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MICROPROPAGATION OF *MELIA AZEDARACH* FROM MATURE TISSUE

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Abstract

Proliferating shoot cultures were established from nodal bud segments excised from a mature *Melia azedarach* tree. Highest proliferation rate was observed in Murashige & Skoog medium supplemented with BAP (1.0 mg/l). Rooting of shoots *in vitro* was achieved in MS medium containing 1.0-2.0 mg/l of IBA. Phenotype of the plantlets was similar to the plant from which the explants were obtained.

Introduction

In vitro micropropagation has now enabled a much broader range of species to be cloned rapidly under highly controlled conditions. The potential was first realised with the reports of Boxus (1974) and Murashige *et al.*, (1974). The method of axillary shoot enhancement has since been applied to many woody dicotyledons. These include fruit trees (Jones *et al.*, 1977; Mathews & Rangan, 1979; Lane, 1978, 1979), chestnut (Rodriguez, 1982; Vietez *et al.*, 1983), teak (Gupta *et al.*, 1982) and conifers (Bonga, 1981). *Melia azedarach* (Dhrek) of the family Meliaceae is a fast growing tree used for timber, firewood, forestry and soil erosion control. Clonal propagation is essential to establish a uniform and high quality plantation. This paper presents procedures for axillary shoot proliferation from field grown mature mother plant.

Material and Methods

Axillary bud explant (5-10 mm) were obtained from a 40 year old *Melia azedarach* tree growing in the P.C.S.I.R. labs. Complex, Lahore. Buds were surface sterilized for 15 minutes in 0.1% $HgCl_2$ containing few drops of wetting agent (Tween 20). After three rinses in sterile distilled water buds were transferred on culture medium containing sucrose 30 g/l which was gelled with 7 g/l Difco agar. pH of the medium was adjusted at 5.7 by the addition of HCl prior to the addition of agar and sterilized @ 15 psi for 15 min. After inoculation of the buds the cultures were incubated at $26 \pm 1^{o}C$ under a 16 h photoperiod at 3k Lux of cool white light produced from fluorescent tubes. To establish axillary bud cultures *in vitro* for shoot growth and proliferation, sterilized axillary bud explants were incubated on MS medium containing the following combinations of PGRs.

A) Kinetin (KIN) at 0.05, 0.1 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0. mg/l.
B) Benzylaminopurine (BAP) at 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 mg/l.
C) Iso-pentenyl adeninie (2ip) at 0.1, 1.0, 2.0, 3.0 and 4.0 mg/l.
D) GA_3 at 0.05, 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l.
E) BAP at 1.0 and GA_3 at 0.1, 0.5, 1.0 and 2.0 mg/l.
F) Napthalene acetic acid (NAA) at 0.1, 2.0, 4.0 and BAP at 0.1, 1.0, 2.0 and 4.0 mg/l.

Proliferated shoots, 2.0-2.5 cm long, were used in experiments to establish the best treatment for rooting under the following treatments:
A) MS salts at full strength and half strength and containing no PGR.
B) MS medium containing Indole acetic acid (IAA) and Indole butyric acid (IBA) at 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l.
C) MS salts in the medium at half strength and containing IBA at 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l.
D) Transference of cultured shoots to auxin-free medium when roots had formed.

Results and Discussion

Shoot growth and proliferation were observed within 15-20 days of establishing the cultures from axillary bud explants taken directly from field grown tree. Shoot proliferation was obtained on media containing BAP (Table 1) but not on those supplemented with KIN alone (Table 2). Shoot proliferation was observed on media

Table 1. Effect of BAP on Shoot Growth and Proliferation of *Melia azedarach*.

BAP (mg/l)	Mean shoot Number Per Culture	Mean shoot Length per Culture (cm)	Callusing
0.05	0.71	2.80	-
0.10	0.71	3.00	-
0.50	8.00	3.80	-
1.00	12.00	4.10	+
1.50	7.80	4.30	+
2.00	1.28	3.90	+
2.50	0.85	2.90	+ +
3.00	0.85	2.20	+ +
4.00	0.42	2.70	+

Data is average of 7 replicates.

Table 2. Effect of Kinetin on Shoot Growth of *Melia azedarach* and its Proliferation.

KIN (mg/l)	Mean Shoot Number per Culture	Mean Shoot Length Per Culture (cm)	Callusing
0.05	0.42	2.90	-
0.10	0.57	3.10	-
0.50	0.57	3.05	-
1.00	0.83	3.58	-
1.50	1.14	4.10	-
2.00	1.42	4.25	-
2.50	1.00	3.90	-
3.00	0.83	1.60	+
4.00	0.42	1.20	+

Data is average of 7 replicates.

containing 0.5-1.5 mg/l BAP, with highest proliferation rate at 1.0 mg/l. Following subculture of shoots on to fresh medium containing 1.0 mg/l BAP as many as 12-15 shoots, with numerous small leaves, per culture were produced (Fig. 1). No callogenesis was observed in explants when 0.05-0.5 mg/l BAP was used. Some soft callus was produced at the base of most explants at higher (1.0-3.0 mg/l) concentration of BAP. The callus dried out later on and did not seem to interfere with shoot proliferation. The inclusion of cytokinin into nutrient media generally has the marked effect of promoting the outgrowth of axillaries in cultured buds (Hussey, 1986). There have been numerous reports where BAP has been used for axillary shoot proliferation. These include *Gladiolus* (Hussey, 1977), sugarbeet (Hussey & Hepher, 1978), *Castanea sativa* (Rodriguez, 1982).

Axillary bud activation was observed in all media tested that contained Kinetin (0.05-4.0 mg/l) but shoot proliferation was not observed even after three subcultures. Multiple shoot formation was observed in some cultures containing 1.5-2.0 mg/l of KIN. Callusing was not observed at lower (0.05-2.5 mg/l) concentrations. Slight callusing was however observed at higher (3.0-4.0) mg/l) of KIN. 2iP (0.1-4.0 mg/l) did not show any shoot growth. Shoots formed in some cultures were thin or delicate and lacked any photosynthetic capability. Similarly GA_3 when used alone (0.1-4.0 mg/l) or with BAP (1.0 mg/l) did not induce any shoot growth or proliferation (Fig. 2).

Rapidly proliferating soft green calli were produced at the base of explants when GA_3 (0.1-4.0 mg/l) alone was used. Quality and quantity of callus improved with increasing concentration of GA_3. Callus growth at the base of explants is undesirable as it will be utilizing nutrients from the medium and there is also the possibility of

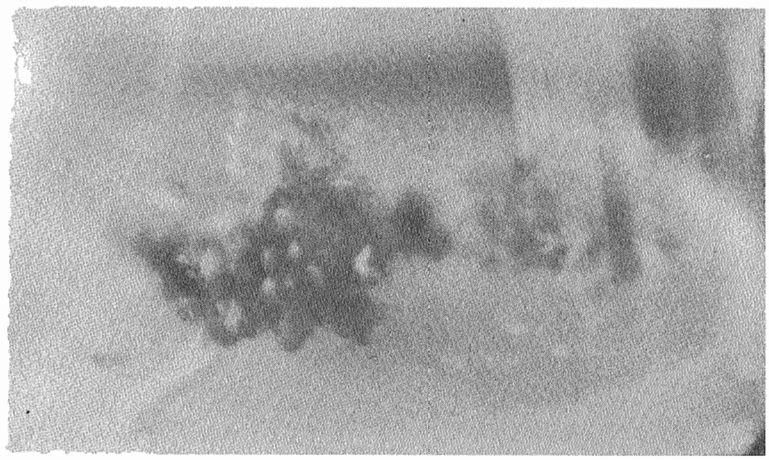

Fig 1. Shoot proliferation of *Melia azedarach* on medium containing 1.0 mg/l of BAP.

Fig 2. Axillary bud activation of *Melia azedarach* on medium containing 3.0 mg/l KIN.

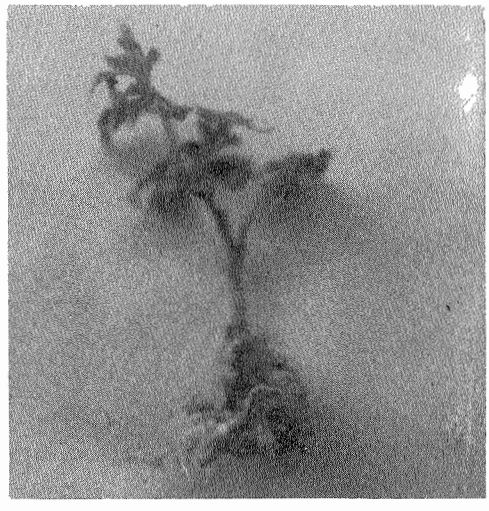

Fig 3. Rooting of cultured *Melia* shoots.

Table 3. Rooting in Cultured shoots of *Melia azedarach*.

Treatments	IBA (mg/l)	% of shoots with roots	Mean root number per shoot	Mean root length per shoot (cm)
MSXI	0.1	0	0	0
	0.5	0	0	0
	1.0	80	3.25	3.00
	2.0	50	2.8	2.60
	4.0	20	2.5	1.75
MSX0.5	1.0	80	3.5	3.0
	2.0	60	2.3	2.8
Transfer to auxin free medium				
MSX1	1.0	80	3.37	3.87
	2.0	50	2.80	3.5

Date is average of 10 replicates.

All results were recorded after 30 days.

Shifted to auxinless medium after 14 days.

inhibitors being produced as the callus senesces (Eliason, 1981). The presence of callus may reduce nutrient uptake by the shoots (Barghchi & Alderson 1983). Use of NAA (0.1-4.0 mg/l) + BAP (0.1-4.0 mg/l) also promoted callusing in the explants and no shoot growth was observed.

Cultured *Melia* shoots (2.0-2.5 cm) did not produce any roots in medium containing MS salts at full strength and half strength and no PGR (Fig. 3). Rooting in cultured shoot was observed in 13-15 days in medium containing IBA (1.0-2.0 mg/l). However best rooting was achieved at 1.0 mg/l of IBA (Table 3). IBA has effectively been used for *in vitro* rooting in many plant species, such as *Robinia pseudoacacia* (Barghchi, 1987), *Alnus cordata* (Barghchi, 1988), *Pseudotsuga menziesii* (Cheng, 1975), *Pinus palustris* (Sommer *et al.*, 1975).

IAA did not induce any rooting in shoots. No significant difference was observed in rooting pattern when IBA (0.1-4.0 mg/l) was used with full strength MS medium or at half strength (Table 3). Root length improved when shoots with induced roots were transferred to auxinless medium. Root initiation and root growth and development are two separate processes. Root development conditions can be inhibitory to the initiation of further root primordia (Lane, 1978; Mullins *et al.*, 1979). All the plantlets so produced looked similar to the plant from which explants were made.

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