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A STUDY OF INCOMPATIBILITY WITHIN THE GENUS *ZANTEDESCHIA*

by

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ABSTRACT

Zantedeschia includes many important varieties for New Zealand cut flower and pot plant production. The genus is divided into two sections. The first section consists of a single species *Z.aethiopica*, the common white calla, and a few selections such as the dwarf 'Childsiana'. The second section includes the remaining five species whose spathes range in colour from ivory, yellow and pink to maroon. Interspecific hybridization following hand pollination readily occurs between species of Section II and all of the coloured *Zantedeschia* cultivars have been derived from this section. *Z.aethiopica*, however, has never been successfully crossed with any of the species from Section II. *Z.aethiopica* has a different flowering period, is more floriferous and more tolerant of *Erwinia* (a soft rot of tubers) than the other species. The ability to produce hybrids from the two sections would greatly increase the potential for improved cultivars. This project was initiated to explore interspecific incompatibility by examining pollen physiology, embryogenesis of compatible and incompatible crosses, and rescue of embryos from incompatible crosses using embryo culture techniques.

Z.aethiopica 'Childsiana' pollen was similar in morphology to pollen from the coloured species of Section II. Pollen from all species examined were oval in shape with a light-yellow, waxy pollen coat. The exine was smooth with no aperture, while the cytoplasm was full of starch grains. The pollen of *Zantedeschia* is trinucleate. In contrast to other trinucleate pollen lower concentrations of sucrose (5-10%) were preferable for pollen germination and high concentration of sucrose (30%) sharply reduced germination. Pollen germination of *Zantedeschia* was unaffected by agar concentration and pollen even germinated well in liquid media. The *in vitro* medium containing sucrose (100 g l^{-1}), boric acid (50 mg l^{-1}), calcium chloride (100 mg l^{-1}) and agar (10 g l^{-1}) with pH 6 was optimal and was selected for further pollen germination studies.

The mature megagametophyte of *Zantedeschia* was a typical seven-celled embryo sac, with a three celled egg apparatus, three antipodal cells and a secondary nucleus formed by the two polar nuclei. The secondary nucleus was present at the chalazal end of the embryo sac before fertilization. Ovules were anatropus i.e. inverted, and had an inner and outer-integument. The apical cells of the nucellar epidermis divided periclinally to form a nucellar cap (or epistase) 4 - 5 cells in thickness.

Control ovaries of unpollinated (Section II) *Zantedeschia* hybrids 'Best Gold' and 'Chromatella' grew for 2 weeks and then started to shrink and turn yellow. These ovaries eventually died 8 to 10 weeks after anthesis. Incompatible crosses had far less ovule development compared with compatible crosses. In incompatible crosses ovary size was much smaller than compatible crosses from four weeks after pollination.

The embryos of compatible crosses had a steady growth rate up to 12 weeks. The embryo was globular at 4 weeks but cotyledon, radicle and a leaf primordium were differentiated by 6 weeks. In the incompatible crosses globular embryos were also found at 4 weeks, but their growth rate was much slower thereafter. Most of the incompatible embryos remained at a globular stage of development. A small percentage of incompatible embryos continued development but the endosperm surrounding these embryos often became necrotic. By 8 weeks after pollination 1-2% of embryos from the *Z. 'Chromatella' X Z. aethiopica 'Childsiana'* were larger than 0.7 mm with distinct cotyledon development. Compatible embryos at this time were about 2 mm long.

The endosperm in compatible crosses did not contain starch during the first two weeks after pollination, but small starch grains were seen in the outer endosperm from week 4 and starch levels increased throughout the endosperm to week 12. In the incompatible crosses starch levels were always lower and starch grains were confined to the outer region of the endosperm; the central region was poorly developed. In the incompatible crosses necrosis of the endosperm and embryo was clearly seen in most ovules from week 6. Even in embryo sacs with well developed embryos, the endosperm had shrunk and the seeds dried by week 8. The arrest of growth of embryos from incompatible crosses appeared to be related to degeneration of the endosperm and failure of nutrient metabolism and transport.

Young seeds were removed from ovaries at various stages (2 to 12 weeks after pollination) and cultured *in vitro*. The embryos from compatible crosses taken at 6 weeks or later grew well *in vitro* on Murashige and Skoog (1962) medium with sucrose (30 g l^{-1}). At this concentration of sucrose most embryos produced shoots, roots and cotyledonary haustorium tissue within one month of culture. High concentrations of sucrose (90 g l^{-1}) delayed growth of most of the cultured embryos.

Most embryos from incompatible crosses (dissected 6 to 10 weeks after pollination) were small (0.1-0.2 mm) and remained globular in culture. Higher concentrations of sucrose ($60\text{-}90 \text{ g l}^{-1}$) did not improve embryo growth irrespective of the age of the embryos. Some incompatible embryos were larger (0.7 mm) when dissected, and in culture these eventually produced shoots but all tissues were albino.

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CHAPTER 1

GENUS OF *ZANTEDESCHIA* REVIEW

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INTRODUCTION

The genus *Zantedeschia* was named by Sprengel in honour of a professor Giavnni Zantedeschi a botanist of Breschia (Traub, 1948) and plants within the genus are commonly known as Arum or Calla lilies.

Zantedeschia is a relatively small genus and all species are native to Africa. Since Sprengel established the genus *Zantedeschia* in 1826, other names have been put forward such as *Arodes*, *Richardia* and *Calla* but the name *Zantedeschia aethiopica* (L.) was upheld by Engler in 1915. From that time *Zantedeschia* has been conserved against later generic names. Traub (1948) revised the genus on the same lines as Engler, and recognized eight species (Table 1).

In a monograph on the genus Letty (1973) has traced the history of the nomenclature and suggests that *Zantedeschia* must be considered the correct designation. Letty (1973) recognised six species with three subspecies (Table 1). She criticized Traub's lack of knowledge of plants in the field and indicated that the following entities should be included within *Zantedeschia albomaculata*: *Z. oculata* (Engl, 1859), *Z. hastata* (Hook.f, 1883), *Z. melanoleuca* (Hook. f, 1869), *Z. angustiloba* (Schott, Engl, 1883), *Z. macrocarpa* (Engl, 1883) *Z. melanoleuca* var *tropicalis* (Brown.N.E, 1901), *Z. chloroleuca* (Engl, 1903) and *Z. melanolenca* var *concolor* (Davy.B, 1924).

Table 1. *Zantedeschia* species and subspecies recognised by Letty (1973) and Traub (1948).

Traub (1948)	Letty (1973)
<i>Z. aethiopica</i>	<i>Z. aethiopica</i>
<i>Z. elliottiana</i>	<i>Z. elliottiana</i>
<i>Z. rehmannii</i>	<i>Z. rehmannii</i>
<i>Z. hastata</i>	<i>Z. pentlandii</i>
<i>Z. melanoleuca</i>	<i>Z. jucunda</i>
<i>Z. angustiloba</i>	<i>Z. albomaculata</i>
<i>Z. albomaculata</i>	Subspecies: <i>Z. albomaculata</i> subsp. <i>albomaculata</i> <i>Z. albomaculata</i> subsp. <i>macrocarpa</i> <i>Z. albomaculata</i> subsp. <i>valida</i>

At present Letty,s (1973) work is accepted.

The species of *Z. albomaculata* show great variation in size, shape and maculation (spotting) of the leaf, the size, shape and colouring of the spathe; and the size of the berries and number of ovules (Letty, 1973).

The *Zantedeschia* species fall into two distinct sections:

- (I) Section I contains one species *Zantedeschia aethiopica*, the white calla lily of florists, which is common on the humid meadows around Capetown and generally in southwest Cape province, South Africa. The natural habitat of the species extends eastward to Natal. The essential characters of *Z. aethiopica* are that it is a robust, evergreen plant up to 60 cm high without a dark purple area inside of the base of the spathe. The spathe is milk-white, and the anthers are bright yellow; the green, unspotted leaves are cordate or hastate (Letty, 1973). The plant is evergreen and does not die down in winter nor does it require a resting period. Flowering occurs in winter from June to November in New Zealand. The female flowers in the lower part of spadix are interspersed with staminate flowers. Following fertilization the fruits enlarge and later turn orange, become soft and then mucilaginous at maturity. The root stock of *Z. aethiopica* is a rhizome (D. Cohen, pers. comm., 1988).

Hybrids of *Z. aethiopica* are rarely found in the wild state (Letty, 1973). White flower spotted forms exist but

the genealogy is unknown (Pers.comm. E. Welsh, 1989, Massey University Horticulture Science, Palmerston North). New (1986) suggests that the different times of maturity of the male and female flowers result in the failure of self fertilization in white calla. Within *Z. aethiopica* manual hybridization and selection has produced several varieties differing in size, scent and chlorophyll content of spathes (e.g. Childsiana, Green Goddess, Gigantea and Fragrans).

(II) Section II consists of the remaining five species, all of which have several unifying characteristics. These species flower during summer. There are no staminate flowers among female flowers on the spadix. The fruit remain firm and green until they finally wither and rupture or decompose on the ground. They have compact corms which require a resting period (Letty 1973). The species from sections I and II can be separated using several characteristics (Table 2).

Z. aethiopica is the easiest species to grow within the genus and it is well suited to New Zealand environmental conditions (Tjia and Funnell, 1985). It has many advantages for cut flower or pot plant production. Unfortunately *Z. aethiopica* is only available in milk white, or in some cultivars, white with green spathes. In Europe and North America in particular they are sold as funeral flowers which restricts sale as a cut flower and pot plant (T.E.Welsh, pers. comm., 1989).

In contrast, species within section II can be hybridized with each other. In New Zealand, Harrison and his associates have produced an outstanding collection of *Zantedeschia* hybrids. The great range of colour, size and form of Harrison strains for cut flowers, small pot plant and garden plants has attracted considerable overseas attention (Amos, 1984)

Zantedeschia cultivars have a very exciting future as a cut flower export crop and are recommended by Action Flower Export Co Ltd. (1987) as one of the more profitable flower export crops for growers at NZ \$ 1/stem for coloured flowers. The total annual export value reached NZ \$ 1,127,599 in 1989 (The Orchardist, 1989).

Zantedeschia are remarkably tolerant and relatively free from most pests and diseases. The major problem affecting the rhizomes or tubers is a soft rot caused by *Erwinia* and *Pseudomonas*. *Erwinia* is thought to be the predominant pathogen (Action Export Co Ltd. 1987). Sensitivity to soft rot disease has led to severe losses from disease either in storage, shipment or production. These problems greatly increase production cost per corm/rhizome. *Z. aethiopica* has many advantages for cut flower or pot plant production. For example it is more resistant to soft rot disease than section II hybrids (e.g. Best Gold, Pink Persuasion, Black Magic, Aztec Gold, Chrohatella, et) (Pers. Comm, D. Cohen, 1988, DSIR Fruit and Trees, Palmerston North; Pers. Comm. P. Long, 1989, Massey university Plant Health. Palmerston North).

Table 2. Key to *Zantedeschia* species.

(after Letty, 1973)

Species	EG ¹	Flowers			Leaves	
		DT ²	Colour	FL ³	Maculate	Shape
<i>Z. aethiopica</i>	yes	No	White	June-Nov	No	Varied ovate-cordate
<i>Z. rehmannii</i>	No	No	White, pink, Dark-maroon	Sep-Feb	No	Lanceolate
<i>Z. jucunda</i>	No	Yes	Golden-yellow	Nov-Jan	Densely	Triangle-hastate
<i>Z. elliottiana</i>	No	No	Golden-yellow	Nov-Feb	Yes	Oblong-hastate
<i>Z. pentlandii</i>	No	Yes	Lemon, chrome	Nov-Dec	Seldom	Oblong-hastate
<i>Z. albomaculata</i>	No	Yes	White, ivory, Pale-yellow,	Oct-Feb	Rare	Triangle-orbicular
<i>Z. albomaculata</i> <i>sub albomaculata</i>	No	Yes	Coral-pink	Nov-Apr	Rare	Oblong-hastate
<i>Z. albomaculata</i> <i>sub macrocarpa</i>	No	Yes	Pale-yellow, Coral-pink	Oct-Feb	Sparsely	Triangle-hastate
<i>Z. albomaculata</i> <i>sub valida</i>	No	Yes	Cream, Ivory-cream	Oct-Mar	No	Ovate-cordate

1, evergreen; 2, dark throat; 3, flowering

The two different sections have complementary characteristics which are attractive for breeding purpose. It would be a great advantage if the genetic characteristics of these two sections could be combined. New coloured cultivars with greater disease resistance and an increased flowering period would give New Zealand the leading edge in this industry.

The barriers between *Z. aethiopica* and the coloured hybrids are unclear. Traub (1948) suggests there may be a distinct gap from the standpoint of gene exchange. Chromosome analysis of *Zantedeschia* species and hybrids (*Z. aethiopica*, *Z. pentlandii*, *Z. rehmannii*, *Z. elliottiana* hybrid, Best gold and *Z. albonaculata*) showed that all have 32 chromosomes. *Z. aethiopica* var. *childsiana* is distinct from other species in only having two sets of metacentric chromosomes the others were subtelecentric. In comparison, most of the chromosomes in the others species (*Z. pentlandii*, *Z. rehmannii*, *Z. elliottiana* hybrid, Best Gold and *Z. albomaculata*) metacentric to submetacentric (Pers. Comm. Jai-Long, 1989, DSIR Fruit and Trees, Palmerston North).

Preliminally experiments had been carried out by Cohen (1988) who crossed a range of summer flowering colour hybrids with *Z. aethiopica* and found that incompatibility was expressed in all crosses. In earlier crosses (Traub, 1948) embryos developed indicating that pollination and fertilization probably occurred. By dissecting these embryo and transferring them to *in vitro* conditions some shoots were successfully obtained. All shoots rescued by embryo culture were albino and died without producing roots (Cohen, 1988). There is limited information on interspecific incompatibility but it is apparent that it is more diverse and much less understood compared with intraspecific incompatibility (Shivanna, 1982). Prefertilization incompatibility is seen as a pollen-pistil rejection response, expressed at number of possible levels in the pistil, from the stigma surface to penetrated embryo sac. It involves reduced pollen germination, slow pollen tube growth or distortion and bursting of pollen tubes (Chen and Gibson, 1974; Kazimierska, 1978a; Heslop-Harrison, 1982).

Postfertilization barriers are common features of interspecific crosses. The genome of the parent species may be physiologically incompatible leading to hybrid seedling fatality, physiological abnormality and sterility (Chen et al., 1983; Shill et al., 1982). More commonly the endosperm aborts resulting in starvation and death of potentially viable embryos (White and Willam 1976). The physiological basis of hybrid endosperm abortion is unknown, but it may be related to disturbance of nutrient transfer patterns among the diploid maternal tissue, the diploid hybrid embryo and the triploid hybrid endosperm (Evans, 1962; Williams and White, 1976).

Recently, Takahashi (1974) and Hogenboom (1975) suggested that interpopulational incompatibility is controlled by incongruity. Incongruity is due to the lack of genetic information in one partner about some relevant aspect of the other. It may also be concerned with a post-fertilization barrier. Thus incongruity represents a passive reject where as incompatibility involves active rejection as a result of S gene action.

The hypotheses explaining interspecific incompatibility are based largely on genetic studies and very little work has been done on the physiological and biochemical aspects.

In this study four aspects will be examined

(1) Pollen physiology of *Zantedeschia*

Several researchers indicate that the two Sections within *Zantedeschia* have incompatible crosses (Traub, 1948; Letty, 1973; Cohen, 1989) but there has been no study of the

differences in pollen structure between these two sections. Pollen may be different in size, exine or internal organization between the two sections. This was investigated by dissecting pollen and using TEM and SEM to examine pollen structure.

The two Sections have different flowering times. It would be a great advantage if pollen could be stored. The pollen grains of *Zantedeschia* are 3-celled (Davis, 1966; Brebaker, 1967). Tricellular pollen is notoriously short-lived whatever the conditions (Heslop-Harrison and Shivanna, 1977). *in vitro* germination of pollen grains is a easy and reliable technique to examine pollen germination ability. To obtain a suitable pollen germination medium different concentrations of sucrose, boric acid, calcium chloride, agar, and various pH and temperature regimes were examined.

(2) Crosses between the two sections of *Zantedeschia* species in the field

Hybridization is possible among all the *Zantedeschia* species with the exception of *Z. aethiopica* (Traub, 1948; Shibuya, 1956). Difficulties in hybridization between the two sections of *Zantedeschia* are compounded by the different flowering times. *Z. aethiopica* naturally flowers in New Zealand during winter from June to December and the coloured callas flower during December and January. Artificial pollinations between the two Sections of *Zantedeschia* were performed. The growth of ovary, ovule, embryo sac and embryo size was measured following dissection of the ovary and ovule every two weeks after pollination.

(3) Comparative embryo development of incompatible and compatible crosses

The detailed morphological study of embryo growth or failure in *Zantedeschia* hybrids is essential in a programme directed towards crop improvement in this genus. The arrested embryo growth and degeneration of the endosperm was clearly observed by using the dissection and stain technique. The processes of megagametogenesis and post-fertilization events in compatible crosses and incompatible crosses within *Zantedeschia* are unknown. The present study, forms a part of a more general post-fertilization embryo and embryo sac development survey. It concerns compatible and incompatible crosses and the development of embryo sacs leading to an explanation of failure in incompatible crosses.

(4) Embryo culture.

Abortive embryos can be rescued by using embryo culture techniques in many interspecies crosses. (Williams 1978. Neal, et al 1983) Embryo abortion occurs as either a pre- or post-fertilization event in interspecific crosses, but in *Zantedeschia* post-zygotic abortion was most common. *in vitro* culture of globular and early-stage embryos has proven to be difficult since these heterotrophic stages have complex nutritional and hormonal requirements (Neal, 1983). Most of *Zantedeschia* embryos from incompatible crosses remained in a globular stage. Osmotic pressure is obviously an important factor for growth of the immature embryo (Neal, 1983; Cook, 1988). This study aimed to rescue embryos from incompatible crosses by evaluating levels of sucrose supplemented in the culture media.

CHAPTER 2

POLLEN PHYSIOLOGY OF *ZANTEDESCHIA*

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