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MICROSATELLITE LETTERS

Development of 11 polymorphic microsatellite markers for *Xylocarpus granatum* (Meliaceae) using next-generation sequencing technology

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Abstract Human impacts have seriously damaged mangroves, and conservation of mangroves will require information on local and regional population genetic structures. Here, we report the development and polymorphism of eleven novel microsatellite markers, developed using next-generation sequencing on 56 samples of widespread mangrove species *Xylocarpus granatum* (Meliaceae) from nine populations across the Indo-West Pacific region. All loci were found to be polymorphic, with the number of alleles per locus ranging from four to 19. In a population from Sabah (Malaysia), the mean observed and expected heterozygosity per locus was 0.59 and 0.58, respectively. No null allele, significant linkage disequilibrium or deviation from Hardy–Weinberg equilibrium was detected among all loci. The eleven markers developed can be valuable tools to conservation genetics of this species across its distributional range.

Keywords Conservation · Genetic diversity · Mangrove · Pyrosequencing · SSR · Indo-West Pacific

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Table 1 Primer sequence, repeat motif, and accession number of eleven microsatellite markers developed in *X. granatum*

Locus	Primer sequence (5′–3′)	Repeat motif	Accession no.
XG01	F: GGTTTTCCCAGTCACGACGCTGATGGGCATGCATTGA	$(TA)_{13}$	AB812880
	R: GTTTCATGGAATTAGCACCTACGGA		
XG05	F: GGTTTTCCCAGTCACGACGCATCAGAGGCACACGAAGAA	$(AT)_{12}$	AB812881
	R: GTTTGTCTTTCGAAGTCGCTGA		
XG07	F: GGTTTTCCCAGTCACGACGGGCCAAGTAAATCAGCAAA	$(AT)_{12}$	AB812882
	R: GTTTGCTTCGCCTCTTTCATCT		
XG08	F: GGTTTTCCCAGTCACGACGCCTCCATGATGGTGATGAT	$(AG)_{12}$	AB812883
	R: GTTTGACGGTGAGGATGCTTATG		
XG10	F: GGTTTTCCCAGTCACGACGGATTCATACACAGATGGTGGA	$(CT)_{11}$	AB812884
	R: GTTTAGTCGGTCGTGCATGGATA		
XG12	F: GGTTTTCCCAGTCACGACGATGATTGAGTCCGTTCCGTC	$(AT)_{11}$	AB812885
	R: GTTTAGCAGAGCAGTCCACTCGAT		
XG16	F: GGTTTTCCCAGTCACGACGATTTGCCAGATCAGTCGGT	$(TC)_{11}$	AB812886
	R: GTTTCAATTTCACGAGTGGATGC		
XG18	F: GGTTTTCCCAGTCACGACGACCGTAGCAATGGTAGGTCG	$(CT)_{11}$	AB812887
	R: GTTTTCAACACGTGTCCTTGCTCT		
XG21	F: GGTTTTCCCAGTCACGACGTCTCTTCCACACCGAAG	$(AG)_{10}$	AB812888
	R: GTTTAGTGCAATCGCTGCTAGGTT		
XG22	F: GGTTTTCCCAGTCACGACGATGAAGGAATTCGTTGTCCG	$(AG)_{10}$	AB812889
	R: GTTTGAGAGTGGGTGGAGAGC		
XG24	F: GGTTTTCCCAGTCACGACGTTTGTCTGAGGCAGGAAT	$(AT)_{10}$	AB812890
	R: GTTTGCGAAAGGATAATCCACCAG		

Forward primers contain U19 tail and reverse primers contain GTTT tail

Mangroves are a characteristic intertidal ecosystem found along tropical and subtropical coastlines. Despite the essential ecosystem services and economic values of mangroves, escalating anthropogenic threats (e.g. land conversion) highlight the growing need for conservation of mangroves. *Xylocarpus granatum* Koenig (Meliaceae) is an important mangrove species from the Indo-West Pacific (IWP) region. Here, we developed and characterized eleven microsatellite markers for *X. granatum* using next-generation sequencing, and tested the level of polymorphism with a set of samples covering its distribution range.

Isolation and characterization of microsatellite markers were conducted using 56 samples from nine populations across the IWP: 24 individuals from Sabah (Malaysia) and four individuals from each of the other eight populations at Quelimane (Mozambique), Ayeyarwady (Myanmar), Klang (Malaysia), Kemaman (Malaysia), Bali (Indonesia), Panay (Philippines), Airai Bay (Palau) and Malatie (Vanuatu).

Total genomic DNA of one individual from Sabah was extracted using DNeasy Plant mini Kit (Qiagen, Hilden, Germany). A DNA library for pyrosequencing was generated by a standard protocol using the GS Junior Titanium Sequencing Kit (Roche Applied Science, Penzberg). Sequencing was carried out with only one-third of the run using Multiplex Indentifier (MID) adaptors on a Roche 454 Genome Sequencer Junior (Roche Applied Science, Penzberg). A total of 43,576 reads with an average of 390 bp were obtained. Microsatellite sequences were selected according to the criteria of Takayama et al. (2011) using QDD v.2.1 (Meglécz et al. 2010). A total of 860 microsatellites with perfect motifs and more than five repeats was obtained. Twenty-four candidate microsatellites with more than nine

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Table 2 Characteristics of eleven microsatellite loci in nine populations of *X. granatum*

Locus	Allele size range (bp)	All	Sabah Malaysia (n = 24)			Quelimane Mozambique (n = 4)	Ayeyarwady Myanmar (n = 4)	Klang Malaysia (n = 4)	Kemaman Malaysia (n = 4)	Bali Indonesia (n = 4)	Panay Philippines (n = 4)	Airai Bay Palau (n = 4)	Malatie Vanuatu (n = 4)
		N_A	N_A	H_O	H_E	N_A	N_A	N_A	N_A	N_A	N_A	N_A	N_A
XG01	142–168	14	7	0.71	0.57	1	3	5	5	1	3	4	3
XG05	244–262	13	7	0.58	0.63	2	3	2	4	2	3	3	2
XG07	157–173	15	6	0.67	0.73	3	3	3	2	4	4	5	3
XG08	204–212	5	5	0.46	0.44	1	1	1	5	1	2	2	1
XG10	142–146	8	3	0.38	0.51	2	1	2	3	4	3	4	1
XG12	136–164	19	12	0.79	0.82	2	3	2	3	4	4	7	2
XG16	110–114	4	2	0.54	0.48	2	3	1	2	2	2	2	1
XG18	220–240	11	8	0.63	0.70	2	5	4	4	3	2	5	1
XG21	122–128	6	4	0.63	0.48	1	4	2	1	2	3	3	1
XG22	239–243	7	3	0.29	0.32	2	3	2	4	2	2	1	2
XG24	265–277	13	6	0.83	0.72	2	4	6	6	4	5	5	2
	Mean	10.5	5.73	0.59	0.58	1.82	3.00	2.73	3.55	2.64	3.00	3.73	1.73

N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

repeats and varying amplicon sizes were selected for subsequent PCR. For these candidate markers, primer designing was carried out by the same method described in Shinmura et al. (2012).

The candidate markers were tested for PCR amplification, reproducibility and the level of polymorphism using the 56 samples described above. Simplex PCR was conducted in a final volume of 5 µl using the Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany) according to the conditions described in Shinmura et al. (2012). PCR products were genotyped with the ABI 3130xl automated DNA sequencer (Applied Biosystems, CA, USA) and GeneMapper v.4.1 (Applied Biosystems, CA, USA).

Eleven of the 24 candidate markers had consistent and reproducible amplification (Tables 1, 2). The nucleotide sequences of the eleven loci were deposited in GenBank and shown in Supplementary materials. The number of alleles per locus (N_A) varied from four to 19, with a mean of 10.5. All populations were polymorphic, with a mean N_A per population ranging from 1.73 to 5.73. The presence of linkage disequilibrium (LD) and null alleles, and the deviation from Hardy–Weinberg equilibrium (HWE) were tested by Micro-checker 2.2.3 (van Oosterhout et al. 2004) and GENEPOP 4.0 (Raymond and Rousset 1995) using 24 samples from Sabah. No significant LD was detected across loci pairs, no null allele was found at any locus and all loci were in HWE ($P < 0.05$). The N_A per locus in the Sabah population varied from two to 12, with an expected heterozygosity ranging from 0.32 to 0.82 (Table 2). The 11 microsatellite markers developed and characterized here will facilitate the studying of the global phylogeography and conservation genetics of *X. granatum*.

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