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Development and characterization of microsatellite loci in *Taihangia rupestris* (Rosaceae), a rare cliff herb[1]

Hong-Wei Wang[2,5], Bing Zhang[3], Zhao-Shan Wang[4], Yue-Qin Cheng[2], and Yong-Zhong Ye[3]

[2]College of Plant Protection, Henan Agricultural University, Zhengzhou 450002, China; [3]College of Life Sciences, Henan Agricultural University, Zhengzhou 450002, China; [4]State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

- *Premise of the study*: Microsatellite primers were developed for the rare *Taihangia rupestris* (Rosaceae) to evaluate genetic diversity, population genetic structure, mating system, and demographic events of this species.
- *Methods and Results:* Ten primer sets were developed using an enriched genomic library and were successfully amplified in *T. rupestris* var. *ciliata* and *T. rupestris* var. *rupestris*. The number of alleles per locus ranged from 2 to 21; the observed and expected heterozygosities ranged from 0.300 to 0.950 and from 0.328 to 0.956, respectively, in the two varieties.
- *Conclusions:* The markers described here will be useful for studies of genetic variation, genetic structure, and mating systems of *T. rupestris*, which are important for the future conservation of this rare species.

Key words: microsatellite marker; population genetics; *Taihangia rupestris*.

Taihangia rupestris Yu & Li, the only species of *Taihangia,* belongs to the family Rosaceae and contains two varieties, *T. rupestris* var. *ciliata* and *T. rupestris* var. *rupestris* (Yu and Li, 1980). It is endemic to China where it has a disjunct distribution and occurs sporadically across the limestone regions of the eastern margin of the Taihang Mountains (Shen et al., 1994). *Taihangia rupestris* is a valuable wildflower resource and has been named "rare cliff flower" in the Taihang Mountains (Xu et al., 2006). In addition, it is an important species in the evolutionary study of the tribe Dryadeae because of its important systematic position (Yu and Li, 1983). However, the number and size of *T. rupestris* populations have decreased rapidly in past decades, and now only a few, small populations of *T. rupestris* var. *ciliata* and *T. rupestris* var. *rupestris* can be found. Therefore, this beautiful wildflower has been registered on the *China Species Red List* (Wang and Xie, 2004). To protect this rare species, it is critical to investigate its population genetics, including its genetic variation, population genetic structure, and mating systems.

Simple sequence repeat (SSR) markers have been used effectively in conservation biology and molecular ecology with the virtues of hyper-variability, codominance, and high reproducibility (Huang et al., 2010; Sharma et al., 2010). Here, we report the development and characterization of 10 polymorphic microsatellite loci, which will facilitate the study of genetic diversity and sustainable conservation for *T. rupestris*.

METHODS AND RESULTS

Total genomic DNA was extracted from silica-gel-dried leaves using the modified CTAB method (Fang et al., 2009).We constructed a microsatellite-enriched library following Tian et al. (2008) with some modifications. Briefly, genomic DNA was digested with *Rsa* I and *XMn* I, and three kinds of single-strand biotinylated microsatellite probes $(CA)_{15}$, $(GA)_{12}$, $(GAT)_{15}$ were further used for target fragment enrichment. After solid culturing, we screened the positive clones by PCR amplification using SP6 and T7 primers, and finally, 36 positive clones were selected and sequenced on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, California, USA), of which 25 (about 70%) contained SSRs.

PCR primers were designed for 18 sequences using program PRIMER 5.0 (http://www.premierbiosoft.com). These primers were tested for polymorphisms in 24 *T. rupestris* individuals that came from 10 different populations. PCR amplifications were performed in 15-μL total volumes containing ~75 ng genomic DNA, 10 μM of each primer, and 1×PCR Mix (Tiangen Biotech, Beijing, China). Microsatellites were amplified under the following conditions: 5 min initial denaturation at 95°C, 35 cycles of 30 s at 94°C, 30 s at 55–64°C (Table 1), 1 min at 72°C, and a final extension of 72°C for 10 min. The products were checked with 2.0% agarose gels. Ten pairs of primers (Table 1) showed single and clear bands in both varieties. The forward or reverse primers screened were labeled with the fluorescent dye (FAM or HEX) for polymorphism detection.

Forty *T. rupestris* individuals were used to characterize the microsatellite loci, of which 20 individuals were sampled from five *T. rupestris* var. *ciliata* populations, and the other 20 individuals from five *T. rupestris* var. *rupestris* populations (Appendices 1, 2). The number of alleles per locus (*Na*), the observed and expected heterozygosity (H_O and H_E),the deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium among loci were analyzed using the computer program Arlequin ver. 3.1 (Excoffier et al., 2005).

Ten primers were successfully amplified in all samples of the two varieties. Similar genetic diversity was found in two varieties, and the number of alleles

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[5] Author for correspondence: whwcas@yahoo.com.cn

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TABLE 1. Characteristics of 10 polymorphic microsatellite loci in *Taihangia rupestris*. Information on each primer pair includes forward and reverse sequences, the repeat motif, size range of the original fragment (bp), annealing temperature (Ta), and GenBank accession number.

Locus	Primer Sequence (5′–3′)	Repeat Motif	Size Range (bp)	Ta (°C)	GenBank Accession No.
THH2	F: GAGCGGTTGTTAGGGTGGT	$(GA)_{10}$	284–300	60	HM140574
	R: GGAGTCCTTTTCAGGCATT				
THH5	F: ACAAAATGTAAGTTAACCACA	$(AG)_{26}$	113–151	55	HM140576
	R: CACCAACAACACTAGAAGTAG				
THH7	F: GTGGTGCTGATTCTGGTGT	$(TC)_{11}$	347–379	60	HM140578
	R: CCTACTGTTGCGGTGGTT				
THH8	F: TACAAGAAGGTAAAGCCACA	$(GA)_{16}$	441–472	55	HM140579
	R: ATGAGCTACAGGTTAAGGGT				
THH14	F: AACTGATGGGAAGGAGG	$(GA)_{22}$	163–229	63	HM140581
	R: CAAAGGATGTTGTGGGA				
THH15	F: CAACAAAAAACCTACACA	$(AG)_9(AG)_9$	250–272	63	HM140582
	R: GAGATAGCCCAGACACTC				
THH19	F: TGGACGATGAAGACAAGAT	$(GA)_{19}$	325–373	60	HM140580
	R: TGGACGAATGACCACTATG				
THH3	F: CTCTTTGCATCCGCTTCT	$(AG)_{22}$	367–429	56	HM140575
	R: CAGGGTTATCGGTGGTGT				
THH6	F: GCTTGGATGAGAATCTAAAGGACT	$(TG)_9$	227–244	60	HM140577
	R: TTGTGAAAATCAGAACTTCGCTC				
THH17	F: CCGACCACCAAATCACTC	$(CA)_5(CA)_6(CA)_6$	320–326	64	HM140583
	R: TCAAGCAATCTCCTTCCG				

TABLE 2. Results of initial primer screening in *Taihangia rupestris*. For each primer pair, the number of alleles (*Na*), average observed heterozygosity (H_O) and expected heterozygosity (H_E) are reported. Sample size within each variety (*N*) is indicated in parentheses. Deviations from Hardy–Weinberg: * $P < 0.01$

	T. rupestris var. *ciliata* (*N* = 20)			*T. rupestris* var. *rupestris* (*N* = 20)		
Locus	*Na*	H_o	H_E	*Na*	H_o	H_E
THH2	9	0.750	0.850	7	0.450	0.576
THH5	9	0.600	0.797	10	0.650	0.724
THH7	13	0.300*	0.892	9	0.350	0.474
THH8	10	0.300*	0.842	11	0.400*	0.812
THH14	10	0.400*	0.791	12	0.350*	0.869
THH15	6	0.450*	0.731	4	0.550	0.704
THH19	15	0.800*	0.921	21	0.900	0.947
THH3	21	0.950	0.945	17	0.850	0.956
THH6	7	0.650	0.768	4	0.450	0.560
THH17	2	0.300	0.328	3	0.350	0.488
Average	10.2	0.550	0.787	9.8	0.530	0.711

per locus ranged from 2 to 21, with a mean of 10.2 and 9.8 in *T. rupestris* var. *rupestris* and *T. rupestris* var. *ciliata*, respectively (Table 2). The observed and expected heterozygosity per locus ranged from 0.300 to 0.950 and from 0.328 to 0.956, respectively. Two loci (THH8, THH14) showed significant deviation from HWE ($P<0.01$) in *T. rupestris* var. *rupestris*, and five loci (THH7, THH8, THH14, THH15, THH19) in *T. rupestris* var. *ciliata*, due to heterozygote deficiency. No significant linkage disequilibrium was detected among all pairs of loci.

CONCLUSIONS

Moderate levels of genetic diversity and the excess in heterozygosity in seven loci suggest that the newly developed 10 polymorphic microsatellite markers described here for *T. rupestris* are suitable for population genetic studies, including evaluation of genetic diversity and population genetic structure, and investigation of mating system and demographic events of this species.

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APPENDIX 1. Geographic localities and sample sizes of *Taihangia rupestris* populations in this study.

Population	Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	Sample Size
T. rupestris var. *rupestris*					
DPB	Dapubu, Henan	35°28′16″	113°19′41″	610	4
YDS	Yidoushui, Henan	35°28′15″	113°23′34″	980	4
LHP	Lianhuapen, Henan	35°44′08″	113°36′25″	1321	4
QPG	Qianpugou, Henan	36°01′23″	113°39′30″	1070	4
XXT	Xiaoxitian, Henan	36°03′06″	113°40′02″	1435	4
Total					20
T. rupestris var. *ciliata*					
HHS	Huanghuashan, Henan	36°05′21″	113°42′07″	1533	4
JG	Jiaogou, Hebei	36°30′20″	113°38′09″	990	4
SSM	Sanshimu, Shanxi	36°40′32″	113°24′18″	767	4
NY	Nanyao, Shanxi	36°45′08″	113°31′27″	1294	4
DLG	Donglinggou, Hebei	36°56′47″	113°46′13″	1496	4
Total					20

APPENDIX 2. Herbarium voucher information.

Taxon; Population; Herbarium voucher accession code.

Taihangia rupestris* var. *rupestris Yu & Li; DPB; 2008-Wang-01; ***Taihangia rupestris var. rupestris*** Yu et Li; YDS; 2008-Wang-02; ***Taihangia rupestris* var. *rupestris*** Yu & Li; LHP; 2008-Wang-03; ***Taihangia rupestris* var. *rupestris*** Yu & Li; QPG; 2008-Wang-04; ***Taihangia rupestris* var. *rupestris*** Yu et Li; XXT; 2008-Wang-05; ***Taihangia rupestris* var. *ciliata*** Yu & Li; HHS; 2008-Wang-06; ***Taihangia rupestris var. ciliata*** Yu & Li; JG; 2008-Wang-07; ***Taihangia rupestris* var. *ciliata*** Yu & Li; SSM; 2008-Wang-08; ***Taihangia rupestris* var. *ciliata*** Yu & Li; NY; 2008-Wang-09; ***Taihangia rupestris* var. *ciliata*** Yu & Li; DLG; 2008-Wang-10.