Development of somatic embryogenesis as a propagation method for ornamental eucalypt hybrids

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Summary
Experiments were conducted to produce plants via tissue culture of ornamental eucalypts, as clonal propagation of superior genotypes is essential to perpetuate horticultural characteristics. The plants chosen for initial study were *E. erythronema, E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii*. Most work was conducted on seedling material, in order to develop suitable methods for the hybrids, of which limited material was available. Successful production of callus, buds, shoots and roots was achieved using the plant growth regulators BAP and NAA. Micropropagation was also successful. No somatic embryos were produced, but this was not seen as a problem, as successful production of plants *in vitro* had been achieved via organogenesis and micropropagation.

Future work is required to harden off plants produced *in vitro*, and to apply the successful techniques to superior hybrids between *E. erythronema* and *E. stricklandii.*

Introduction
The objective of the project is to investigate tissue culture techniques, such as somatic embryogenesis, as propagation methods for ornamental hybrid eucalypts (Arruda et al 2000, Berney 2000, Watt et al 200, 2003). A breeding programme is underway at the University of Adelaide to develop superior eucalypt cultivars for ornamental horticulture, particularly for cut stem production (Delaporte et al 2001). The research was conducted on the species *E. erythronema, E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii*, such as hybrid *Eucalyptus* 'Urrbrae Gem' (Delaporte et al 2001). Research needs to be focused towards finding optimum media, plant growth regulators and environmental conditions necessary for successful propagation, as the development of a rapid, economic and reliable method of propagation for ornamental hybrid eucalypts is essential for their commercial development. In addition, observation of tissue cultured material at a microscopic level will provide insights into organisation of callus and the development of normal and abnormal organogenesis.

Materials and methods
The plant material used was open pollinated seedlings of *Eucalyptus erythronema, E. stricklandii* and *E.* "Urrbrae Gem", located in the Waite Arboretum of the University of Adelaide, with limited work conducted on clonal material of *E.* "Urrbrae Gem" and of other *E. erythronema* x *E. stricklandii* hybrids from the University of Adelaide eucalypt breeding programme.

Three tissue culture pathways were investigated, somatic embryogenesis, organogenesis and micropropagation (Watt et al 2003).

Experiments
Experiment 1. Effect of plant growth regulators on different genotypes and tissue types
Seeds of *E. erythronema, E. stricklandii* and *E*. 'Urrbrae Gem" were sterilised and germinated *in vitro*. Hypocotyl, cotyledon and leaf explants were harvested from 2-3 week old seedlings and placed on MS medium. The auxins were NAA at 0, 5 and 10 µM, and 2,4-D at 0, 5 and 10 µM. The cytokinins were BAP at 0, 1, 5 µM and kinetin at 0 and 5 µM. The explants were placed on medium in 30 mL polycarbonate tubes with 10 mL medium per tube in a growth room at 24^{o}C with 16 hours low light at 11 microeinsteins per m^{2} per second, with 10 replicates per treatment. Data collected were callus growth, root number, shoot number and bud number and data were analysed by ANOVA.

Experiment 2. Effect of low concentration of BAP on bud and shoot regeneration from different genotypes and tissue types
Leaf and apex explants were taken from 2-3 week old seedlings of *E. erythronema, E. stricklandii* and *E*. 'Urrbrae Gem", and from *in vitro* grown shoots of *E. erythronema* x *E. stricklandii* hybrid 2.5. BAP levels were 0, 0.1, 0.25, 0.5 and 1 µM. Other conditions were as for Experiment 1.

Experiment 3. Effect of auxins on organogenesis from different genotypes and tissue types
This experiment tested a technique known to produce somatic embryos in *Eucalyptus globulus* (Pinto et al 2002). Apices and 3 day old cotyledons were taken from seedlings of *E. erythronema, E. stricklandii* and *E*. 'Urrbrae Gem", and apices were taken from in vitro shots of *E. erythronema x E. stricklandii* hybrid 2.5. NAA was used at 0, 4.5, 16, 26 and 80 µM for 1 - 4 weeks after which explants were subcultured to medium lacking plant growth regulators.

Experiment 4. Micropropagation
Nodal explants were taken from seedlings of *E*. 'Urrbrae Gem' germinated *in vitro*. These were multiplied on MS medium and shoots were transferred to medium with IBA for root initiation.

Results
Experiment 1. Effect of plant growth regulators on different genotypes and tissue types
Organogenesis was achieved from leaf explants with the following combinations of plant growth regulators:
Callus: 1 µM BAP and 5 µM NAA
Callus: 5 µM BAP and 5 µM NAA
Buds and shoots: 1 µM BAP alone
Callus: 5 µM BAP and 5 µM NAA
Roots: 5 µM NAA alone
Roots: 10 µM NAA alone

Callus, roots, buds and shoots were produced, and microscopy showed that the shoots had normal meristematic and vascular organisation. No somatic embryos were produced.

Organogenesis from hypocotyls and cotyledons was varied. Low levels of BAP were effective, so the next experiment investigated even lower levels.

Experiment 2. Effect of low concentration of BAP on bud and shoot regeneration from different genotypes and tissue types
Apices were more responsive than leaves, with good shoot growth and less callus than in the previous experiment. No somatic embryos were observed.

Experiment 3. Effect of auxins on organogenesis from different genotypes and tissue types
No somatic embryos were produced, but shoots and roots developed producing plantlets after 1 week on 80 µM NAA.

Experiment 4. Micropropagation
In vitro shots were established from nodal explants. These were rooted with IBA.

Discussion

No previous research had been conducted on the ornamental species *E. erythronema, E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii* (Watt et al 2003). Hence, the first experiment was conducted to determine the best combination of plant growth regulators for these species. Leaf tissue was more responsive than hypocotyl, which was more responsive than cotyledon. Seedlings of *Eucalyptus* 'Urrbrae Gem ' were more similar in response to the female parent *E. erythronema* than to the male parent *E. stricklandii*. They produced callus, buds, shoots and roots, whereas *E. stricklandii* produced fewer shoots. 2,4-D produced poor callus with no shoots and few roots. Kinetin was generally less effective than BAP.

Because low levels of BAP were effective, it was decided to try lower levels, and to include apices in Experiment 2. Apices were more responsive than leaves, and better shoots with less callus were produced. These shoots were then rooted with IBA.

Experiment 3 followed a method shown to produce somatic embryos in *Eucalyptus globulus* (Pinto et al 2002), using high levels of NAA and 2,4-D. Cotyledons produced callus and roots and apices produced shoots and roots, but no somatic embryos were produced. It appears that the species species *E. erythronema, E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii* are not as responsive as some other eucalypt species in the production of somatic embryos.

Leaves and apices were the most responsive in tissue culture. Callus from mature tissue produced only more callus and did not undergo organogenesis.

Conclusions

- Plants with shoots and roots were produced *in vitro* via organogenesis
- Plants with shoots and roots were produced *in vitro* via micropropagation

- No somatic embryos were produced

Future work

Further research is required to harden off plants produced *in vitro*, and to apply the successful techniques to superior hybrids between *E. erythronema* and *E. stricklandii.*

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