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Research note

Somatic embryogenesis and plant regeneration from callus culture of *Acacia catechu* - a multipurpose leguminous tree

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Abstract

Plant regeneration via somatic embryogenesis was achieved from callus derived from immature cotyledons of *Acacia catechu* Willd. on Woody Plant Medium (WPM) supplemented with 13.9 μM kinetin and 2.7 μM 1-naphthaleneacetic acid. The addition of 0.9–3.5 mM L-proline to the medium influenced development of somatic embryos and also promoted secondary somatic embryogenesis. The light-green somatic embryos germinated on half-strength MS medium supplemented with 2% (w/v) sucrose. Somatic embryos germinated into plantlets that were acclimatized in the greenhouse and subsequently transferred to the field.

Acacia catechu Willd., a tree legume (9–12 m high), is used for afforestation and reclamation due to its easy adaptability and rapid growth rate even under degraded and wasteland conditions in the tropics (Anonymous 1985). Somatic embryogenesis offers an efficient system for mass clonal propagation (Ammirato 1989; Attree & Fowke 1993). Somatic embryogenesis has been achieved in a number of angiosperms but success has been limited with woody species (Radojevic 1988; Ammirato 1989), which tend to be recalcitrant. Somatic embryogenesis has been achieved with several *Acacia* species (Jones *et al.* 1990; Zhao *et al.* 1990). We report for the first time *in vitro* regeneration via somatic embryogenesis of *Acacia catechu*.

Immature green pods of *Acacia catechu* (40 days after flowering) were collected from an elite tree growing in the Regional Plant Resource Centre at Bhubaneswar, India. The pods were treated with 2% (v/v) solution of the detergent 'Teepol' (Labolene) (Glindia, India) for 10 min, disinfested with 0.1% (w/v) aqueous mercuric chloride solution for 20 min. and rinsed three or four times with sterile double distilled water. The cotyledons without the embryonic axis were cut into smaller segments (5×8 mm), aseptically inoculated onto Murashige & Skoog (1962) (MS) or Woody Plant Medium (WPM, Lloyd & Mc Cown 1981) semisolid basal media with 3% (w/v) sucrose supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2, 4-D; 2.3-13.6 μM), indole-3-acetic acid (IAA; 2.9-17.1 μM), 1-naphthaleneacetic acid (NAA; 2.7-21.5 μM) and indole-3-butyric acid (IBA; 2.5-14.8 μM) alone or in combination with benzyladenine (BA; 2.2-13.3 μM) or kinetin (2.3-18.6 μM) for induction of callus. The pH of the media was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl solution prior to adding 0.8% (w/v) agar (Qualigen, India). To avoid browning of the medium, 18.4 μM ascorbic acid was added to the media during the initial culture. Routinely, 15 ml of molten medium was dispensed into a culture tube (25×150 mm) that was then plugged with non-absorbent cotton wrapped in one layer of cheese cloth. The culture tubes were steam sterilized at 121 °C at 104 kPa for 15 min. For callus induction the initial cultures were incubated at 25±2 °C either in the dark or in the light (16-h photoperiod, 50 μmol m^{-2} s^{-1}, Philips cool white fluorescent lamps) for 4 weeks. Callus was maintained on fresh medium with the same composition and the same conditions by subculturing at 4-week intervals. After the first subculture, the friable calli (200±20 mg

Table 1. Somatic embryogenesis in callus culture *Acacia catechu* on various media after 4 weeks of culture

WPM + growth regulators (μM)		Cultures forming embryogenic callus (%±S.E.)*	Number of somatic embryos per embryo genic culture (Mean±S.E.)*
Kinetin	NAA		
0	0	0	0
2.3	0	0	0
2.3	2.7	0	0
4.6	2.7	0	0
9.3	2.7	32.2±0.5	16.2±0.2
13.9	1.3	36.6±0.2	12.4±0.4
13.9	2.7	48.1±0.7	24.5±0.8
18.6	1.3	46.2±0.4	22.3±0.1
18.6	2.7	37.2±0.4	18.3±0.3

* Mean±standard error of two experiments; each treatment consisted of 20 replicates.

Table 2. Effect of induction medium (WPM + 13.9 μM kinetin + 2.7 μM NAA + 3% sucrose) supplemented with L-proline or L-glutamine on somatic embryogenesis of *Acacia catechu* after 4 weeks of culture.

Amino acids		Embryogenic callus per culture (%±S.E.)*	Number of somatic embryos per culture (Mean±S.E.)*
L-proline (mM)	L-glutamine (mM)		
0	0	48.4±0.7	23.4±0.2
0.9	0	52.8±0.1	26.5±0.7
1.7	0	64.6±0.2	38.2±0.2
2.6	0	72.2±0.8	63.5±0.4
3.5	0	96.7±0.5	102.8±0.8
4.3	0	32.6±0.2	20.2±0.3
0	0.7	50.2±0.4	38.2±0.5
0	1.4	46.4±0.8	22.4±0.4
0	2.1	34.4±0.5	12.4±0.4
0	2.7	26.6±0.4	8.9±0.9
0	3.4	8.2±0.4	2.2±0.2

* Mean±standard error of two experiments; each treatment consisted of 20 replicates.

fresh weight) were transferred to different basal nutrient media (MS or WPM) with 3% sucrose (w/v) supplemented with different concentrations and combinations of NAA (0.0–10.7 μM) and 2,4-D (0.0–9.0 μM) in combination with BA (0.0–13.3 μM) or kinetin (0.0–18.6 μM). The cultures were incubated at 25±2 °C and a 16-h photoperiod as noted above. For development and multiplication of somatic embryos, the embryogenic calli were subcultured on WPM + 13.9 μM kinetin + 2.7 μM NAA + 3% sucrose (w/v). The addition of L-proline (0.9–4.3 mM) or L-glutamine (0.7–3.4 mM) to the induction medium was investigated for possible effects on maintenance of long-term cultures and production of secondary somatic embryos. The cultures were examined after 4 weeks and morphological changes were recorded on the basis of visual observations. The effect of different treatments was quantified as the percentage of calli producing somatic embryos and the number of somatic embryos per cul-

ture. Each treatment had 20 replicates and the experiment was conducted twice.

To promote maturation and germination of somatic embryos, the embryos were transferred to germination medium (1/2 MS without growth regulators + 2% (w/v) sucrose) and incubated under the same culture conditions as noted earlier. Well-rooted plantlets (5–7 cm tall) were transferred to 5 cm earthen pots containing sterile garden soil:sand:cowdung (2:1:1) (v/v) and kept in a climate-controlled greenhouse for establishment.

Cotyledonary segments expanded on all media 8-10 days after inoculation. Callus formed on the entire surface and developed into a mass of callus after 16-18 days of culture depending on the auxin and cytokinin concentration in the culture media. Most of the media supplemented by either NAA or 2,4-D along with kinetin or BA stimulated rapid proliferation of callus. Inclusion of IBA or IAA alone or in combination with kinetin or BA in the culture medium slowed the rate of callus proliferation (data not shown). The most callus formation was obtained with WPM + 4.6 μM kinetin + 21.5 μM NAA + 3% sucrose and callus formed faster in the dark than in the 16-h photoperiod. The explants cultured on WPM containing 2,4-D produced friable yellowish-brown callus, whereas callus formed on WPM containing NAA was more compact and deep yellowish.

Somatic embryogenesis occurred only with WPM mineral salts supplemented with kinetin and NAA (Table 1). L-proline stimulated proliferation of embryogenic callus only at 1.7–3.5 mM (Table 2); L-glutamine did not stimulate embryogenic callus proliferation. The rate of proliferation of embryogenic calli continued when the calli were subcultured on fresh induction medium with a similar composition at 4-week intervals. Within 4 weeks of transfer to the induction medium, somatic embryos developed that were globular, heart-shaped and cotyledonary stage; 60 to 70% of the somatic embryos were morphologically normal showing distinct cotyledons and a radicle. The embryos were loosely attached to the mother callus with a short suspensor-like structure at the basal end that subsequently developed into a long tap-root system. Clusters of three or four somatic embryos also arose from the basal region of other embryos indicating secondary somatic embryogenesis (Maheswaran & Williams 1986; Ammirato 1987). Secondary somatic embryogenesis was achieved by repeatedly subculturing the embryogenic mass at 4-week intervals on fresh medium with L-proline; the cultures were maintained for 18 months without any loss in the regenerative capacity (Ammirato 1989; Radojevic 1988).

To promote germination, the isolated somatic embryos were inoculated onto a germination medium comprising half-strength MS basal medium + 2% (w/v) sucrose without growth regulators. Within 6-7 days after transfer, the greenish white somatic embryos germinated into complete plantlets with distinct shoot and root systems. The well-rooted plantlets were acclimatized in the climate-controlled greenhouse and subsequently established in the field.

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