

Short Communications

Direct organogenesis in *Datura metel* L. from *in vitro* and *in vivo* nodal explants

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In vitro plant regeneration was achieved in *Datura metel* from nodal explants collected from both *in vitro* germinated seedlings and field grown plants (*in vivo*). The explants were cultured on MS medium with BAP (0.5-3.0 mg/l) and NAA (0.5 mg/l). The nodal explants isolated from *in vivo* source exhibited a greater number of healthy multiple shoots than *in vitro*. BAP at 3 mg/l with NAA at 0.5 mg/l was found to be optimal for regeneration of shootlets.

Keywords: *Datura metel*, medicinal, *in vitro* regeneration, nodal explants, BAP, organogenesis

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The Indian systems of medicine predominantly use plant based raw materials in most of their preparations and formulations. The efficacy of these systems thus mainly depends upon the use of pure and genuine raw materials in the manufacture of drugs. The increase in demand for herbal medicines has resulted in the problem of availability of sufficient quantity of medicinal plants for the manufacture of genuine drugs¹. As a result of continuous exploitation of these plants and absence of regular developmental programmes in the forestry and agricultural sector, the prerequisite is the production of large quantity of plants within a short time through non-conventional methods like plant tissue culture.

Datura metel L. (Family, *Solanaceae*) is a fast depleting valuable medicinal plant containing alkaloids like hyoscyamine, hyoscyne, atropine, etc². It is a reputed drug for dog bites and respiratory ailments^{3,4}. The present study was undertaken to evolve a repro-

ducible protocol of micropropagation for the mass production of *D. metel* plants from nodal explants.

The seeds were collected from field grown plants near the research station. Uniform seeds were washed with soap water and 0.1% (w/v) bavistin for 5 min and then washed thoroughly with running tap water and dried in the sunlight. For quick germination, the dried seeds were scarified and surface sterilized with 0.1% HgCl₂ for 3 min, followed by three washings with sterile double distilled water inside the laminar air flow chamber. Then the seeds were inoculated on MS⁵ solidified agar medium devoid of any growth regulators for germination. Nodal explants were collected from 20-day-old *in vitro* grown healthy seedlings and also from 2-month-old *in vivo* plants. *In vitro* and surface sterilized *in vivo* nodal explants were inoculated on MS medium containing 3% (w/v) sucrose, BAP (0.5-3.5 mg/l) and NAA (0.5 mg/l). The medium (pH, 5.8), after addition of 0.8% (w/v) agar, was autoclaved at 121°C for 15 min. The cultures were incubated under cool white fluorescent light with 16 hrs photoperiod.

The nodal explants from *in vitro* germinated plants were cultured on MS medium supplemented with various concentrations of BAP (0.5-3.5 mg/l) in combination with 0.5 mg/l of NAA. After 15 days of incubation, numerous green shoot buds emerged from the basal regions (Fig. 1a). The percentage of shoot proliferation was high (70%) at 3.0 mg/l BAP + 0.5 mg/l NAA and decreased gradually with the decreasing concentration of BAP (Table 1). In the present study, 3.0 mg/l BAP + 0.5 mg/l NAA showed better response than the other concentration, whereas in *Datura insignis*, cytokinin (1.0 mg/l BA) alone was effective for multiple shoot induction⁶.

Nodal explants from *in vivo* source were cultured on MS medium fortified with BAP (0.5-3.5 mg/l) in combination with 0.5 mg/l NAA. The nodal explants from *in vivo* source exhibited a greater number of healthy multiple shoot buds (Fig. 1b) than the *in vitro* nodal explants. Of the various concentrations, 3.0 mg/l BAP with 0.5 mg/l NAA showed 83.3% shoot bud proliferation and highest number (20) of shootlet regeneration (Table 1). Thus, *in vivo* nodal explants were more responsive than nodal explants of *in vitro* origin. This report is in accordance with that of

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Fig. 1—Plant regeneration in *D. metel*: a, Multiple shoot induction from *in vitro* nodal explants; b, Multiple shoot induction from *in vivo* nodal explant; c, Rooted plantlet; & d, Hardened plantlet in plastic cup

*Solanum viarum*⁷, where the nodal explants collected from the field grown plants produced the highest number of shoots on MS medium supplemented with BAP (2.0 mg/l) alone.

Regenerated shootlets from *in vitro* and *in vivo* nodal explants were sub cultured on the same medium for elongation and then excised and transferred to root induction medium, which contained IBA (0.5-3.0 mg/l). Rhizogenesis was observed between 10-15 days after transfer to the rooting medium (Fig. 1c). The highest rooting response was obtained at 2.0 mg/l IBA from *in vivo* nodal regenerants (80%) followed by *in vitro* nodal explants (66.6%). In the present investigation, 2.0 mg/l IBA was found to be optimal for root induction (Table 2). This result is in accordance with that of *Hybanthus ennaespermus*⁸ and *D. metel*⁹.

The rooted plantlets were removed from the culture tubes and washed in running tap water to completely remove the agar. Then the plantlets were transferred to vermiculite and nourished with half strength MS liquid medium (Fig. 1d). After two weeks, the plantlets were transferred to polythene bags, which contained a soil mixture of red soil and sand in the ratio of 1:1 mixed with tap water. Nearly 90% of the micropropagated plantlets were successfully transferred to the field.

Table 1—Effect of different concentrations of BAP and NAA on shoot proliferation from nodal explants

Concentration (mg/l)		Percentage of shoot proliferation		Number of shoots per culture mean±SD		Shoot length (cm) mean ± SD	
BAP	NAA	Node (<i>in vitro</i>)	Node (<i>in vivo</i>)	Node (<i>in vitro</i>)	Node (<i>in vivo</i>)	Node (<i>in vitro</i>)	Node (<i>in vivo</i>)
0.5	0.5	*	*	*	*	*	*
1.0	0.5	16.7±0.58	36.7±1.53	2.7±0.58	6.0±0.0	5.1±0.59	4.5±0.21
1.5	0.5	36.7±1.52	40.0±1.73	4.7±0.58	6.3±2.1	5.3±0.31	4.9±0.20
2.0	0.5	50.0±1.0	56.7±0.58	6.3±1.2	9.7±2.5	6.0±0.20	5.7±0.40
2.5	0.5	63.3±0.58	63.3±0.58	10.7±1.53	15.0±3.0	6.6±0.72	6.1±0.21
3.0	0.5	70.0±1.0	83.3±2.1	13.7±1.53	20.0±2.0	7.4±0.62	7.0±0.25
3.5	0.5	46.7±1.15	50.0±1.0	9.7±1.53	6.7±2.1	5.7±0.38	6.4±0.32

*No response

Table 2—Effect of different concentrations of IBA on root induction from regenerated shoots

Concentration of IBA (mg/l)	Node (<i>in vitro</i>)		Node (<i>in vivo</i>)	
	Percentage of rooting	Number of roots per shoot mean ± SD	Percentage of rooting	Number of roots per shoot mean ± SD
0.5	16.7	23.±0.58	23.3	2.0±1.0
1.0	46.7	4.0±1.0	43.3	5.0±1.0
1.5	63.3	6.7±1.15	70.0	7.3±0.58
2.0	66.6	9.3±0.58	80.0	10.0±1.0
2.5	50.0	7.0±0.0	60.0	8.0±1.0
3.0	26.7	2.7±0.58	40.0	3.7±1.53

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