(白花ミヤマキリシマ×サツキ‘博多白’)×キレンゲツツジの雑種個体における形態とRAPDマーカーの遺伝性について

誌名	園藝學會雑誌
ISSN	00137626
著者名	小林,伸雄 赤羽,勝 半田,高 高柳,謙治
発行元	園藝學會
巻/号	65巻1号
掲載ページ	p. 145-153
発行年月	1996年6月

J. Japan. Soc. Hort. Sci. 65(1) : 145-153. 1996.

Inheritance of Morphological Characters and RAPD Markers in Intersubgeneric Hybrids of Azalea, (*Rhododendron kiusianum* Makino × *R. indicum* (L.) Sweet) ×*R. japonicum* (A. Gray) Suringer f. *flavum* Nakai

Nobuo Kobayashi[1], Masaru Akabane[2], Takashi Handa[1] and Kenji Takayanagi[1]
[1]*Institute of Agriculture & Forestry, University of Tsukuba, Tsukuba, Ibaraki 305*
[2]*2469-5 Yasuzuka, Mibu-machi, Tochigi 321-02*

Summary

The inheritance of morphological characters and RAPD (random amplified polymorphic DNA) markers among the intersubgeneric hybrids of (*Rhododendron kiusianum* Makino × *R. indicum* (L.) Sweet) ×*R. japonicum* (A. Gray) Suringer f. *flavum* Nakai was investigated to clarify the usefulness of RAPD markers in the breeding program of yellow-flowered evergreen azalea (Akabane, 1993).

Tree forms of the hybrids were similar to *R. japonicum* f. *flavum*. Leaf morphology of the hybrids was intermediate to that of the parents. Microscopic observation of foliar trichomes revealed that all the hybrids had long hairs similar to those of *R. japonicum* f. *flavum*. The short hair trait of *R. japonicum* f. *flavum*. was observed in some hybrids. The degree of defoliation in winter varied among the hybrids.

Seven out of 16 primers, generated a total of 28 RAPD bands. The hybrids and their parents could be distinguished by band patterns. Inheritance of RAPD bands was confirmed by Southern hybridization.

Variations in morphology and polymorphisms of RAPD pattern among the hybrids were attributed to the heterozygosity and the phylogenetic distance between the parental species. The hybrids that had more paternal RAPD bands tended to exhibit more patrilineal morphological characters. This suggests that some RAPD markers and morphological characters may be linked.

Introduction

Azalea breeders have always dreamed of producing yellow-flowered evergreen azalea (Noguchi, 1930). There is no yellow-flowered azalea in the subgenus *Tsutsusi* which includes the evergreen azaleas ; therefore, the yellow color trait must be introduced from species of other subgenera through interspecific hybridization. *Rhododendron japonicum* (A. Gray) Suringer f. *flavum* Nakai in subgenus *Pentanthera* (Yamazaki, 1989) is a deciduous azalea that produces yellow flowers attributable to the presence of a carotenoid (Spethmann, 1980). Thus, this species is promising as one of the parents for breeding yellow-flowered azaleas. Hybridization of this species with evergreen azalea has been tried, but the few progenies obtained were albino (Akabane, 1971; Noguchi, 1930, 1932).

Recently, Akabane (1993) succeeded in obtaining intersubgeneric hybrids by crossing white-flowered hybrids, *R. kiusianum* Makino cv. 'Shirobana-miyamakirishima' × *R. indicum* (L.) Sweet cv. 'Hakata-jiro' of subgenus *Tsutsusi*, with yellow-flowered azalea (*R. japonicum* f. *flavum*). The morphological characters of these parents are obviously different, so that their hybrids are expected to show a wide range of morphologic and genetic variations.

Received for publication 21 August 1995.

A part of this paper was presented at the 1993 and 1995 spring meetings of the Japanese Society for Horticultural Science.

This work was partly supported by a grant-in-aid (No. 06404007) from the Ministry of Education, Culture, Sports and Science.

This study was conducted to investigate the inheritance of morphological characters including tree forms, leaf morphology, and defoliation in intersubgeneric hybrids of *Rhododendron*. RAPD analysis was used to disclose the complicated genomic constitutions of the hybrids and the inheritance patterns of the RAPD markers.

Materials and Methods

Plant Materials

In this study, we used 10 hybrids of '9129' line ('Hybrid No. 1'×*R. japonicum* f. *flavum* 'No. 1') and 5 hybrids of '9137' line ('Hybrid No. 11' × *R. japonicum* f. *flavum* 'No. 1') which were obtained by the crosses conducted in 1991 and planted in the following year (Akabane, 1993) (Table 1).

The maternal plants, 'Hybrid No. 1' and 'Hybrid No. 11', are white-flowered F_1 hybrids derived from the cross between *R. kiusianum* 'Shirobana-miyamakirishima', a white-flowered cultivar, and *R. indicum* 'Hakata-jiro', a white-flowered cultivar of unknown garden origin.

The pollen parent is *R. japonicum* f. *flavum* 'No. 1' which is a yellow-flowered selection of this predominantly orange to orange-red flowered species.

Morphological analysis

Tree form, plant height, numbers of branches in primary and secondary branching, leaf size, and leaf shape of each plant were investigated in early November, 1994. Defoliation was observed at the end of December, 1994. Microscopic evaluation of foliar trichomes was made in early June, 1995. The leaf width/leaf length ratio (W/L) was calculated.

DNA extraction

Newly expanded leaves of each plant were collected in early July, 1994 and the samples were stored at −80°C until needed. Total genomic DNA was extracted from 150 mg of frozen leaf sample by a modified CTAB method (Greenwood et al., 1989 ; Kobayashi et al., 1995 a, b).

After RNase treatment, both quality and quantity of DNA were determined by a UV spectrophotometer at 260 nm wave length.

DNA amplification

Sixteen arbitrary decamer primers (OPERON Technologies U.S.A.) which showed obvious polymorphisms in cultivar identification of azalea (Kobayashi et al., 1995 a, b) were used for PCR amplification (Williams et al., 1990). The reaction mixtures (10 μl) containing 10 ng genomic DNA, 0.5 μM primers, 0.1 mM dNTPs, 2 mM $MgCl_2$, 1 × the original reaction buffer, and 0.2 unit Ampli Taq DNA polymerase (Perkin Elmer Cetus-Takara) were prepared. DNA was amplified with DNA thermal cycler PJ-2000 (Perkin Elmer Cetus-Takara) by the following program ; 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C, and followed by 10 min at 72 °C. The amplified products were electrophoresed in 1.5 % agarose gels with 1 × TAE ; the resulting gel was stained with ethidium bromide and photographed under UV light. All reactions were repeated at least twice. The number of RAPD bands was counted and the proportion of bands inherited from each parent was calculated.

Southern hybridization

Amplified DNA was electrophoresed as described above and transferred to Hybond N^+ nylon blotting membrane (Amersham) using a transfer solution containing 0.4 M NaOH and 0.6 M NaCl.

The selected two RAPD fragments of the parents were excised from 0.8 % agarose gel, labeled with ECL kit (Amersham) and used as probes. Hybridization and washes were performed at 42 °C in a hybridization oven according to the manufacturer's instructions (Amersham). The membranes were exposed to RX x-ray film (Fuji) at room temperature.

Results and Discussion

Morphological characters (Table 1)

The parents, *R. japonicum* f. *flavum* 'No. 1' and 'Hybrid No. 1' or 'Hybrid No. 11', were morphologically different ; their hybrid progenies exhibited some morphological variations.

In this hybrid population, loosely branched shrubs were classified as '*R. japonicum*' type, densely branched and spreading shrubs similar to *R. kiusianum* and *R. indicum* as 'hybrid' type, and shrubs intermediate to the two forms as 'intermediate' type. Tree forms of lines '9129' and '9137' varied from 'intermediate' to '*R. japonicum*'

Table 1. Morphological characters of the parents and their hybrids.

Code #	Parents and hybrids	Tree form	Plant height (cm)	Number of branches		Leaf characters						Defoliation degree
				Primary	Secondary	Length (cm)	Width (cm)	W/L Ratio	Shape	Hair type	Short hair	
	Parents											
1	*Rhododendron kiusianum* ' Shirobana-miyamakirishima'	——	—	—	—	1.70	0.83	0.49	elliptic	seta	—[y]	3[x]
2	*R. indicum* ' Hakata-jiro'	——	—	—	—	2.45	1.12	0.46	elliptic	seta	—	0
3	' Hybrids No. 1' (#1×#2)	hyb[z]	—	—	—	2.45	1.12	0.46	elliptic	seta	—	1
4	' Hybrids No.11' (#1×#2)	hyb	—	—	—	2.05	1.16	0.57	elliptic	seta	—	2
5	*R. japonicum* ' No.1'	jap	—	—	—	8.58	2.51	0.29	narrow obovate	long	+	4
	Hybrids											
6	'9129- 1' (#3×#5)	jap	32	12	2	3.49	1.26	0.36	narrow elliptic	long	—	3
7	'9129- 2' (#3×#5)	jap	39	10	1	4.17	1.48	0.36	narrow elliptic	long	—	2
8	'9129- 3' (#3×#5)	jap	27	4	0	3.19	1.15	0.37	narrow elliptic	long	+	3
9	'9129- 4' (#3×#5)	mid	23	5	0	2.78	1.05	0.38	narrow elliptic	long	+	2
10	'9129- 5' (#3×#5)	mid	22	6	0	3.99	1.43	0.36	narrow elliptic	long	—	3
11	'9129- 6' (#3×#5)	jap	24	6	2	3.12	1.32	0.43	elliptic	long	+	3
12	'9129- 7' (#3×#5)	jap	20	4	2	2.66	1.18	0.45	elliptic	long	+	3
13	'9129-12' (#3×#5)	jap	23	6	2	4.09	1.35	0.33	narrow elliptic	long	+	3
14	'9129-14' (#3×#5)	mid	21	10	2	3.06	1.14	0.37	narrow elliptic	long	—	2
15	'9129-15' (#3×#5)	jap	20	7	0	3.05	1.17	0.39	narrow elliptic	long	+	2
16	'9137- 1' (#4×#5)	jap	49	11	5	4.06	1.80	0.44	elliptic	long	+	2
17	'9137- 2' (#4×#5)	jap	20	11	2	3.52	1.33	0.38	narrow elliptic	long	—	4
18	'9137- 3' (#4×#5)	mid	16	8	6	2.58	1.19	0.46	elliptic	long	—	2
19	'9137- 4' (#4×#5)	jap	18	7	0	2.87	1.29	0.45	elliptic	long	—	3
20	'9137- 5' (#4×#5)	mid	15	11	5	3.31	1.32	0.40	narrow elliptic	long	+	2

[z] hyb ; Hybrid No.1 or No.11 type, jap ; *R. japonicum* type, mid ; intermediate type of Hybrid No.1 or No.11 and *R. japonicum*.

[y] +; present, —; absent

[x] 0; almost no defoliation (*R. indicum*), 1; slight defoliation (Hybrid No.1), 2; intermediate (Hybrid No.11), 3; severe defoliation (*R. kiusianum*), 4; complete defoliation (*R. japonicum*). Data was collected on 23 Dec., 1994.

types ; no maternal 'hybrid' type was observed. Plant height and number of branches varied among the hybrids. Leaf length and width of the hybrids tended to be similar to their maternal parents, 'Hybrid No. 1' or 'Hybrid No. 11'. Leaf shapes of 'Hybrid No. 1' and 'Hybrid No. 11' were elliptic ; that of *R. japonicum* f. *flavum* 'No. 1' was narrow obovate ; those of the hybrids ranged from elliptic to narrow elliptic. Leaf W/L ratio of the hybrids was equal to the midparental average. Microscopic observation of foliar trichomes, revealed that 'Hybrid No. 1' and 'Hybrid No. 11' and their pa-

Table 2. Sequences of primers that generated polymorphic bands in this study.

Primer No.	Sequence
OPK-11	5′-AATGCCCCAG-3′
OPK-12	5′-TGGCCCTCAC-3′
OPK-19	5′-CACAGGCGGA-3′
OPM- 9	5′-GTCTTGCGGA-3′
OPM-11	5′-GTCCACTGTG-3′
OPO- 6	5′-CCACGGGAAG-3′
OPO-19	5′-GGTGCACGTT-3′

Table 3. Survey of 28 RAPD bands in this study.

Primer	size (bp)	Code #[z] of the parents and their hybrids																				
		1	2	3	6	7	8	9	10	11	12	13	14	15	5	4	16	17	18	19	20	5
OPK-11	1200	−[y]	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	+
	700	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	−	−
	650	−	−	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	−	+	+
	600	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−
	570	−	+	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	+	−	+	−
	500	+	+	+	−	−	−	−	+	+	−	−	−	−	−	+	+	+	+	−	+	−
	450	+	−	+	+	+	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−
OPK-12	1000	+	−	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−
	950	−	+	+	+	−	+	+	+	−	+	−	+	−	−	−	−	−	−	−	−	−
	750	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	−
	700	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
OPK-19	930	+	−	+	+	−	−	+	+	+	−	−	−	+	−	+	+	−	−	−	−	−
	850	+	+	+	−	+	+	−	−	−	+	+	+	−	−	+	−	+	+	+	+	−
	700	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	−
	600	−	−	−	+	−	+	+	−	+	−	+	−	−	+	−	+	+	+	+	+	+
	500	−	+	+	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	400	+	−	+	−	+	+	−	−	+	+	−	−	−	−	+	−	+	+	+	+	−
OPM-9	1450	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
	950	+	−	+	−	+	+	+	−	+	+	−	+	+	−	+	−	−	+	+	+	−
	550	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
OPM-11	800	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
	700	−	−	−	+	+	+	+	+	−	+	+	+	−	+	−	+	+	+	+	+	+
OPO-6	700	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	−
	650	−	−	+	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
	550	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
OPO-19	1000	+	−	+	−	−	+	+	−	−	+	+	+	−	−	+	+	+	+	−	−	−
	850	+	+	+	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	+	−	−
	800	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Total number of bands		14	8	16	18	18	21	21	17	16	20	17	19	15	10	13	16	19	21	18	18	10

[z] Code # corresponds to Table 1.
[y] + means present, − means absent

rents which belong to subgenus *Tsutsusi* had seta hairs, whereas *R. japonicum* f. *flavum* 'No. 1' had both long and short hair. Most hybrids had long hairs similar to *R. japonicum* f. *flavum* 'No. 1' ; a few had short hairs. No seta hair was observed among the hybrids. The degree of defoliation also varied among the hybrids. These hybrids have not yet blossomed.

The heterozygosity and genetic distance between the parents can account for these morphological variations among the hybrids.

RAPD analysis

Out of 16 primers tested, 7 primers (Table 2) generated a total of 28 RAPD bands ; all hybrids and their parents used in this study could be distinguished by their band patterns (Table 3).

RAPD patterns of *R. japonicum* f. *flavum* 'No .1' were quite different from those of the other parents, whereas *R. kiusianum* and *R. indicum* had several common RAPD bands (Table 3, Figs. 1-A, 2-A) which reflect the genetic distances among them.

The presence or absence of each RAPD band varied among the hybrids. RAPD markers are known as mostly dominant genetic markers inherited in a Mendelian fashion (Williams et al., 1990). The inheritance of RAPD markers has been reported in several field crops and arboreal species (Carlson et al., 1991; Harada et al., 1993; Heum

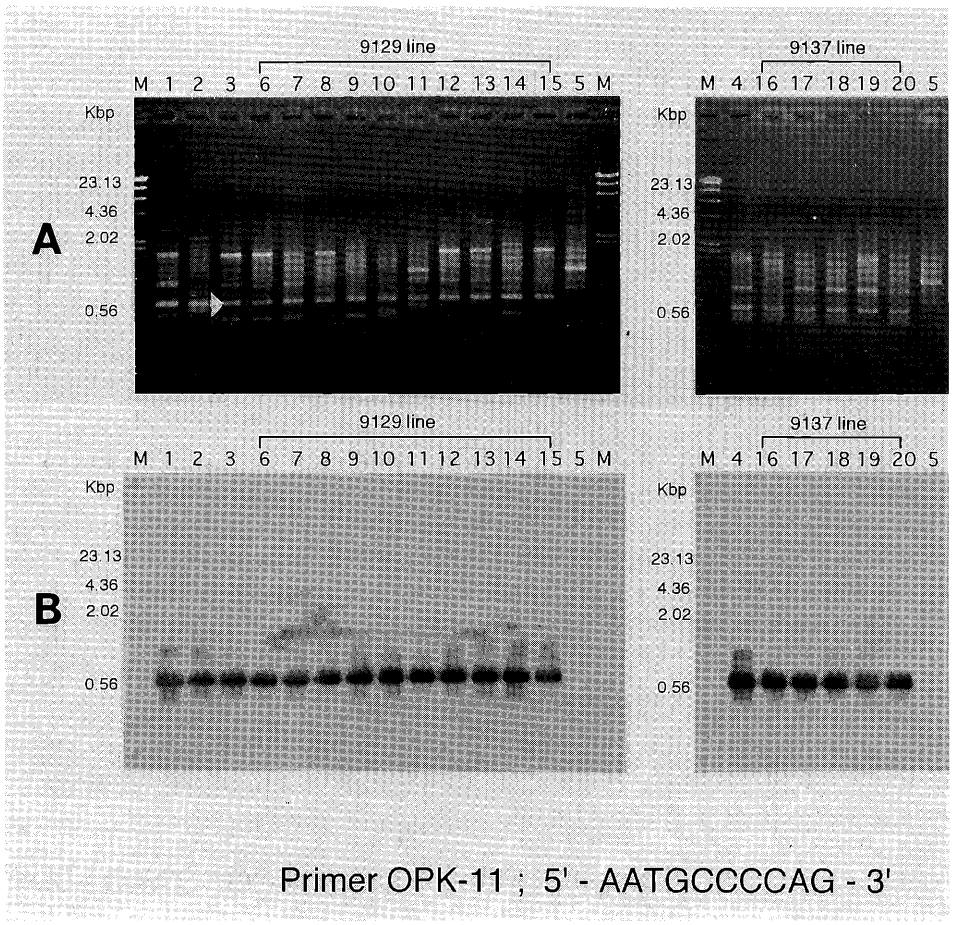

Fig. 1. A; RAPD pattern of parents, '9129' and '9137' lines generated by primer OPK-11. Lane No. corresponds to Code # of Table 1. Lane M is a molecular weight marker (Hind Ⅲ digested-lambda DNA).
B; Southern blot hybridization of RAPD pattern in Fig. 1-A with a labeled 600 bp RAPD fragment (Fig. 1-A, arrowhead).

and Helentjaris, 1993 ; Hosaka and Hanneman, 1994 ; Roy et al., 1992 ; Tinker et al., 1993). In this study, most of the RAPD bands in the hybrids were confirmed to follow this rule, although a 570-bp product of '9129-6' generated by primer OPK-11 and a 650-bp product of 'Hybrid No. 1' generated by primer OPO-6 were the exceptions. Polymorphisms among the hybrids may suggest the heterozygosity of their parents (Williams et al., 1990).

The ratios of bands inherited from the pollen parent (*R. japonicum* f. *flavum* 'No .1') were higher than those from the maternal ones ('Hybrid No. 1' and 'Hybrid No. 11') in all progenies except '9137-3' (Table 4). The morphology of the hybrids also tended to be more similar to the pollen parent. Hybrid '9137-3', which had the highest ratio of heritable bands from its maternal parent ('Hybrid No. 11'), exhibited more matrilineal morphology (Table 1). These results suggest that RAPD markers and morphological characters may be linked.

Southern analysis

In order to provide further molecular proof of inheritance of RAPD markers, RAPD products generated by primers OPK-11 (Fig. 1-A) and OPO-6 (Fig. 2-A) were transferred to membranes. The selected bands, specific to each of the parents, were then labelled as probes and used for South-

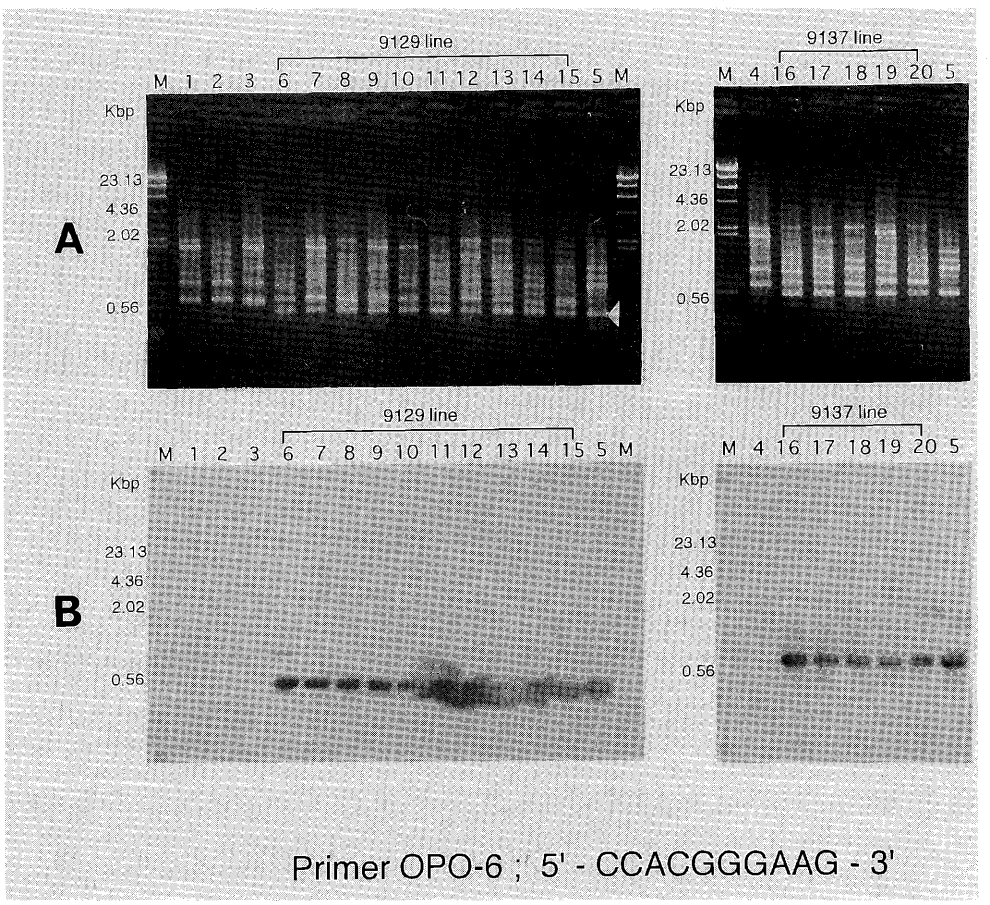

Fig. 2. A; RAPD pattern of parents, '9129' and '9137' lines generated by primer OPO-6. Lane No. corresponds to Code # of Table 1. Lane M is a molecular weight marker (Hind III digested-lambda DNA).
B; Southern blot hybridization of RAPD pattern in Fig. 2-A with a labeled 550 bp RAPD fragment (Fig. 2-A, arrowhead).

Table 4. Number of RAPD bands and the ratio of bands in the hybrids inherited from either parent.

Code #	Parents and hybrids	Total number of RAPD bands	Number of RAPD bands inherited from the following parents				
			Code#3	Code#4	Code#5	Code#1	Code#2
	Parents						
1	*Rhododendron kiusianum* 'Shirobana-miyamakirishima'	14	——	——	——	——	——
2	*R. indicum* 'Hakata-jiro'	8	——	——	——	——	——
3	'Hybrid No. 1' (#1×#2)	16	——	——	——	13(0.93)	7(0.88)
4	'Hybrid No.11' (#1×#2)	13	——	——	——	12(0.86)	6(0.75)
5	*R. japonicum* 'No.1'	10	——	——	——	——	——
	Hybrids						
6	'9129- 1' (#3×#5)	18	9(0.56)[z]	——	9(0.90)	7(0.50)	4(0.50)
7	'9129- 2' (#3×#5)	18	10(0.65)	——	8(0.80)	9(0.64)	4(0.50)
8	'9129- 3' (#3×#5)	21	12(0.75)	——	9(0.90)	9(0.64)	5(0.63)
9	'9129- 4' (#3×#5)	21	12(0.75)	——	9(0.90)	9(0.64)	4(0.50)
10	'9129- 5' (#3×#5)	17	9(0.56)	——	8(0.80)	8(0.57)	4(0.50)
11	'9129- 6' (#3×#5)	16	8(0.50)	——	8(0.80)	8(0.57)	5(0.63)
12	'9129- 7' (#3×#5)	20	12(0.75)	——	8(0.80)	10(0.71)	5(0.63)
13	'9129-12' (#3×#5)	17	8(0.50)	——	9(0.90)	8(0.57)	3(0.38)
14	'9129-14' (#3×#5)	19	11(0.69)	——	8(0.80)	8(0.57)	4(0.50)
15	'9129-15' (#3×#5)	15	8(0.50)	——	7(0.70)	7(0.50)	3(0.38)
16	'9137- 1' (#4×#5)	16	——	7(0.54)	9(0.90)	6(0.43)	4(0.50)
17	'9137- 2' (#4×#5)	19	——	10(0.77)	9(0.90)	9(0.64)	6(0.75)
18	'9137- 3' (#4×#5)	21	——	12(0.92)	9(0.90)	11(0.79)	6(0.75)
19	'9137- 4' (#4×#5)	18	——	9(0.69)	9(0.90)	9(0.64)	4(0.50)
20	'9137- 5' (#4×#5)	18	——	9(0.69)	9(0.90)	8(0.57)	5(0.63)

[z] Number in parenthesis ; Inherited ratio of bands = $\frac{\text{number of band in the hybrid inherited from the parent}}{\text{total number of bands in the parent}}$

ern hybridization.

OPK-11, a 600-bp product of 'Hybrid No. 1' (lane 3), indicated by an arrowhead in Fig. 1-A, was excised and labelled as a probe. Fig. 1-B shows that the labelled probe clearly hybridized with the bands of the maternal parental line (lanes 1, 2, 3 and 4) and all the hybrids of '9129' and '9137' lines (lanes 6-15 and 16-20), but not that of *R. japonicum* f. *flavum* 'No. 1' (lane 5). This result matches the expectation from the RAPD pattern of Fig. 1-A and proves that the 600-bp RAPD fragment is a dominant marker of the maternal parent.

OPO-6, a 550-bp product of *R. japonicum* f. *flavum* 'No. 1', indicated by an arrowhead in Fig. 2-A, when used as a probe hybridized with the bands of *R. japonicum* f. *flavum* 'No. 1' (lane 5) and those of hybrids of '9129' and '9137' lines (lanes 6-15 and 16-20), but not with those of the maternal parental line (lane 1, 2, 3 and 4) (Fig. 2-B). This result was expected from the RAPD pattern of Fig. 2-A and reveals that the 550-bp RAPD fragment of *R. japonicum* f. *flavum* 'No. 1' is a dominant marker of the paternal parents.

In this experiment, we tried to associate the morphological characteristics with the RAPD band patterns among the intersubgeneric hybrids between 'Hybrid No. 1' or 'Hybrid No. 11' (maternal parent) and *R. japonicum* f. *flavum* 'No. 1' (pollen parent). Morphological variations among the intersubgeneric hybrids reflected the heterozygosity of the parents. Using the unique RAPD bands and the amplification patterns generated from the parents and their hybrids for genetic analyses, correlations between RAPD markers and morphological characters were indicated in this study.

Although carotenoids are generally inherited recessively, we expect some of these hybrids to produce yellow flowers, because most of the hybrids had the morphology and RAPD bands similar to those of the yellow-flowered paternal parent.

Acknowledgement

We are grateful to Ms. I. Ohshima of the Institute of Agriculture & Forestry, University of Tsukuba for her technical advice of Southern analysis.

Literature Cited

Akabane, M. 1971. Wide cross of genus *Rhododendron*. Recent advances in Breeding 12 : 83-89. (In Japanese).

Akabane, M. 1993. On the cross compatibility F_1-plants (*Rhododendron kiusianum* Mak. × *R. indicum* Sweet) and *R. japonicum* Suringer. J. Japan. Soc. Hort. Sci. 62 (Suppl. 1) : 344-345. (In Japanese).

Carlson, J. E., L. K. Tulsieram, J. C. Glaubitz, V. W. K. Luk, C. Kauffeldt and R. Rutledge. 1991. Segregation of random amplified DNA markers in F_1 progeny of conifers. Theor. Appl. Genet. 83 : 194-200.

Greenwood, M. S., C. A. Hopper and K. W. Hutchison. 1989. Mutation in Larch 1. Effect of age on shoot growth, foliar characteristics and DNA methylation. Plant Physiol. 90 : 406-412.

Harada, T., K. Matsukawa, T. Sato, R. Ishikawa, M. Niizeki and K. Saito. 1993. DNA-RAPDs detect genetic variation and paternity in *Malus*. Euphytica 65 : 87-91.

Heun, M. and T. Helentjaris. 1993. Inheritance of RAPDs in F_1 hybrids of corn. Theor. Appl. Genet. 85 : 961-968.

Hosaka, K. and R. E. Hanneman, Jr. 1994. Random amplified polymorphic DNA markers detected in a segregating hybrid population of *Solanum chacoense* ×*S. phureja*. Japan. J. Genet. : 53-66.

Kobayashi, N., R. Takeuchi, T. Handa and K. Takayanagi. 1995a. Cultivar identification of azalea with RAPD method. J. Japan. Soc. Hort. Sci. 64 : 611-616.

Kobayashi, N., M. Akabane, T. Handa and K. Takayanagi. 1995b. Studies on phylogeny of *Rhododendron* L. and origin of horticultural azalea. 3. Inheritance of morphological characters and RAPD markers in hybrids of (*Rhododendron kiusianum* Makino × *R. indicum* (L.) Sweet) × *R. japonicum* (A. Gray) Suringer, J. Japan. Soc. Hort. Sci. 64 (Suppl. 1) : 344-345. (In Japanese).

Noguchi, Y. 1930. How to breed yellow-flowered azalea. Nippon Engei Zassi. 42 : 1-6. (In Japanese).

Noguchi, Y. 1932. Studies on the species crosses of Japanese *Rhododendron*. 1. On the crossability between various species and the cotylendon color of F_1 seedlings. Japan. J. Bot. 6 : 103-126.

Spethmann, W. 1980. Flavonoids and carotenoids of rhododendron flowers and their significance for the classification of the genus *Rhododendron*. p. 247-275. In : J. L. Luteyn and M. E. O'Brien (eds.). Contributions toward a classification of rhododendron. New York Bot. Garden, New York.

Roy, A., N. Frascaria, J. Mackay and J. Bousquet. 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. Theor. Appl. Genet. 85 : 173-180.

Tinker, N. A., M. G. Fortin and D. E. Mather. 1993. Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. 85 : 976-984.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Acid Res. 18 : 6531-6535.

Yamazaki, T. 1989. Wild flowers of Japan. Woody plants II. p. 122-156. Heibonsha, Tokyo. (In Japanese).

(白花ミヤマキリシマ×サツキ'博多白')×キレンゲツツジの雑種個体における形態とRAPDマーカーの遺伝性について

小林伸雄[1]・赤羽　勝[2]・半田　高[1]・高柳謙治[1]

[1]筑波大学農林学系　305　茨城県つくば市天王台1-1-1

[2]栃木県下都賀郡壬生町安塚2469-5　321-02

摘　要

'白花ミヤマキリシマ'とサツキ'博多白'の雑種2個体('Hybrid No. 1'および'No. 11')にキレンゲツツジ'No. 1'を黄色花の常緑性ツツジの作出を目標に交雑した亜属間雑種個体(3年生)を材料として，形態学的特徴とRAPDマーカーの遺伝性について検討した．

'9129'系('Hybrid No. 1'×キレンゲツツジ'No. 1')および，'9137'系('Hybrid No. 11'×キレンゲツツジ'No. 1')とも外観上の樹形はレンゲツツジ型に偏り，葉の大きさや形状は両親の形態の範囲内で雑種個体ごとに様々であった．顕微鏡による葉の毛じの観察ではすべての雑種個体でレンゲツツジ型の長毛を有していたが，レンゲツツジ特有の短毛についてはその有無が分離した．落葉の程度も両親の中間で個体差が見られた．

RAPD分析では，7プライマーで28本の多型バンドが得られ，本研究に用いた20個体をすべて識別できた．雑種個体が有するバンドのほとんどは明白にバンドパターンが異なる両親から遺伝していた．さらにサザン分析によりバンドの遺伝性を証明した．

これらの雑種における多様な形態変異やRAPDパターンの多型は，両親のヘテロ性や遠縁交雑によるものと思われる．また，全体に花粉親のレンゲツツジに偏った形態を示す雑種個体ではレンゲツツジ由来のRAPDバンドを有する比率が高く，逆に，母親の'Hybrid No. 11'由来のバンドの比率が最も高い'9137-3'の形態形質はより母親に近いことからもRAPDマーカーと形態との関連性が示唆された．