Fagus sylvatica L. by RAPD. Theor. Appl. Genet., 97: 211-219.

Grzywacz, A. 1995. More important infection diseases (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 371-415.

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

Heinze, B., Westcott, R., Schmidt, J., Glössl, J. 1996. Application of random amplified polymorphic DNA (RAPD) to detect genetic variation in Norway spruce. New Forests, 11: 173-184.

Heuertz, M. 2003. Population genetic structure in common ash: a focus on southeastern European genetic resources. PhD thesis, Free University of Brussels, Belgium and the Public Research Centre - Gabriel Lippmann, Luxembourg.

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

Hick, M., Adams, D., O'Keefe, S., Macdonald, E., Hodgetts, R. 1998. The development of RAPD and microsatellite markers in lodgepole pine (*Pinus contorta* var. *latifolia*). Genome, 41: 797-805.

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

Karolewski, P. 1995. Resistance against abiotic factors (in Polish). In: Nasze drzewa lesne: Monografie Popularnonaukowe Tom 17: Jesion wyniosly – *Fraxinus excelsior* L. [Our forest trees: popular monograph. Volume 17: European ash - *Fraxinus excelsior* L.] (ed. Bugala W.), Institute of Dendrology, Sorus Eds., Poznan-Kórnik, Poland, pp. 442-468.

Kremer, A. 1994. Diversité génotypique et variabilité des caractčres phénotypiques chez les arbres forestiers. Génétique Sélection Evolution, 26, suppl. 1, pp. 105-123.

Kremer, A., Kleinschmit, J., Cottrell, J., Cundall, E. P., Deans, J.D., Ducousso, A., Konig, A.O., Lowe, A.J., Munro, R.C., Petit, R.J. 2002. Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection between genetic diversity in European oaks? Forest Ecology and Management, 156: 75-85.

Krzakowa, M. and Przybyl, K. 2002. Peroxidase polymorphism in ash tree (*Fraxinus excelsior* L.). In: Plant Peroxidases. Acosta M., Rodriguez-Lopez J. N; and Pedreno M. A., eds. University of Murcia. Spain, pp. 147-151.

Latta R.G. and Mitton J. B. 1997. A comparison of population differentiation across four classes of gene marker in Limber Pine (*Pinus flexilis* James). Genetics, 146: 1153-1163.

Le Corre, V., Dumolin-Lapčgue, S., Kremer, A. 1997. Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: role of history and geography. Mol. Ecol., 6: 519-529.

Lefort, F., Brachet, S., Frascaria-Lacoste, N., Edwards, K.J., Douglas, G.C. 1999. Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae). Mol. Ecol., 8: 1075-1092.

Leibenguth, F. and Shoghi, F. 1998. Analysis of random amplified polymorphic DNA markers in three conifer species. Silvae Genetica, 47(2-3): 120-126.

Lewontin, R.C. 1972. The apportionment of human diversity. Evol. Biol., 6: 381—398.

Mosseler, A., Major, J.E., Rajora, O.P. 2003. Old-growth red spruce forest as reservoirs of genetic diversity and reproductive fitness. Theor. Appl. Genet., 106: 931-937.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

Nkongolo, K.K., Michael, P., Gratton, W.S. 2002. Identification and characterization of RAPD markers inferring genetic relationships among Pine species. Genome, 45: 51-58.

Nowakowska J. 2003. Genetic diversity of Scots pine (*Pinus sylvestris* L.) Polish provenances based on RAPD analysis. Sylwan, 11: 26-37.

Palmé, A.E., Su, Q., Rautenberg, A., Manni, F., Lascoux, M. 2003. Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. Molecular Ecology, 12: 201-212.

Petit, R.J., Pineau, E., Demesure, B., Bacilieri, R., Ducousso, A., Kremer, A. 1997. Chloroplast DNA footprints of postglacial recolonization by oaks. Proc. Natl. Acad. Sci. USA, 94: 9996-10001.

Pliūra, A. and Heuertz, M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute, Rome, Italy, 6 pages.

Primack, R.B. 1998. Essentials of conservation biology. Sunderland, Massachusetts, USA, Sinauer Associates Publishers, pp. 660.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Przybyl, K. 2002a. Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. For. Path., 32: 387-394.

Przybyl, K. 2002b. Mycobiota of thin roots showing decay of *Fraxinus excelsior* young trees. Dendrobiology, 48: 65-69.

Rajora, O.P. and Rahman, M.H. 2003. Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (*Populus x canadensis*) cultivars. Theor. Appl. Genet., 106: 470-477.

Raquin, C., Brachet, S., Jeandroz, S., Vedel, F., Frascaria-Lacoste, N. 2002. Combined analyses of microsatellite and RAPD markers demonstrate possible hybridization between *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl. Forest Genetics, 9(2): 111-114.

Ratkiewicz, M. and Borkowska, A. 2002. Use of molecular markers in ecology (in Polish). Wiadomosci ekologiczne, 2: 99-121.

Skov, E. 1998. Are RAPD-markers reproducible between different laboratories? A case study of *Picea abies* (L.) Karst. Silvae Genetica, 47(5-6): 283-287.

Sun, G. Bond, M., Nass, H., Martin, R., Dong, Z. 2003. RAPD polymorphism in spring wheat cultivars and lines with different level of *Fusarium* resistance. Theor. Appl. Genet., 106: 1059-1067.

Thill, A. 1970. Le frźne et sa culture. Presses Agronomiques de Gembleoux, Eds J. Duculot, S.A., Gembloux, Belgium.

Troggio, M., DiMasso, E., Leonadri, S., Ceroni, M., Bucci, G., Piovani, P., Menozzi, P. 1996. Inheritance of RAPD and IISSR markers and population parameters es-

timation in European beech (*Fagus sylvatica* L.) Forest Genetics, 3(4): 173-181.

Yeh, F.C. and Boyle, T.J.B. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian J. Bot., 129: 157.

Zanetto, A. and Kremer, A. 2000. Geographical structure of gene diversity in *Quercus petraea* Matt. Liebl. I. Monolocus patterns of variation. Heredity, 75: 506-517.

Zvingila, D., Verbylaite, R., Abraitis, R., Kuusiene, S., Ozolincius, R. 2002. Assessment of genetic diversity in plus tree clones of *Pinus sylvestris* L. using RAPD markers. Baltic Forestry, 8 (2): 2-7.

Received 04 March 2004

ГЕНЕТИЧЕСКАЯ ИЗМЕНЧИВОСТЬ ВНУТРИ И МЕЖДУ ПОПУЛЯЦИЯМИ ЯСЕНЯ ОБЫКНОВЕННОГО (*FRAXINUS EXCELSIOR* L.) В ПОЛЬСКИХ И ЛИТОВСКИХ ПОПУЛЯЦИЯХ, ОСНОВАННЫХ НА АНАЛИЗЕ RAPD

Ю. Новаковска, С. Яблонский, Р. Моцкелюнайте, Й. Бенек

Резюме

Эта работа характеризует генетическое разнообразие происхождения ясеня обыкновенного методом маркеров произвольной амплификаций ДНК (RAPD). Всего 183 деревья ясеня были генотипизированы с 12 праймеров деревьев Operon. В польских образцах среднее число и эффективное число аллель в 97% полиморфических локусах было n_a=1.970 и n_e=1.301, в литовских образцах, соответственно, в 95.2% полиморфических локусах было n_a=1.952 and n_e=1.337 среднее число и эффективное число аллель. Высокая генетическая изменчивость была обнаружена как и польских (H_S=0.166 и G_{ST}=0.198), так и в литовских (соответственно, H_S=0.195 и G_{ST}=0.122) популяциях. Эти коэфициенты были ниже по своему значению коэфициентов разнообразия популяции (H_T=0.207 в польских и, соответственно, H_T=0.222 – в литовских). Было обнаружено, что четыре популяции ясеня из северных, южных и северо-западных регионов Польшы отличались от одной популяции из северо-востока страны. Эти результаты были обсуждены сравнивая с данными генетической изменчивости из базы данных листьев дуба и бука.