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Development of Microsatellites in *Labisia pumila* (Myrsinaceae), an economically important Malaysian herb

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- **Premise of the study:** The exploitation of *Labisia pumila* for commercial demand is gradually increasing. It is therefore important that conservation is prioritized to ensure sustainable utilization. We developed microsatellites for *L. pumila* var. *alata* and evaluated their polymorphism across var. *alata*, var. *pumila*, and var. *lanceolata*.

- **Methods and Results:** Ten polymorphic microsatellites of *L. pumila* were developed using the magnetic bead hybridization selection approach. A total of 84, 48, and 66 alleles were observed in *L. pumila* var. *alata*, *pumila*, and var. *lanceolata*, respectively. The species is likely a tetraploid, with the majority of the loci exhibiting up to four alleles per individual.

- **Conclusions:** This is the first report on the development of microsatellites in *L. pumila*. The microsatellites will provide a good basis for investigating the population genetics of the species and will serve as a useful tool for DNA profiling.

**Key words:** kacip fatimah; *Labisia pumila*; medicinal plant; microsatellites; Myrsinaceae; tetraploid.

*Labisia pumila* (Blume) Fern.-Vill. (Myrsinaceae) is a small understory shrub that is widely distributed in the tropical forests of Malaysia, Indonesia, Thailand, the Philippines, and Myanmar (Sunarno, 2005). Eight varieties are recognized (Sunarno, 2005), of which *L. pumila* var. *pumila*, *L. pumila* var. *alata* (Scheff.) Mez, and *L. pumila* var. *lanceolata* (Scheff.) Mez are commonly found in Malaysia. These varieties are morphologically distinct from one another in terms of their petiole and leaf characteristics. Among the Malay communities, these varieties are collectively known as kacip fatimah, which has long been used as the traditional medicine for the treatment of pre- and post-partum complications, menstrual disorders, dysentery, rheumatism, flatulence, and gonorrhea (Burkill, 1966; Jaganath, 2000).

To date, the exploitation of *L. pumila* for commercial demand, particularly in the pharmacological and cosmeceutical applications, is gradually increasing. Attention to conservation should therefore be prioritized to ensure sustainable utilization. Despite the importance of *L. pumila*, the availability of genetic information for the species is still very limited. Only two genetic variability studies of the species have been reported using dominant markers (Bhore et al., 2009; Farah Fazwa et al., 2013). In this study, we report the development of 10 microsatellite loci in *L. pumila* var. *alata*, and we evaluate their polymorphism across var. *alata*, var. *pumila*, and var. *lanceolata*. Microsatellites are preferred markers because of the nature of their codominant inheritance, high abundance, extent of allelic diversity, and the ease of assessing the size variation by PCR with pairs of flanking primers (Weising et al., 2005).

**METHODS AND RESULTS**

Leaf samples of 25, 20, and four individuals of *L. pumila* var. *alata*, *pumila*, and var. *lanceolata*, respectively, were obtained from Pasoh Forest Reserve (2°58′N, 102′18′E). An additional six individuals of var. *lanceolata* were obtained from the Ethnobotanical Garden of the Forest Research Institute Malaysia (FRIM). The voucher specimens of these three varieties were deposited in FRIM Herbarium (KEP; barcode numbers 223663–223665). Total genomic DNA was extracted from fresh leaves of *L. pumila* using a modified cetyltrimethylammonium bromide (CTAB) protocol (Murray and Thompson, 1980) and further purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Penzberg, Germany).

A genomic library enriched for dinucleotide CT and GT was constructed following the approach of Kijas et al. (1994). Approximately 5 μg of genomic DNA was obtained from an individual of *L. pumila* var. *alata* from the Ethnobotanical Garden of FRIM. After digestion with *Nde*II (Promega Corporation, Madison, Wisconsin, USA), the digested fragments were electrophoresed on 2% agarose gels with a 100-bp DNA ladder (New England Biolabs, Ipswich, Massachusetts, USA). Fragments of 300–1000 bp were excised and ligated into *Sac*IIA1 cassettes (TaKaRa Bio, Otsu, Shiga, Japan). After ligation, the nicks were repaired using DNA polymerase I (TaKaRa Bio). The cassette-ligated DNA was enriched for microsatellite repeats via hybridization to 5′-biotinylated (CT)15 and (GT)15 probes and retrieved using magnetic beads coated with streptavidin (Promega Corporation). The selectively recovered hybrids were reamplified using C1 cassette primers, digested with *Nde*II, cloned into pUC118 Bam HI/BAP vector (TaKaRa Bio), and transformed into QIAGEN EZ Competent Cells (QIAGEN GmbH, Hilden, Germany). Insert-containing clones were selected by blue/white screening on Luria–Bertani (LB) agar plates containing 100 μg/mL ampicillin, 50 μM isopropyl-β-D-thiogalactopyranoside (IPTG), and 80 μg/mL X-gal. Plasmid DNAs of a total of 608 clones were amplified using the Illustra TempliPhi Amplification Kit (GE Healthcare, Piscataway, New Jersey, USA).
Piscataway, New Jersey, USA) and sequenced using BigDye Terminator Sequencing Kit version 3.1 (Applied Biosystems, Foster City, California, USA) on an ABI 3130xl Genetic Analyzer (Applied Biosystems) with the following cycling conditions: 4 min at 94°C; 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s; followed by a final extension of 30 min at 72°C. Twenty-nine primer pairs that showed specific amplification products of expected fragment size were selected for fluorescent labeling at the 5’-end of the forward primers with either 6-FAM or HEX. These primers were further screened using 25 samples of L. pumila var. lanceolata, L. pumila var. alata, and var. lanceolata, respectively (Table 2). H_e ranged from 0.039 to 0.857, 0.000 to 0.793, and 0.000 to 0.874 in L. pumila var. alata, var. pumila, and var. lanceolata, respectively. Notably, locus Lpu15 was found to be monomorphic in varieties pumila and lanceolata.

**Note:** T_a = annealing temperature.
*Monomorphic microsatellites.

**TABLE 1.** Description of 10 polymorphic and three monomorphic microsatellites screened in *Labisia pumila* var. alata.

| Locus | Repeat motif | Primer sequence (5’→3’) | T_a (°C) | Allele size range (bp) | Fluorescent label | GenBank accession no. |
|-------|--------------|--------------------------|----------|------------------------|-----------------|----------------------|
| Lpu02 | (GT)_20      | F: GCAGAAGGAGGTTAGTGTG    | 50       | 197–235                | HEX             | KF318311             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu08a| (GC)_15      | F: AAGGAAATATTTATACCAACCT | 100      | 103–132                | HEX             | KF318312             |
|       |              | R: AAGGAAATATTTATACCAACCT |          |                        |                 |                      |
| Lpu08b| (TC)_21      | F: CTCTTGCTTCTTGTTGTTA    | 50       | 178–211                | HEX             | KF318312             |
|       |              | R: AAGGAAATATTTATACCAACCT |          |                        |                 |                      |
| Lpu13 | (TG)_11      | F: GCAGAAGGAGGTTAGTGTG    | 50       | 303–342                | HEX             | KF318313             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu15 | (TTTC)_3     | F: GCAGAAGGAGGTTAGTGTG    | 50       | 198–213                | 6-FAM           | KF318314             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu16a| (CAG)_31     | F: GCAGAAGGAGGTTAGTGTG    | 50       | 93–107                 | 6-FAM           | KF318315             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu16b| (GTAT)_20    | F: CTAGGAGGTTAGTGTGTTA    | 50       | 69–112                 | 6-FAM           | KF318315             |
|       |              | R: TCAGGAGGTTAGTGTGTTA    |          |                        |                 |                      |
| Lpu21a| (GA)_26      | F: GCAGAAGGAGGTTAGTGTG    | 50       | 175–207                | HEX             | KF318316             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu21b| (GA)_15      | F: GCAGAAGGAGGTTAGTGTG    | 50       | 388–415                | 6-FAM           | KF318316             |
|       |              | R: AAATTATAAGGCCCACAGAG   |          |                        |                 |                      |
| Lpu38 | (CA)_30      | F: TCCACTACTGCTCAGATGTCG  | 50       | 75–83                  | HEX             | KF318317             |
|       |              | R: GCTTTGAAGGTTGCGGGTAGT  |          |                        |                 |                      |
| Lpu06a| (CA)_20      | F: TAAAGCCACACATATCAATC   | 50       | 148                    | HEX             | KF318318             |
|       |              | R: GCTTTGAAGGTTGCGGGTAGT  |          |                        |                 |                      |
| Lpu20a| (CTC)_30     | F: CATCCGCTACCAATACGCA    | 50       | 315                    | 6-FAM           | KF318319             |
|       |              | R: GCTTTGAAGGTTGCGGGTAGT  |          |                        |                 |                      |
| Lpu24a| (GGAATT)_31  | F: CATATTGTGTGATGGATTAG   | 50       | 159                    | 6-FAM           | KF318320             |
|       |              | R: GCCAAATTCTACAAATTTATA  |          |                        |                 |                      |

were calculated for biallelic phenotypes (AAAB and AABB) (De Walt et al., 2011). Expected heterozygosity (H_e) was calculated using ATETRA 1.2 (Van Puyvelde et al., 2010), with 10,000 Monte Carlo simulations. A total of 84, 48, and 66 alleles were observed in L. pumila var. alata, var. pumila, and var. lanceolata, respectively (Table 2). H_e ranged from 0.039 to 0.857, 0.000 to 0.793, and 0.000 to 0.874 in L. pumila var. alata, var. pumila, and var. lanceolata, respectively. Notably, locus Lpu15 was found to be monomorphic in varieties pumila and lanceolata.

![Fig. 1. Electropherogram showing four alleles amplified from an individual at loci Lpu02 and Lpu21a.](http://www.bioone.org/loi/apps)

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TABLE 2. Genetic properties of 10 microsatellites of Labisia pumila across varieties alata, pumila, and lanceolata.

| Locus   | A     | $H_e$ (min) | $H_e$ (max) | $H_o$ |
|---------|-------|-------------|-------------|-------|
| Lpu02   | 11    | 0.940       | 0.953       | 0.857 |
| Lpu08a  | 9     | 0.880       | 0.880       | 0.793 |
| Lpu08b  | 11    | 0.940       | 0.940       | 0.834 |
| Lpu13   | 10    | 0.706       | 0.753       | 0.848 |
| Lpu15   | 2     | 0.020       | 0.027       | 0.039 |
| Lpu16a  | 5     | 0.280       | 0.340       | 0.412 |
| Lpu16b  | 10    | 0.973       | 0.973       | 0.853 |
| Lpu21a  | 11    | 0.940       | 0.940       | 0.834 |
| Lpu21b  | 10    | 0.972       | 0.972       | 0.843 |
| Lpu38   | 5     | 0.806       | 0.820       | 0.731 |

Note: $A$ = number of alleles; $H_e$ = expected heterozygosity; $H_o$ (min) = minimum observed heterozygosity; $H_o$ (max) = maximum observed heterozygosity; $n$ = number of individuals.

CONCLUSIONS

This is the first report on the development of microsatellites in L. pumila. The observed levels of polymorphism and genetic diversity suggest that, apart from monomorphic loci, these microsatellites can serve as useful tools for DNA profiling and population genetic studies of the species.

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