MICROPROPAGATION AND GENETIC TRANSFORMATION OF

VERTICORDIA GRANDIS

BY

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Summary

Verticordia grandis (Scarlet feather flower) is the subject of this study. It is a member of the family Myrtaceae and has a brilliant display of red flowers on long upright stems. The horticultural potential of V. grandis as a cut flower and for pot culture is recognised in the industry. However, supply is limited due to difficulties of propagating the plant. In particular, the induction of roots on explants in vitro has limited the successful commercial micropropagation of this species.

A system has been established for the micropropagation of V. grandis. Protocols were developed for the initiation, shoot proliferation and root induction of V. grandis explants, in vitro. The sterilisation treatment successfully decontaminated 82% of the explants. Shoot multiplication rates of at least 3 fold were obtained on the multiplication medium. In addition, the location of meristematic cells at the leaf petiole region indicated that shoot proliferation from leaf discs was possible. The root induction medium consistently produced 100% rooting of explants. Anatomical investigations confirmed that roots were connected to stem vascular elements. Therefore the methods developed were suitable for commercial production. These methods are essential requirements for nursery production and are also important prerequisites for transformation studies.

Rooted plantlets were successfully transferred to glasshouse conditions. Survival rates were influenced by both the plant genotype and the time of year of out-planting. Soil conditions critical for the successful pot culture of V. grandis included adequate drainage (air filled porosity of 20%), slightly acidic medium (pH 5.5-6) and low supplementary nutrient application. V. grandis plants responded favourably to high illumination and low humidity levels. A foliar 6-benzyladenine (BA) spray (210mg/l) in combination with apical bud removal increased the number of lateral shoots and were useful in improving the shape of the plants for commercial pot culture.
The micropropagation and out-planting protocols developed for *V. grandis* were utilised in *Agrobacterium* mediated transformation investigations. Initially, susceptibility trials using wild type *A. rhizogenes* strains were employed. Results obtained from these investigations indicated that wild type *A. rhizogenes* does not induce hairy root formation in *V. grandis* stem explants. However, *V. grandis* is susceptible to infection with *A. rhizogenes* as determined by gall formation and opine analysis.

A system has been established for the genetic transformation of *V. grandis*. This system utilises the method developed for plantlet regeneration from leaf discs. Leaf discs were inoculated with an *Agrobacterium* strain containing a marker gene for antibiotic resistance (NPT II gene) and a reporter gene (GUS) for screening of transformants. The transformation results were confirmed by PCR and Southern hybridisation. These results represent the first example of genetic engineering of a plant from the Myrtaceae.

This thesis describes, therefore, the establishment of *in vitro* organ culture techniques for the propagation of *V. grandis* and for the establishment and pot culture of plants in the glasshouse and demonstrates the application of the leaf disc regeneration method for the transformation and regeneration of *V. grandis*. 
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