

Callus Induction and Micropropagation from Different Explants of *Tylophora indica* Wight and Arn. (Antamul)

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Abstract

Node, internode and leaf explants of *Tylophora indica* had the potential to produce calli on Murashige and Skoog (MS) medium supplemented with 2, 4-D (2.0 mg/liter) + KIN (0.1 mg/liter) + CH (200 mg/liter). Calli of different explant origin showed variable growth responses on combinations of hormones. The shoots were favorably formed indirectly from the calli of leaf explant and directly from the node explant. The maximum number of shoots was produced from the calli sub-cultured on MS + KIN (1.0 mg/liter) + IAA (0.5 mg/liter). The roots were initiated from the shoots on MS medium supplemented with IBA (3.0 mg/liter).

Key words : Callus, *In vitro* culture, Micropropagation, *Tylophora indica*.

Tylophora indica Wight and Arn. (Antamul), a medicinal plant belonging to family Asclepiadaceae, is a perennial twining or climbing herb. The roots and leaves of the plant have been used for treatment of asthma, whooping cough, dysentery and diarrhoea since long (1). These are also effective in rheumatic and gouty pains and hydrophobia. Extract of stem and leaves show significant anti-cancer activity (2) and roots contain tylophorinidine an alkaloid with anti-tumor activity (3). Some other alkaloids such as tyloidicines (4) and tyloindane (5) have also been extracted from aerial parts of the plant. Presently only wild populations of this species are exploited for various purposes and commercial plantations have not been widely attempted due to which this medicinal species declined fast and is now listed as a species vulnerable to extinction. In view of this, the present study was conducted to initiate and maintain the calli produced from tissues of various plant parts (node, internode and leaf) and to define the nutritional conditions for regeneration and large scale multiplication of this species.

Methods

The node, internode and leaf explants obtained from over ten year old, mature plants of *Tylophora*

indica were washed with 5.0% teepol solution (10 min) followed by washing with running tap water. These were then surface sterilized with 70% alcohol for 2 min followed by 0.1% mercuric chloride (HgCl₂) for another 2 min and subsequent washed with sterilized double distilled water. Surface sterilized explants were cut into small pieces (1 cm) and inoculated immediately into the medium, aseptically. Two to three explants were placed in each culture bottle. The MS (6) medium containing 3% sucrose and 0.8% agar along with growth regulators was poured into culture bottles. The pH of the medium was adjusted to 5.8, prior to addition of agar and autoclaving at 121 C, 1.2 kg/cm² pressure for 15 min. MS basal medium was used without additives and supplemented with different concentrations of BAP, KIN, 2, 4-D, IAA and NAA alone and KIN in combination with 2, 4-D and CH for callus induction.

The calli so obtained were maintained and multiplied through sub-culturing on the fresh medium having composition similar to induction medium, after every four-week. For differentiation and regeneration, eight-week old calli derived from different explant sources were sub-cultured on MS medium supplemented with different concentrations of KIN and IAA. Nodal explants were placed directly on MS medium supplemented with different concentrations

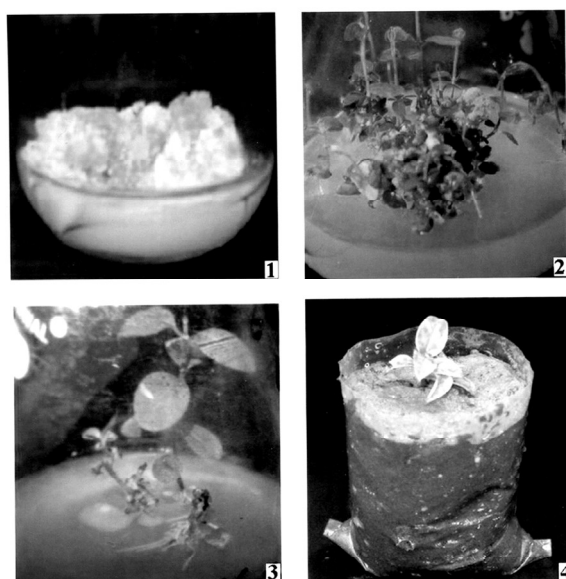


Figure 1. Callus obtained from leaf explants on MS medium supplemented with 2, 4-D (2.0 mg/l) + KIN (0.1) + CH (200 mg/l). **Figure 2.** Juvenile shoots from callus on MS medium supplemented with KIN (1.0 mg/l) + IAA (0.5). **Figure 3.** Induction of roots from *in vitro* regenerated shoots on MS medium supplemented with IBA (3.0 mg/l). **Figure 4.** Transferred plant in polythene bag containing sterilized sand and vermin compost (3 : 1) in the green house.

of BAP. All the cultures were incubated at 26 ± 2 C under 16 h of white fluorescent light photoperiod (intensity of 2000 lux) and 8 h of darkness.

The shoots formed from calli, and directly from the nodal explants (4–5 cm long) were cut and sub-cultured on MS medium supplemented with different IBA concentrations for rooting. After rooting the plantlets were transferred into small polythene bags containing soil and vermi-compost (3 : 1). Plants were

supplied with $\frac{1}{2}$ strength MS salt solution, kept in the green house for hardening and acclimatization for one month and then transferred to field.

Results and Discussion

MS medium without growth regulators failed to induce callusing from any of the explant. Similarly, BAP, KIN, 2, 4-D, IAA and NAA alone were not

Table 1. Effect of different concentrations of 2, 4-D, KIN and CH supplemented to MS medium on callusing from different explants of *T. indica* after 28 days of inoculation. + Poor, ++ Moderate, +++Good.

Growth regulator (mg/l)	Leaf		Internode		Node	
	Callus formation frequency (%)	Callus growth	Callus formation frequency (%)	Callus growth	Callus formation frequency (%)	Callus growth
2, 4-D (1.0) + KIN (0.1) + CH (100)	96	++	64	++	78	++
2, 4-D (1.0) + KIN (0.5) + CH (100)	90	+	60	+	64	++
2, 4-D (2.0) + KIN (0.1) + CH (100)	88	+++	58	++	92	++
2, 4-D (2.0) + KIN (0.5) + CH (100)	72	++	52	+	74	++
2, 4-D (1.0) + KIN (0.1) + CH (200)	92	++	92	++	90	+
2, 4-D (1.0) + KIN (0.5) + CH (200)	86	+	90	++	80	+
2, 4-D (2.0) + KIN (0.1) + CH (200)	100	+++	96	+++	96	+++
2, 4-D (2.0) + KIN (0.5) + CH (200)	78	++	88	+	84	++

Table 2. Effect of different concentrations of KIN and IAA supplemented to MS medium on leaf callus of *T. indica* for shoots regeneration after 42 days of inoculation. + Poor, ++ Moderate, +++ Good.

Growth regulator (mg/l)	Callus growth	Green patches formation	Days taken for shoots formation	No. of shoots
KIN (0.5) + IAA (0.1)	C ⁺	G ⁻	25—30	5
	(0.5) C ⁺⁺	G ⁺⁺	25—30	30
	(1.0) C ⁺⁺	G ⁺	25—30	30
KIN (1.0) + IAA (0.1)	(0.1) C ⁺	G ⁻	20—25	10
	(0.5) C ⁺	G ⁺⁺⁺	15—20	35
	(1.0) C ⁺	G ⁺	20—25	20
KIN (2.0) + IAA (0.1)	(0.1) C ⁺	G ⁻	20—25	5
	(0.5) C ⁺	G ⁺	25—30	15
	(1.0) C ⁺	G ⁺	25—30	15

able to induce callusing (data not shown). However, in MS medium supplemented with different concentrations of 2, 4-D, KIN and CH, callus formation was evident from curling and enlargement of the explants to various degrees. Maximum callusing was noticed in 2.0 mg/l 2, 4-D + 0.1 mg/liter KIN + 200 mg/liter CH for all the explants. The frequency of callus induction was 100% for leaf and 96% for node and internodal segments. But the maximum callus with good growth was obtained from leaf as compared to the node and internodal explants (Table 1, Fig. 1). The role of auxin, cytokinin and CH has been reported by Abdel-Rahim et al. (7) in date palm. Similarly, 2, 4-D was the auxin choice for initiation and growth of callus in many palnt species (8) ; 2, 4-D and kinetin gave the highest value of fresh weight of callus from *Datura metal* (9).

Calli from different explant sources were transferred to MS medium with variable concentrations of KIN (0.5—2.0 mg/liter) + IAA (0.1—1.0 mg/liter). However, the morphogenesis was achieved only with the calli of leaf explants within 15—30 days (Table 2)

Table 3. Effect of different concentrations of BAP on shoot regeneration from node explants of *T. indica* after 28 days of inoculation.

Growth regulator (mg/l)	Shoots sprouting frequency (%)	No. of shoots/explant	Shoot length (cm)
BAP (1.0)	100	1—2	4—5
(2.0)	100	4—6	1—2
(3.0)	100	2—3	1—2

as compared to calli of node and internode explants which did not show any morphogenesis up to six-weeks (data not shown). For leaf calli, all the combinations were effective for morphogenesis (Table 2, Fig. 2), however, the efficiency varied with the concentrations of KIN and IAA. Maximum number of shoots (35) formation was achieved only with 1.0 mg/liter KIN and 0.5 mg/liter IAA within 15—20 days of inoculation (Fig. 2). Such variability in the regeneration potential by the explants has also been reported in the *Tamarindus* (10), jute species (11) and peanut (12). In the present study, the leaf callus was most efficient to produce a considerable number of shoots.

The regeneration of shoots was also achieved directly from the nodal explants (Table 3) and the maximum number of shoot buds proliferated on 2.0 mg/liter of BAP. Lower and higher concentrations of BAP produced less number of shoots. The reason for the shoot proliferation directly from the explant like nodal segment could be attributed to the presence of pre-existing primordial which proliferated into shoots

Table 4. Influence of different concentrations of IBA on rooting of *in vitro* formed shoots of *T. indica* after 28 days of inoculation. ++ Moderate, +++ Good.

Growth regulator (mg/l)	No. of roots/shoot	Length of roots
IBA (1.0)	1—2	+++
(2.0)	3—4	++
(3.0)	4—6	++
(4.0)	4—6	++

under adequate amount of cytokinin supplied in the nutrient medium which nullify the effect of apical dominance and thus enhanced the proliferation of lateral buds from the axis. This supports the studies of Rajkumar (13) in *Camellia sinensis*, Harikrishan and Hariharan (14) in *Plumbago rosea* and Babber et al. (15) in *Cardiospermum halicacabum*.

The shoots when separated and implanted on root initiation media (Table 4) resulted in roots initiation. Maximum number of roots was initiated on 3.0 mg/liter of IBA (Fig. 3). This indicated that exogenous auxins are required for rooting. IBA is most commonly used to induce rooting (16—20). After the roots formation, the plantlets were transferred to polythene bags containing soil and vermin compost mixture (3 : 1) and ½ MS solution (Fig. 4). The survival percentage of the plants was 85%. Similar acclimatization procedure for successful establishment of *in vitro* regenerated plants has been demonstrated by Purohit et al. (16), Beekheet (21), Singh et al. (22).

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