NOTE

ISOLATION AND CHARACTERISATION OF EIGHT MICROSATELLITE MARKERS IN *PARASERIANTHES FALCATARIA*, A FAST-GROWING TROPICAL LEGUMINOUS TREE SPECIES

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Paraserianthes falcataria is a fast-growing leguminous tree species with widespread natural distribution in tropical lowland to mountainous habitats in areas ranging from the Moluccas to the Solomon Islands (Fosberg 1965, Nielsen et al. 1983, Parrotta 1990). Three subspecies are recognised, namely, subsp. *falcataria*, distributed in the Moluccas Islands and New Guinea; subsp. *solomonensis* distributed in the Bismarck Archipelago and Solomon Islands; and subsp. *fulva*, endemic to the central mountainous part of New Guinea (Nielsen et al. 1983). Individuals grow up to 40 m tall and > 100 cm in diameter. Its wood is used for manufacturing furniture, cabinets, plywood and particleboard (Rojo et al. 1993). This species is widely planted in the tropics (Rojo et al. 1993) and it is one of the most commonly used species in plantations in Indonesia (Cossalter & Pye-Smith 2003, Siregar et al. 2007). To promote sustainable tropical forestry with fast-growing species, an intensive *P. falcataria* breeding programme was started in 2003 and a seed orchard was established in East Java, Indonesia with funds from the Japanese Ministry of Environment.

Genetic diversity has potential of sustaining forest health in the face of environmental change (Mosseler et al. 2003). It also provides buffers in production plantations against vulnerability to pests and climatic extremes, together with a range of genes that may have value in future breeding programme (Ledig 1986). Therefore, conservation of gene resources of useful tree species is essential not only for ecosystem stability but also for human economy. Genetic diversity of *P. falcataria* plantations in Indonesia has been evaluated in several studies (Seido et al. 1993, Suharyanto et al. 2003), but results were not enough to clarify the overall genetic diversity in Indonesia in spite of the importance of this species. Although single nucleotide polymorphism (SNP) markers have been developed in *P. falcataria* (Yuskianti & Shiraishi 2010), microsatellite markers are still informative to study population genetics because of high polymorphisms. Therefore, in the present study, we isolated microsatellite markers that should be valuable for exploring the genetic diversity and structure of *P. falcataria* populations.

Total DNA was extracted from fresh *P. falcataria* leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Zhou et al. 1999) after which microsatellite regions were isolated with a dual-suppression polymerase chain reaction (PCR) technique (Lian & Hogetsu 2002). To construct adaptor-ligated DNA libraries, DNA was digested using the restriction enzymes *Eco*RV, *Ssp*I, *Alu*I, *Afa*I, *Hinc*II or *Hae*III. The resulting fragments were then ligated to an unequal-length adaptor, consisting of a 48-mer (5'GTAATACGACTCACTATAGGGCACGCGTG GTCGACGGCCCGGGCTGGT3') and an 8-mer (5'ACCAGCCC-NH23') with the 3'-end capped by an amino residue, using a DNA ligation kit.

In the first step, the fragments, each flanked by a microsatellite at one end, were amplified from the *Ssp*I or *Hae*III library using $(AC)_{10}$ primer and the adaptor primer AP2 (5'CTATAGGGCACGCGTGGT3'). The amplified fragments were cloned using a pT7Blue Perfectly Blunt Cloning Kit, then 66 of the cloned fragments were amplified using the U19 forward and M13 reverse primers. The length of PCR products was checked by electrophoresis and 15

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products with lengths between 300 and 500 bp were chosen. The chosen PCR products were sequenced directly by T7 or U19 primers labelled with Texas Red using a thermo sequenase pre-mixed cycle sequencing kit and 10 products were successfully sequenced.

An initiating primer (IP1) was designed from the region flanking the repeat in each of the fragment. A second initiating primer (IP2) was designed for nested PCR based on the sequences between IP1 and the repeat, together with AP1 (5'CCATCGTAATACGACTCA CTATAGGGC3') and AP2 adapter primers. Primary nested PCR amplification was conducted with each DNA library using the IP1 and AP1 primers, followed by secondary amplification using the IP2 and AP2 primers, together with the primary PCR products which were diluted 100 fold. Single fragments were most frequently amplified from the libraries. Individual fragments were subcloned and sequenced as described above. A third initiating primer (IP3) was designed for each locus, based on the newly identified sequence between the AP2 binding site and microsatellite. IP1/IP3 or IP2/IP3 primer pairs were used as simple sequence repeat (SSR) markers. In total, eight primer pairs were isolated and their characteristics were checked using 23 seedlings collected in Java Island.

The characteristics of these loci are presented in Table 1. The number of alleles per locus detected ranged between 2 and 10 (average 5.9). The program FSTAT version 2.9.3 (1995) was employed to calculate expected and observed heterozygosities and to test for Hardy–Weinberg equilibrium and linkage disequilibrium at the eight polymorphic loci. The expected heterozygosities ranged from 0.202 to 0.845 (Table 1). Only one locus, pafa06, deviated significantly from Hardy–Weinberg equilibrium. The genetic bias of seed materials from a few mother trees possibly might produce this result. This needed to be further confirmed by more analysis of populations. No pairwise combination showed significant linkage disequilibrium. Thus, these microsatellite loci are useful for studies on the population genetics structure and diversity of *P. falcataria*.

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Table1 Characteristics of the eight microsatellite loci in *Paraserianthes falcataria*: locus name, repeat motif, primer sequences, length of franking region, optimal annealing temperature (Ta), number of alleles, heterozgyogosity (expected/observed) (He/Ho) and GenBank accession no

Locus name	Repeat	Primer sequence (5'-3')	Franking region (bp)	Ta (°C)*	Number of alleles*	Allele size (bp)*	He */ Ho	GenBank accession no.
Pafa02	$A_9(CA)_9$	F; (NED) CTCTTGTACGTTTCTGAATGC R; AGGCGTATACATTTACAAATC	82 12	54	6	149-163	0.682/0.522	AB751367
Pafa03	$(CA)_8$	F; (VIC) AACTTTGATTTGGGAGCTTG R; ACCACTTCCTTAATTACCGTG	36 60	54	6	149-165	0.764/0.652	AB751368
Pafa04	$(CT)_5(AT)_6$	F; (VIC) CTACACAAATTACCACATGC R; GGGATTATAAAGGAGACCATTGTGGTG	66 56	58	2	191-193	0.202/0.043	AB751369
Pafa05	$(AC)_9$	F; (FAM) TACCGAATTACTTAGTGCAG R; ATTAATAGCTTCTGACCGAAG	94 16	52	3	172-176	0.584/0.522	AB751370
Pafa06	$(AC)_{13}$	F; (FAM) TGCGAATTCCTTATCTGAAC R; GAGTATTATTTCCCAAAGTGGTGAGGG	37 93	54	8	170-208	0.787/0.435	AB751371
Pafa07	$(AC)_9$	F; (FAM) TAACCAAATGGTATTGCATG R; TCAGTACTCAGTACCTGGTAC	57 7	54	6	122-134	0.744/0.870	AB751372
Pafa08	$(AC)_{13}$	F; (PET) AAGTCAGCTGTAAGTCATTG R; GAGCAGAGAAAAAAGCTGATG	119 17	54	10	204-234	0.845/0.957	AB751373
Pafa10	$(ATG)_{12}$	F; (NED) ACGAGTACCCCGTTATTTTG R; TGTGGTAATTGCAGTGTTAG	9 92	54	6	171-185	0.817/0.783	AB751374

*Saito et al. (2005)

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