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RAPD 분석을 통한 적수오, 백수오 및 이엽우피소 다품종 동시감별용 SCAR 유전자 감별마커 개발

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Development of SCAR Markers for the Discrimination of *Polygonum multiflorum*, *Cynanchum wilfordii* and Cynanchum auriculatum Based on RAPD.

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Objectives

The Polygoni Multiflori Radix ('Hasuoh' in Korean) and Cynanchi Wilfordii Radix ('Baeksuoh' in Korean) is prescribed as the root of *Polygonum multiflorum* Thunberg and *Cynanchum wilfordii* Hemsley, respectively, in Korean Pharmacopoeia. However, Cynanchi Wilfordii Radix was distributed as Polygoni Multiflori Radix in the market because of its similar Korean name, and the root of Cynanchum auriculatum Royle ex Wight was also distributed as Cynanchi Wilfordii Radix because of the morphological similarity of the aerial parts and herbal states. Therefor, the correct authentication of these radixes is difficult, depending on the inspection of morphological features. In an attempt to find a method for discriminating among these three radixes, we applied the recently developed technique of random amplified polymorphic DNA (RAPD) among *Polygonum multiflorum, Cynanchum wilfordii* and Cynanchum auriculatum at the genomic level, and further developed a reliable SCAR (Sequence Characterized Amplified Region) marker for the identification of these three varieties.

Materials and Methods

○ Plant Materials

- *Polygonum multiflorum* Thunberg, *Cynanchum wilfordii* Hemsley and Cynanchum auriculatum Royle ex Wight.

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○ Methods

- DNA Extraction: Total genomic DNA was extracted from plants and herbal states with a Plant genomic DNA Prep Kit (Solgent, Korea).
- RAPD analysis: The PCR was carried out according to the method of Williams *et al.*, using 24 10-mer RAPD primers from Operon kits A and C (Operon, Germany) and DNA Engine Dyad Cycler (Bio-Rad, USA). The PCR amplification products were separated in the 1.5% agarose gel and the species-specific amplicons were rescued using Gel Extraction Kit (Solgent, Korea).
- Sequence analysis: species-specific RAPD amplicons were cloned into the pGEM-Teasy vector and the sequences were determined from the both strands using an automatic DNA sequencer with T7 and SP6 primers.

Results

To find a reliable method for discriminating among the medicinal herbs similar in morphological features, we have applied the technique of RAPD at the genomic level. Several species-specific PCR products were selected to develop the molecular discrimination marker of *P. multiflorum, C. wilfordii* and C. auriculatum. In the analysis of these sequences, we obtained specific primer region for the identification of *P. multiflorum* from three varieties and developed three kinds of SCAR markers which produced 240 bp, 460 bp and 340 bp *P. multiflorum* specific DNA fragment, respectively. Moreover, we developed three SCAR markers for the both *C. wilfordii* and C. auriculatum amplified *C. wilfordii* and C. auriculatum specific DNA fragment at 196 bp, 110 bp and 407 bp, respectively, and one of them for C. auriculatum amplified 300 bp C. auriculatum specific DNA fragment. Furthermore, we established the genetic markers for discrimination of three varieties all at once by the application of multiplex-PCR methods with the combination of primers designed for the SCAR markers. These genetic markers are very useful to prevent the distribution of adulterates because it could not only classify the three medicinal herbs but also authenticate the existence of the adulterate.

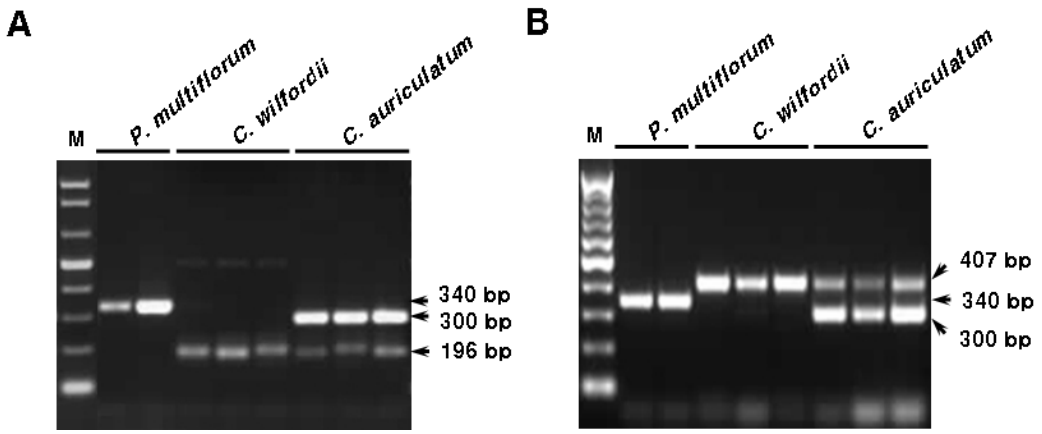

Figure 1. Development of multi-species discrimination SCAR marker for *P. multiflorum, C. wilfordii* and C. auriculatum.