African Journal of Biotechnology Vol. 10(70), pp. 15805-15807, 9 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1877
ISSN 1684–5315 © 2011 Academic Journals

Short Communication

Identification of *Meconopsis* species by a DNA barcode sequence: The nuclear internal transcribed spacer (ITS) region of ribosomal deoxyribonucleic acid (DNA)

Rong Li and Zhiling Dao*

Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China.

Accepted 23 September, 2011

Deoxyribonucleic acid (DNA) barcoding is a novel technology that uses a standard DNA sequence to facilitate species identification. Species identification is necessary for the authentication of traditional plant based medicines. Although a consensus has not been agreed regarding which DNA sequences can be used as the best plant barcode, the internal transcribed spacer (ITS) region of nuclear ribosomal DNA has been tested and applied extensively in recent years. In this study, we used the ITS region to test its possibility to serve as barcodes for *Meconopsis* species which are widely used in traditional Tibetan medicines that are used throughout the Himalayas. We sequenced 14 accessions representing four *Meconopsis* species. The result shows ITS sequences were found to be unique for each species, with the interspecies percentages of nucleotide differences averaging 5.1% and ranging from 0.2 to 9.6%. In contrast, the intraspecies percentages of nucleotide differences among the *Meconopsis* species studied ranged from 0 to 1.9%. Our results indicate that the ITS region can be used as a barcode to distinguish *Meconopsis* species.

Key words: DNA barcoding, species identification, *Meconopsis*, ITS region.

INTRODUCTRON

DNA barcoding technology has gained worldwide attention in the scientific community because it can provide rapid, accurate and automatable species identification using a standardized DNA sequence (Hebert et al., 2003; Schindel and Miller, 2005; Miller, 2007). Most workers agree that the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene is an effective DNA barcode in most animal species (Hebert, 2003). However, in plants, the evolutionary ratio in CO1 gene are slow, which barely can offer enough informative sites for identifying plant species (Chase et al., 2005, 2007; Kress et al., 2005; Pennisi, 2007). Thanks for the blooming in recent molecular phylogeny work, multiple sequences both the nuclear and cytoplasm DNA sequences were explored and tested for their usefulness as DNA barcodes (Kress et al., 2005; Chase et al., 2007; Shaw et al., 2007). The ITS region of nuclear ribosomal DNA is one of the barcode sequences extensively applied in plant (Chase et al., 2005; Kress et al., 2005).

In traditional Chinese medicine, the crude plants were usually dried and most lost the diagnostic features for identification, therefore, the barcoding technology is extremely useful in this situation to authenticate the origin of these herbal medicines (Sucher and Carles, 2008). 'Lüronghao' (the genus *Meconopsis* Viguier) is one of the most widely used groups of herbs in traditional Tibetan medicine in China and other Himalayan countries. Their roots are used to treat various diseases such as inflammation, pain, hepatitis and tuberculosis (Luo, 1997). Many of the 43 *Meconopsis* species native to China are used as 'Lüronghao' (Zhang and Christopher, 2008). The high market value and high demand for 'Lüronghao' compared to other medicinal plants, has led

*Corresponding author. E-mail: daozhl@mail.kib.ac.cn. Tel: +86-871-5223137. Fax +86-871-5223533.

Abbreviations: **DNA**, Deoxyribonucleic acid; **ITS**, internal transcribed spacer; **PCR**, polymerase chain reaction; **dNTPs**, deoxynucleotide triphosphates; **PEG**, polyethylene glycol; **TAE,** tris-acetate- ethylenediaminetetraacetic acid.

Table 1. Plant materials used in this study.

Taxon	Locality	Voucher number	Accession number
Meconopsis impedita Prain	Xiangcheng, Sichuan	Li and Dao 36	JF411027
Meconopsis impedita Prain	Xiangcheng, Sichuan	Li and Dao 39	JF411028
Meconopsis integrifolia (Maximowicz) Franchet	Shangri-La, Yunnan	Li and Dao 11A	JF411029
Meconopsis integrifolia (Maximowicz) Franchet	Shangri-La, Yunnan	Li and Dao 11B	JF411030
Meconopsis integrifolia (Maximowicz) Franchet	Shangri-La, Yunnan	Li and Dao 31	JF411031
Meconopsis integrifolia (Maximowicz) Franchet	Xiangcheng, Sichuan	Li and Dao 43	JF411032
Meconopsis lancifolia (Franchet) Franchet ex Prain	Xiangcheng, Sichuan	Li and Dao 44	JF411033
Meconopsis racemosa Maximowicz	Shangri-La, Yunnan	Li and Dao 08A	JF411034
Meconopsis racemosa Maximowicz	Shangri-La, Yunnan	Li and Dao 08B	JF411035
Meconopsis racemosa Maximowicz	Shangri-La, Yunnan	Li and Dao 08C	JF411036
Meconopsis racemosa Maximowicz	Xiangcheng, Sichuan	Li and Dao 40	JF411037
Meconopsis racemosa Maximowicz	Xiangcheng, Sichuan	Li and Dao 42	JF411038
Meconopsis racemosa Maximowicz	Xiangcheng, Sichuan	Li and Dao 49	JF411039
Meconopsis racemosa Maximowicz	Xiangcheng, Sichuan	Li and Dao 58	JF411040

to substantial adulterations with other *Meconopsis* species resulting in serious confusion in the identification and variation in the quality of this traditional medicine. Various criteria and methods have been developed to authenticate medicinal *Meconopsis* species, including the use of morphological characteristics (Gan and Yang, 1995; Wang et al., 2003), chemical profiles (Zhou et al., 2009), and DNA microarrays (Sulaiman and Hasnain, 1996). DNA barcoding technology uses a universal sequence across a wide range of taxa, allowing quick and cheap species authentication. In this study, we sequenced the ITS region of four medicinal *Meconopsis* species to investigate its utility as an effective, accurate marker for identification of *Meconopsis* species.

MATERIALS AND METHODS

Plant materials

Experimental materials were collected from the Yunnan Province and Sichuan Province, People's Republic of China (Table 1) and 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 using the DNeasy plant mini kits (QIAGEN, Mississauga, Ontario) following the manufacturer's protocol. The ITS region was amplified using primers ITS4 and ITS5 (White et al., 1990). PCR amplifications were performed in a 25 µl volume containing 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTP), 0.4 mM of each primer, 1 U of *Taq* polymerase (Bioline), and about 10 to 50 ng of 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 × Tris-acetate- ethylenediaminetetraacetic acid (TAE) buffer. The PCR products were purified using the polyethylene glycol (PEG) precipitation procedure following the protocol (Sambrook et al., 1989). Cycle sequencing was conducted using BigDye 3.1 reagents and 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 Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, Michigan) was used to evaluate 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 analyzed using the program MEGA 4.0 (Tamura et al., 2007).

RESULTS AND DISCUSSION

Specifically, a fragment of about 685 bp was amplified and sequenced from the samples, which included ITS1, 5.8S and ITS2 regions. All sequences generated in this study have been submitted to GenBank (Table 1). The alignment of all sequences was available from the corresponding author.

The results show that the ITS region from 14 accessions representing four *Meconopsis* species were 684 to 685 bp in length. The interspecies percentages of nucleotide differences in the ITS region of all the four *Meconopsis* species ranged from 0.2 to 9.6%, with an average of 5.1%. In contrast, the intraspecies percentages of nucleotide differences among the four *Meconopsis* species were very low, ranging from 0 (*M. impedita*), to 0.7% (*M. integrifolia*), and 0 to 1.9% (*M.

racemosa). Our sequence data showed that the average percentage difference of *Meconopsis* ITS region was 5.1%. Moreover, each *Meconopsis* species in this study was found to have a unique sequence in the ITS region, allowing its use in authentication of *Meconopsis* species and other potentially adulterant species on the market. Several methods have been used to authenticate traditional Chinese medicines, such as PCR-based, sequencing-based and hybridization-based methods (Yip et al., 2007). However, these methods are unpractical, and poorly identify the species, or are too expensive and time consuming. Undoubtedly, DNA barcoding represents another powerful and rapid technology for species identification. Certainly, the construction of a DNA barcoding database of medicinal plants will greatly facilitate this effort in the long term (Sucher and Carles, 2008).

ACKNOWLEDGMENTS

We thank Dr. Philip Thomas from the Royal Botanic Garden Edinburgh, United Kingdom for revising an earlier version of this manuscript. The study was supported by the knowledge innovation engineering of the Chinese Academy of Sciences (2010KIBA06).

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