

# IMPLICATIONS FOR THE USE OF FOREST REPRODUCTIVE MATERIAL OF COMMON ASH (*FRAXINUS EXCELSIOR* L.) IN SLOVENIA BASED ON THE ANALYSIS OF NUCLEAR MICROSATELLITES

## PRIJEDLOZI ZA UPOTREBU ŠUMSKOG REPRODUKCIJSKOG MATERIJALA OBIČNOG JASENA (*FRAXINUS EXCELSIOR* L.) U SLOVENIJI NA BAZI ANALIZE JEZGRINIH MIKROSATELITA

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### Summary

Assumption that forest reproductive material is better adapted to local conditions is the basis of current forest policy that promotes the use of local material. Genetic diversity and structure of two approved seed stands and three non-approved stands of *Fraxinus excelsior* L. were analysed with nuclear microsatellites to get genetically based support for the use of its reproductive material in Slovenia. Genetic diversity was high ( $H_E = 0.80$ ) and differentiation between populations measured as  $F_{ST}$  ( $F_{ST} = 0.018$ ) low to nonexistent when measured with genetic distances. Calculated allelic indices for seed stands were the same or a bit above the Slovenian average with two exceptions. Based on the analysis of five microsatellite loci, no restrictions for transferring forest reproductive material within the studied range can be presented. However, collection of forest reproductive material from seed stand Rodik should follow good seed collection practices to ensure high genetic diversity of reproductive material.

KEY WORDS: *Fraxinus excelsior*, common ash, Slovenia, microsatellites, genetic diversity, genetic structure, forest reproductive material

### Introduction

#### Uvod

Common ash (*Fraxinus excelsior* L.) is the most widespread and commercially important of the three ash species in Slovenia. It grows from lowlands to the forest border,

even though it is rarely observed at elevations above 1 000 m (Brus 2008). It is a highly outcrossing wind pollinated species with complex polygamous breeding system. Trees with male, female and hermaphroditic flowers are observed (FRAXIGEN 2005). Although self-fertilisation in common

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ash is possible, selfed seeds, which have lower genetic variability, may not survive because of inbreeding depression. Levels of selfing and seed set are similar in female and hermaphrodite trees. It is therefore unlikely that seed collection from hermaphrodite trees would result in the use of selfed seed or reduced levels of genetic diversity (FRAXIGEN 2005). Dispersal of pollen and succeeding siring of seed strongly depend on the density of the stand, the proportion of trees flowering and the landscape. Majority of common ash pollen travels over short distances, approximately 45 m (70 m in non-mast years) (FRAXIGEN 2005) to approximately 330 m between isolated patches of common ash in the barren landscape of Scotland (Bacles et al. 2005), although a proportion of pollen travels over substantial distances (Bacles and Ennos 2008). Mast years do not occur regularly and abundant flowering does not necessarily mean an abundant seed crop, especially if the weather is unsuitable for wind pollination or late frosts destroy the flowers (FRAXIGEN 2005). The fully developed seeds start to disperse by wind in the autumn. Seed dormancy usually lasts for two to six winters. Stored seed requires combined warm-cold stratification to germinate (Pliūra and Heuertz 2003).

Common ash usually regenerates naturally, but when and where natural regeneration fails, it is exceedingly important to use site adapted reproductive material of high genetic diversity to preserve natural genetic variability of the species, ensure its future adaptability and reduce the costs of artificial regeneration through successful seedling establishment, especially under changed environmental and disease conditions (Rajora and Mosseler 2001, Koskela et al. 2007). Local genotypes are assumed to be better adapted to local conditions due to natural selection, although initial results of reciprocal transplant experiments in Britain show no evidence of home site advantage for British provenances of common ash, while common ash from continental Europe is poorly adapted to British climate (Boshier and Stewart 2005). In order to ensure site adaptability, which in common and narrow-leaved ash seems to stretch over large distances (Boshier and Stewart 2005, Bogdan et al. 2007), the whole territory of Slovenia represents one provenance region delineated into four altitudinal belts for common ash (Kutnar et al. 2002). Transfers of ash reproductive material between altitudinal belts should be avoided, except in strictly defined cases (Rules on the designation... 2003). Reductions in genetic diversity (i.e. variation within populations / species that is attributable to differences in units of hereditary information) can predispose populations / species / forests to environment-related decline in health, productivity and its ability to reproduce (Rajora and Mosseler 2001, Hubert and Cottrell 2007, Hosius et al. 2006). Here common as well as low frequency and rare alleles are important because the first represent current genetic potential of the populations and the latter two latent genetic potential

(Rajora and Mosseler 2001, Hattemer 1995). Forestry practices such as silvicultural system, regeneration method, thinning as well as seed harvesting and processing can greatly affect genetic diversity within the stand. Collection of seed from a small number of trees spaced closely together, where the bulk of seed was collected from only a part of all selected trees can substantially reduce genetic diversity of the seed lot, which can be further reduced during careless seed processing and culling of young plants in the nursery (Hosius et al. 2006, Schmidt 2000).

When talking about genetic diversity, sample size is very important. Mean square error estimates of resampled data from a common ash stand in France indicated that more than 300 individuals are necessary for accurate measures of allelic richness (number of alleles, number of effective alleles) while estimation of expected heterozygosity (gene diversity) requires smaller samples (< 30). Intermediate sample sizes are needed for accurate estimation of the inbreeding coefficient (Miyamoto et al. 2008).

We have used nuclear microsatellites to investigate patterns of neutral genetic diversity and population genetic structure of common ash in five populations from Slovenia. In particular we were interested in the following questions: (1) Are the two analysed approved seed stands better suited for collection of forest reproductive material from a genetic perspective than non-approved stands? (2) Is the existing one provenance region for the whole country sufficient for common ash? We are however not addressing the question connected with climate change, in which the principle 'local is best' based on assumption that natural selection has optimised populations to their local environment, endorsed by the Helsinki guidelines (MCPFE 1993), European (1999/105/EC) and Slovenian (Rules on the designation... 2003) legislation, is questioned with respect to the oncoming climate change.

## Materials and Methods

### Materijal i metode

#### Plant material, DNA isolation and microsatellite analysis – Biljni materijal, izolacija DNA i analiza mikrosatelita

Twigs of 148 *Fraxinus excelsior* trees from five populations were sampled (Table 1). Genomic DNA was extracted from approximately 15 × 2 mm large strip of cambium using 2 % CTAB extraction buffer as described by Doyle and Doyle (1990). Microsatellite analysis was performed with five primer pairs (FEMSATEL4, FEMSATEL11, FEMSATEL16, FEMSATEL19, M2-30) developed by Lefort et al. (1999) and Brachet et al. (1999). PCR reactions were performed in a GeneAmp® 9700 thermocycler in a reaction mix containing 2 mM MgCl<sub>2</sub>, 0.2 U *Taq* polymerase, 1× PCR buffer, 0.4 mM

**Table 1:** Overview of the sampled populations of *Fraxinus excelsior***Tablica 1:** Pregled uzorkovanih populacija običnog jasena

Population Populacija	Longitude E Zemljopisna dužina V	Latitude N Zemljopisna širina S	Elevation [m] Nadmorska visina [m]	No. of trees sampled Broj analiziranih stabala	Origin Izvor
Dolsko	14°40'34"	46°5'12"	260	28	autochthonous
Grofija	14°13'21"	46°20'24"	450	30	autochthonous
Razpotje	14°54'53"	46°8'45"	320	30	autochthonous
Rodik	13°59'3"	45°37'35"	550	30	non-autochthonous <sup>1</sup>
Soteska	14°2'13"	46°17'57"	485	30	autochthonous

<sup>1</sup> Population Rodik was planted from seedlings raised in local nurseries. However, no record on the origin of the seed used in the nurseries exists.

of each primer, 0.2 mm of each dNTP and 1 µl of genomic DNA directly from extraction in a total reaction volume of 10 µl. Amplification conditions, with the exception of the annealing temperature for primer pair M2-30, which was 57 °C, were as described by Heuertz et al. (2001). PCR products were multiplexed together with 0.4 µl of internal size standard GS-400 HD ROX and 12 µl of deionised formamide. Positive and negative controls were used to verify the accuracy of the reactions, amplification conditions and fragment analysis. Fragment analysis was performed in ABI-PRISM 310. Sizing and genotyping were carried out using accompanying software GeneMapper.

### Data analysis – Analiza podataka

The number of alleles per locus  $A$ , number of alleles with frequency equal to or higher than 0.05  $A_{\geq 0.05}$ , number of effective alleles  $A_E$ , number of private alleles  $A_{PRIV}$ , proportion of observed heterozygotes  $H_O$  were calculated using GenALEX 6 (Peakall and Smouse 2006) and gene diversity  $H_E$  as well as total gene diversity  $H_T$  with FSTAT (Goudet 1995). Wright's inbreeding coefficient  $F_{IS}$ ,  $F_{ST}$  (relative differentiation based on allele identity) and  $R_{ST}$  (relative differentiation based on allele size) and their statistical significance were calculated using SpaGeDi (Hardy and Vekemans 2002). For testing statistical significance, 20000 permutations were used. Contribution of stepwise mutations on genetic structure, that is, whether parameter  $R_{ST}$  is better suited for the analysis of the given dataset that  $F_{ST}$ , was tested with the same programme (20000 permutations). Additionally, distance based clustering methods were used in an attempt to detect genetic structure between populations. Neighbour-joining and UPGMA trees were constructed, based on Cavalli-Sforza and Edwards chord distance  $D_C$  (1967, cit. after Takezaki and Nei 1996) and Nei's standard genetic distance  $D_S$  (Nei 1972). The chord distance  $D_C$  is thought to be best for the construction of tree topology, while  $D_S$  and Goldstein's  $(\delta\mu)^2$  (Goldstein et al. 1995) are better for the estimation of branch lengths (Takezaki and Nei 1996). Goldstein's distance  $(\delta\mu)^2$ , designed specifically for microsatellites, was not used, because no contribution of stepwise mutations

was observed for our dataset (see results). Programme Populations 1.2.30 (Langella 1999) was used for the calculation of genetic distances and tree construction. The bootstrap value was set to 10000.

Isolation by distance, i.e. positive relationship between geographical and genetic distances, between sampled populations was estimated using the Mantel test implemented in Genepop 4.0 (Rousset 2008). We performed 20000 random permutations between the matrix of pair-wise genetic differences between populations calculated as  $F_{ST} / (1 - F_{ST})$  and the matrix of the natural logarithm of geographic distance. Under isolation by distance, the values of pair-wise  $F_{ST} / (1 - F_{ST})$  ratios are expected to increase linearly with the logarithm of distance in a two-dimensional model (Rousset 1997).

Sequential Bonferroni corrections according to Rice (1989) for testing multiple comparisons were applied where appropriate to reduce the total type I error to 0.05, 0.01 and 0.001.

Data was managed and input files in different formats prepared with MolekBase (<http://www.gozdis.si/index.php?id=151>).

## Results

### Rezultati

#### Genetic diversity – Genetski diverzitet

All five microsatellite loci scored were highly polymorphic, displaying a high number of alleles (from 13 to 42 per locus). Total gene diversities ranged between 0.459 on locus FEMSATL16 to 0.960 on locus M2-30 (Table 2).

High levels of genetic diversity were observed in the analysed populations (Figure 1) with mean number of alleles per population and locus between 12.8 (Razpotje) and 15.6 (Soteska). Totally 38 private alleles, i.e. alleles only present in one population, were found in the dataset, ranging from four in population Razpotje to 12 in population Rodik and from 6 on locus M2-30 to 9 on loci FEMSATL4 and 11. No

**Table 2:** Allelic diversity of microsatellite loci scored in *Fraxinus excelsior*.  $N$ , number of alleles;  $H_T$ , total gene diversity;  $F_{IS}$ , Wright's inbreeding coefficient. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; all other values were not statistically significant.

**Tablica 2:** Diverzitet alela za mikrosatelitne lokuse običnog jasena.  $N$ , broj alela;  $H_T$ , ukupni genetski diverzitet;  $F_{IS}$ , Wrightov koeficijent inbridinga. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ostale vrijednosti nisu statistički signifikantne.

Locus / Lokus	N	HT	FIS	FST	RST
FEMSATL4	29	0.842	0.033	0.006	-0.012
FEMSATL11	24	0.878	0.029	0.031***	0.014
FEMSATL16	13	0.459	0.438***	0.008	0.027
FEMSATL19	22	0.903	0.045	0.017***	-0.003
M2-30	42	0.960	0.119***	0.021***	-0.016
Multilocus		0.809	0.102	0.018***	-0.007

locally common alleles, i.e. alleles with frequency equal to or higher than 0.05, were found in one population only. Locally common alleles that were shared among at least two populations, were observed in all five populations.

Analysed allele indices for seed stands were slightly above the Slovenian average (mean values based on all five analysed populations), except for the number of private alleles in seed stand Grofija and the observed number of alleles in seed stand Rodik.

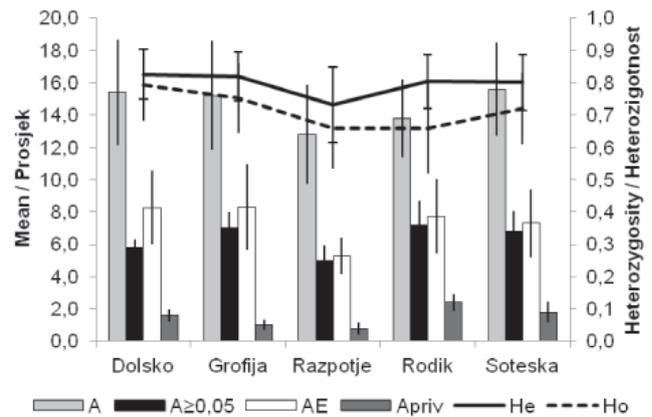
Mean observed heterozygosity ( $H_O = 0.72$ ) was lower than the expected heterozygosity ( $H_E = 0.80$ ), causing a significant positive mean inbreeding coefficient ( $F_{IS} = 0.102$ ,  $P = 0.000$ ). Overall inbreeding coefficient decreased to 0.058 after omitting locus FEMSATL16 from the analysis, which showed substantial deficiency of heterozygotes (results not shown), but remained significantly different from zero ( $P = 0.000$ ).

### Genetic structure – Genetska struktura

Differentiation between common ash populations based on allele size ( $R_{ST} = -0.007$ ) was not significantly higher than differentiation based on allele identity ( $F_{ST} = 0.018$ ;  $P = 0.993$ ). The null hypothesis that stepwise mutations do not contribute to genetic differentiation could not be rejected even for individual loci. Therefore only information based on allele identity ( $F_{ST}$ ) was considered further.

Population Razpotje was significantly different from all other populations based on pair-wise  $F_{ST}$  values, which were between 0.023 and 0.040. Except from additional differentiation between populations Grofija and Soteska, other pairs of populations did not differ among each other (Table 3).

In contrast to pair-wise  $F_{ST}$  values, which take into account two populations at a time, distance based methods use all the data simultaneously. The latter failed to detect differentiation between populations. Bootstrap values were low, never exceeding 50 %, and branch lengths short (Figure 2).



**Figure 1:** Mean values of allele indices for *Fraxinus excelsior* at five microsatellite loci.  $A$ , number of alleles per locus;  $A_{\geq 0.05}$ , number of alleles with frequency equal to or higher than 0.05;  $A_E$ , number of effective alleles;  $A_{PRIV}$ , number of private alleles;  $H_O$ , proportion of observed heterozygosity;  $H_E$ , gene diversity

**Slika 1:** Prosječne vrijednosti alelnih indeksa za obični jase na bazi analize pet mikrosatelitnih lokusa.  $A$ , broj alela po lokusu;  $A_{\geq 0.05}$ , broj alela s frekvencijom jednakom ili višom od 0.05;  $A_E$ , broj efektivnih alela;  $A_{PRIV}$ , broj privatnih alela;  $H_O$ , zapažena heterozigotnost;  $H_E$ , očekivana heterozigotnost

**Table 3:** Matrix of pair-wise  $F_{ST}$  values between five common ash populations based on the analysis of five loci. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; all other values are not statistically significant.

**Tablica 3:** Matrica parnih vrijednosti  $F_{ST}$  između pet populacija običnog jasena izračunata na bazi analize pet lokusa. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ostale vrijednosti nisu statistički signifikantne

FST	Dolsko	Grofija	Razpotje	Rodik	Soteska
Dolsko		0.006	0.023***	0.005	0.005
Grofija			0.032***	0.007	0.013*
Razpotje				0.034***	0.040***
Rodik					0.010

Differentiation between populations measured as  $F_{ST} / (1 - F_{ST})$  did not increase significantly with the natural logarithm of geographical distance between common ash populations ( $P = 0.330$ ). Therefore no isolation by distance among the analysed populations can be reported.

### Discussion

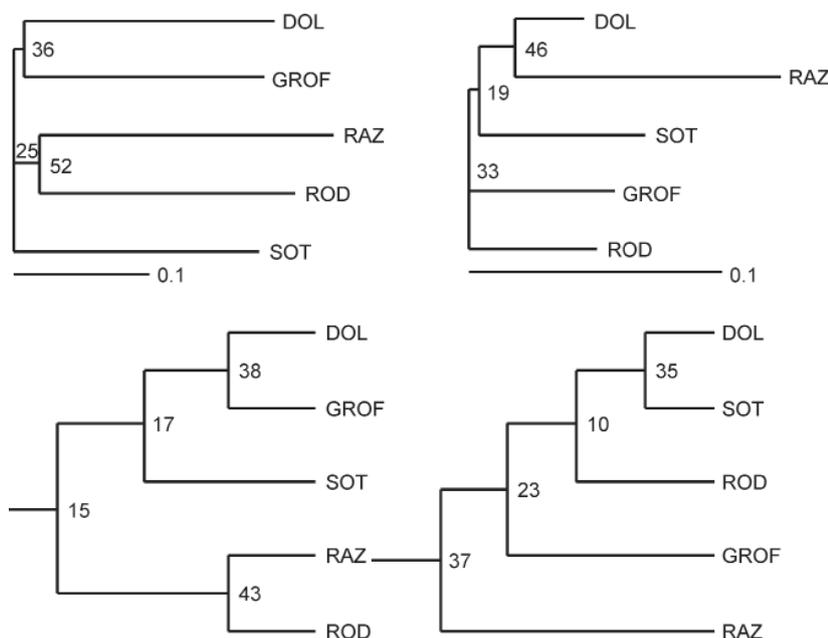
#### Rasprava

the overall pattern of genetic diversity observed at microsatellite loci of common ash was comparable to that seen in other common ash studies in Europe. Mean genetic diversity in the five studied populations was high ( $H_E = 0.80$ ) and comparable to that obtained in other common ash studies where  $H_E$  ranged between 0.73 and 0.82 (Ballian et al. 2008, Ferrazzini et al. 2007, Heuertz et al. 2004, Heuertz et al. 2003, Heuertz et al. 2001, Sutherland et al. 2010). On the other hand, differentiation between populations was low. Null alleles did not substantially affect the estimation of

population differentiation as the difference between  $F_{ST}$  and  $F_{ST}$  corrected for null alleles was by one order of magnitude lower than both of the calculated indices (data not shown). Differentiation between populations measured as  $F_{ST}$  ( $F_{ST} = 0.018$ ,  $P = 0.000$ ), was so far the lowest in Europe. It was lower than the one obtained for populations of common ash in Bosnia (0.023; Ballian et al. 2008), Britain (0.025; Sutherland et al. 2010), western and central Europe (0.027; Heuertz et al. 2004), Italy (0.049; Ferrazzini et al. 2007), Bulgaria (0.087; Heuertz et al. 2001) and at the European level (0.076; Heuertz et al. 2004). Though statistically significant, the differentiation between populations measured as  $F_{ST}$  might not be biologically significant, because variation of adaptive loci is not necessarily correlated to highly variable loci such as microsatellites used here (Hedrick 1999). Even after omitting population Rodik of non-autochthonous origin from the dataset, the  $F_{ST}$  value remained the same, showing that population Rodik comes from the same gene pool as the other analysed populations. Construction of neighbor-joining and UPGMA trees also failed to detect structure among populations as well as no positive relationship between geographical and genetic distances was observed. Lack of differentiation between populations is most likely explained by the fact that common ash is a wind pollinated tree species, where pollen and seed can travel over substantial distances (Bacles et al. 2005, Bacles et al. 2006, Bacles and Ennos 2008), the largest between any of the studied population pairs being 92 km.

The level of inbreeding is an important indicator for the quality of a seed stand since heterozygotes are thought to be more resilient to environmental stress (Namkoong 1998, Farris and Mitton 1984). Therefore homozygosity should

be taken into consideration when selecting seed stands to ensure seed quality, long-term survival of seedlings/trees and their adaptability. In the case of seed stand Rodik, which is of non-autochthonous origin, the positive inbreeding coefficient ( $F_{IS} = 0.182$ ,  $P = 0.000$ ), indicating excess of homozygotes, was high and statistically significantly different from zero and remained so also after the exclusion of locus FEMSATL16 ( $F_{IS} = 0.106$ ,  $P = 0.001$ ). In the same stand, number of alleles and observed heterozygosity were lower than the average over all five stands, while gene diversity and number of effective alleles were little above the average. The results, especially high inbreeding coefficient, could be explained with the artificial establishment of the stand with reproductive material that had possibly a narrower genetic base in comparison to natural regeneration. The observed inbreeding coefficient in this population was nevertheless lower than the one found in common ash populations from France (Morand et al. 2002) and Italy (Ferrazzini et al. 2007). According to Miyamoto et al. (2008), results based on the sample size of 30 individual ash trees are biased for estimation of the number of alleles and number of effective alleles, but in our case the first index was under and the latter above the Slovenian average. For collection of forest reproductive material from seed stand Rodik good seed collection practice is therefore advised to evade establishment of new forests with restricted genetic diversity and poor adaptive potential. Collection of approximately equal amounts of seed from as many as possible, within the stand equally spaced trees, is essential to maximise genetic diversity of the reproductive material. Number of alleles, effective alleles, observed and expected heterozygosity in seed stand Grofija were higher than the Slovenian



**Figure 2:** Neighbour-joining (top) and UPGMA (bottom) trees based on  $D_c$  (left) and  $D_s$  (right) genetic distances **Slika 2:** Metoda sparivanje susjeda (gore) i UPGMA (dole) stabla na bazi genetskih udaljenosti  $D_c$  (lijevo) i  $D_s$  (desno)

average and low inbreeding coefficient was not statistically significantly different from zero. By all analysed indices, seed stand Grofija is suitable for collection of forest reproductive material.

Population Razpotje is not an appropriate candidate for a seed stand because all indices used to describe genetic diversity were under the Slovenian average. Lower genetic diversity of this population is probably the reason for statistically significant differentiation from other populations (Table 3), although the same differentiation was not observed with genetic distances. Most likely explanation for the observed properties is the placement of the stand, which was located alongside a water stream and was longer than wider compared to other populations with more or less equal width and length of the stand.

According to the analysis of five microsatellite loci, the seed stand of non-autochthonous origin Rodik (which was presumably planted with seedlings from the neighbouring stands), does not differ from other analysed stands (except from the stand Razpotje based on the pair-wise  $F_{ST}$  values). Its origin is therefore most likely within the same gene pool as the remaining four populations or at least within the studied range of common ash, as was assumed at the time of its approval (The Slovenian national... 2010). Whether the gene pool is limited to the studied range remains open. One way of verifying the borders of the gene pool is to compare alleles from our study to the ones in the existing studies in at least northern Italy and Bosnia with the help of positive controls used during laboratory procedures, sizing and genotyping. If no adaptive differences are discovered, transfer of forest reproductive material within the same gene pool is possible, even if it extends beyond Slovenian borders. Also, mixing of seed lots from different approved seed stands, or from the same seed stand, produced in different ripening years, might be considered for improving of genetic diversity; however, caution would be needed to avoid including hybrids or seeds from related species, or seeds with reduced genetic diversity (Valadon 2009). All mixing is subjected to an official control of the share of original seed lots.

Based on the results of this study, analysed populations are genetically similar, at least when neutral variation is considered. No obstacle to liberal transfer of forest reproductive material in western and central Slovenia is therefore present. It must be however noted that in the present study no adaptive traits were analysed. Additionally, individuals with intermediate signs between common and narrow-leaved ash were observed in ash stands in the Sub-Pannonian region (Jarni 2009, Westergren 2010). These were not included in the here presented genetic analysis. In the light of possible hybrids in this region, it is strongly advised to consider each case individually, before taking the decision of

transferring common ash forest reproductive material between Sub-Pannonian and other Slovenian provenance or ecological regions. Apart from potential hybrids, selection of site appropriate provenances is necessary also because common ash from riparian forests is more tolerant to waterlogging than common ash from mountainous environment, indicating genetic adaptation to a reduction of oxygen in common ash from riparian forests (Jaeger et al. 2009). The same study also showed that net assimilation of narrow-leaved ash (*Fraxinus angustifolia* Vahl), a species with higher flooding tolerance than common ash, remained unaffected during waterlogging, while slight reduction was observed for riparian provenance and a strong one for mountainous provenance.

## Conclusion

### Zaključak

Overall pattern of nuclear genetic diversity of the studied populations of common ash in Slovenia gives a picture of high diversity belonging to a single gene pool. Based on the analysis of five microsatellite loci, no restrictions for transferring forest reproductive material within the studied range (western, south-western and central Slovenia) can be put forward. However, forest reproductive material from seed stand Rodik should be collected in a way that ensures its wide genetic base.

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## Sažetak

Obični jasen (*Fraxinus excelsior* L.) je najraširenija i ekonomski najvažnija vrsta jasena u Sloveniji. Obično se prirodno obnavlja, ali kada prirodna obnova nije moguća, bitno je da za umjetnu obnovu osiguramo staništu prilagođen šumski reprodukcijski materijal većeg genetskog diverziteta. On nam omogućava prilagodbu na promjenljive klimatske uvjete, otpornost na gljive i insekte te učinkovitu obnovu šuma (Rajora i Mosseler 2001, Koskela i sur. 2007). Stoga je bitno da prilikom sakupljanja i manipulacije sjemenom ne smanjimo njegov genetski diverzitet (Hosius et al. 2006, Schmidt 2000). Uporaba lokalnog reprodukcijskog materijala predstavlja aktualnu šumarsku politiku, jer bi zbog prirodne selekcije lokalni genotipovi trebali biti najbolje prilagođeni lokalnim uvjetima, u kojima uspijevaju.

Pomoću jezgrinih mikrosatelita istraživali smo genetski diverzitet i strukturu pet populacija običnog jasena u Sloveniji. Željeli smo odgovoriti na sljedeća pitanja: (1) Jesu li sa genetske perspektive analizirane sjemenske sastojine običnog jasena kvalitetnije za sakupljanje reprodukcijskog materijala nego nesjemenske sastojine? (2) Da li je za obični jasen dovoljna jedna provenijencijska regija koja obuhvaća cijelu Sloveniju?

Pomoću pet jezgrinih mikrosatelita (FEMSATEL 4, 11, 16, 19 i M2-30) analizirali smo DNA 148 stabala običnog jasena iz pet populacija (tablica 1). Za verifikaciju nekontaminacije i točnosti lančane reakcije s polimerazom i fragmentne analize upotrijebili smo pozitivne i negativne kontrole. Pomoću programa GenAEx 6 (Peakall i Smouse 2006), FSTAT (Goudet 1995) i SpaGeDi (Hardy i Vekemans 2002) analizirali smo sljedeće indekse genetskog diverziteta: broj alela po lokusu  $A$ , broj alela s frekvencijom jednakom ili većom od  $0.05 A_{\geq 0.05}$ , broj efektivnih alela  $A_E$ , broj privatnih alela  $A_{PRIV}$ , zapaženu heterozigotnost  $H_O$ , genetski diverzitet  $H_E$ , ukupni genetski diverzitet  $H_T$  i Wrightov koeficijent inbridinga  $F_{IS}$ . Genetsku strukturu analizirali smo pomoću indeksa  $F_{ST}$ ,  $R_{ST}$  i genetskih udaljenosti te izradom filogenetskih stabala. Programom Genepop 4.0 (Rousset 2008) analizirali smo i korelaciju između genetskih i geografskih udaljenosti između parova populacija. Statističku značajnost izračunatih indeksa testirali smo permutacijama.

Svih pet analiziranih lokusa bilo je vrlo polimorfno. Prosječni genetski diverzitet bio je visok ( $H_T = 0.809$ ), broj alela po populaciji bio je između 12.8 i 15.6. Ukupno smo otkrili 38 privatnih alela. Lokalnih općih alela (alela sa frekvencijom jednakom ili većim od 0.05), a koji se pojavljuju u samo jednoj od pet populacija, nismo otkrili. Alelni indeksi za sjemenske sastojine su sa dvije iznimke bili jednaki ili nešto iznad slovenskog prosjeka. Prosječna zapažena heterozigotnost ( $H_O = 0.72$ ) bila je niža od očekivane heterozigotnosti ili genetskog diverziteta ( $H_E = 0.80$ ). To je rezultiralo u pozitivnom i statističko značajnom koeficijentu inbridinga ( $F_{IS} = 0.102$ ,  $P = 0.000$  (tablica 2)). Na ispitivanoj razini mutacije nisu doprinijele diferencijaciji populacija ( $R_{ST} = -0.007$ ;  $F_{ST} = 0.018$ ;  $P = 0.993$ ). Zasnovano na parnim vrijednostima  $F_{ST}$ , od ostalih razlikovala se populacija Razpotje, ali ne i pomoću analize genetskih udaljenosti  $D_C$  i  $D_S$ . Isto tako nismo utvrdili veze između genetskih i geografskih udaljenosti.

Genetski diverzitet ispitivanih populacija bio je sličan onima u Bosni i Hercegovini (Ballian i sur. 2008), Bugarskoj (Heuertz i sur. 2001), Rumunji (Heuertz i sur. 2003), Italiji (Ferrazzini i sur. 2007), Velikoj Britaniji (Sutherland i sur. 2010) kao i onoj na razini Europe (Heuertz i sur. 2004), dok je diferencijacija između slovenskih populacija bila najmanja u Europi. Male razlike između populacija najvjerojatnije su rezultat učinkovitog širenja peludi i sjemena. Koeficijent inbridinga bio je posebno visok u sjemenskoj sastojini Rodik, što tumačimo umjetnim nastankom te sastojine iz reprodukcijskog materijala uske genetske baze. Iako rezultati indeksa broja

alela i broja efektivnih alela kod analize malih uzorka mogu biti pristrani, prvi indeks za sjemensku sastojinu Rodik bio je ispod, a drugi iznad slovenskog prosjeka. Da bi izbjegli reprodukcijски materijal malog genetskog diverziteta u sjemenskoj sastojini Rodik, predlažemo sakupljanje sjemena sa što većeg broja stabala, koja su između sebe približno jednako udaljena, a sa svakog stabla sakupiti približno jednaku količinu sjemena. Indeksi  $A$ ,  $A_E$ ,  $H_O$  i  $H_E$  za sjemensku sastojinu Grofija su iznad slovenskog prosjeka. Zbog manjeg genetskog diverziteta sastojina Razpotje nije odgovarajući kandidat za sjemensku sastojinu. Analizom genetske strukture utvrdili smo da sjemenska sastojina neautohtonog izvora Rodik pripada istom genetskom bazenu kao i ostale populacije. Unutar tog bazena reprodukcijски materijal običnog jasena može se prenositi, ako između populacija nema razlika u adaptaciji na uvjete okolina. Granice genetskog bazena možemo utvrditi uspoređivanjem rezultata genetskih analiza običnog jasena u sjevernoj Italiji i Bosni i Hercegovini pomoću standardizacije pozitivnih kontrola.

Na osnovi dobivenih rezultata, koji nisu povezani sa adaptacijskim svojstvima, analizirane populacije su slične, što omogućava liberalan prijenos reprodukcijskog materijala unutar istraživanog areala/genetskog bazena. Istodobno potreban je poseban oprez kod prijenosa reprodukcijskog materijala iz Prekmurja u središnju Sloveniju, jer su u Prekmurju zapaženi hibridi između običnog i poljskog jasena (Westergren i sur., u pripremi). Prekmurske populacije nisu uključene u ovu studiju. Pri sakupljanju sjemena u sjemenskoj sastojini Rodik treba pratiti pravila dobre prakse produkcije sjemena.

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KLJUČNE RIJEČI: *Fraxinus excelsior*, obični jasen, Slovenija, mikrosateliti, genetski diverzitet, genetska struktura, šumski reprodukcijски materijal