

## Analysis of Genetic Relationship of Native *Iris* species Plants using RAPD

Young-Hee Ahn

Department of Applied Plant Science, Chung-Ang University, Ansung 456-756, Korea  
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This study was carried out to provide the basic data for an identifying system for *Iris* species distributed in Korean market from complete analysing of genetic relationship between three native *Iris* species and one cultivar bred from the native *Iris* plant. RAPD analysis of genetic relationship among 4 *Iris* species was possible. According to the RAPD analysis, they were divided into two groups. Among 4 *Iris* species used in this study, *Iris laevigata* 'Veriegata', *Iris laevigata* and *Iris setosa* were classified into the same group since they had many similarities even though the habitat of *Iris laevigata* in Korean peninsula is restricted mainly in the south and *Iris setosa* is naturally inhabited in the northern part of Kangwondo. The value for the dissimilarity index of *Iris laevigata* and *Iris laevigata* 'Veriegata' was 6.757. The value for the dissimilarity index of *Iris laevigata* and *Iris dichotoma* was 95.000, so that they were genetically the farthest among them since the genetic relationship between two species are separated far if the value of the dissimilarity index is close to 100.

Key Words : *Iris* species, Genetic relationship, RAPD, Two groups, Dissimilarity index

### 1. Introduction

*Iris* species are monocotyledonous and perennial herbaceous plants belonging to Iridaceae<sup>1)</sup>. There are about 300 species of upright, rhizomatous or bulbous, sometimes fleshy-rooted perennials found in a wide range habitats from the temperate zone to the subarctic zone in the Northern hemisphere<sup>2)</sup>.

Because flowers and leaves of *Iris* species are very beautiful, these plants are generally cultivated as flowering plants in order to sell their flowers, or replant in pots or flower gardens. About 10 species including *Iris rossii* have been found in Korea. As international exchanges of many plant species have become active recently, various *Iris* species originally inhabited in Europe, such as *Iris pseudoacorus*, have been brought in Korea and distributed. Especially many garden species containing various acquired characters have been imported into Korea and planted as landscape plants. These garden species have been distributed indiscreetly over Korea

without defining importing channel or breeding process accurately, even without analyzing what kinds of genetic characters they have been acquired or identifying which cultivars they are.

In general *Iris* species are the vegetatively propagated plants by division or dividing, but many *Iris* species also propagate by seed since they seed very well. However, they become to lose their value as horticultural cultivars because nursery plants propagated by seed have undefined genetic characters from the natural crossing. For the native and cultivated *Iris* species in Korea, therefore, an accurate survey should be done promptly in order to classify how many cultivars are grown at present and a precise identification method based on their characters should be established as well.

*Iris* is very difficult plant species to identify by external appearance since appearance of these plants is very simple. Because of this reason, RAPD analysis is an effective and quick method to identify genetic variation among plant species or individuals in the same species by amplifying specific DNA regions in a short time<sup>3)</sup>. Study of genetic relationship among same plant species by RAPD analysis was already reported by Ahn *et al.*<sup>4)</sup> with a *Taraxacum*. RAPD analysis has

Corresponding Author : Young-Hee Ahn, Department of Applied Plant Science, Chung-Ang University, Ansung 456-756, Korea  
Phone: +82-31-670-3041  
E-mail: ahn3041@post.cau.ac.kr

been used widely in many areas to identify genetic variation or to analyze genetic relationship among many plant species because it is a simple and quick method for the DNA polymorphism with arbitrary primers that can be easily synthesized *in vitro*<sup>5)</sup>. Wild *Iris* plants used in this study were *Iris dichotoma*, *Iris laevigata* and *Iris setosa* that are specific *Iris*es naturally inhabited in Daechungdo or Mt. Odae, Korea. Especially *Iris laevigata* is a species widely populated in Siberia, northeastern area of China and Japan including Korea. *Iris laevigata* 'Variegata' is a cultivar developed for a horticultural purpose and used internationally for greening in damp area. In this study, therefore, analysis of genetic relationship among 4 *Iris*es that have known distributed area, character and breeding process was conducted by RAPD. This study was carried out to provide the basic data for an identifying system for *Iris* species distributed in Korean market from complete analysing of genetic relationship between three native *Iris* species and one cultivar bred from the native *Iris*

plant.

## 2. Materials and Methods

Our study was carried out from August 2002 to June 2004. Four *Iris* plants used in this study were listed in Table 1. The samples of *Iris setosa* and *Iris laevigata* were collected in Mt. Odae and Mt. Chiri accordingly. *Iris dichotoma* and *Iris laevigata* 'Variegata' were collected at Pyungkang Botanical Garden located in Pochungun, Kyungkido. After keeping collected fresh leaves of each species at 4°C for 72h to remove all carbohydrates, they were frozen by liquid nitrogen and stored in -72°C deep freezer for RAPD analysis.

Primers used in PCR were the random primers in the primer kit supplied by Operon Technologies Inc.(Alameda, CA). Nucleotide sequences and GC content of each primer were shown in Table 2. PCR conditions for analysis of genetic variation between *Iris* plants using the random primers are as follows; Mixture of 50 ng of each genomic DNA, 2.5 $\mu$ l of

Table 1. Morphological studies of three native *Iris* species and one cultivar

Scientific name	Plant height (cm)	Flower		Leaf		
		Blooming period	Color	Length (cm)	Width (cm)	Color
<i>Iris laevigata</i>	50 - 90	May - June	Pansy purple	50 - 90	1 - 3	Green
<i>Iris laevigata</i> 'Variegata'	about 70	May - June	Pansy purple	50 - 80	1 - 3	White variegated plant
<i>Iris dichotoma</i>	about 80	June - July	Blue purple	30 - 50	1 - 2	Green
<i>Iris setosa</i>	about 70	Aug. - Sep.	Blue or purple	20 - 30	2 - 2.5	Green

Table 2. List of arbitrary 10-mer primers used in the RAPD study

Operon Primer No.	Primer Sequence	GC Content(%)
OPA	5'-AGGGGTCTTG-3	60
	5'-GGTCCCTGAC-3	70
OPB	5'-GTTTCGCTCC-3	60
OPC	5'-GTGAGGCGTC-3	70
OPD	5'-GGACCCAACC-3	70
OPF	5'-ACGGATCCTG-3	60
OPK	5'-CATTCGAGCC-3	60
OPO	5'-ACGTAGCGTC-3	60
	5'-CTGTTGCTAC-3	50
OPS	5'-CTACTGCGCT-3	60
OPAW	5'-ACCTAGGGGA-3	60
	5'-CTGCTTCGAG-3	60

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10X reaction buffer, 2 $\mu$ l of 2mM MgCl<sub>2</sub>, 2 $\mu$ l of 2mM dNTPs, 20ng of each random primer and 1 unit of Taq polymerase were made and dH<sub>2</sub>O was added to make the final volume of 25 $\mu$ l. After 5min of predenaturation at 95°C, amplification reactions were carried out on the Primus 96-Plus (MWG-Biotech Inc., Germany) subjected to 55 cycles of PCR as follows; 95°C, 1min denaturation; 35°C, 2min annealing; 72°C, 2min extension<sup>6)</sup>.

Amplification products were analysed by electrophoresis on 1.5% of agarose gel containing 0.1  $\mu$ g/ml of ethidium bromide and visualized by UV transilluminator. The DNA fingerprints were photographed with Polaroid print film. Molecular weight of each amplified DNA fragment was determined by lambda-Hind III standard marker. Based on the bands recorded as present or absent, a binary matrix was obtained. The binary matrix was transformed into a similarity matrix according to the Nei and Li's method<sup>7)</sup> using Treecon<sup>8)</sup>. From this matrix, phenogram was obtained by cluster analysis, UPGMA method (Unweighted pair-group method with arithmetic average), and a bootstrap analysis<sup>9)</sup> was performed on it as the percent of 100 replication.

### 3. Results and Discussion

Morphological character of three native *Iris*, *Iris setosa*, *Iris dichotoma* and *Iris laevigata*, and one cultivar, *Iris laevigata* 'Veriegata' was examined. Flower color of *Iris dichotoma* is pink or violet, but flower color of *Iris setosa* is dark purple. Flower color of *Iris laevigata* is very similar to *Iris setosa*, but it is

little light. Flower shape and color of *Iris laevigata* 'Veriegata' were similar to *Iris laevigata*, but its color is relatively light. In the central region of Korea, blooming period of *Iris laevigata* is the earliest time, May through June, and *Iris laevigata* 'Veriegata' is May through July. Blooming period of *Iris dichotoma* is August through September, which is the latest time among them. Leaves' shape of *Iris laevigata* and *Iris laevigata* 'Veriegata' is 40-60cm that is longer than others and *Iris setosa* is 30-50cm. But, leaves' shape of *Iris dichotoma* is 20-30cm, which is the shortest leaves among them. Since *Iris laevigata* 'Veriegata' is a horticultural cultivar developed from *Iris laevigata*, they have similar morphological characteristics and white striped patterns on their leaves<sup>1)</sup>.

To analysis genetic relationship among four Irises, primer screening using 100 10mer-arbitrary primers supplied by Operon Technologies Inc.(Alameda, CA) was conducted to find primers that showed in polymorphism. A total of 41 polymorphic bands were generated using 30 primers selected by primer screening(Fig. 1). From this data, RAPD analysis of genetic relationship among 4 Irises was possible. According to the RAPD analysis, they were divided into two groups. The result showed that there was not different in the genetic variation between *Iris laevigata* 'Veriegata', a horticultural cultivar developed for the white striped patterns on their leaves as an ornamental value from *Iris laevigata*, and its original species, *Iris laevigata*. From comparing to these two plants during cultivating, it was easy to know that plant's height,

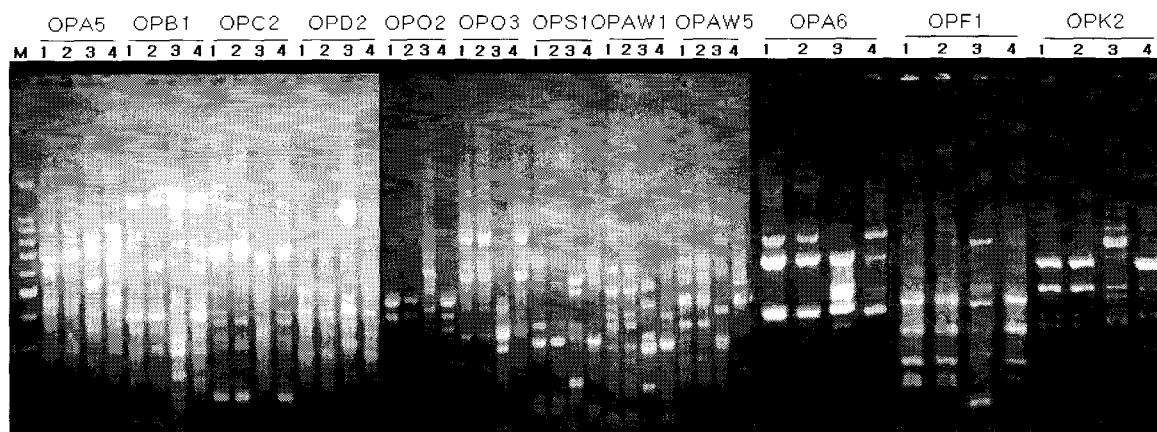


Fig. 1. RAPD band profiles of 3 *Iris* native species and one cultivar separated on the 1.5% agarose gel. M : DNA size marker( $\lambda$ -HindIII).

1: *Iris laevigata*, 2: *Iris laevigata* 'Veriegata', 3: *Iris dichotoma*, 4: *Iris setosa*

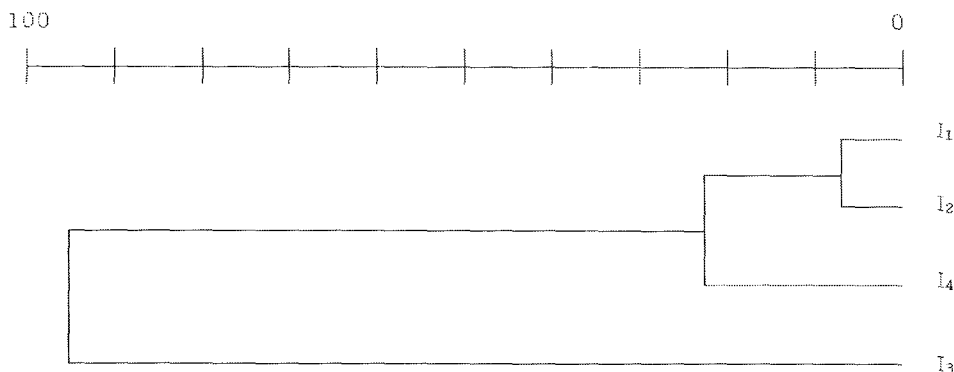


Fig. 2. Phenogram generated from Nie and Li's coefficient demonstrating the relationship between 3 native *Iris* species and one cultivar based on RAPD. Dissimilarity index indicates the genetic distance.

I<sub>1</sub>: *Iris laevigata*, I<sub>2</sub>: *Iris laevigata* 'Variegata', I<sub>3</sub>: *Iris dichotoma*, I<sub>4</sub>: *Iris setosa*

blooming period and flower colors were very similar each other except leaves' color and the white striped patterns on their leaves. Among 4 Irises used in this study, *Iris laevigata* 'Variegata', *Iris laevigata* and *Iris setosa* were classified into the same group since they had many similarities even though the habitat of *Iris laevigata* in Korean peninsular is restricted mainly in the south and *Iris setosa* is naturally inhabited in the northern part of Kangwondo (Fig. 2). However, the morphological characteristics of flowers of *Iris laevigata* and *Iris setosa* were similar in appearance. Because habitat of *Iris dichotoma* classified into another group is restricted in Daechungdo and Baekryungdo located in the western sea of Korea and isolated from other species, it has several specific regional characteristics. Since flower's shape and color and leaf's shape of *Iris dichotoma* were completely different from 3 other species, the RAPD analysis of 4 Irises showed the similar result that expected.

The dissimilarity index for 4 Irises was determined (Table 3). The value for the dissimilarity index of *Iris laevigata* and *Iris laevigata* 'Variegata' was 6.757. Because the similarity value between each species was determined by the dissimilarity index, *Iris laevigata* and *Iris laevigata* 'Variegata' were genetically closed each other. The value for the dissimilarity index of *Iris laevigata* and *Iris dichotoma* was 95.000, so that they were genetically the farthest among them since the genetic relationship between two species are separated far if the value of the dissimilarity index is close to 100<sup>(10)</sup>.

Table 3. Dissimilarity index (matrix) between 3 native *Iris* species and one cultivar

	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	I <sub>4</sub>
I <sub>1</sub>	0.000	6.757	95.000	20.000
I <sub>2</sub>	6.757	0.000	94.937	25.874
I <sub>3</sub>	95.000	94.937	0.000	93.548
I <sub>4</sub>	20.000	25.874	93.548	0.000

I<sub>1</sub>: *Iris laevigata*, I<sub>2</sub>: *Iris laevigata* 'Variegata', I<sub>3</sub>: *Iris dichotoma*, I<sub>4</sub>: *Iris setosa*

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