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Authentication of *Seok-Chang-Po* by the Development of RAPD-derived SCAR Markers and Multiplex-PCR Analysis

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RAPD 유래 SCAR 마커 및 Multiplex-PCR 분석을 통한 석창포 감별

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Objectives

Medicinal materials that originate from members of the genus *Acorus*, family of Araceae, including *Acorus gramineus* SOLAND., *A. calamus* L., *A. tatarinowii* SCHOTT., and *A. gramineus* SOLAND. var. *pusilus* ENGL., represent a good example of the complications that can arise in herbal medicine. The aerial parts and rhizomes of these plants show similar morphological characteristics and the national pharmacopoeia differently prescribes the original plant species of the herbal medicine in Asian traditional medicines. Unfortunately, due to morphological similarities in the rhizomes and few visual differences in the processed herbal drugs, it is very difficult to discriminate correctly between *Acorus* species. Therefore, we employed molecular genetic tools to develop a reliable method for distinguishing the four *Acorus* Plant species and authentic herbal medicines in appropriate substitutes and adulterants.

Materials and Methods

○ Plant Materials

- Sixteen samples of four *Acorus* medicinal plant species, *Acorus gramineus* SOLAND., *A. calamus* L., *A. tatarinowii* SCHOTT., and *A. gramineus* SOLAND. var. *pusilus* ENGL., were collected from different native habitats in Korea and China.

○ Methods

- DNA Extraction: Genomic DNA was extracted from fresh leaves and herbal medicines using DNeasy® Plant Mini Kits (QIAGEN, CA, USA), according to the manufacturer's protocol.

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- RAPD analysis: The PCR was carried out according to the method of Williams *et al.*, using 34 10-mer RAPD primers from Operon RAPD kits (Operon Technologies Inc., CA, USA) and the PCR amplification products were separated in the 1.5% agarose gel.
- Development of SCAR marker: SCAR primers were prepared from the nucleotide sequences of species-specific RAPD amplicons and amplification specificity were verified. The amplification conditions were: pre-denaturation at 95° C for 5min, followed by 30 cycles of 95° C for 1 min, 53° C for 30 s and 72° C for 2 min, and a final extension at 72° C for 5 min.

Results and Discussion

We developed several SCAR markers for the identification of four *Acorus* species, *Acorus gramineus*, *A. calamus*, *A. tatarinowii,* and *A. gramineus* var*. pusilus*, by the comparative analysis of species-specific RAPD amplicons. Furthermore, we established a rapid molecular marker by combining the primers from each SCAR markers that can be used for the simultaneous discrimination of these four species. These results provide useful tools that can distinguish the four *Acorus* plants species and elucidate authentic herbal materials from inauthentic substitutes and adulterants.

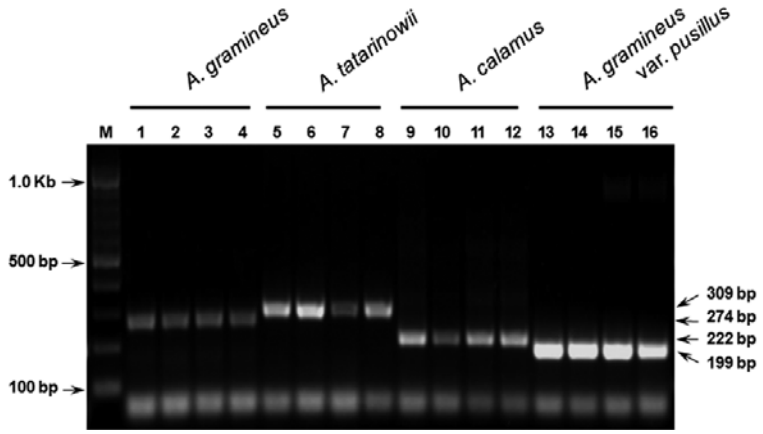

Fig. 1. Development of a rapid authentication SCAR marker using a combination of SCAR primers in a single multiplex-PCR. Multiplex-PCR products were generated from a combination of the following SCAR primers. Arrowheads to the right of the panels indicate the precise sizes of the SCAR markers. M represents a 100-bp DNA ladder.