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Evaluation of Pre-fertilization Barriers by Observation of Pollen Tube Growth and Attempts for Overcoming Post-fertilization Barriers in Intergeneric Hybridization between *Alstroemeria* and *Bomarea* by Ovule Culture

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Abstract

In the flower market there is a continuous demand for new cultivars of *Alstroemeria* as an ornamental flower. Many interspecific hybrids of *Alstroemeria* are utilized for commercial cultivars. However there is no report about intergeneric hybridization between *Alstroemeria* and *Bomarea*. We are investigating the possibility of creating intergeneric hybrids between *Alstroemeria* and *Bomarea*. Moreover, ovule culture conditions were examined for overcoming post-fertilization barriers after pollination of *Bomarea* pollen grains to *A. aurea*, *A. pelegrina* var. *rosea* and *A. magenta*. The frequency of pollen tube entry into ovules was compared in the pistil of *A. aurea*, *A. pelegrina* var. *rosea* and *A. magenta* after pollination of *B. coccinea* pollen grains. Pollen tubes in *Alstroemeria* ovaries were observed with aniline blue staining 48 hours after pollination. The frequencies of pollen tube entry into ovules were 0.3%, 5.6% and 10.0% in *A. aurea*, *A. pelegrina* var. *rosea* and *A. magenta*, respectively. For ovule culture, pollinated ovaries were harvested 3 and 7 days after pollination, and the ovules were cultured on 2 g l⁻¹ gellan gum-solidified MS medium with or without gibberellic acid, and supplemented with sucrose at different concentrations (30, 60, 80 or 100 g l⁻¹). As a result, 3 plantlets were obtained in *A. pelegrina* var. *rosea* × *B. coccinea* cultured on MS medium supplemented with 80 g l⁻¹ sucrose. Although pollen tubes of *B. coccinea* were reached to *A. aurea* ovules, this combination might have stronger pre-fertilization barriers than those of *A. pelegrina* var. *rosea* and *A. magenta*. It supposed to be different intensity of pre-fertilization barriers among species. Our data also suggested that sucrose concentration at 80 g l⁻¹ in culture medium was effective to obtain progenies after intergeneric pollination. Confirmation of hybrid natures for the plantlets is now in progress.

INTRODUCTION

In recent years, *Alstroemeria* has become a popular and important cut flower species because it is available in a variety of colours and has good vase life. There is always a demand for new cultivars of ornamental flower species. Therefore various

cultivars have been produced. Some interspecific hybrids of *Alstroemeria* species have been produced using ovule culture (Buitendijk et al., 1995; De Jeu and Jacobsen, 1995; Lu and Bridgen, 1996, Ishikawa et al., 1997, 2001). By conducting detailed studies on interspecific hybridization via ovule culture. Shinoda and Murata (2003) reported the possibilities and limitations for producing hybrids in 15 *Alstroemeria* species.

It has been reported that the genera *Alstroemeria* and *Bomarea*, which belong to the family Alstroemeriaceae, include approximately 75 and 120 species, respectively (Hofreiter and Rodriguez, 2006). Thus, *Bomarea* shows genetic diversity, and *Bomarea* species are desirable candidates to expand *Alstroemeria* variations. For utilizing *Bomarea* species, intergeneric hybridization is required by overcoming distant crosses.

Previously, we found that there was a post-fertilization barrier between *A. aurea* and *B. coccinea* (Kashihara and Hoshino, 2008). The objectives of our study were to investigate species-dependant differences of the frequency of pollen tube entry into ovules after intergeneric pollination and to examine ovule culture conditions for overcoming post-fertilization barriers in order to introduce novel characters into *Alstroemeria*.

MATERIALS AND METHODS

Plant materials

Alstroemeria aurea, *A. pelegrina* var. *rosea*, *A. magenta* and *Bomarea coccinea* were used in this study and grown in a greenhouse of Hokkaido University.

Pre-fertilization barriers

The frequency of pollen tube entry into ovules was compared in the pistil of *A. aurea*, *A. pelegrina* var. *rosea* and *A. magenta* after pollination of *B. coccinea* pollen grains. Stigmatic pollination was applied. Pollen types were frozen pollen grains. Pollen tubes in *Alstroemeria* ovaries were observed with aniline blue staining 48 hours after pollination.

Post-fertilization barriers

Pollinated ovaries were harvested 3 and 7 days after cross pollination (DAP), and the ovules were cultured on 2 g l⁻¹ gellan gum-solidified MS medium (Murashige and Skoog, 1962) with or without gibberellic acid (GA), and supplemented with sucrose at different concentrations (30, 60, 80 or 100 g l⁻¹). Cultures were kept at 20 °C under the light condition.

The relative DNA contents of nuclei isolated from leaf tissues were measured using a flow cytometer PA (Partec, GmbH, Münster, Germany).

RESULTS AND DISCUSSION

Pre-fertilization barriers

The frequencies of pollen tube entry into ovules were 0.3%, 5.6% and 10.0% in *A. aurea*, *A. pelegrina* var. *rosea* and *A. magenta*, respectively (Table 1). *A. aurea* has strong pre-fertilization barrier compared with another 2 species. Pollen tubes of *B. coccinea* were reached to *A. aurea* ovules however this combination might have stronger pre-fertilization barriers than those of *A. pelegrina* and *A. magenta*. It supposed to be different intensity of pre-fertilization barriers among species.

Post-fertilization barriers

Three plantlets (1 from 7 DAP and 2 from 3 DAP) were obtained in *A. pelegrina* × *B. coccinea* cultured on MS medium supplemented with 80 g l⁻¹ sucrose (Table 2, 3 and Figure 1). However, no germination was observed in *A. aurea* × *B. coccinea* and *A. magenta* × *B. coccinea* both 7 DAP (Table 4 and 5) and 3 DAP (data not shown). Some germinated ovules were obtained in *A. magenta* × *B. coccinea* with sucrose 60 g l⁻¹ and 80 g l⁻¹ in 7 DAP under dark condition (data not shown). To overcome post-fertilization barriers, our data suggest that sucrose concentration at 80 g l⁻¹ in culture medium was effective to obtain progenies after intergeneric pollination. In ovule culture between *A. pelegrina* × *B. coccinea*, developmental stage of ovules after pollination involved in the germination. In our experiments, ovules at 3 DAP might be useful. Gibberellic acid seemed to have no influence on these ovule cultures.

The basic chromosome numbers of *Alstroemeria* and *Bomarea* were $x=8$ and $x=9$, respectively. In preliminary experiments using flow cytometric analysis, the fluorescence intensity of the obtained progeny showed higher than that of *A. pelegrina*, an ovule parent, and seemed to be close to that of *B. coccinea*, an pollen parent (Figure 2). This indicated that the progeny was derived from intergeneric cross pollination. At present, we make attempt to acclimatize these plantlets and to assess hybrid nature by DNA analysis.

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Tables

Table 1. Frequency of pollen tube entry into ovules 48 hours after intergeneric pollination.

Cross-combinations	No. of ovaries observed	No. of ovules observed	No. of pollen tube entry	Frequency (%)
<i>A. aurea</i> × <i>B. coccinea</i>	23	504	2	0.3
<i>A. pelegrina</i> × <i>B. coccinea</i>	3	89	5	5.6
<i>A. magenta</i> × <i>B. coccinea</i>	1	30	3	10.0

Table 2. Ovule culture 3 days after pollination between *A. pelegrina* × *B. coccinea*.

Medium	No. of ovaries	No. of ovules	No. of germination	Percentage of germination ¹	No. of plantlets
Sucrose 30 g l ⁻¹	2	48	1	2.0	0
Sucrose 60 g l ⁻¹	2	43	1	2.3	0
Sucrose 80 g l ⁻¹	3	67	5	7.4	2
Sucrose 100 g l ⁻¹	2	41	0	0.0	0
Sucrose 30 g l ⁻¹ GA 0.5 mg l ⁻¹	2	50	0	0.0	0
Sucrose 30 g l ⁻¹ GA 1.0 mg l ⁻¹	3	69	0	0.0	0

¹Number of germination / No. of ovules × 100.

Table 3. Ovule culture 7 days after pollination between *A. pelegrina* × *B. coccinea*.

Medium	No. of ovaries	No. of ovules	No. of germination	Percentage of germination ¹	No. of plantlets
Sucrose 30 g l ⁻¹	6	112	0	0.0	0
Sucrose 60 g l ⁻¹	7	154	2	1.3	0
Sucrose 80 g l ⁻¹	6	123	5	4.1	1
Sucrose 100 g l ⁻¹	6	125	0	0.0	0
Sucrose 30 g l ⁻¹ GA 0.5 mg l ⁻¹	7	135	0	0.0	0
Sucrose 30 g l ⁻¹ GA 1.0 mg l ⁻¹	8	161	2	1.2	0

¹Number of germination / No. of ovules × 100.

Table 4. Ovule culture 7 days after pollination between *A. aurea* × *B. coccinea*.

Medium	No. of ovaries	No. of ovules	No. of germination	Percentage of germination ¹	No. of plantlets
Sucrose 30 g l ⁻¹	4	96	0	0.0	0
Sucrose 60 g l ⁻¹	5	99	0	0.0	0
Sucrose 80 g l ⁻¹	5	105	0	0.0	0
Sucrose 100 g l ⁻¹	6	121	0	0.0	0
Sucrose 30 g l ⁻¹ GA 0.5 mg l ⁻¹	4	75	0	0.0	0
Sucrose 30 g l ⁻¹ GA 1.0 mg l ⁻¹	4	95	0	0.0	0

¹Number of germination / No. of ovules × 100.

Table 5. Ovule culture 7 days after pollination between *A. magenta* × *B. coccinea*.

Medium	No. of ovaries	No. of ovules	No. of germination	Percentage of germination ¹	No. of plantlets
Sucrose 30 g l ⁻¹	6	167	0	0.0	0
Sucrose 60 g l ⁻¹	2	60	0	0.0	0
Sucrose 80 g l ⁻¹	3	83	0	0.0	0
Sucrose 100 g l ⁻¹	2	51	0	0.0	0
Sucrose 30 g l ⁻¹ GA 0.5 mg l ⁻¹	3	92	0	0.0	0
Sucrose 30 g l ⁻¹ GA 1.0 mg l ⁻¹	3	83	0	0.0	0

¹Number of germination / No. of ovules × 100.

Figures



Figure 1. Obtained plantlet derived from ovule (3 DAP) from *A. pelegrina* × *B. coccinea*, which was cultured in vitro on the medium supplemented with 80 g l⁻¹ sucrose.

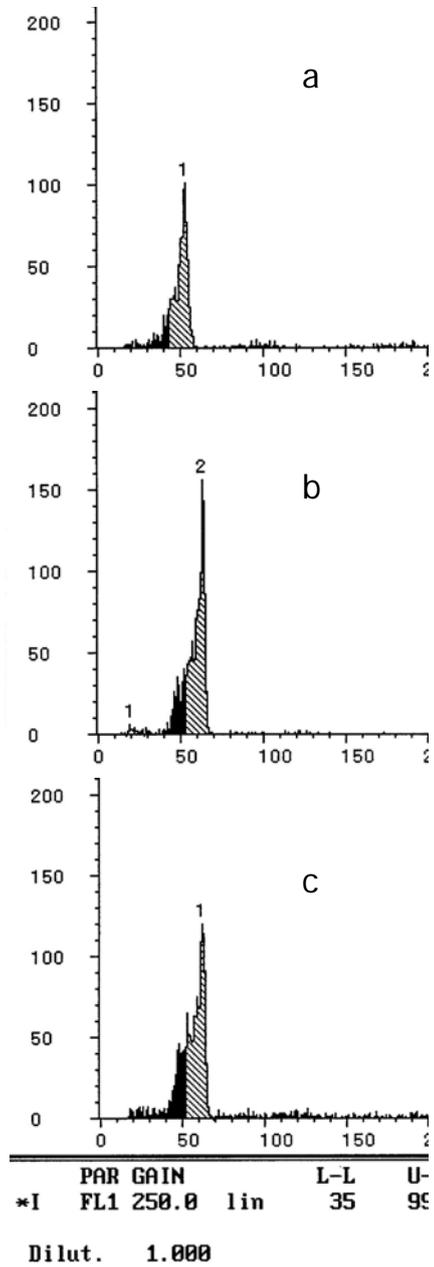


Figure 2. Flow cytometric profiles of *A. pelegrina* (a), *B. coccinea* (b) and putative hybrid of the cross *A. pelegrina* × *B. coccinea* (c).