Micropropagation of *Acacia chundra* (Roxb.) DC.

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ABSTRACT: An *in vitro* propagation of an economic leguminous tree, *Acacia chundra*, has been standardized. Induction of bud sprout was obtained from shoot tip and nodal explants derived from *in vitro* grown plants of *A. chundra* on the Murashige and Skoog (MS) basal medium supplemented with 6-benzylaminopurine (BA) (1.0 mg/l) and 20 mg/l adenine sulfate (Ads). The rate of multiplication was obtained on MS medium supplemented with 1.5 mg/l BA, 0.01 to 0.05 mg/l (indole-3-acetic acid) IAA and 50 mg/l Ads. The multiplication rate varied from 3 to 6 shoots depending on the growth regulators used. Excised shoots were rooted on half-strength MS basal salts supplemented with 0.25 mg/l indole-3-butyric acid (IBA) or IAA and 20 g/l (w/v) sucrose after 10 to 12 days of culture. The micropropagated plantlets have been acclimatized and successfully transferred to soil.

Keywords: growth regulators; *in vitro*; legume tree; micropropagation

Micropropagation of tree species offers a rapid means to produce clonal planting stock for afforestation, woody biomass production and conservation of elite germplasm. In general, the woody plants are difficult to regenerate under *in vitro* conditions but previously some successes were achieved in a few leguminous tree species (Tomar, Gupta 1988; Mittal et al. 1989; Raghava Swamy et al. 1992; Upreti, Dhar 1996; Monteuuis, Bon 2000; Nanda et al. 2004). There were some successful reports on development of plantlets from callus raised through seedling shoot tip cultures and from cotyledonary buds and nodal explant (Tomar, Gupta 1988; Raghava Swamy et al. 1992). Micropropagation of different *Acacia* species has been reported by various researchers (Dewan et al. 1992; Beck et al. 2000; Beck, Dunlop 2001; Vengadesan et al. 2002, 2003). *Acacia chundra* (Roxb.) DC. (family *Leguminosae*) is an economic multipurpose legume tree distributed in Indian Peninsula and mostly occurring in Maharashtra, Gujarat, Rajsthan and Tamilnadu on dry and rocky soils (Anonymous 1985). It plays an important role in the afforestation schemes. Besides, this tree is used as a source of pulpwood production, timber, fodder, gum and various medicinal properties. It is also called Cal Khair or red Kutch (http://www.cabicompendium.org). It is considered to be a good fodder tree and is extensively lopped to feed goats and cattle on the basis of crude protein, crude fibre and tannin contents. Different parts of the plant are used for medicinal purposes. The bark is reported to be useful in the treatment of snakebites. The trees yield a gum and are regarded as the best substitute for Gum Arabic (Anonymous 1985). Conventional methods of propagation of *A. chundra* are difficult due to recalcitrant seeds as well as short seed viability. *In vitro* culture techniques have a great potential for clonal propagation of superior genotypes of this legume species for reforestation programmes as well as utilization in paper industries. So far, there has been no report on *in vitro* shoot multiplication of *A. chundra*. This paper describes a successful protocol on *in vitro* clonal propagation of *A. chundra* from apical and nodal explants of *in vitro* grown plants.

MATERIALS AND METHODS

Explant source. Semi-mature seeds of *Acacia chundra* were collected from reserve forest and washed with 5% (v/v) detergent solution Teepol (Qualigen, Mumbai, India) for 10 min. Subsequently, it was surface sterilized with 0.1% (w/v) aqueous mercuric chloride solution for 20 min and washed 4–5 times in sterile distilled water. The seeds were soaked with sterile blotting paper and cultured aseptically in MS basal medium without growth regulator. After 15 days of seed germination, the shoot tips and nodal explants were used as explant source.

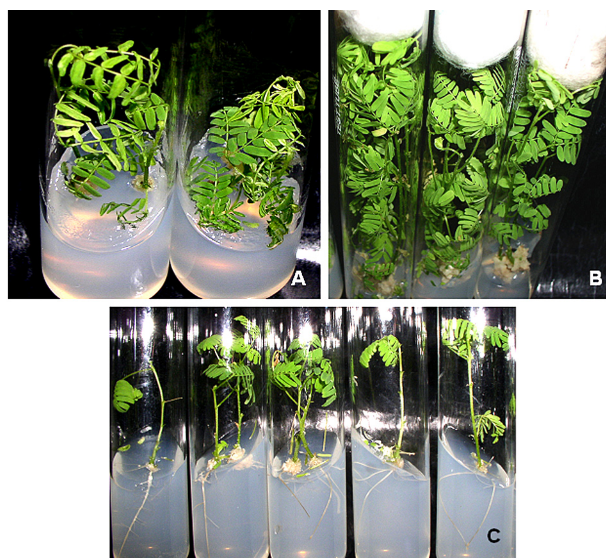

Fig. 1. Micropropagation of *Acacia chundra*: A – development of shoot from apical meristems of *A. chundra* on MS medium supplemented with 1.5 mg/l BA, 50 mg/l Ads, 0.25 mg/l IAA and 3% (w/v) sucrose after 2 weeks of culture. B – induction of multiple shoots from apical meristems of *A. chundra* on MS medium supplemented with 1.5 mg/l BA, 0.05 mg/l IAA, 50 mg/l Ads and 3% (w/v) sucrose after 4 weeks of culture. C – induction of rooting from microshoots of *A. chundra* on ½ MS medium supplemented with 0.25 mg/l IBA and 2% sucrose after 2 weeks of culture

Culture condition. Both shoot tips and nodal explants were cultured on Murashige and Skoog (1962) (MS) medium supplemented with different concentrations of 6-benzylaminopurine (BA: 0, 0.5, 1.0, 1.5 and 2.0 mg/l), kinetin (Kn: 0, 0.5, 1.0, 1.5 and 2.0 mg/l), adenine sulfate (Ads: 0, 25, 50, 100 mg/l), indole-3-acetic acid (IAA: 0, 0.01, 0.05, 0.10, 0.25 mg/l) or 1-naphthylacetic acid (NAA: 0, 0.01, 0.05, 0.10, 0.25 mg/l) and gibberellic acid (GA_3: 0, 0.5, 1.0, 1.5 mg/l) alone or in combination. All media were adjusted to pH 5.7 using 0.1N HCl or 0.1N NaOH before autoclaving. The medium was supplied with 0.8% (w/v) agar (Qualigen, India). 20 ml of molten medium was dispensed into a culture tube (25 × 150 mm) and plugged with nonabsorbent cotton wrapped in one layer of cheesecloth. The cultures were steam sterilized at 1.06 kg/cm^2 for 15 min. All cultures were incubated in 16 h light/8 h dark photoperiod under light intensity of 50 µE/m^2/s provided by cool white fluorescent light (Philips, India) at 25 ± 2°C with 55% relative humidity. Each treatment had 20 culture tubes and the experiment was repeated thrice. The effects of different treatments were quantified as the mean number of multiple shoots per culture. The data were statistically analyzed by the Duncan multiple range test (Harter 1960).

Induction of rooting. *In vitro* raised shoots measuring about 2–3 cm grown in multiplication medium were excised and cultured on half-strength MS basal salts supplemented with 0, 0.01, 0.25, 0.5 and 1.0 mg/l of IAA, IBA and NAA alone or in combination for induction of rooting. In case of rooting experiment, 10 replicates per treatment were taken and the experiment was repeated thrice. The cultures were examined every week and the morphological changes were recorded on the basis of visual observations. The effects of different treatments were quantified as the mean percentage of rooting.

Acclimatization. Rooted plantlets were removed from the culture tube. After thorough washing of the roots under tap water, the plantlets were transferred to sterile sandy soil at the ratio of 1:1 (garden soil: sand) into earthen pots and kept under high humidity (70%) in a greenhouse with the temperature of 30 ± 2°C for acclimatization.

Table 1. Effect of various growth regulators on development of shoot buds from nodal explants of *Acacia chundra* after two weeks of culture

Growth regulator (mg/l)				% of bud sprouting (mean ± SE)*
BA	Ads	IAA	NAA	
0	0	0	0	0
0.5	0	0	0	26.8 ± 1.0
1.0	0	0	0	46.2 ± 1.2
1.5	0	0	0	62.8 ± 1.1
1.0	25	0	0	54.6 ± 0.8
1.0	50	0	0	60.6 ± 1.0
1.0	50	0.10	0	72.4 ± 1.3
1.0	50	0.25	0	74.2 ± 1.1 +
1.5	50	0.25	0	78.6 ± 0.9 +
1.5	50	0	0.10	68.4 ± 1.0
1.5	50	0	0.25	70.6 ± 1.1 +
1.0	50	0.5	0	64.8 ± 1.0 +
1.5	50	0	0.5	58.6 ± 0.8 +

*20 replicate per treatment; repeated thrice. Means having the same letters are not significantly different by Duncan's multiple range tests at the level of 5%, + callusing at the basal end

RESULTS AND DISCUSSION

Shoot multiplication

The present investigation was carried out to explore the morphogenic potential of *A. chundra* by using different combinations of growth regulators. The nodal explants resumed new bud growth by proliferating the axillary shoot within 1–2 weeks of culture (Fig. 1A). Out of the various cytokinins tested, BA was the most effective for inducing bud break and growth in axillary meristems. Kinetin alone or in combination did not show any positive response on bud growth (data not shown). No bud sprout was obtained in media without growth regulator. The result also showed that the medium supplemented with Ads alone or in combination with BA sustains positive response on bud sprout (Table 1). The maximum bud break was observed in nodal explants cultured on MS medium supplemented with 1.5 mg/l BA, 50 mg/l Ads and 0.25 mg/l IAA. A little callus was obtained at the basal end of the explants. Within 4 weeks of culture, the axillary meristems elongated up to 2–3 cm in height. Similar results were reported in other legume trees (Mittal et al. 1989; Upreti, Dhar 1996). Regarding shoot multiplication, among the twenty combinations tried (BA, Ads, IAA and NAA), the combination of BA (1.5 mg/l), Ads (50 mg/l) and IAA (0.05 mg/l) proved to be the most effective treatment for promoting shoot multiplication (3–5 shoots/explant, each shoot having 3 to 4 nodes) within 4 weeks of subculture (Table 2, Fig. 1B). Addition of GA_3 in the multiplication medium did not show any positive effect on shoot multiplication. The number of shoots was further enhanced by repeated subculture on the same medium after excising the shoots from original explants. The interaction of two cytokinins with low concentration of auxin enhancing shoot multiplication has been reported by many authors (Dhar, Upreti 1999; Beck, Dunlop 2001; Nanda et al. 2004). The inclusion of higher concentration of auxin (either IAA or NAA) into the cytokinin-rich medium inhibited not only shoot multiplication but also produced some compact callus at the base of the explants. Similar observations were made in *Pterocarpus santalinus* (Lakshmi Sita et al. 1992), in *A. auriculiformis* (Mittal et al. 1989) and in *A. mangium* (Nanda et al. 2004). Vengadesan et al. (2003) achieved shoot multiplication from nodal explants of 10-year-old tree of *A. sinuata* by using 8.9µM BA, 2.5µM thidiazuron and 135.7µM adenine sulfate.

Induction of rooting

Elongated shoots derived from nodal explants were separated and cultured on half strength basal MS medium supplemented with IAA, IBA and NAA alone or in combination for induction of rooting. Of the three auxins tested, IBA and IAA induced rooting (Table 3). The optimum concentration was 0.25 mg/l of IBA or IAA and it resulted in 75 to 80% of root initiation within 8–10 days of culture (Fig. 1C). At higher concentrations (1.0–1.5 mg/l) of IBA or IAA, the percentage of rooting was reduced and callus formation was obtained at the basal cut

Table 2. Effect of cytokinins and auxins on shoot multiplication from nodal explants of *Acacia chundra* after 4 weeks of culture

Growth regulators (mg/l)				Avg No. of multiple shoots/ explant (mean ± SE)*	Avg length of shoots (cm) (mean ± SE)*
BA	Ads	IAA	NAA		
0	0	0	0	0	0
1.0	50	0	0	2.34 ± 0.6	1.21 ± 0.4
1.5	50	0	0	3.31 ± 0.7	1.43 ± 0.5
1.5	50	0.01	0	3.25 ± 0.8	3.12 ± 0.4
1.5	50	0.05	0	4.21 ± 0.5	3.56 ± 0.6
1.5	50	0.25	0	3.82 ± 0.6 +	3.11 ± 0.5 +
2.0	50	0.01	0	3.45 ± 0.7 +	2.88 ± 0.4 +
2.0	50	0.05	0	3.66 ± 0.8 +	3.14 ± 0.5 +
1.5	50	0	0.01	2.32 ± 0.6	1.46 ± 0.4
1.5	50	0	0.05	2.86 ± 0.7	1.76 ± 0.5
1.5	50	0	0.25	2.65 ± 0.6 +	2.0 ± 0.4 +

*20 replicates/treatment; repeated thrice. Shoots with less than 0.25 cm length were not taken into account. Means having the same letters are not significantly different by Duncan's multiple range test at the level of 5%, + callusing at the basal end

Table 3. Effect of IAA, NAA and IBA on rooting of *Acacia chundra* after two weeks of culture

Auxin concentration (mg/l)			% of rooting (mean ± SE)*
IAA	IBA	NAA	
0	0	0	0
0.10	0	0	30.8 ± 2.1
0.25	0	0	74.5 ± 1.2
0.50	0	0	56.4 ± 1.3 +
0	0.10	0	45.8 ± 1.0
0	0.25	0	80.4 ± 1.5
0	0.50	0	68.8 ± 0.9 +
0	0	0.10	52.5 ± 0.8
0	0	0.25	60.2 ± 1.1
0	0	0.50	36.6 ± 0.5 +
0.10	0.10	0	58.4 ± 0.8
0.10	0.25	0	72.6 ± 1.1
0.25	0.10	0	64.6 ± 1.2

+ Callusing at the basal end

end. The use of either IAA or IBA in the culture medium influences the higher rate of root induction from microshoots of different *Acacia* species (Dewan et al. 1992; Beck et al. 2000; Beck, Dunlop 2001; Vengadesan et al. 2002, 2003; Nanda et al. 2004). The percentage of rooting was decreased on medium containing different concentrations of NAA. The combination of either IAA + NAA, IAA + IBA or IBA + NAA did not show any positive response on rooting, rather it formed callus at the cut end of the excised shoot and also the shoot became yellowish (Table 3). These observations have also been reported for other woody species (Skolmen 1986; Yadav et al. 1990; Upreti, Dhar 1996). Vengadesan et al. (2002) achieved 55% rooting from microshoots of *A. sinuata* by using half-strength MS medium supplemented with 7.36µM IBA. Monteuuis and Bon (2000) reported that exposing the microshoots to 4–6µM IAA or IBA in darkness significantly increased rooting of the mature clone. *In vitro* grown rooted plants (Fig. 1C) were removed from the adhering gel, transplanted to earthen pots and kept under high humidity for acclimatization. About 65% of the plantlets survived under greenhouse condition after one month of transfer. This study illustrates a successful micropropagation system for *A. chundra*, a leguminous economic tree.

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Mikrorozmnožování dřeviny *Acacia chundra* (Roxb.) DC.

ABSTRAKT: U hospodářsky významné luskoviny a dřeviny *Acacia chundra* byl standardizován postup klonového rozmnožování v podmínkách *in vitro*. Indukce tvorby výhonů byla docílena z apikálních explantátů a z nodů *A. chundra* pěstovaných v podmínkách *in vitro* na základním médiu Murashige a Skoog (MS) s dodáním 6-benzylaminopurinu (BA) (1,0 mg/l) a 20 mg/l adenin sulfátu (Ads). Intenzivního množení bylo dosaženo na médiu MS s dodáním 1,5 mg/l BA, 0,01 až 0,05 mg/l kyseliny β-indolyloctové (IAA) a 50 mg/l Ads. Počet vytvořených výhonů kolísal od tří do šesti v závislosti na použitých regulátorech růstu. Oddělené výhony byly zakořeňovány na základním médiu MS s poloviční koncentrací solí a s dodáním 0,25 mg/l kyseliny indolylmáselné (IBA) nebo IAA a 20 g/l sacharózy po 10 až 12 dnech pěstování. Takto získané rostlinky z mikrorozmnožování byly aklimatizovány a úspěšně vysazeny do zeminy.

Klíčová slova: regulátory růstu; *in vitro*; leguminózní dřevina; mikrorozmnožování

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