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Micropropagation of *Eucalyptus* Species

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***Abstract.* Two cold-tolerant species (*Eucalyptus macarthurii* Deane et Maiden and *E. smithii* R.T. Baker), a cold-tolerant hybrid (*E. macarthurii*×*E. grandis* Hill ex Maiden), and *E. saligna* Sm. were propagated in vitro from nodal explants collected from field-grown seedlings and from clonal hedges. Shoot growth was initiated on modified Murashige and Skoog (MS) medium containing BA at 0.1 mg·liter^{-1}. Modified MS medium with BA (0.2 mg·liter^{-1}) and NAA (0.01 mg·liter^{-1}) was most effective in promoting shoot proliferation. Root initiation was achieved on half-strength modified MS medium with 2 mg IBA/liter. Rooted plants were hardened and established in the field. Chemical names used: N-(phenylmethyl)-1EZ-purin-6-amine (BA); 2-(1-naphthyl)acetic acid (NAA); 1H-indole-3-butyric acid (IBA).**

The increasing demand for forest products has resulted in the need for greater yields from plantations. Vegetative propagation can provide clonal plantations of superior trees that produce greater yields than seedling-propagated plantations (Zobel and Ikemori, 1983). Unfortunately, many of the *Eucalyptus* L' Her. species grown commercially, particularly the cold-tolerant ones, do not propagate easily by cuttings (McComb and Wroth, 1986). This study reports on the in vitro micropropagation of two cold-tolerant eucalyptus species (*Eucalyptus macarthurii* and *E. smithii*), a cold-tolerant hybrid (*E. macarthurii* × *E. grandis*) (MG25), and *E. saligna,* which is somewhat cold-tolerant.

Shoots were collected from vigorously growing l-year-old seedlings and l-year-old clonal hedges. The ortets of the clonal hedges were 5- to 10-year-old mature trees. Stock plants were sprayed with methyl[1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate (benomyl) (1 g·liter^{-1}) twice weekly for 2 weeks before harvesting shoots. The fungicidal pretreatment was essential for effective decontamination of explants (data not shown).

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Shoot lengths of two to three nodes were surface-disinfested by five rinses in sterile deionized water before and after a 15-min treatment with 0.02'% $HgCl_2$ containing two or three drops of Tween 20 per 100 ml. Explants consisting of a single node with leaves trimmed near the base were placed upright in 25 × 100-mm glass vials containing 10 ml sterile initiation medium (IM). The IM consisted of Murashige and Skoog (MS) (1962) inorganic salts and vitamins and (all per liter) 1 g polyvinylpyrrolidone (PVP) (M.W. ±70,000), 20 g sucrose, and 0.1 mg BA. The medium was adjusted to pH 5.8 before the addition of 2 g Gelrite/liter (Labretoria, Pretoria) and autoclave at 120C for 20 min. Cultures were placed in the dark for 5 days at 25C and then transferred to a 16-h photoperiod under cool-white fluorescent light at 20 μmol·s^{-1}·m^{-2} and a constant 25C.

The number of surviving explants varied greatly between trials and depended largely on the condition of the stock plants. Stock plants producing young, healthy, vigorously growing shoots gave the best results. Shoots developed from axillary buds within 4 weeks in more than 90% of the surviving explants. These were transferred to shoot multiplication medium (SMM) consisting of IM modified with (all per liter) 0.1 mg calcium pantothenate, 0.1 mg biotin, 0.1 g myo-inositol, and 0.01 mg NAA, and various concentrations of either BA (0.2, 0.5, or 1.0 mg·liter^{-1}) or N-phenyl-N´-1,2,3,-thiadiazol-5-urea (thidiazuron; 0.1 or 0.2 mg·liter^{-1}). Twenty-one explants were used for each treatment.

Shoot -proliferation for each species and treatment was determined as the change in shoot number over 28 days. Significance was determined by two-way analysis of variance and Tukey's highest significant difference range test. BA (0.2 mg·liter^{-1}) with NAA (0.01 mg·liter^{-1}) proved to be the most effective treatment for promoting shoot multiplication for all species (Fig. 1). Explants on thidiazuron-amended media produced prolific callus that engulfed the developing shoots. Thidiazuron has been reported as a substitute for adenine-type cytokinins in micropropagation of woody species (van Niewkerk et al., 1986) and usually has higher biological activity than the most active adenine-type cytokinins (Mok et al., 1987). The concentrations of thidiazuron used in our trials were probably too high; however, lower concentrations were not tested, as BA at 0.2 mg·liter^{-1} gave satisfactory results.

No significant differences were found in the rates of shoot multiplication between species when compared over a total of 15 subcultures on media containing 0.2 mg BA/liter and 0.01 mg NAA/liter, using one-way analysis of variance. and Tukey's range test ($P = 0.05$). The mean rates of shoot proliferation for *E. macarthurii, E. smithii, E. saligna,* and the hybrid MG25 were 3.0 ± 0.5, 2.5 ± 0.5, 3.4 ± 0.4, and 2.8 ± 0.5, respectively. A few shoots (1 % to 5%) became vitrified during each subculture, and callus formed at the nodes of some shoots, which resulted in apical senescence and leaf drop. These problems have also been reported for other *Eucalyptus* spp. (Franclet and Boulay, 1982) and could be minimized by precluding affected shoots from further subcultures. Although Gelrite has been reported to induce vitrification in some tissue cultures (Loreti and Pasqualetto, 1986), *E. grandis* shoot cultures were found to grow better on Gelrite than on agar (S. MacRae and J. Van Staden, unpublished data).

Elongated shoots (<20 mm) were transferred to root initiation medium that consisted of half-strength MS inorganic salts and

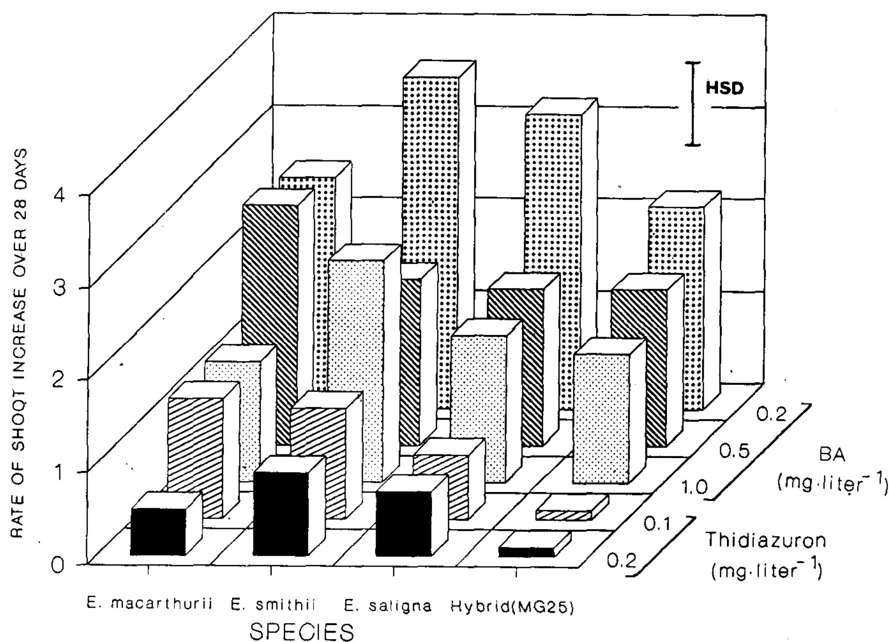

Fig. 1. Rates of axillary shoot proliferation from nodal explants of *Eucalyptus* spp. on modified Murashige and Skoog medium containing 0.01 mg NAA/liter and various BA and thidiazuron concentrations. Values represent means of 21 samples. Separation by two-way analysis of variance, Tukey's range test, $P = 0.05$.

vitamins, and (all per liter) 1 g PVP, 0.1 mg calcium pantothenate, 0.1 mg biotin, 0.1 g myo-inositol, 15 g sucrose, 2 mg IBA, and 2 g Gelrite, pH 5.8. Cultures were placed in darkness for 72 h and then transferred to a 16-h photoperiod of lower light intensity (5 $\mu mol \cdot s^{-1} \cdot m^{-2}$) at 25C.

Roots became visible after 10 to 14 days in light. Roots often formed on small callus growths at the edges of leaves in contact with the medium; these plants could not be hardened. 'Therefore, the lower leaves on microcuttings should be removed before transfer to root initiation medium. Rooting ability of shoots varied 'greatly for all species when comparing shoots from four sequential subcultures. Differences in rooting ability between species were significant *(P* = 0.05) in two out of four trials, and the range in rooting percentage was 0%-67% for *E. macarthurii,* 2%-30% for *E. smithii,* 21%-100% for *E. saligna,* and 9%-86% for the hybrid MG25.

Rooted shoots were transferred to a sterile potting mixture of 1 sand :1 bark :1 perlite (by volume) and hardened by gradually reducing the amount of intermittent mist (initially 5 sec every 15 rein) over 2 weeks. More than 80% of the plantlets with suitable root systems survived. Established micropropagated plants had the same appearance as their ortets.

The technique for rooting of shoots requires further investigation, as *E. macarthurii* and *E. smithii* rooted poorly. These two species also root poorly from coppice cuttings (unpublished data). The micropropagation system described has potential either as a system for mass production of ramets for plantations or for the establishment of clonal hedges from which propagation by cuttings can be performed.

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