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Isolation and characterization of 23 microsatellite loci in Astragalus *camptodontus* (Leguminosae), a traditional medicinal plant in Yunnan province

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ABSTRACT

Astragalus camptodontus is a traditional medicinal plant in Yunnan province, and it is used as one of substitutes for the Chinese medicine Astragali radix, namely "Huangqi". Twenty-three microsatellite loci were developed from its nuclear genome using a fast isolation protocol by amplified fragment length polymorphism of sequences containing repeats (FIASCO). Two wild populations collected from Northwest Yunnan were used to assess polymorphism of each locus. The number of alleles per locus ranged from 2 to 5 with a mean of 4.2. Values for H_O and H_E ranged from 0.042 to 0.875 and from 0.254 to 0.793, with average of 0.403 and 0.616, respectively. These polymorphic loci will facilitate further studies on genetic diversity, population structure and gene flow of *A. camptodontus* and its allied species.

1. Introduction

Astragalus camptodontus Franch. is a perennial herb and it is mainly distributed in Yunnan, Sichuan and Xizang provinces (Fu, 1993). It grows in alpine meadow at altitudes ranging from 2500 m to 3800 m elevation. Radix of this species and several related species are used as local substitutes of the certified product, namely Astragali radix (Huangqi) (Qian et al., 1997). Due to severe collecting and habitat destruction, wild resource of the species has been becoming endangered in recent years.

To provide effective conservation for endangered species, it is critical to investigate their genetic diversity and spatial population structure (Allendorf and Liukart, 2006). Compared with common molecular markers such as RAPD, ISSR and AFLP, microsatellite showed obvious advantages, including hyper-variability, co-dominance and high reproducibility (Powell et al., 1996). However, microsatellite loci developed from nuclear genome of *Astragalus* L. were very limited (Chen et al., 2011; King et al., 2012). In this study, we developed and characterized 23 micorsatellite loci with polymorphism for *A. camptodontus* using a fast isolation protocol by amplified fragment length polymorphism of sequences containing repeats (FIASCO). (Zane et al., 2002).

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2. Materials and methods

2.1. Samples collection and DNA extraction

A total of 24 individuals of *A. camptodontus* were collected from LJ (Yuhu village, Lijiang: 27°02'N, 100°13'E, 2735 m) and ZD (Geza village, Zhongdian: 27°53′N, 99°44′E, 3306 m) populations in NW Yunnan province. Fresh leaves were dried by silica gel in fieldwork. Total genomic DNA was extracted from dry leaves using modified CTAB method (Doyle and Doyle, 1987).

2.2. Isolation and development of SSR primers

Microsatellite loci were developed and isolated according to the method of FIASCO (Zane et al., 2002). Approximately 500 ng of total genomic DNA was digested with *Mse*I enzyme (New England Biolabs, Beverly, MA, USA), and then fragments were ligated to the *Mse*I AFLP adaptor pair (5′–TAC TCA GGA CTC AT–3′/5′– GAC GAT GAG TCC TGA G–3′) at 37°C for 2 h with T4 DNA ligase (Fermentas, Burlington, Ontario, Canada). A diluted digestion-ligation mixture (1:10) was amplified with adaptor-specific primers *Mse*I-N (5′–GAT GAG TCC TGA GTA AN-3′) by the following program: 95 °C for 3 min, 30 cycles of 94 °C for 30 s, 53 °C for 60 s, 72 °C for 60 s followed by an elongation step of 5 min at 72 °C. Amplified DNA fragments (200–800 bp) were enriched for microsatellite repeats by magnetic bead selection with 5′-biotinylated $(AC)_{15}$ and $(AG)_{15}$ probes, respectively. Captured fragments were re-amplified with adaptor-specific primer. Then PCR products were purified using an EZNA Gel Extraction Kit (Omega Bio-Tek, Guangzhou, China).

The purified PCR products with enriched microsatellite repeats were ligated into the pGEM-T vector (Promega, USA), and then transformed into DH5α cells (TaKaRa, Dalian, China). Recombinant clones were screened by blue/white selection and positive clones were tested by PCR with $(AC)_{10}$/$(AG)_{10}$ and T7/Sp6 Primers. Clones with positive inserts were sequenced with an ABI PRISM 3730XL DNA sequencer (Applied Biosystems, Foster City, California, USA). Program Oligo 6.0 was used to design locus – specific primers for those microsatellite sequences possessing sufficient flanking regions (Rychlik, 2010).

PCR amplification for all microsatellite loci were performed in 20 μl of reaction system containing 30–50 ng genomic DNA, 0.6 μM of each primer, 7.5 μl 2 × *Taq* PCR MasterMix (Tiangen, Beijing, China). It were conducted under the following program: 95 °C for 3 min, 30 cycles of 94 °C for 30 s, with the annealing temperature optimized for each specific primer for 30 s (Table 1), 72 °C for 60 s, and a final extension step at 72 °C for 7 min. Amplified fragments were separated on 8% polyacrylamide denaturing gels with a 20-bp ladder molecular size standard (Fermentas, Burlington, Ontario, Canada) by silver staining.

2.3. Data analysis

Microsatellite data was analysed by program GENEPOP 6.0 for the following parameters: observed heterozygosity (H_O), expected heterozygosity (H_E) and departure from Hardy–Weinberg Equilibrium (HWE) for 23 polymorphic loci (Raymond and Rousset, 1995).

Table 1
Characteristics of 25 microsatellite loci in *Astragalus camptodontus*.

Locus	Primer sequence (5′-3′)	Repeat motif	Size range (bp)	Ta (°C)	GenBank accession No.
De005[a]	F: TGAACTGTATCCATTTGGTG R: CTTATCGCACATCCCCCTAC	$(TG)^{9}$	150–178	51	JX993153
De006[a]	F: TTAGAAGAGCCAATCAACAG R: AAAGGATAGACACGAGGAGG	$(TG)^{11}$	274–310	50	JX993154
De010[a]	F: ATCACTCACTCAACCAACAA R: ACAGTTACACACAGGAGATG	$(TC)^{10}$	152–162	54	JX993155
De016[a]	F: GATGAGCGTGATGTGTTTGG R: TACACCTACATTGATGAAAGA	$(AG)^{19}$	211–233	49	JX993156
De020[a]	F: TACGCTTTCTTGGTGTGATG R: ATTGGATGCTTTGTTGTAGA	$(AG)^{9}$	190–208	50	JX993157
De022	F: GCTTTCATTTTGTTCTGGAT R: ACCTCACTCTCTCACACTTG	$(AG)^{18}$	234	50	JX993158
De030[a]	F: GTTTGTCCGCATCTCATCTC R: GGTAATCCGAATCCATCCAT	$(TC)^{10}$	219–283	52	JX993159
De040[a]	F: GACCTTTTGTAGCGTTTGAC R: GAAATGAGAGGAGGAAATGG	$(TC)^{14}$	100–133	54	JX993160
De041[a]	F: TCCTGAGTAAATAAAATCCC R: TATATCTGCCTTGGCTGGTC	$(TC)^{10}$	136–154	45	JX993161
De047[a]	F: CAACCCAGATTTTCCCCATT R: ACCAAAGGGCTCACATTCAC	$(AG)^{18}$	194–206	61	JX993162
De049[a]	F: GAGTCCTGAGTAAGTGAAGAT R: TTCGTCAAGGGTCATTACAT	$(TG)^{14}$	144–176	47	JX993163
De051[a]	F: GGAGTAGAAGAGGGAAAGCA R: AGGAAGAGAAGATGAAACAACAG	$(TC)^{9}$	213–217	50	JX993164
De052[a]	F: AAACCAAGAAACAGGAAACT	$(AG)^{27}$	175–205	57	JX993165

(*continued on next page*)

Table 1 (*continued*)

Locus	Primer sequence (5′-3′)	Repeat motif	Size range (bp)	Ta (°C)	GenBank accession No.
	R: AGAAAAACAATGTCAAGAGG				
De062[a]	F: CATCTCTTGTAACCTCTCTC	$(TC)^{13}$	133–165	55	JX993166
	R: ATTAGTCGTCCATCCCTTCG				
De063[a]	F: ATCAATGCCAAAAGAGAGAG	$(AG)^{15}$	172–182	52	JX993167
	R: TCAGTTTCATTCCCTCATTC				
De066	F: CACCATAATCAACGCCACTC	$(TC)^{14}$	173	53	JX993168
	R: GGGAGGGAAAATAACTAACC				
De068[a]	F: GCTAACAACAAGAACGCAAT	$(AG)^{22}$	264–304	59	JX993169
	R: TGACTCCACTATACAAATCC				
De072[a]	F: CCTTTCTCACTAACCCTAAT	$(TTC)^{13}$	178–200	49	JX993170
	R: CAACAGGAACGCACATAGCA				
De075[a]	F: TGGAGCATACAACACAAACA	$(TC)^{16}$	197–213	52	JX993171
	R: TCATTTCATCTGCGGGTAAC				
De079[a]	F: GAGAGGGAAAGAAAAGAATG	$(AG)^{12}$	257–267	60	JX993172
	R: ATGATGCTTTAGAATTGACG				
De081[a]	F: AACACCACAACAACAAATCG	$(AG)^{12}$	260–294	49	JX993173
	R: CTCACAAGCATAGGTTCTCT				
De082[a]	F: TGTCTCCTCTCGGCAATCA	$(TC)^{16}$	116–148	52	JX993174
	R: TTAGGTCAAAATTCAGGGTA				
De087[a]	F: TGAAGAGGAGGATGAAATGA	$(TC)^{11}$	179–187	52	JX993175
	R: TGCCCAAAAACAACAAGAAT				
De088[a]	F: CCACTTACAAGAGCACCTAT	$(AG)^{15}$	228–246	55	JX993176
	R: ATCTCAAACAAACACATCCT				
De097[a]	F: TTCCATTCTCCCTTCCCTCT	$(AG)^{7}\ldots(AG)^{8}$	191–199	55	JX993177
	R: CGCTGTTCCTCCTCTCTTCT				

Note: [a] displayed polymorphisms in *Astragalus camptodontus*; Ta, PCR annealing temperature.

Table 2
Result of 23 polymorphic microsatellite loci screening with 24 individuals of *Astragalus camptodontus*.

Locus	N_A	H_O	H_E	Locus	N_A	H_O	H_E
De005[a]	5	0.739	0.776	De062	4	0.429	0.652
De006[a]	5	0.875	0.681	De063[a]	5	0.458	0.751
De010[a]	3	0.042	0.254	De068[a]	4	0.625	0.732
De016[a]	5	0.542	0.712	De072[a]	4	0.875	0.744
De020[a]	5	0.167	0.715	De075[a]	5	0.417	0.698
De030	5	0.375	0.444	De079[a]	5	0.238	0.685
De040	5	0.583	0.684	De081[a]	3	0.286	0.595
De041	4	0.458	0.684	De082[a]	4	0.083	0.511
De047[a]	3	0.250	0.520	De087[a]	3	0.091	0.417
De049[a]	5	0.458	0.609	De088[a]	4	0.450	0.706
De051[a]	2	0.125	0.395	De097[a]	3	0.083	0.421
De052[a]	5	0.625	0.793				

Note: N_A, number of alleles revealed; H_O, observed heterozygosity; H_E, expected heterozygosity.
[a] Statistically significant deviation from Hardy–Weinberg equilibrium (HEW) ($P < 0.01$).

3. Results and discussion

A total of 278 clones with positive inserts were sequenced, among which 184 sequences were found to contain simple sequence repeats (SSR). Finally, 100 sequences that had appropriate microsatellite and enough flanking region were selected for primer design. In these primers, 25 amplified target regions successfully and 23 of them displayed polymorphism (Table 1).

The number of alleles ranged from 2 to 5 in all individuals sampled from the studied populations in this study. Values for H_O and H_E ranged from 0.042 to 0.875 and from 0.254 to 0.793, with average of 0.403 and 0.616, respectively (Table 2). Among the 23 polymorphism loci, 19 showed significant deviation from Hardy–Weinberg Equilibrium (HEW) ($p < 0.01$), probably due to heterozygote deficiency or limited sampling. High discriminatory power of these microsatellite loci suggested that they should be suitable for researches on genetic diversity and population structure in this species and its allied species. This work will help to develop viable strategies for the conservation and management of *A. camptodontus*.

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