Molecular Ecology Notes (2003) **3**, 380–383 doi: 10.1046/j.1471-8286.2003.00456.x

PRIMER NOTE

A set of cross-species amplifying microsatellite markers developed from DNA sequence databanks in *Picea* (Pinaceae)

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Abstract

Seven codominant genetic markers (of which six are located in gene untranslated transcribed regions) were developed from *Picea* DNA sequences available in databanks. Primers were designed to specifically amplify by polymerase chain reaction tri-, di- or mononucleotide repeated motifs. They provided single locus length polymorphism on a representative sample of 93 *Picea abies* individuals. The usefulness of each locus in mapping, population genetics or gene flow studies was assessed. A weak geographical genetic differentiation was revealed. The loci were also amplified in *P. glauca*, *P. engelmannii* and *P. omorika* demonstrating potential transferability across *Picea* species.

Keywords: DNA sequence databanks, EST, *Picea*, SSR

Received 3 December 2002; revision received 2 February 2003; accepted 2 April 2003

In the last decade, the demand for codominant DNA markers, especially codominant gene markers, has increased remarkably. Such locus-specific and multiallelic markers offer promising applications in population genetics, genetic mapping or marker-assisted selection when linkage between genes and traits can be established. The identification of gene–trait associations is indeed an important challenge at the present time (McKay & Latta 2002).

Spruces (genus *Picea*) are conifers occurring naturally in plains and medium mountains over the northern hemisphere. In this genus, which includes several economically and ecologically important species such as *Picea abies* (L.) Karst or *P. glauca* (Moench) Voss., only a few sets of markers located in genes have been made available (Perry & Bousquet 1998; Scotti *et al*. 2000; Schubert *et al*. 2001). Moreover, to date, no more than about 20 of these loci have been shown to be polymorphic in population analysis of different *Picea* species.

In this work, we present a set of new microsatellite loci (Table 1) developed from *Picea* DNA sequences available in public databanks (July 2002). To be fully useful, codominant markers have to be between-species transferable for results comparison (i.e. comparative mapping) and must not display null-alleles which reduce their utility in population genetics. A database including all *Picea* DNA sequences available in GeneBank was created. Accessions displaying at least five, six or 10 repeats of tri-, di- or mononucleotide motifs, respectively, were identified using the FINDPATTERNS software (GCG package, http://www.accelrys.com/products/gcg_wisconsin_package/). Thirteen were selected corresponding to mRNAs (AF172094, AJ133748, X79281, L47609, L42464, X91487, L42465 and L47116) and nontranscribed repeated DNA regions (AF305145, AF100429, AF150993, AF104485 and AF104487). Primers were designed in the flanking regions of these repeated motifs. To examine the potential of these loci for genetic studies, they were tested on a representative tree sample including 93 individuals collected over the three geographical areas (Alpine, Hercyno-Carpathian and Baltico-Nordic domains) usually recognized within the natural range of Norway spruce [International Union of Forest Research Organisation (IUFRO) test, Amance forest, referenced in Collignon *et al*. (2002)]. Marker inheritance was assessed from their segregation in an F1 mapping population (TH787F × Sire5 progeny). Finally, to test their within-genus transferability, polymerase chain reaction (PCR) amplification of the loci was performed on one individual

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Table 1 Primer pairs providing length polymorphism in the tree sample (93 individuals) and sequence characteristics of the corresponding DNA regions

Code	Primers (5′ → 3′)	EMBL Accession no.	Sequence characteristic	Microsatellite motif	Allele size range (bp)*
	F: †GTGAGTGTTGGGAGAGTCAC	AF172094	*P. rubens* actin mRNA	$(GCT)_6$	83–101
prGB1	R: AGGTATCGATCCTGCTCGTC			5′ UTR	
	F: †AGTGATTAAACTCCTGACCAC	AJ133748	*P. abies* mRNA for major	$(AT)_{11}$	117–153
paGB3	R: CACTGAATACACCCATTATCC		intrinsic protein (aquaporin)	3′ UTR	
	F: †CCATTGCGGAGAACCCAGAG	L47609	*P. glauca* heat shock-like protein	$(AT)_9$	88–106
pgGB5	R: CGCAGAACAATGAATCTCCAC		(hsp18.2-like) mRNA	5′ UTR	
	F: †GATGATGAATTTCAGAGGCATC	L42464	*P. glauca* Em-like protein (EMB44)	$(T)_{10}$	149–172
pgGB7	R: GTACCAATTAAGAGAGAATGCC		mRNA	3′ UTR	
	F: AGCATGTACAAAATGAAGATTCTC	AF100429	*P. abies* clone PA12H2 repetitive	$(AT)_4(TG)_{16}$	254–350
paGB8	R: †CCCTTTAGTGTTTTCTCTTTCTAC		DNA sequence		
	F: †GCAAGATATTCTAATGTTGGG	L42465	*P. glauca* late embryo abundance	$(AT)_6$	104–114
pgGB11	R: GTATAAAAGACTGAACTTTCATATC		protein (lea) mRNA	3′ UTR	
	F: †GTTTACGAAGGGAATGCGGG	L47116	*P. glauca* late embryogenesis abundant	$(T)_{10}$	111–121
pgGB16	R: CTGGTTGGGAAAGCTCACGAG		protein (EMB6) mRNA	3′ UTR	

*In *Picea abies*.

†Primer labelled with the fluorochrome IRD800.

Primer pairs amplifying monomorphic loci are not shown. UTR, Untranslated transcribed region.

Table 2 Characterization of the seven polymorphic loci: polymerase chain reaction (PCR) conditions used, level of polymorphism revealed in the tree sample, discriminating power of each locus (*D*; in percentage), expected (H_E) and observed (H_O) heterozygosities, between-population differentiation (F_{ST}) at each locus, map location (Acheré *et al*., unpublished) and transferability to other *Picea* species

								Transferability		
Locus	PCR conditions used	No. of alleles	*D*	H_O	H_E	F_{ST}†	LG	*Po*	*Pe*	*Pg*
prGB1	T_a = 55 °C, 2.5 mM $MgCl_2$	4	6.4	0.033	0.033	0.00ns	—	+	++	+
paGB3	T_a = 55 °C, 2.5 mM $MgCl_2$	9	92.0	0.652	0.764	0.00ns	12	++	+	+
pgGB5	T_a = 45 °C, 5 mM $MgCl_2$, DMSO 5%	10	90.7	0.753	0.763	0.00ns	7	+	++	++
pgGB7	T_a = 55 °C, 2.5 mM $MgCl_2$	8	76.9	0.565	0.570	0.034**	10	+	++	++
paGB8	T_a = 55 °C, 2.5 mM $MgCl_2$	43	99.9	0.914	0.963	0.015**	10	++	+	++
pgGB11	T_a = 55 °C, 3.5 mM $MgCl_2$	3	8.4	0.043	0.041	0.038*	—	+	+	+
pgGB16	T_a = 55 °C, 2.5 mM $MgCl_2$	3	12.3	0.064	0.063	0.00ns	—	+	+	+

DMSO, Dimethyl sulphoxide.
†Significance of each F_{ST} value: ns, nonsignificant; *$P < 0.05$; **$P < 0.01$.
LG, Linkage group no.; *Po*, *Picea omorika*; *Pe*, *P. engelmannii*; *Pg*, *P. glauca*; T_a, annealing temperature; +, amplification of a single allele (homozygote); ++, amplification of two alleles (heterozygote).

of three other *Picea* species: *P. omorika* (Pancic) Purkyne, *P. engelmannii* Parry ex Engelm. and *P. glauca*.

DNAs were extracted using a cetyltrimethylammonium bromide method (Collignon *et al*. 2002). The PCR reaction mixtures contained 50 ng of DNA template, 1× reaction buffer, 0.2 mM dNTPs, 0.2 µmol of each oligonucleotide primer (one 5′ labelled with the IRD800 fluorochrome; MWG-Biotech) and 0.75 U of DNA polymerase (Invitrogen) in a total volume of 25 µL. Specific PCR conditions for selected primer pairs are reported in Table 2. Reaction mixtures were incubated in a thermocycler (I-cycler; Bio-Rad) firstly for 4 min at 94 °C and then for 36 cycles consisting of 1 min at 94 °C, 1 min at the defined annealing temperature (Table 2) and 1 min at 72 °C. The last cycle was followed by a 6-min extension at 72 °C. Electrophoresis of PCR products was carried out on a denaturing 6.5% polyacrylamide gel using an automated sequencer (4200; LiCor). The discriminating power (*D*) corresponding to the probability that two randomly chosen profiles would appear different (Tessier *et al*. 1999) was computed for each polymorphic locus. A linkage disequilibrium test between each pair of loci and deviation of observed genotypes from Hardy–Weinberg equilibrium were computed in each geographical area with the GENEPOP software (version 3.2a; Raymond & Rousset 1995). *F*-statistics (F_{IS}, F_{IT} and F_{ST}) within and among the three geographical areas of *P. abies* were estimated using FSTAT (Goudet 1995).

All tested primer pairs produced clear amplification profiles. Six primer pairs failed to reveal length polymorphism in the tree sample (Accession nos AF305145, X79281, AF150993, X91487, AF104485 and AF104487). Conversely, seven loci displayed length polymorphism with three to 43 alleles (Tables 1 and 2). Polymorphism in *pgGB7* and *pgGB16* was due to 1–2-bp indels probably in the SSR motif and to 10–20-bp indels probably in the regions flanking the SSR motif.

A low discriminating power (Table 2) was revealed for *prGB1*, *pgGB11* and *pgGB16* which, in addition, displayed a low allelic diversity and rare alleles potentially interesting for gene flow measurements. *D* was higher for the four other loci (Table 2) which appeared, therefore, to be appropriate for parentage analysis. These loci were assigned to linkage group on a saturated genetic map (Table 2).

On the analysed tree sample, the linkage disequilibrium test between each pair of loci in each geographical area did not reveal significant non–random association ($P > 0.05$). Furthermore, a weak intergeographical area differentiation was revealed ($F_{ST} = 0.012$, $P < 0.01$; Table 2) reflecting long-distance gene flow by pollen in this species. Deviation from Hardy–Weinberg equilibrium and F_{IS} were not significant when assessed in each geographical area and did not reveal heterozygote deficiency, suggesting an absence or a low frequency of null-allele.

The seven loci were successfully used in the other *Picea* species (Table 2). A few new alleles were revealed (*pgGB7*-161 and *pgGB11*-78 in *P. omorika*, *pgGB7*-158 and *prGB1*-95 in *P. engelmannii*). This important information may be useful for further selection of markers amplifying across the genus *Picea*. The increasing availability of sequences in databanks will certainly stimulate the development of new codominant markers in genes which will be the tools of choice for future investigations.

Acknowledgements

We thank V. Jorge for her help in the research of DNA accessions.

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