Cross-Compatibility and the Ploidy of Progenies in Crosses between Diploid and Tetraploid Cyclamen (Cyclamen persicum Mill.)

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Summary

Cross-compatibility and characteristics of F1 progenies in intraspecific crosses between diploid and tetraploid cyclamen (Cyclamen persicum Mill.) were investigated.

1. Although many pollen tubes reached the ovules in reciprocal crosses between diploid and tetraploid cultivars, only a few fully developed seeds were obtained and the seedlings derived therefrom were all tetraploids.

2. More fully developed tetraploidy seeds were obtained when plants such as the diploid cultivar 'Pure White' having numerous giant pollen grains are used as pollen parent. Therefore, we hypothesize that fertilization of a 2x-egg by a giant pollen grain is the mechanism whereby tetraploid progenies originate in 4x×2x cyclamen crosses.

3. Progenies from crosses between diploid and tetraploid cyclamen cultivars are predicted to remain tetraploid in further generations because F2 progenies are all tetraploids.

Introduction

Cyclamen (Cyclamen persicum Mill.) is one of the most popular potted plants and the only commercial species among 19 Cyclamen species.

Legro (1959) reported that there were diploid with 2n = 48 chromosomes, tetraploid with 2n = 96 chromosomes, and tetraploidy aneuploid with 2n=90, 92, 94 and 95 chromosomes among cyclamen cultivars; triploid cyclamen has not been observed.

Presently, there are diploid and tetraploid in the cyclamen cultivars with desirable characteristics in each ploidy, e.g., the yellow-flowered one (Miyajima et al., 1991) has been limited only to diploid cultivars, but a few picotees have been observed in diploids. If a cross between diploid and tetraploid is possible, the breeding of triploid cultivars that have both diploid and tetraploid characteristics can be expected.

It has been reported that most F1 progenies from crosses between diploid and tetraploid were tetraploid (Kappert, 1941; Legro, 1959; Wellensiek, 1955) and the origin of the tetraploidy progenies is not clear. Although a few triploid cyclamen were obtained, there are no triploid cyclamen cultivars at present. Moreover, the cross-compatibility in the crosses was scarcely discussed in the earlier papers.

This study aims 1) to confirm the cross-compatibility in reciprocal crosses between diploid and tetraploid cyclamen cultivars and the ploidy of the F1 progenies, and 2) to clarify the mechanism of tetraploid in the crosses. We made crosses between diploid and tetraploid cultivars and observed pollen tube growth to examine for cross-compatibility. Characteristics of F1 progenies from the crosses between diploids and tetraploids were also observed to examine their ploidy.

Materials and Methods

Experiment 1

Selfs and reciprocal crosses between a diploid, 'Pure White' and two tetraploid cultivars, 'Lilac' and 'Victoria' were made between January and February 1990 in the greenhouse of Kyushu Uni-
The flowers of pistillate parents were emasculated and covered with paper bags about 7 days before anthesis. All pollinated flowers were re-covered with paper bags to prevent contamination by undesirable pollen. At that time, the pollen viability of pollen parents was recorded by staining with aceto-carmine at least 3000 pollen grains per plant. The pollen diameter was recorded on 300 viable pollen grains per plant. Mature seeds were harvested 3~4 months after pollination and visually classified into two groups: (1) fully developed and (2) partially developed or empty.

To study pollen tube growth, pollinated pistils were collected 4 days after pollination, fixed in FAA (formalin : acetic acid : 50 % ethanol 1:1:18, v/v/v) for 24 hours, transferred to 70 % ethanol, and stored at 5 °C for microscopic observation. The styles were separated from the ovaries, softened at 60 °C for 60 minutes in 1 N NaOH, and stained by aniline blue dissolved in 0.1 N K₃PO₄ (Kho and Bear, 1968; Martin, 1959) at least for 15 minutes; they were then squashed and observed under a fluorescence microscope (Olympus VANOX) equipped with a high pressure mercury vapor lamp (HBO: 100 W) and a combined filter (BG-12, DM-50, O-530) providing blue-ultraviolet (530~320 nm) excitation and 530 nm barrier filtration.

**Experiment 2**

The fully developed hybrid seeds were sown in the greenhouse in October 1990 or in 100 ml-flasks in April 1991. The method of sowing in 100 ml-flasks was the same as reported earlier (Takamura et al., 1993). The pollen viability, pollen diameter, guard cell size, and chromosome number of the plants grown in the greenhouse were examined in December 1991. Selfing was carried out in January 1992. The chromosome number of the seedlings incubated in the flasks was counted in June 1991. Chromosome counts were made by observing mitotic metaphase in root tip squashes. Root tips were fixed in 2 mM 8-hydroxyquinoline at 18 °C for 5 hours and then transferred to acetic acid- ethanol (1:3, v/v) for 12 hours. The samples were stored in 70 % ethanol. They were softened in 1 N HCl at 60 °C for 5 minutes and stained by lacto propionic orcein. The method of pollen viability test was the same as described in Experiment 1.

Some seeds obtained by the selfing of the F₁ progenies were sown and the chromosome number of F₂ progenies was counted by observing the mitotic metaphase in root tip squashes.

**Results**

**Experiment 1**

Although pollen germinated and their tubes grew, many reaching the ovules within 4 days after pollination, differences in fruit set and seed formation among the crosses occurred (Table 1). More than 70 % of pollinated flowers fruited and

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of flowers pollinated</th>
<th>No. of fruits obtained</th>
<th>No. of seeds$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2x self-pollinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Pure White'</td>
<td>70</td>
<td>54 (77.1)$^v$</td>
<td>1874 (34.7)$^x$</td>
</tr>
<tr>
<td>4x self-pollinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Lilac'</td>
<td>50</td>
<td>40 (80.0)</td>
<td>1172 (29.3)</td>
</tr>
<tr>
<td>'Victoria'</td>
<td>55</td>
<td>39 (70.9)</td>
<td>885 (22.7)</td>
</tr>
<tr>
<td>2x × 4x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Pure White' × 'Lilac'</td>
<td>137</td>
<td>64 (46.7)</td>
<td>15 (0.2)</td>
</tr>
<tr>
<td>'Pure White' × 'Victoria'</td>
<td>139</td>
<td>60 (43.2)</td>
<td>17 (0.3)</td>
</tr>
<tr>
<td>4x × 2x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Lilac' × 'Pure White'</td>
<td>150</td>
<td>22 (14.7)</td>
<td>32 (1.5)</td>
</tr>
<tr>
<td>'Victoria' × 'Pure White'</td>
<td>196</td>
<td>25 (12.8)</td>
<td>28 (1.1)</td>
</tr>
</tbody>
</table>

$^a$ FD: Fully developed seeds. PD: Partially developed seeds. E: Empty seeds.

$^v$ Percent fruits obtained.

$^x$ No. of seeds per fruit.
more than 20 seeds per fruit were obtained in the selfing of both diploid and tetraploid cultivars.

In $2x \times 4x$, 43 % ('Pure White' × 'Victoria') and 47 % ('Pure White' × 'Lilac') of the flowers set fruit, but there were only 0.3 fully developed seeds per fruit; many seeds had no embryos. In the $4x \times 2x$ crosses, fruiting occurred in less than 15 % of pollinated flowers and the number of fully developed seeds was less than 1.5 per fruit.

Fruiting and seed formation differed depending on the individual pollen parents in $4x \times 2x$ crosses (Fig. 1). Pollen diameter measurements revealed that all 'Pure White' plants used as pollen parent produced giant pollen grains (Fig. 2), but there were differences in their frequency among individuals (Fig. 3). In $4x \times 2x$ crosses, more fully developed seeds were obtained in the crosses with 'Pure White'-W2 with many giant pollen grains than with 'Pure White' having few giant pollen grains like W5 (Figs. 1 and 3). In $2x \times 4x$ crosses, some diploid pistillate plants did not form fully developed seeds but in those which formed seeds, differences among individuals in seed formation were indistinct because of low cross-compatibility in $2x \times 4x$ crosses (data not shown).

**Experiment 2**

The germination rate of the fully developed hybrid seeds from reciprocal crosses was very high; the rate being almost equal to that obtained in seeds resulting from the selfing of diploid or tetraploids (Table 2). Petal color of the plants from $2x \times 4x$ and $4x \times 2x$ was similar to the neutral tints of the parents (data not shown). The pollen viability was more than 90 % in all $F_1$ progenies obtained by $2x \times 4x$ and $4x \times 2x$ crosses, which is equal to that of the plants from the selfing of diploid or tetraploids (Table 3). The pollen diameter and guard cell size of the $F_1$ progenies were larger than those of plants obtained by the selfing of diploid and nearly equal to the plants from the selfing of tetraploid. The chromosome number of the $F_1$ progenies was 96 or near 96 (Table 4).

When $F_1$ progenies obtained by crosses between diploid and tetraploid were selfed, more than 83 % of pollinated flowers set and produced more than 45 seeds per fruit (Table 5). The values were nearly equal to those derived from selfing of diploids or tetraploids. All $F_2$ progenies were tetraploids (data not shown).

**Discussion**

The chromosome number, guard cell size, and pollen characteristics of $F_1$ progenies obtained by reciprocal crosses between diploid and tetraploid cyclamens indicated that the $F_1$ progenies were
Tetraploid progenies have been obtained by intraspecific or interspecific crosses between diploids and tetraploids not only in Cyclamen (Kappert, 1941; Legro, 1959; Wellensiek, 1955) but also in Brassica (Nishiyama and Inamori, 1953), Campanula (Gairdner and Darlington, 1931), Solanum (Koopmans and Burg, 1952; Prakken and Swaminathan, 1952) and Citrus (Cameron and Burnett, 1978; Esen and Soost, 1972; Oiyama and Okudai, 1988).

We obtained only a few F1 seeds from reciprocal crosses between the diploid and tetraploid cyclamens although the pollen tube reached the ovule in all combinations; the F1 progenies were all tetraploids. We expected that crosses between diploid and tetraploid parents would yield a few triploid zygotes or that most of them become aborted; however, only tetraploid zygotes were formed and developed by some unknown factors. The differences in seed set percentage between 2x × 4x and 4x × 2x crosses is attributed to the difference in fertilization rate and/or in embryo development because the number of partially developed and/or empty seeds in 2x × 4x was much more than in 4x × 2x. No triploid progenies were obtained in this study though a few triploids were obtained in earlier reports (Kappert, 1941; Legro, 1959; Wellensiek, 1955). The environmental condition and genetic background may account for the discrepancy among researchers regarding the formation of triploid progenies. This hypothesis
Legro (1959) postulated that dispermy is the probable mechanism causing tetraploid F1 progenies in 4x×2x cyclamen crosses. However, we found that more fully developed tetraploidy seeds were obtained when plants producing more giant pollen grains were used as pollen parent in 4x×2x cyclamen cross. Therefore, we postulate that in 4x×2x cyclamen crosses, fertilization of a 2x-egg from tetraploid pistillate parent by a giant pollen grain of diploid pollen parents is the mechanism by which tetraploid progenies originate.

Esen and Soost (1972) reported that unexpected tetraploid formation in 2x×4x Citrus crosses depended upon the diploid pistillate parents, explaining that the tetraploid progenies were the result of 2x-eggs in diploid pistillate parents. In our 2x×4x cyclamen crosses, some pistillate parents did not form fully developed seeds, whereas others did. There is a possibility that tetraploid progenies from 2x×4x cyclamen crosses are consequences of 2x-egg being present in diploid pistillate parents; it is difficult to prove that from our data.

Wellensiek (1955) and Legro (1959) reported that tetraploid F1 progenies obtained by crosses between diploid and tetraploid cyclamen cultivars remained tetraploids in further generations and behaved as autotetraploids. We came to the same conclusion because pollen fertility of F1 progenies was equal to diploid and tetraploid cultivars and all F2 progenies were tetraploids.

Acknowledgments

Gratitude is expressed to Prof. emeritus Dr. K. Fujieda of Kyushu University for his valuable advice, and the authors thank Mr. T. Kage for his valuable assistance.
Literature Cited


シクラメンの二倍体と四倍体との交雑における交雑和合性と後代の倍性数

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摘要

シクラメンの二倍体と四倍体との交雑における交雑和合性とF₁の特性を調査した。

1）交雑の正逆を問わず、二倍体と四倍体との交雑では多くの花粉管が胚珠に到達したにも関わらず、完全種子はわずかしか得られず、しかもそれからの実生はすべて四倍性であった。

2）二倍体品種 'ピュアホワイト' には巨大花粉を分離する株が存在した。また、4 x 2 x の交雑において、より多くの巨大花粉を分離する株を花粉親として用いた場合に、より多くの四倍性完全種子が得られた。これらのことから、4 x 2 x の交雑において四倍体の後代が得られる機構として、巨大花粉と 2 x 卵細胞との間での受精が考えられた。

3）F₁を自殖して得られた F₂ の倍数性を調査したところ調査した個体はすべて四倍性であり、シクラメンの二倍体と四倍体との交雑で得られた後代は四倍性に固定されることが示唆された。

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