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Intraspecific Morphological Characteristics and Genetic Diversity of Korean *Calanthe*

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Abstract - The present study researched morphological characteristics and analyzed the genetic diversity by using RAPD in *Calanthe* species, native plant in Jeju, Korea. Twenty-six samples were selected by flower color, and 19 horticultural traits were investigated to study morphological characteristics. The *C. discolor* had the smallest leaf, the length and width of dorsal sepal, lateral sepal, petal, central lip, lateral lip, and flower stalk length were shortest and/or smallest except the spur and ovary length in *Calanthe* species, but those of *Calanthe discolor* for. *sieboldii* (Dence.) Ohwi (*Calanthe discolor* for. *sieboldii*) were the largest and/or biggest, and those of variants were the intermediate between *C. discolor* and *C. discolor* for. *sieboldii*, but spur length was the longest in *C. discolor*, the shortest in *C. discolor* for. *sieboldii*, and intermediate in the variants. Ovary length in *C. discolor* was shortest and *C. discolor* for. *sieboldii* and variants were similar with each other. The flower colors of *C. discolor* were brownish red, the value of CIE Lab was between 40 and 50. The flower color of *C. discolor* for. *sieboldii* was yellowish; the value of CIE Lab was between 110 and 130. And variants had various colors between 50 and 70 in the value of CIE Lab. After analyzing multiple band patterns of PCR products, 154 bands were selected as polymorphic RAPD markers. The analysis of Genetic distance of *Calanthe* species using RAPD showed that *C. discolor* and *C. discolor* for. *sieboldii* are more distant from each other than variants, and demonstrated the fact that genetic position of variants is between the other two species.

Key words - *Calanthe*, Morphological character, CIE Lab, RAPD

Introduction

Genus *Calanthe* includes about two hundreds species which taxa were distributed in south China, South Korea, Japan, South East Asia, Australia, South Africa, and Middle America in template and tropical areas (Hotsunimi *et al*., 1989; Yoon, 1990). Five species, *Calanthe coreana* Nakai, *C. discolor* Lindl., *C. replexa* Maxim., *C. striata* R. Br. for. *sieboldii* Ohwi. and *C. discolor* Lindl. var. *bicolor* Makino, are indigenous to South Korea (Hotsunimi *et al*., 1989; Yoon, 1990). However, there has been little systematic research on *Calanthe* species. Recently, interest in *Calanthe* species has increased in light of recognition of horticultural values of gorgeous flower color, fragrance of flower, and long flowering period. This result means that various flower colors of variants were thought to be originated from natural hybridization between *C. discolor* and *C. discolor* for. *sieboldii*. Random amplified polymorphic DNA (RAPD) analysis is a technique for amplification of specific segments of genomic DNA using random arbitrary primers (Williams *et al*., 1990; Perez *et al*., 1998). The RAPD technique provides a faster and easier approach for exploring genetic polymorphism, requires only small amounts of DNA, and involves no radioactivity (Hu and Quiros, 1991; Koller *et al*., 1993; Yang and Quiros, 1993; Stiles *et al*., 1993). The current analysis assessed the genetic diversity within and among populations (Fisher and Matthies, 1998; Fisher *et al*., 2000; Belaj *et al*., 2002; Song *et al*., 2002), and elucidated the phylogenetic relationship among cultivated varieties (Dweikat *et al*., 1993; Han *et al*., 1998). Our study was conducted to investigate

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morphological characters, to analyze the genetic diversity and phylogenetic relationship by using RAPD in *Calanthe* species native to Korea.

Materials and Methods

Plant materials

Plant materials were selected by flower color at habitat at Jeju-do in Korea. Total twenty-six plants included three *C. discolor*, three *C. discolor* for. *sieboldii* and twenty variants which have different flower color. These selected plants were used flower color analysis.

Morphological characters investigation

Morphological characteristics survey was conducted from middle April after flowering and survey methods were as follows. The number of new leaves counted after eliminating old leaves completely. Leaf length and leaf width was measured in the longest leaf of the new leaves. The number of flowers was counted after flowering of all the flowers. Length and width were measured in dorsal sepal, lateral sepal, petal, central lip, and lateral lip, respectively. The length of the spur, ovary, and flower stalk was measured. Flower color was measured using a color meter (Micro S-5, Technidyne corporation, USA), which presents color value in Commission Internationale de I'Eclairage (CIE) color system (Table 1; Munsell, 1923).

Analysis of relationship at *Calanthe*

Total genomic DNA was extracted from young and healthy leaves of plant individuals using the protocol of Paterson *et al*. (1983). DNA was dissolved to appropriate dilution in TE buffer and quantified in a Spectrophotometer. One hundred 10-mer random arbitrary primers of OPA, OPB, OPC, OPD, and OPE-set were obtained from Operon Technologies (California, USA). PCR was performed based on the standard protocol of Williams *et al*. (1990). DNA amplification reactions were performed in a volume of 20 ㎕ reaction solution containing 5 ng template DNA, 5 pico mole primer, and 20 mM dNTP mixture (dATP, dTTP, dGTP, dCTP, Neurotics, Korea), 2.0 unit *Taq* DNA Polymerase (Neurotics, Korea), Operon 10-mer (10 nucleotides) primer, 10 × reaction buffer, and sterilized water. Amplification reaction was performed in a program temperature control system (PC-707, ASTEC, Japan). DNA was amplified using the following program: preheating at 95℃ for 2 minutes, 1 cycle involving 95℃ (denaturation) for 1 minute, 36℃ (annealing) for 1 minute, and 72℃ (extension) for 2 minutes. A total of 45 cycles were operated and then 7 minutes at 72℃ in the last cycle. Aliquots of 10 ㎕ of DNA products from PCR amplification were loaded on 0.8% agarose gels for electrophoresis in 1×TAE buffer. Gels were stained with ethidium bromide and photographed under UV light with Polaroid film 667. Each polymorphic fragment detected by RAPD analysis was treated as a unit character which was quantified by 1 for presence of fragment and 0 for absence of fragment from 0 to 2,000 base pair. Phylogenetic similarity coefficients of each strain were quantified using the NTSYS (ver. 2.11; Rohlf, 1998) computer program. A cluster analysis was done using the unweighted pair group method with arithmetic (UPGMA).

Table 1. Equation of color expression on CIE L*a*b* system[z].

Equation	Meaning of value
$\triangle L^* = L2^* - L1^*$	Difference of lightness
$\triangle a^* = a2^* - a1^*$	Red - Green
$\triangle b^* = b2^* - b1^*$	Yellow - Blue
$\triangle E^* = (\triangle L^{*2} + \triangle a^{*2} + \triangle b^{*2})^{1/2}$	Difference of color

[z]The three parameters in the model represent the lightness of the color (L*, L*=0 yields black and L*=100 indicates white), its position between magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicates blue and positive values indicate yellow) (Munsell, 1923).

Results and Discussion

Twenty-six samples were selected by flower color and 19 horticultural traits were investigated to study morphological characteristics of *Calanthe* species native to Korea (Table 2). The number of leaves was 3 to 4. There was no difference between 2 species and variants. Leaf length was the longest in *C. discolor* for. *sieboldii*, the shortest in *C. discolor*, and variants were intermediate between *C. discolor* and *C. discolor* for. *sieboldii*. Leaf width was widest in *C. discolor* for. *sieboldii*, the narrowest was *C. discolor*, variants were intermediate between *C. discolor* and *C. discolor* for. *sieboldii*. The number of flowers was about 15 in *C. discolor* and *C. discolor* for. *sieboldii*, but some samples had 20 to 24 flowers. Flower stalk length showed that *C. discolor* was around 25 cm, and *C. discolor* for. *sieboldii* was more than 30 cm. The petal length of *C. discolor* was around 15 mm, *C. discolor* for. *sieboldii* was around 25 mm, and variants were between *C. discolor* and *C. discolor* for. *sieboldii*. Petal width showed that *C. discolor* was around 5 mm, *C. discolor* for.

Table 2. Morphological characteristics of *Calanthe* species and variants.

Sample no.*	LN (ea)	LL (㎝)	LW (㎝)	FN (ea)	FSL (㎝)	PL (㎜)	PW (㎜)	DSL (㎜)	DSW (㎜)
1	3.6 ± 0.5*	21.5 ± 3.9	7.2 ± 1.0	11.0 ± 3.7	26.4 ± 6.3	13.7 ± 2.1	5.0 ± 0.6	14.9 ± 1.9	7.5 ± 1.0
2	3.6 ± 0.5	20.5 ± 2.7	7.3 ± 1.2	12.0 ± 2.3	26.6 ± 6.5	15.6 ± 2.0	5.4 ± 0.5	15.2 ± 2.3	7.8 ± 0.8
3	3.6 ± 1.2	23.4 ± 4.0	6.9 ± 1.2	10.5 ± 3.0	23.5 ± 5.3	13.6 ± 1.8	5.2 ± 0.4	15.0 ± 2.1	6.9 ± 0.9
4	3.3 ± 1.0	34.5 ± 3.8	8.5 ± 1.2	12.9 ± 3.4	34.5 ± 7.8	25.7 ± 2.2	8.8 ± 0.8	23.2 ± 3.2	13.2 ± 0.8
5	3.5 ± 0.6	30.2 ± 4.0	8.4 ± 2.7	13.0 ± 3.3	32.5 ± 8.0	22.9 ± 2.4	8.8 ± 0.7	28.6 ± 2.9	12.6 ± 1.0
6	3.2 ± 0.4	32.3 ± 3.9	8.6 ± 2.0	13.5 ± 3.6	33.5 ± 6.9	25.5 ± 1.9	8.8 ± 0.9	24.3 ± 3.5	12.6 ± 0.7
7	4.3 ± 1.2	33.2 ± 2.1	12.1 ± 1.2	14.7 ± 2.5	31.6 ± 6.0	22.6 ± 1.0	9.0 ± 1.4	25.4 ± 1.7	21.1 ± 0.4
8	3.6 ± 0.5	30.3 ± 3.8	8.8 ± 1.2	12.9 ± 3.4	31.8 ± 7.8	23.6 ± 1.9	8.3 ± 0.8	26.7 ± 2.3	11.8 ± 0.8
9	3.3 ± 0.6	26.8 ± 4.3	8.7 ± 2.7	11.0 ± 1.7	30.5 ± 9.9	19.6 ± 1.0	7.7 ± 0.5	20.0 ± 1.5	10.8 ± 0.8
10	3.5 ± 0.4	29.3 ± 2.4	9.5 ± 1.3	11.5 ± 2.8	34.9 ± 6.7	24.4 ± 1.4	9.2 ± 0.8	28.0 ± 1.0	12.1 ± 0.7
11	3.2 ± 0.5	23.6 ± 3.5	7.8 ± 0.7	14.7 ± 3.4	32.4 ± 3.3	17.3 ± 1.2	5.8 ± 0.5	19.0 ± 1.2	10.0 ± 0.6
12	3.3 ± 0.5	24.7 ± 2.0	7.9 ± 1.2	11.8 ± 2.9	30.0 ± 5.0	19.2 ± 1.6	6.8 ± 0.8	20.5 ± 2.5	10.0 ± 1.0
13	3.5 ± 0.5	19.6 ± 3.6	5.2 ± 1.3	12.8 ± 2.0	29.4 ± 3.0	15.5 ± 1.3	7.4 ± 0.8	17.2 ± 1.3	9.9 ± 1.2
14	3.7 ± 0.5	27.4 ± 0.9	7.3 ± 1.1	13.7 ± 3.3	31.7 ± 5.5	17.7 ± 2.0	6.7 ± 0.7	19.2 ± 2.5	9.7 ± 1.0
15	3.5 ± 0.4	26.7 ± 3.7	7.6 ± 1.0	12.5 ± 2.5	28.5 ± 4.2	19.3 ± 1.0	5.8 ± 0.3	20.0 ± 1.8	10.2 ± 1.6
16	3.2 ± 0.4	27.4 ± 3.1	8.3 ± 1.0	14.8 ± 4.6	34.1 ± 6.5	17.1 ± 2.3	6.8 ± 0.4	18.6 ± 3.2	10.1 ± 1.4
17	3.5 ± 0.6	26.1 ± 3.1	7.2 ± 0.4	19.8 ± 4.5	34.4 ± 4.5	18.5 ± 0.9	6.6 ± 0.4	20.3 ± 0.8	9.9 ± 0.4
18	4.0 ± 0.0	27.4 ± 3.5	8.1 ± 1.0	19.5 ± 2.5	33.7 ± 1.5	18.6 ± 0.7	6.9 ± 0.5	21.2 ± 1.6	10.3 ± 0.4
19	3.5 ± 0.4	31.4 ± 3.4	9.8 ± 1.3	12.1 ± 1.5	43.4 ± 5.3	23.5 ± 2.4	9.7 ± 1.2	27.1 ± 0.6	11.5 ± 0.6
20	3.7 ± 0.6	24.9 ± 2.9	6.9 ± 0.9	12.5 ± 2.0	36.4 ± 7.2	17.3 ± 0.9	7.7 ± 0.8	18.4 ± 0.7	11.8 ± 0.4
21	3.5 ± 0.5	23.4 ± 2.8	6.1 ± 0.7	10.0 ± 1.8	24.5 ± 4.3	14.3 ± 0.8	4.8 ± 0.3	14.4 ± 0.6	7.7 ± 0.8
22	3.5 ± 0.5	26.4 ± 3.4	7.8 ± 1.0	11.5 ± 1.6	35.4 ± 5.8	15.9 ± 1.0	5.2 ± 0.2	16.7 ± 0.5	8.9 ± 1.0
23	3.3 ± 0.4	21.6 ± 3.5	6.4 ± 0.8	10.5 ± 1.5	22.3 ± 3.5	13.1 ± 1.1	5.4 ± 0.2	14.5 ± 0.9	9.0 ± 0.6
24	3.3 ± 0.5	24.6 ± 2.0	5.9 ± 0.4	19.8 ± 4.6	32.9 ± 3.2	12.3 ± 1.0	5.1 ± 0.4	14.1 ± 0.8	7.1 ± 0.9
25	3.3 ± 0.5	24.8 ± 1.4	7.2 ± 0.7	11.4 ± 0.8	28.6 ± 4.2	14.1 ± 0.5	4.8 ± 0.5	15.4 ± 0.4	7.8 ± 0.2
26	3.5 ± 0.7	16.5 ± 1.3	5.3 ± 0.6	7.0 ± 1.0	17.2 ± 3.3	12.8 ± 1.2	4.4 ± 0.2	13.5 ± 1.2	6.6 ± 1.1

*1~3: *C. discolor*, 4~6: *C. discolor* for. *sieboldii*, 7~26: variants. Figures were represented as mean value ± standard deviation. LN: number of leaves, LL: leaf length, LW: leaf width, FN: no of flowers, FSL: flower stalk length, PL: petal length, PW: petal width, DSL: dorsal sepal length, DSW: dorsal sepal width, LSL: lateral sepal length, LSW: lateral sepal width, CLL: central lip length, CLW: central lip width, LLL: lateral lip length, LLW: lateral lip width, SL: spur length, OL: ovary length.

Table 2. continued.

Sample no. *	LSL (㎜)	LSW (㎜)	CLL (㎜)	CLW (㎜)	LLL (㎜)	LLW (㎜)	SL (㎜)	OL (㎜)
1	15.5 ± 2.2	5.7 ± 0.8	9.2 ± 0.7	5.0 ± 1.0	8.1 ± 0.9	7.9 ± 0.3	13.8 ± 1.4	15.0 ± 2.2
2	16.9 ± 2.5	5.7 ± 0.6	10.0 ± 0.9	5.6 ± 0.6	8.4 ± 0.4	7.0 ± 0.9	13.3 ± 1.2	15.2 ± 2.0
3	16.2 ± 3.0	5.2 ± 0.4	9.9 ± 0.6	4.8 ± 0.8	8.0 ± 0.8	7.2 ± 0.5	14.2 ± 1.0	16.3 ± 1.8
4	30.8 ± 3.3	10.2 ± 1.0	17.3 ± 1.6	9.4 ± 0.6	15.0 ± 1.4	10.3 ± 1.0	11.5 ± 1.0	15.3 ± 1.5
5	25.2 ± 2.0	11.1 ± 0.9	17.3 ± 1.5	8.9 ± 1.5	13.4 ± 1.5	10.3 ± 0.9	10.7 ± 1.1	14.8 ± 1.7
6	30.4 ± 2.8	10.6 ± 1.2	17.5 ± 1.8	9.3 ± 2.0	14.8 ± 4.2	11.3 ± 1.0	10.9 ± 0.8	16.2 ± 1.8
7	26.1 ± 1.4	9.9 ± 0.8	17.7 ± 2.8	9.5 ± 2.7	14.9 ± 1.4	11.4 ± 0.6	10.6 ± 1.2	18.7 ± 3.2
8	27.6 ± 2.4	9.8 ± 0.6	17.0 ± 2.9	9.5 ± 1.7	14.3 ± 2.0	11.3 ± 1.2	10.8 ± 0.6	17.0 ± 1.5
9	22.2 ± 7.1	8.9 ± 0.7	14.2 ± 2.5	7.2 ± 2.0	10.6 ± 2.6	9.2 ± 0.8	12.9 ± 0.9	18.2 ± 0.5
10	29.0 ± 1.1	10.0 ± 0.5	16.9 ± 1.7	9.3 ± 0.7	14.1 ± 0.8	10.1 ± 1.4	12.5 ± 1.2	18.1 ± 2.1
11	19.9 ± 1.3	7.5 ± 1.0	12.5 ± 1.6	7.3 ± 0.8	11.7 ± 1.8	10.4 ± 1.0	13.1 ± 1.1	16.0 ± 1.1
12	21.2 ± 1.2	7.7 ± 0.9	13.6 ± 1.8	6.7 ± 1.0	10.2 ± 1.0	6.4 ± 0.6	9.1 ± 0.8	18.1 ± 0.3
13	17.8 ± 1.3	7.6 ± 0.8	12.1 ± 1.6	5.7 ± 0.6	11.2 ± 0.5	10.5 ± 0.4	12.3 ± 1.1	17.0 ± 0.5
14	19.1 ± 2.2	7.4 ± 0.7	12.4 ± 1.5	8.8 ± 1.0	11.7 ± 0.8	8.0 ± 0.6	13.7 ± 1.3	15.3 ± 1.4
15	21.8 ± 3.3	7.2 ± 1.5	13.5 ± 1.4	7.2 ± 0.5	13.7 ± 0.2	11.5 ± 0.4	12.1 ± 1.2	14.7 ± 0.7
16	20.4 ± 2.0	7.7 ± 1.2	13.8 ± 1.2	7.9 ± 0.4	13.1 ± 1.2	10.8 ± 0.8	13.1 ± 0.8	14.6 ± 0.4
17	21.9 ± 1.8	7.4 ± 0.4	14.1 ± 1.0	7.6 ± 0.2	13.1 ± 1.1	11.7 ± 0.7	12.8 ± 0.8	16.8 ± 1.1
18	21.4 ± 1.4	7.6 ± 0.6	14.7 ± 1.8	8.7 ± 1.0	11.6 ± 0.6	9.8 ± 0.2	14.0 ± 1.1	19.3 ± 0.9
19	29.8 ± 1.6	9.7 ± 0.9	18.8 ± 1.8	9.5 ± 2.0	14.6 ± 0.8	13.0 ± 1.0	13.7 ± 0.9	17.5 ± 0.8
20	20.1 ± 0.8	7.9 ± 0.6	13.2 ± 0.6	6.1 ± 0.6	10.7 ± 1.3	9.0 ± 0.3	12.7 ± 0.8	17.2 ± 1.5
21	16.0 ± 1.1	5.7 ± 0.3	11.4 ± 0.5	5.9 ± 0.3	9.7 ± 0.4	8.5 ± 0.5	17.1 ± 1.3	19.5 ± 1.7
22	17.5 ± 1.1	6.2 ± 0.4	12.6 ± 0.8	6.1 ± 0.2	11.5 ± 0.5	7.9 ± 0.8	13.8 ± 1.2	17.0 ± 0.7
23	15.0 ± 0.7	6.0 ± 0.5	10.1 ± 1.1	6.0 ± 0.4	9.1 ± 0.9	7.8 ± 0.9	16.9 ± 1.4	15.0 ± 1.3
24	15.2 ± 0.3	5.6 ± 0.3	9.7 ± 0.6	4.9 ± 0.5	8.8 ± 0.8	6.3 ± 0.5	11.5 ± 0.8	15.2 ± 1.1
25	16.7 ± 0.6	5.8 ± 0.4	11.1 ± 0.4	6.9 ± 0.4	9.9 ± 0.5	8.7 ± 0.5	12.6 ± 1.0	11.7 ± 0.3
26	14.7 ± 1.2	5.1 ± 0.8	9.4 ± 0.4	4.1 ± 0.4	7.8 ± 0.3	6.3 ± 0.2	13.3 ± 1.3	14.7 ± 1.8

sieboldii was 8 to 9 mm, and variants were 6 to 7 mm. The dorsal sepal length of *C. discolor* was around 15 mm, *C. discolor* for. *sieboldii* around 25 mm, and variants around 20 mm. The dorsal sepal width had the same tendency as dorsal sepal length. The lateral sepal length of *C. discolor* was 15 to 16 mm, *C. discolor* for. *sieboldii* around 30 mm, and variants around 20 mm. The lateral sepal width was around 5 mm in *C. discolor*, around 10 mm in *C. discolor* for. *sieboldii*, and 7 to 8 mm in the variants. The central lip length was around 10 mm in *C. discolor*, 15 to 18 mm in *C. discolor* for. *sieboldii*, and 12 to 14 mm in variants. The central lip width was 5 mm in *C. discolor*, 9 mm in *C. discolor* for. *sieboldii*, and 6 to 8 mm in variants. Lateral lip length was 8 mm in *C. discolor*, 14 to 15 mm in *C. discolor* for. *sieboldii*, and 10 to 13 mm in variants. The lateral lip width was 7 to 8 mm in *C. discolor*, and 10 to 11 mm in *C. discolor* for. *sieboldii* and variants. The spur length was 13 to 14 mm in *C. discolor*, 10 to 11 mm in *C. discolor* for. *sieboldii*, and similar or shorter than *C. discolor* in variants. The ovary length showed that *C. discolor* was 15 to 16 mm, *C. discolor* for. *sieboldii* was 14 to 16 mm, and variants were similar with two species. The flower color of *C. discolor* was dark purplish red or brownish red. The L* (lightness) value was 30 to 40, a* (Red-Green) value was 0 to 20, b* (Yellow to Blue) was 20 to 30, and the CIE Lab value was 40 to 50. *C. discolor* for. *sieboldii* flowers was yellow or bright yellow. The L* value was above 80, a* value was -10, b* value was around 80, and the CIE Lab value was 110 to 130. The value of CIE Lab of variants was between 50 and 70. The CIE Lab value of lip color was 90 in white lip and 110 to 120 in yellow lip. Morphological character, especially flower color, is customarily investigated using Munsell (1923). This investigation method is not accurate because it depends on the sense of human sight. In the current study, we used a color meter (Brightimeter Micro S-5, Technidyne corporation, USA), which presents a color value according to the CIE (CIE; Commission Internationale de I'Eclairage) color system. This color measuring system can present us with an accurate and scientific color value of flowers of the *Calanthe* species. We believe that this trial using a color meter to measure flower color is the first such study. We are sure that morphological characters of flowers and other organs were measured exactly because they were measured in at least 10 or more samples of the same size and colored plants. We believe that flower color, morphological character, and measuring manner will become the standard of horticultural and botanical studies in the future. Most studies used Munsell (1923) to measure flower color. However, the current study was the first to measure flower color using a color meter in *Calanthe* species. This data will be useful as the basis of flower color study in *Calanthe* species. Hyun *et al*. (1999)

Table 3. CIE Lab value on color space of each flower in *Calanthe* species and variants.

Sample no.*	L, a, b color space of flower			CIE Lab	L, a, b color space of lip			CIE Lab
	L^z	a^y	b^x		L	a	b	
1	48.61	1.22	30.83	57.6	90.40	-9.81	15.56	92.3
2	32.80	0.77	28.29	43.3	90.44	-9.67	15.44	92.3
3	31.41	20.55	35.82	51.9	90.38	-9.57	15.39	92.2
4	87.94	-10.11	79.34	118.9	87.33	-9.88	78.68	118.0
5	87.50	-9.51	89.26	125.4	87.31	-9.89	78.61	117.9
6	87.85	-10.04	79.30	118.8	87.28	-9.78	78.60	117.9
7	82.39	-1.20	88.44	120.9	91.49	-6.66	20.95	94.1
8	83.14	-9.78	93.40	125.4	87.06	-9.60	90.92	126.2
9	72.85	16.22	64.51	98.6	86.65	-16.04	79.45	118.6
10	62.63	-15.31	65.80	92.1	86.68	-16.10	79.48	118.7
11	51.61	1.00	59.62	78.9	87.34	-10.32	62.90	108.1
12	41.67	17.48	31.37	55.0	43.29	13.20	42.94	62.4
13	30.23	7.06	28.12	41.9	72.09	-7.41	80.22	108.1
14	31.44	20.38	35.25	51.4	87.33	-9.76	78.57	117.9
15	41.59	17.50	31.40	55.0	91.85	-10.40	50.61	105.4
16	35.40	15.71	37.55	53.9	87.24	-10.40	62.85	108.0
17	51.69	17.45	63.08	83.4	87.31	-9.74	78.46	117.8
18	72.28	9.40	95.93	120.5	81.56	-9.29	99.66	129.1
19	31.39	20.42	35.74	51.8	87.16	-10.34	62.75	107.9
20	41.68	17.40	31.18	54.9	81.90	-8.75	77.66	113.2
21	41.26	14.32	13.69	45.8	91.20	-4.35	9.28	91.8
22	60.93	1.01	58.98	84.8	91.88	-4.84	10.49	92.6
23	52.62	18.79	34.99	65.9	91.88	-4.86	10.32	92.6
24	30.38	7.42	13.73	34.2	91.49	-10.21	24.20	95.2
25	52.09	19.77	27.75	62.2	92.14	-12.62	35.87	99.7
26	86.69	-15.99	79.63	118.8	91.64	-10.49	16.10	93.6

*1~3: *C. discolor*, 4~6: *C. discolor* for. *sieboldii*, 7~26: variants. ^{z}represent value of lightness. ^{y}represent value of red to green. ^{x}represent value of yellow to blue.

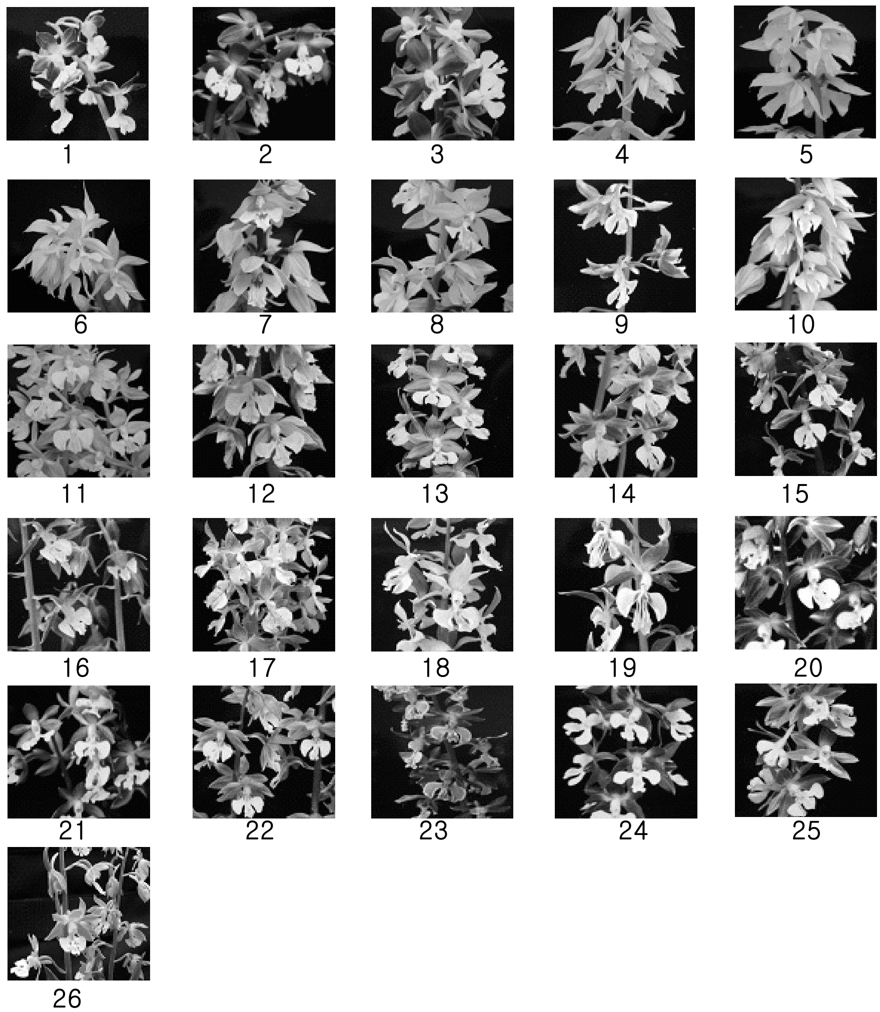

Fig. 1. Flowers of *Calanthe* species used for this study. 1~3: *C. discolor*, 4~6: *C. discolor* for. *sieboldii*, 7~26: variants.

investigated leaf length and width, sepal length and width, petal length and width, lip length and width, spur length, and ovary length in *C. discolor*, *C. discolor* for. *sieboldii*, and *C. bicolor* which is a putative hybrid between *C. discolor* and *C. discolor* for. *sieboldii*. Leaf length was longest in *C. discolor* for. *sieboldii*, around 26 cm, the shortest in *C. discolor*, and *C. bicolor* was intermediate between the two species. The length and width of dorsal and lateral sepal, petal, and central and lateral lip were longest and widest in *C. discolor* for. *sieboldii*, the shortest in *C. discolor*, and intermediate in *C. bicolor*. The spur length was the longest was *C. discolor*, the shortest in *C. discolor* for. *sieboldii*; *C. bicolor* was intermediate between the two species. The ovary length of *Calanthe* species was the longest in *C. discolor* for. *sieboldii*, the shortest in *C. discolor*, and *C. bicolor* was between the two species. These results are similar with our studies. However, Kim and Kim (1989) reported that length and width of leaf were the longest in putative hybrid between *C. discolor* and *C. discolor* for. *sieboldii*, the shortest in *C. discolor*. In addition, in spur length, *C. discolor* was the shortest. The result of spur length disagreed with Hyun *et al*. (1999) and our study which was thought that plant materials had some problems in step of selection or errors in measurement.

The genetic relationship of 3 of *Calanthe discolor*, 3 of *C. discolor* for. *sieboldii*, and 20 of variants was investigated using RAPD. One hundred Operon primers were used to analyze the relationship of *Calanthe* species using RAPD analysis. The number of amplified bands for each ten-mer primer varied from 2 to 8, with an average of around 4 bands per primer accounting for a total of 305 bands from 87 primers. After analyzing the multiple band patterns of the PCR products, 154 bands of 305 bands were selected as polymorphic RAPD markers. The size of the amplified products ranged from 0.5 kb to 2.0 kb (Fig. 2). The dendrogram was constructed to the 154 polymorphic bands by the NTSYS (ver. 2.11; Rohlf, 1998) program (Fig. 3). The dendrogram is separated into two major branches. One branch contained the majority of *C. discolor* for. *sieboldii*, which are closely related with about 70% similarity. This group consists of yellow and large flowered plants. The other branch contained two branches which were divided into *C. discolor* and variants. The branch of *C. discolor* is closely related with about 90%. This group consists of plants with dark brown and small flower. The genetic similarities between *C. discolor* and *C. discolor* for. *sieboldii* was about 52%. It was thought that these 2 species are almost genetically different species, because the genetic distance is very far between 2 species and they have different morphological characteristics, such as flower color and flower size. The average genetic similarities between *C. discolor* and variants were 70%, and that of between *C. discolor* for. *sieboldii* and variants was 65%. These differences of genetic similarities indicate that the genetic position of variants is between *C. discolor* and *C. discolor* for. *sieboldii*, and variants is more genetically similar with *C. discolor* than *C. discolor* for. *sieboldii*. Orchid and Life (1990) reported that *Calanthe* species have been found in the flowering season in their habitats and that the different colored flowers originated *C. discolor* and *C. discolor* for. *sieboldii*. These factors of variants appearance indicate that there were high possibility to cross naturally between *C. discolor* and *C. discolor* for. *sieboldii* in their habitat. Our results appear to confirm isozyme studies by Hyun (1997) and Kim *et al*. (1990) which indicate genetic diversity associated with the origin of *C. discolor*, *C. discolor* for. *sieboldii*, and variants. On the other hand, RAPDs were able to distinguish among *Calanthe* species which were found to be monomorphic with isozymes. The *Calanthe* species fingerprinted in this study were similar enough in appearance to be grouped together taxonomically; however, their DNA fingerprints indicate significant genetic diversity. RAPD markers appear to be a good choice for assessing genetic relationships in *Calanthe* species with polymorphism levels sufficiently high to establish informative fingerprints with relatively few markers. The highly informative primers identified in our fingerprinting studies will be useful in future

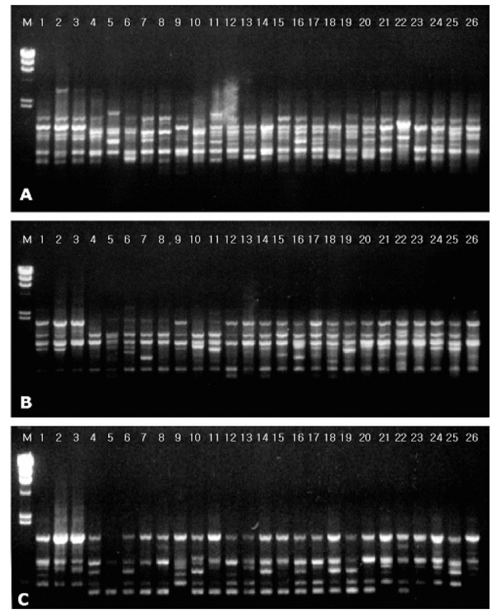

Fig. 2. RAPD profiles resulted from different *Calanthe* species using Operon primer OPC 08(A), OPD 03(B), and OPE 09(C). M: λ / Hind III (Expressed band's size is 23.1, 9.4, 6.5, 4.4, 2.3, 2.0, and 0.6 kb in order downward from upside), 1~3: *C. discolor*, native plant in Jeju, 4~6: *C. C. discolor* for. *Sieboldii*, native plant in Jeju, 7~26: variants.

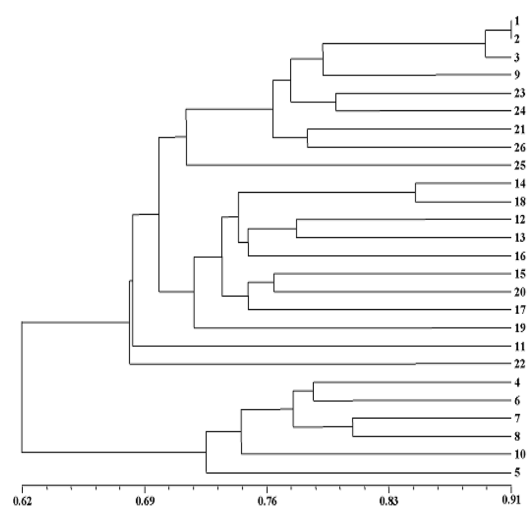

Fig. 3. UPGMA of genotype for *Calanthe* species and variants based on RPPD analysis. Coefficient value is the similarity of each other samples. 1~3: *C. discolor*, 4~6: *C. discolor* for. *sieboldii*, 7~26: variants.

genetic analysis to establish evolutionary and dendrogram. RAPDs are currently used routinely by plant breeders to identify genetic variation (Keil and Griffin, 1994; Lashermes *et al*., 1996; Perron *et al*., 1995), to locate regions of the genome linked to agronomical important genes (Reiter *et al*., 1992; Martin *et al*., 1991; Michelmore *et al*., 1991; Pillay and Kenny, 1996), and to facilitate introgression of desirable genes into commercial crops (Stuber, 1992; Lavi *et al*., 1994). The all variants was originated from *C. discolor* except the 7, 8, and 10 variant. We plan to use these markers in genetic populations being developed to tag genes associated with tool of flower color selection in *Calanthe* species.

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