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Comparison of the Juvenile Period of Interspecific–Cross Seedlings in Evergreen Azaleas

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We evaluated the time to the first flowering of the crossed seedlings of five species and one variety of evergreen azaleas (*Rhododendron* spp.) to obtain the basic understanding for controlling of the juvenility. It required three years after crossing for the first flowering in all crosses and over five years for obtaining of 70% flowering plants in most crosses. The length of the juvenile period of the seedlings was different by cross parent, whereas the difference by cross direction was not affected. The juvenile period was the longest in the crossed seedlings with *R. simsii*, and less than 35% seedlings flowered five years after crossing in some crosses.

INTRODUCTION

Many cultivated varieties of Japanese azaleas (*Rhododendron* spp.) have been brought up through cross breeding among evergreen species (Kunishige and Kobayashi, 1980). The time to flowering from seed sowing is long in this plant, which prevents from efficient cross breeding. In the cross breeding of azalea, mature seeds harvested six months after crossing are sown immediately in fall or sown in next spring. It is said that several years are required for the first flowering in azaleas (Doorenbos, 1955). However, the knowledge of juvenile period of crossed seedlings is scanty and it must be of assistance in shortening the juvenile period in breeding. The objective of this study is to investigate the time to the first flowering of crossed seedlings in several evergreen azaleas for controlling of the juvenility of the offspring.

MATERIALS AND METHODS

Interspecific crossing among five species and one variety of evergreen azaleas (Table 1) was carried out in April 1996. Flowers of seed parents were emasculated two or three days before anthesis, and the pistils were covered with paper bags to prevent any pollen from contamination. The stigmas were pollinated with fresh pollen and the emasculated flowers were isolated in paper bags for one month.

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Table 1. *Rhododendron* species used as parents.

Species	Code
R. kaempferi	KAE
R. kaempferi var. *macrogemma*	MCR
R. kiusianum	KIU
R. simsii	SIM
R. tosaense	TOS
R. transiens	TRA

Capsules were harvested six months after crossing and air–dried at 25 °C for two weeks. Seeds were taken out of the capsules, soaked in 50 mgl^{-1} of GA_3 under dark condition at 25 °C for 24 hrs, and sown on sphagnum. They were incubated at 25 °C under a continuous light with cool–white fluorescent lamps of 25 $\mu molm^{-2}s^{-1}$ PPFD.

In July 1997, all seedlings having about 10 leaves were transplanted into polyvinyl pots (7.5 cm in diameter, 6.5 cm in height) filled with pumice, and cultivated in a greenhouse with 50% shade at the experimental field of Kyushu University, Fukuoka, Japan. Lateral shoots ($<$2 cm) of all the seedlings were removed to promote the growth of the main stem in 1997, and then no pinching treatment was given. In June 1998, the seedlings with sufficient vegetative growth were transplanted to polyvinyl pots (12 cm in diameter, 10 cm in height) containing pumice and cultivated in the same greenhouse. The seedlings were fertilized with 1000 times diluted OK–F–1 (N=15%, P_2O_5=8%, K_2O=17%, Otsuka Kagaku Co., Tokyo) weekly during May and October until 1998 and with solid organic fertilizer (10 g per pot every two months) thereafter. From July 1997 to May 2001, the mean monthly temperature ranged from 6.4 °C to 27.6 °C.

Flowering was recorded in every spring of 1998–2001 in all the crosses.

RESULTS

No seedlings flowered in the spring of 1998 (two years after crossing) in any cross combination. In the spring of 1999 (three years after crossing), the flowering percentage of the seedlings varied by the cross combinations (Table 2). The percentage was highest (90%) in the cross of *R. tosaense* × *R. transiens*, and about half of the seedlings set flowers in four crosses; *R. kaempferi* × *R. kiusianum*, *R. kiusianum* × *R. kaempferi*, *R. kiusianum* × *R. tosaense* and *R. transiens* × *R. kaempferi* var. *macrogemma*. Three crosses of *R. kaempferi* var. *macrogemma* × *R. simsii*, *R. kiusianum* × *R. transiens* and *R. tosaense* × *R. kiusianum*, however, did not flower yet. Cross direction did not affect the percentage in most crosses, excepting two reciprocal crosses between *R. tosaense* and *R. transiens* (28.6% vs. 90.0%) and between *R. kiusianum* and *R. tosaense* (0% vs. 45.8%). The averages were 15–35% in the crosses with *R. kaempferi*, *R. kaempferi* var. *macrogemma*, *R. kiusianum*, *R. tosaense* and *R. transiens*, whereas with *R. simsii*, they were only 5–7%. Totally, the average in all the seedlings was 21.4%.

Flowering was observed in all the crosses in the spring of 2000 (four years after crossing) (Table 3). The flowering percentages were apparently different among cross

Table 2. Percentage of interspecific–cross seedlings with flowering in the spring of 1999 (three years after crossing).

Female	Male												Ave.
	KAE		MCR		KIU		SIM		TOS		TRA		
KAE			38.2	(34)	45.6	(79)	3.3	(30)	4.5	(22)	8.3	(12)	20.0
MCR	38.7	(31)			9.1	(33)	0	(35)	19.4	(31)	36.4	(33)	20.7
KIU	56.6	(53)	20.0	(10)			17.1	(35)	45.8	(24)	0	(35)	27.9
SIM	3.2	(31)	2.6	(38)	11.1	(27)			6.3	(32)	3.2	(31)	5.3
TOS	–[a]		–		0	(23)	9.7	(31)			90.0	(10)	33.2
TRA	21.4	(14)	43.5	(23)	32.3	(31)	3.1	(32)	28.6	(21)			25.8
Ave.	30.0		26.1		19.6		6.6		15.7		27.6		21.4

The value in parenthesis indicates the number of investigated seedlings.
[a] Not investigated.

Table 3. Percentage of interspecific–cross seedlings with flowering in the spring of 2000 (four years after crossing).

Female	Male						Ave.
	KAE	MCR	KIU	SIM	TOS	TRA	
KAE		73.5	87.3	40.0	59.1	66.7	65.3
MCR	74.2		45.5	25.7	61.3	54.5	52.2
KIU	90.6	50.0		45.7	66.7	28.6	56.3
SIM	25.8	42.1	40.7		53.1	29.0	38.1
TOS	–[a]	–	78.3	45.2		100	74.5
TRA	78.6	56.5	51.6	18.8	47.6		50.6
Ave.	67.3	55.5	60.7	35.1	57.6	55.8	54.9

The number of investigated seedlings is indicated in Table 2.
[a] Not investigated.

combinations; from 18.8% in *R. transiens* × *R. simsii* to 100% in *R. tosaense* × *R. transiens*. Seedlings with flowering were obtained at the rate of >70% in seven crosses, in which four crosses were two reciprocal crosses between *R. kaempferi* and *R. kaempferi* var. *macrogemma* and between *R. kaempferi* and *R. kiusianum*. The averages were 38.1% and 35.1% in the crosses using *R. simsii* as seed and pollen parents, respectively, and the values were lower than those in the other crosses. Totally, more than half of the seedlings flowered (54.9%).

About half of the seedlings flowered in the most crosses with *R. simsii* five years after crossing, while 70% seedlings set flowers in the most of the other crosses (Table 4). The average was particularly high (>90%) in the reciprocal crosses between *R. kaempferi* and *R. kiusianum*, *R. kaempferi* var. *macrogemma* × *R. kaempferi* and *R. tosaense* × *R. transiens*.

The difference of the flowering percentages between reciprocal crosses was below 20% in all cross combinations throughout the three years, except for three reciprocal crosses between *R. kiusianum* and *R. tosaense*, between *R. kiusianum* and *R. transiens*, and between *R. tosaense* and *R. transiens*.

Table 4. Percentage of interspecific–cross seedlings with flowering in the spring of 2001 (five years after crossing).

Female	Male						Ave.
	KAE	MCR	KIU	SIM	TOS	TRA	
KAE		79.4	91.1	46.7	59.1	83.3	71.9
MCR	96.8		66.7	48.6	80.6	72.7	73.1
KIU	90.6	70.0		65.7	75.0	71.4	74.5
SIM	32.3	55.3	66.7		62.5	58.1	55.0
TOS	–[a]	–	87.0	54.8		100	80.6
TRA	78.6	69.6	87.1	34.4	61.9		66.3
Ave.	74.6	68.6	79.7	50.0	67.8	77.1	69.5

The number of investigated seedlings is indicated in Table 2.
[a] Not investigated.

DISCUSSION

It was confirmed that it took at least three years after crossing for the first flowering of the seedlings from immediate sowing of mature seeds in azaleas, and that it required five years to obtain more than 70% flowering for assessing the flower characteristics of the progenies.

Offspring in Scots pine between early–flowering and late–flowering parents generally produced smaller proportions of precocious flowering, which was especially observed in the crosses using early–flowering parent as male (Teich and Holst, 1969). A large number of early–flowering hybrids are, on the other hand, obtained in pear, especially in using early–flowering parent as female (Zielinski, 1963). It is reported in apple that juvenile period of crossed seedlings parallels to that of seed parent (Way, 1971). These results indicate that cross direction participates the flowering age of progenies. An opposite example is, however, confirmed in garden stock, that flowering time of offspring is intermediate, but a little close to the early–flowering parent, irrespective of cross direction (Ecker *et al.*, 1994). Regarding the effect of cross direction on the juvenile period of progenies, our results are similar to those in garden stock, and cross direction may not be considered in breeding program of azaleas.

The length of the juvenile period in all the crosses with *R. simsii* was longer than that of the other crosses in the present study even cultured under the same environmental conditions. It is considered that *R. simsii* has longer juvenile period than other species, and that long juvenility inherit dominantly, as shown in Scots pine (Teich and Holst, 1969). *Rhododendron simsii* distributes in Iriomote Island, where the climate is subtropical, while the others used in this study are temperate species (Yamazaki, 1996). Since the present study was conducted in temperate area, the temperature might not be enough for the first flowering in the crossed seedlings with *R. simsii*.

We conclude that the selection of parents may strongly influence the efficiency of breeding program, progenies in the crosses with subtropical species require the long period for the first flowering in temperate area, and other measures like ovule culture (Michishita *et al.*, 2001) or rapid growth treatment (Doorenbos, 1955) should be effectively taken in the azalea breeding.

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