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Plants obtained from the Khair tree (*Acacia catechu* Willd.) using mature nodal segments

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Abstract An in vitro method for obtaining plants of *Acacia catechu* has been developed using nodal explants from mature 'elite' trees growing in the field. Maximum shoot bud development (eight to ten) from a single explant was achieved on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP) (4.0 mg/l) and α-naphthaleneacetic acid (0.5 mg/l). Addition of adenine sulphate (25.0 mg/l), ascorbic acid (20.0 mg/l) and glutamine (150.0 mg/l) to the medium was found beneficial for maximum shoot bud induction. The shoot buds developed into healthy and sturdy shoots on MS medium containing BAP and kinetin at 1.0 mg/l. Excised shoots were rooted on 1/4-strength MS medium with indole-3-acetic acid at 3.0 mg/l and 1.5% sucrose to obtain complete plants.

Key words *Acacia catechu* · Clonal propagation · Mature nodal explants

Abbreviations *BAP* 6-Benzylaminopurine · *IAA* Indole-3-acetic acid · *IBA* Indole-3-butyric acid · *Kn* 6-Furfurylaminopurine · *NAA* α-Naphthaleneacetic acid · *MS* Murashige and Skoog medium

Introduction

The Khair or Cutch tree (*Acacia catechu* Willd.) is a multipurpose leguminous tree of the Indian desert providing katha, tannin, kheersal, fodder and wood. It is used for afforestation and reclamation due to its easy adaptability and rapid growth rate even under degraded and wasteland conditions (Anonymous 1985). Vegetative propagation is generally done by coppicing which depends upon the age and vigour of the tree and season of cutting, and is generally an irregular procedure.

Micropropagation is an alternative to the conventional method of vegetative propagation with the objective of enhancing the rate of multiplication. Initiation of tissue culture (Rout et al. 1995) resulted in somatic embryogenesis and plant regeneration from callus cultures derived from immature cotyledons of *A. catechu*. However, improved technology for vegetative propagation of mature 'elite' trees of *A. catechu* is highly desirable. The present communication describes a method for rapid multiplication of *A. catechu* from mature explants.

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Materials and methods

Nodal segments of *A. catechu* Willd. were collected from mature 'elite' trees and used as explants. These were kept under running tap water for 20–30 min, washed thoroughly with 2% commercial detergent (Extran solution), rinsed in sterile distilled water and pretreated with chilled, sterile antioxidant solution [ascorbic acid (100 mg/l), citric acid (100 mg/l), polyvinylpyrrolidone (100 mg/l)] for 1 h. Subsequently, the explants were surface-sterilized with 0.1% mercuric chloride followed by several rinses in sterile distilled water. The sterilized nodal explants were transferred to Murashige and Skoog (1962) (MS) medium supplemented with growth regulators at various concentrations and combinations: 6-benzylaminopurine (BAP) and/or kinetin (Kn) at 1.0–6.0 mg/l, indole-3-acetic acid (IAA)/α-naphthaleneacetic acid (NAA) at 0.1–1.0 mg/l. Various additives – adenine sulphate, ascorbic acid and glutamine – at different concentrations were also added to the medium. The pH of the media was adjusted to 5.8 before autoclaving at 15 psi for 20 min. The cultures were incubated at 28±2°C under a 16-h photoperiod, 40 $\mu E\ m^{-2}\ s^{-1}$ light intensity and 50–60% relative humidity for 4 weeks. Shoots 3–4 cm long were excised for rooting. For rooting, 1/4-, 1/2- and full-strength MS medium supplemented with IAA, indole-3-butyric acid (IBA) or NAA at different concentrations (1.0–6.0 mg/l) was used. All experiments were repeated twice and six replicates per treatment were taken. The data have been analysed using Student's t-statistics at 5% significance.

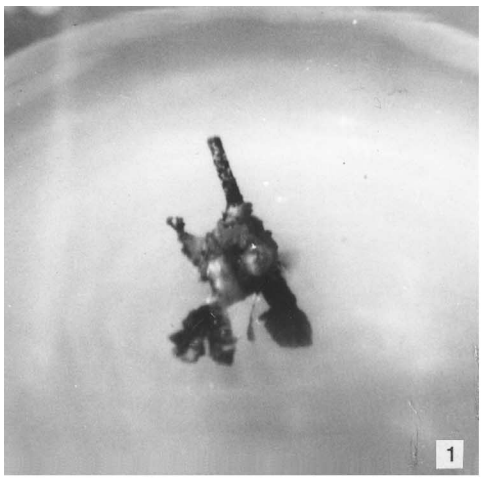

Fig. 1 Shoot bud induction from a nodal shoot segment on MS medium + BAP (4.0 mg/l)+NAA (0.5 mg/l)+ascorbic acid (20.0 mg/l), adenine sulphate (25.0 mg/l) and glutamine (150.0 mg/l) (×4)

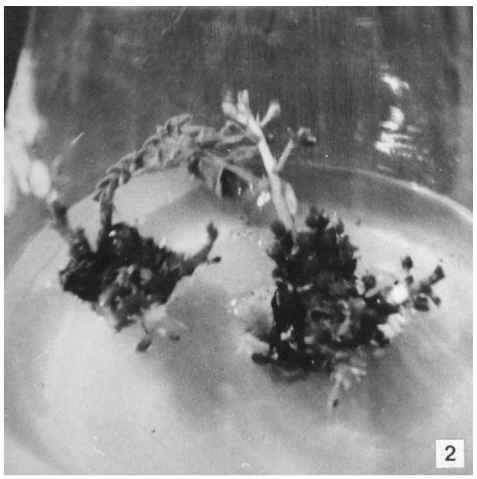

Fig. 2 Shoot bud proliferation on medium described in Fig. 1 (×4)

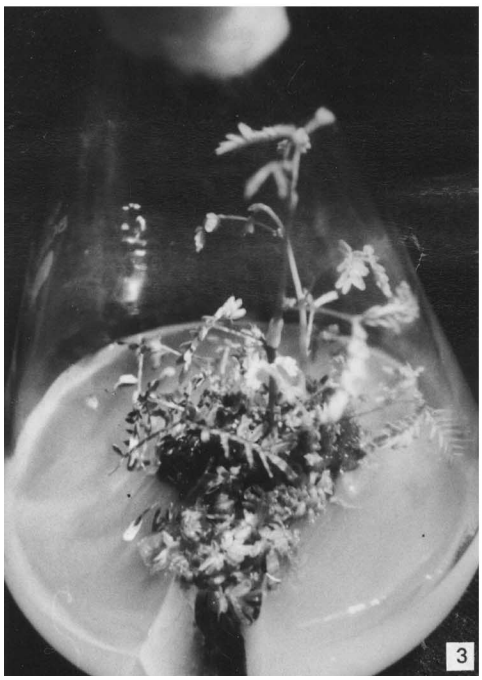

Fig. 3 Shoot elongation on MS medium containing BAP and Kn (1.0 mg/l each) (×6)

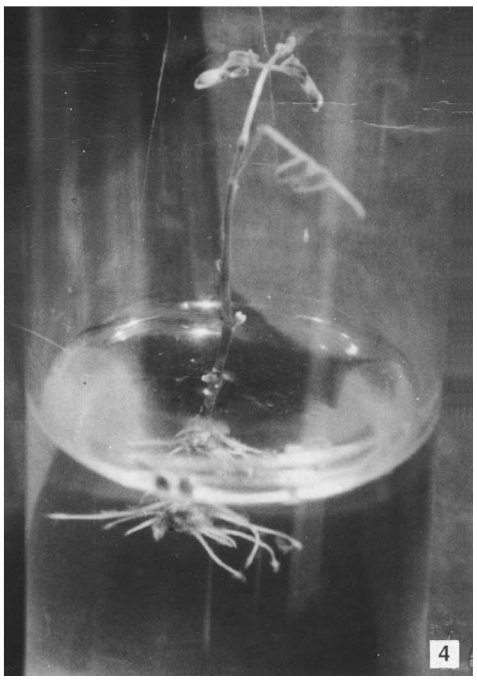

Fig. 4 A rooted shoot of *A. catechu* on 1/4 MS medium + IAA (3.0 mg/l) + sucrose (1.5%) (×6)

Results and discussion

Nodal shoot segments proved to be excellent explants for multiple shoot formation. Shoot buds emerged from pre-existing meristems or buds after 3–4 weeks of incubation (Fig. 1). The nodal explants inoculated on MS medium responded differently to various cytokinins singly or in combination (Table 1) and maximum shoot multiplication (8.66±1.71) per explant was achieved on MS medium fortified with BAP (4.0 mg/l) and NAA (0.5 mg/l) along with other additives – adenine sulphate (25.0 mg/l), ascorbic acid (20.0 mg/l) and glutamine (150.0 mg/l) (Fig. 2). When kinetin was used as the sole cytokinin (1.0–6.0 mg/l), a maximum of 4.16±0.79 shoot buds were produced at a concentration of 4.0 mg/l. Cytokinins (BAP and Kn) when used in combination (1.0–3.0 mg/l) in the medium did not further improve shoot bud induction.

Using BAP at 4.0 mg/l as optimum for maximum shoot bud induction, different auxins (IAA and NAA) at 0.5–2.0 mg/l were added to the medium along with BAP. Low levels of NAA (0.5 mg/l) had a synergistic effect on shoot bud induction while higher concentrations (1.0–2.0 mg/l) were not found beneficial, and there was callus formation at the base of the explant. The formation of axillary buds in response to MS medium+BAP (4.0 mg/l) and IAA (0.5–2.0 mg/l) showed that IAA at all levels was not effective in enhancing shoot bud production and, again, callus was formed at the base of the explant. Since the maximum number of shoot buds was initiated in the presence of BAP (4.0 mg/l) and NAA (0.5 mg/l), this medium was designated ‘shoot bud induction medium’. Similar results were obtained by Swamy et al. (1992) for

Table 1 Effect of cytokinins on axillary bud proliferation. Results are expressed as the mean±SE (95% confidence limits)

Cytokinin (mg/l)		Number of shoot buds per explant
Kn	BAP	
0	–	–
1.0	–	–
2.0	–	0.66±0.54
3.0	–	2.5 ±0.57
4.0	–	4.16±0.79
5.0	–	3.0 ±0.66
6.0	–	1.33±0.54
–	1.0	1.16±0.79
–	2.0	2.66±0.85
–	3.0	5.33±1.08
–	4.0	8.66±1.71
–	5.0	4.33±0.85
–	6.0	2.83±0.74
1.0	1.0	1.83±1.22
1.5	1.5	2.33±1.27
2.0	2.0	4.66±0.85
2.5	2.5	3.16±0.79
3.0	3.0	1.83±0.42

Dalbergia latifolia and Purohit et al. (1994) for *Wrightia tomentosa*.

The shoot buds produced on this induction medium did not develop further and remained stunted structures which failed to elongate on the same medium. Therefore, for elongation and development into healthy shoots, shoot buds were subcultured on MS medium containing both BAP and kinetin (1.0 mg/l each) (Fig. 3). BAP and kinetin in combination, though not ideal for shoot bud induction, were effective in converting shoot buds into sturdy and healthy shoots. Therefore, this medium was designated 'shoot bud elongation medium'. A similar observation was made by Barve and Mehta (1993) with *Commiphora wightii*.

The shoot segments of *A. catechu* exhibited browning of the explant and medium due to leaching of phenolics. Supplementing the medium with ascorbic acid at a concentration of 20.0 mg/l reduced the leaching. The addition of adenine sulphate (25.0 mg/l) showed a synergistic effect on shoot bud induction and shoot quality. A problem of leaf fall was mitigated to a large extent when glutamine (150.0 mg/l) was added to the medium.

Healthy and sturdy shoots (3–4 cm long) were transferred to rooting medium containing different concentrations of MS salts (1/4, 1/2 and full strength) and different auxins (IAA, IBA and NAA) applied singly at various concentrations. After 4 weeks of incubation, in response to IBA (1.0–6.0 mg/l), no rooting took place, but profuse callus formation occurred at the base of the shoots. Only 20–30% rooting was observed on NAA (5.0 mg/l). Better rooting (60–80%) was achieved on 1/4 MS medium supplemented with IAA (3.0 mg/l) (Fig. 4). In *A. nilotica* (Dewan et al. 1992) and *Woodfordia fruticosa* (Krishnan and Seeni 1994), rooting was also obtained on IAA-augmented medium.

There was intermittent callus formation at the junction of root and shoot. To reduce the amount of callus, the sucrose concentration was reduced to 1.5% from 3.0%. Sucrose at reduced concentrations (1.5%) has also been found optimal for other trees such as *Feronia limonia* (Purohit and Tak 1992) and *A. auriculiformis* (Das et al. 1993).

Thus, eight to ten true-to-type plantlets of *A. catechu* were raised in vitro from a single mature nodal explant and could be transferred to pots.

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