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A micropropagation system for *Eucalyptus dunnii* x *Eucalyptus* sp.

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Introduction

A *Eucalyptus dunnii* hybrid was first noticed during seedling production in 1984. This spontaneous hybrid originated from a seed production area of *E. dunnii* and its characteristics, such as leaf color, shape, wax content and stem color were distinct from the type. Although the hybrid growth potential cannot yet be determined, early tree selection revealed 2 important features: 1) tolerance to frost comparable to that of the parental species, which is a major factor to consider in establishing *Eucalyptus* in the southern region of Brazil; 2) the hybrid can be easily propagated by stem cuttings, as opposed to *E. dunnii*. While this method is successful, *in vitro* propagation would reduce propagation stock requirements and would also be a rapid method for mass clonal propagation. The development of a system to micropropagate *Eucalyptus dunnii* x *Eucalyptus* sp. was the objective of this study.

Materials and Methods

Nodal segments of *E. dunnii* x *Eucalyptus* sp. were collected from rooted cuttings actively growing in an open greenhouse. After leaf removal, segments were surface-sterilized in 1% NaClO plus 2 drops of Tween-20/100 ml for 15 min followed by 3 rinses in autoclaved distilled and deionized water. The basal medium for initiation consisted of Murashige and Skoog (1962) salts plus vitamins as described by Gamborg and Wetter (1975), 3% sucrose with BAP (benzylaminopurine) and IBA (indole butyric acid) at 0.1 $mg \cdot l^{-1}$. The pH of the medium was adjusted to 5.7 prior to the addition of 6 $g \cdot l^{-1}$ of Bacto-agar. At the multiplication stage, the medium combinations of BAP and KIN (kinetin) (0.1, 0.5 and 1.0 $mg \cdot l^{-1}$) and IBA (0.01, 0.05 and 0.1 $mg \cdot l^{-1}$) were used. For the shoot elongation experiment, single shoots or clusters of shoots were placed on a medium containing the treatments shown in Table I. Cultures in each treatment were incubated under 16 h/8 h light/dark photoperiod (around 1200 lux), at 25°C. After 60 d in culture, shoot height was recorded. Roots were initiated on Knop (1985) or on White (1954) medium supplemented with IBA (0.5, 1.0 and 1.5 $mg \cdot l^{-1}$) and riboflavin (0 and 5 $g \cdot l^{-1}$). All experiments (unless otherwise stated) were completely randomized with a variable number of replicates in each stage. Prior to transfer to greenhouse conditions, the plantlets in the culture vessels were established in 50 cm^3 polypropylene containers with a mixture of vermiculite and sterile soil (1:2.3, v/v) under high humidity in the greenhouse. After 2 wk in this environment, the humidity was gradually reduced to ambient conditions.

Table I. Shoot elongation treatments.

T1 = MS
T2 = T1 + activated charcoal (AC) (5 g·l^{-1})
T3 = T2 + GA_3 (0.1 mg·l^{-1})
T4 = T3 + BAP (0.01 mg·l^{-1})
T5 = T4 + IBA (0.01 mg·l^{-1})
T6 = T3 + BAP (0.05 mg·l^{-1}) + IBA (0.05 mg·l^{-1})
T7 = T3 + BAP (0.1 mg·l^{-1}) + IBA (0.05 mg·l^{-1})

Table II. The influence of BAP, KIN and IBA levels on the shoot multiplication rate per culture of *E. dunnii* x *Eucalyptus* sp.

IBA (mg·l^{-1})	*BAP (mg·l^{-1})*			*KIN (mg·l^{-1})*		
	0.1	*0.5*	*1.0*	*0.1*	*0.5*	*1.0*
0.01	1.1	2.13	1.0	1.0	1.0	1.0
0.05	1.3	8.0	1.0	1.0	6.0	2.0
0.1	2.0	1.9	1.0	2.0	1.0	2.5

Results

Optimum shoot multiplication rate was obtained on the medium containing 0.5 mg·l^{-1} BAP and 0.05 mg·l^{-1} IBA (Table II). A rate of 8 shoots per explant was developed within 30 d. When KIN was used, multiplication rates were lower than those with BAP. Nevertheless, the same optimal levels as those determined with BAP were found with KIN (6:1) (Table II).

Shoot elongation was influenced by the treatment (Figs. 1 and 2). When only MS medium was used, shoot elongation did not occur (Fig. 1). The single addition of AC, GA_3, BAP or IBA or both at 0.01 mg·l^{-1} to the MS medium, increased shoot length. Although no significant difference was observed among these treatments, single additions were not as effective in promoting shoot development as they were when combined (T6). On the other hand, increasing the BAP level (T7) resulted in shoots shorter than those subjected to T6. Shoots cultured in the dark were more elongated than those grown under 16 h light photoperiod (Fig. 1). Inoculation procedures affected shoot growth only in the treatments in which all promoters were used (Fig. 2). Under those treatments (T6 and T7), single shoot inoculation produced longer shoots compared to clusters. Despite this treatment, shoots in cluster gave a higher yield of less developed shoots, but these were still of adequate size for rooting.

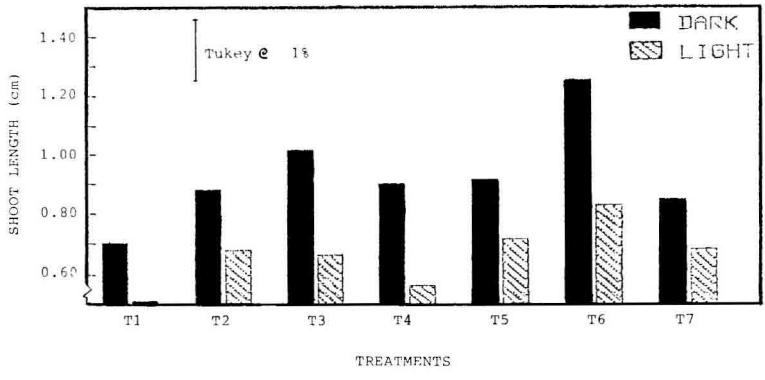

Fig. 1. The influence of several growth promoting substances on the shoot development of *E. dunnii* x *Eucalyptus* sp.

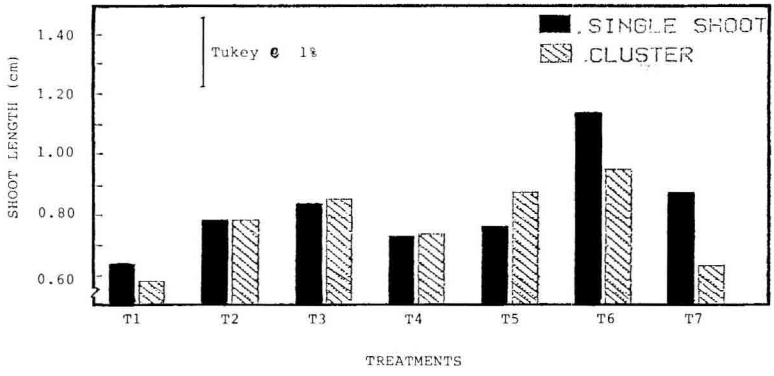

Fig. 2. The influence of the inoculation method on shoot development of *E. dunnii* x *Eucalyptus* sp. in each treatment group.

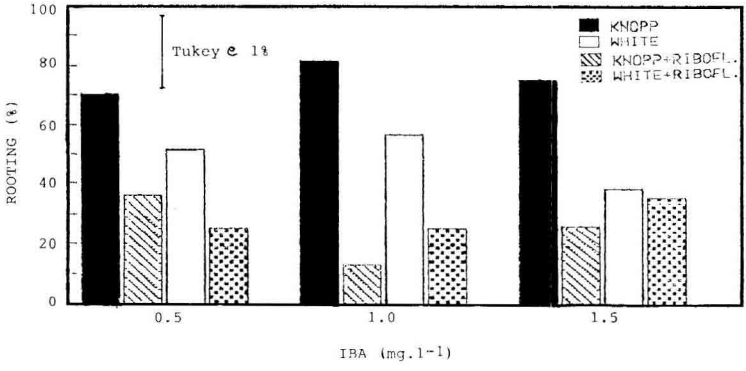

Fig. 3. Rooting percentage of *E. dunnii* x *Eucalyptus* sp. as affected by 2 media, 3 levels of IBA in the presence and the absence of riboflavin.

Rooting occurred in all treatments but it was greatest on Knop medium with 1.0 $mg \cdot l^{-1}$ IBA (Fig. 3). On this medium, rooting was 82% compared to 60% observed on White medium at the same IBA concentration. Rooting was also stimulated by an IBA concentration up to 1.0 $mg \cdot l^{-1}$. As the concentration of IBA increased beyond 1.0 $mg \cdot l^{-1}$, root formation decreased. Similarly, rooting was also reduced when riboflavin (5.0 $mg \cdot l^{-1}$) was included in the medium. Survival of regenerated plantlets was 90% after 2 wk in the greenhouse under high humidity.

Discussion and Conclusion

Micropropagation is a viable system for mass propagation of *E. dunnii* x *Eucalyptus* sp. High shoot multiplication rates were obtained with 0.5 $mg \cdot l^{-1}$ BAP or KIN, combined with 0.05 $mg \cdot l^{-1}$ IBA. In this

treatment combination, BAP was more effective in promoting shoot proliferation than KIN was. At the elongation stage, the addition of AC, GA_3, BAP (0.05 $mg \cdot l^{-1}$) and IBA (0.05 $mg \cdot l^{-1}$) in the MS medium produced the best shoot growth. Although incubation in the dark increased shoot length, light incubated shoots grew more readily than dark incubated ones. Similarly, elongation was better when shoots were inoculated on medium individually as opposed to clusters. Inoculation of clusters, however, yielded many elongated shoots with an adequate root system. The best percentage of rooting was obtained on Knop medium with IBA at 1.0 $m \cdot l^{-1}$. Shoots rooted better on Knop than White medium, regardless of the IBA level. Addition of riboflavin to the rooting medium has been reported to improve root formation through the modification of root morphology. Fewer and longer roots were produced in the presence of riboflavin and these roots developed towards the medium, whereas those initiated in the absence of riboflavin grew on the surface of the medium (De Fossard, 1981). In the present study, the addition of riboflavin reduced the rooting capacity of the explants and promoted the development of an undesireable root system with fewer roots, which were more brittle than those without riboflavin in all treatment combinations. The success of the establishment of the regenerated plantlets (90% survival) under greenhouse conditions indicates, once more, the potential of this method to propagate *E. dunnii* x *Eucalyptus* sp. hybrids.

References

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