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TECHNICAL NOTE

Development of microsatellite loci for *Syzygium paniculatum* (Myrtaceae), a rare polyembryonic rainforest tree

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Abstract Fourteen novel microsatellite loci were developed for the rare polyembryonic and potentially polyploid rainforest tree *Syzygium paniculatum* Gaertn. Markers were characterized using 40 individuals from populations along the NSW coast. The mean number of alleles amplified per locus was three (range 1–7, $H_E = 0.54$ and $H' = 0.85$). Low allelic variation may be explained by the polyembryonic nature of *S. paniculatum* seeds. These markers provide the opportunity to use nuclear microsatellite markers to study the population genetic structure and breeding system in *S. paniculatum* and related species.

Keywords *Syzygium* · Microsatellite loci · Polyembryony · Apomixis · Polyploidy · Clonal

Syzygium paniculatum Gaertn. is a rare rainforest tree of the Myrtaceae family, endemic to a narrow coastal strip of New South Wales, Australia. *S. paniculatum* produces polyembryonic seeds, however, little else is known about its reproductive biology. Seeds of *S. paniculatum* are likely to be apomictic, originating clonally from maternal tissues, as this is evident in other polyembryonic species of *Syzygium* (Nic Lughadha and Proença 1996; Sahai and Roy 1962; Roy 1961 as *Eugenia*). If so, the population genetic structure of the species is likely to be affected.

We sought to develop molecular microsatellite markers that could be used to understand the reproductive and population biology within *S. paniculatum*. Genomic DNA was extracted and purified from leaf material from one individual from The Entrance Peninsula (New South Wales, S 33° 18′ 29.4″, E 151° 31′ 18.3″) using QIAGEN DNeasy Plant mini kit. A microsatellite AG-enriched library was constructed using the method of Glenn and Schable (2005). Polymerase chain reactions (PCRs) were performed on 96 clones and sequencing using M13 universal primers revealed 65 containing microsatellites. BioEdit v5.0.6 (Hall 1999) was used to edit sequences. Primer 3 (Rozen and Skalesky 2000) and NetPrimer (PREMIER Biosoft International) were principally used for primer design. Fourteen primer pairs were designed (Table 1) and to facilitate fluorescent labelling of PCR products an M13 tag (5′-TGTAAAACGACGGCCAGT-3′) was attached to the 5′ end of all forward primers (Schuelke 2000). These were tested for amplification and polymorphism in *S. paniculatum*, six other *Syzygium* species as well as three related taxa (Table 2).

Microsatellite loci were amplified simultaneously using the Qiagen multiplex PCR Kit in 10 μL volumes each containing 5 μL 2× QIAGEN Multiplex PCR Master Mix, 0.05 μM each forward primer, 0.2 μM each reverse primer, 0.3–0.4 μM of M13 forward primer (with fluorescent dye attached) and approximately 5 ng of DNA. Reaction conditions: one cycle of 95°C for 15 min; 30 cycles of 94°C for 30 s, 60°C for 90 s, 72°C for 60 s; eight cycles of 94°C for 30 s, 53°C for 90 s, 72°C for

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Table 1 Fourteen microsatellite loci characterized for *Syzygium paniculatum* across 40 individuals representing 11 populations

Locus	GenBank	Primer sequence[a] (5′–3′)	Motif	Size (bp)	N	N_A	N_A^{IND}	H_E	H'
SP33BGT	HQ213973	F: GTGGCAGAGAGGGAGAGGAA R: CGACACAGCAAGATTTGGTC	(TC)4 TT (TC)12	142	40	4	1–4	0.680	1.270
SP38BGT	HQ213974	F: GTCTCCTTCATGCTCCCTAT R: AGTGCCTGTGCTTCACCCTC	(AG)8	300	40	4	2–3	0.628	1.017
SP42.1BGT[c,d]	HQ213975	F: CGAAGCAGAGAGGCAGCT R: AACCCAACTCGCAATACT	(AG)9	214	30	3	3	–	–
SP52.1BGT	HQ213976	F: ATCGCTGTCTGGCTAACGAA R: TCTCTTTCTGGCTGGTGTGA	(GAA)10	244	40	3	2	0.482	0.675
SP54BGT	HQ213977	F: TTCCCTTACTGGCTTGGTTG R: TGGCAGATTTATGGACAGCA	(CT)11	165	40	6	3	0.658	1.085
SP56BGT	HQ213978	F: CTGTGAATAACCGTGCCAT R: GACGAATATCGGGTCTTGAA	(AG)11	141	40	2	2	0.498	0.720
SP75BGT	HQ213979	F: GTCTTCTCAGAGTAGATATGGA R: GAGGCACGAGAGGAGTTTC	(AG)3 GGG (AG)17	166	30	4	4	–	–
SP83BGT	HQ213980	F: GCCAGGCTACTATCGACTT R: CGGTTCTTCAGCGACTAC	(CT)9	249	40	4	2–3	0.511	0.743
SP85.1BGT	HQ213981	F: AGGAGTGGATTCGGAGT R: ACAGTGGAGCAAAGAAGCAC	(AG)6 A (AG)6	238	49	5	1–2	0.410	0.590
SP94BGT[b,c]	HQ213982	F: GTCCTTGACCTCTTTATCCTTC R: CAAATCTAACATCCGCTCTGC	(CT)2 TT (CT)16	195	29	1	1	–	–
SP100BGT	HQ213983	F: CCCTTTCTTCAACTTCGCCTTA R: GGAATCTTGTTTTGCCTCGCCTGTG	(GA)7	196	30	2	2	0.490	0.702
SP109BGT[b,c]	HQ213984	F: CTTGTTTATCGCTTCCTTGC R: CGAGTGTTGATCTGTGTGC	(GA)12	122	39	1	1	–	–
SP113BGT[c,d]	HQ213985	F: CAGACGGGGATGAGGACCT R: TTCTCTCATCAATCCTCATCG	(TA)5 (N)26 (AG)10	267	34	7	2–4	–	–
SP116BGT[b]	HQ213986	F: TGTCATCCTTGCGAGAAATC R: CACTCCTTGCCTCTCCATTA	(CT)3 CC (CT)6	291	40	2	1	–	–

N Number of individuals amplified, N_A number of alleles, N_A^{IND} range of alleles per individual, H_E expected heterozygosity, H' Shannon–Weiner diversity index

[a] M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) was appended to the 5′ end of all F primers

[b] No Heterozygosity in populations tested

[c] Did not amplify well across all populations tested, allele count uncertain

[d] Alleles uncertain due to weak amplification

60 s; one cycle of 60°C for 30 min and 25°C for 1 min. Genotyping was performed on an ABI 3730 Capillary Sequencer (Applied Biosystems). GeneMapper version 3.7 (Applied Biosystems) was used to investigate and score alleles for each locus.

After optimisation for amplification and genotyping, markers were characterized (Table 1) using 40 individuals in total, 20 from The Entrance Peninsula and four each from five other *S. paniculatum* populations representing the entire range of *S. paniculatum* along the NSW coast. The number of alleles amplified per locus in *S. paniculatum* ranged from one to seven, with a mean of three. All 14 loci were genotyped in all 40 individuals; however, six loci either did not amplify across all populations or produced no heterozygosity in populations tested. The remaining loci showed heterozygosity and most showed variation across populations of *S. paniculatum* (Table 1).

One to four alleles were observed per locus in each individual indicating polyploidy which precludes the calculation of a range of standard genetic statistics, including deviations from Hardy–Weinberg and linkage equilibria. ATETRA (Van Puyvelde et al. 2010) was used to estimate expected heterozygosity (H_E), which ranged from 0.41 to 0.68 (mean $H_E = 0.54$), and Shannon Weiner diversity index (H') which ranged from 0.59 to 1.27 (mean $H' = 0.85$). Low allelic variation observed may be explained by the polyembryonic and potentially clonal nature of *S. paniculatum* seeds. Most loci were successfully amplified across the other species and

Table 2 Cross species amplification of microsatellite loci developed for *S. paniculatum*

Species/locus	SP33BGT	SP38BGT	SP43.1BGT	SP52.1BGT	SP54BGT	SP56BGT	SP75BGT	SP83BGT	SP85.1BGT	SP94BGT	SP100BGT	SP109BGT	SP113BGT	SP116BGT
Syzygium paniculatum	b	b	a	b	b	b	b	b	b	a	b	b	a	b
Syzygium corynanthum	c	c	c	c	–	c	c	c	c	–	–	c	–	–
Syzygium francisii	c	a	–	–	–	c	a	–	c	c	–	–	–	–
Syzygium jambos	c	c	c	c	–	–	a	–	c	–	–	c	c	–
Syzygium luehmannii	c	–	a	–	–	c	a	–	c	c	–	–	–	–
Syzygium moorei	c	c	c	–	–	–	a	–	–	–	–	c	–	–
Acmena ingens	c	–	c	–	–	–	c	–	c	c	–	c	–	–
Anetholea anisata	c	c	c	c	c	–	a	–	c	–	–	–	c	–
Waterhousea floribunda	a	–	a	–	–	–	–	–	c	a	–	–	–	–

a Successful amplification, *b* amplification and observed variation in *S. paniculatum*, *c* amplification and observed variation among species, – unsuccessful amplification

were found to be polymorphic when compared to *S. paniculatum* (Table 2).

We aim to use these markers to conduct a fine scale study of the spatial distribution of genotypes across the geographic range of *S. paniculatum*. The markers can also be used to investigate the reproductive biology of the species to determine whether polyembryonic seeds of *S. paniculatum* are formed asexually by apomixis and whether apomixis is likely to occur in all individuals. This will be helpful in developing a long term conservation plan for this rare species. These loci can also be used to investigate the population and reproductive biology of other *Syzygium* species, some of which are of economic significance in Asia, as well as for closely related Myrtaceous species.

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