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EVALUATION OF GENETIC DIVERSITY OF POPLAR GENOTYPES BY RAPD AND AP-PCR ANALYSIS*

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RAPD (randomly amplified polymorphic DNA) and AP-PCR (arbitrarily primed PCR) were utilized to establish the genetic diversity of 19 *Populus* genotypes. A set of 40 primers of random sequence was tested, of which 35 exhibited polymorphism. Eighteen primers generated 162 easily detectable bands between 250 and 2500 base pairs in size, sufficient to distinguish between the genotypes. Similarity measures, cluster and multidimensional scaling analysis were performed to evaluate the RAPD and AP-PCR data. Our study demonstrated that in most instances similarity in the RAPD and AP-PCR banding patterns reflected the relationship due to origin. Nineteen primers gave a species or hybrid-specific pattern. One primer generated a specific pattern in *P. euramericana*. Ten primers produced specific fragments in VIF (*P. alba*), 4 primers in KOR (*P. pyramidialis* × *P. berolinensis*) and 4 primers in UNA and RAS (*P. trichocarpa* × *P. deltoides*). The results of this study demonstrated that RAPD or AP-PCR can be used to distinguish between poplar genotypes.

Keywords: Poplar – RAPD – AP-PCR – polymorphism – cluster analysis

INTRODUCTION

Amplification of DNA sequences by using polymerase chain reaction (PCR) is currently applied to many organisms to study populations and taxonomy, to tag major genes, and to construct genetic maps. The advantages of PCR-based DNA markers over the RFLPs (restriction fragment length polymorphism) are their rapidity, their simplicity, and the need for only small amounts of DNA [4].

The recent development of the PCR to amplify DNA, and the use of randomly amplified polymorphic DNA (RAPD) [15] or arbitrarily primed PCR (AP-PCR) [14], has resulted in a potentially useful tool for poplar genotype discriminations. RAPD analysis is widely used to reveal inter- or intraspecific variation, and to study taxonomic relationships within the genus *Populus* [2]. The RAPD technique can be successfully applied to determine the genetic fidelity of micropropagated poplar plants or somaclones [6, 11]. Liu et al. [8] analyzed 55 poplar clones representing 8 species

*Dedicated to Professor Lajos Ferenczy on the occasion of his 70th birthday.

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and hybrids by means of RAPD and AP-PCR. Cervera et al. [3] used an AFLP (amplified fragment length polymorphism) technique for the clonal identification of 3 *Populus* species. *Populus deltoides* allele specific primers were applied for analysis of the introgression in *Populus nigra* [5].

DNA fingerprints by RAPD markers were chosen in the present study for the genotype differentiation and individual clonal characterization of *Populus*.

The aim of this work was the development of a simple and reliable method to differentiate various poplar genotypes (clones, hybrids, etc.). Based on these achievements, we have started tests of the whole Hungarian sortiment in order to evaluate the molecular diversity and the agreement with the botanical classification.

MATERIALS AND METHODS

Plant materials

Nineteen poplar clones representing *P. euramericana* (15 clones) and *P. alba* (1 clone) species, and *P. trichocarpa* × *P. deltoides* (2 clones) and *P. pyramidialis* × *P. berolinensis* (1 clone) hybrids were analyzed. The origins of the poplar genotypes subjected to RAPD analyses are listed in Table 1. The samples were collected at the Sárvár Research Station, Hungarian Forest Research Institute.

DNA extraction

DNA was isolated from lyophilized leaves (200–300 mg) using 2% CTAB buffer (100 mM Tris-HCl, pH 8.0, 1.4 mM NaCl, 20 mM EDTA, 2% hexadecyltrimethylammonium bromide, 0.2% 2-mercaptoethanol). The leaf tissue was ground to a powder in liquid nitrogen, mixed with 5 ml CTAB extraction buffer and incubated at 65 °C for 60 min. The samples were extracted with 5 ml of chloroform/isoamyl alcohol (24 : 1 v/v) and the aqueous phase was mixed with 2/3 volume of isopropanol [1]. Nucleic acid was recovered by centrifugation, washed with 70% ethanol, dried and resuspended in TE_1 (10 mM Tris, 1 mM EDTA) buffer. Single-strand RNA was digested with 1 μg/μl RNAse for 30 min at 37 °C. The quality of the isolated DNA was controlled on a 0.8% agarose gel stained with ethidium bromide.

Polymerase chain reaction

RAPD and AP-PCR analyses were performed with 36 decamers (RAPD) and 4 (AP-PCR) primers (Table 2). PCR amplification was carried out in 25 μl of a reaction mixture containing 10–20 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 2 mM $MgCl_2$, 0.75 μM dNTPs, 60 nM primer, and 1.5 U of Taq DNA polymerase (Promega). Cycling was carried out in a Perkin-Elmer 9600 thermocycler:

Table 1
Poplar *(Populus* spp.) clones used in the experiments and their origins. The first 10 samples are male, while samples 11–19 are female

No.	Name of clone	Abbreviation	Species and hybrid	No.	Name of clone	Abbreviation	Species and hybrid
1	Robusta[1]	ROB	*P. euramericana*	11	I214[2]	I21	*P. euramericana*
2	Blanc. Poitou[1]	BDP	*P. euramericana*	12	Agathe[3]	AGA	*P. euramericana*
3	I154[2]	I15	*P. euramericana*	13	Pannónia[4]	PAN	*P. euramericana*
4	I273[2]	I27	*P. euramericana*	14	Parvifol[2]	PAR	*P. euramericana*
5	I45/41[2]	I45	*P. euramericana*	15	H328[4]	H32	*P. euramericana*
6	Kopecky[3]	KOP	*P. euramericana*	16	Bl[2]	BL	*P. euramericana*
7	Tripló[2]	TRI	*P. euramericana*	17	Kornik[6]	KOR	*P. pyramidialis* × *P. berolinensis*
8	Sudár[3]	SUD	*P. euramericana*	18	Raspalje[5]	RAS	*P. trichocarpa* × *P. deltoides*
9	Koltay[4]	KOL	*P. euramericana*	19	Villa. franca[2]	VIF	*P. alba*
10	Unal[5]	UNA	*P. trichocarpa* × *P. deltoides*				

Origin: [1]France,[2] Italy, [3]The Netherlands, [4]Hungary, [5]Belgium, [6]Poland.

Table 2
Sequences and sources of primers used in the RAPD and AP-PCR analyses

Primer	Sequence (5'-3')	References	Primer	Sequence (5'-3')	References
OPA02	TGCCGAGCTG	Operon Technologies[1]	OPJ19	GGACACCACT	Operon Technologies[1]
OPA08	GTGACGTAGG	Operon Technologies[1]	OPJ20	AAGCGGCCTC	Operon Technologies[1]
OPA09	GGGTAACGCC	Operon Technologies[1]	OPK02	GTCTCCGCAA	Operon Technologies[1]
OPA11	CAATCGCCGT	Operon Technologies[1]	OPO08	CCTCCAGTGT	Operon Technologies[1]
OPA13	CAGCACCCAC	Operon Technologies[1]	OPP08	GTCCCGTTAC	Operon Technologies[1]
OPA14	TCTGTGCTGG	Operon Technologies[1]	OPQ14	GGACGCTTCA	Operon Technologies[1]
OPA17	GACCGCTTGT	Operon Technologies[1]	OPU08	GGCGAAGGTT	Operon Technologies[1]
OPA18	AGGTGACCGT	Operon Technologies[1]	OPX11	GGAGCCTCAG	Operon Technologies[1]
OPA19	CAAACGTCGG	Operon Technologies[1]	OPX18	GACTAGGTGG	Operon Technologies[1]
OPB05	TGCGCCCTTC	Operon Technologies[1]	OPAB09	GGGCGACTAC	Operon Technologies[1]
OPB06	TGCTCTGCCC	Operon Technologies[1]	OPAI21	CACGCGAACC	Operon Technologies[1]
OPB07	GGTGACGCAG	Operon Technologies[1]	OPAL20	AGGAGTCGGA	Operon Technologies[1]
OPC15	GACGGATCAG	Operon Technologies[1]	UBC354	CTAGAGGCCG	UBC[2]
OPD05	TGAGCGGACA	Operon Technologies[1]	NO08	ATCCGCGTTC	Sakamoto et al. [12]
OPD12	CACCGTATCC	Operon Technologies[1]	NO11	ACGGCATATG	Sakamoto et al. [12]
OPJ01	CCCGGCATAA	Operon Technologies[1]	PAL2	CCAGGTGGACC	Own construction
OPJ05	CTCCATGGGG	Operon Technologies[1]	E9	GACGAGATCTACGC	Own construction
OPJ06	TCGTTCCGCA	Operon Technologies[1]	E10	CAAACTCGGAACCA	Own construction
OPJ09	TGAGCCTCAC	Operon Technologies[1]	E11	ATGGGTCTTGCAGAG	Own construction
OPJ17	ACGCCAGTTC	Operon Technologies[1]	E12	GCGTAGATCTCGTC	Own construction

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Table 3
Matrix of Jaccard coefficients of similarity between the 19 poplar genotypes. The minimum and maximum values are highlighted

	ROB	BDP	I15	I27	I45	KOP	TRI	SUD	KOL	UNA	I21	AGA	PAN	PAR	H32	BL	KOR	RAS	VIF
ROB	1																		
BDP	.703	1																	
I15	.724	.770	1																
I27	.724	.735	.813	1															
I45	.793	.680	.717	.717	1														
KOP	.777	.690	.664	.649	.720	1													
TRI	.707	.718	**.832**	.794	.735	.679	1												
SUD	.815	.716	.737	.737	.750	.738	.792	1											
KOL	.723	.625	.705	.721	.716	.771	.772	.718	1										
UNA	.618	.573	.619	.532	.596	.573	.560	.556	.522	1									
I21	.753	.745	.768	.699	.781	.750	.750	.696	.698	.660	1								
AGA	.787	.722	.726	.726	.798	.712	.745	.761	.673	.600	.793	1							
PAN	.800	.673	.743	.760	.773	.743	.778	.776	.740	.623	.788	.766	1						
PAR	.694	.657	.693	.660	.740	.667	.660	.690	.600	.606	.720	.713	.680	1					
H32	.789	.745	.733	.750	.745	.783	.768	.784	.714	.583	.778	.774	.806	.720	1				
BL	.796	.733	.792	.720	.750	.738	.773	.789	.670	.600	.802	.761	.758	.724	.821	1			
KOR	.623	.565	.566	.553	.602	.632	.595	.606	.611	.479	.589	.561	.613	.568	.604	.591	1		
RAS	.660	.582	.583	.527	.621	.637	.555	.579	.558	.637	.607	.626	.587	.600	.578	.625	.568	1	
VIF	.414	.387	.409	.385	.374	.419	.397	.391	.415	**.350**	.370	.369	.378	.383	.393	.416	.460	.445	1

1 cycle at 94 °C for 2 min; 40 cycles at 94 °C for 10 sec, at 36 °C (RAPD) or 44 °C (AP-PCR) for 30 sec, and at 72 °C for 1 min; and 1 cycle at 72 °C for 2 min. Electrophoresis was performed in 1.2% agarose (FMC) gels. The gel was stained with ethidium bromide or SYBR® Gold nucleic acid gel stain (Molecular Probes) and photographed with a Polaroid instant camera.

Analysis of PCR-amplification profiles

PCR-amplification profiles were scored as present (1) or absent (0). The similarity was analyzed on the basis of the number of shared amplification products according to [9]. A dendrogram based on similarity coefficients was generated with SYN-TAX Version 5.0 [10] by means of the unweighted pair group method of arithmetic means (UPGMA). For each group, estimates of similarity between all pairs of varieties were summarized in matrix form. Multidimensional scaling analysis of 19 genotypes (objects) was conducted by using the statistical software package SPSS 8.0.

RESULTS AND DISCUSSION

Of the 40 primers (36 RAPD and 4 AP-PCR oligonucleotides) tested, 35 furnished evidence of polymorphism. The number of fragments produced ranged from 1 (OPJ05) to 16 (OPB05, OPJ08). The size of the bands varied from 250 to 2500 base pairs (bp). Examples of the RAPD products produced and analyzed for the 19 poplar genotypes are shown in Fig. 1.

Eighteen primers were selected for cluster analysis, which yielded a total of 162 detectable bands with sufficient intensity to allow the determination of their presence or absence in samples. Other polymorphic primers generated patterns which were either faint and difficult to detect or not reproducible. UPGMA cluster analysis, calculated from RAPD and AP-PCR data, gave a dendrogram (Fig. 2) which indicated that the distance separating VIF is greater than the distance between all other genotypes. The genotypes were divided into 4 main clusters. The first cluster included the genotype VIF *(P. alba)*. The second cluster was composed of the hybrids UNA *(P. trichocarpa × P. deltoides)* and RAS *(P. trichocarpa × P. deltoides)*. The third cluster contained only one hybrid KOR *(P. pyramidialis × P. berolinensis)*. The last group included *P. euramericana* clones. The four groups that could be separated with the cluster analysis followed the origin of the clones well. Further sub-groups could be separated within group 4, in which the *P. euramericana* clones belonged. The clones ROB, SUD, PAN, I21, H32, BL, I45 and AGA formed a separate sub-group. Similarly, distinct sub-groups could be developed from I15, TR1 and I27, and KOP and KOL clones. In group 4, BDP and PAR were stand-alone clones, i.e. they did not belong in any sub-group. The most deviant clone in this group was PAR, which differed most morphologically from the other clones.

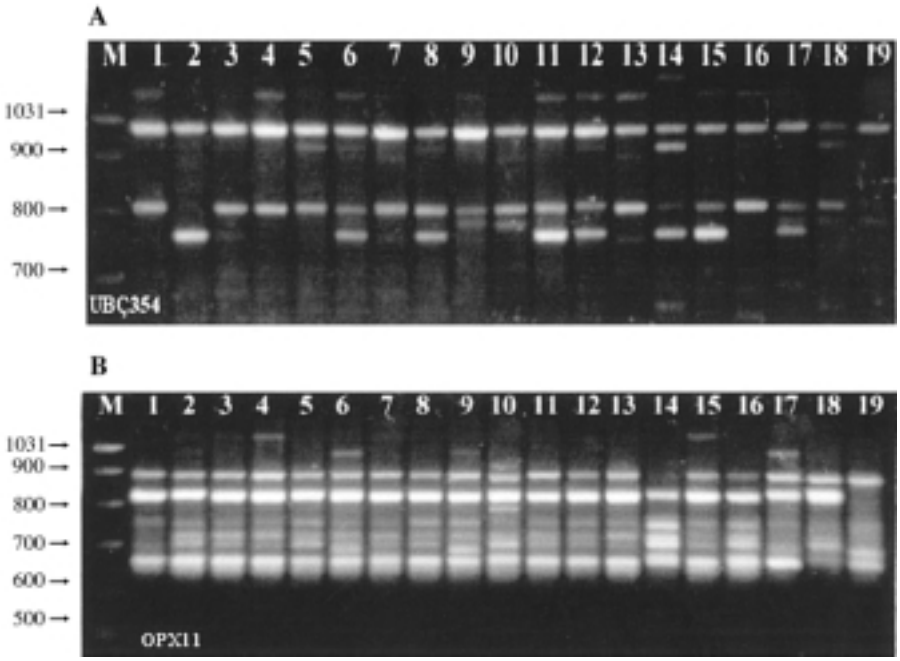

Fig. 1. Patterns of RAPD fragments of 19 poplar genotypes (Table 1) generated with primers UBC354 (A) and OPX11 (B). M = 100 bp DNA ladder (Fermentas)

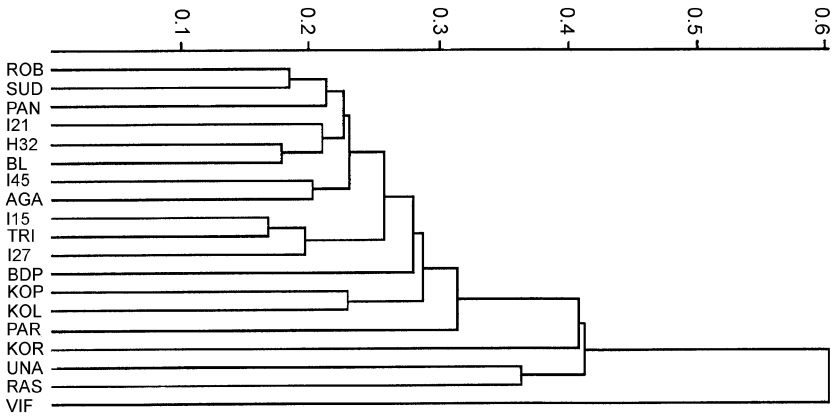

Fig. 2. Dendrogram of the poplar genotypes (Table 1) analyzed on the bases of UPGMA cluster analysis (SYN-TAX; Podani [10]) of genetic similarity estimates (Jaccard coefficient)

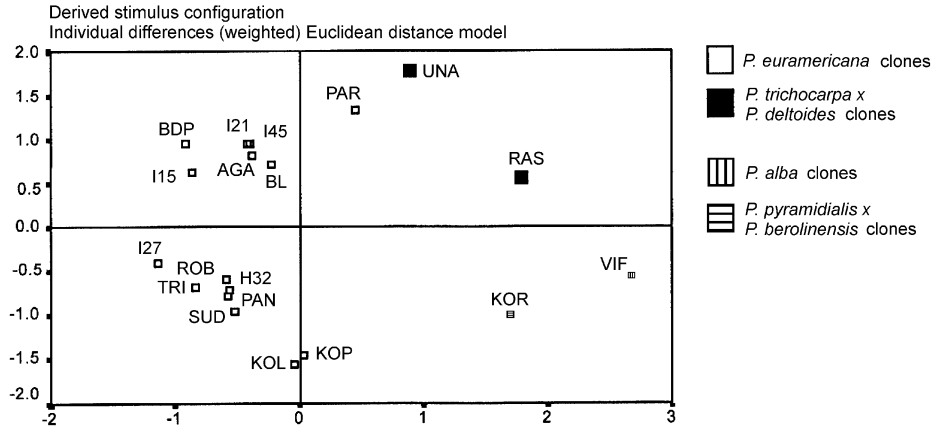

Fig. 3. Multidimensional scaling analysis of poplar genotypes calculated from PCR data corresponding to 162 polymorphic bands

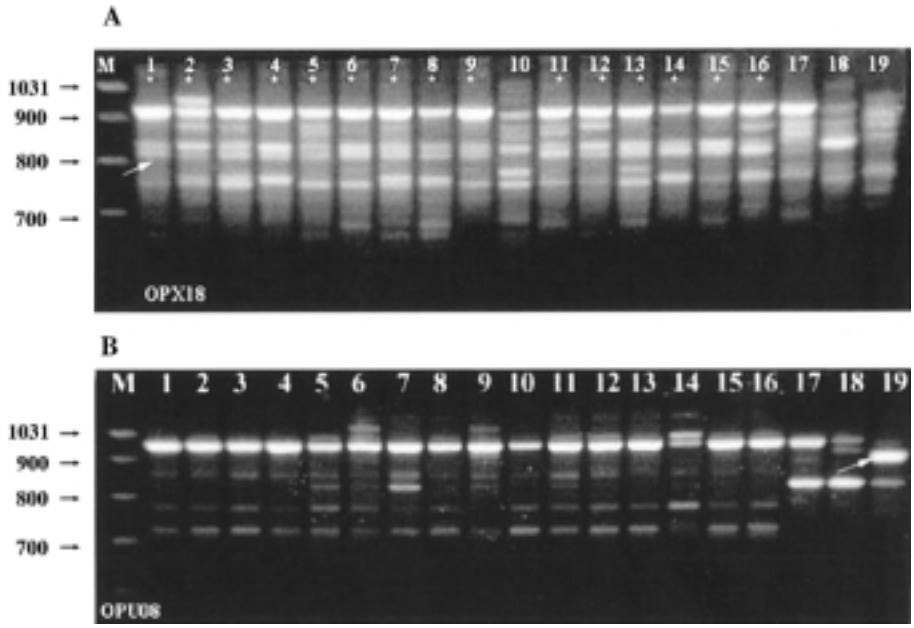

Fig. 4. Species-specific fragments. Arrows indicate specific fragments. Fig. 4/A: Species-specific fragment generated with OPX18 primer in *P. euramericana.* Asterisks indicate *P. euramericana* clones. Fig. 4/B: Specific fragment generated with OPU08 primer in *P. alba.* M = 100 bp DNA ladder (Fermentas). For the numbering of the samples, see Table 1

The Jaccard coefficients of similarity between the 19 poplar genotypes are given in Table 3. The range of similarity coefficients varied from 0.350 to 0.832. As expected, VIF had the lowest similarity coefficient (range 0.350–0.460). In our results, the least genetic distance, i.e. the largest similarity, was observed between I15 and TRI (83% similarity). Castiglione et al. [2] studied 10 species (32 clones) belonging in the 3 main *Populus* sections, *Aigeiros*, *Tacamahaca* and *Leuce,* by RAPD and AP-PCR. Of the 32 genotypes they analyzed, 5 (TRI, I15, I45, I21 and VIF) were present in our experiments. One cluster of *P. euramericana* clones proved to agree well with our observations. Similarly as in our results, Castiglione et al. [2] detected a very small genetic distance between TRI and I15. The male clone used as pollen donor in the crossing that gave rise to TRI was in fact a tetraploid clone obtained by colchicine treatment of the apical meristems of I15 [13]. They also found that I45 and I21 belonged in the same sub-group and their genetic distance was relatively short, which agreed well with our results. The most distant clone in our tests and those of Castiglione et al. [2] was VIF. There was no relationship between the origin of the clones and the RAPD and AP-PCR analysis pattern.

Genotypes were reflected in the multidimensional scaling (MDS) analysis (Fig. 3) as well. The MDS analysis of the 19 genotypes (objects) was generated in the Euclidean model (n = 2), with algorithmic options: Maximum Iterations – 30; Convergence Criterion – 00100; Minimum S-stress – 00500. The MDS plot illustrates the relationship between the poplar genotypes in terms of RAPD and AP-PCR banding patterns.

Nineteen primers gave a species or hybrid-specific pattern (Fig. 4). The *P. euramericana*-specific pattern could be generated with one primer. Ten primers generated specific fragments in VIF (*P. alba*), 4 primers in KOR *(P. pyramidialis* × *P. berolinensis)* and 4 primers in UNA and RAS *(P. trichocarpa* × *P. deltoides).* Similar experiments were carried out by Lin et al. [7], who analyzed 55 poplar clones representing 8 species and hybrids. Of the 17 primers tested, only 4 were needed to distinguish between all 55 poplar clones according to species and hybrids.

The first 10 clones of the 19 poplar genotypes used in this study were male, and the other 9 were female (Table 1). No sex-linked marker was found with the tested primers.

To summarize, RAPD markers were shown to be useful in the identification of *Populus* species (or clones) and for estimation of the genetic distances in poplar. We further wish to use the RAPD method to test the whole Hungarian sortiment in order to evaluate the molecular diversity and the agreement with the botanical classification.

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