Topola N^{o} 179/180 (2007) str. 71-79

UDK: **582.681.81:577.2**

Prethodno saopštenje *Preliminary report*

OVERVIEW OF DNA BASED STUDIES OF GENETIC VARIABILITY IN POPLARS

Vladislava Galovic[1], Sasa Orlovic[1]

Abstract: Development of DNA markers in forest genetics has overcome the limitations on the number of the used to date morphological and biochemical markers and provided the tools that study variation in coding, non-coding and highly variable regions of both nuclear and organelle (chloroplast and mitochondrial) genomes. In phylogeografic and phylogenetic studies of tree populations as well as gene flow, organelle genomes and highly variable genetic markers proved to be highly informative.

Many genetic markers belong to so-called anonymous DNA marker type. Techniques based on these markers are: microsatellites or simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), and amplified fragment length polymorphisms (AFLPs). These marker types generally measure neutral DNA variation. They proved to be very useful (with different efficiency) in the analyses of phylogenetic relationships, population structure, mating system, gene flow, parental assignment, introgressive hybridization, marker-aided selection and genetic linkage. However, anonymous-DNA markers are not useful for measuring adaptive genetic diversity where newly developed marker system named expressed sequence tag polymorphisms (ESTPs) could lead to further progress.

The need of implementation of those DNA based, powerful, new and highly informative methods in forest genetic research have arisen recently concerning high interest in genetic variability and fingerprinting of poplar species.

Key words: molecular markers, genetic variability, Poplar

[1] Vladislava Galović, naučni saradnik, Dr Saša Orlović naučni savetnik, Istraživačko razvojni Institut za nizijsko šumarstvo i životnu sredinu, Antona Čehova 13, 21000 Novi Sad

PREGLED DNK BAZIRANIH ISTRAŽIVANJA GENETIČKE VARIJABILNOSTI KOD TOPOLA

Vladislava Galovic[1], Sasa Orlovic[1]

***Izvod**: Razvoj DNK markera je uticao da se u genetici šumskih vrsta prevaziđu ograničenja uzrokovana morfološkim i biohemijskim markerima obezbedjujući moćno oruđe koje omogućuje istraživanja varijabilnosti u kodirajućim, ne-kodirajućim i visoko varijabilnim regionima nuklearnih genoma kao i genoma organela (hloroplastni i mitohondijalni).U oblastima filogenetskih i filogeografskih istraživanja populacija drvenastih vrsta kao i istraživanjima protoka gena, genomi organela kao i visoko varijabilni genetički markeri su se pokazali kao veoma informativni.*

Mnogi genetički markeri, koji se u svetu široko primenjuju, pripadaju takozvanim anonimnim DNK marker tipovima. Tehnike bazirane na ovim markerima su: simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA, (RAPDs) and amplified fragment length polymorphisms (AFLPs). Ovi tipovi markera generalno mere neutralnu DNK varijabilnost. Pomenuti marker sistemi su se pokazali kao veoma korisni (sa različitom efikasnošću) u filogenetskim analizama, u istraživanjima populacionih struktura, različitih sistema ukrštanja, protoka gena, određivanja parentalnih karakteristika, introgresivne hibridizacije, markerima asistiranoj selekciji i povezanosti gena.

Medjutim, svi navedeni marker sistemi nisu korisni jedino u oblasti istraživanja konzervacije i adaptivnog genetičkog diveziteta gde novorazvijeni marker sistem pod imenom expressed sequence tag polymorphisms ili ESTPs može da omogući visoko informativne podatke.

Ukazala se potreba da se ove moćne, nove i visoko informativne metode implementiraju u istraživanja genetičke varijabilnosti kao i određivanju lične karte različitih vrsta topole.

***Ključne reči:** molekularni markeri, genetička variabilnost, topola*

1. INTRODUCTION

Preservance of genetic variability is the main task for maintenence of the adaptive potential in species. Characterisation of diversity in forest species has been mainly based on morphological and biochemical traits. Due to recent developements in the field of molecular genetics, a variety of different techniques has been invented in order to reveal genetic variation on molecular level.

Reliable information on the distribution of genetic variation is a prerequisite for breeding and conservation program in forest tree species. Until recently, field and laboratory techniques employed morphological and biochemical markers in order to estimate genetic diversity and mating system parameters from population surveys.

Morphological markers are proved to be difficult to use in forest genetics due to different reasons: recessive in nature, (therefore heterozygotes are not identifiable); those markers are usually mutations and they often confer a deleterious phenotype to the organism; they are exhibited epistatic effect or pleiotrophy or their expression may be affected by environmental conditions and also confer a phenotype that is only apparent at one stage of an organism's development.

However application of the isozymes, as biochemical marker, is proved to be limited by the number of enzyme loci, relatively low levels of variability and the fact that they only reveal variation in protein coding genes and that only a minor proportion of modifications of the quaternary structure of the proteins can be detected by electrophoresis on starch gel.

Since the advent of recombinant DNA technology in population genetics in the mid - 1980's, the number of genetic markers available for population genetic studies of forest tree species has increased enormously.

Development of DNA markers has overcome the limitations on the number of variable loci and provided the tools to study variation in coding, non-coding and highly variable regions of both nuclear and organelle (chloroplast and mitochondrial) genomes. Molecular markers gives new dimension to genetic and population studies, as well as to breeding practice, protection of breeder's rights, while the methods of gene manipulation enabled fast introduction of desired genes in interesting genotypes, which is of special importance in breeding of perennial wood species (Kovačević et al., 2002).

In phylogeografic and phylogenetic studies of tree populations as well as gene flow, organelle genomes and highly variable genetic markers proved to be highly informative.

Many modern genetic markers belong to anonymous DNA marker type such as microsatellites or simple sequence repeats (SSRs), restriction fragment length polymorphisms (PFLPs), random amplified polymorphic DNA (RAPDs), and amplified fragment length polymorphisms (AFLPs). These marker types generally measure neutral DNA variation. They proved to be very useful (with different efficiency) in the analyses of phylogenetic relationships, population structure, mating system, gene flow, parental assignment, introgressive hybridization, marker-aided selection and genetic linkage (Vendramin and Hansen, 2005).

But all those are not useful for measuring adaptive genetic diversity (Krutovsky and Neale, 2005). The ideal marker for estimating adaptive variation should meet the following: it has to be directly involved in genetic control of adaptive traits; to identify DNA sequence and its function and could easily identify allelic variation. Such promising new marker that satisfies most or all of these criteria emerged recently as a result of new, modern science – genomics.

The newly developed marker system named expressed sequence tag polymorphisms (ESTPs) mostly reveals genetic variation within genes, although variation can be found in both coding and non-coding regions of genes. Thus ESTPs, at present, stands for the most informative techniques in terms of gene function.

Genomics as a new science is not much discussed in forest genetics. This science studies the whole genome by integrating traditional genetic disciplines such as population, quantitative and molecular genetics with new technologies in molecular biology, DNA analysis, bioinformatics and automated robotic systems. A number of genomics subdisciplines (such as: structural, functional, comparative, statistical and associative genomics) can be combined to provide a powerful approach to broad

studying adaptive genetic diversity. Thus genetic conservation and adaptive genetic diversity can benefit from new achievements in genomics.

This paper has the intention to present the part of the international study results concerning application of the DNA based technologies in forest genetics research concerning the implementation of those techniques in the area of genetic diversity of poplar clones and fingerprinting of poplar hybrids in our research work.

2. OVERVIEW OF DNA BASED STUDIES

Genus *Populus* belongs to the willow, family *Salicaceae* and contains at least 35 species of trees, along with a number of natural hybrids. Black poplar species (*Populus nigra* L.) has a wide geographical distribution ranging from Central and South Europe to Central Asia and North Africa (Zsuffa, 1974). It is a pioneer tree species of riparian ecosystems and there, in softwood floodplain forests, represents a keystone species as it is highly adapted to water dynamics and sediment movement (Storme, submitted for publication).

As the fast growing species, ease for clonal propagation and strong heterosis upon interspecific hybridization, poplars have become a tree species of prime economic importance worldwide. As the outcome, *P. nigra* along with few species native to Europe or North America such as *P. deltoides* and *P. trichocarpa* plays a central role in poplar breeding programs and has contributed to many successful interspecific hybrids (Frison et al., 1995, and Weisgerber and Han, 2001). Thus hybrids *P.x euramericana* have found its utility as a source of timber whereas *P. nigra* 'Italica' is used as a windbreak or for landscaping purposes. Hybrid Pannonia (*P.x euramericana)* was successfully raised for a high growth vigor (Orlović et al., 2006).

However, in the last centuries, large areas of its natural habitat have been lost due to changes in the management of riverbanks involving drainage, more intensive grazing and more frequent felling of trees (Lefevre et al., 1998). Autochthonous species *P. nigra* is additionally endangered by intercrossing events with other species of the section *Aigeiros* .

Over the past 50 years, in order to optimize the genetic diversity and enable them to adapt to changing environmental conditions, gene bank collections for *P. nigra* have been set up in most European countries mainly for breeding purposes (Heinze, 1997; Cottrell et al., 2002**).**

Because of its small genome size (550 Mb; 19 chromosomes) and its amenability for genetic transformation, poplar has become a model system for fundamental research on trees (Stettler et al., 1996) and in May 2002, the U. S. department of Energy (DOE) initiated an international project to sequence the genome of a Poplar tree within 18 month. A female *Populus trichocarpa*, named Nisqually-1 (after its habitat along Washington state river) was chosen for the sequencing project. The draft sequence is now publicly available (www.genome.jgi-psf.org/poplar0/poplar0.home.html) and the International Populus Genome Consortium

(IPGC) has been founded to help guide post-sequencing activities in poplar (www.oml.gov/ipgc/). Finally in 2004 its entire genome has been sequenced.

Environmental influences on morphology and phenotype differences between juvenile and mature characters make it difficult to discriminate between genetically different individuals on the basis of morphological characters alone.

Biochemical and molecular markers were turn out to be the most suitable markers in order to detect hybrids and duplicated accessions and to establish the extent of genetic variation and the levels of heterozygosity (Karp et al., 1997). Isozymes have been used in several studies of black poplar and they have been successful in distinguishing the different populus species and their hybrids (Guzina, 1980; Legionnet et al., 1997, Janssen, 1997, Heinze, 1998.).

Storme et al. (in press) used 3 AFLP and 5 SSR primer combinations as well as 11 isozyme systems in order to evaluate the existing genetic diversity of *P. nigra* within *ex sity* collections of 675 *P. nigra* accessions from 9 European gene bank collections. In this study those authors found isozyme markers very successful in detection of the hybrid populations. For genetic diversity they used SSR markers (expressed in terms of % polymorphic loci, effective number of alleles and Nei's expected heterozygosity) and AFLP (gene diversity) markers. Implication from processed data was that *P. nigra* shows moderate level of genetic differentiation between the regions. Most unique alleles were present in Danube region in Austria, the Rhone region in France, in Italy, the Rijn region in the Netherlands and in the Ebro region in Spain. The diversity was largest in southern countries and the clustering, PCA analysis, was according to topography.

Cervera et al. (2001) used Populus species for interspecific crosses (*P. deltoids* x *P trichocarpa* and *P. deltoids* x *P. nigra*) and their backcrosses to set a dense genetic linkage maps based on AFLP and SSR markers.

Rahman and Rajora (2000) were developed and characterized new SSR markers for 8 loci in *P. tremuloides* from its partial genomic library. DNA markers were examined by determining polymorphisms in 38 *P. tremuloides* individuals. They proved microsatellite DNA markers to be very useful for assisting various genetic, breeding, biotechnology, genome mapping, conservation and sustainable forest management programs in poplars.

Since accurate identification of Populus clones and cultivars is essential for effective selection, breeding, and genetic resource management programs, the same authors (Rahman and Rajora, 2002) but in 2002 used 10 SSR loci for genetic fingerprinting and differentiation of 96 clones/cultivars and varieties belonging to six Populus species (*P. deltoides, P. nigra, P. balsamifera, P. trichocarpa, P. grandidentata, and P maximowiczii*) from three sections of the genus. The unit of cultivation and breeding in poplars is a clone, and individual cultivars are normally represented by a single clone. The authors stated that all 96 clones/cultivars could be uniquely fingerprinted based on their single- or multilocus microsatellite genotypes. Results showed that *Populus nigra* var. italica clones were genetically differentiated from the *P. nigra* var. nigra clones. The authors concluded that microsatellite DNA

markers could be useful in genetic fingerprinting, identification, classification, certification, and registration of clones, clultivars, and varieties as well as genetic resource management and protection of plant breeders' rights in Populus.

In 2003, the same authors (Rahman and Rajora, 2003) combined three markers, one biochemical (allozyme) and two molecular (10 SSR loci and 248 RAPD loci) for DNA fingerprinting and differentiation of 17 widely grown *Populus x canadensis* syn. *Populus x euramericana* (interspecific *Populus deltoides x Populus nigra* hybrids) cultivars ("Baden 431", "Blanc du Poitou", "Canada Blanc", "Dorskamp 925", "Eugenei", "Gelrica", "Grandis", "Heidemij", "I-55/56", "I-132/56", "I-214", "Jacometti", "Ostia", "Regenerata", "Robusta", "Steckby" and "Zurich 03/3"), and determination of their genetic interrelationships. Informativeness of microsatellite and RAPD markers was also evaluated in comparison with allozyme markers for clone/cultivar identification in P. x canadensis. Informativeness of microsatellite and RAPD markers was also evaluated in comparison with allozyme markers for clone/cultivar identification in *P. x canadensis*.

High microsatellite DNA and RAPD genetic diversity was observed in the sampled cultivars. Overall, microsatellite DNA markers were the most informative for DNA fingerprinting of *P. x canadensis* cultivars. On the per locus basis, microsatellites were about six-times more informative than RAPD markers and about nine-times more informative than allozyme markers. However, on the per primer basis, RAPD markers were more informative. Both the microsatellite and RAPD data suggest that the cultivars "Baden 431", "Heidemij", "Robusta" and "Steckby" are genetically closely related. The inter-cultivar genetic relationships from microsatellite DNA and RAPD markers were consistent with those observed from allozyme markers, and were in general agreement with their speculated origin. Microsatellite DNA and RAPD markers could be used for clone and cultivar identification, varietal control and registration, and stock handling in *P. x canadensis*.

Aknowledge all said above, we orientated our research work to one of the wide used molecular systems, AFLPs and SSRs in order to distinguish genetic variability among poplar clones in different locations in Serbia and also to established the fingerprint of newly developed hybrids resulted from breeding activities in the Institute of Loweland Forestry and Environment, Novi Sad. We are planning to employ AFLP markers including 4 different AFLP primer combinations and 15 SSR markers in order to achieve our goal.

REFERENCES

Cervera M.T., Storme V., Ivens B., Gusmao J., Liu B. H., Hostyn V., Slycken J. V., Montagu M. V., Boerjan W., 2001: Dense Genetic Linkage Maps of Three Populus Species (Populus deltoides, P. Nigra and P. Trichocarpa) based on AFLP and Microsatellite Markers. Genetics158: 787-809.

Cottrell, J.E., Tabbener, H.E., G.I. Forrest G.I. (2002): Distribution of variation in British black poplar: the role of human management. IN: (eds BC van Dam, S Bordács) Genetic diversity in river populations of European Black poplar - implications for riparian ecosystem management. Proceedings of an international symposium held in Szekszárd, Hungary, from 16-20 May, 2001.

Frison, E., Lefèvre F., De Vries S., Turok J., compilers. (1995) *Populus nigra* network. Report of the first meeting, 3-5 October 1994, Izmit, Turkey. IPGRI, Rome, Italy.

Guzina V. (1980): Procena genetskog varijabiliteta jasike (*Populus tremula* L.) pomoću polimorfizma izoperoksidaza. Zbornik radova Instituta za topolarstvo Novi Sad, knjiga broj 9.

Heinze, B. (1997) *Populus nigra* in Austria: rare, endangered, not recognized? IN: (Turok J., Lefèvre F., de Vries S. and Tóth B., compilers) Euforgen *Populus nigra* Network. Report of the third meeting. 5-7 October 1996, Sávár, Hungary. Pp 34-41.

Heinze, B. (1998) PCR-based chloroplast DNA assays for the identification of native *Populus nigra* and introduced poplar hybrids in Europe. For. Genet. 5, pp 31-38.

Janssen, A. (1997): Unterscheidung der beiden Schwarzpappelarten *Populus nigra* L und *P. deltoides* March sowie ihrer Arthybride *P. x euramericana* (Dode) Guinier mit Hilfe von Isoenzymmmustern. Die Holzzucht 51:pp 17-23.

Karp, A.K. Edwards J., Bruford M., Funk S., Vosman B. (1997): Molecular technologies for biodiversity evaluation: opportunities and challenges. Nature Biotechnol. 15: pp 625-628

Kovačević B., Orlović S., Aleksić J., (2002): Mogućnost primene biotehnologije u šumarstvu. Zbornik radova naučnog skupa "Savetovanje o biotehnologiji u Vojvodini", 12-13 septembar, Novi Sad. pp: 127-131.

Krutovsky K. V. and D. B. Neale, 2005: Conservation and Management of forest genetic Resources in Europe. Chapter 4: Forest genomics and new molecular genetic approaches to measuring and conserving adaptive genetic diversity. Arbora publishers, Zvolen.

Lefèvre, F., Legionnet A., de Vries, S., and Turok J. (1998) Strategies for the conservation of a pioneer tree species, *Populus nigra* L., in Europe. Genetics Selection Evolution 30 (Suppl. 1): S181-S196.

Legionnet, A., Faivre-Rampant P., Villar, M., and Lefèvre, F. (1997): Sexual and asexual reproduction in natural stands of *Populus nigra*. *Bot Acta* 110: pp 257-263.

Orlovic, S. Pilipovic, A., Galic, Z., Ivanisevic, P., Radosavljevic, N., (2006): Results of poplar clone testing in field experiments. Genetika, 38 (3): 257-264.

Rahman MH, Rajora OP., 2000: Microsatellite DNA markers in *Populus tremuloides.* Genome, 43: 293-297.

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

M.H. Rahman, O.P. Rajora, 2002: Microsatellite DNA fingerprinting, differentiation, and genetic relationships of clones, cultivars, and varieties of six poplar species from three sections of the genus Populus, Genome 45:1083–1094.

Storme, V., A. Vanden Broeck, B. Ivens, D. Halfmaerten, J. Van Slycken, S. Castiglione, F. Grassi, T. Fossati, J.E. Cottrell, H.E. Tabbener, F. Lefèvre, C. Saintagne, S. Fluch, V. Krystufek, K. Burg, S. Bordács, K. Gebhardt, B.Vornam, A. Poh, N. Alba, D. Agúndez, J. Bovenschen, B.C. van Dam, J. van der Schoot, B. Vosman, W. Boerjan, M.J.M. Smulders, 2007: Ex-situ conservation of Black poplar in Europe: genetic diversity in nine gene bank collection and their value for nature development. Submitted for publication

Tabor G. M., T. L. Kubisiak, N. B. Klopfenstein, R. B. Hall, and H. S. McNabb Jr., 2000: Bulked Segregant Analysis Identifies Molecular Markers Linked to *Melampsora medusae* Resistance in *Populus deltoids*. Phytopathology: 90 (9): 1039-1042.

Vendramin G. G. and O. K. Hansen, 2005: *In*: Conservation and Management of forest genetic Resources in Europe. Chapter 4: genetic techniques and their applications in conservation and management of forest genetic resources. Arbora publishers, Zvolen, 2005.

Zsuffa, L. (1974) The genetics of Populus nigra L. Annales Forestales 6/2 Academia scientiarum et artium slavorum meridionalium Zagreb.

Weisgerber H. and Y.F. Han, 2001: Diversity and breeding potential of poplar species in China, Forest Chron. 77: 227–237.

Rezime

PREGLED DNK BAZIRANIH ISTRAŽIVANJA GENETIČKE VARIJABILNOSTI KOD TOPOLA

Vladislava Galovic[1], Sasa Orlovic[1]

U različitim oblastima genetičkih istraživanja veoma često se primenjuju biohemijski i molekularni pristupi. Većina pomenutih istraživanja su usmerena na različite marker sisteme kao što su izozimi, AFLP, SSR i ESTP. Ovi markeri su bili veoma uspešni u određivanju genetičkog diverziteta kod divljih populacija određujući postojanje polimorfizma između pojedinih individua, različitih vrsta i njihovih hibrida. Ovi sistemi takođe omogućuju formiranje genetičke mape kao i potvrđivanje pripadnosti određenom genotipu putem genetičkog fingerprintinga u cilju zaštite oplemenjivačkih prava.

Razvijanje DNK markera omogućilo je prevazilaženje ograničenja kod brojnih varijabilnih lokusa obezbeđujući oruđe za istraživanje u oblasti kodirajućih, nekodirajućih i visoko varijabilnih regiona kako kod nuklearnih tako i kod genoma organela (hloroplastni i mitohondrijalni). DNK markeri su potvrdili visoku informativnost u oblasti filogeografskih i filogenetičkih studija populacije drveća kao i u oblasti istraživanja protoka gena i genoma organela.

U okviru razvijanja nove nauke, genomike, razvijen je i novi marker sistem pod imenom expressed sequence tag polymorphisms (ESTPs) koji se pokazao kao moćno oruđe u istraživanju genetičkih varijabilnosti unutar gena bilo da se on nalazi u kodirajućem ili nekodirajućem regionu . Trenutno se ovaj marker sistem smatra jednim od najinformativnijih od do sada razvijenih u istraživanju funkcije gena. Ovim marker sistemom će biti omogućena istraživanja u okviru ataptivnog genetičkog diveziteta i genetičke konzervacije.

Ovaj rad je napisan u nameri da sumira deo internacionalnih studija koje se odnose na aplikaciju novih tehnologija u genetičkim istraživanjima kod različitih vrsta topola kao i da se ova objavljena iskustva implementiraju u istraživačke programe Instituta za nizijsko šumarstvo i životnu sredinu.