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Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in *Angelica sinensis* (Oliv.) Diels varieties of China

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ABSTRACT

Background: Angelica sinensis is a well-known traditional Chinese medicinal plant. We aimed to assess the genetic diversity and relationships in *A. sinensis* cultivars collected from different locations of China and also some other *Angelica* species.

Results: We employed an improved random amplified polymorphic DNA (RAPD) technique for the amplification of DNA materials from ten *Angelica* cultivars, and the results were verified by inter-simple sequence repeat (ISSR) analysis. Twenty six RAPD primers were used for RAPD, and the amplified bands were found highly polymorphic (96%). Each primer amplified 8–14 bands with an average of 10.25. The cluster dendrogram showed that the similarity coefficients ranged from 0.41 to 0.92. The similarity coefficients were higher among different cultivars of *A. sinensis*, and lower among different species. Twenty ISSR primers were used for the amplification, and each primer generated 6–10 bands with an average of 7.2 bands per primer. The cluster dendrogram showed that the similarity coefficients ranged from 0.35 to 0.89.

Conclusions: This study genetically characterized the *Angelica* species, which might have a significant contribution to the genetic and ecological conservation of this important medicinal plant. Also, this study indicates that the improved RAPD and ISSR analyses are important and potent molecular tools for the study of genetic diversity and authentication of organisms.

1. Introduction

The herb *Angelica sinensis* (Oliv.) Diels, also *A. sinensis*, from Apiaceae family, has a long history of usage in Traditional Chinese Medicine (TCM). This plant is indigenous to China, and locally known as 'Dang gui' (当归). *A. sinensis* is often called 'female ginseng', as its major usage is in female health related diseases [1,2,3]. In addition to effect in ameliorating female reproductive complications, *A. sinensis* also possess a number of other medicinal or health beneficial activities [4,5]. For example, different extracts or specific active ingredients from *A. sinensis* show potent anticancer activities [6,7]. Extracted natural products from *A. sinensis* have been proved successful also against cardiovascular complications, hepatic diseases, inflammation, etc. [4,8,9,10]. Although the traditional medicinal formula of *A. sinensis* is available in many countries, the plant is indigenous to China only. It is suspected that it may face extinction threat in future. Molecular studies and genetic characterization of this plant might be a useful tool for the ecological and genetic conservation of this valuable medicinal plant.

A. sinensis is mainly cultivated in Sichuan, Gansu, Hubei and Yunnan of P.R. China. The cultivars native to Min county of Gansu are famous for their officinal components and good qualities. Due to the slightly different morphological characteristics of *Angelica* in their dried roots, several other species are usually considered to be "Dang gui" by error. The species of *Angelica acutiloba* and *Levisticum officinale* are the main adulterants or substitutes for *A. sinensis* in China. *L. officinale* is native to southwest Asia and Europe, and was introduced into China in 1957 [11]. More comprehensive molecular systematic studies show that *L. officinale* and *A. sinensis* are sister taxa. *A. acutiloba* native to Japan and Korea, is distributed in the northeast of China. The root is used as a substitute for the crude drug of *A. sinensis*; however, the substitutes and adulterants have little medicinal importance. Inevitably, the confusion may compromise the genuine resources and therapeutic effect of this TCM, and even imperil the safety of consumers.

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In the era of modern biotechnology, several molecular techniques have been developed for the genetic studies and characterizations of different organisms, among which random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), and simple sequence repeat (SSR) analysis are well established and widely used [12,13,14,15]. RAPD is a more reliable molecular technique for the genetic characterization of organisms, especially of plants. However, this technique was reported to have some limitations like poorer reproducibility and lower production. We have developed an improved method of RAPD analysis by prolonging the RAMP time, and hence name RAMP-PCR instead of traditional RAPD-PCR [16].

There are few reports on the genetic characterization of *A. sinensis*, among which most are very preliminary works and are more than decade long old [17,18]. In this study, we have analyzed ten samples of *Angelica* cultivars and adulterants by using improved RAPD analysis and then verified the results by using ISSR analysis. The detailed genetic characterization and distance analysis among the samples might have significant roles in the genetic and ecological conservation of *Angelica* species.

2. Materials and methods

2.1. Experimental materials and reagents

The RAPD primers (SBS Genetech Corporation, Table 1), ISSR primers (UBC Primer Set #9, Table 2), 2 × PCR Taq MasterMix (TianGen Biotech Co. Ltd, Beijing, China) and DNA Markers (Takara Biotechnology Co. Ltd, Dalian, China) were reported previously [12]. Other reagents were analytical grade reagents as described at our previous experiments [16,19].

2.2. Collection of Angelica samples

A total of ten *Angelica* cultivars, including seven of *A. sinensis* from Minxian and Pingliang of Gansu, Mianyang and Jiuzhaigou of Sichuan, Lijiang of Yunnan, Linzhi of Tibet and Enshi of Hubei, and *A. acutiloba* (Sieb. et Zucc.) Kitag. from Yanji of Jilin, *A. acutiloba* from Aba of Sichuan, and *L. officinale* Koch from Changchun of Jilin, were collected from different geographic locations of China in this study (Fig. 1) (Table 1). The fresh leaf materials were sampled in fields and identified by Prof. Pixian Shui. The plants are deposited in the medicinal botanical nursery of Luzhou Medical College.

2.3. Extraction of DNA

Total genomic DNA was extracted from silica-gel-dried leaves and commercially crude materials using a modified CTAB (Cetyl trimethylammonium bromide) method described previously [12,20,21,22]. *Angelica* plant materials were first fixed in fixing solutions containing chloroform, PVP, and 2-hydroxy-1-ethanethiol (but without liquid nitrogen), and then ground into tiny pieces by silica (SiO_2) for the extraction of DNA. DNA quality was checked by a 0.8% agarose gel electrophoresis and spectrophotometry [19]. The final concentration of all DNA samples was adjusted to 10 ng/μL for PCR, and stored at -20°C till use.

Table 1
Sources of RAPD samples.

Sample	Species	Source	No.
MX	*A. sinensis* (Oliv.) Diels	Minxian, Ganshu	001
YJ	*A. acutiloba* (Sieb. & Zucc.) Kitag.	Yanji, Jilin	002
AB	*A. acutiloba* (Sieb. & Zucc.) Kitag.	Aba, Sichuan	003
PL	*A. sinensis* (Oliv.) Diels	Pingliang, Gansu	004
HB	*A. sinensis* (Oliv.) Diels	Enshi, Hubei	005
JZ	*A. sinensis* (Oliv.) Diels	Jiuzhaigou, Sichuan	006
EP	*L. officinale* Koch	Changchun, Jilin	007
LJ	*A. sinensis* (Oliv.) Diels	Lijiang, Yunnan	008
MY	*A. sinensis* (Oliv.) Diels	Mianyang, Sichuan	009
LS	*A. sinensis* (Oliv.) Diels	Linzhi, Tibet	010

Table 2
Sequences of RAPD primers.

Primer	Sequence 5′-3′
SBS-A1	CAGGCCCTTC
SBS-A3	AGTCAGCCAC
SBS-I1	ACCTGGACAC
SBS-I3	CCGCCTAGTC
SBS-I15	TCATCCGAGG
SBS-M8	TCTGTTCCCC
SBS-M18	CACCATCCGT
SBS-N6	GAGACGCACA
SBS-N19	GTCCGTACTG
SBS-Q16	AAGCGACCTG
SBS-A2	TGCCGAGCTG
SBS-A14	CAATCGCCGT
SBS-I2	GGAGGAGAGG
SBS-I14	TGACGGCGGT
SBS-M6	CTGGGCAACT
SBS-M13	GGTGGTCAAG
SBS-N2	ACCAGGGGCA
SBS-N14	TCGTGCGGGT
SBS-N20	GGTGCTCCGT
SBS-Q20	TCGCCCAGTC

2.4. RAPD-PCR

Twenty six SBS primers, purchased from SBS Genetech Corporation (Beijing, China) were initially evaluated for polymorphism by improved RAPD analysis, among which twenty four primers amplified DNA well with polymorphic profiles for data analysis (Table 2). Contents of the PCR system (10 μL) were prepared as follows: 1 μL of primers (2.5 μmoL/L), 1 μL (10 ng) of *Angelica* species DNA template, 5 μL of 2 × PCR Taq Mastermix and 3 μL of ddH_2O. The PCR condition was as follows: Initial denaturation at 95°C for 90 s, followed by 40 cycles of 40 s at 94°C, 60 s at 36°C, 90 s at 72°C, and final extension of 5 min at 72°C. PCR of each accession was executed in a machine of "Applied Biosystems Veriti® 96-Well Thermal Cycler" (Life Technology, USA). The RAMP rate from annealing to extension was adjusted from 2.5°C/s (100% ramp rate) to 0.125°C/s (5% ramp rate) for *Angelica* cultivars using our previously established ramp PCR conditions [12], to compare the resolution and production of the two methods in the present study. All the PCRs were repeated three times for each of the 10 samples.

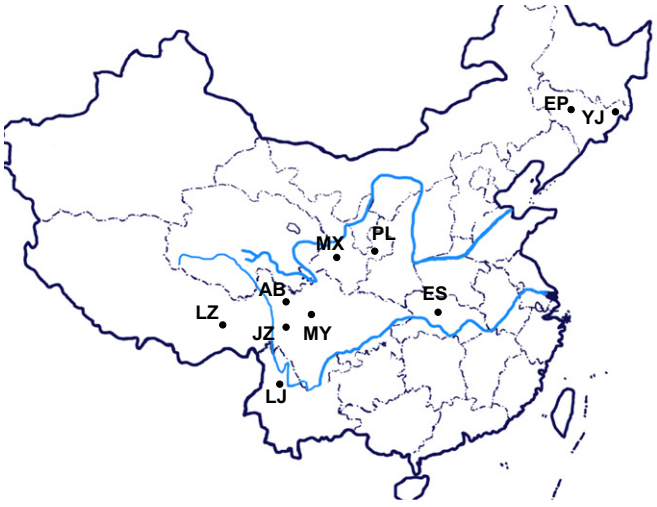

Fig. 1. The localities of samples of *Angelica* cultivars from different regions in China. Spots in dark blue indicate cities and lines in light blue indicate the Yellow River (up) and the Yangtze River (down).