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**INTERSPECIFIC HYBRIDISATION AND
MOLECULAR CHARACTERISATION OF HYBRIDS
IN THE GENUS ZANTEDESCHIA**

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Abstract

The genus *Zantedeschia* consists of two sections: a section containing *Z. aethiopica*, a white-flowered, evergreen species and a second section containing five winter-dormant species. A new species, *Z. odorata*, was recently described which does not fit into either of the two sections. Chromosome karyotypes of five species and two hybrid cultivars were prepared. Karyotypes are distinct between the two sections but not distinct within the second section. The karyotype of *Z. odorata* falls between the two sections although it is more closely related to *Z. aethiopica*.

Colchicine treatment of multiplying shoots *in vitro* produced tetraploid plants from eight cultivars and two species of the second section of the genus. Most of these plants were pure tetraploids. *Z. aethiopica* did not multiply *in vitro*. Colchicine treatment of *Z. aethiopica* 'Childsiana' germinating seed produced a few tetraploid plants and many diploid/tetraploid chimeric plants. A screening procedure for tetraploids using stomatal measurements, with confirmation by chromosome counting, was demonstrated to be an efficient and accurate way to identify tetraploids. Triploid plants were produced from two diploid/tetraploid crosses with the aid of *in vitro* embryo culture.

Crosses were made between the two sections using a number of species and genotypes at the diploid and tetraploid levels. In these crosses, endosperms were watery and transparent and embryos were small (in most cases less than 0.3 mm). Embryos embedded within endosperms were cultured because the embryos were too small to be cultured separately from the endosperms. From these cultures, over one hundred hybrid embryos were rescued. These hybrids were all albino. In an electron microscopy study, it was found that the plastids of these albino hybrids had no prolamellar body in the dark nor grana in the light.

Z. odorata was tested as a bridge for gene transfer between the two sections because it falls in between them. Hybridisation between *Z. odorata* and *Z. aethiopica* produced a number of virescent, albino and chimeric (green/albino) hybrids following embryo culture and seed germination. Hybrid production was much easier, however, when *Z. aethiopica* was used as the maternal parent. All hybrids rescued from crosses between *Z. odorata* and the second section of the genus were albino. This study also demonstrated that *Z. odorata* stigmas can receive pollen from the same spadix. *Z.*

odorata embryos become dormant before the seeds matured. Plants of *Z. odorata* did not produce any flowers unless they were treated with gibberellic acid (GA). However, two or three flowers per plant were produced when 50 ppm GA₃ or GA₄ + 7 was applied to tubers as a pre-planting treatment.

A partial library was constructed with total leaf DNA of *Z. aethiopica* 'Childsiana'. A species-specific nuclear DNA clone, pZAC3, was isolated by differentially screening this library with radioactively-labelled total DNA of different species. This clone was characterised by restriction enzyme mapping and RFLP (restriction fragment length polymorphism) analysis. By RFLP analysis, an apple rDNA clone differentiated between the sections of the genus. The apple rDNA clone and pZAC3 were successfully used for hybrid identification. From this library, six plastid (pt) DNA clones were also isolated by hybridisation with kiwifruit ptDNA clones. Using these ptDNA clones, RFLP bands were identified to differentiate between species and, in one case, between genotypes within a species. Biparental ptDNA inheritance and a ptDNA deletion were detected in the albino hybrids between the two sections with these ptDNA clones. The data from RFLP analyses gives the first molecular data on the phylogeny of *Zantedeschia* and indicates that *Z. odorata* is distinct from the previously-described two sections and falls in between the two sections.

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