Compatibility of Interspecific Hybridization in *Zantedeschia*Takashi Kubo^{1,3*}, Koji Inaba^{2,3} and Genjiro Mori⁴¹Department of Horticulture and Landscape Architecture, Hokkaido College, Senshu University, Bibai 079–0197, Japan²Kyoto Prefectural Institute of Agriculture, Wakunari, Amarube-cho, Kameoka 621–0806, Japan³Kyoto Prefectural Institute of Agricultural Biotechnology, Oji, Kitainayazuma, Seika-cho, Soraku 619–0244, Japan⁴Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-Cho, Saka 599–8531, Japan

Two cultivars of *Zantedeschia aethiopica* (group 1) and 21 cultivars in other *Zantedeschia* spp. (group 2) were hybridized to investigate the cross-compatibility of each combination. From a total of 757 crosses from 37 hybrid combinations that were attempted, 3,144 resulting embryos were cultured. As a result, 175 embryos representing 26 combinations formed shoots, but all hybrids were albino and viable only in vitro. Some hybrids had plastids of both parents, but no relationship between the appearance of albino hybrids and plastid inheritance could be detected.

Key Words: embryo culture, interspecific hybrid, *Zantedeschia*.

Introduction

The genus *Zantedeschia*, which contains six species and two sub-species, is divided into two groups (Funnell, 1993). The group 1 contains only *Z. aethiopica*, an evergreen spp. in which the inflorescence appears in late winter to late spring in its native habitat. Group 2 contains the remaining five species, which are deciduous and flower in summer.

The species, belonging to group 2, have various spathe colors, whereas *Z. aethiopica* has only white, pink, or green spathes with a long flowering period. 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) obtained fertilized embryos of these incompatible crosses by using an embryo-rescue technique, but all hybrids were albino and viable only in vitro.

Yao et al. (1995) obtained albino hybrids by using only 4 cross-combinations. The hybrids, viable autotrophically, may be obtained from the crosses of more combination patterns. In this study, we performed crosses between 2 cultivars of *Z. aethiopica* and 21 cultivars in group 2 to obtain hybrids from more combination patterns and investigated their compatibility. Incompatibility between the genome and plastome could be one of the factors to cause this dysfunction (Michishita et al., 2002). Hence, the relationship between

the appearance of albino hybrids and plastid inheritance were also investigated.

Materials and Methods

Investigation of the compatibility of interspecific hybridization

Crosses were performed between 2 cultivars of *Z. aethiopica* and 21 cultivars in group 2. Plants were grown in greenhouses to achieve simultaneous flowering. The maternal plants were emasculated and fresh pollen applied to the stigma. After pollination, inflorescences were covered with waxed paper bag to prevent contamination.

The ovaries that were harvested 6 to 8 weeks after pollination were surface-sterilized with sodium hypochlorite solution (active chloride: about 2%) for 15 min and rinsed thrice with sterilized water. The embryos were cultured in Murashige and Skoog (1962) (MS) medium that contained 2 g·L⁻¹ gelrite, 30 g·L⁻¹ sucrose, 1 mg·L⁻¹ BA, and 0.1 mg·L⁻¹ NAA. The fertilized embryos were separated from the endosperms and cultured on the media at 25°C in the dark. Embryos that were too small to be separated from the endosperm were cultured together. When the embryos had grown more than 5 mm, they were transferred to a 16-h photoperiod illuminated by cool white fluorescent light (40 μmol·m⁻²·s⁻¹) and kept at 25°C. The embryos that formed shoots were transferred to 1/2 MS medium to induce rooting.

Investigation of plastid inheritance

The sizes of PCR products amplified from DNA of the progeny by using a primer pair specific to *atpH* (*atpH*-1 and *atpH*-2) of the plastid DNA were investigated to identify which parent's plastid was inherited. *atpH*-1 (5'-TTGACCAACTCCAGGTCCAA-3') and *atpH*-2 (5'-CCGCAGCTTATATAGGCGAA-

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designed from plastid DNA of *Spinacia oleracea* (Tsumura et al., 1996) amplify about 600- and 900-bp fragments in *Z. aethiopica* and cultivars in group 2, respectively. 20 ng of total DNA that was extracted from leaf tissues by a modified CTAB method (Rogers and Bendich, 1985) was added to a reaction mixture (10 µL) containing 1 mM of each primer, 0.2 mM of each dNTP, 0.25 units of Taq (TAKARA Co., Kyoto, Japan), and 1 µL of $\times 10$ buffer for Taq. Amplification was carried out in a thermal cycler (Applied Biosystem 9600, Applied Biosystem, California, USA) for 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 2 min at 60°C and 2 min at 72°C, and ending with 7 min incubation at 72°C.

Results

Investigation of the compatibility of interspecific hybridization

A total of 757 crosses from 37 cross-combinations were attempted from which 3,144 embryos were cultured (Table 1). Overall, 175 embryos formed shoots from crosses of 26 combination patterns, and the percentages of embryos that formed shoots were 1.1–20.0%. But, these shoots were barely pigmented even under illumination; 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; these were transplanted into soil mixture, and then placed in a greenhouse controlled at 25°C. These hybrids survived for about one month, but remained albino and never unfolded new leaves. All albino hybrids were identified as interspecific hybrids by RAPD analysis (data not shown).

Investigation of plastid inheritance

By investigating six albino hybrids derived from 'Wedding March' $\times$ 'Black Eyed Beauty', and 'Wedding March' $\times$ 'Pink Superba', biparental inheritance of plastid DNA was detected in 3 hybrids derived from 'Wedding March' $\times$ 'Black Eyed Beauty' and one from 'Wedding March' $\times$ 'Pink Superba' (Fig. 1).

Discussion

Yao et al. (1995) previously obtained interspecific hybrids from 4 combination patterns, *Z. aethiopica* $\times$ *Z. elliottiana* and 3 hybrid cultivars of group 2 $\times$ *Z. aethiopica*. In this study, hybrids from 2 cultivars of *Z. aethiopica* $\times$ *Z. rehmanii* 'Pink Superba' were obtained, but these were albino and viable only in vitro, as with those of *Z. aethiopica* $\times$ *Z. elliottiana* obtained by Yao et al. (1995). Furthermore, though we also obtained hybrids from crosses using even 21 hybrid cultivars of group 2 as paternal or maternal parent, all progenies were albino. These results imply that there is a genetic

Table 1. The results from interspecific hybridization in *Zantedeschia*.

Parents		Number of crosses attempted	Number of embryos cultured	Number of embryos forming shoot	Percentage of embryos forming shoot (%)	Hybrid shoot colour
Maternal	Paternal					
'Wedding March'	'Black Eyed Beauty'	68	664	43	6.5	albino
	'Pink Superba'	99	552	40	7.2	albino
	'Chianti'	26	111	13	11.7	albino
	'Golden Giant'	29	131	9	6.9	albino
	'Black Magic'	36	449	7	1.6	albino
	'Parfait'	18	98	5	5.1	albino
	'Sunshine'	43	113	5	4.4	albino
	'Soft Glow'	19	62	4	6.5	albino
	'Carmine Red'	18	15	3	20.0	albino
	'Majestic Red'	13	78	2	2.6	albino
	'Florex Gold'	11	11	2	18.2	albino
	'Neone Amour'	31	28	2	7.1	albino
	'Deep Rose Gem'	34	93	1	1.1	albino
	'Millennium Gold'	11	22	0	0.0	—
	'Hazel Mary'	4	10	0	0.0	—
	'Mango'	13	6	0	0.0	—
	'Crystal Glow'	19	10	0	0.0	—
	'Crystal Brash'	17	8	0	0.0	—
	'Lip Stick'	22	8	0	0.0	—
	'Pink Superba'	47	109	6	5.5	albino
	'Black Eyed Beauty'	49	139	3	2.2	albino
	'Chianti'	11	43	3	7.0	albino
	'Parfait'	6	37	2	5.4	albino
	'Golden Giant'	24	16	1	6.3	albino
	'Lip Stick'	4	28	1	3.6	albino
	'Soft Glow'	11	8	1	12.5	albino
	'Majestic Red'	7	8	1	12.5	albino
'Childsiana'	'Black Magic'	22	46	0	0.0	—
	'Super Gem'	2	1	0	0.0	—
	'Crystal Glow'	5	9	0	0.0	—
	'Deep Rose Gem'	3	7	0	0.0	—
	'Carmine Red'	3	4	0	0.0	—
	'Wedding March'	2	15	2	13.3	albino
	'Childsiana'	15	121	8	6.6	albino
	'Black Eyed Beauty'	6	31	5	16.1	albino
	'Best Gold'	5	31	5	16.1	albino
	'Mango'	4	22	1	4.5	albino
Total		757	3144	175	5.7

Fig. 1. Gel plates showing the PCR products amplified using a primer pair specific to *atpH*. Lane M, Size marker derived from ϕ X174/*Hae*III; Lanes 1 and 9, *Z. aethiopica* 'Wedding March'; Lane 2, *Z. hybrida* 'Black Eyed Beauty'; Lanes 3–8, albino hybrids derived from 'Wedding March' $\times$ 'Black Eyed Beauty'; Lane 10, *Z. hybrida* 'Pink Superba'; Lanes 11–16, albino hybrids derived from 'Wedding March' $\times$ 'Pink Superba'. Fragments (a) and (b) are specific to plastids of species in group 2 and *Z. aethiopica*, respectively.

barrier between *Z. aethiopica* and species of group 2. Inoue (1996) reported that the hybrid lethality in interspecific hybrid between *Nicotiana gossei* and *N. tabacum* was avoided by irradiating pollen with ion beams which induced gene deletion. Attempts could be made to remove the factors causing postfertilization barriers by inducing mutation on pollen or albino hybrids.

The dysfunctions of chlorophyll synthesis were observed in interspecific hybrids of *Impatiens* (Arisumi, 1985) and *Rhododendron* (Ureshino and Miyajima, 2002). Incompatibility between the genome and plastome could be one of the factors causing this dysfunction (Michishita et al., 2002). It was previously shown that, in *Zantedeschia*, plastids could be inherited from both parents (Yao et al., 1994). Snijder (2004) showed that diminution of chlorophylls in hybrids, derived from interspecific crosses among group 2 *Zantedeschia* species, was caused by incompatibility between the genome and plastome. Biparental inheritance of plastids was also detected in some hybrids obtained in this study (Fig. 1). But, no relationship between the appearance of albino hybrids and plastid inheritance was detected, which implies that a hybrid genome was incompatible to both parental plastids, or that the dysfunction of chlorophyll synthesis was caused by other factors, such as the hybrid genome in itself or incompatibility between genome and mitochondrial genome.

In this study, no compatible combinations were detected, because all hybrids were albino that survived only in vitro. Further studies are required to elucidate the causes for the appearance of albino hybrids.

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カラー種間交雑における交雑和合性の調査

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Zantedeschia aethiopica (グループ 1) およびグループ 1 以外の *Zantedeschia* 属 5 種 (グループ 2) に属する種間における交雑和合性について調査するため、グループ 1 を 2 品種、グループ 2 を 21 品種用いて交雑を行った。37 組み合わせの交雑より得られた 3144 個の雑種胚を培養し、26 の交雑組み合わせから 175 個の雑種個体を得た。しかし、得られた全ての雑種個体はアルビノ個体であり、独立栄養環境下での生育は不可能であった。なお、いくつかの雑種個体でプラスチドの両性遺伝が認められたが、このことと雑種個体の色との関係は見出せなかった。