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A set of novel microsatellite markers developed for an economically important tree, *Dracontomelon duperreanum*, in China

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ABSTRACT. *Dracontomelon duperreanum*, the most representative species of the family Anacardiaceae, is an important multipurpose tree in China and Vietnam. However, no genetic diversity studies have been reported on this species. In this study, we identified 11 microsatellite markers for *D. duperreanum* by using the restriction-site-associated DNA sequencing (RAD-seq) method and examined their polymorphisms in 22 samples obtained from the South China Botanical Garden, South China. We could detect only two or three alleles for each microsatellite marker. The mean observed and expected heterozygosities were 0.41 and 0.39, respectively, which were lower than those reported for the species with similar life history forms. These relatively low genetic diversities in this common plant species are unexpected and might have resulted from its extensive cultivation. To our knowledge, this is the first report of microsatellite markers in the genus *Dracontomelon*. These microsatellite markers will be valuable for studying the genetic

diversities and structures in *D. duperreanum* and other *Dracontomelon* species.

Key words: *Dracontomelon duperreanum*; Genetic diversity; Microsatellite marker development; Polymorphism; Restriction-site-associated DNA sequencing; Subtropical China

INTRODUCTION

Dracontomelon duperreanum, a broad-leaved evergreen tree, belongs to the family Anacardiaceae. It is naturally distributed in South China and Vietnam (Zheng and Ming, 1980). Because the surface of its seed shows some characters like a human face, it is called "Ren Mian Zi" meaning human face, in China (Yu, 1987) (Figure 1).

Figure 1. Pictures showing the trees, leaves and seeds of *Dracontomelon duperreanum* planted in the South China Botanical Garden, Guangzhou, China.

Dracontomelon duperreanum is an important multipurpose tree. It grows in different kinds of soil and can tolerate low temperatures (Zhou et al., 2008). It has a straight trunk with strong buttresses and can grow up to 20-m tall (Xiao and Feng, 2014). Its crowns are dense and spreading (Zhang and Ma, 2005). It is seldom attacked by insects and diseases and stands strong in the winds and polluted air (Lu et al., 2005). All these features make it suitable for planting in gardens and along streets (Yu, 1987; Xiao and Feng, 2014). It has been reported that *D. duperreanum* can be a host species of *Santalum album*, a famous aromatic tree native to southern China (Lu et al., 2014). Its fruits are edible and can be eaten raw or preserved as jams and conserves (Wang et al., 2016b). Its seed oil can be used for making soap or lubricating oil (Luo and Liu, 2007). Its wood is dense, lustrous, corrosion-resistant, and odor-free, and therefore suitable for making furniture. It is also a Chinese traditional medicinal plant (Liang, 1988; Zhang and Ma, 2005). Its fruits, barks, roots, and leaves can be used as medicine to strengthen stomach, relieve pain, and promote wound healing (Su et al., 2008). Its bark contains flavonoids (Weng et al., 2016), which have been recently confirmed to have anticancer activity (Chahar et al., 2011). Its leaf extracts contain many antimicrobial components, such as petroleum ether, chloroform, ethyl acetate, and n-butanol, which act against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (Su and Nong, 2010). In addition, its leaf extracts can inhibit the activity of *Microcystis aeruginosa*, an algal species that causes harmful algal blooms (Wang et al., 2016a). However, in the natural environment, owing to the low content of polyphenols in its leaves, its litters can be easily decomposed, supporting material cycling and energy flow in the ecosystem (Yan et al., 2007; Guan et al., 2008).

Genetic diversity is the amount of variation in the heritable material maintained within and among species; it represents the adaptive and evolutionary potential of a species and has great economic, environmental, and scientific values (Loo et al., 2014). However, no genetic diversities have been reported for this valuable multipurpose tree yet, and such information is necessary for the efficient management of its genetic resources. Therefore, in this study, we developed a set of microsatellite markers that can be used to study the genetic diversities and population structures of this economically important species.

MATERIAL AND METHODS

Mature leaves of two trees, one from Dinghushan Natural Reserve (DHS) and the other from the South China Botanical Garden (SCBG), were collected and put in liquid nitrogen immediately. These two sampling sites are located in Guangdong province of South China. Subsequently, DNA was extracted from one leaf of each sample using a modified cetyl trimethylammonium bromide (CTAB) method (Doyle, 1991) and dissolved in 50 μL (Tris-EDTA) TE solution. The concentration and quality of DNA were assessed using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Each DNA sample was then diluted to a final concentration of 50 ng/μL, and stored at -20°C until further use. The restriction-site-associated DNA sequencing (RAD-seq) libraries for these two DNA samples were constructed using 300 ng of each DNA sample and subsequently subjected to DNA sequencing using a HiSeq2500 sequencer (Illumina, Inc., San Diego, CA, USA). After trimming adapters and removing polymerase chain reaction (PCR) duplicates, we obtained 31,418,522 and 22,947,686 clean sequences for DHS and SCBG samples, respectively. By further removing the low-quality sequences, we assembled the remaining sequences using the software STACK 1.24 (Catchen et al., 2011; Catchen et al., 2013). For the assembled

sequences, we used MSATCOMMANDER 0.8.2 (Faircloth, 2008) to identify the dinucleotide and trinucleotide motifs with a minimum of nine and eight repeats, of which 49 could be used to design primers.

To test the availability of these microsatellite loci, we initially used one individual from SCBG to conduct PCR amplification. For each microsatellite locus, PCR was then carried out in a 20-μL reaction mixture containing 50 ng template DNA, 2 μL 10X PCR Buffer (Mg^{2+} plus), 0.4 μL 10 mM dNTPs, 1 μL forward and reverse primers (5 μM), and 1 U *Taq* DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, Liaoning, China). PCR amplification was programmed for initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, and a final extension at 72°C for 10 min. Amplified products were electrophoretically separated using 2% agarose gels. Thirty-seven microsatellite loci gave successful results of the target regions. Because all loci performed well at the annealing temperature of 55°C, we did not optimize them further to reduce the experimental cost. These loci were then used to examine their polymorphisms in 22 individuals from SCBG. Unlike the samples used for RAD-seq library constructions, the samples collected from these trees were put into the sealed bags containing silica gels, but they were subjected to the same DNA extraction, assessment, and storage procedures. Their PCR amplification products were electrophoretically separated on ABI 3730 sequencer (Applied Biosystems, Carlsbad, CA, USA), and their product sizes were determined using the ABI GeneMapper Software 4.1. Finally, eleven microsatellites showed stable and clear polymorphism.

The genetic diversity of these microsatellite loci was computed in GENEPOP 4.3 (Rousset, 2008). We also used this software to assess the deviation from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) among all pairs of loci. The levels of significance for HWE and LD tests were adjusted by using the sequential Bonferroni correction (Holm, 1979).

RESULTS AND DISCUSSION

The number of alleles per locus varied from two to three (Table 1). The H_O and H_E values varied from 0.105 to 0.800 and from 0.102 to 0.663, respectively. No locus exhibited significant departure from HWE. Significant LD was found in only five locus pairs without Bonferroni correction and in three loci pairs (RMZ-8 with RMZ-1, RMZ-25, and RMZ-45) after Bonferroni correction.

Using the same RAD-seq method, we obtained 63 polymorphic loci from 170 candidate loci (37.1%) in an endangered species, *Bretschneidera sinensis*, in a previous study (Li et al., 2016), but we could obtain only 11 polymorphic loci among the 49 loci (29.7%) identified in this study. In addition, the highest number of alleles per locus was three in *D. duperreanum* and 16 in *B. sinensis* with 30 samples from one population. In *B. sinensis*, we found that 37 loci had more than three alleles each, and five loci had more than 10 alleles each. Because *D. duperreanum* is a common and widely distributed species in South China, such small number of alleles was unexpected.

Low genetic diversity in *D. duperreanum* was further confirmed when we compared its genetic diversity with the genetic diversities of other species with similar life history forms. According to Nybom (2004), the mean H_O and H_E were 0.63 and 0.68 for long-lived perennials, 0.65 and 0.65 for regionally distributed species, 0.63 and 0.65 for outcrossing

Table 1. Characteristics of 11 microsatellite markers in *Dracontomelon duperreanum*. Annealing temperature for all loci is 55°C. Forward primers for all loci are labeled with 6-fluorescein amidite (FAM).

Locus	Repeat motifs of individual applied to RAD sequencing	Primer sequences (5'→3')	Size range (bp)	*A*	H_O	H_E	*F*	EMBL/GenBank accession No.*
RMZ-1	$(CT)_9$	F: TCAGTAACTAATGTTCTCCACAAG R: ACTAGACCAATAGGCATGTCTTC	217-233	3	0.800	0.569	-0.4206	LT671604
RMZ-2	$(AT)_{11}$	F: TGGCTTAGCTCTTACGGCTC R: TCCAATGCTCACAGTGCTATC	229-233	2	0.150	0.142	-0.0556	LT671605
RMZ-8	$(ATT)_9$	F: ATCCTACAAATGCATCTCAGTTC R: GGGATGAGAGGGTCAAGGC	157-160	2	0.318	0.460	0.3131	LT671606
RMZ-12	$(CT)_{10}$	F: GCGTGCCCGAAGAAACTC R: TTCCCTTGTGCATAAACGTG	261-263	2	0.474	0.422	-0.1250	LT671607
RMZ-25	$(TA)_9$	F: GGCTCAGCATACTTTGGGAC R: GATTCCGGAGCCATACTGC	152-154	2	0.150	0.358	0.5870	LT671609
RMZ-28	$(AG)_{10}$	F: TGAAACCCTTTAGAGTATGCTTG R: GGACATCTTGCTTGGAGGC	184-186	2	0.300	0.663	-0.1515	LT671611
RMZ-29	$(AG)_{12}$	F: ACTCACCTATTTCCTTGGCTG R: GAGCGGGAAGCCTTAGACC	209-227	2	0.105	0.102	-0.0286	LT671612
RMZ-33	$(AT)_9$	F: AATTCATCCACGCAACGGG R: GGCAAATGTGAATGCTCACG	211-213	2	0.650	0.481	-0.3646	LT671613
RMZ-45	$(CA)_{13}$	F: CGTCATTGAGGACACTAGACC R: TGGTACTTGGGATGAAACTTAGG	185-187	2	0.400	0.492	0.1915	LT671614
RMZ-46	$(TA)_{10}$	F: TCGCAATTGTTTCTGAAGGG R: TCATGGGATGAATTGGTGGC	159-177	3	0.316	0.383	0.1787	KY304004
RMZ-48	$(AT)_{13}$	F: GGAGGGTGAAAGCTACTATGTTC R: GCCATTAGCTGCTTCCTCG	201-205	2	0.600	0.431	-0.4074	LT671615

A: number of alleles; H_O: observed heterozygosity; H_E: unbiased expected heterozygosity; *F*: fixation index; RAD sequencing: Restriction site-associated DNA sequencing; EMBL: European Molecular Biology Laboratory. *Full sequence information will be released on June 15, 2017.

species, and 0.50 and 0.47 for species with gravity seed-dispersal, respectively. These values were higher than the H_O (0.41) and H_E (0.39) values obtained for *D. duperreanum* in this study. One possible reason for the low genetic diversity in *D. duperreanum* might be that the samples we studied were obtained from the South China Botanical Garden, and they might have been obtained from some cultivated resources harboring low genetic diversity. A deficiency of genetic variation in cultivated plant is a common phenomenon due to modern plant breeding (Ahuja and Jain, 2015). However, further studies are necessary to investigate if the genetic diversities in the wild *D. duperreanum* populations are also low.

To the best of our knowledge, this is the first report of the development of microsatellite markers in the genus *Dracontomelon*. These markers will be valuable for studying the genetic diversities and structures in *D. duperreanum* and other *Dracontomelon* species.

Conflicts of interest

The authors declare no conflict of interest.

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