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DNA 분석법에 의한 한약재 상엽의 종감별

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Authentification of Morus Folium using DNA Analysis Techniques

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Objectives

Morus Folium (Sang-yeop in Korean), the dried leaf of *Morus alba* Line, is one of the most important Oriental medicinal plants used in the Oriental medicine. Traditional authentication methods of medicinal plants, which have relied on morphological and histological differences, are limited and quite often unreliable. In comparison, DNA analysis by molecular techniques is highly accepted for the proper identification of medicinal plants, because genotype based analysis is not influenced by growth stages and environmental conditions of the plants. In this study, we developed a DNA technique to discriminate *M. alba* and *M. cathayan* from *M. tricuspidata*, by analyzing the mitochondrial *nad*7 intron 2 region.

Materials and Methods

○ Plant Materials

Plant samples of *M. alba*, *M. cathayan*, and *M. tricuspidata* were collected from Korea local market, Plant DNA Bank (http://pdbk.korea.ac.kr/), and The Wild Plant Seed Bank (http://seedbank.pdrc.re.kr/), respectively.

○ Methods

The collected leaves were frozen in liquid nitrogen and ground into fine powder. Genomic DNA was isolated and purified using a Plant DNA extraction kit (Gene All, General bio system, Seoul, Korea). The primer pairs used for amplification of *nad*7 intron2 region were *nad*7/2. PCR amplification was performed in a total volume of 20 µl, and the reaction mixture consisted of each of the primers at a concentration of 0.5 µM, 50 ng of template DNA, and 10 µl of 2X PCR premix (Genotech, South Korea). The amplification profile consisted of 1 pre-denaturation cycle of 4 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C, and a final extension at 72°C for 7 min. The PCR products were migrated on a 1.0% agarose gel electrophoresis and detected by ethidium bromide staining under UV.

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Results and Discussion

Molecular discrimination of *M. cathayana*, *M. alba*, and *M. tricuspidata* was conducted using multiplex PCR with the five primers which described above. The combination of five specific primers, as shown in Fig. 1, yielded expected amplicons for different species. All the three species generated a universal band of 686 bp with primer PCF and DBR, which provide a positive control to show that the PCR amplification works properly. As expected, *M. cathayana* and *M. tricuspidata* yielded their specific amplicons with sizes of 325 bp and 488 bp, which generated by their specific primer sets, GBF-GBR and DBF-DBR, respectively. Therefore, *M. alba*, *M. cathayana*, and *M. tricuspidata* can be clearly differentiated from each other by the developed multiplex PCR system. This method is reliable, time-saving, and can be used for numerous repeated tests of many medicinal plants. We strongly recommend the methodology presented in this study can be adapted for authentication of other medicinal materials.

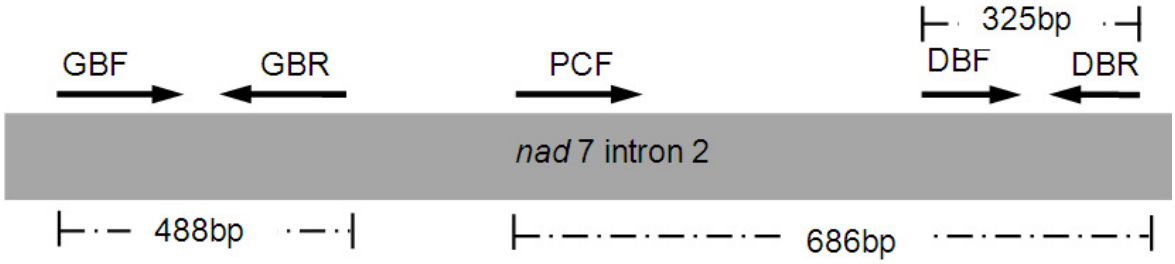

Fig. 1. Schematic diagrams of the primers used in multiplex PCR.

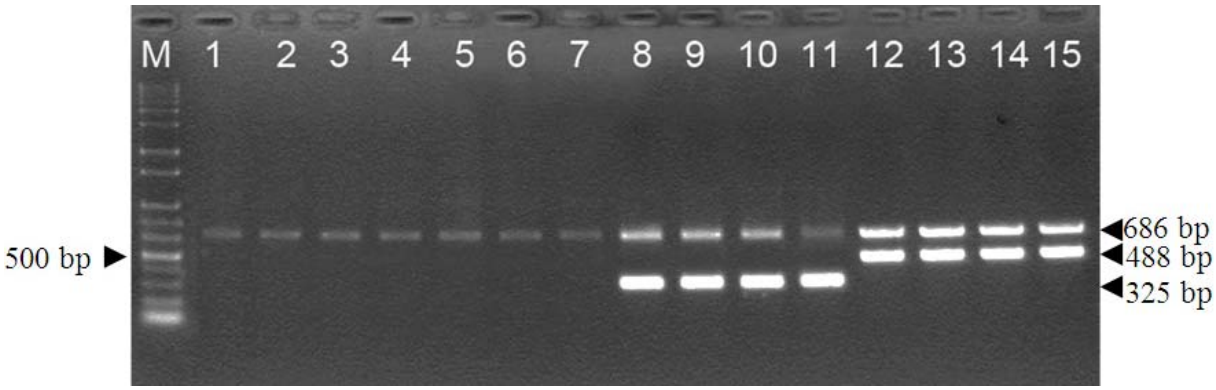

Fig. 2. Gel image of multiplex-PCR products. Lane M: 1000 bp DNA ladder; lane 1-7: *M. alba*; lane 8-11: *M. cathayana*; lane 12-15: *M. tricuspidata*.