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Micropropagation of *Eucalyptus dunnii* Maid.

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Introduction

Among the *Eucalyptus* recommended for reforestation in the southern region of Brazil, *Eucalyptus dunnii* Maid. has been the most promising due to its rapid growth, stem straightness and frost tolerance. The establishment of tree improvement programs and extensive plantations, however, have been restrained due to both low seed production (Graça, 1987) and low rooting capacity when propagated by stem cuttings.

The high multiplication rates which can be obtained with micropropagation techniques can assist in overcoming these problems as plant production is rapidly increased.

Although several eucalypt species have been *in vitro* propagated (Hartney, 1982), work with *E. dunnii* has not been reported. This paper describes a micropropagation technique for rejuvenated *E. dunnii.*

Materials and Methods

Nodal segments of *E. dunnii* (about 1 cm long) containing one node from greenhouse grown plants derived from stem cuttings were used as explants. Explants were disinfected by 30 min immersion in 0.5 $g \cdot l^{-1}$ 'Benomyl', immediately followed by soaking for 5 min in 1.5% (v/v) commercial detergent and for 15 min in 1% sodium hypochlorite. The disinfectants were removed by 3 successive rinses in autoclaved and bidistilled water.

In the multiplication stage, explants were cultured on MS medium (Murashige and Skoog, 1962) containing the following substances (mg/l): myoinositol (100), nicotinic acid (0.25), pyridoxine·HCl (0.25), thiamine·HCl (0.5), glycine (2), adenine sulfate (20), sucrose (30,000) and Difco Bacto-agar (6,000). Growth regulator treatments were 6-benzylaminopurine (BAP) at 0.1, 0.5 and 1.0 $mg \cdot l^{-1}$ combined with indole-3-butyric acid (IBA) at 0.01 and 0.1 $mg \cdot l^{-1}$. The pH was adjusted to 5.8. Cultures were maintained under 16 h/8 h light/dark photoperiod and 25 ± 2°C; after 30 and 60 d, shoot numbers per explant were recorded.

For shoot elongation experiments, individual shoots or clusters were cultured on media containing MS salts (T_1) or one-half MS salts (T_2). These treatments were combined with 0.1 $mg \cdot l^{-1} GA_3$ (T_3 and T_4, respectively) or with 1.0 $mg \cdot l^{-1}$ GA_3 to give T_5 and T_6, respectively. All treatments were supplemented with 0.1 $mg \cdot l^{-1}$ BAP and 0.01 $mg \cdot l^{-1}$ IBA. Further shoot elongation was investigated by comparing the best elongation treatment obtained in the above experiment with the treatments described in Table I.

Elongated shoots (2.0–2.5 cm in length) were subcultured on a medium containing 1/4 x MS salts at 0.5, 1.0 or 1.5 $mg \cdot l^{-1}$ IBA and 10 $mg \cdot l^{-1}$

Table I. Shoot elongation treatments.

T_1	MS + B_5[a] + 5.0 g·l^{-1} AC[b] + 0.05 mg·l^{-1} BAP + 0.05 mg·l^{-1} IBA
T_2	Gonçalves' medium[c] + 0.5 mg·l^{-1} BAP + 1.0 mg·l^{-1} IAA[d]
T_3	MS/2 + 38 mM ($N-NO_3$) + 0.1 mg·l^{-1} BAP + 0.01 mg·l^{-1} IBA
T_4	MS/2 + 0.1 mg·l^{-1} BAP + 0.01 mg·l^{-1} IBA
T_5	MS + 0.1 mg·l^{-1} BAP + 0.01 mg·l^{-1} IBA + 1.0 mg·l^{-1} GA_3 + 2.0 g·l^{-1} AC
T_6	MS/2 + 0.1 mg·l^{-1} BAP + 0.01 mg·l^{-1} IBA + 1.0 mg·l^{-1} GA_3 + 2.0 g·l^{-1} AC

[a] Gamborg *et al.* (1968).
[b] AC = activated charcoal.
[c] Gonçalves (1983).
[d] Indole-3-acetic acid.

thiamine·HCl to initiate roots. Cultures were maintained under darkness for the 1st wk, followed by 16/8 h light/dark photoperiod with light intensity of 1000 lux.

All experiments were conducted twice using a randomized design with number of replicates per treatment variable to the stage. Cultures (unless otherwise stated) were maintained under a 16/8 h light/dark photoperiod and 25 ± 2°C.

Results

At the end of 30 d, maximum shoot production occurred at 0.5 mg·l^{-1} BAP and 0.01 mg·l^{-1} IBA (Fig. 1). In this treatment, an average of 10 shoots developed per explant. Increasing BAP from 0.5 to 1.0 mg·l^{-1} decreased axillary shoot formation at both auxin concentrations. A similar pattern was observed when these shoots were subcultured for an additional 30 d (Fig. 2). In this subculture, shoot proliferation was greatest at 0.5 mg·l^{-1} BAP and 0.1 mg·l^{-1} IBA. Up to 35 shoots developed per explant within 60 d. However, at 0.5 mg·l^{-1} BAP, no differences were observed in axillary shoot number between the auxin concentrations.

During the multiplication stage, not all shoots elongated sufficiently to be rooted. These shoots were then transferred into elongation media. Shoot elongation was greatest when cultured on half-strength MS medium containing 0.1 mg·l^{-1} BAP

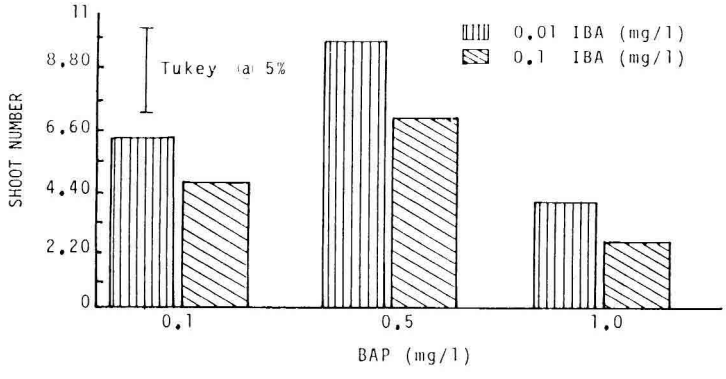

Fig. 1. Influence of BAP and IBA levels on shoot proliferation of *E. dunnii* after 30 d.

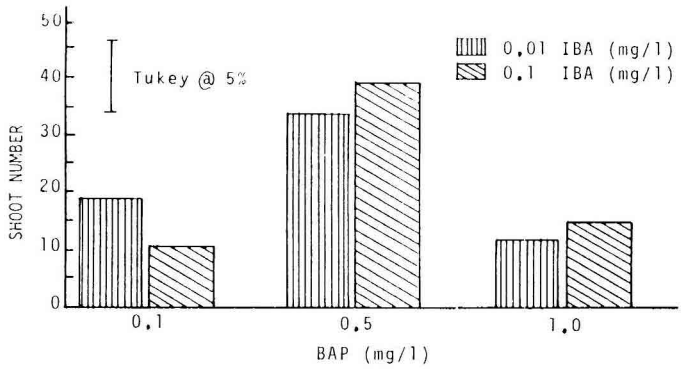

Fig. 2. Influence of BAP and IBA levels on shoot proliferation of *E. dunnii* after 60 d.

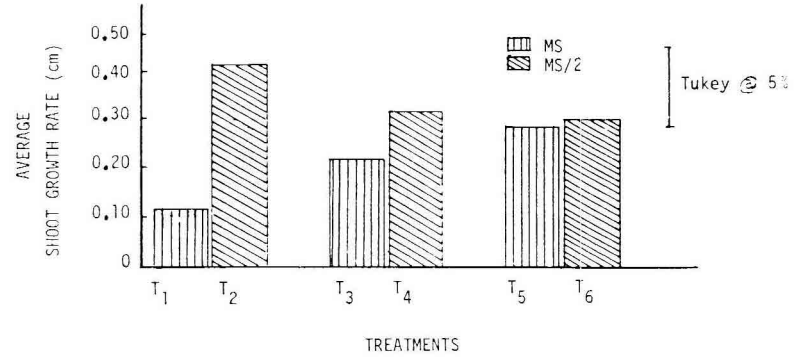

Fig. 3. Influence of MS salt concentrations and GA_3 levels on shoot growth of *E. dunnii* after 15 d.

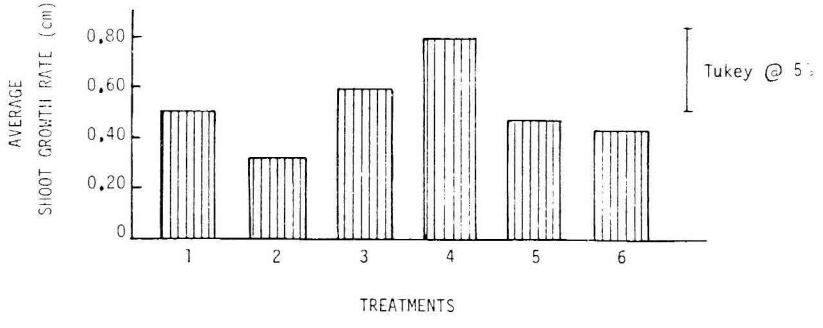

Fig. 4. Influence of different media on shoot elongation of *E. dunnii*.

and 0.01 mg·l^{-1} IBA. Additions of GA_3, at both concentrations, did not induce as much growth as culturing on half-strength MS medium alone. Using this medium, an increase of 0.4 cm in length was obtained within 15 d. Nevertheless, shoots were longer when cultured on medium containing GA_3, compared to those grown on MS medium alone (Fig. 3). Similarly, additions of activated charcoal and GA_3, nitrogen or using different salt formulations and concentrations were not as effective on shoot elongation as half-strength MS medium (Fig. 4).

Roots initiated on elongated shoots on 1/4 MS salts containing IBA. A higher percentage of shoots formed roots at 1.0 mg·l^{-1} IBA than at other concentrations.

Discussion and Conclusion

Large-scale micropropagation of *E. dunnii* is feasible due to its high multiplication rates. Maximum shoot proliferation was achieved on MS medium containing 0.5 mg·l^{-1} BAP and 0.01 mg·l^{-1} IBA. Up to 35 shoots developed per explant within 60 d. Shoot length obtained during the multiplication stage was not sufficient for root initiation. Therefore, an elongation stage was needed.

At the elongation stage, reducing MS salts to half caused the greatest increase in shoot growth. When GA_3 was added to a half- or full-strength MS medium, shoot elongation did not improve significantly. Also, shoots grown on medium containing GA_3 were less vigorous and the leaves became lanceolate instead of round, as were those grown without GA_3. This was observed in individual and cluster-inoculated shoots.

The addition of activated charcoal to a medium containing GA_3 and other growth regulators was reported to cause elongation and to recover the morphological characteristics of the species (Franclet and Boulay, 1982). In the present study, the addition of activated charcoal and growth regulators reduced shoot elongation and caused leaf browning. An even greater reduction of shoot growth was also observed when shoots were cultured on Gonçalves' medium containing 0.5 mg·l^{-1} BAP and 1.0 mg·l^{-1} IAA.

Rooting occurred on 58% of the elongated shoots at 1.0 mg·l^{-1} IBA. This low rooting percentage and the poor quality of

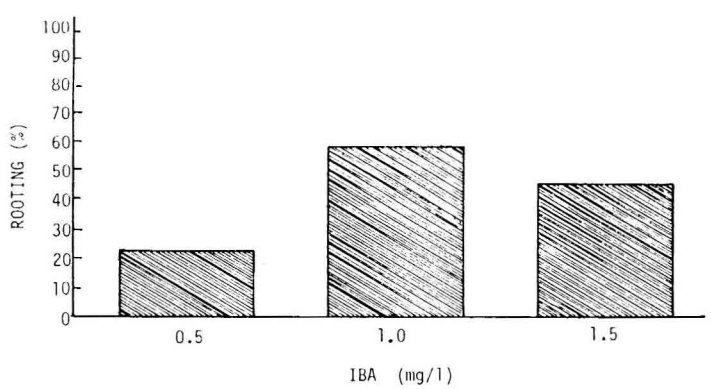

Fig. 5. Rooting of *E. dunnii* shoots as a function of IBA levels.

the root system are the main factors limiting the successful establishment of *E. dunnii*.

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