American Journal of Botany

American Journal of Botany: e000–e000. 2011.

AJB Primer Notes & Protocols in the Plant Sciences

Isolation and characterization of 20 new microsatellite loci in *Coriaria nepalensis* (Coriariaceae)[1]

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- *Premise of the study*: Microsatellite markers were developed for *Coriaria nepalensis,* a traditional Chinese medicinal and ornamental plant, to investigate its genetic diversity and population genetic structure as well as its evolutionary history.
- *Methods and Results*: Twenty-eight dinucleotide microsatellite loci were identified in *C. nepalensis*, 20 of which showed polymorphism among five populations. The expected heterozygosities were 0–1 (mean 0.469).
- *Conclusions*: These markers may be useful for further investigation of the population genetics, systematics, and phylogeography of *Coriaria nepalensis*.

Key words: *Coriaria nepalensis*; genetic diversity; microsatellite; polymorphism.

Coriaria is the only genus of Coriariaceae and is widely distributed across four separate areas of the world: East Asia, the Philippines and Pacific islands (New Zealand), Central and South America (from Mexico to Chile), and the Mediterranean. There are three species of *Coriaria* in China. One of these, *Coriaria nepalensis* Wall., is a common native shrub species in the central Himalayan region and Southwest China with an elevation of 200–3200 m (Min & Brach, 2008). It is a pioneer species in secondary forests in hilly regions and is a common ornamental plant. *Coriaria nepalensis* is a traditional Chinese medicinal plant used to treat numbness, toothaches, traumatic injuries, and acute conjunctivitis (Dictionary of Traditional Chinese Medicine, 1977). Previous research on *C. nepalensis* has focused on the cytogenetics (diploid, $2n = 40$) (Oginuma et al., 1991), chemical constituents (Shen et al., 2004), and molecular phylogeny (Yokoyama et al., 2000). Comparatively little work has been done on its population genetics. Therefore, in this study, 20 polymorphic microsatellite loci of *C. nepalensis* were developed as potential tools to investigate the genetic structure and genetic diversity of *C. nepalensis* populations. The classification of *Coriaria* species is controversial, with unresolved issues about species affinities and the number of constituting species. Therefore, identifying polymorphic microsatellite loci may provide a helpful tool to increase knowledge about the taxonomy and phylogeography of *Coriaria* and produce useful insights about its biogeography.

[1]Manuscript received 31 December 2010; revision accepted 26 January 2011.

This study was supported by grants from the National Natural Science Foundation of China (NSFC 30900085 to J. H. Chen), Yunnan Natural Science Foundation (2010CD109 to J. H. Chen), the Xibuzhiguang (Light in Western China) project of the Chinese Academy of Sciences (to J. H. Chen).

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doi:10.3732/ajb.1100001

METHODS AND RESULTS

Genomic DNA was extracted from leaf tissue using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). We constructed an enriched partial genomic library for the repeat motif (AG)n, and then isolated simple sequence repeats (SSRs) using the protocol suggested by Hauswaldt and Glenn (2003). Restriction enzymes RsaI and BSt1 (Fermentas, Burlington, Ontario, Canada) was used to completely digest 500 ng of genomic DNA. The DNA was then ligated to SuperSNX linkers. For enrichment, the ligated products were hybridized with a 5′-biotinylated probe $(AG)_{15}$ in the 100-µl hybridization solution (12× SSC and 0.2% SDS) at 95°C for 5 min, quickly ramped to 48°C, and maintained this temperature for 2 h. Hybridized DNA was then mixed with Streptavidin-coated magnetic beads (Promega, Madison, Wisconsin, USA). Captured DNA was recovered as the template to amplify using the SuperSNX-Forward primer (5′-GTTTAAGGCCTAGCTAGCAGAATC-3′). PCR products were purified with a PCR products purification kit (TIANGEN, Beijing, China), and then these fragments containing microsatellite loci were cloned using pMD18-T vector (Takara, Dalian, Liaoning, China) according to the manufacturer's instructions and propagated in an *E. coli* DH5α strain. Positive clones were amplified using $(AG)_{10}$ and M13 primers. PCR products ranging from 200 to 800 bp were selected for sequencing. Of the 81 clones sequenced, 80 contained potential microsatellite motifs. Of these, 42 clones with unique microsatellites were selected for designing primers using Primer 5.0 (Clarke & Gorley, 2001).

Polymorphism was evaluated in 45 individuals of *C. nepalensis* from five populations in the Yunnan Province of Southwest China. These included 11 individuals from the ZXS population, nine from BG, 10 from LJ, eight from ML, and seven from SM (Table 1). Voucher specimens were deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences. PCR was performed in 20-µL volume using a PTC0200 thermal cycler (BIO-RAD, Foster City, California, USA). Each reaction was performed using 20 ng of genomic DNA, 1 µM of each dNTP, 1µM of each primer, 1× *Taq* buffer (100 mM Tris–HCl, pH8.8, 2.0 mM $MgCl_2$, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20) and 1 U of *Taq* polymerase (Takara, Dalian, Liaoning, China). The PCR program was: initial denaturing step at 94°C for 3 min, 30 cycles of 94°C for 30 s each, primer-specific annealing temperature of 50–58°C for 30 s, 72°C for 60 s, and a final extension step at 72°C for 8 min. The PCR products were electrophoresed in denaturing 6% polyacrylamide gels using a 25-bp DNA ladder molecular size standard (Fermentas, Burlington, Ontario, Canada) to estimate allele sizes by silver staining.

Of the 42 new primers, 28 primer pairs successfully amplified products with expected size. Twenty of them displayed polymorphism across populations,

TABLE 1. Sample information of *Coriaria nepalensis*

Population	Location	Position	Altitude (m)	Sample size	Voucher (Herbarium)
ZXS	Zixi Shan, Chuxiong, Yunnan Province	25°04′15.5″N, 101°25′17.5″E	2016	11	*JH Chen 1051* (KUN)
BG	Botanic Garden, Kunming, Yunnan Province	25°08′28.05″N, 102°44′29.69″E	1961	9	*JH Chen 1052* (KUN)
LJ	Yulong Snow mountain, Lijiang, Yunnan Province	27°00′31.82″N, 100°11′52.63″E	2870	10	*JH Chen 1053* (KUN)
ML	Malong River, Chuxiong, Yunnan Province	24°31′15.4″N, 101°34′42.2″E	1912	8	*JH Chen 1054* (KUN)
SM	Aziyin, Songming, Yunnan Province	25°32′17.84″N, 102°51′12.41″E	1812	7	*JH Chen 1055* (KUN)

TABLE 2. Characteristics of 28 identified microsatellite loci for *Coriaria nepalensis* Wall.

Primer	Repeat motif	Primer sequence (5′–3′)	Allele Size range (bp)	T_a (°C)	Locus (GenBank Accession No.)
CJH21	$(AG)_9$	F: ATTCTCCATTACTGCTCCTG R: TACCTCCAAATCAACACCTC	338–346	56	GU564484
CJH 49	$(AGA)_8(TC)_7$	F: TTGGGGAAAGATGAAAAGGT R: TTGTGCGTAAGGGATAGAAA	301–309	50	GU564483
CJH 93	$(CT)_6(AG)_6$	F: GGTTGTGAGGACGAATAAGG R: TTTGAAGCAATAATGGCAGA	386–394	50	GU564485
CJH 33	$(TC)_9$	F: ATTAACTTCGTTTTCCTCAA R: TGTCCACTCCTTTACTATTT	350–354	50	GU564486
CJH 52	$(TCTT)_3(GA)_5$	F: ACAATTACAGCACCACCATC R: CGTTTCAATCGCATCTATCT	236–250	58	GU564487
CJH 79	$(TCTATC)_2(CACAAA)_2$	F: TTTTCCCTCACAATCTTCAA R: ATTATTCTCCGACGATTTCT	206–220	52	GU564488
CJH 83	$(AT)_6(AT)_4$	F: ATCACGAAGACTGCCACAAA R: TGCGGTTACAGTAGAAGAAG	299–303	50	GU564489
CJH 92	$(TC)_{10}$	F: TTGCGACGAAGTTTGCTCAG R: TCTCGCTCCTTCCTTTCCAG	338–344	52	GU564490
CJH 2	$(GA)_8$	F: TTGCTCTTCTTTTATTGCTT R: AAAGAGGTCATGCTTACGAT	391–401	52	GU564491
CJH 3	$(CT)_{18}$	F: TTAATCCCAACTTAGCTTCT R: CACTCCATATCCCCTTCCCT	339–345	52	GU564492
CJH 20	$(TC)_8$	F: AAAGGAACGGAAAGACAAGC R: TTCAATCAAGAATCGAGGAG	240–248	54	GU564493
CJH 22	$(TCCC)_2(CT)_4$	F: GCGTTGAGTGGCAAATAAGT R: GGCAACAGAAGAAAAGGAAA	348–358	58	GU564494
CJH 24	$(TC)_4(CT)_4(CT)_4$	F: CCGGTGCGCTTGTTTGTTAG R: CTCCGCCTTGTTTCACTTTG	296–302	50	GU564495
CJH 42	$(AG)_{10}$	F: GCCGTCTTTGTAGATGAGTG R: TGCTAAACGCCTAAAGGATA	304–316	50	GU564496
CJH 45	$(TC)_{13}$	F: AGACAGAGCTTCTGCGTTTC R: GACCTTCTCGACAGCATCAT	291–301	52	GU564497
CJH 56	$(TC)_9$	F: ACTGGGATTAAAGAAGAAGG R: AGCTCAAGGCTAGGGAAGAG	262–266	50	GU564498
CJH 57	$(GA)_{10}$	F: AGCCTTCCAGCTTCTTTTC R: TTTTCCACCCTCTTATGCA	339–351	50	GU564499
CJH 63	$(CT)_{10}$	F: AGATTGGGAAGTAGGGAATT R: CTCTTTGCCTCTTTGTTGTG	339–345	50	GU564500
CJH 82	$(AG)_9$	F: TTGGAGTCCAACTCCGTCAC R: CAGAGCATAAGCATAGAAGC	396–402	50	GU564501
CJH 83	$(AG)_9$	F: CCATTTGTTACAGCTCGTA R: TGTCTTGGGAAGTGGGATT	273–279	50	GU564502
CJH 12*	$(CT)_8$	F: AAGTGACCAGGACCCGAAGA R: GAAATAGACGCCGAAGGAGC	375	56	HQ896723
CJH 67*	$(TC)_6(TC)_4$	F: CACAACACTCACCAAACAAA R: CGGTAAAAGAGGAAACAGGA	236	52	HQ896724
CJH 69*	$(CT)_5(GAGT)_3$	F: CAGTTCCCACTTTCATTTCC R: ATGCTCCTCCTACTCCTTCG	339	50	HQ896725
CJH 58*	$(CT)_6$	F: ATTCTACCTCGGTTTCTCAG R: TTATCTTCTTGTGCCATTTC	290	50	HQ896726
CJH 68*	$(GA)_{10}(GA)_4$	F: TCCGTCCGATCTGATTCTGC R: TCTTCTTGGGCTTGGTGGTG	295	50	HQ896727
CJH 16*	$(CT)_6(TC)_6$	F: TCCAGATCGGATACCTAACG R: AAGCAATTTCTTACCGCAGA	289	50	HQ896728
CJH 59*	$(AG)_6(GA)_5$	F: TTCTGCAAGCGAAGTATTT R: ATCTTCCATTGGCACTTAC	256	50	HQ896729
CJH 4*	$(TC)_8$	F: CCCTGAAACAGAGGAAACT R: GAGTAAACAGAAGCGTGCA	303	50	HQ896730

Ta, annealing temperature; * monomorphic microsatellite marker.

TABLE 3. Results of initial primer-screening in *Coriaria nepalensis* Wall.

	ZXS (*N* = *11*)		BG (*N* = *9*)		LJ (*N* = *10*)		ML (*N* = *8*)		SM (*N* = *7*)		
Locus	A	H_E	A	H_E	A	H_E	A	H_E	A	H_E	HWE (*P*-value)
GU564484	3	0.5111	3	0.6667	3	0.6667	2	0.6667	2	0.5556	0.0121
GU564483	4	0.6444	2	0.5303	2	0.3030	2	0.6667	3	0.6664	0.7083
GU564485	2	0.5556	2	0.5455	2	0.5303	2	0.5000	2	0.5334	0.0001*
GU564486	2	0.3556	1	0	3	0.6818	1	0	2	0.3556	0.0005*
GU564487	1	0	2	0.4849	1	0	2	0.5000	1	0	1.0000
GU564488	3	0.5111	2	0.5455	2	0.4849	2	0.6667	3	0.5111	1.0000
GU564489	2	0.4667	2	0.5455	2	0.1667	2	0.6667	1	0	1.0000
GU564490	2	0.3556	2	0.1667	3	0.3182	1	0	3	0.7111	0.0202
GU564491	2	0.3556	4	0.7727	3	0.3182	2	0.6667	2	0.4667	0.3788
GU564492	3	0.6444	5	0.8030	2	0.5455	3	0.8334	3	0.6444	0.0000*
GU564493	3	0.7111	3	0.5455	3	0.6818	3	0.8334	3	0.7111	0.0000*
GU564494	3	0.6000	3	0.5303	3	0.5303	2	0.6667	2	0.3556	0.0295
GU564495	3	0.5111	2	0.5455	2	0.5303	2	0.6667	2	0.5556	0.0004*
GU564496	3	0.6222	4	0.6364	4	0.7727	2	0.6667	3	0.6889	0.0801
GU564497	3	0.5111	4	0.6515	2	0.3030	3	0.8334	4	0.7111	0.2776
GU564498	2	0.2000	2	0.4091	2	0.1667	2	0.5000	2	0.2000	1.0000
GU564499	2	0.2000	4	0.7727	3	0.6667	4	1.0000	3	0.5111	0.0837
GU564500	2	0.3556	3	0.3182	1	0	1	0	2	0.2000	1.0000
GU564501	2	0.3556	1	0	3	0.4394	1	0	2	0.3556	0.0035*
GU564502	2	0.3556	2	0.5455	2	0.5303	1	0	2	0.3556	0.0543

A, number of alleles; H_E, expected heterozygosity; statistically significant deviation from Hardy–Weinberg equilibrium is indicated by * ($P < 0.01$).

whereas eight primer pairs displayed monomorphism (Table 2). The number of alleles per locus, expected heterozygosity (H_E), and deviation form Hardy–Weinberg equilibrium (HWE) were assessed using GENEPOP version 3.4 (Raymond & Rousset, 1995). The number of alleles per locus ranged from 2 to 5 with an average of 3.2 (Table 3). The expected heterozygosities (H_E) ranged from 0 to 1, with an average of 0.469 (Table 3). Among the 20 polymorphic microsatellite markers, six loci showed significant deviation from Hardy–Weinberg equilibrium ($P < 0.01$) (Table 3). These were due to deficiency of heterozygotes or the limited sample size. Tests for linkage disequilibrium were run in FSTAT version 2.9.3.2 (Goudet, 1995). In additional, no loci showed significant linkage disequilibrium between locus pairs after the Bonferroni correction.

CONCLUSIONS

Twenty of the 42 microsatellite makers are polymorphic. These newly developed nuclear microsatellite markers will be a useful tool for studying the population genetics of *Coriaria nepalensis*. In additional, the set of novel makers are also helpful for further studies of the systematics, phylogeography and evolutionary history of *Coriaria*.

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