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Isolation and characterization of 18 polymorphic microsatellite markers for the "Female Ginseng" *Angelica sinensis* (Apiaceae) and cross-species amplification

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ABSTRACT

Angelica sinensis (Apiaceae) is an endangered alpine herb that is widely used as a medicinal plant in traditional Chinese medicine (TCM). Wild populations of *A. sinensis* have become quite rare in China. Thus, population genetics studies of this species are urgently needed for its effective conservation and sustainable use. However, to date, no microsatellite loci have been isolated in *A. sinensis*. To address this issue, we isolated 18 polymorphic loci and genotyped 120 individuals collected from 6 populations. The number of alleles per locus ranged from 1.2 to 5.5, and the average was 2.4. The observed and expected heterozygosity per locus for a population varied, respectively, from 0.000 to 0.983 (averaged at 0.198) and from 0.066 to 0.661 (averaged at 0.333). Deviation from the Hardy–Weinberg equilibrium ($p < 0.01$) was observed for 4 to 14 loci in various populations. These microsatellite markers were cross-amplified in 10 species affinis, and 7 loci were successfully amplified in all species. These microsatellite markers are useful for genetic studies, the conservation management of *A. sinensis*, and identification of *A. sinensis*.

1. Introduction

Angelica sinensis (Oliv.) Diels (Apiaceae), widely known as "Female Ginseng", is an important medicinal herb endemic to China, and it is the most widely used and the best known among the many species of *Angelica* encountered in traditional Chinese medicine (TCM) (Zhang et al., 2012). *A. sinensis* is frequently used in TCM because practitioners believe it supports the cardiovascular system, thrombolysis, channel regulation, and digestion and has analgesic properties. In addition to its medicinal uses, *A. sinensis* is also used as a health food, a cosmetic and a dietary supplement in Asia, Europe and America

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(Filipiak-Szok et al., 2014). Owing to important medicinal value, wild *A. sinensis* has been over-exploited and become quite rare in China during over 2000 years (Zhang et al., 2012). Cultivation of *A. sinensis* was initiated to meet increasing demand of human since 1000 ago (Kou, 1990). Long-term cultivation maybe leads to domestication bottleneck. Therefore, genetic diversity and genetic structure of this species should be investigated for effective conservation and sustainable utilization.

In addition, the *materia medica* of *Angelica* are often misused or intentionally substituted in the TCM market to lower production cost, and five species of *Angelica* are used as folk substitutes (Yuan et al., 2015). However, discriminating *Angelica* species is very difficult because of their complex and controversial taxonomic history (Feng et al., 2009). Novel molecular should be developed to identify the species and *materia medica* within *Angelica*.

In recent decades, microsatellite or simple sequences repeat (SSR) markers have become widely used genetic markers for population genetics, genome mapping, conservation genetics and other studies because of their high abundance, high level of polymorphism, codominance and transferability across studies (Wang et al., 2012; Hsu et al., 2013; Zhang et al., 2014). However, no microsatellite loci from *A. sinensis* have been isolated to date, which greatly impedes studies of genetic diversity and population structure in this medicinal plant. To overcome this impediment, we isolated and characterized 18 polymorphic microsatellite loci from *A. sinensis* using next-generation sequencing (NGS) technology.

2. Materials and methods

2.1. Plant materials and DNA extraction

A total of 120 individuals from six populations (each with 20 individuals) of *A. sinensis* in cultivated fields were tested for polymorphisms (Table 1). Total genomic DNA was extracted using the standard cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) from silica-gel-dried leaves.

2.2. Development of microsatellite markers

Genomic DNA was pooled from ten *A. sinensis* individuals for the sequencing of 1/6 plate on the Roche 454 Genome Sequencer FLX Titanium platform (Rothberg and Leamon, 2008). Library construction and sequencing followed Roche 454 standard protocols. The NGSQC toolkit version 2.3.3 (Dai et al., 2010) was used to control and check the quality of raw sequencing data with the default parameter settings. Sequences shorter than 100 bp or with an average Phred quality score of lower than 20 were discarded. Then sequences were divided into clusters using usearch version 7.0.1090 (Edgar, 2010) with the identity threshold of 1.0. Then, microsatellites were screened out from the representative sequences of clusters with the following criteria: at least 6, 5, 5, 5 and 5 repeat units for di-, tri-, tetra-, penta- and hexa-nucleotide SSR, respectively, using the MISA package (Thiel, 2014) and PCR primer pairs were designed for each microsatellite loci with Primer3 (Rozen and Skaletsky, 1999) with default parameter settings.

2.3. SSR marker validation and genotyping

A subset of 100 microsatellites was selected for further experimental validation. PCR amplification was performed on DNA extracted from four individuals of *A. sinensis*. Only microsatellites that consistently produced specific amplification products across individuals were further evaluated for genetic polymorphism using all 120 individuals. The PCR reaction was performed in final volumes of 25 μl containing 2.5 μl 10 × PCR buffer, 2.0 μl dNTPs, 0.5 μl BSA, 0.3 μl Taq DNA Polymerase

Table 1
Sample location for each population and 10 species affinis of *Angelica sinensis*. Sample size, location and coordinate are indicated.

Species	Location	Species code	Sample size	Latitude	Longitude
Angelica sinensis	Yunnan, China	YNZY	20	N 25°49′31″	E 104°06′46″
	Gansu, China	GSZX	20	N 34°41′45″	E 104°22′0″
	Gansu, China	GSMX	20	N 34°21′2″	E 104°36′54″
	Gansu, China	GSLT	20	N 34°40′37″	E 103°24′47″
	Gansu, China	GSZN	20	N 34°39′2″	E 103°27′2″
	Gansu, China	GSWS	20	N 34°27′3″	E 104°55′0″
Angelica acutiloba	Jilin, China	Aac	4	N 41°47′03″	E 126°23′42″
Angelica anomala	Jilin, China	Aan	4	N 43°06′28″	E 128°54′12″
Angelica apaensis	Xizang, China	Aap	4	N 27°36′24″	E 94°29′57″
Angelica cartilaginomarginata	Jiangsu, China	Aca	4	N 32°03′17″	E 118°49′59″
Angelica dahurica	Gansu, China	Ada	4	N 35°05′19″	E 104°24′52″
Angelica paeoniifolia	Xizang, China	Apa	4	N 27°36′24″	E 94°29′57″
Angelica tianmuensis	Zhejiang, China	Ati	4	N 30°20′12″	E 119°26′05″
Heracleum millefolium	Xizang, China	Hmi	4	N 29°37′23″	E 94°21′26″
Levisticum officinale	Gansu, China	Lof	4	N 35°05′19″	E 104°24′52″
Ligusticum striatum	Sichuan, China	Lst	4	N 30°50′36″	E 104°06′26″