Tissue Culture of Ornamental Eucalypts *Eucalyptus erythronema, E. stricklandii* and their hybrids

Pauline Glocke

B. Ag. Sc (Hons, Horticultural Science)

Submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

Department of Agriculture and Wine

Waite Agricultural Research Institute

University of Adelaide

October 2004

TABLE OF CONTENTS

Abstract

This research project was carried out using the ornamental species *E. erythronema, E. stricklandii*, their natural interspecific hybrid *E.* 'Urrbrae Gem', and artificial hybrids of these two species, with the aim of identifying a clonal propagation method for hybrid ornamental eucalypts using the tissue culture techniques of micropropagation, organogenesis and somatic embryogenesis.

Seeds of *E. erythronema, E. stricklandii*, and *E.* 'Urrbrae Gem' were decontaminated and germinated in culture for experiments on callus growth and response to plant growth regulators. Murashige and Skoog (1962) medium supplemented with 1 μM BAP, 10 μM NAA, 20 g^{-1} sucrose, gelled with 7 g^{-1} Phytagel® proved the most suitable callus growth medium when compared to those of Gamborg (1968), Lloyd and McCown (woody plant medium) (1980), Almehdi and Parfitt (1996) and de Fossard(1976) (*Eucalyptus)*.

A range and combination of plant growth regulators was used to investigate organogenesis, somatic embryogenesis and axillary shoot proliferation. A broad based experiment was first conducted to assess the response of the hybrid and its parental species to wide concentrations of commonly used auxins and cytokinins known to induce organogenesis and somatic embryogenesis. Callus growth was consistent across all genotypes and tissue types with soft and spongy callus growth in the presence of 2,4-D and hard and friable callus in the presence of NAA, while callus growth was greater with auxin or auxin/cytokinin (BAP or kinetin) combinations compared to cytokinin alone. Healthy root development was observed from treatments with NAA, while roots that developed from

treatments with 2,4-D turned brown within 12 weeks. Bud development was observed with BAP alone or in combination with NAA with large bud clusters observed on *E. erythronema* and *E.* 'Urrbrae Gem' explants. Shoots were observed in treatments with NAA alone, or in combination with BAP or kinetin, but not in treatments with 2,4-D. No somatic embryos were observed with any treatment or taxon. Light microscopy of explants treated with 2,4-D alone showed calli consisting only of large parenchyma cells, while 2,4-D in combination with a cytokinin produced smaller parenchyma cells, with areas of vascular tissue and spherical growths. BAP, kinetin and NAA alone or in combination produced an increase in vascular tissue. Shoot and root initiation appeared to occur within the callus with connection to the vascular tissue.

Shoot organogenesis from *E. erythronema, E. stricklandii* and *E.* 'Urrbrae Gem' seedlings and *E. erythronema* x *E. stricklandii* hybrid 2.5 was further investigated using apex and leaf explants with 1 μM and lower levels of BAP. Apex and leaf explants of *E. erythronema* and *E.* 'Urrbrae Gem' developed clusters of buds from the apex base and leaf petiole while apex and leaf explants of *E. stricklandii* and hybrid 2.5 produced individual buds. Bud and shoot development was greater on apex than leaf explants with 0.5 and 1 μM BAP producing the greatest shoot numbers. Light microscopy showed that meristem development had occurred within the callus and bud structures, but there were few shoot primordia considering the amount of meristem development (meristemoids).

Somatic embryogenesis was further investigated in juvenile ad callus tissue using NAA, NAA/2,4-D combinations, and heat and smoked water treatments. Somatic embryogenesis

was not observed macroscopically with 2,4-D and NAA treatments. Globular somatic embryo structures were observed on apex explants of *E.* 'Urrbrae Gem' seedlings at 2 wk on MS medium supplemented with 16.11 μM NAA but there was no development beyond the globular stage. Areas of defined callus development, which did not contain typical embryogenic cells, were observed on all treatments and genotypes. Root development was observed from all auxin treatments but was less on explants treated with 5.37 μM NAA/ 4.5 μM 2,4-D compared to NAA alone while roots and shoots developed simultaneously on apex explants after culture for 1 wk on MS medium supplemented with 80.55 μM NAA. Heat and smoked water were detrimental to *E.* 'Urrbrae Gem' callus and no somatic embryogenesis was observed.

Explants from field trees of *E. erythronema, E. stricklandii*, *E.* 'Urrbrae Gem', *E. erythronema* x *E. stricklandii* hybrids 20E, 20P, 20R, 20T, 20V, 21A, 21G, 21U and potted plants of *E. erythronema* x *E. stricklandii* hybrids 35.2 and 2.5 growing under glasshouse conditions, were harvested and initiated into culture. Of the selected plants only explants from glasshouse grown material could be initiated into culture and multiplied. *E. erythronema* x *E. stricklandii* hybrids 35.2 and 2.5 were multiplied by axillary shoot proliferation over 3 yr on Quoirin and Lepoivre medium (1977) with 2.2 μM BAP, 0.5 μM NAA and 0.5 μM GA_3, 20 g^{-1} sucrose, gelled with 7 g^{-1} Phytagel®. However root growth proved difficult as shoots developed short thick roots that failed to elongate and the plants could not be hardened off. By using zeatin in the multiplication medium and activated charcoal after the IBA pulse elongated roots suitable for hardening off were developed. By comparison seedlings of *E.* 'Urrbrae Gem' were successfully germinated in culture and

multiplied by axillary shoot proliferation using WPM, 2.2 μM BAP, 0.5 μM NAA and 0.5 μM GA_3, 20 g^{-1} sucrose, gelled with 7 g^{-1} Phytagel®. They were rooted on ½WPM, 20 μM IBA, 10 g^{-1} sucrose gelled with 7 g^{-1} Phytagel® for 7 d, followed by subculture to PGR free medium. Following root initiation and growth plants were hardened off to the external environment.

In conclusion genetic differences were observed between genotypes, in regards to initiation into culture, and response to nutrient medium, and plant growth regulators, with differences observed in explant deterioration, medium exudates, callus growth and appearance, somatic embryogenesis, bud, shoot and root organogenesis, axillary shoot development, and rooting of shoots. For organogenesis, juvenile explants were more responsive in culture than mature explants and potted plants more responsive than field plants for micropropagation. Shoot and root organogenesis was achieved from juvenile explants while shoot explants of *E. erythronema* x *E. stricklandii* hybrids were successfully micropropagated by axillary shoot proliferation. Somatic embryogenesis was observed with *E.* 'Urrbrae Gem' seedlings but was blocked at the globular stage. This research has provided a greater understanding of the clonal propagation requirements via tissue culture techniques for *E. erythronema, E. stricklandii* and their hybrids.