Molecular Ecology Notes (2001) **1**, 297–299

PRIMER NOTE

Microsatellite primers for *Sorbus torminalis* and related species

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Abstract

This study reports the cloning and characterization of nine microsatellite primer pairs in a scattered woody species (*Sorbus torminalis*), and shows their potential for further use in 36 species of the *Maloideae*, a *Rosaceae* subfamily containing important fruit crop and ornamental species. These primers were designed from a microsatellite library constructed from genomic DNA of *S. torminalis* and enriched for CA and GA repeats. Genotyping 48 *S. torminalis* of a natural population with the six best markers yielded a mean of 10.7 alleles per locus, and an expectation of exclusion probability for paternity analysis greater than 0.993.

Keywords: enrichment, forest tree, *Maloideae*, *Rosaceae*, *Sorbus torminalis*

Received 14 May 2001; revision received 12 June 2001; accepted 28 June 2001

The wild service tree (*Sorbus torminalis* L. Crantz) is a fruit tree, which is very appreciated in the wood industry (furniture veneering), and is characterized by a scattered distribution (usual densities range from 0.5 to 30 individuals/hectare). In order to understand the population biology of this valuable species, different genetic studies were performed throughout its natural range using allozymic and chlorosplast DNA markers (Demesure *et al*. 2000; Oddou *et al*. 2001). However, appropriate and codominant multiallelic DNA markers, such as microsatellites are not yet available for estimating current levels of gene flow within a set of populations, a relevant issue in this intensively logged species with low population size. This study reports the characterization of nine microsatellite primer pairs in *S. torminalis*, and shows their potential for further use in various species of the *Maloideae*, a subfamily of *Rosaceae*, containing important forest and fruit crop tree species, as well as many ornamentals.

DNA was extracted from dormant buds following a robust protocol described in Oddou *et al*. (2001). For *S. torminalis*, a microsatellite library enriched for CA and GA repeats was constructed from genomic DNA as described by Edwards *et al*. (1996) and modified by Butcher *et al*. (2000). After two rounds of enrichment, polymerase chain reaction (PCR) products were cloned using Topo TA cloning kit (Invitrogen The Netherlands) according to the manufacturer's instructions. One hundred clones were sequenced, giving a total of 28 different clones containing a microsatellite repeat.

Primers were designed for nine simple sequence repeats (SSRs) (Table 1) using the computer program PRIMER (version 5.0, Whitehead Institute for Biomedical Research). PCR was carried out in a MJ Research PTC100 Thermal Cycler, using 0.75 units of *Taq* Polymerase (Appligene), and approximately 5 ng of genomic DNA in a total volume of 12.5 μL containing 200 μM of each nucleotide, 1.25 μL of *Taq* Polymerase 10× (Appligene), 1.5 mM of $MgCl_2$, 0.2 μM of each primer and 0.125 mg of BSA. Either the forward or reverse primer of each pair was fluoresced-labelled with TEXAS-RED (noted TR), HEX or 6-FAM (Table 1). After a preliminary denaturation at 94 °C for 4 min, PCR was performed for 35 cycles under the following conditions: 45 s at 94 °C, 45 s at the annealing temperature (Table 1), and 1 min at 70 °C, with a final extension step of 5 min at 70 °C. Then, 5 μL of PCR product were diluted in 5 μL of sterile H_2O and 10 μL of loading buffer (95% formamide, 10 mM NaOH, 0.05% bromophenol blue and 0.05% xylene cyanol). At this step, two different PCR products (labelled with different dyes) were multiplexed. These dilutions were heated for 5 min at 94 °C and quickly cooled on ice. Finally, 3.5 μL of denatured SSR fragments were loaded onto 28 cm denaturating gels containing 8% acrylamide:bisacrylamide (19:1), 6 M urea and 1× TBE (89 mM TRIS, 89 mM boric acid,

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Table 1 Characteristics of nine *Sorbus torminalis* microsatellite loci, with repeat motif and PCR product size (bp) of the sequence allele, and optimal annealing temperature (T_a). For the sample of 48 adult trees, we give the number of alleles observed (k) with their range size, the observed and expected heterozygosity (H_O and H_E) and the EMBL accession number (No)

Locus	Primer sequence	Fluorescent dye	Cloned repeat	Size (bp)	Size range	T_a (°C)	k	H_O	H_E	No
MSS1	ATGTTCGGTAGTCATCCCCT	—	GA (15)	162	150–174	59	10	0.85	0.81	AJ313035
	GCTCAGATAGCCACTCCCC	TR								
MSS3	TGTAATTTTCCGTTATTTCGCT	—	CA (12)	176	—	51	1	—	—	AJ313036
	TCGCAAGTAACTATGAGTGGG	TR								
MSS4	AAGTGGTATTTGAGGGTGGG	—	GT = CA (15)	282	282–300	59	3	0.56	0.67	AJ313037
	GTATGTAATGTGCCTTCGTGC	TR								
MSS5	CCCCAACAACATTTTTCTCC	—	GA (19)	138	130–146	60	7	0.75	0.72	AJ313038
	CCTCTCGCTCTTTGCCTCT	HEX								
MSS6	CGAAACTCAAAAACGAAATCAA	—	GT = CA (14)	258	252–332	56	16	0.94	0.88	AJ313039
	ACGGGAGAGAAACTCAAGACC	6-FAM								
MSS9	AAGTTTTCAAGCCATTTTCATT	—	CT = GA (19)	216	216–256	57	13	0.88	0.89	AJ313040
	CTTCACCATTTTTGTTGTGTGT	HEX								
MSS10	TCTCATCTTGGTAACCCCTCT	—	CT = GA (28)	243	250–280	57	5	0.52	0.65	AJ313041
	CCGACTATTCCCCTTACTTCT	HEX								
MSS13	TATGCGTCTTTCCATTCCG	TR	CA (12)	250	252–266	56	6	0.73	0.68	AJ313042
	GCGTTTGACTCACTCAGATTTG	—								
MSS16	CTCCCCTTGTGTGATGCC	—	CT = GA (28)	186	154–210	57	18	0.94	0.92	AJ313043
	TTGCCCTCAAAGAATGCC	TR								

2 mM EDTA). Electrophoresis was performed on Jumbo Gel system (CBS) using 1× TBE running buffer, with run parameters of 70 W per plate. Banding patterns were detected with an FM-BioII scanner (Hitashi Software).

To test these nine microsatellite loci for polymorphism, 48 wild service trees from Rambouillet forest (Yvelines, France) were genotyped (Table 1). Three loci were eliminated from further analyses in *S. torminalis*: MSS10 showed multibanding pattern, the MSS3 PCR product was non-specific (monomorphic banding pattern of shorter size than expected), and MSS4 exhibited only three alleles. The six loci left yielded 70 alleles with a mean of 10.7 alleles per locus, and a multilocus expectation of exclusion probability (calculated as described in Gerber *et al*. 2000) greater than 0.993 (for a single parent). Together, these loci provide theoretically enough variation to resolve paternity analyses in *S. torminalis* natural populations.

Twenty-three species from the genus *Sorbus* and 13 *Maloideae* species (1–4 individuals of each species) were tested for cross-species and cross-genera amplification with the nine *S. torminalis* microsatellite markers (Table 2). Note that PCR was performed at optimal annealing temperature determined for *S. torminalis*. A high rate of multibanding pattern was observed (26% of the species), notably with MSS3, MSS10 and MSS4. Letting alone those three markers, 63% of the six best *S. torminalis* primers are directly transferable (i.e. mono-locus amplification, with or without polymorphism) within *Sorbus*, 44% within *Malus*, 61% within *Pyrus*, and 16% to *Mespilus germanicus*. It is not possible to predict marker transferability into a given species on the basis of its phylogenetic relations with *S. torminalis* alone. For instance, MSS13 was more conserved within *Sorbus*, but MSS6 was more transferable to *Pyrus* than to *Sorbus*.

Acknowledgements

This project was supported by the Office National des Forêts. We thank the arboreta of Les Barres, Melles and Chevreloup for providing us with *Sorbus*, *Pyrus*, *Malus* and *Mespilus* samples.

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Table 2 Microsatellites screening in 36 species of Rosaceae (classified according to *McAllister 1998; or †Phipps *et al*. 1990). PCR was performed for *n* individuals in each species, leading either to locus-specific amplification (+), with possibly polymorphism (+ +) or to nonspecific amplification (m) or to weak/absence of amplification (–). With MSS3, when we could easily distinguish between a nonspecific monomorphic locus, next to the expected polymorphic locus, we characterized the polymorphism of the expected locus (noted after m)

Genus	Section	Subsection	Species	*n*	mss1	mss3	mss4	mss5	mss6	mss9	mss10	mss13	mss16
*Sorbus**	*S. torminalis*		*S. torminalis*	48	+ +	m + +	+ +	+ +	+ +	+ +	m	+ +	+ +
	S. aria		*S. aria*	4	+ +	m + +	m	+ +	+ +	–	m	+ +	+
			S. graeca	3	+ +	m + +	m	+ +	+	–	m	+ +	+ +
	hybrids		*S. latifolia*	3	+	m + +	m	–	+ +	–	m	m	+ +
			S. hybrida	3	+	m + +	m	m	+ +	–	m	+ +	m
			S. mougeotti	3	+ +	m	m	+ +	+ +	–	m	+ +	m
	S. domestica		*S. domestica*	3	–	m +	–	+	+	–	–	+	+ +
	S. aucuparia	*aucuparia*	*S. aucuparia*	3	+ +	m + +	m	–	+	–	m	+ +	+ +
			S. pohuanensis	3	+ +	m + +	m	+ +	+	–	m	+ +	+
		commixta	*S. americana*	3	+ +	m + +	m	+ +	–	–	m	m	+ +
			S. decora	3	+ +	m + +	m	+	+ +	–	m	m	+ +
			S. scopulina	1	+	m +	–	+	–	–	m	+	+
		other	*S. sambucifolia*	2	+ +	m + +	m	+ +	+ +	–	m	+ +	+
		vilmorini	*S. vilmorini*	2	+	m +	m	+ +	+	–	–	m	+
		koehneana	*S. cashmiriana*	1	+	m +	m	–	+	–	m	+	m
			S. prattii	1	–	–	–	m	–	–	–	+	–
		hupehensis	*S. hupehensis*	3	+	m +	m	+ +	+ +	–	m	+	+
			S. forestii	3	+	m + +	+	–	–	–	+ +	m	+
		hybrids	*S. meinichii*	3	+ +	m + +	m	+	–	–	m	m	+ +
			S. x arnoldiana	1	+	m +	m	–	+ +	–	m	+	+
	Micromeles		*S. alnifolia*	3	+	m + +	m	+ +	–	–	+ +	+ +	+ +
			S. megalocarpa	3	+	m +	m	+ +	+ +	–	+	+ +	–
			S. aronioides	1	+	m +	+	+	–	–	m	+	+
	S. chamaemespilus	*S. chamaemespilus*	*S. chamaemespilus*	1	–	m + +	–	–	+	–	–	–	m
Malus†	*Malus*	*Malus*	*M. eleyi*	3	–	m	+ +	+ +	+	–	+	+	+ +
		Baccatae	*M. baccata*	1	–	m	–	+	+	–	–	–	–
			M. hupehensis	3	–	m	+ +	+ +	+	–	+ +	+	+ +
			M. rockii	3	–	m	+	m	+	–	+	–	m
		Florentinae	*M. florentina*	2	–	m	+	+ +	+	–	+	–	–
		Kansuenses	*M. fusca*	3	–	m	+ +	+ +	+ +	–	+ +	–	+ +
Pyrus†	*Pashia*	*Pashia*	*P. betulaefolia*	2	+	m	+	+ +	+	–	–	–	+ +
			P. calleryana	4	+	m	+ +	+ +	+	–	m	+ +	+ +
	Pontica	*Pontica*	*P. amygdaliformis*	3	+	m	+	+	+	–	–	–	+
			P. salicifolia	3	+	m	+	+ +	+ +	–	+ +	–	+
	Pyrus		*P. balansae*	1	+	m	+	m	–	–	+	–	+ +
			P. pyraster	3	+ +	m	+ +	m	+	–	m	–	+ +
			Mespilus germanica	1	–	m	–	–	+	–	–	–	–