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DEVELOPMENT OF MICROSATTELITE LOCI FOR THE RIPARIAN TREE SPECIES

**Melaleuca argentea** (Myrtaceae) USING 454 SEQUENCING

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**Key words:** 454 GS-FLX; Melaleuca argentea; northern Australia; nuclear SSR markers; riparian tree; shotgun sequencing.

**Premise of the study:** Microsatellite primers were developed for *Melaleuca argentea* (Myrtaceae) to evaluate genetic diversity and population genetic structure of this broadly distributed northern Australian riparian tree species.

**Methods and Results:** 454 GS-FLX shotgun sequencing was used to obtain 5860 sequences containing putative microsatellite motifs. Two multiplex PCR reactions were optimized to genotype 11 polymorphic microsatellite loci. These were screened for variation in individuals from two populations in the Pilbara region, northwestern Western Australia. Overall, observed heterozygosities ranged from 0.27 to 0.86 (mean = 0.52) and the number of alleles per locus ranged from two to 13 (average = 4.3).

**Conclusions:** These microsatellite loci will be useful in future studies of the evolutionary history and population and spatial genetic structure in *M. argentea*, and inform the development of seed sourcing strategies for the species.

**METHODS AND RESULTS**

Genomic DNA (5 μg) was isolated from the leaf tissue of one individual of *M. argentea* following the protocol of Glauzi et al. (2001), modified with the addition of a wash buffer (Wagner et al., 1987). The DNA was then sent to the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia) for shotgun sequencing on a Roche 454 GS-FLX sequencer with titanium chemistry (Roche Applied Science, Indianapolis, Indiana, USA) following Gardner et al. (2011). The sample occupied 12.5% of a plate and produced 274,938 individual sequences, with an average read length of 362 bp, of which 5860 contained microsatellites. We used the program QDD version 1 (Meglacz et al., 2010) to screen the raw sequences for those that contained eight or more di-, tri-, tetra-, or pentabase repeats, remove redundant sequences, and design primers (automated in QDD using Primer3 [Rozen and Skaletsky, 2000]). Software running parameters were set to default values with the exception of PCR product lengths, which was set to 90–450 bp. Primer pairs were designed for 548 different loci and from these we excluded immediately all loci that contained imperfect repeats, had a greater than 2°C difference between the forward and reverse primer annealing temperatures, a GC content less than 40% or greater than 60%, polynucleotide runs of four or more runs in the flanking regions, and short repeat motifs within the flanking region or primer sequence. From the remaining 355 loci, we randomly chose for further development 32 loci containing either dinucleotide or trinucleotide repeats (GenBank accession no.: JX424003–JX424034). These 32 loci were trialed for amplification with the cost-effective approach of Schuelke (2000) using a QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany). Loci were amplified in individual, 20-μL reactions containing 10 μL QIAGEN Multiplex PCR Master Mix, 2.5 μL Q-solution; forward primer (with sequence tag at 5′ end, unlabeled) 0.05 μM, reverse primer (unlabeled) 0.2 μM, and 5′ sequence tag (labeled; unique to primer) 0.2 μM; 10–50 ng DNA; plus sterile H2O to 20 μL. PCR cycling was performed in a Corbett Gradient Palm-Cycler (Corbett Life Science, Sydney, Australia) according to the manufacturer’s protocol as follows: *Taq* activation at 95°C for 15 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 90 s, and extension at 72°C for 90 s; followed by a final extension at 60°C for 30 min. PCR products were visualized...
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TABLE 1. Characteristics of 11 nuclear microsatellite primers developed in Melaleuca argentea.a,b

| Locus   | Primer sequences (5′–3′) | Repeat motif | Size range (bp)c | Label (Multiplex)c | GenBank accession no. |
|---------|-------------------------|--------------|------------------|-------------------|----------------------|
| KPMA07  | F: CAGCGACTGATCTCTTTGA  | (AG)11       | 119–127          | FAM (1)           | JX424909             |
|         | R: AAATAGTAGCGGCCGAGGC  | (GGG)8       | 126–144          |                   |                      |
| KPMA11  | F: ACCATGCTAACCAACCAAC  | (GCA)6       | 202–208          | VIC (1)           | JX424106             |
|         | R: AGCCAGGCGGCAACAGAT   | (AGA)5       | 132–140          |                   |                      |
| KPMA16  | F: CTTGACCGCTTAGGTTGTTT   | (CT)13       | 238–258          | NED (2)           | JX424027             |
|         | R: CCTGGTGGGTGCTTGCCCTAAT | (GTG)8     | 208–216          |                   |                      |
| KPMA20  | F: TTAGGAGGTCGTTTGCTGACCA  | (GCA)6   | 221–230          | PET (1)           | JX424022             |
|         | R: TAGCTAATTGGCGTCCCCAC  | (GGG)6       | 126–138          |                   |                      |
| KPMA23  | F: ATGTAAGGCGCCGCAACAGC  | (GCG)6       | 142–188          | NED (2)           | JX424027             |
|         | R: CCCCTTCCTGCACTCTTCTTCT | (CTT)11    | 238–258          |                   |                      |
| KPMA25  | F: GTCCATTGCTTGGCTCCTCAT | (CTT)9      | 123–140          |                   |                      |
|         | R: CCGTCTCCCTAAGGTTCTCCC | (AG)10     | 208–216          |                   |                      |
| KPMA26  | F: GATCTGAGCGGCAAGATGAG  | (CTT)9      | 123–140          |                   |                      |
|         | R: TAGCTGCTTCAGAAAGTGCCA | (GGA)9     | 142–188          |                   |                      |
| KPMA28  | F: ACCAAACATTTTCCCCTCTAG | (GCA)6     | 152–172          | PET (2)           | JX424030             |
|         | R: TATAGGCGCTCTTCTCAAGGC | (GGA)9     |                   |                   |                      |

Note

a All values are based on samples from two populations in the Pilbara region of Western Australia (WW-BO; UTM coordinates 720216E 7448488N, n = 25; WW-LP: UTM coordinates 725022E 7462878N, n = 21).

b An annealing temperature of 56°C was used for all primers.

c Forward primer sequences do not include the 5′-forward tail.

Table 2. Results of primer screening in two populations of Melaleuca argentea.a

| Locus   | WW-BO       | WW-LP       |
|---------|-------------|-------------|
|         | A  | Hs  | A  | Hs  |
| KPMA07  | 5  | 0.61* | 5  | 0.23* |
| KPMA11  | 3  | 0.44  | 3  | 0.33  |
| KPMA14  | 2  | 0.58  | 4  | 0.44  |
| KPMA16  | 3  | 0.58  | 3  | 0.55  |
| KPMA17  | 3  | 0.56  | 4  | 0.38  |
| KPMA18  | 3  | 0.44  | 3  | 0.65  |
| KPMA20  | 2  | 0.56  | 5  | 0.57  |
| KPMA25  | 5  | 0.51  | 10 | 0.52  |
| KPMA26  | 6  | 0.72  | 6  | 0.86  |
| KPMA28  | 6  | 0.64  | 6  | 0.76  |

Note: A = number of alleles; Hs = expected heterozygosity; Hr = observed heterozygosity.

a All values are based on samples from two populations in the Pilbara region of Western Australia (WW-BO: UTM coordinates 720216E 7448488N, n = 25; WW-LP: UTM coordinates 725022E 7462878N, n = 21).

b Significant deviations from Hardy–Weinberg equilibrium (*P < 0.01) after correction for multiple tests (sequential Bonferroni procedure) are reported.

**CONCLUSIONS**

The microsatellite loci presented in this study will be useful in the examination of the population genetic structure of *M. argentea*, a species that may be impacted by hydrological changes associated with mining in the Pilbara region of northwestern Western Australia. Specifically, research is planned that will examine genetic connectivity between geographically proximate riparian systems, which will inform the development of seed sourcing guidelines for the species.
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