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Identification of *Cynanchum wilfordi* based on *trnL-F* sequences and application of the molecular marker

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trnL-F 영역의 염기서열을 이용한 백하수오의 기원식물 동정 및 분자마커 적용

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Objectives

This study was conducted to identify the exact original plant and to develop the molecular marker from *C. wilfordi* and *C. auriculatum.*

Materials and Methods

○ Materials

The material were collected from fresh leaves and purchased from a commercial supplier in Korea and China.

○ Methods

The genomic DNA of each sample was extracted according to the manual for the genomic DNA plant kit (Macherey-Nagel, Germany). Primers *trnF* and *trnL* described by Taberlet *et al.* (1991) were used to amplify the *trnL-F* intergenic spacer of chloroplast DNA. Also, the PCR was carried out according to the Williams *et al.*(1990) methods using UBC primer. The nucleotide sequences of the resulting inserted DNA fragments were determined by an automatic DNA sequencer (ABI, 3730 Applied Biosystems, U.S.A.).

Results

At the comparative analysis based on the sequence of *trnL-F* intergenic spacer, the *C. wilfordi* and *C. auriculatum* showed 4 bp base variations, and the inter-individual sequences of two species separately showed 100% homology. According to the results, *C. wilfordi, C. auriculatum* were divided into two groups. Furthermore, we attempted to discriminate both *C. wilfordi* and *C. auriculatum* using the sequence characterized amplified region marker based on the RAPD-PCR product. This results provided that species-specific markers showed a authentic methods for the discrimination of *C. wilfordi* and *C. auriculatum.*

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Table 1. Materials used in this study

No.	Species	Sample state	Serial No. of KIOM	Locality
1	*Cynanchum wilfordi*	Leaf	16-1-1	Yangpyeong, Korea
2		Leaf	16-3-1	Suwon, Korea
3		Leaf	16-4-1	Daegu, Korea
4		Leaf	1999-0759	Plant DNA bank, Korea
5		Leaf	2000-1188	Plant DNA bank, Korea
6		Leaf	2002-0392	Plant DNA bank, Korea
7		Dried sample	10G1011	Yeongcheon, Korea
8		Dried sample	10G1012	Andong, Korea
9	*Cynanchum auriculatum*	Leaf	16-3-3	Beijing, China
10		Leaf	16-5-1	Daegu, Korea
11		Dried sample	H-1-4	Commercial, Korea
12		Dried sample	10G1013	Yeongju, Korea
13		Dried sample	10G1014	Commercial, China
14	*Metaplexs japonica*	Leaf	16-6-1	Daejeon, Korea
15		Leaf	16-6-2	Daejeon, Korea
16		Leaf	16-6-3	Ulleung, Korea

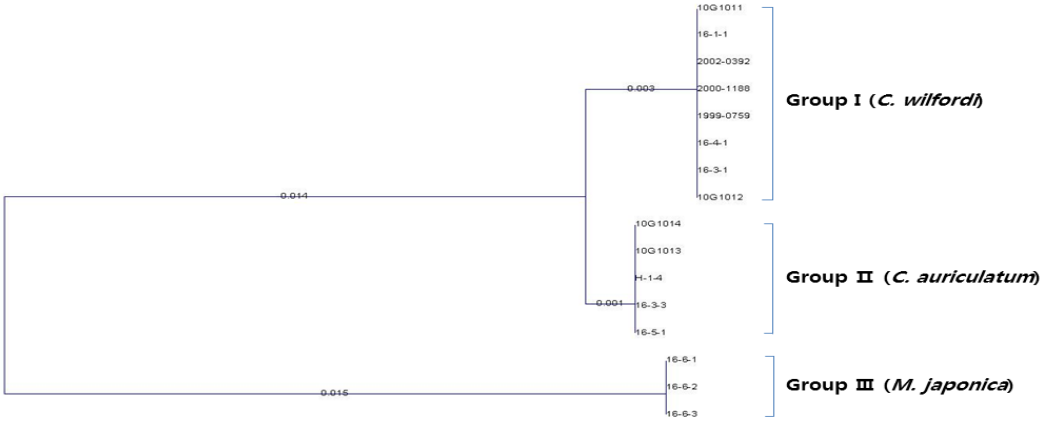

Fig. 1. Classification of *C. wilfordi*, *C. auriculatum* and *M. japonica* in the sequences based on the *trnL-F* region of cpDNA.

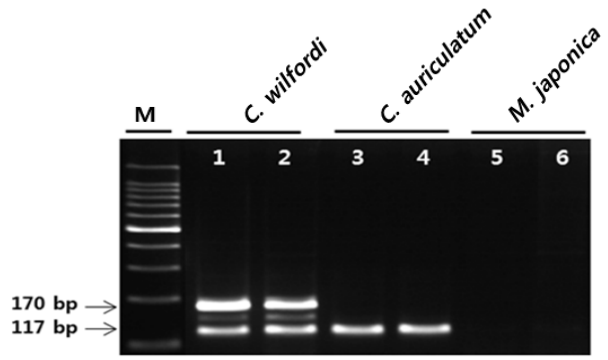

Fig. 2. Multiplex PCR amplification products of *C. wilfordi*, *C. auriculatum* and *M. japonica* using the combination of designed primer pair (CW and CAW), M; 100 bp DNA ladder.