Varietal and Individual Differences in Cross-compatibility in the $2x \times 4x$ Crosses of Cyclamen (*Cyclamen persicum* Mill.)

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Summary

Varietal and individual differences in cross-compatibility in the $2x \times 4x$ crosses of cyclamen, particularly those of diploid pistillate parents, were investigated. A factor of tetraploid formation and efficient methods to obtain progenies in the crosses are discussed.

Different cross-compatibilities, depending upon the pistillate parents, were observed in the $2x \times 4x$ crosses, whereas pollen parents scarcely affected fruiting and seed production. There were individual and varietal differences, and all progenies in the crosses showed tetraploidy. All the diploid pistillate parents producing tetraploid progenies through crossing had giant pollen grains. These results suggest the possibility that fertilization between a reduced male gamete from a tetraploid plant and an unreduced female gamete from a diploid plant results in tetraploid progenies.

The individual and varietal differences of cross-compatibility among parents also suggest that the selection of diploid pistillate parents decides the formation of seeds. Thus, the selection of diploid pistillate cultivars and individuals, which can produce the tetraploid progenies, should be effective in the inter-ploidy cross breeding of cyclamen.

Key Words: cyclamen, inter-ploidy cross, cross-compatibility, individual and varietal differences, unreduced gametes.

Introduction

There are diploid and tetraploid cultivars containing aneu-tetraploid in cyclamen (*Cyclamen persicum* Mill.). The triploid cultivar in cyclamen has never been observed so far, although sesquidiploids between *C. persicum* and its wild relatives have been bred through the ovule culture (Ishizaka and Uematsu, 1992, 1995; Ishizaka, 1996). Commonly, the polyploidy breeding in ornamental plants is effective for improving characteristics. Since there are valuable characteristics in each ploidy of cyclamen, hybridization between the diploid and tetraploid seems to be useful for breeding the new cultivars and/or triploids. However, the cross breeding between the diploids and tetraploids is difficult because of the low cross-compatibility in the reciprocal crosses (Takamura and Miyajima, 1996a). Moreover, only few tetraploid seeds were formed in these reciprocal crosses

because of the degeneration of the zygotic embryo (Takamura and Miyajima, 1996a, b). Although the formation of the unexpected tetraploid progenies in cyclamen was reported by Kappert (1941), Wellensiek (1955) and Legro (1959), the mechanism of the tetraploid formation remains unclear. The mechanism in $2x \times 4x$ crosses may differ from that in $4x \times 2x$ crosses, because the ploidy of pistillate and pollen parent in the $2x \times 4x$ differs from the ploidy in the $4x \times 2x$. Therefore, factors on the tetraploid formation in the $2x \times 4x$ and $4x \times 2x$ crosses should be investigated separately.

Esen and Soost (1972) reported that the formation of tetraploid progenies in the $2x \times 4x$ crosses of *Citrus* depended on the diploid pistillate parents; they concluded that the main factor of tetraploid formation in the crosses is the formation of unreduced female gametes in the diploid. In cyclamen, the cross-compatibilities in the $4x \times 2x$ crosses depended on the diploid pollen parents (Takamura and Miyajima, 1992, 1996a). These facts suggest that tetraploid-progeny formation in the crosses between diploid and tetraploid may be attributed to the diploid parents rather than the tetraploids. However, the varietal and/or individual differences between cross-compatibility in the $2x \times 4x$ crosses of cyclamen have never been investigated. In this study, such differences, particularly those caused by diploid pistillate parents, were investigated to find efficient methods for obtaining desirable progenies, and the factor of unexpected tetraploid production in the crosses was also

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estimated.

Materials and Methods

Four diploid cultivars, 'Anneke', 'Wase Murasaki', 'Pure White', and 'Kage Yellow' were used as pistillate parents. Ten plants from each cultivar were hand-pollinated with a diploid 'Pure White' and tetraploids 'Victoria', 'Lilac', and 'Bonfire'. The percentage of giant pollen grains in all the plants used as pollen parents was 0–0.1%.

In January 1992, 1993, and 1994, the pollinations were carried out seven days after emasculation, according to our earlier report (Takamura and Miyajima, 1996a) in a greenhouse; all pollinated flowers were covered with paper bags to prevent contamination by undesirable pollen grains. When pollen grains were collected from some flowers of the pistillate parents which were not emasculated and pollinated, pollen viability and diameters were recorded. The pollen viability was assessed by staining fresh pollen in 0.1% aceto-carmin. Seeds were extracted three to four months after pollination (at fruit maturity).

All the hybrid seeds obtained by the $2x \times 4x$ crosses, except the seeds from 'Kage Yellow' $\times$ 'Lilac' crosses,

and ten seeds from each of the $2x \times 2x$ crosses were sown in the greenhouse in October 1992, 1993, and 1994 and allowed to mature into flowering plants. For assessment of the ploidy levels of F_1 progenies, their pollen viability, pollen diameter, guard cell size, and chromosome number of root-tip cells were recorded. The observations were carried out in the same manner as reported previously (Takamura and Miyajima, 1996a).

For the cytological observation, all seeds obtained by 'Kage Yellow' $\times$ 'Lilac' crosses and those of diploid and tetraploid cultivars were fixed in FAA (formalin: acetic acid: 50% ethanole, 1: 1: 18, v/v/v). After 24 hours the seeds were transferred to 70% ethanol and stored at 5 °C until use. They were dehydrated in a butyl alcohol series and embedded in paraffin (melting point 58–60 °C). The paraffin embedded seeds were sectioned at 10 or 15 μ m and the sections were stained with hematoxylin.

Results and Discussion

We have already reported the difficulty in obtaining fully-developed hybrid seeds in the $2x \times 4x$ cyclamen crosses of 'Pure White' $\times$ 'Lilac' and 'Pure White' $\times$ 'Victoria' (Takamura and Miyajima, 1996a). In this

Table 1. Seed set in the crosses of $2x \times 4x$ and $2x \times 2x$ cyclamen cultivars.

Pistillate parent	Pollen parent	Year	No. of flowers pollinated	No. of fruits obtained	No. of fully developed seeds	Fully developed seeds per fruit
2x × 4x						
‘Anneke’	‘Victoria’	1992	100	44(0) ^z	0	0
		1993	100	46(2)	2	0.0 ± 0.0
‘Wase Murasaki’	‘Lilac’	1992	100	39(2)	2	0.1 ± 0.0
	‘Bonfire’	1993	100	49(2)	3	0.1 ± 0.0
	‘Victoria’	1992	100	51(0)	0	0
		1993	100	45(0)	0	0
	‘Lilac’	1992	100	45(0)	0	0
	‘Bonfire’	1993	100	38(0)	0	0
‘Pure White’	‘Victoria’	1992	100	41(6)	8	0.2 ± 0.1
		1993	100	45(6)	9	0.2 ± 0.1
	‘Lilac’	1992	100	39(5)	6	0.2 ± 0.1
	‘Bonfire’	1993	100	43(10)	11	0.3 ± 0.1
‘Kage Yellow’	‘Victoria’	1993	100	40(21)	35	0.9 ± 0.2
		1994	100	33(17)	34	1.0 ± 0.3
	‘Bonfire’	1993	100	38(21)	40	1.1 ± 0.2
	‘Lilac’	1994	100	44(24)	60	1.4 ± 0.3

2x × 2x						
‘Anneke’	‘Pure White’	1992	100	79(79)	3471	44.5 ± 2.3
		1993	100	74(74)	3112	42.1 ± 2.0
‘Wase Murasaki’	‘Pure White’	1992	100	78(78)	3231	41.4 ± 2.3
		1993	100	78(78)	2884	37.0 ± 2.2
‘Pure White’	‘Pure White’	1992	100	86(86)	2664	31.0 ± 1.6
		1993	100	83(83)	2978	36.3 ± 2.0
‘Kage Yellow’	‘Pure White’	1993	100	71(71)	2201	31.0 ± 1.6
		1994	100	77(77)	2187	28.4 ± 2.2

^z No. of fruit with fully developed seeds

study, the $2x \times 4x$ crosses also set seeds poorly, as compared with the $2x \times 2x$ crosses (Table 1). Less than 50% of fruiting and only two fully-developed seeds per fruit were recorded in the $2x \times 4x$ crosses, whereas more than 70% of pollinated flowers in the $2x \times 2x$ crosses set fruits, yielding more than 30 fully-developed seeds per fruit.

Different cross-compatibilities, depending upon the pistillate parents in the $2x \times 4x$ crosses, are shown in Table 1. However, pollen parents scarcely affect fruiting and seed production in the crosses, e.g., more than 50% of fruits had fully-developed seeds in the crosses with 'Kage Yellow' as pistillate parent, whereas no seeds were obtained with 'Wase Murasaki'. Fewer seeds were obtained in crosses with 'Anneke' and 'Pure White' than with 'Kage Yellow'. It is difficult to attribute these results to the differences in seed-production ability among pistillate parents, because more fully-developed seeds were obtained in the $2x \times 2x$ crosses of 'Wase Murasaki' $\times$ 'Pure White' and 'Anneke' $\times$ 'Pure White' than from 'Kage Yellow' $\times$ 'Pure White'.

Individual differences in seed-production ability in the $2x \times 4x$ crosses were observed even between the pistillate-parent plants in same cultivars. Independently of pollen parents, the ability of pistillate individuals to form fully-developed seeds were fixed. Only one of 'Anneke' plants and three of 'Pure White' plants formed seeds in the crosses with three tetraploid-cultivars as pollen parents (Figs. 1 and 2). In crosses of 'Kage Yellow' $\times$ three tetraploid-cultivars, nine pistillate individuals formed seeds and their number of seed per fruit was also stable (Fig. 3). One of the nine plants did not form seeds in crosses of 'Kage Yellow' $\times$ 'Victoria' in 1993, which might be due to the lower fruiting percentage caused by unknown factors.

Takamura and Miyajima (1996a) reported that all F_1 progenies in the $2x \times 4x$ cyclamen crosses of 'Pure White' $\times$ 'Lilac' and 'Pure White' $\times$ 'Victoria' were not triploidy but tetraploidy. In this study, we also found that the progenies obtained in the $2x \times 4x$ crosses were tetraploids (Table 2). These tetraploid progenies were neutral tints of the parents (data not shown) which suggest that the fixed pistillateparent plants forming fully-developed seeds produced unexpected tetraploid progenies. The formation of unexpected tetraploids in $2x \times 4x$ crosses has been reported in many plants, such as *Dactylis* (Carroll and Borrill, 1965), *Sorghum* (Endrizzi, 1957; Hadley, 1958), *Zea* (Alexander and Beckett, 1963; Rhoades and Dempsey, 1966), *Campanula* (Gairdner and Darlington, 1931), *Solanum* (Koopmans and van der Burg 1952), *Citrus* (Tachikawa et al., 1961; Esen and Soost, 1972), and *Primula* (Skiebe, 1958). However, almost all of them reported that F_1 progenies in $2x \times 4x$ crosses were either triploids or tetraploids which suggests that the zygotes of triploid cyclamen degenerated in the process of embryo development as reported previously (Takamura and Miyajima, 1996b) and that

Fig. 1. Individual differences in cross-compatibility among $2x$ 'Anneke' $\times$ $4x$ cultivars and between 'Anneke' $\times$ $2x$ 'Pure White'.

□ ; number of seeds in 1992, □ ; seed set percentage in 1992
 ■ ; number of seeds in 1993, ◆ ; seed set percentage in 1993.
 vertical bar ; standard error.

^z Individuals.

^y Percentage of giant pollen grains in the individual.

only the tetraploid zygotes survived.

Esen and Soost (1972) attributed the unexpected *Citrus* tetraploids from the $2x \times 4x$ crosses to the diploid female gametes in the diploid pistillate parents, because the endosperm of the tetraploids was hexaploid and the frequency of tetraploids was dependent on the pistillate parents. This study also shows that the formation of fully-developed tetraploid seeds in $2x \times 4x$ cyclamen crosses was dependent on the pistillate parents. The tetraploid seed formation was usually constant when a

Fig. 2. Individual differences in cross-compatibility among 2x 'Pure White' × 4x cultivars and between 'Pure White' × 2x 'Pure White'.

□ ; number of seeds in 1992, □ ; seed set percentage in 1992
 ▨ ; number of seeds in 1993, ◆ ; seed set percentage in 1993.
 vertical bar ; standard error.

^z Individuals.

^y Percentage of giant pollen grains in the individual.

given diploid pistillate individual was pollinated with any tetraploid pollen cultivars, but the number varied with diploid pistillate individuals when pollinated with a given tetraploid cultivar (Figs. 1, 2 and 3). In addition, all pistillate parents forming fully-developed seeds in the 2x × 4x crosses had giant pollen grains, which are considered as unreduced male gametes (Takamura and Miyajima, 1994); no diploid without the giant pollen grain produced seeds (Fig. 4). This finding suggests that

Fig. 3. Individual differences in cross-compatibility among 2x 'Kage Yellow' × 4x cultivars and between 'Kage Yellow' × 2x 'Pure White'.

▨ ; number of seeds in 1993, ◆ ; seed set percentage in 1993
 ▨ ; number of seeds in 1994, ○ ; seed set percentage in 1994.
 vertical bar ; standard error.

^z Individuals.

^y Percentage of giant pollen grains in the individual.

the production of tetraploid progenies is dependent on the formation of unreduced gametes by the diploid pistillate parents. The cell size and appearance of embryo and endosperm of the tetraploid seed from 2x × 4x crosses of 'Kage Yellow' × 'Lilac' were similar to those of tetraploid seed from 4x × 4x crosses (data not shown). Therefore, there is a possibility that the endosperm of tetraploid seed from 2x × 4x crosses was hexaploid, but this supposition was not confirmed. Nevertheless, we postulate that the fertilization between

Table 2. Ploidy level of F_1 progenies in the crosses of $2x \times 4x$ and $2x \times 2x$ cyclamen cultivars.

Pistillate parent	Pollen parent	Year	No. of progenies observed	No. of plants with indicated ploidy		
				2x	3x	4x ^z
2x × 4x						
‘Anneke’	‘Victoria’	1993	1	0	0	1
	‘Lilac’	1992	2	0	0	2
	‘Bonfire’	1993	3	0	0	3
‘Pure White’	‘Victoria’	1992	6	0	0	6
		1993	7	0	0	7
	‘Lilac’	1992	5	0	0	5
	‘Bonfire’	1993	9	0	0	9
‘Kage Yellow’	‘Victoria’	1993	26	0	0	26
		1994	24	0	0	24
	‘Bonfire’	1993	32	0	0	32

2x × 2x						
‘Anneke’	‘Pure White’	1992	10	10	0	0
		1993	10	10	0	0
‘Wase Murasaki’	‘Pure White’	1992	10	10	0	0
		1993	10	10	0	0
‘Pure White’	‘Pure White’	1992	10	10	0	0
		1993	10	10	0	0
‘Kage Yellow’	‘Pure White’	1993	10	10	0	0
		1994	10	10	0	0

^z In some plants, chromosome number was 94 – 96 but the precise number couldn't been confirmed.

Fig. 4. Correlation between the percentage of giant pollen grains in diploid cyclamens and the production of fully-developed seeds in the $2x \times 4x$ crosses.

a reduced pollen grain from a tetraploid and an unreduced egg cell from a diploid is the mechanism whereby tetraploid progenies in $2x \times 4x$ cyclamen cross are derived.

The results of this study suggest that the pistillate parents in $2x \times 4x$ cyclamen crosses control the formation of seeds and that only tetraploid progenies can be obtained when certain fixed diploids that produce un-

reduced female gametes are selected as pistillate parents because obtaining triploid progenies is difficult. It might be possible to select the diploids that produce the progenies in the $2x \times 4x$ crosses by investigating the frequency of giant pollen grains in the diploids. The unique phenotypes are observed in each ploidy of cyclamen; for example, a yellow-flowered phenotype (Miyajima et al., 1991) in the diploid and petals with a picotee in the tetraploid. The valuable characteristics in a diploid, which have never existed in tetraploid cultivars, will be introduced into tetraploid cultivars by the crosses between a diploid and a tetraploid. Breeding by the crosses between a diploid and a tetraploid, therefore, seems to be useful for developing new cyclamen cultivars through selection of fixed diploids, which consistently produce tetraploid seeds in the $2x \times 4x$ crosses.

In cyclamen, all F_1 progenies from $4x \times 2x$ crosses are also tetraploid (Takamura and Miyajima, 1996a), thus, we have started an investigation on the mechanism of tetraploid formation.

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シクラメンの $2x \times 4x$ 交雑における交雑和合性の品種および個体間差異

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摘 要

シクラメンの $2x \times 4x$ の交雑において効率よく雑種個体を得る方法を検討するために、その交雑和合性の品種および個体間差異、特に二倍体種子親の違いによる差異を調査した。また、その交雑における四倍体雑種形成の要因についても考察を行った。

シクラメンの $2x \times 4x$ の交雑においては、花粉親の四倍体株に関係なく種子親の二倍体株によってその交雑和合性が異なり、その差異は、品種間だけではなく個体間にまで及んだ。従って、シクラメンの $2x \times 4x$ の交雑における交雑和合性は、その二倍体種子親の遺伝子型に大きく影響されることが

明らかとなった。なお、その交雑で形成された雑種はすべて四倍体であり、しかもこれら四倍体雑種を形成した二倍体種子親はすべて巨大花粉を有していた。

本実験の結果、種子親品種ならびに個体を選抜することにより、 $2x \times 4x$ の交雑において比較的確実に、しかも多くの F_1 が得られるものと示唆された。また、シクラメンの $2x \times 4x$ の交雑における四倍体雑種形成に、二倍体の非還元性配偶子が関与している可能性も示唆された。

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