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Characterization of Polymorphic Microsatellite Markers Isolated from Genomic DNA of *Elaeocarpus decipiens* Hemsly (Elaeocarpaceae)

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Abstract

The development of compound microsatellite markers was conducted in *Elaeocarpus decipiens* to investigate genetic diversity and population genetic structure of this species. Eighteen microsatellite markers that were successfully amplified showed polymorphism when tested on 35 individuals from three populations in Chinese mainland. Overall, the number of alleles per locus ranged from 4 to 11, with an average of 7.06 alleles per locus. These results indicate that these microsatellite markers are adequate for detecting and characterizing population genetic structure and genetic diversity in *E. decipiens*. Of these primers, only four could be successfully transferred to *E. sylvestris* and *E. japonicus*.

Keywords: *Elaeocarpus decipiens*; Microsatellite markers; Genomic DNA; Genetic diversity

Introduction

Elaeocarpus decipiens is an evergreen, broad-leaved, woody species of the Elaeocarpaceae family with a disjunct distribution in south of Chinese mainland, the Ryukyu Archipelago and Taiwan. Currently, most of the efforts have been focused on the germplasm, breeding and cultivation of this species [1]. The study of population genetic diversity, population genetic structure and population ecology of this species is insufficient and limited. However, population genetic analysis of this disjunct plant will potentially provide insights into the geographic structure of genetic diversity that reflects the evolutionary history of *E. decipiens*. To assess gene flow across the populations and to infer biogeographic patterns, we developed microsatellite markers for this species, for which none were available previously. Additionally, these loci were tested for cross-amplification in *E. sylvestris* and *E. japonicas*.

Materials and Methods

Genomic DNA of *E. decipiens* was extracted from fresh leaves using a modified CTAB (cetyltrimethyl ammonium bromide) method [2]. An adaptor-ligated DNA library was constructed following the protocol of Lian et al. [3]. Briefly, total genomic DNA (10 μg) was digested with a blunt-end restriction enzyme, EcoRV (Takara, Dalian, Liaoning, China), and the restricted fragments were ligated to an unequal-length adaptor, using DNA Ligation Kit Version 2.0 (Takara, Dalian, Liaoning, China). Then, fragments flanked by a microsatellite at one end were amplified from the EcoRV DNA library using compound SSR primer $(AC)_6(AG)_5$ and an adaptor primer AP2 (5′-CTATAGGGCACGCGTGGT-3′). The recovered DNA was ligated into a pGEM-T vector (Promega, Madison, Wisconsin, USA), and transformed into DH5α competent cells (Takara, Dalian, Liaoning, China). Transformants were cultured on selective agar media with ampicillin, X-Gal and IPTG, for blue/white colony selection. After PCR-tested for insert size of the white colonies, a total of 144 clones were found to contain $(AC)_6(AG)_n$ compound SSR motifs. 55 sequences were too short to design primer. And 89 clones proved suitable for primer design using PREMIER version 5.0 [4]. These primers were tested for polymorphism in *E. decipiens*. A total of 53 out of the 89 primer pairs tested successfully amplified the target fragments. PCR was performed in 10-μL reaction volumes containing 30-50 ng/μL of template DNA, 0.25 unit *Taq* DNA polymerase (TaKaRa, Dalian, Liaoning, China), 1 μL 10×PCR buffer, 0.5 μL of 2.5 mM $MgCl_2$,1 μL of 2.5 mM dNTPs, 0.05 μL bovine serum albumin (BSA) (TaKaRa, Dalian, Liaoning, China), and 0.6 μL of each 10 μM primer. The thermal profile used was initial denaturing for 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s of annealing at the optimized annealing temperature (Table 1), 1 min 30 s of elongation at 72°C, ending with a 10-min extension at 72°C. The forward primer of each pair was labeled with a fluorescent dye (6-FAM). Products were resolved using an ABI 3730 sequencer (Applied Biosystems), along with a fluorescently labeled internal size standard (GeneScan 500 LIZ Size Standard; Applied Biosystems), and the samples were genotyped using GENEMAPPER version 4.0 (Applied Biosystems).

Polymorphisms of these primers were assessed in 35 natural individuals of *E. decipiens* collected from Jinggang Mountain (JG, 26°35’19” N, 114°07’39” E), Laohunao Mountain (LHN, 27°13’18” N, 116°00’43” E) and Tongbo Mountain (TB, 28°04’57” N, 118°14’18” E). Voucher specimens for the sampled populations are stored at the Herbarium of Nanchang University (JXU). Parameters of genetic diversity including the expected heterozygosity (*He*) and observed heterozygosity (*Ho*), number of alleles (*A*) per locus, tests for linkage disequilibrium (LD), and deviation from Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP version 4.0.7 [5]. In addition, CERVUS version 3.0.3 [6] was employed to calculate the value of polymorphic information content (*PIC*).

Results

Eighteen out of the 53 loci were identified as polymorphisms and generated consistent amplification products of the expected size range (Table 1). These loci contained 4 to 11 alleles in the 35 individuals, with He and Ho ranging from 0.685 to 0.909 and from 0.583 to 0.917, respectively. On average, the PIC were 0.747 (range: 0.627-0.854), 0.756 (range: 0.654-0.845) and 0.751 (range: 0.605-0.850) for populations

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Locus	Repeat	Primer sequence (5'–3')	s	*Ta* (C°)	*A*	GenBank	Cross-amplification
Ed1	$(AC)_6(AG)_{17}$	F: ACACACACACACAGAGAGAGAG R: CTGATGTTGCCACGGAGT	277 265-303	54	10	JX193598	
Ed2	$(AC)_6(AG)_7$	F: ACACACACACACAGAGAGAGAG R: TCAAAACACAAAAAAACTCA	171 167-194	52	9	JX193599	
Ed3	$(AC)_6(AG)_6$	F: ACACACACACACAGAGAGAGAG R: ATACAAATTGAACAAGGGCTTA	314 312-330	53	8	JX193600	
Ed4	$(AC)_6(AG)_6$	F: ACACACACACACAGAGAGAGAG R: AGTTTGAGGCTTTATTCAGTTT	213 211-225	54	5	JX193601	
Ed5	$(AC)_6(AG)_7$	F: ACACACACACACAGAGAGAGAG R: ACAGGGTTCTTGCTATTTCA	187 185-218	53	7	JX193602	
Ed6	$(AC)_6(AG)_9$	F: ACACACACACACAGAGAGAGAG R: GCCACCAATCCTTGAACCT	175 169-212	54	9	JX193603	a, b
Ed7	$(AC)_6(AG)_7$	F: ACACACACACACAGAGAGAGAG R: TGTCATTGATGGGAAAAACT	291 289-334	53	10	JX193604	
Ed8	$(AC)_6(AG)_{11}$	F: ACACACACACACAGAGAGAGAG R: AAATGTCATAATCAAAAAGCAG	134 124-154	51	9	JX193605	
Ed9	$(AC)_6(AG)_6$	F: ACACACACACACAGAGAGAGAG R: TGATTCTTGATGTCCTTCTATT	179 177-199	54	5	JX193606	a
Ed10	$(AC)_6(AG)_9$	F: ACACACACACACAGAGAGAGAG R: GCTTTTGAGGGCTATTGATG	216 208-232	54	8	JX193607	
Ed11	$(AC)_6(AG)_{11}$	F: ACACACACACACAGAGAGAGAG R: CATCACCTTTTTCCCTATCA	402 394-418	53	5	JX193608	a, b
Ed12	$(AC)_6(AG)_6$	F: ACACACACACACAGAGAGAGAG R: TCGGGAATGAAAAAAAATAG	159 157-204	53	5	JX193609	a, b
Ed13	$(AC)_6(AG)_6$ $CG(AG)_5$	F: ACACACACACACAGAGAGAGAG R: GGGAGATAGAGATAGAGACG	184 174-199	55	4	JX193610	
Ed14	$(AC)_6(AG)_6$	F: ACACACACACACAGAGAGAGAG R: ATTTCATTTGGTGGGCTTT	284 284-308	55	4	JX193611	
Ed15	$(AC)_6(AG)_8$	F: ACACACACACACAGAGAGAGAG R: ATCCTTTTTAGATTTCGTTTTA	195 190-223	54	5	JX193612	
Ed16	$(AC)_6(AG)_{11}$	F: ACACACACACACAGAGAGAGAG R:TACCACATAAACAAACCATT	386 376-411	54	9	JX193613	
Ed17	$(AC)_6(AG)_{12}$	F: ACACACACACACAGAGAGAGAG R:TTATCAAAAAATCAACAAAT	291 280-322	53	11	JX193614	
Ed18	$(AC)_6(AG)_6$ $AC(AG)_3$	F: ACACACACACACAGAGAGAGAG R:CGGTTATGCCACGGACTT	277 271-298	56	4	JX193615	

Notes: The a and b represent the primers that can successfully amplify in *E. sylvestris* and *E. japonicus*

Table 1: Characteristics of 18 compound microsatellite loci developed for *E. decipiens*. Shown for each locus are the locus name, the forward (F) and reverse (R) primer sequence, the optimized annealing temperature (Ta), allele size ranges, the total number of alleles per locus (A) and the GenBank accession number. Size ranges and the total number of alleles include all values detected within three *E. decipiens* populations used in this study (Table 2).

Locus	Population JG (11)				Population LHN(12)				Population TB(12)			
	A	*Ho*	*He*	*PIC*	*A*	*Ho*	*He*	*PIC*	*A*	*Ho*	*He*	*PIC*
Ed1**	9	0.818	0.905	0.849	8	0.583	0.848	0.789	8	0.750	0.859	0.800
Ed2*	6	0.727	0.823	0.753	8	0.833	0.899	0.845	8	0.750	0.884	0.828
$Ed3^{n.s}$	5	0.818	0.797	0.720	6	0.917	0.855	0.794	7	0.667	0.837	0.777
$Ed4^{n.s}$	5	0.909	0.801	0.726	5	0.750	0.815	0.745	5	0.750	0.797	0.723
$Ed5^{n.s}$	7	0.727	0.874	0.813	5	0.833	0.815	0.746	6	0.750	0.797	0.731
Ed6*	7	0.818	0.831	0.765	7	0.750	0.804	0.738	8	0.750	0.855	0.797
Ed7*	7	0.818	0.866	0.803	7	0.833	0.884	0.828	7	0.667	0.833	0.770
$Ed8^{n.s}$	7	0.818	0.857	0.794	7	0.750	0.870	0.811	7	0.750	0.804	0.740
$Ed9^{n.s}$	5	0.818	0.827	0.756	5	0.750	0.812	0.741	5	0.750	0.819	0.750
Ed10**	7	0.818	0.840	0.773	6	0.667	0.841	0.778	7	0.583	0.866	0.808
$Ed11^{n.s}$	5	0.818	0.779	0.700	5	0.667	0.815	0.745	5	0.750	0.786	0.716
$Ed12^{n.s}$	5	0.818	0.766	0.687	5	0.583	0.808	0.737	5	0.750	0.830	0.762
$Ed13^{n.s}$	4	0.818	0.723	0.627	4	0.750	0.736	0.654	4	0.833	0.685	0.605
$Ed14^{n.s}$	4	0.818	0.775	0.691	4	0.750	0.764	0.683	4	0.833	0.764	0.683
$Ed15^{n.s}$	4	0.818	0.775	0.691	4	0.750	0.757	0.677	5	0.750	0.808	0.737
$Ed16^{n.s}$	9	0.818	0.853	0.791	6	0.750	0.851	0.790	6	0.750	0.819	0.753
Ed17**	10	0.818	0.909	0.854	7	0.833	0.884	0.828	9	0.667	0.902	0.850
$Ed18^{n.s}$	4	0.818	0.740	0.651	4	0.833	0.761	0.678	4	0.750	0.772	0.691

Notes: *, ** and ***, significant departures from Hardy–Weinberg equilibrium at $P<0.05$, $P<0.01$, $P<0.001$, respectively.
n.s.= not significant.

Table 2: Results of initial primer screening in three populations of *E decipiens*. Shown are locus name, the number of alleles per locus (*A*), mean values of observed (*Ho*) and expected (*He*) heterozygosity, and polymorphism information content (*PIC*). The sample size for each population is shown in parentheses.

in JG, LHN and TB Mountains, respectively (Table 2). Six loci (Ed1, Ed2, Ed6, Ed7, Ed10 and Ed17) significantly deviated from HWE ($P<0.05$) due to heterozygote deficiency. In addition, significant linkage disequilibrium (LD) was not detected between any pair of loci. Microsatellite loci were all identified and their respective sequences were deposited in GenBank (Accession Nos. JX193598–JX193615). Details about the 18 microsatellite loci and their variability across the 35 individuals were summarized in Table 1. Additionally, cross-amplification of the 18 prime pairs was performed in 2 individuals of *E. sylvestris* and *E. japonicus*. Of these primers, only four (Ed6, Ed9, Ed11 and Ed12) could be successfully transferred to the tested species (Table 1).

Conclusion

The approach used in this study substantially reduces time in comparison with the FIASCO (Fast Isolation by APLR of Sequences Containing Repeats) protocol. Because a common fluorescent compound SSR primer can be used in polymorphism analyses for different loci and different species and the fluorescent primer is rather expensive, this may save investigation costs [7]. These polymorphic microsatellite markers of *E. decipiens* should represent a useful tool to assess patterns of geographical molecular variation in *E. decipiens* at the population level, and across the species' ranges in south of Chinese mainland, Taiwan and the Ryukyu Archipelago. Moreover, studies have shown that microsatellite primers developed in one species could be cross-amplified in related taxa [8]. However, only three and four loci were successfully amplified in *E. japonicus* and *E. sylvestris*, respectively. Even so, cross-species amplification in *E. sylvestris* and *E. japonicus* has opened an opportunity for comparative studies among these species. In addition, the use of these markers will facilitate the follow up introgression of favorable variation from *E. sylvestris* and *E. japonicus* into *E. decipiens*.

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