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PRIMER NOTE

Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.)

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Abstract

Using an enrichment procedure, we have cloned microsatellite repeats from black poplar (*Populus nigra* L.) and developed primers for microsatellite marker analysis. Ten primer pairs, mostly for trinucleotide repeats, produced polymorphic fragments in *P. nigra*. Some of them also showed amplification in other poplar species. (*P. deltoides*, *P. tricocarpa*, *P. tremula*, *P. tremuloides*, *P. candicans*, *P. lasiocarpa*). The best six loci were tested on 23 *P. nigra* genotypes collected across Europe. The microsatellites produced up to 12 alleles per locus in this set, with observed heterozygosity between 0.32 and 0.91.

Keywords: allelic ladder, genetic diversity, sequence-tagged microsatellite site (STMS) simple sequence repeat

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Black poplar (*Populus nigra* L.) is a pioneer tree species of riparian ecosystems. Large areas of its natural habitat have been lost due to management of riverbanks, intensive grazing and wood cutting. During restoration of the natural borders of rivers, existing black poplar stands may act as source population for recolonization of floodplains. Therefore, it is important to know how much genetic variation in black poplar is still present along different rivers (Cottrell *et al*. 1997; Arens *et al*. 1998; Winfield *et al*. 1998). To establish the extent of genetic variation, levels of heterozygosity, and dispersal characteristics of black poplar, codominant microsatellite markers would be an excellent choice. Microsatellite primers have been developed by Dayanandan *et al*. (1998) for trembling aspen (*P. tremuloides*), but these were not useful in black poplar. A large number of microsatellites developed for *P. trichocarpa* are listed in the Poplar Molecular Genetics Cooperative database (http://poplar2.cfr.washington.edu/pmgc). These are mostly (GA) dinucleotide repeats, of which some give a product in black poplar. To increase the number of simple sequence repeat (SSR) loci for black poplar, we isolated microsatellite loci using an enrichment procedure (Van de Wiel *et al*. 1999; Arens *et al*. 2000), designed primers, and tested the markers on genotypes from all over Europe. Initially, markers were designed and tested for dinucleotide repeats (Van der Schoot *et al*. 2000). Here, we describe the markers resulting from the trinucleotide enrichments.

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Microsatellite-enriched libraries were produced by a selective hybridization procedure (Karagyozov *et al*. 1993; modified by Van de Wiel *et al*. 1999) on genomic DNA of *P. nigra* clone IBN1749 digested with *Alu*I, as described by Van der Schoot *et al*. (2000). Twenty-one unique microsatellite repeats were obtained with hybridizations to pools of synthetic trinucleotide oligonucleotides. Primers were designed using Lasergene (DNAstar) and synthesized by Isogen (Maarssen, The Netherlands). The markers were tested using two different polymerase chain reaction (PCR) protocols and three annealing temperatures (details in Table 1) on a Hybaid TouchDown thermal cycling system. Amplification was performed in 20 μL containing 75 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 0.01% (w/v) Tween 20, 100 μM of each dNTP, 200 nM of each primer, 16 ng template DNA in sterile distilled water and 0.1 unit Goldstar DNA polymerase (Eurogentec). Amplification products were mixed with an equal volume of 8 M urea containing 10 mM NaOH and 0.05% bromophenolblue, denatured for 5 min at 80 °C, separated on 6% denaturation polyacrylamide gels (National Diagnostics), and visualized with silver staining (Promega Silver Sequence DNA Sequencing System). Quality of the fragments generated by the best amplification protocol was judged according to Smulders *et al*. (1997).

The most polymorphic primer pairs were WPMS13 and WPMS19, and they amplified 10–12 different alleles

Table 1 Characterization of 10 polymorphic microsatellite markers in *Populus nigra*

Microsatellite locus	Primer sequences forward, reverse, 5′–3′	Amplification conditions*	Repeat†	Expected Product length (observed range)	Quality of patterns in *P. nigra*‡	Number of alleles among 23 genotypes§	H_O	EMBL Nucleotide Sequence Database Accession no.
WPMS13	GATCCTGAACAATGTCGTACTTC ACGATAACCTGCGAGAAATGT	60NP	$(GT)_{22}$	141 (111–150)	3	10¶		AJ297286
WPMS14	CAGCCGCAGCCACTGAGAAATC GCCTGCTGAGAAGACTGCCTTGAC	60LP	$(CGT)_{28-3}$	245 (215–287)	1	12	0.91	AJ297287
WPMS15	CAACAAACCATCAATGAAGAAGAC AGAGGGTGTTGGGGGTGACTA	60LP	$(CCT)_{14-3}$	216 (201–219)	1	6	0.32	AJ297288
WPMS16	CTCGTACTATTTCCGATGATGACC AGATTATTAGGTGGGCCAAGGACT	55LP	$(GTC)_8(ATCCTC)_5$	158 (140–161)	1	7	0.57	AJ297289
WPMS17	ACATCCGCCAATGCTTCGGTGTTT GTGACGGTGGTGGCGGATTTTCTT	60LP	$(CAC)_{15-1}$	146 (119–152)	4	6	0.68	AJ297290
WPMS18	CTTCACATAGGACATAGCAGCATC CACCAGAGTCATCACCAGTTATTG	60LP	$(GTG)_{13}$	245 (219–248)	1	7	0.87	AJ297291
WPMS19	AGCCACAGCAAATTCAGATGATGC CCTGCTGAGAAGACTGCCTTGACA	65NP	$(CAG)_{28-3}$	204 (174–252)	3	12¶		AJ297292
WPMS20	GTGCGCACATCTATGACTATCG ATCTTGTAATTCTCCGGGCATCT	60NP	$(TTCTGG)_8$	252 (222–252)	1	6	0.91	AJ297293
WPMS21	TGCTGATGCAAAAGATTTAG TTGGAACTTCAACATTCAGAT	55LP	$(GCT)_{45-12}$	242 (287–326)	4	5¶		AJ297294
WPMS22	ACATGCTACGTGTTTGGAATG ATCGTATGGATGTAATTGTCTTA	55LP	$(TGA)_{23}$	129 (100–135)	3	6¶		AJ297295

*55, 60, 65, temperature; NP protocol, 1 cycle 3 min 94 °C, 30 cycles (5 s 94 °C, 15 s at the annealing temp., 60 s 72 °C) 10 min 72 °C; LP protocol: 1 cycle 3 min 94 °C, 30 cycles (45 s 94 °C, 45 s at the annealing temp., 105 s 72 °C) 10 min 72 °C.

†'–'denotes mismatch.

‡Quality 1, weak stutter bands, well scorable; quality 2, 'stutter' bands present, but product still scorable; quality 3, ladders of bands of equal intensity, making scoring difficult; quality 4, bands of unexpected sizes also present.

§Cultivar Italica and 22 trees from the EUFORGEN Core Collection (Turok *et al*. 1998): IBW-N004 and IBW-N009 (BEL), SEEFAR-SVICHTOV-N2 (BGR), FBS-215/63-JUG-1 and FBS-87/65-OFFENBERG-1 (DEU), SIA-PA1 and SIA-LUC2 (ESP), FCR-HUNTINGTON and FCRA-HOBSONS-CONDUIT (UK), FF-V336 and FF-V408 (HRV), ERTI-3–3-1 (HUN), ISP-N068 (ITA), IBN-1238 and IBN-1792 (NL), LVU-BAKA and LVU-IVACHNOVA (SKV), KAE-N.90.013 (TUR), IZT-NS002 and IZT-NS001 (YUG), VULHM-88044 (CZ) and ICAS-4 (ROM).

¶In 12 of these 23 trees.

Table 2 Amplification of microsatellite markers in other *Populus* species

Microsatellite locus	Amplification in other *Populus* species*
WPMS14	D, T, Ta, To
WPMS15	D, T, Ta, To, C, L
WPMS16	D, T, Ta, To, C, L
WPMS17	D, T, Ta, To, C, L
WPMS18	D
WPMS20	D, T, Ta, To, C, L

*D = *P. deltoides*, T = *P. trichocarpa*, Ta = *P. tremula*, To = *P. tremuloides*, C = *P. candicans*, and L = *P. lasiocarpa*.

in only 12 plants; however, these were also the ones with many stutter bands, which may hamper unambiguous scoring under the conditions of amplification and detection that we used. The degree of polymorphism for the best six primer pairs was tested using a set of 23 genotypes representing diversity across west and middle Europe. These primer pairs amplified between six and 12 alleles each. Observed heterozygosity was high (0.57–0.91), with the exception of WPMS15 (which had a heterozygosity of only 0.32), where one allele was present in a high frequency. Alleles of loci WPMS14 through to WPMS19 differed by 3 bp or multiples of 3 bp in size, and formed continuous or nearly continuous allelic ladders for WPMS15, WPMS16 and WPMS18. The allelic ladders spanned up to 27 (WPMS19) repeat units. The alleles of WPMS16, which contains a trinucleotide and a hexanucleotide repeat, formed a trinucleotide repeat ladder. The alleles of WPMS20 formed a continuous hexanucleotide allelic ladder, in accordance with the repeat present in the fragment. It was not a problem to identify each individual tree based on its own unique multilocus genotype. The six loci were also tested on other Populus species (Table 2). Most loci amplified fragments in all species, producing products in the same size range, often consisting of new alleles.

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