

Full Length Research Paper

Molecular authentication of the traditional Tibetan medicinal plant, *Meconopsis impedita*

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The root of *Meconopsis impedita* is one of the most widely used traditional Tibetan medicinal plants. However, it is often confused with *Meconopsis racemosa* whose roots may be used as a substitute. To establish a DNA polymorphism-based assay for the identification of *M. impedita*, the chloroplast *rps16* intron region of 11 accessions representing *M. impedita* and *M. racemosa* were sequenced and analyzed. Based on the unique sequence of the *rps16* intron region between the two species, one pair of species-specific primers was designed and used for the rapid PCR identification of *M. impedita*.

Key words: *Meconopsis impedita*, the *rps16* intron, species-specific PCR, molecular authentication.

INTRODUCTION

The root of *Meconopsis impedita* Prain (called Oubei in Tibetan language) is a well-known and widely used traditional Tibetan medicine in China and other Himalayan countries. It has been used to treat various diseases such as inflammation, pain, hepatitis and tuberculosis (Luo, 1997). However, in different parts of China, it is often confused with *Meconopsis racemosa* Maximowicz whose roots are often substituted. This can lead to inconsistent therapeutic effects. Conventionally, the identification of *M. impedita* relies mainly on morphological characteristics (Gan and Yang, 1995; Wang et al., 2003) and chemical profiles (Zhou et al., 2009). The identification guidelines of these methods are based on generic phenotypes such as appearance and chemical components, which are sensitive to intrinsic and extrinsic factors (Joshi et al., 2004). Moreover, these methods either rely on experienced experts or involve expensive laboratory equipment (Carles et al., 2001). Thus, it is critical to establish a more reliable and convenient technique to identify *M. impedita* and *M. racemosa*.

The DNA-based polymorphism assay may offer an alternative method for the identification of herbal medicines (Xu et al., 2007; Xue et al., 2007a, b; Yao et al., 2009; Zheng et al., 2009; He et al., 2010; Pang et al.,

2010). In angiosperms, the chloroplast *rps16* intron is widely used as molecular marker for species authentication and polygenetic analysis among genera, species or even populations (Nie et al., 2010; Xie et al., 2010; Xu et al., 2010). In this study, the *rps16* intron regions of 11 accessions representing *M. impedita* and *M. racemosa* were sequenced and compared to explore the possibility of using it to differentiate the two species.

MATERIALS AND METHODS

Plant materials

The experimental materials were collected from the Yunnan Province and Sichuan Province, of People's Republic of China (Table 1) and were identified by the first author using the key for *Meconopsis* in the Flora of China (Zhang and Christopher, 2008). The voucher samples were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

The total DNA was extracted from about 15 mg silica-gel dried leaf material or the roots purchased at the market using the DNeasy plant mini kits (QIAGEN, Mississauga, Ontario) following the manufacturer's protocol. The *rps16* intron was amplified using primers F and R2 (Oxelman et al., 1997; Andersson and Rova, 1999). PCR amplifications were performed in a 25 µl volume containing 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.4 mM of each primer, 1 U of *Taq* polymerase (Bioline) and about 10 to 50 ng of

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Table 1. Plant materials used in this study.

Taxon	Locality	Voucher No	Length (bp)	Accession number
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	Li and Dao 36	777	JF411041
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	Li and Dao 39	777	JF411042
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	Li and Dao 38	777	JF411043
<i>Meconopsis racemosa</i>	Shangri-La, Yunnan	Li and Dao 08A	812	JF411044
<i>Meconopsis racemosa</i>	Shangri-La, Yunnan	Li and Dao 08B	812	JF411045
<i>Meconopsis racemosa</i>	Shangri-La, Yunnan	Li and Dao 08C	812	JF411046
<i>Meconopsis racemosa</i>	Xiangcheng, Sichuan	Li and Dao 40	812	JF411047
<i>Meconopsis racemosa</i>	Xiangcheng, Sichuan	Li and Dao 42	811	JF411048
<i>Meconopsis racemosa</i>	Xiangcheng, Sichuan	Li and Dao 48	811	JF411049
<i>Meconopsis racemosa</i>	Xiangcheng, Sichuan	Li and Dao 49	811	JF411050
<i>Meconopsis racemosa</i>	Xiangcheng, Sichuan	Li and Dao 58	811	JF411051
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	XC 03*		
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	XC 06*		
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	XC 07*		
<i>Meconopsis impedita</i>	Xiangcheng, Sichuan	XC 09*		

*, Purchased from the market.

DNA template under the following conditions: 3 min at 95°C, followed by 37 cycles of 20 s at 94°C, 30 s at 50°C and 40 s at 72°C, and then a final 5 min extension at 72°C. Electrophoresis was performed for the amplified DNA fragments on 1.0% agarose gels in 0.5 × TAE buffer. The PCR products were purified using the polyethylene glycol (PEG) precipitation procedure following the protocol of Sambrook et al. (1989). Cycle sequencing was conducted using BigDye 3.1 reagents and was carried out using the following profile: 35 cycles of 97°C for 15 s, 50°C for 5 s and 60°C for 4 min. The products of cycle-sequencing reactions were cleaned using the Sephadex columns (Amersham Pharmacia Biotech, Piscataway, New Jersey). The sequences were generated on ABI prism 3730XL capillary sequencer (Applied Biosystems, Foster City, California) using amplification primers.

Data analysis

The program Sequencer 4.5 (Gene Codes Corporation, Ann Arbor, Michigan) was used to evaluate the chromatograms for base confirmation and to edit contiguous sequences. Sequences were initially aligned with ClustalX version 1.83, followed by manual adjustments on Se-Al v2.0a11 and were analyzed using the program MEGA 4.0 (Tamura et al., 2007).

RESULTS AND DISCUSSION

Sequence divergence of the *rps16* intron between *M. impedita* and *M. racemosa*

The *rps16* intron regions of the 11 accessions representing *M. impedita* and *M. racemosa* were amplified and sequenced. All the sequences generated in this study have been submitted to the GenBank (Table 1). The alignment of all the sequences was available from the corresponding author. The results showed that the *rps16* intron regions from the 11 accessions representing *M. impedita* and *M. racemosa* were 777 to 812 bp in

length. The interspecies percentages of nucleotide differences in the *rps16* intron region of *M. impedita* and *M. racemosa* ranged from 1.8 to 2.0%, with an average of 1.9%. In contrast, the intraspecies percentages of nucleotide differences among the 2 *Meconopsis* species were very low, ranging from 0% (*M. impedita*), and 0% to 0.6% (*M. racemosa*). Furthermore, *M. impedita* and *M. racemosa* were found to have unique sequences in the *rps16* intron region, respectively. For *M. impedita*, the unique sequences were located between 82 and 102 and between 657 and 679; a separation of 554 bp and in *M. racemosa*, the unique sequences were located between 82 and 102 and between 692 and 714; a separation of 589 bp. It is therefore feasible to easily distinguish the two species at the DNA level.

Reliability and sensitivity of diagnostic species-specific primers

Based on the unique sequence analysis of the *rps16* intron region between the two species, one pair of *M. impedita* species-specific primers P1 (5'-TAT ATA GTT CCA AAT ATA TTG-3') and P2 (5'-GCT CGA GCA GAA AGT ATT GAT TA-3') were designed to enable rapid and accurate distinction between *M. impedita* and *M. racemosa* (Figure 1). Then, the diagnostic primers were used to amplify the template DNA. Only the segments of the *M. impedita* (554 bp) could be amplified using species-specific primers, while the *M. racemosa* was negative. The commercially prepared crude drugs (the root of *M. impedita*) purchased at the market were also tested. Even though the extracted genomic DNA was severely degraded, the desired PCR products could still be amplified when the annealing temperature was

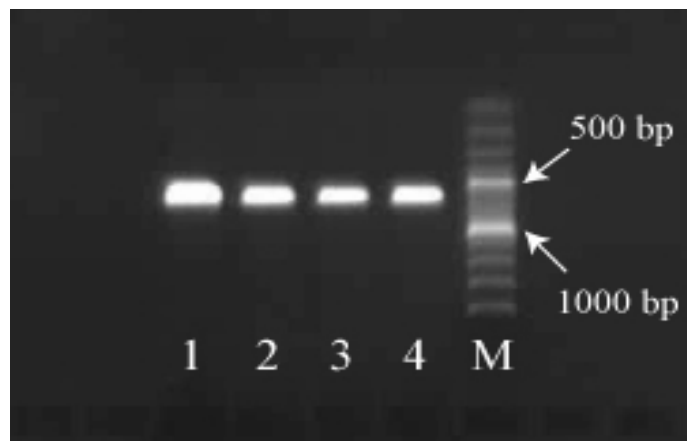


Figure 2. Agarose gel image of the PCR products derived with species-specific primers P1 and P2 for the commercial samples of *M. impedita*. Lane 1 = XC 03; lane 2 = XC 06; lane 3 = XC 07; lane 4 = XC 09; lane M = DNA marker.

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