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TECHNICAL NOTE

Isolation and characterization of microsatellite loci in *Sorbus aria* (Rosaceae)

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Abstract *Sorbus aria (L)* Crantz (Common Whitebeam) is native to Europe, east of the Balkans and in North Africa; it is also present in the Canary Islands. To evaluate the genetic diversity in natural populations of this vulnerable species, nine novel polymorphic microsatellite markers were isolated from enriched libraries. Microsatellite loci were screened in 97 individuals from La Palma (Canary Islands) and Sierra Nevada (Granada, Spain). Examination of the microsatellite profiles shows that *S. aria* individuals have up to three alleles per locus. The cloned sequences in microsatellite loci confirmed the polyploidy status of the plants. The number of alleles ranged from 5 to 14 per locus. The phenotype diversities across loci (H'_T) ranging from 0.653 to 0.847.

Keywords *Sorbus aria* · Microsatellite · Canary Islands · Genetic diversity · Conservation

The genus *Sorbus* L. (Rosaceae) includes small to medium sized trees from the North Temperate Zone. They are closely related to the commercial genus *Malus* and *Pyrus* (Robertson et al. 1991; Campbell et al. 1995).

Sorbus aria (L) Crantz (Common Whitebeam) is native to Europe, east of the Balkans and in North Africa. It is distributed in mountain zones throughout almost all of Europe and part of Asia, from the Iberian Peninsula and Ireland to the Himalayas. It is also present in the Canary Islands. The trees are isolated and alone despite their widespread distribution; for this reason UICN has listed it as a "vulnerable species" (Cabezudo et al. 2000; Chester et al. 2007).

The European samples of *Sorbus aria* have been reported as diploids although the subgenus *Aria* (or *S. aria* aggregate) contains apomictic triploid and tetraploid species (Nelson-Jones et al. 2002). However, little is known about the genetic populations in the Canary Islands which have only been found on La Palma and Tenerife.

In this paper, we describe the isolation and characterization of 9 microsatellite markers in *Sorbus aria* and we indicate their effectiveness in identifying patterns of genetic diversity.

Genomic DNA for the development of markers and subsequent surveys were extracted from leaf tissue of 97 samples from La Palma (45) and Sierra Nevada (52) populations using a modified CTAB protocol (Doyle and Doyle 1987).

Microsatellite loci were developed by ATG Genetic Inc. using biotin/streptavidin protocol (Khasa et al. 2000). Briefly, genomic DNA was digested with restriction endonucleases (Hae III or Rsa I with PshA I). A synthetic adaptor M28/M29 was added to the ends of the genomic DNA's by T4 DNA ligase. Two rounds of hybridization with 5′biotin-labeled oligonucleotide (TGn and GAn) and capture by streptavidin-coated magnetic beads (Dynabeads, Dynal GmbH) were carried out. The enriched genomic products were amplified using adaptor primer M28 and were cloned into plasmid vectors (pGEM3Z+, Promega). Positive microsatellite clones were identified by dot blot hybridization with appropriate mixes of biotin labelled SSR oligonucleotides.

Sequences were obtained by amplifying an aliquot of frozen bacterial culture from positive hybridizing colonies

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using M13 universal forward and reverse primers, treated with Exonuclease I and Shrimp alkaline phosphatase, and then sequenced from both orientations using ABI3730 capillary electrophoresis (NAPS Service, University of British Columbia). For 18 microsatellite loci isolated from these libraries, PCR primers complementary to the flanking regions of loci were designed with c. 40% GC and avoiding palindromic sequence motifs. Ten microsatellites were scored as "useful" based on good amplification of polymorphic sized bands from single copy genomic target but only nine have given PCR polymorphic results and were considered robust and predictable enough for further analyses (Table 1).

Each 25 µl PCR reaction contained approximately 20 ng of DNA, 10 pmol of each primer, as well as PCR Master Mix (Reddy-Mix, ABgene, Surrey, UK) that included 0.625 units of Taq DNA polymerase, 75 mM Tris–HCl, 20 mM $(NH4)_2SO_4$, 0.01% Tween20, 1.5 mM de $MgCl_2$, and 0.2 mM of each dNTP. Forward primers were colour-labeled at the 5′-end with 6-FAM, PET, NED or VIC.

In general, amplifications were carried out using the following thermal cycling conditions: 3 min denaturation at 95°C, 35 cycles of 30 s denaturation at 95°C, 30 s at annealing temperature, and 1.5 min elongation at 72°C; followed by 5 min elongation at 72°C. The products were detected using an ABI 3100 GENETIC ANALYZER and fragment sizes were determined using GENESCAN v. 2.02 and GENOTYPER v. 1.1 (Applied Biosystems, Inc.).

Examination of the microsatellite profiles shows that *S. aria* individuals have up to three alleles per locus. The cloned sequences in microsatellite loci confirmed that the polyploidy status of the plants. We are not able to determinate the exact number of copies of each allele because we do not know allele dosage in those individuals with partial heterozygoty. We identified allele peak profiles at each locus and assigned a phenotype to each individual.

Analysis of *S. aria* microsatellites used the phenotype-based statistics in the FDASH program (Obbard et al. 2006) that can measures diversity in terms of the total number of allelic phenotypes in the population. Because allopolyploids are derived from interspecific hybridization and therefore comprise at least two different genomes, this program assumes the sharing of alleles between isoloci must be owing to common ancestry. So, using a single allelic-phenotype diversity statistic (H') that measures diversity as the average number of alleles by which pairs of individuals differ plus a population differentiation measure (F'_{ST}) which is analogous to $F_{ST;}$ we can capture essential information regarding genetic diversity in polyploids.

The nine *S. aria* microsatellite loci are highly variable with a mean of 9.255 alleles/locus and phenotype diversities across loci (H'_T) ranging from 0.653 to 0.847 with a mean value of 0.742; F_{st} mean value was 0.428 (Table 2).

Table 1 Primer sequences and characteristics of nine microsatellite loci from natural populations of *Sorbus aria*

Locus	Repeat motif	PCR primer sequence (5′→3′)	T_a (°C)	Size of cloned allele (size–range) in bp	Fluorescent label	GeneBank accession no
SA01[a]	$(GA)_{13}$	F: ATGGAGTTGAGCTCCACATC R: GGTGGAGGGACAATTGTGTC	60	229 (212–254)	6-FAM (blue)	FN563114
SA02[b]	$(GA)_{16}$	F: CTAGGTATCATCTCCGACCA R: ACGTAGCACTGAATGGTATAG	60	293 (270–325)	NED (yellow)	FN563115
SA03[a]	$(GA)_{12}$	F: CACTTCTTCCTGCTGTTTGG R: ACTACTGCTACTTCTGTGGG	60	234 (206–249)	VIC (green)	FN563116
SA06[a]	$(GA)_{32}$	F: ATTTGATCCATGTGCGACTGCA R: TGCAGCGGTTGCAGATTGCA	60	297 (248–297)	PET (red)	FN563117
SA07[a]	$(GA)_{15}$	F: ACGTTTTCAGTATGATGGCC R: CTTCGCAGTTCATTAAGCAC	60	334 (325–349)	6-FAM (blue)	FN563118
SA08[b]	$(CT)_{16}$	F: CAGAGAGAGTGCACTGCCT R: GAATTCTTGGCAGTTTGCCT	60	249 (233–287)	6-FAM (blue)	FN563119
SA09[a,c]	$(AG)_{17}$	F: CTTGTTGGACGGATTTCTTC R: CCAATACTTGAGTAGCATAC	60	174 (161–197)	NED (yellow)	FN563120
SA14[a]	$(TC)_{30}$	F: ATGGATTTAGGTTAACAGTTGTC R: GAGGTAAAACCTACCAGTATAC	57	203 (197–232)	PET (red)	FN563121
SA19.1[a]	$(GA)_{24}$	F: AAGTTTACAAGAGTGTGTTCAG′ R: GAATTCATGAAAGCAGCTAATG	60	241 (212–250)	VIC (green)	FN563122

T_a = Annealing temperature

[a] PCR Master Mix (AB gene) $MgCl_2$ 1.5 mM, [b] PCR Master Mix (AB gene) $MgCl_2$ 2.5 mM. [c] Final elongation: 30 min, 72°C

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Table 2 Patterns of variability at individual microsatellite and genetic diversity analysis across all populations of *Sorbus aria*

Locus	*A*	*AvA*	*P*	*AvP*	H'_S	H'_T	F'_{ST}
SA01	5.000	3.033	5.000	2.462	0.295	0.653	0.547
SA02	9.000	5.680	7.000	3.680	0.342	0.681	0.498
SA03	10.000	6.602	7.000	3.269	0.449	0.734	0.388
SA06	9.000	4.897	8.000	3.897	0.473	0.763	0.380
SA07	8.000	5.875	11.000	5.688	0.627	0.847	0.260
SA08	10.000	5.062	12.000	5.639	0.468	0.804	0.418
SA09	10.000	5.680	6.000	3.144	0.358	0.690	0.480
SA14	14.000	7.588	12.000	5.887	0.495	0.808	0.387
SA19,1	8.000	4.211	7.000	2.758	0.350	0.693	0.496
Average across loci	9.255	5.422	8.367	4.065	0.429	0.742	0.428

A total number of alleles, *AvA* average number of different allele per sample, *P* total number of phenotypes seen, *AvP* average number phenotypes per sample, H'_S phenotype diversity within sample, H'_T phenotype diversity across all samples, $F'_{ST} = (H'_T - H'_S)/H'_T$ measure of genetic differentiation among populations

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