MICROSATELLITE MARKERS IN RHODIOLA (CRASSULACEAE), A MEDICINAL HERB GENUS WIDELY USED IN TRADITIONAL CHINESE MEDICINE

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• Premise of the study: Microsatellite loci are described for Rhodiola, a medicinal herb genus widely used in traditional Chinese medicine.

• Methods and Results: A total of 17 polymorphic microsatellite primer pairs were developed using the combined biotin capture method. The number of alleles per locus ranged from one to 12 across 192 individuals from R. bupleuroides, R. crenulata, R. fastigiata, and R. sacra, and the mean observed and expected heterozygosities ranged from 0.177 to 0.412 and from 0.363 to 0.578, respectively.

• Conclusions: The results demonstrate the potential use of this new set of microsatellite markers for genotyping individuals and estimating genetic diversity in Rhodiola.

Key words: Crassulaceae; medicinal plants; microsatellites; Rhodiola bupleuroides; Rhodiola crenulata; Rhodiola fastigiata; Rhodiola sacra.

METHODS AND RESULTS

Four species of Rhodiola (R. bupleuroides (Wull. ex Hook, f. & Thomson) S. H. Fu, R. crenulata, R. fastigiata (Hook, f. & Thomson) S. H. Fu, and R. sacra (Pra in ex Raym.-Hamet) S. H. Fu) were collected from the Qinghai–Tibet Plateau, China. Genomic DNA from four individuals of two populations per species were isolated and then pooled to construct microsatellite libraries. Voucher information for the sampled populations is provided in Table 1. DNA was extracted from dry leaves using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987), digested by RsaI and XbaI (New England Biolabs, Beijing, China), and then ligated with Super SNX24 adapters (5′-GGTTAGGCCC-TAGCTAGCTAGCAGAATC-3′ and 5′-gGATCTGCTAGCAGGCCTATACAAAA-3′) (Sangon Biotech, Shanghai, China) by T4 DNA ligase (TaKaRa Biotechnology Co., Dalian, Liaoing, China) (Glenn and Schable, 2005). Digested, adapter-ligated DNA fragments containing potential microsatellite loci were hybridized to 5′-biotinylated oligonucleotides (CCCA, (AAG)6, (AOG)6, (CT)13, (AGG)6, (AC)13, and (ATC)6, and microsatellite-rich hybridized genomic fragments were recovered by capture with streptavidin-conjugated magnetic Dynabeads (Invitrogen, Carlsbad, California, USA). Captured molecules were amplified with SNX24 adapter-specific primers, ligated into pMD18-T, and transformed into the competent Escherichia coli DH5α host cells (TIANGEN Biotech, Beijing, China). Positive clones were identified by PCR amplification with M13 primers (Sangong Biotech, Shanghai, China). The PCR reactions were performed in a Biometra thermocycler (Biometra, Goettingen, Germany) with a denaturation period of 4 min at 94°C; followed by 30 cycles of 1 min at 94°C, 45 s at 55°C, and 40 s at 72°C; and a final extension of 10 min at 72°C. Reactions were carried out in a volume of 20 μL containing 9.6 μL double-distilled water, 2 μL 10× Taq reaction buffer, 2.4 μL dNTP, 2.4 μL MgCl2, 1.2 μL M13 forward primers, 1.2 μL M13 reverse primers, 0.2 μL 0.5 U/μL Taq DNA polymerase (Aidlab Biotechnologies Co. Ltd., Beijing, China), and 1 μL template DNA. A total of 243 positive clones were sequenced on an ABI 377XL DNA sequencer (Applied Biosystems, Foster City, California, USA), and 145 (59.7%) were found to contain simple sequence repeats (SSRs). DNA sequence alignments and primer design were performed using Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA).

Primer pairs were synthesized for 66 microsatellite sequences containing a repeat region of 20–24 bases and initially screened using four samples from each Rhodiola species. After PCR optimization, including gradient PCR for testing...