Embryo Culture for the Production of Interspecific Hybrids in *Zantedeschia*

Takashi Kubo[1]; Koji Inaba[2]; Genjiro Mori[3]

Abstract

To establish an optimum method for the production of interspecific hybrids between *Zantedeschia aethiopica* (group 1) and *Zantedeschia* spp. except *Z. aethiopica* (group 2), the development processes of hybrid embryos and endosperms after fertilization were observed, and a suitable medium for embryo rescue was studied. Interspecific hybrids were obtained efficiently when embryos excised from ovaries 6 - 8 weeks after pollination were cultured on MS medium containing 0.1 mg・liter^{-1} NAA and 1.0 mg・liter^{-1} BA. But, all hybrids were albino and only viable *in vitro* and so further studies are required to conquer the postfertilization barrier.

Key words: embryo culture, interspecific hybridization, *Zantedeschia*, BA, NAA

1. Introduction

Zantedeschia plants (Araceae), commonly known as calla lilies, are native to South Africa (Funnell, 1993) and due to their attractive spathes, commonly referred to as flowers, they, along with their hybrids, are popular as cut flowers and potted plants. The genus *Zantedeschia*, which contains six species and two sub-species, is divided into two groups (Funnell, 1993). The first (group 1) contains only *Z. aethiopica*, the foliage of which does not die down in winter and the inflorescence of which occurs in late winter to late spring in its native habit. The other (group 2) contains the remaining five species, which typically exhibit complete foliage senescence in winter and flowers in summer.

The species belonging to group 2 have various spathe colors, while *Z. aethiopica* has a much longer flowering period but only white, pink or green spathes. Hybrids with characters from both groups are therefore very attractive for plant breeders. Interspecific hybrids are readily achieved between species within group 2; however, because of postfertilization barriers, no successful crosses have been achieved between species in groups 1 and 2 (Traub, 1948). Yao et al. (1995) rescued fertilized embryos of these incompatible crosses using an embryo culture technique, but all hybrids were albino and viable only *in vitro*.

Yao et al. (1995) obtained albino hybrids using crosses of only 4 combination patterns. Establishment of the efficient method for embryo rescue is required to investigate the hybrids from more combination patterns. In this study, we determined the optimum embryo isolation time and medium conditions for embryo rescue.

2. Materials and methods

2.1. Plant material and pollination

Z. aethiopica 'Wedding March' as maternal parent and, *Z. hybrida* (group 2) 'Black Eyed Beauty' and 'Pink Superba' as paternal parents were used. Plants were grown in greenhouses to achieve simultaneous flowering. 'Wedding March' was emasculated by removing the male zone. Pollinations were performed using fresh pollen only and after pollination, inflorescences were covered with waxed paper bags.

2.2. Observation of fertilized embryos and endosperms

Ovaries were sampled weekly from 4 to 10 weeks after pollination. Embryos and endosperms were removed from these ovaries and observed under a stereoscope.

2.3. Effect of embryo development stage and the medium on embryo rescue

Ovaries were harvested at 6 to 8 weeks after pollination. The surfaces of the ovaries were then sterilized with sodium hypochlorite solution (active chloride: about 2%) for 15 min and rinsed three times with sterilized water. The medium used for embryo culture was Murashige and Skoog (1962) (MS) medium containing 2 g・liter^{-1} gelrite, 30 g・liter^{-1} sucrose, and 0, 1, 2, 5 mg・liter^{-1} BA or 1 mg・liter^{-1} BA and 0, 0.1, 1 mg・liter^{-1} NAA. The fertilized embryos were removed from the endosperms and cultured on the media. Embryos that were too small to be separated from the endosperm were cultured together with the endosperm. A total of 16 - 45 embryos were cultured for each treatment at 25°C in the dark. After 8 weeks, the percentages of shoot formation were recorded.

[1] Hokkaido College, Senshu University, Bibai, Hokkaido, 0779-0197, Japan: e-mail: kubo@senshu-hc.ac.jp
[2] Kyoto Prefectural Institute of Agriculture, Wakunari, Amarube-cho, Kameoka 621-0806, Japan
[3] Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-Cho, Sakai 599-8531, Japan

3. Results

3.1. Observation of fertilized embryos

Embryos in self-pollinated *Z. aethiopica* 'Wedding March' were globular and about 0.3 mm in diameter 4 weeks after pollination (Table 1). They then started elongating and developed to over 3 mm 8 weeks after pollination. Endosperms were translucent and jelly-like 4 weeks after pollination, becoming gradually firmer and white, and completely matured 8 weeks after pollination.

Embryos in crosses performed with 'Wedding March' as the maternal and 'Black Eyed Beauty' or 'Pink Superba' as the paternal plants were similar to those of self-pollinated 'Wedding March' 4 weeks after pollination. But, most embryos failed to elongate and expanded while still globular (Figure 1a). Only about 10% of embryos elongated, but elongation stopped 6 to 8 weeks after pollination, not reaching over 1.0 mm (Figure 1b). Endosperms didn't solidify and started to degenerate 5 to 6 weeks after pollination. All ovaries lost their endosperms by 10 weeks after pollination.

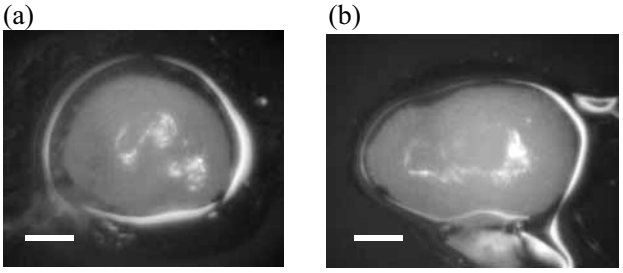

Fig.1. Hybrid embryos of *Z. aethiopica* 'Wedding' × 'Black Eyed Beauty' at 5 (a) and 7 weeks (b) after pollination. Bars = 100 μ m.

3.2. Effect of embryo development stage and the medium on embryo rescue

Most of the globular embryos less than 0.2 mm in diameter didn't develop on any media (Tables 2 and 3.). On the other hand, larger globular embryos (0.2 - 0.5 mm and 0.5 - 1.0 mm in diameter) responded to medium and a few formed single or multiple shoots at about 6 weeks after starting the culture. Spheroidical embryos, which started to elongate developed more successfully than globular embryos, and about 2-4 times more spheroidical embryos than globular embryos formed shoots.

Supplementation of the medium with BA was required for shoot development of the embryos (Table 2.). Embryos at all development stages developed shoots on MS medium containing 1 mg·liter^{-1} BA, but medium supplemented with 2 mg·liter^{-1} BA inhibited development of globular embryos. When the BA concentration was increased to 5 mg · liter^{-1}, development of spheroidical and globular embryos was inhibited. Supplementation with 0.1 mg·liter^{-1} NAA to MS medium containing 1 mg·liter^{-1} BA promoted embryo development (Table 3.), but supplementation with 1 mg·liter^{-1} NAA inhibited development. The highest percentage of embryos that formed shoots was identified on medium containing 1 mg·liter^{-1} BA and 0.1 mg·liter^{-1} NAA.

All shoots formed from the embryos of interspecific crosses were barely pigmented even under illumination, and the SPAD value of these leaves was less than 2.0. Furthermore, these albino shoots grew very slowly and barely rooted. Several plantlets rooted and grew over 5 cm and were transplanted into soil mixture then placed in a greenhouse controlled at 25°C. These hybrids survived for about one month, but remained albino and never unfolded new leaves.

Table 1. Morphological change of fetilized embryo and endosperm.

Development of the embryo and state of endsperm	Weeks after pollination					
	4	5	6	7	8	10
'Wedding March' self pollination						
shape of embryo	globular	spheroidical	spheroidical	rod-like	rod-like	rod-like
length (mm) of embryo	less than 0.2	0.2～1.0	0.2～1.0	1.0～3.0	1.0～3.0	more than 3.0
state of endosprerm	jelly	solid	solid	solid	solid	solid
percentage of ovaries that lost endsperm	0	0	0	0	0	0
'Wedding March'×'Black Eye Beauty'						
shape of embryo	globular	globular	globular	globular, spheroidical	-	-
length (mm) of embryo	less than 0.2	0.2～0.5	0.2～1.0	0.2～1.0		
state of endosprerm	jelly	jelly	jelly	jelly		
percentage of ovaries that lost endsperm	14.3	25.0	50.0	57.1	100.0	100.0
'Wedding March'×'Pink Superba'						
shape of embryo	globular	globular	globular	globular, spheroidical	globular, spheroidical	-
length (mm) of embryo	less than 0.2	0.2～0.5	0.2～1.0	0.2～1.0	0.2～1.0	
state of endosprerm	jelly	jelly	jelly	jelly	jelly	
percentage of ovaries that lost endsperm	0.0	35.3	37.5	69.2	87.5	100.0

Table 2. Percentage of embryos forming shoot on MS medium cotaining 0, 1, 2, 5 mg·liter^{-1} BA.

Stage of embryo		BA (mg·liter^{-1})			
form	size(mm)	0	1	2	5
'Wedding March'×'Black Eye Beauty'					
spheroidical	0.5 - 1.0	0.0	29.8	12.9	0.0
globular	0.5 - 1.0	0.0	3.5	5.0	0.0
	0.2 - 0.5	0.0	1.9	0.0	0.0
	less than 0.2^{z}	0.0	3.3	0.0	0.0
'Wedding March'×'Pink Superba'					
spheroidical	0.5 - 1.0	0.0	39.9	13.8	0.0
globular	0.5 - 1.0	0.0	12.7	0.0	0.0
	0.2 - 0.5	0.0	0.0	0.0	0.0
	less than 0.2	0.0	0.0	0.0	0.0

^{Z}The embryos were too small to separate from endosperm, so the embryos were embeded on medium with endosperm.

Table 3. Percentage of embryos forming shoot on MS medium cotaining 1 mg·liter^{-1} BA and 0, 0.1 or 1.0 mg·liter^{-1} NAA.

Stage of embryo		NAA (mg·liter^{-1})		
form	size(mm)	0	0.1	1
'Wedding March'×'Black Eye Beauty'				
spheroidical	0.5 - 1.0	29.8	42.9	25.0
globular	0.5 - 1.0	3.5	15.7	0.0
	0.2 - 0.5	1.9	9.3	3.6
	less than 0.2^{z}	3.3	0.0	0.0
'Wedding March'×'Pink Superba'				
spheroidical	0.5 - 1.0	39.9	53.0	6.4
globular	0.5 - 1.0	12.7	1.7	0.0
	0.2 - 0.5	0.0	11.7	1.7
	less than 0.2	0.0	0.0	0.0

^{Z}The embryos were too small to separate from endosperm, so the embryos were embeded on medium with endosperm.

4. Discussion

The embryos obtained from interspecific crosses between groups 1 and 2 stopped developing halfway (Table 1). Similar postfertilization barriers have been observed in distant crosses between various plants (Sharma et al., 1996). Generally, the higher developed embryos formed shoots easier (Hadley and Openshaw, 1980). In *Zantedeschia*, the hybrid embryos stopped developing about 7 - 8 weeks after pollination, but the degeneration of endosperms started at about 5 - 6 weeks after pollination (Table 1). These results indicated that the suitable isolation time of ovaries was about 6 - 8 weeks after pollination.

Cytokinins usually inhibit the growth of immature embryos (Sharma et al., 1996), but embryos obtained from interspecific crosses of *Zantedeschia* required BA for shoot formation. NAA further enhanced shoot formation. Similar effects of auxin have been detected in various plants (Sharma et al., 1996). Optimum concentrations of BA and NAA for embryo shoot formation were 1.0 and 0.1 mg·liter^{-1}, respectively.

In this study, all shoots formed from the embryos of interspecific crosses were albino, as with those of Yao et al. (1995). Similar dysfunctions of chlorophyll synthesis were observed in interspecific hybrids of *Trifolium* (Hovin, 1962; Przywara et al., 1989), *Impatiens* (Arisumi, 1985) and *Rhododendron* (Ureshino and Miyajima, 2002). Yao et al. (1995) obtained interspecific hybrids from 4 combination patterns, and we also performed only 2 combination patterns. Further investigations for the hybrids from more combination patterns were required.

The present study indicated that the optimum method for obtaining interspecific hybrids is to excise embryos from ovaries at 6 - 8 weeks after pollination and culture on MS medium containing 0.1 mg·liter^{-1} NAA and 1.0 mg·liter^{-1} BA.

5. Reference

Arisumi, T. (1985) Rescuing abortive *Impatiens* hybrids thorough aseptic culture of ovules. J. Am. Hort. Sci. 110: 273-276.

Funnell, K.A. (1993) *Zantedeschia*. p. 683-704. In: A. De Hortogh and M. Le Nard (eds.). The physiology of flower bulbs. Elsevier, Amsterdam-London-New York-Tokyo.

Hadley, H. H. and S. J. Openshaw. (1980) Interspecific and intergeneric hybridization. p. 133-159. In: W. R. Fehr and H. H. Hadley (eds.). Hybridization of crop plants. American Society of Agronomy and Crop Science Society of America, Madison..

Hovin, A. W. (1962) Interspecies hybridization between *Trifolium repens* with *T. nigrescens* Viv. and analysis of hybrid meiosis. Crop Sci. 2: 252-254.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Przywara, L., D. W. R. White, P. M. Sanders and D. Maher. (1989) Interspecific hybridization of *Trifolium repens* with *T. hybridum* using in ovulo embryo and embryo culture. Ann. Bot. 64: 613-624.

Sharma, D. R., R. Kur and K. Kumar. (1996) Embryo rescue in plants – a review. Euphytica. 89: 325-337.

Traub, H. P. (1948) The genus *Zantedeschia*. Plant life 4: 9-32.

Uresshino K. and I. Miyajima. (2002) The study on the relationship between leaf colors and ptDNA inheritance in intersectional cross of *Rhododendron kiusianum* × *R. japonicum* f. *flavum*, Resulting in an unexpected triploid progeny. J. Japan. Soc. Hort. Sci. 71: 214-219.

Yao, J. L., D. Cohen and R. E. Rowland. (1995) Interspecific albino and variegated hybrids in the genus *Zantedeschia*. Plant Sci. 109: 199-206.