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MICROSATELLITE LETTERS

Isolation and characterization of 18 microsatellites for *Aconitum vilmorinianum* Kom. (Ranunculaceae) using next-generation sequencing technology

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Abstract *Aconitum vilmorinianum* is a very important Chinese traditional medicinal plant, which is one of the main raw materials in Yunnan Baiyao as an analgesic and anti-inflammatory agent. Recently, it has been threatened by overexploitation and human disturbances. Genetic background of this species is essential for the efficient conservation and rational utilization. Therefore, a total of 18 novel microsatellite markers were developed for *A. vilmorinianum* using next-generation sequencing technology. The number of alleles per locus ranged from 1 to 5, with a mean of 2.560, and the observed and expected heterozygosities ranged from 0 to 1.000 and from 0 to 0.733, respectively. These polymorphic microsatellite loci will be especially useful for genetics studies of this important medicinal plant.

Keywords *Aconitum vilmorinianum* · Microsatellite markers · Genetics · Medicinal plant

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Aconitum L. (Ranunculaceae) is a large genus with about 300 species and mainly distributes in the temperate regions of the Northern Hemisphere. About 76 *Aconitum* species in China are poisonous and have been used as medicinal plants mainly for treatment of rheumatoid arthritis and various types of pains (Xiao et al. 2006). These species have similar organoleptic characteristics with different toxicities and chemical constituents. *Aconitum vilmorinianum* Kom. is a climbing perennial herb endemic to China, and wildly distributed in the montane shrubland of Yunnan, Sichuan and Guizhou Provinces. The tuberous mother roots of *A. vilmorinianum* ('Huangcaowu', used by Chinese druggists), is one of the main raw materials in Yunnan Baiyao, which is Chinese Class-1 protected traditional medicine and has been honored as 'trauma panacea' for more than a century (Li et al. 2009). *Aconitum vilmorinianum* has been used as a popular analgesic and anti-inflammatory agent (Li et al. 2013) for long, and commonly used as a substitute of Aconiti Radix and Aconiti Kusnezoffii Radix (Wan et al. 2007). Due to years of overharvesting and anthropogenic disturbances, the wild resources of *A. vilmorinianum* have reduced dramatically. Therefore, effective conservation and rational utilization strategies are urgently needed, based on comprehensive evaluation of the genetic variation and structure of this species In the present study, we isolated 18 novel microsatellite markers from the genome of this important medicinal plant using next-generation sequencing techniques.

Genomic DNA was isolated from approximately 10 mg of each leaf sample using DNeasy Plant Mini Kit (QIAGEN). Extracted DNA was used for a library preparation with a NEBNext *R* DNA Library Prep Master Mix Set for Illumina. The sequencing was performed on the MiSeq Benchtop Sequencer (Illumina) using the 2 × 250 bp read mode. The obtained data was assembled using CLCGenomicWorkbench

Table 1 Descriptive statistics over all loci for the two natural populations of *Aconitum vilmorinianum*

Locus name	NCBI GenBank accession no.	Primer sequence (5′-3′)	Repeat motif	Size (bp)	Ta (°C)	Kunming			Wuding		
						N_a	H_o	H_e	N_a	H_o	H_e
Av_7306	KP275580	F:AGTTTCCATATTTTCGTG R:GGAGTTCTACAAGGTTTT	$(AGA)_5$	141	45	3	0.417	0.351	3	0.250	0.226
Av_11592[a]	KP275583	F:CTAAACGACCGACCTTGT R:GTTGTTCTGCTGCCTCTT	$(TC)_6$	165	51	3	0.750	0.601	4	0.818	0.682
Av_13712	KP275585	F:AGCCGTGTCACTCTATCC R:AGGACGAGTTATTTCTGC	$(TTA)_5$	472	49	2	0.000	0.153	2	0.000	0.278
Av_19762	KP275590	F:TAGTCCGTGCTTGTATGA R:CGGTGTTTAGAAGGTTAG	$(GA)_6$	142	47	2	0.083	0.080	3	0.750	0.538
Av_32854	KP275593	F:GGTGCCTTATCTGCGTGT R:TCTTCTCGGGTCTTCTCC	$(TTC)_7$	99	47	4	0.583	0.656	4	0.500	0.642
Av_33511[a]	KP275594	F:CCGCCAAATGTCTAATCG R:CAGGAGGGAGTGGTTGAT	$(GT)_5$	108	50	2	0.400	0.320	3	1.000	0.569
Av_38645	KP275598	F:TGAGACGGTTGTGGCTGT R:AGGTGAAACGAAAGGTGG	$(GA)_6$	145	53	2	0.167	0.153	2	0.417	0.330
Av_38749	KP275599	F:AAAGGAGGATGGGTCAAA R:CAAGTTGTTCGCAGTGTTC	$(CT)_5$	231	52	3	0.833	0.531	3	0.500	0.559
Av_39204	KP275600	F:GTGACTCAATGGTATGGG R:AAGGAGTATGTAGGCAACC	$(CTT)_6$	235	47	2	0.250	0.497	3	0.500	0.573
Av_40439	KP275601	F:AATCTCCCACCTTGTTCT R:AAGCATCGGTAAGTCGTT	$(GA)_{11}$	233	48	5	0.500	0.733	5	0.333	0.604
Av_43058	KP275605	F:TTCTCCCAGTGCTGACCT R:CCCCTGAAACTCCTAACA	$(AC)_6$	192	51	2	0.583	0.413	2	0.417	0.330
Av_52852	KP275610	F:TTCAGTTTTCCCTGTTGT R:TGGTTGTCTTGGTTCTTA	$(TGA)_5$	127	43	3	0.667	0.538	2	0.583	0.497
Av_54680	KP275611	F:ACATGGACCAATTCCCAC R:TCATCCCAGATTCCCAGTG	$(ATT)_6$	228	48	3	0.417	0.542	3	0.417	0.344
Av_62869	KP275615	F:ATCTTAAACCTCCAACCC R:TTCTTTCTCAAGGCAATC	$(TTC)_5$	168	48	2	0.333	0.375	2	0.667	0.444
Av_70958	KP275618	F:CAAACCATTACTGCTACT R:GATAACTATTCCTCACCT	$(GAA)_5$	115	43	2	0.417	0.469	2	0.333	0.486
Av_77766	KP275622	F:ACTCCACGCACTTCAACA R:CAGGATTTCAACCAGCAA	$(TC)_6$	155	50	2	0.333	0.278	2	0.167	0.153
Av_83799	KP275624	F:CAAGATTAGGCGTCGGTA R:CCACTCATCCGTGTTATT	$(GT)_6$	186	47	1	0.000	0.000	3	0.417	0.351
Av_84257	KP275625	F:CCAGGGTCAGCATCTTCTT R:CACTCAGTAATCCGAAACG	$(TC)_5$	238	52	3	0.333	0.497	3	0.250	0.569

Ta PCR annealing temperature, N_a number of allelesC revealed, H_o observed heterozygosity, H_e expected heterozygosity

[a] Indicate significant departures from Hardy–Weinberg equilibrium in Wuding population ($p < 0.01$)

(CLCBio) into 87,846 contigs and the microsatellites were then detected using QDD 2.1 Beta (Meglecz et al. 2010). A total of 2204 contigs contained at least one microsatellite of which 51 loci were selected for initial screening. In all, 24 individuals from two populations (Kunming and Wuding, Yunnan Province) were screened for polymorphisms at these loci.

PCR reactions were performed in 15 μL reaction containing 30–50 ng genomic DNA, 0.6 μM of each primer, 7.5 μL 2 × Taq PCR MasterMix [Tiangen (Tiangen Biotech, Beijing China); 0.1 U Taq polymerase/μL, 0.5 mM dNTP each, 20 mM Tris–HCl (PH 8.3), 100 mM KCl, 3 mM $MgCl_2$]. PCR amplifications were conducted on ABI thermocycler under the following conditions: 95 °C for

3 min followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at the optimized annealing temperature for each specific primer (Table 1; each primer pair was tested separately) for 30 s, extention at 72 °C for 45 s, and a final extension step at 72 °C for 7 min. PCR products were separated and visualized using QIAxcel of capillary gel electrophoresis system (QIAGEN, Irvine, California, USA).

Of the 51 primers, 33 primers showed monomorphism and 18 primer pairs displayed polymorphisms, and all the sequences were deposited in GenBank (Table 1). The genetic statistics were calculated using the package GENEPOP (version 4.0). The number of alleles per locus (N_a) ranged from 1 to 5, with a mean of 2.560. In the investigated populations, the observed (H_o) and expected (H_e) heterozygosities ranged from 0 to 1.000 and from 0 to 0.733, with averages of 0.639 and 0.590, respectively (Table 1). Two loci (AV_11592 and AV_33511) were significantly deviated from Hardy–Weinberg equilibrium in Wuding population. Av_19762 and Av_83799 showed no signal of genetic variation in the population Kunming, probably due to the deficit in heterozygotes. Significant pairwise linkage disequilibrium was detected for only two pairs of loci: AV_33511 and AV_70958 (p value < 0.01), AV_11592 and AV_52852 (p value < 0.001). These 18 polymorphic microsatellite loci developed in this study would provide valuable tools to assess genetic diversity and population structure of *A. vilmorinianum*, which would faciliate the strategy establishment for appropriate resource exploitation and management for this important Chinese medicinal plant.

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