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Theses of Doctoral (PhD) Dissertation

Genetic investigation on natural populations of native *Leuce* poplar species in the Transdanubian region

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Introduction and topic issue

The density of natural populations of native poplar species has been reduced significantly over the past centuries due to river regulation works along our major rivers, the hybrid poplar cultivation affecting the natural habitats and the forest owners' and managers' preference for hybrid poplars. These species are important members of valuable forest communities therefore the survey of the genetic diversity of their populations is essential for nature protection and forest tree breeding as well.

The author selected trees in the main Transdanubian natural populations of white poplar and aspen and prepared leaf samples for genetic and morphological investigations from each tree. The main questions which he focused on during the investigations are the following:

- Does the leaf shape analysis based on elliptic Fourier descriptors enable for segregation of the individuals of white poplar and aspen? Does it suitable for identification of hybrid forms as well?
- Which populations have the highest genetic diversity in the case of the two species?
- What is the level of within- and between-population differentiation of the Transdanubian populations of both species?
- What phylogenetic connection exists between the Transdanubian populations of the species?
- What is the level of introgression from parent species in natural grey poplar individuals?

Methods applied

For leaf morphological analysis, Shape 1.3 software package has been used which enables for investigation of many biological shapes, using elliptic Fourier descriptors. During the leaf morphological investigation 2095 leaves of 439 sampled trees were analysed (3-5 leaves from each tree). The author carried out the principal component analysis of the calculated elliptic Fourier descriptors. The discriminant and canonical analysis of individuals characterized by the means of their 20 principal components average was carried out by Statistica 6.0 software.

The survey of the genetic diversity of each population was carried out with 3 molecular genetic methods. For the survey of diversity of nuclear DNA 6 SSR (*Single Sequence Repeat*) and 2 RAPD (*Random Amplified Polymorphic DNA*) primers were used. For analysing the chloroplast DNA, which is inherited by the poplars maternally, the author used 4 PCR-RFLP (*Polimerase Chain Reaction-Restriction Fragment Length Polymorphism*) primer – restriction endonuclease combination. The allelic patterns were evaluated by the use of GenAlEx 6.5 software.

Results and discussion

As the results of the principal component analysis showed the rate of leaf length and leaf width had the main effect on the variance of the leaf shape (its proportion from the total variance was 68,25 %). Significant analysis was carried out using principal components. During this analysis two principal components (PC2, PC3) showed significant difference between the species and the hybrids. Neither the first two (PC1, PC2) nor the two significant principal components were able to separate the species and identify the hybrids. The author carried out canonical analysis with the principal component scores of the individuals. In the scatterplot which was constructed by the canonical scores (CV1, CV2) of the individuals, the individuals of both species were concentrated in separate groups. Therefore it can be stated that leaf shape described by elliptic Fourier descriptors is able to separate individuals of white poplar and aspen. The group of the individuals of grey poplar overlapped partly with the aggregations of white poplar and aspen but the overlap was higher towards the direction of white poplar. It corresponds the results of the earlier taxonomic investigations.

Species identification was controlled on the one hand by the results of the discriminant analysis, and on the other hand by the allele frequencies deriving from SSR analysis. Based on these data 160 white poplar, 152 aspen and 23 grey poplar trees were appointed for the further investigations.

The following populations showed the highest genetic variation during the SSR analysis using 6 polymorphic markers: Belső-Somogy, Zselic, Szigetköz in the case of white poplar, and Bakony, Zselic, Belső-Somogy in the case of aspen. The values of F and G statistics showed very low genetic differentiation within and among populations of both species in comparison with results of past investigations as well. This can be interpreted by the strong gene flow between the populations. The *Complete linkage* dendrogram constructed by Statistica 6.0 software using Nei genetic distance showed only partial relationships between genetic and geographic distance of the populations.

High genetic diversity was found using 2 polymorphic RAPD markers in the populations of Belső-Somogy, Villányi Mountains and Zselic in the case of white poplar and Belső-Somogy, Villányi Mountains, Zselic and Mecsek in the case of aspen. The dendrogram constructed using Nei genetic distance matrix did not show relationships between genetic and geographic distance of the populations.

Chloroplast DNA diversity of the populations of white poplar and aspen was investigated by PCR-RFLP method. 12 haplotypes were found in white poplar and 9 haplotypes in aspen populations, using 4 primer pair – restriction enzymes combinations. The genetic diversity based on haplotype distribution and diversity deriving from PCR-RFLP analysis was high in the white poplar populations of Szigetköz, Zselic, Keszthelyi Mountains and Dráva basin, and in the aspen populations of Bakony and Villányi Mountains. Median-joining network constructed by Network 4.6.1.2 phylogenetic software using allelic data showed a complex relationship between white poplar haplotypes observed. Based on the structure of the network and the haplotype data of the populations, it is probable that the white poplar colonised the Transdanubian region from different directions after the last glacial along the main rivers and through lowland areas. Similar tendency in the case of aspen could not be stated. Based on the haplotype data of the populations Bakony, Zselic and Mecsek it can be assumed that they were refugial populations and took part in the recolonisation of the Transdanubian region primarily. Among the 21 investigated grey poplar individuals 11 were found white poplar and 10 which were aspen haplotype carriers.

The genetic ancestry of grey poplars identified during genetic and leaf morphologic analysis was analysed by Structure 2.1 software. The author defined two groups of grey poplar: the first group included trees which proved to be grey poplars consistently by each method (23 individuals), and the second group consisted of trees, which proved to be grey poplar based on at least one method (123 individuals). The analysis of ancestry was carried out on both groups. Only two groups could be defined by the allele frequency data of the 437 individuals using Structure 2.1 software. Therefore the software was not made probable the existence of a separate group between the two species in genetic sense. This result supports the outcomes of former observations and investigations that is the grey poplar individuals are transitional forms between the parent species in genetic and morphologic sense as well. The probability of belonging to each species was evaluated of every individual using Structure 2.1 software. This assignment probably provided opportunity to evaluate the introgression from direction of parent species. The witnessed existence and the high proportion of aspen origin haplotypes in F1 grey poplars found during comparison of results of structure and PCR-RFLP analysis shows balanced maternal contribution to the offspring. The results confirmed the former observation that first-generation grey poplar individuals backcross primarily with white poplar parents, promoting the intergradation of the hybrid swarm towards the white poplar. The backcross with aspen parents and the survival of the resulting genotypes might be suppressed by multiple factors (flowering phenology, cytoplasmatic incompatibility, low vigour).

Thesis

1. Leaf shape analysis based on elliptic Fourier descriptors is able to separate individuals of white poplar and aspen.
2. The results of SSR analysis showed very low genetic differentiation within and among populations of both species, which refer to strong gene flow between the populations.
3. The analysis of nuclear DNA using two molecular genetic methods failed to detect relationship between the genetic and geographic distance of each population of both species.

4. Haplotype diversity and ancestry relationships between different haplotypes make different phylogenetic way probable in the case of the two poplar species during their postglacial recolonisation in Transdanubia.
5. It was observed that there was a higher introgression between identified grey poplar individuals from the direction of white poplar parent, which confirms the previous observation that the first-generation grey poplars backcross in nature in higher proportion to white poplar.
6. There were identified aspen origin haplotypes between the first-generation grey poplars in high proportion. Therefore it can be assumed that both *alba* × *tremula* and *tremula* × *alba* hybrids rise in nearly the same proportion in hybrid zones.

Practical application of the results

The diverse Transdanubian populations of white poplar and aspen should serve as basis of gene conservation of nature protection and forest tree breeding, and can provide information for regulation of propagation material management. It can be suggested to use genetically diverse populations for establishment of seed-orchards in the case of both species in connection with the development of two regions of provenance (north and south) in the Transdanubian region. In order to conserve the genetic diversity, the protection of white poplar populations of Szigetköz, Belső-Somogy, Zselic, Dráva basin, and aspen populations of Bakony, Belső-Somogy, Zselic, Mecsek has a special importance.

List of written publications, posters and presentations related to the topic of the PhD dissertation

Written publications

Benke A., Cseke K., Borovics A. (2009): A szőke nyárak szekciójába tartozó őshonos fafajaink genetikai állományának felmérése a Dunántúlon. Konferencia kiadvány, XV. Növénynemesítési Tudományos Napok, Budapest, p. 26-30. [Survey of genetic diversity of native tree species belonging to section *Leuce* in Transdanubian region]

Benke A., Cseke K., Takács R., Kámpel J., Borovics A. (2010): Hagyományos és molekuláris genetikai módszerekkel támogatott nyárnemesítés a bioenergetika szolgálatában. Mezőgazdasági Technika, 51: 8-10. [Traditional and novel poplar breeding methods supported by molecular genetic in service of bioenergy]

Benke A., Cseke K., Borovics A. (2011): Dunántúli *Leuce* nyár populációk genetikai vizsgálata RAPD és cpDNS markerekkel. Erdészettudományi Közlemények, 1: 83-93. [Genetic investigation of transdanubian *Leuce* poplar populations using RAPD and cpDNA markers]

Cseke K., Benke A., Borovics A. (2011): Nyár genotípusok azonosítása DNS ujjlenyomatuk alapján. Erdészettudományi Közlemények, 1: 107-114. [Identification of poplar genotypes based on their genetic fingerprints]

Benke A., Cseke K. (2011): Leuce szekcióba tartozó hazai nyár fajok dunántúli állományainak populációgenetikai vizsgálata RAPD, PCR-RFLP és SSR markerekkel. A Nyugat-magyarországi Egyetem Savaria Egyetemi Központ Tudományos Közleményei, 18: 41-45. [Population genetic investigation of Transdanubian populations of native poplar species belonging to section *Leuce* using RAPD, PCR-RFLP and SSR markers]

Benke A., Cseke K., Borovics A. (2013): Őshonos nyárfajok genetikai változatossága a Dunántúlon. Konferencia kiadvány, Alföldi Erdőkért Egyesület Kutatói Nap, Lakitelek, p. 46-50. [Genetic diversity of native poplar species in Transdanubia]

Posters

Benke A., Cseke K., Csintalan Zs., Borovics A. (2007): Hazai nyárak populációgenetikai és élettani vizsgálata az erdészeti célú felhasználás fejlesztése céljából. Poszter, XIII. Növénynemesítési Tudományos Napok, Budapest, 2007. március 12. [Population genetic and physiological investigation of native poplars in order to promote forestry utilization]

Cseke K., Benke A., Borovics A. (2007): Nyár klónok genetikai azonosítása. Poszter, XIII. Növénynemesítési Tudományos Napok, Budapest, 2007. március 12. [Genetic identification of poplar clones]

Benke A., Cseke K., Borovics A. (2008): Dunántúli fehér és rezgő nyár állományok populációgenetikai vizsgálatának előzetes eredményei. Alföldi Erdőkért Egyesület Kutatói Nap, Szeged, 2008. november 6. [Preliminary results of population genetic investigation of transdanubian white poplar and aspen stands]

Benke A., Cseke K., Borovics A. (2011): Őshonos nyárak populációgenetikai és növényélettani vizsgálata. Poszter, Az erdők helye a vidékstratégiában – 2011. az erdők nemzetközi éve Konferencia, Budapest, 2011. november 21. [Population genetic and plant physiological investigation of native poplars]

Presentations

Benke A., Cseke K., Borovics A. (2009): A szőke nyárak szekciójába tartozó őshonos fafajaink genetikai állományának felmérése a Dunántúlon. XV. Növénynemesítési Tudományos Napok, Budapest, 2009. március 17. [Survey of genetic diversity of native tree species belonging to section *Leuce* in Transdanubian region]

Benke A., Cseke K., Takács R., Kámpel J., Borovics A. (2010): Hagyományos és molekuláris genetikai módszerekkel támogatott nyárnemesítés a bioenergetika szolgálatában. Fenntartható bioenergia termelés – fiatal kutatók a bioenergetikában szakmai konferencia és kiállítás, Gödöllő, 2009. október 29. [Traditional and novel poplar breeding methods supported by molecular genetic in service of bioenergy]

Benke A., Cseke K. (2011): Leuce szekcióba tartozó hazai nyár fajok dunántúli állományainak populációgenetikai vizsgálata RAPD, PCR-RFLP és SSR markerekkel. Euroregionális Természettudományi Konferencia, Szombathely, 2011. január 26. [Population genetic investigation of Transdanubian populations of native poplar species belonging to section *Leuce* using RAPD, PCR-RFLP and SSR markers]

Benke A., Cseke, K. (2011): Molekuláris genetikai markerek alkalmazása hazai nyárak hibridizációs viszonyainak vizsgálatában. XVII. Növénynemesítési Tudományos Napok, Budapest, 2011. április 27. [Application of molecular genetic markers in investigation of hybridization relationships of native poplars]

Benke A., Cseke K., Borovics A. (2013): Őshonos nyárfajok genetikai változatossága a Dunántúlon. Alföldi Erdőkért Egyesület Kutatói Nap, Lakitelek, 2013. november 15. [Genetic diversity of native poplar species in Transdanubia]