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Molecular Cytogenetics and Development of SCAR Marker for Distinguishing Three Medicinal Species: *Cnidium officinale, Ligusticum chuangxiong* and *Angelica polymorpha*

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Objectives

Cnidium officinale, *Ligusticum chuanxiong* and *Angelica polymorpha*, belonging to *Umbelliferae*, are traditional medicinal plants which have been used in East Asia for a long time. Two species, *C. officinale* and *L. chuanxiong*, are currently cultivated, while *A. polymorpha* is a wild plant. To date, studies have been focused on the chemical component, pharmacological effect and molecular identification in these species. Although three species are evidently different in botanical characteristics, cytological data and major constituents, the dried roots (rhizome) of their plants have long been treated as same medicinal herb in the market.

In the present work, FISH/GISH, ITS/NTS sequence and RFLP and SCAR markers were used to elucidate molecular and cytogenetic relationships among the three medicinal species.

Materials and Methods

○ Plant materials

C. officinale Makino and *L. chuanxiong* Hort (Ginseng and Medicinal Plants Research Institute, Chungbuk Prov., Korea).

A. polymorpha Maxim (Korea National Arboretum, Gyeonggi-do Prov., Korea).

○ Methods

FISH(fluorescence *in situ* hybridization)

GISH(genomic *in situ* hybridization)

ITS (internal transcribed spacer)/NTS (non - transcribed spacer)

RFLP of ITS sequence, SCAR marker

Results

Cytogenetically, *C. officinale* and *A. polymorpha* were found to be diploid with 2n=2x=22, but *L. chuanxiong* had a triploid chromosome number (2n=3x=33). FISH analysis revealed that each two pairs of the 45S- and 5S rRNA genes were detected on the different chromosomes of *C. officinale*, and that triplet of the rRNA genes were localized on the same loci of the homologue in *L. chuanxiong*. GISH conducted using a genomic DNA probe detected strong cross-hybridization of genomes between

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C. officinale and *L. chuanxiong*, while no distinct GISH signal between *C. officinale* and *A. polymorpha* was observed. ITS and NTS data also revealed a very high sequence homology (95~96%) between *C. officinale* and *L. chuanxiong*. The RFLP profile of the rDNA-ITS was able to confirm the genetic distance among species. An effective species-specific SCAR marker for the identification of the wild plant, *A. polymorpha*, was also developed. Taken together, the results of this study suggest two possibilities; (1) *L. chuanxiong* originated from *C. officinale*, or (2) *L. Chuanxiong* and *C. officinale* diverged from the same parental plant.

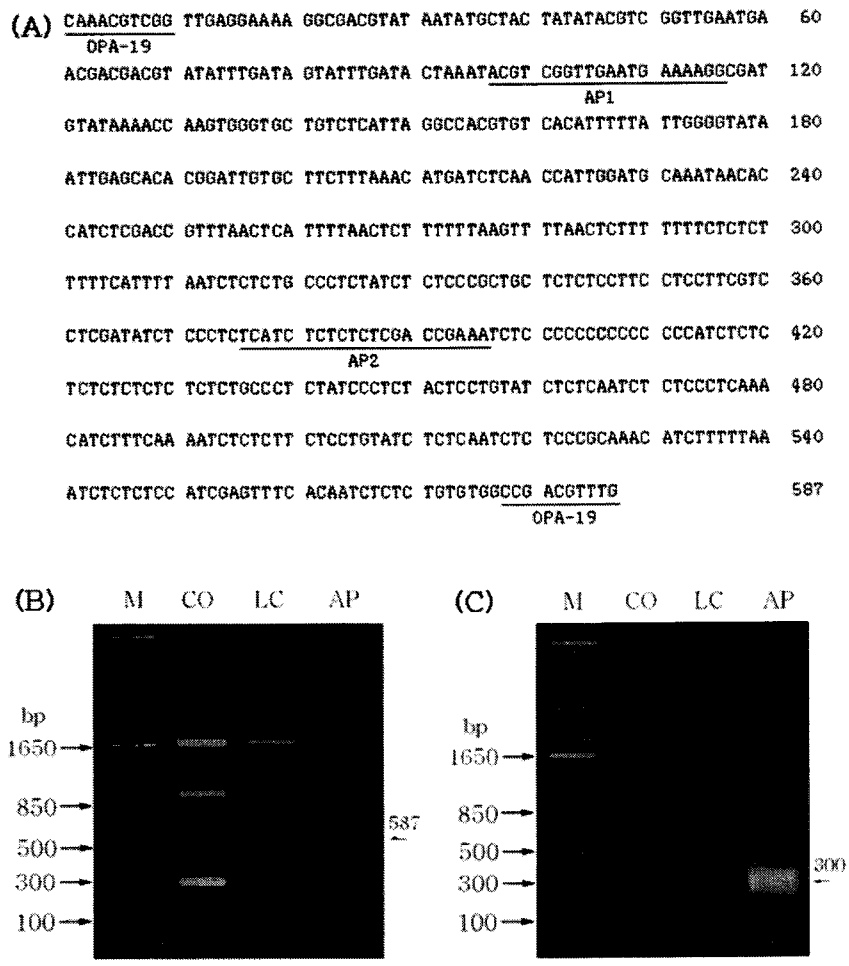

Fig. 1. Nucleotide sequence of the RAPD amplicon, AP19a, produced from *A. polymorpha* and development of a SCAR marker. (A) A 587 bp-AP19a sequence and SCAR primers (AP1 and AP2, underlined). (B) RAPD profiles of the three species, *C. officinale* (CO), *L. chuanxiong* (LC) and *A. polymorpha* (AP), amplified using the OPA-19 single primer. (C) PCR amplification of a species-specific SCAR fragment (300 bp) for *A. polymorpha*. Lane M, DNA size marker.