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Discrimination of the Genus *Leontopodium* Species (Gentianales: Asteraceae) Based on RAPD

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Abstract

Korean *L. leiolepis* of the genus *Leontopodium* could be discriminate from the foreign *L. alpinum* using random amplified polymorphic DNA (RAPD). Among the 12 URP markers used for the detection, the URP-5 marker and the URP-7 marker detected polymorphic DNA bands, ranging from 400-1000 bp in the size of amplified DNA fragments.

Key Words: *Leontopodium*, RAPD, universal rice primer (URP), *Leontopodium leiolepis*

Introduction

The genus *Leontopodium* belongs to the family Asteraceae (the daisy or sunflower family) and comprises approximately 30-40 species which are mainly distributed in Asia (Himalayas, Altai Mountains, Siberia, Japan, China, and Korea), with a major centre of biodiversity on the Tibetan Plateau. In Europe, the two species of *L. alpinum* (known as the common 'Edelweiss') and *L. nivale* are recognized to date (Blöcha et al. 2010; Safer et al. 2011; Khela 2013). *Leontopodium alpinum* is distributed in the Pyrenees, the Alps, the Carpathians and the Balkan peninsula, whereas *L. nivale* is locally distributed in the Central Apennines in Italy and the Pirin Mountains in Bulgaria (Blöcha et al. 2010; Safer et al. 2011; Khela 2013). The genus *Leontopodium* which can be found in Korea comprises 6 species of *L. leontopodioides*, *L. leiolepis*, *L. coreanum*, *L. japonicum*, *L. hallaisanense*, *L. seorakensis*, and four species of them (*L. leiolepis*, *L. coreanum*, *L. hallaisanense*, and *L. seorakensis*) are native to Korea. *Leontopodium hallaisanense* is distributed in high region of the Hallasan National Park, which is located in the southernmost island of South Korea. Distribution of *L. seorakensis* is restricted to only the Seoraksan National Park and it is recently described as a new species (Lee and Choi 2011; KBIC 2014, Lim et al. 2012).

It is relatively hard to identify species of the genus *Leontopodium*, because of the morphological similarity of the *Leontopodium* species. Thus, the objective of this study is to discriminate the two Korean endemic species of *L. leiolepis* and *L. japonicum* from a foreign species *L. alpinum* using random amplification of polymorphic DNA (RAPD) markers.

Materials and Methods

A total of nine individuals of *L. leiolepis* (5 individuals), *L. japonicum* (one individual), and *L. alpinum* (3 individuals) were used for this study. Genomic DNA was extracted from

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the plant tissue samples using DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) and manufacturer-supplied protocols. The RAPD was carried out with of 12 universal rice primer (URP) markers. We used the temperature profile for PCR amplification as in Table 1. The URP - PCR products were electrophoresed on a 1.2% agarose gel in TBE buffer, visualized by staining with ethidium bromide and photographed using a Gel Documentation System.

Table 1. Temperature profile for RAPD-PCR amplification

Step	Condition	Temp.	Time	Cycle
Pre-denaturation		95°C	5 min	
Amplification	Denaturation	95°C	1 min	40 cycles from 30°C to 58°C by 0.7°C increase at annealing step of every cycle
	Annealing	30-58°C	1 min	
	Extension	72°C	2 min	
Final extension		72°C	7 min	

Table 2. Sequences of the URP markers produced the polymorphic bands in this study

URP marker No	Sequence (5'-3')
5	GGCAA GCTGGTGGGAGGTAC
7	GGTGAACAGTGAGATGAACC

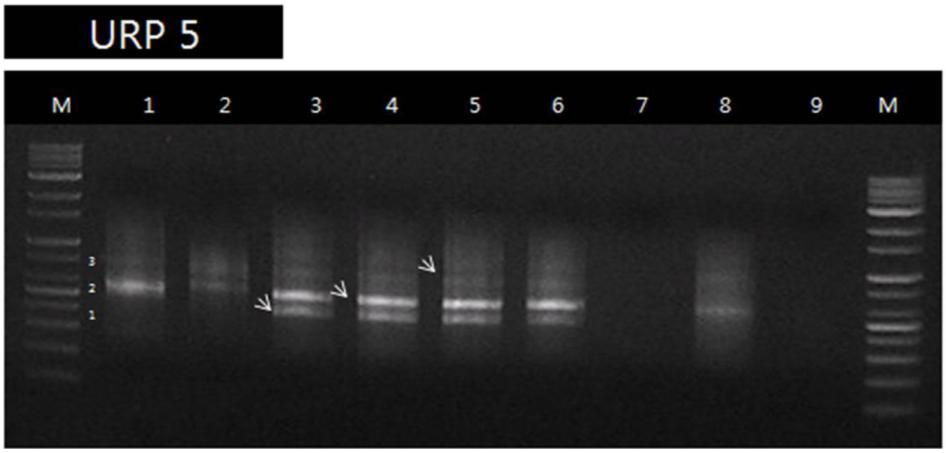

Fig. 1. Band patterns produced by PCR amplification using URP-5 marker. Informattion on samples and species is shown in Table 3.

Table 3. Labeling of bands produced by PCR amplification using URP-5 marker

Sample No.	Species	Collection Locality	Band label
1	*L. alpinum*	–	2/3
2	*L. leiolepis*	Chilseongbong, Seoraksan National Park	2/3
3	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1/2/3
4	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1/2/3
5	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1/2/3
6	*L. leiolepis*	Towangseong, Seoraksan National Park	1/2/3
7	*L. japonicum*	–	–
8	*L. alpinum*	–	2/3
9	*L. alpinum*	–	–

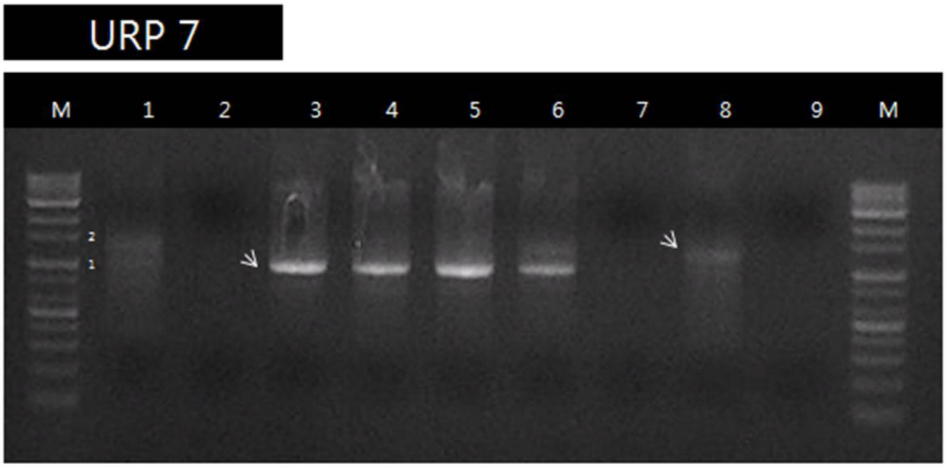

Fig. 2. Band patterns produced by PCR amplification using URP-7 marker. Informattion on samples and species is shown in Table 4.

Table 4. Labeling of bands produced by PCR amplification using URP-7 marker

Sample No.	Species	Collection Locality	Band label
1	*L. alpinum*	–	2
2	*L. leiolepis*	Chilseongbong, Seoraksan National Park	–
3	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1
4	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1
5	*L. leiolepis*	Chilseongbong, Seoraksan National Park	1
6	*L. leiolepis*	Towangseong, Seoraksan National Park	1/2
7	*L. japonicum*	–	–
8	*L. alpinum*	–	2
9	*L. alpinum*	–	–

Results and Discussion

We could produce polymorphic bands from two (URP-5 and URP-7) of 12 URP markers and their sequences are shown in Table 2.

In the PCR amplification using the URP-5 marker, two clear bands (RAPD band-1/2) were produced in the Korean *L. leiolepis* samples, whereas a few weak bands were observed in *L. alpinum* (Fig. 1, Table 3).

The RAPD band-1 which corresponds size of around 400 bp was observed in all the other *L. leiolepis* except *L. leiolepis* (Sample No. 2), whereas the RAPD band was absent in all *L. alpinum* and *L. japonicum* (Sample No. 7). The RAPD band-2/-3 were present in all *L. leiolepis* and most *L. alpinum* samples. Thus, the RAPD band-1 will be used as the PCR-marker for discriminating *L. leiolepis* from *L. alpinum*.

In the PCR amplification using the URP-7 marker, a clear RAPD band-1 which corresponds to size of around 1000 bp was observed in all the other *L. leiolepis* except *L. leiolepis* (Sample No. 2), whereas the RAPD band was absent in all *L. alpinum* and *L. japonicum* (Sample No. 7) (Fig. 2, Table 4). Thus, the RAPD band-1 produced from the URP-7 marker will be used as the PCR-marker for discriminating *L. leiolepis* from *L. alpinum*. Sample No. 2, 7, and 9 produced weak bands or no bands, probably because of small amounts of genomic DNA. The results of this work can help discriminate *L. leiolepis* from the other species within the genus using the two markers.

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