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In vitro plant regeneration using adventitious roots as explants in *Tylophora indica*

Abdul Nayeem*, Rashmi M. Panchaksharadhya and Vedamurthy A. Basappa

P. G. Department of Biotechnology, The Oxford College of Science, HSR Layout, Bangalore

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ABSTRACT

In vitro regeneration of plants using adventitious roots as explants in T. indica has been studied. In the present study, various auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), indole butyric acid (IBA) and naphthalene acetic acid (NAA) alone were tested at different concentrations on Murashige and Skoog (MS) medium with 3% sucrose. Maximum shoot regeneration (25.0) per treatment was achieved when adventitious roots were cultured with IBA at 2.0 mg/L concentration. Shoot bud elongation was achieved on MS full strength with BAP at 0.1 mg/L and rooting was on MS half strength medium with IAA at 0.2 mg/L concentration. Plants were established in a potting substrate. The present protocol will serve as alternative mean for in vitro clonal propagation of this endangered plant of immense medicinal value.

Keywords: Organogenesis, *T. indica*, Medicinal plant, Auxins, Tissue culture.

INTRODUCTION

Tylophora indica (Burm.f.) Merrill, a member of Asclepiadaceae is an important indigenous medicinal plant found in restricted localities in Indian sub continent. The roots possess stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic properties and are used for the treatment of asthma, bronchitis, whooping cough, dysentery, diarrhea and in rheumatic pains. The pharmacological investigations have confirmed the anti-asthmatic effects of its leaf extracts [1] and the plant contains several phenanthroindolizidine alkaloids [2]. The major alkaloid tylophorine has been reported to have immunosuppressive, anti-inflammatory [3] and antitumor [4] properties. Thus the plant is in great demand for the production of traditional and modern medicines. Therefore, the availability of a reliable, *in vitro* clonal propagation system would provide an alternative method of propagation to meet the pharmaceutical needs and also for effective conservation of this important plant species.

Documented literature reveals that there are limited reports on *in vitro* propagation of *T. Indica*. Although micropropagation of *T. indica* by axillary shoot induction and adventitious shoot production [5, 6] and callus-mediated somatic embryogenesis from leaf [7, 8] and inter nodal [9] explants have been previously reported, further improvement of the techniques is a continuous process.

Root tissue has been proven to be highly regenerative explant and is relatively easy to maintain and manipulate *in vitro* [10, 11]. Besides, shoots developing on roots of the same plant have been reported to be genetically uniform and the method, an alternative way for clonal propagation [5]. The present report communicates a reproducible protocol for regeneration of well developed and healthy *T. indica* plantlets via adventitious root culture. The findings will be useful in conservation and mass multiplication of this endangered medicinal plant.

MATERIALS AND METHODS

Explant Source

In our previous study on induction of adventitious roots in *T. Indica* [12], adventitious roots were successfully induced from leaf explants on MS medium supplemented with 1.0 mg/L IAA. Induced adventitious roots were further multiplied, subcultured and maintained on MS medium. Six weeks old *in vitro* developed adventitious roots were used as an explant material for shoot organogenesis. Aseptically removed adventitious roots were repeatedly washed in sterile distilled water to free them from agar adhering to the surface. Subsequently they were inoculated on the culture medium for shoot organogenesis.

Medium and Culture conditions

MS medium [13] supplemented with phytohormones and 3% sucrose was solidified with 0.8% agar (w/v) and the pH of medium was adjusted to 5.8 before adding agar and sterilized by autoclaving at 15 lbs (1.8 kg/cm2) pressure at 121 ^{0}C for 15 minutes. Cultures were incubated at 25 ± 1^{0}C for 16 hours in light (illuminated by 40 watt fluorescent tubes, 50 µE m^{-2} s^{-1}) and for 8 hours in dark cycle.

***In vitro* shoot Organogenesis**

T. indica adventitious roots were inoculated on MS medium containing varying concentrations of phytohormones viz 2, 4-dichlorophenoxyacetic acid (2, 4-D; 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg), indole butyric acid (IBA; 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg) and α-naphthalene acetic acid (NAA; 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg) alone. In each experiment 30 explants were cultured and all the experiments repeated thrice. MS medium lacking plant growth regulators served as control. Data on average shoot number per explant was recorded after an interval of 4 weeks. Once the culture conditions for optimum shoot induction from root explants were established, routine subculturing of developing shoots was carried out at an interval of every three week onto fresh medium.

Elongation and Rooting medium

Shoot buds obtained from initiation medium were separated and cultured individually on elongation medium consisting of full strength MS with 0.1 mg/L BAP. The cultures were maintained at above mentioned standard culture room conditions. The induction of roots was obtained on MS half strength medium supplemented with 0.2 mg/L IAA.

Acclimatization

Rooted plantlets were gently washed under running tap water and transferred to small pots containing sterile coco-peat, sand, and soil [1:1:1 (v/v)] and covered with perforated polythene bags and maintained in culture room conditions. The plantlets were suitably irrigated with MS quarter strength nutrients every alternate day. The polythene bags were periodically withdrawn to acclimatize the plantlets and when new leaves started emerging the bags were completely removed. Hardened plantlets were subsequently transferred to pots containing soil: sand: manure (1:1:1) and shifted to green house for further growth and development.

Statistical Analysis

Experiments were done in triplicate and results were subjected to analysis of variance (*ANOVA*) and mean values were separated according to Duncan's multiple range test at $P = 0.05$.

RESULTS AND DISCUSSION

Various concentrations of plant growth regulators were tested on adventitious root explants from 0.5 mg/L to 10 mg L^{-1}. The root explants turned green within one week of culture (Fig. A). Shoot bud formation was direct process or sometimes associated with callus formation (Fig. B and C). After 4 weeks in culture, well developed shoot buds were observed in root explants (Fig. D). The highest induction of organogenesis of 25 plantlets per treatment was observed in adventitious roots after four weeks in 2 mg/L IBA (Table 1).

In the present experiment, the development of shoots was greatly affected by the type and concentration of growth regulator in the medium. The organogenesis in *T. indica* occurred mostly in culture medium containing IBA. In the absence of IBA, no response was observed and the higher concentration (10 mg/L IBA) was not favorable to organogenesis. No shoot regeneration was observed in the media with NAA at concentrations higher than 1.0 mg L^{-1}. There was no response in the treatments which involved 2, 4-D as growth regulator. Elongation of regenerated shoots was achieved on full strength MS medium with 0.1 mg/L BAP (Fig. E). The elongated shoots were rooted on MS half strength medium supplemented with 0.2 mg/L IAA. The rooted plantlets, when transferred to pots (Fig. F), survived at 80% survival rate.

Our experiments indicate that adventitious root cultures of *T. indica* have the potential to form adventitious buds/shoots. In anatomical analysis of *in vitro* wild tomato (*Lycopersicon peruvianum, L. chilense* and *L. hirsutum*), the formation of buds in the roots, which can also occur direct or indirectly, depending on the genotype used as reported by Peres *et al* [14]. *In vitro* shoot initiation from root segments has been reported in *Dalbergia* sissoo [15]; *Citrus* sinensis [16]; *Citrus mitis* [17]; *Averrhoa carambola* [18] and *Clitoria ternatea* [19]. Plant growth regulator IBA has been reported to involve in shoot induction and proliferation in *Aegle marmelos* [20]; *Aloe Vera* [21]; *Catharanthus roseus* [22]; *Rosa miniature* [23].

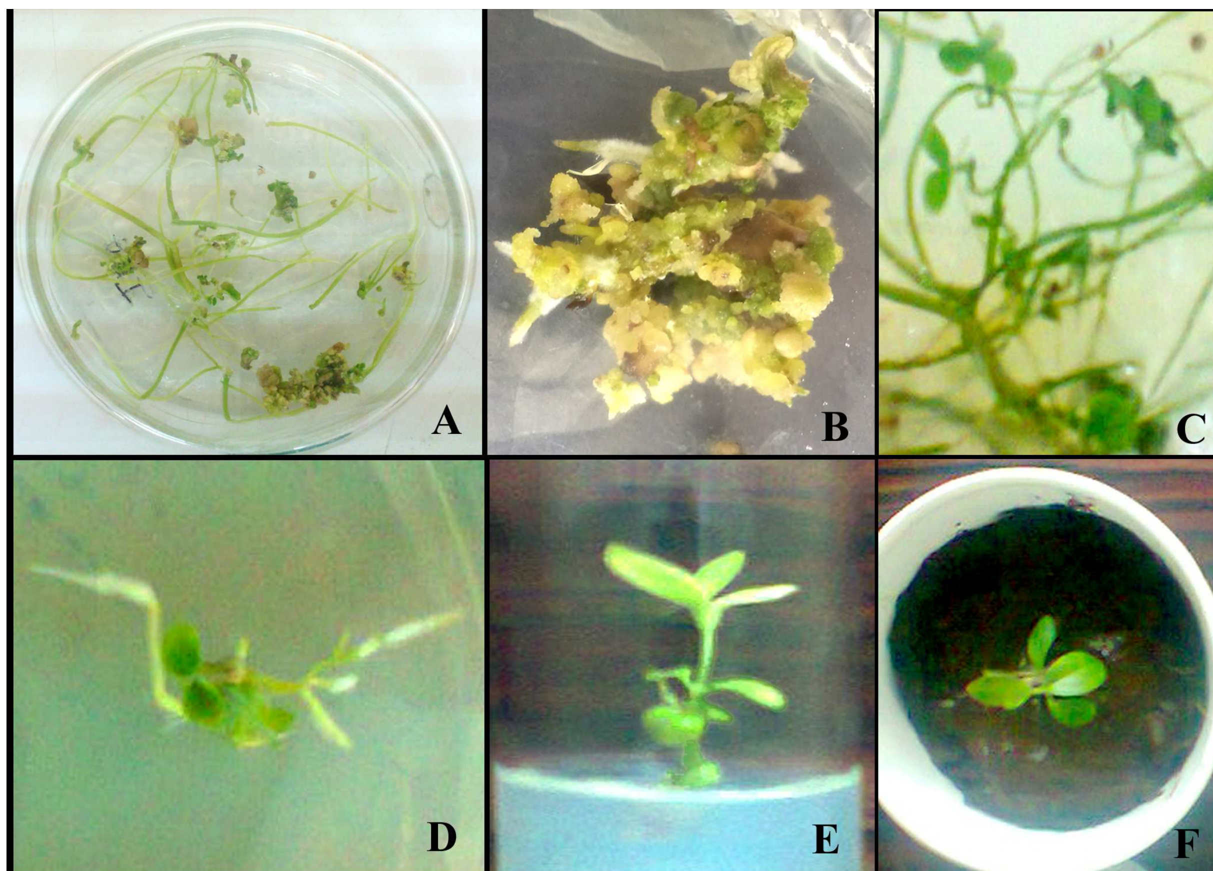

Fig. A: Root explants turned green after one week of culture
Fig. B: Callus formation from root explants showing small shoot buds
Fig. C: Direct shoot bud formation from root explants
Fig. D: shoot buds after four weeks in culture
Fig. E: Elongated shoots on elongation medium
Fig. F: Rooted plantlets transferred to small pots

In the present study, root explants were taken from *in vitro* adventitious roots growing in auxin-rich medium. On placing the explants in another auxin-rich medium may be an inhibiting factor for shoot bud differentiation while facilitating lateral root formation. Contrastingly, in our study IBA when tried at different concentrations was found to be more responsive and induced adventitious shoot buds. The endogenous concentration of cytokinin may have influenced the response in the present study. Further studies are needed to study this contrasting response in *T. indica* and explore the new alternative means for *in vitro* clonal propagation of this endangered medicinal plant.

Table 1. Effect of auxins on shoot regeneration using adventitious roots as explants in *Tylophora indica* [a]

Phytohormone	Concentration (mg/L)	Number of explants (roots) cultured	% of explants forming shoot buds	Mean number of shoots per treatment[b]
NAA	0.0	30	0.00	0.00 e
	0.5	30	8.00	0.00 e
	1.0	30	15.00	1.33 d
	2.0	30	11.00	0.00 e
	5.0	30	6.00	0.00 e
	10.0	30	0.00	0.00 e
IBA	0.0	30	10.00	0.00 e
	0.5	30	20.00	1.33 d
	1.0	30	35.00	3.33 c
	2.0	30	64.00	25.00 a
	5.0	30	30.00	11.00 b
	10.0	30	15.00	1.66 d
2,4-D	0.0	30	0.00	0.00 e
	0.5	30	0.00	0.00 e
	1.0	30	0.00	0.00 e
	2.0	30	2.00	0.00 e
	5.0	30	7.00	0.00 e
	10.0	30	0.00	0.00 e

[a] *Data were collected after 4 weeks of culture.*

[b]*Mean values followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.*

CONCLUSION

This study has revealed the potential of *in vitro* adventitious roots as a source tissue for micropropagation in *T. indica*. The explants can be easily and regularly obtained from established root cultures and do not require disinfection treatment. The present protocol will therefore serve as alternative means for *in vitro* clonal propagation of this endangered plant with immense medicinal value.

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