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Clonal propagation of *Tylophora indica* - A medicinal plant

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An efficient protocol has been developed for clonal propagation from the of internodal regions of *Tylophora indica* (Burm F.) Merrill through successive *in vitro* trails. Basal medium of Murashige and Skoog, (1962) added with the combination of different levels of cytokinin and auxin molarity and concentrations were prepared. The effective suitable combinations were 3.25µM/l BA + 1.50 µM /l IAA, 12.25 µM/l KN +3.75 µM/l IBA and 1.25 µM/l Zeatin + 2.75 µM/l for calli, shoots elongation and roots formed in the inter nodal region of *Tylophora indica* respectively. The highest weight of calli formed in the optimum effective combination of 3.25µM/l BA + 1.50 µM /l IAA was observed in 8 week old cultures. The best effective combinations for shoot proliferation were 3.25µM/l BA + 1.50 µM /l IAA and 12.25 µM/l KN +3.75 µM/l IBA. The optimum rooting auxin concentration was 1.25 µM/l Zeatin + 2.75 µM/l for root formation. The present report serves as an effective protocol for the mass multiplication of *Tylophora indica* (Burm F.) Merrill in future.

Tylophora indica /micropropagation

Asclepiadaceae has approximately 250 genera and 2000 species chiefly of tropical and subtropical regions of both hemispheres (Sundell, 1993). *Tylophora indica* (Burm f.) Merrill (Asclepiadaceae) is a threatened medicinal climber shrub native to plains and hill forests of eastern and southern India up to an altitude of 900m. It forms dense patches in the forest in moist and humid conditions in open hill slopes and narrow valleys. It is also cultivated for its medicinal uses. The plant shows stunted growth in the areas with lesser rainfall.

Tylophora indica (Burm f.) Merrill has been traditionally exploited by tribes from various regions of India (Anonymous, 1976). It is highly demanded for the production of traditional medicines and treatment of various diseases such as bronchial asthma, inflammation, bronchitis, allergies, rheumatism, dermatitis, antipsoriasis, seborrhea, anaphylactic, leucopenia and as an inhibitor of the Schultz-Dale reaction (Sharma et al. 2010). The plant contains several phenanthroindolizidine alkaloids (Gellert, 1982) and pharmacological investigations have confirmed the anti-asthmatic effects of its leaf extracts (Shivpuri et al. 1972).

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The major alkaloid present "tylophorine" has been reported to have immunosuppressive, anti-inflammatory (Gopalakrishnan et al. 1980) and antitumor (Donaldson et al. 1968) properties. The powdered leaves, stems and roots also contain

other minor alkaloids (Rao and Wilson, 1971) including tylophorinine, cryptopleurine, antofine and ficuseptine C which are pharmacologically active and anticancer tylophorinidine has also been isolated from the roots of three-year old plant (Mulchandani et al. 1971).

Due to the ever growing demand of herbal medicines, the availability of medicinal plants is not enough to manufacture herbal medicines by pharmaceutical companies. Micropropagation offers a great potential for large scale multiplication of such useful species and limits the subsequent exploitation (Boro et al. 1998). A previouslyreport on the protocol of micropropagation of leaf explants (Faisal and Anis, 2003), nodal segment (Faisal et al. 2007; Sharma and Chandel, 1992), explants on Murashige and Skoog, (1962) basal medium mass propagation of *Tylophora indica* were found. We have presently developed an efficient protocol for highly efficient regeneration of *Tylophora indica*, which would be highly useful for pharmaceutical companies.

Materials and Methods

Plant materials

Tylophora indica (Burm F.) Merrill were collected from Southern Western Ghats of Tamil Nadu, India and maintained in the green house. The explants of internode (0.5-1cm) were washed in running tap water for 30min and followed by surface sterilized with 70% ethanol for 50s, rinsed with sterile double distilled water and treated with 0.1% $HgCl_2$ for 2min and finally rinse in sterile double distilled water two times for 5min. These sterilized explants were inoculated on basal medium.

Preparation of rooting and shooting medium

After surface sterilization, the explants were inoculated on Murashige and Skoog, (1962) medium added with cytokinins and auxin concentration are BA (2.25-4.25µM)+ IAA (1.15-2.50µM), KN (10.25 -12.50µM)+ IBA (2.25-4.00 µM) and Zeatin (0.25 - 1.75 µM)+ NAA(1.25-3.25µM). The culture medium was supplemented with 3% sucrose (w/v) and solidifying agent 0.8% agar (Himedia, India). The pH of the medium was adjusted to 5.7 with 0.1M NaOH or 0.1M HCl after the addition of growth regulators, prior to the addition of 0.8% agar (Himedia, India). The medium was autoclaved at 121°C, for 30 min. All the cultures were maintained in sterilized culture room at 26 ± 2°C, under 16/8h light regime provided by cool white fluorescent light ($60\mu mol^{-2}s^{-1}$ light intensity) and with 55 - 60% relative humidity.

Transplantation

After the root formation, the plantlets were removed from the test tube and transplanted in to substrate containing 1: 1 sand pots. Equal parts of leaf moulds, sand, peat moss and loam from the plants were transplanted to the field conditions.

Data collection

For each experiment, a minimum of 3 replicates were taken. Observations of the culture were made every week and data collections of the response of shoot proliferation and root initiation after six week old culture were taken.

Results and Discussion

In the present study, an efficient protocol was developed from the mass multiplications of internode regions of *T. indica in vitro* condition. We have developed the protocol using Murashige and Skoog (MS) basal medium contained various concentration of cytokinin and auxin (Table-1). Table-1 showed callus response and calli weight, percentage of shoots proliferation and root ignition observed after six week old culture. Evidence of the results callus responses, shoot proliferation and root formation are seen in Plate-1. The suitable combinations that provoked results are 3.25µM/l BA + 1.50 µM /l IAA, 12.25 µM/l KN +3.75 µM/l IBA and 1.25 µM/l Zeatin + 2.75 µM/l for calli formed from the internodal regions of *Tylophora indica* (Plate-1A, B&C). The highest weight of calli formed in the optimum effective combination of 3.25µM/l BA + 1.50 µM /l IAA was observed in eight week old cultures.

The best effective combination for shoots proliferation were 3.25µM/l BA + 1.50 µM /l IAA and 12.25 µM/l KN +3.75 µM/l IBA (Plate-1D). These combinations showed 100 percentage responses to shoots proliferation in individual cultures. Our results agree to previous reports showed that the same combinations, which was suitable for *in vitro* raising several plants including *Ensete superbum, Solieria filiformis, Rauvolfia serpentina, Hyptis suaveolens, Capparis deciduas, Citrullus vulgaris*, *Zehneria scabra* and *Gymnema sylvestre* (Kulkarni et al. 2002; Yokoya and Handro, 2002; Nishikoshta and Bansal, 2002; John Britto et al. 2001; Deora and Shekhawat, 1995; Dong and Jia, 1991; Reddy et al. 1998; Anand and Jeyachandran, 2004.

Table- 1. Shoots and rooting media for mass clonal propagation of *Tylophora indica* (Burm F.) Merrill

Concentrations (µM)/L		Calli		% of shoots proliferation	% of roots initiation
Cytokinin/ auxin		% of Calli formation	Calli wt (mg)		
BA	IAA				
2.25	1.15	60	267	90	20
2.50	1.25	70	345	100	20
3.25	1.50	100	427	100	10
4.00	2.25	80	210	90	10
4.25	2.50	80	198	90	10
KN IB	**A**				
10.25	2.25	50	234	70	20
11.50	2.75	70	213	90	30
11.75	3.00	90	325	100	10
12.25	3.75	100	412	70	20
12.50	4.00	90	391	80	10
Zeatin NAA					
0.25	1.25	60	218	20	90
0.50	2.50	90	119	50	80
1.25	2.75	100	219	40	100
1.50	3.00	80	234	10	70
1.75	3.25	80	253	10	80

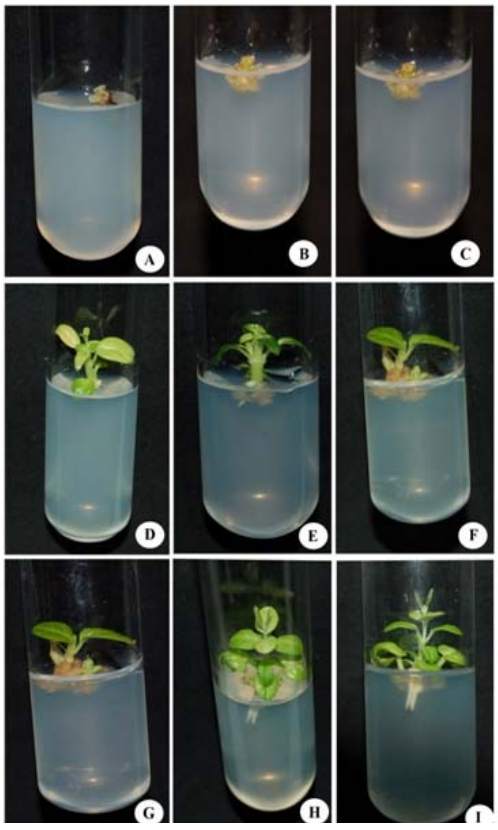

Plate-1. *In vitro* culture of *T. indica*

The optimum rooting auxins concentration was 1.25µM/l Zeatin + 2.75 µM/l NAA (Plate-1E). Root formation of *T. indica* internodal region derived from shoots on sub-culturing in rooting media showed significant higher rooting percentage (100%) at the optimum level of 1.25µM/l Zeatin + 2.75 µM/l NAA. Zeatin combination with an increase in the level of NAA was insignificant in increasing the rooting percentage seen on table-1. 100 percentages of root initiation was formed in the combination of 1.25µM/l Zeatin + 2.75 µM/l NAA as given in plate-1H & I.

Supplement of combination of BA+IAA in the basal media showed multiple shoot and root formation in *T. indica*. BA and KN as a cytokinin was found inhibitory at higher levels for multiple shoot induction (Plate-1H&I). Combination of NAA and zeatin were used for multiple root formation of *T. indica*. Similarly Faisal and Anis, (2003) has showed the dedifferentiated callogenic propagation route via adventitious shoot development in *T. indica.* In the present study, internode is used in different combination of cytokinins and auxins. It has produced the 100 percentage results on the shoots and roots formation. Thus an effective protocol for the micropropagation of *T. indica* was developed.

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