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N. Chomchalow
N. Supakamnerd
N. Sukhvibul

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Interspecific Hybridization in the Genus Globba Using In Vitro Embryo Culture

P. Nountaswasti
Faculty of Agricultural Production
Maejo University, Chiang Mai
Thailand

P. Sukasathan
Queen Sirikit Botanic Gardens
Chiang Mai
Thailand

Keywords: reciprocal crosses, embryo rescue, developmental potentials, fertilization barriers

Abstract

Interspecific hybridization had been carried out among four species of Globba, G. schomburgkii, G. magnifica, G. rosea, and G. globulifera. Direct and reciprocal crosses were made. Two crosses, G. rosea x G. globulifera and G. rosea x G. magnifica, produced fruits in both direct and reciprocal crosses, whereas the other crosses produced fruits only in the direct crosses. An embryo rescue technique was used to obtain interspecific hybrids. Twenty days after pollination (DAP), embryos showed high percentage of germination for all crosses. A total of 11 interspecific hybrid plants were produced in 12 combinations. All hybrid seedlings obtained were successfully transplanted to soil and these seedlings grew normally. Interspecific hybrids showed distinct developmental potentials and characteristics.

INTRODUCTION

The genus Globba is a member of the family Zingiberaceae and consists of more than 100 species. Globba species are distributed throughout tropical and parts of subtropical Asia. Thailand is one of its origins (Larsen, 1996). Although Thailand has high genetic diversity of Globba and exports some species as commercial ornamental bulbs, presently there is no breeding program of Globba. Interspecific hybridization is a procedure to increase the genetic variability of cultivated plants. Novelty in ornamental traits is particularly important for development of the cultivars (Mii, 2009). For bulbous crops, interspecific hybridization is an effective method for the introduction of desired characteristics into breeding material (Van Tuyl and DeJeu, 1997). Nevertheless, the possibilities for interspecific crosses are limited by various pre-fertilization and post-fertilization barriers. To circumvent post-fertilization barriers, embryo culture (Smith, 1944) has been used. In this present study, the interspecific hybridization barriers were investigated and an embryo rescue technique was used to produce the hybrids.

MATERIALS AND METHODS

Plant Materials and Cross Pollination

Four species (one genotype per species) of Globba, viz., G. schomburgkii, G. magnifica, G. rosea, and G. globulifera, were used in this study. All plants were grown in 12-inch plastic pots under 50% shaded plastic greenhouse. The direct and reciprocal crosses were done to investigate the crossing ability in each cross. Flowers of male parents were emasculated before pollination by pointed forceps and hand-pollinated with fresh pollen of male parents at 7:00-10:00 am.

Pollen Germination and Pollen Tube Growth in the Style

To determine pollen germination, pollen grains from blooming flowers were collected and immediately germinated in a culture medium (0.3 mg L^-1 (CaNO)3, 0.14 mg L^-1 MgSO4, 0.05 mg L^-1 H3BO3, and 100 mg L^-1 sucrose) (Baloch et al., 2001), using the hanging drop technique. A pollen grain was considered as germinate when the tube had grown to a length of approximately twice the diameter of the pollen grain. Germinated
pollen grains were counted under a light microscope after 5-10 hours incubation. For each genotype, three replicates with approximately 100 pollen grains per replication were counted.

To determine pollen tube growth in the style, pistils from each cross were fixed in FAA 24 hours after pollination. For fluorescent microscopic observation, the fixed pistils were washed with distilled water, softened in 4 N NaOH overnight under room temperature, and then split longitudinally. They were stained with 0.1% (w/v) aniline blue in 0.1 M K2PO4 (Martin, 1959) and squashed by cover slips. Pollen germination on the stigma and elongation within the styles were observed by using a fluorescent microscope (Nikon ECLIPSE 80 i).

Embryo Culture

Fruits were collected 20 days after pollination (DAP). They were disinfested by dipping in 70% ethanol for 3-5 s, then in 1% sodium hypochlorite for 15 min. One drop of Tween-20 was added to 100 ml sodium hypochlorite solution. The fruits were washed three times for 10 min in sterilized water. The embryos were excised from the seeds and cultured on a modified MS medium containing 1 mg L-1 BA, 1 mg L-1 GA and 0.1 mg L-1 NAA. The cultures were maintained at 25°C under a 16 h-photoperiod of 36 μmol m-2 s-1 light from cool white fluorescent lamps. Embryos that developed normally were subcultured onto a fresh medium. Seedlings were transplanted into 4-inch plastic pots and acclimatized for two weeks, then transplant into 8 inch plastic pots.

RESULTS AND DISCUSSION

The pollens of G. globulifera, G. magnifica, G. rosea and G. schomburgkii germinated well (63.55, 77.11 and 71.43%, respectively), whereas G. schomburgkii pollen had very low germination percentage (1.12%) (Fig. 1). An investigation of pollen germination in the style revealed that pollen generated from crosses between G. globulifera and G. magnifica, G. globulifera and G. rosea, and G. magnifica and G. rosea could germinate well in the style in both direct and reciprocal crosses. In crosses between G. schomburgkii and either G. globulifera, G. magnifica or G. rosea, the pollen of G. schomburgkii did not germinate on the stigma surface whereas in reciprocal crosses, the pollen germinated and grew well. This result showed that there was no pre-fertilization barrier when G. schomburgkii was a female parent (Fig. 2). As expected, the pollen of G. schomburgkii did not germinate on the stigma surface of female parents because pollen of G. schomburgkii had a low viability (Fig. 1). This observation revealed that although fertilization itself might occur normally, a post-fertilization barrier arose because the seeds abortion always found in mature fruits (Fig. 3B and C). Interspecific hybrid embryos from these crosses probably aborted due to the lack of endosperm development, endosperm toxic to embryo, chromosomal abnormality in embryo or developmental failure in early stages of cell differentiation as suggested in several hybridization between distantly related species (Cooper and Brink, 1940).

The crosses between G. rosea and G. globulifera, and G. rosea and G. magnifica produced fruits in both direct and reciprocal crosses, while the crosses between G. magnifica and G. globulifera, G. schomburgkii and G. globulifera, G. schomburgkii and G. magnifica, and G. schomburgkii and G. rosea produced fruit only in direct cross (Table 1). Although G. schomburgkii × G. globulifera could produce fruit with a seed, the embryo did not develop into a plant (Table 1).

Observation of seed development in fruit found that the interspecific hybridization seeds showed severe abortion symptoms in 30 DAP fruits. In 20 DAP fruits, some seed abortion symptoms were found but not severe (Fig. 3). In addition, the seed development observation found that embryos of globba were visible in 18-20 DAP seeds. For this reason, embryos from 20 DAP seeds were used for embryo rescue. Seedlings germinated from cultured embryos within three weeks. The interspecific hybrids showed intermediate characteristics between parents. The G. rosea × G. globulifera hybrids of had short inflorescence with tuft bract and many cup shape bracts (Fig. 4E). The G. magnifica ×

### ACKNOWLEDGEMENT

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<tr>
<td>---------</td>
</tr>
<tr>
<td>G. rosea x G. globulifera</td>
</tr>
<tr>
<td>G. rosea x G. globulifera</td>
</tr>
<tr>
<td>G. rosea x G. globulifera</td>
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$G. \text{globulifera}$ hybrids had longer inflorescence than $G. \text{rosea} \times G. \text{globulifera}$, with larger bracts on the upper part of the inflorescence (Fig. 4F). The inflorescence of $G. \text{rosea} \times G. \text{magnifica}$ hybrids resembled the inflorescence of $G. \text{magnifica}$, but contained cap shape bracts and cover flowers like $G. \text{rosea}$ (Fig. 4G). $G. \text{schomburgkii} \times G. \text{magnifica}$ hybrids had inflorescences like $G. \text{schomburgkii}$, but bracts were larger than $G. \text{schomburgkii}$. They had green-pink bracts which were a mixture of the bract colour of the parents (Fig. 4H). The hybrids showed variation in characteristic and color of inflorescence and bracts depending on their parents.

ACKNOWLEDGEMENT

The work was supported by the Office of the National Research Council of Thailand.

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Tables

Table 1. Result of interspecific crosses among four Globba species.

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<tr>
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<th>No. pollinated flowers</th>
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<td>$G. \text{globulifera} \times G. \text{magnifica}$</td>
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<td>$G. \text{magnifica} \times G. \text{globulifera}$</td>
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<td>3</td>
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<td>$G. \text{globulifera} \times G. \text{rosea}$</td>
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<td>2.94</td>
<td>10</td>
<td>10</td>
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<td>$G. \text{rosea} \times G. \text{globulifera}$</td>
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Fig. 1. Pollen germination percentage of *Globba glabulifera*, *G. magnifica*, *G. rosea* and *G. schomburgkii*, in a germination medium.

Fig. 2. Pollen germination in the style of *Globba*. (A) Rare pollen of *G. magnifica* germinated on *G. schomburgkii* stigma surface; (B) Pollen of *G. rosea* germinated and pollen tube grown normally in *G. magnifica* style (arrows indicate the pollen tubes).
G. rosea and G. magnifica germinated and indicate the pollen.

Fig. 3. A) Interspecific globba fruit 30 DAP; B) Interspecific globba fruit 20 DAP the early sign of seeds abortion occur; C) Interspecific globba fruit 30 DAP showed severe symptom of seed abortion; D) Embryo after culture for one week; E) Embryo developed to seedling within three weeks (Bar = 1 mm).
Fig. 4. Inflorescence characteristics of (A) G. globulifera, (B) G. magnifica, (C) G. rosea, (D) G. schomburgkii, (E) G. rosea × G. globulifera, (F) G. magnifica × G. globulifera, (G) G. magnifica × G. rosea, and (H) G. magnifica × G. schomburgkii.