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Micropropagation of *Leucaena Leucocephala* ([Lam] de Wit)

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Summary

Auxillary buds of *Leucaena leucocephala* were cultured in vitro on a modified Murashige and Skoog basal media (MS). Cultures utilizing MS media or MS supplemented with IAA produced single shoots and normal root development. MS supplemented with BAP + NAA produced multiple shoots and no roots; and after transfer to MS + IBA + K, abnormal roots. High levels of 2,4-D (3 mg/l) prevented both shoot and root formation. Survival of plantlets after transfer to soil was significantly lower in those cultured in MS supplemented with growth regulators. Plantlets cultured on MS produced the greatest number of normal transplants and were not significantly different from those obtained by standard horticultural practices.

Introduction

Members of genus *Leucaena* have received much attention in the past 5-10 years as multi-use leguminous trees with potential for biomass, forage, and timber production (Hill, 1971; National Research Council, 1984). In South Texas, three species of Leucaena (*L.*

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leucocephala, L. pulverulenta, and *L. retusa*) have become naturalized and may offer some potential as a forage resource for semi-arid rangelands.

While some problems are associated with the genus, sufficient genetic variability appears to exist for future improvements. This will potentially involve interspecific hybridization; however, while interspecific crosses are possible (Sorenson and Brewbaker, 1984), the progeny are generally poor seed producers or partially sterile. Therefore, a rapid and efficient technique for the propagation of superior germplasm is needed.

Procedures

Two-month old greenhouse-grown plants of *Leucaena leucocephala* (cv K 28 and K 67) were used as explant sources. Stem sections about 10-20 mm long containing single auxillary buds were removed, washed with detergent, washed 1 minute in 70 percent alcohol, rinsed in sterile distilled water, and then placed in a 20 percent v/v solution of household bleach (1 percent NaOCl) for 25 minutes in a laminar flow hood. After several rinses in sterile water, material was left to air-dry for 15 minutes before transfer.

Media were prepared using standard Murashige-Skoog salt solutions (Murashige and Skoog, 1962) to which 20 g/l sucrose and 7 g/l agar (Difco Bacto Agar) were added. Four treatments were used:

1. MS: Basal media + O growth regulators.
2. MS + 2,4-D: Basal media + 3 mg/l 2,4-Dicholorphenoxyacetic acid.
3. MS + IAA: Basal media + 3 mg/l Indole-3-acetic acid.
4. MS + BAP + NAA: Basal media + 3 mg/l 6-Benzylaminopurine and 0.6 mg/l-Naphthaleneacetic acid.

Fifty replicates were used for each genotype-treatment combination. Sections were transferred aseptically to 20 x 150 mm culture tubes containing 20 ml of media and incubated at 30° ± 1°C with a 16-hour photoperiod (1.4J cm^{-2} sec^{-1}) for 6 weeks. Cultures were then evaluated for shoot and root production.

Cultures producing rooted plantlets were inoculated with rhizobia (Nitrogen-Leucaena Spec. 1) and transferred to a soil medium. Shoots from MS + BAP + NAA were transferred to a rooting medium as described by Goyal et al. (1985) containing 3 mg/l Indole-3-butyric acid (IBA) and 0.05 mg/l Kinetin (K). Rooted plantlets from this medium were also transferred to soil. Surviving transplants were counted after 6 weeks.

Results and Discussion

A high percentage of shoot production was observed in all treatment groups with the exception of Treatment 2 (3 mg/l 2,4-d), in which few cultures exhibited shoots (Table 1). A single shoot and root were produced from explants on Treatments 1 (MS) and 3 (MS + IAA). Growth in both treatments appeared normal, with bud break occurring within 7 days and rooting within 21 days. The apparent ineffectiveness of IAA to elicit a response may have been due to the instability of IAA (Dunlap and Kresovich, pers. comm.).

TABLE 1. COMPARISON OF FREQUENCIES OF ROOT AND SHOOT FORMATION OF CULTURED AXILLARY BUDS OF TWO VARIETIES OF LEUCAENA LEUCOCEPHALA IN DIFFERENT MEDIA TYPES

Media	Genotype	Explants producing shoots percent	Means/number shoots/explant	Explants producing roots percent
MS	K 67	100	1	37
MS	K 28	91	1	46
MS + 2, 4-D	K 67	3	1	0
MS + 2, 4-D	K 28	5	1	0
MS + IAA	K 67	100	1	34
MS + IAA	K 28	92	1	37
MS + BAP + NAA	K 67	67	5.9	0 (*44)
MS + BAP + NAA	K 28	80	6.3	0 (*46)

*After transfer to rooting medial, MS + IBA mg/1 + K 0.05 mg/1.

Multiple shoots were formed with Treatment 4 (MS + NAA + BAP), as 57 shoots were produced per bud (Table 1). Histological examination of the axillary buds has indicated that multiple meristems are located in this region (Hussey and Boogher, unpubl.), therefore multiple shoot production is a function of alleviating apical dominance versus *de novo* production of new shoots.

Transfer of shoots from Treatment 4 to rooting medium resulted in the production of highly branched and thickened roots. While the rooting success in this treatment was similar to that observed for Treatments 1 and 3, ultimate plant survival was greatly reduced (Table 2).

The total number of intact plants recovered from soil was greatest for Treatment 1 (Table 2). While Treatment 3 produced shoots and roots which appeared normal, fewer transplants were recovered.

With the high costs associated with in vitro propagation, and the relatively poor rooting response; it appears that in vitro propagation offers little advantage over traditional horticultural propagation techniques. The direct rooting of vegetative cuttings in a mist bed, has been demonstrated to be more successful than in vitro methods (Hu and Liu, 1981).

TABLE 2. PLANTLETS RECOVERED FROM DIFFERENT MEDIA TREATMENTS AFTER TRANSFER TO SOIL

Media	Genotype	Percent Recovered
MS	K 28	46.2
MS	K 67	41.7
MS + IAA	K 28	22.2
MS + IAA	K67	i2.5
MS + IBA + K	K 28	7.7
MS + IBA + K	K 67	0

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