International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 25-10-2024, Accepted: 24-11-2024, Published: 09-12-2024
Volume 9, Issue 12, 2024, Page No. 11-15

Micropropagation studies from nodal and internodal explants of *Tylophora indica.* (Burm. f.) Merrill

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Abstract
This review provides an in-depth and comprehensive overview of the in micropropagation studies of *Tylophora* species, which have medicinal properties. It is a threatened medicinal plant (climber) of the family Asclepiadaceae. Here in the present study we developed an efficient micropropagation protocol from nodal and internodal explants of *Tylophora indica*. micropropagation is an alternative method of propagation of the threatened and endangered plant which can aid its conservation. The nodal and inter-nodal explants were cultured on MS medium containing different concentration and combinations of growth regulators like cytokines, 6- benzyl amino purine (BAP) and Kn. Multiple shoot buds were regenerated successfully from the nodal explants which were efficiently rooted on half strength MS medium supplemented with 6- benzyl amino purine (BAP) and Indole- 3-butyric acid (IBA). The regenerated plantlets were successfully transferred to the glasshouse, acclimatized and transferred to the field.

Keywords: *Tylophora indica*, asthma herb, micropropagation, nodal and internode explants, MS Medium

Introduction
Tylophora indica (Burm. f.) Merrill, commonly called Antamul or Indian ipecac, is an important medicinal plant belonging to the family Asclepiadaceae. It is a perennial, woody, climbing shrub and is found on plains, hilly slopes and the outskirts of the forests of eastern and southern India. The plant is used as folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis (CSIR 2003) [5]. It also seems to be a good traditional medical remedy for psoriasis, seborrhoea, anaphylaxis, leucopenia and it is an inhibitor of the Schultz– Dale reaction. The powdered leaves and roots contain the alkaloids tylophorine (responsible for strong anti-inflammatory action; Gopalakrishnan *et al*. 1980) [8] and tylophorinine. The roots also contain a potential anticancer alkaloid, tylophorinidine (Mulchandani *et al.* 1971) [12]. Several pharmaceutical companies (Acron Chemicals, Mumbai, India; Sabinsa Corporation, Piscataway, NJ, USA) are marketing T. indica extracts as antiasthmatic herbal drugs. The lack of proper cultivation practices and the indiscriminate way in which this plant is collected from its natural habitat pose a serious threat to its existence in the wild.
Moreover, propagation, either by seed or by vegetative cuttings, is rather difficult. Stem cuttings failed to produce proper root when treated with different growth regulators (Chandrasekhar *et al*. 2006) [2, 3]. Propagation through tissue culture offers a viable alternative for this species because it can also be used as a complimentary strategy for conservation and utilization of genetic resources. Further, *in vitro* plant regeneration through axillary bud culture is an easy and economic way of obtaining a large number of consistently uniform and true-to-type plants within a short span of time. So far a single report on the micropropagation of T. indica via axillary shoot proliferation has been reported (Sharma and Chandel 1992) [19]; this approach produced only a few shoots. There is an obvious need to develop an efficient regeneration system for effective conservation and rapid multiplication in order to meet market demands and to replenish highly impoverished populations. The objectives of the study reported here were to (1) optimize the culture conditions applied for the initiation and proliferation of shoots from nodal explants of T. indica through enhanced axillary branching, and (2) induce rooting in micro shoots and establish the plantlets in outdoor conditions. The present study was carried out to standardize efficient protocols for micropropagation *Tylophora indica* (Burm. f.) Merrill via nodal and internode explants.

Materials and methods
Material
Plant material of the *Tylophora indica* (Burm. f.) Merrill. In addition to their use in micropropagation studies, the plants were also planted in the Botanical Garden of Department of Botany, PVKN Govt Degree College (A), Chitoor, Andhra Pradesh and a plantation was raised in the form of a green hedge. *Tylophora indica* is a slow growing, perennial climber of tropical and subtropical regions leaves and stem spiral opposite petiolate leaves, entire, smooth shiny, varying in shape and size according to their age. Further, a good protocol for micropropagation was developed to aid in its multiplication and conservation.

Micropropagation studies
Tylophora indica plants were subjected to *in vitro* propagation and a good protocol for micropropagation was developed to aid in its multiplication and conservation. The micropropagation studies comprised the culture of nodal and internodal explants on different culture media under standard growth conditions. The nodal and intermodal explants were collected from mature and healthy field grown plants. They were washed under running tap water

for 15 min followed by soaking in 0.1 % (v/v) liquid detergent Tween-20 for 5 min and then subsequently washed with tap water. The explants were then soaked in 70% ethanol (C_2H_5OH) for 5 min followed by washing with water. Finally the explants were surface sterilized with 0.1% solution of mercuric chloride ($HgCL_2$) for 3 to 5 min followed by thorough rinsing in sterile distilled water (DH_2O). A total of thirty explants were inoculated in culture tubes containing MS medium (Murashige and Skoog medium) augmented with 2 % sucrose and 0.8 % agar and different combinations and concentrations of various plant growth regulators. The experiment was carried out in triplicates. Prior to that, the pH of the medium was adjusted to 5.8, autoclaved at 121ºC for 15 lbs / cm^2 for 15 min and allowed to cool before inoculation. The culture media comprised of the following: MS + BAP (0.5-3.5mg/l), MS + Kn (0.5-3.5mg/l0 and MS + BAP + Kn (0.5- 2.5 mg/l) + (0.5- 2.5 mg/l). All the inoculated cultures were incubated in sterile growth room under controlled conditions of 22 ± 1º C temperature, 75 % humidity and 2000 lux illumination of 16 hr / 8 hr L/D cycle. The 2 cm long regenerated shoots were transferred to root inducing media comprising half MS medium supplemented with MS + IBA (0.5 to 3.0 mg/l), BAP (0.5 to 3.5mg/l) and MS + IBA+BAP (1.0+2.0 mg/l). The regenerated plantlets were later transplanted to pots containing a mixture of soil and vermicomposting in the ratio of 2:1. The plantlets were gradually acclimatized on the laboratory bench by covering with a plastic bag with holes (to maintain high humidity), which were opened up gradually over a period of one week. The plants in the pots were moved to the glasshouse to a shaded area and gradually acclimatized.

Results and discussion

Initiation of shoot

Initiation of shoot, any part of organism size in 4-6 mm were shifted in supplemented culture medium by means of an assortment conc. of growth hormone of BA moreover unaccompanied otherwise within combinatorus through Kn. It was used 20 times intended in every treatment in addition to every experimentation be frequent this in addition to associate culture be finished the period of 84 days. For shoot initiation BAP conc. used from 0.5-3.5 mg/l, more number of shoots developed in 2.0 mg/l and length of shoot 0.94 cm. In Kn the conc. used from 0.5-3.5 mg/l high number of shoots obtained in the conc. of 2 mg/l in addition to the shoot length 1.45 cm. Out of these single phytohormones best results were obtained in MS medium containing BAP, more amount of shoots famed and also shoot length is high. The development of BAP showed alone or in combination by means of Kn was effected of shoot formation. MS medium with the combination of 2.0 mg/l BAP + 1.5 mg/l Kn where in the shoots number are improved up toward 13 and length of the shoots were 0.88 cm per culture (Table-1 and fig-1).

Rooting

The elongated regenerated shoots with fully expanded leaves were transferred to the rooting medium. Efficient rooting is important for successful transplantation and survival of the *in vitro* regenerated plantlets. Among different auxins tested BAP an IBA promoted the induction of roots efficiently. Shots obtained from the test tube experiment proliferated lateral buds were implanted in supplemented medium by means of different concentration of BAP and IBA (0.5 to 3 mg/l)/ (0.5 to 3 mg/l) for rooting. Maximum root induction percentage was observed in medium fortified with BAP+IBA (1.0+2.0 mg/l). The induction was gradually with increasing concentration of auxin. The mean number of roots ex plantlets was recorded after 95 days of initiation for the micro cuttings obtained from nodal segments.

In plants regenerated from nodal region mean roots number 4.2 was recorded. Length of Root about 3.42 cm in nodal region was observed (Table-2 and fig-3). Similarly BAP nodal explants mean number of roots 3.6 cm, in 1.5 mg/l conc. and root length of about 3.5 cm was observed. Finally the total plant transferred to green house for acclimatization.

Table 1: Shoot regeneration from nodal explants *Tylophora indica* on MS medium consisting of BAP+Kn after 8 weeks of culture

BAP+Kn conc.(mg/l)	No. of shoots mean	No. Of shoots ± SD	Mean Length of shoots(cm)	Length of shoot ± SD
05+0.5	1.4	1.14	0.48	0.54
1.0+1.0	2.2	1.30	0.4	0.70
2.0+1.5	5.8	1.92	0.88	1.14
2.0+2.0	2.8	1.30	0.64	0.83
2.0+2.5	2.4	0.54	0.48	0.54

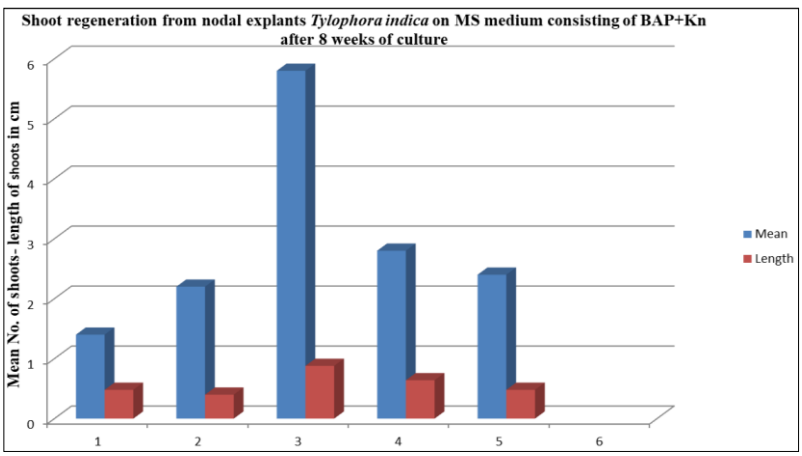

Fig 1

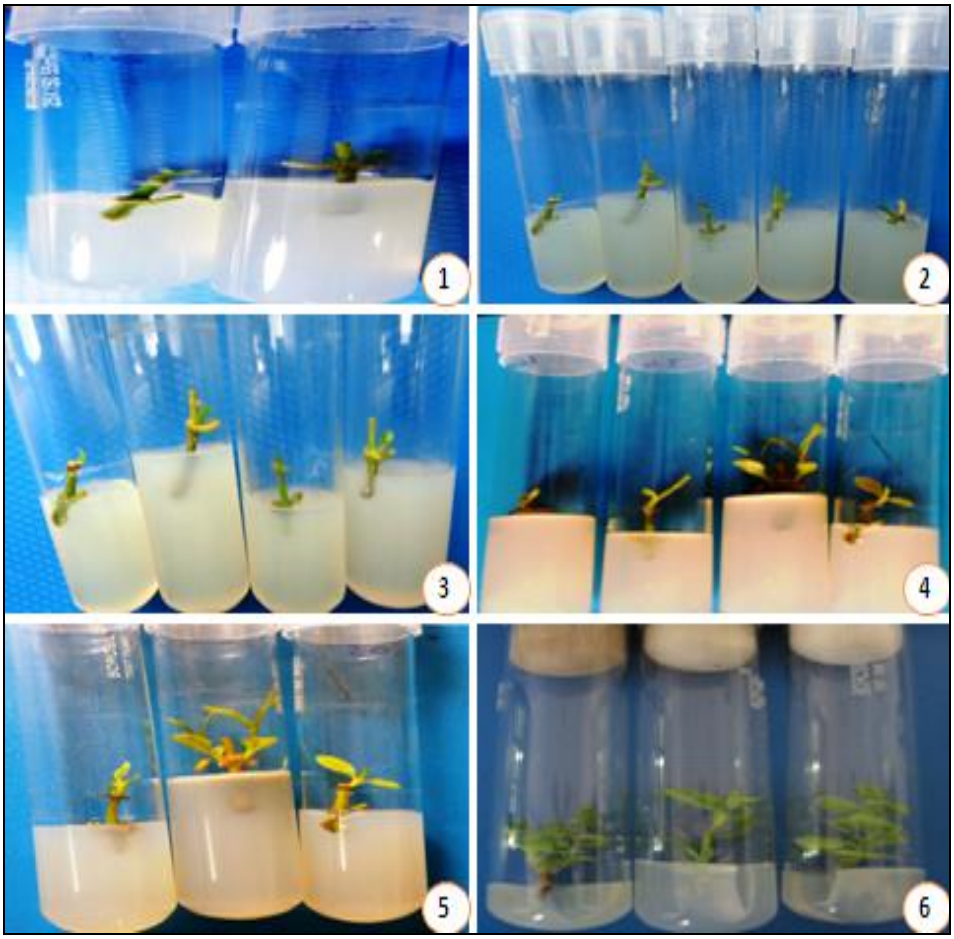

Fig 2: 1-3. Inoculation of nodal explants of *Tylophora indica* 4-5. Shoot regeneration from nodal explants, 15 days after inoculation, 6. Multiple shoots, 45 days after inoculation

Table 2: MS medium consisting of BAP+IBA for root development *in vitro* raised roots after 8 weeks of culture

Hormone conc.(mg/l) BAP+IBA	No. of roots mean	No. Of roots ± SD	Mean Length of roots(cm)	Length of root ± SD
0.5+.1.0	1.4	0.54	1.64	0.50
1.0+1.5	1.6	0.54	1.72	0.58
1.0+2.0	4.2	1.30	3.42	0.83
1.0+2.5	2.0	0.70	2.12	0.39
1.0+3.0	1.8	0.83	1.97	0.37

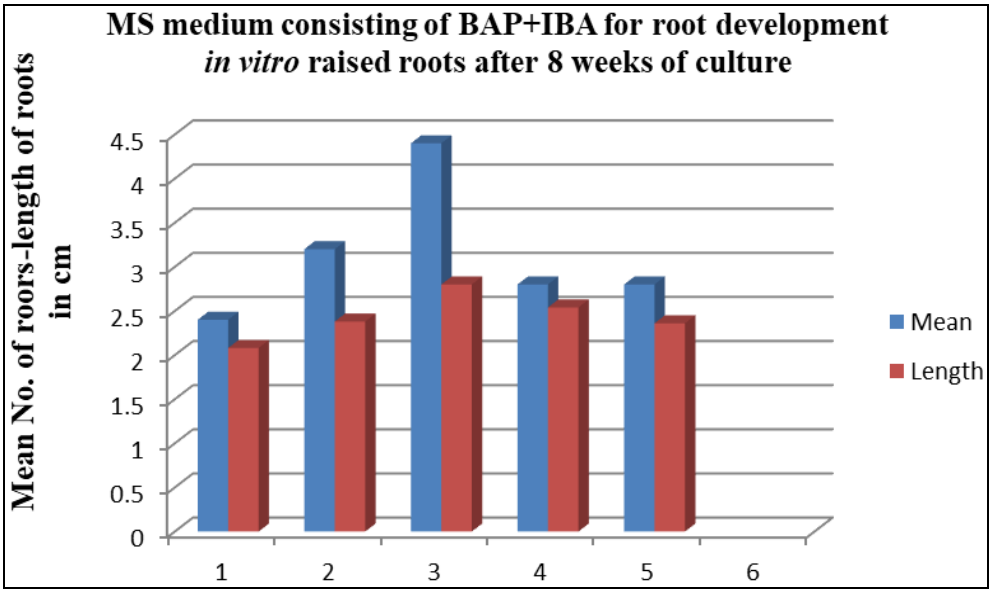

Fig 3

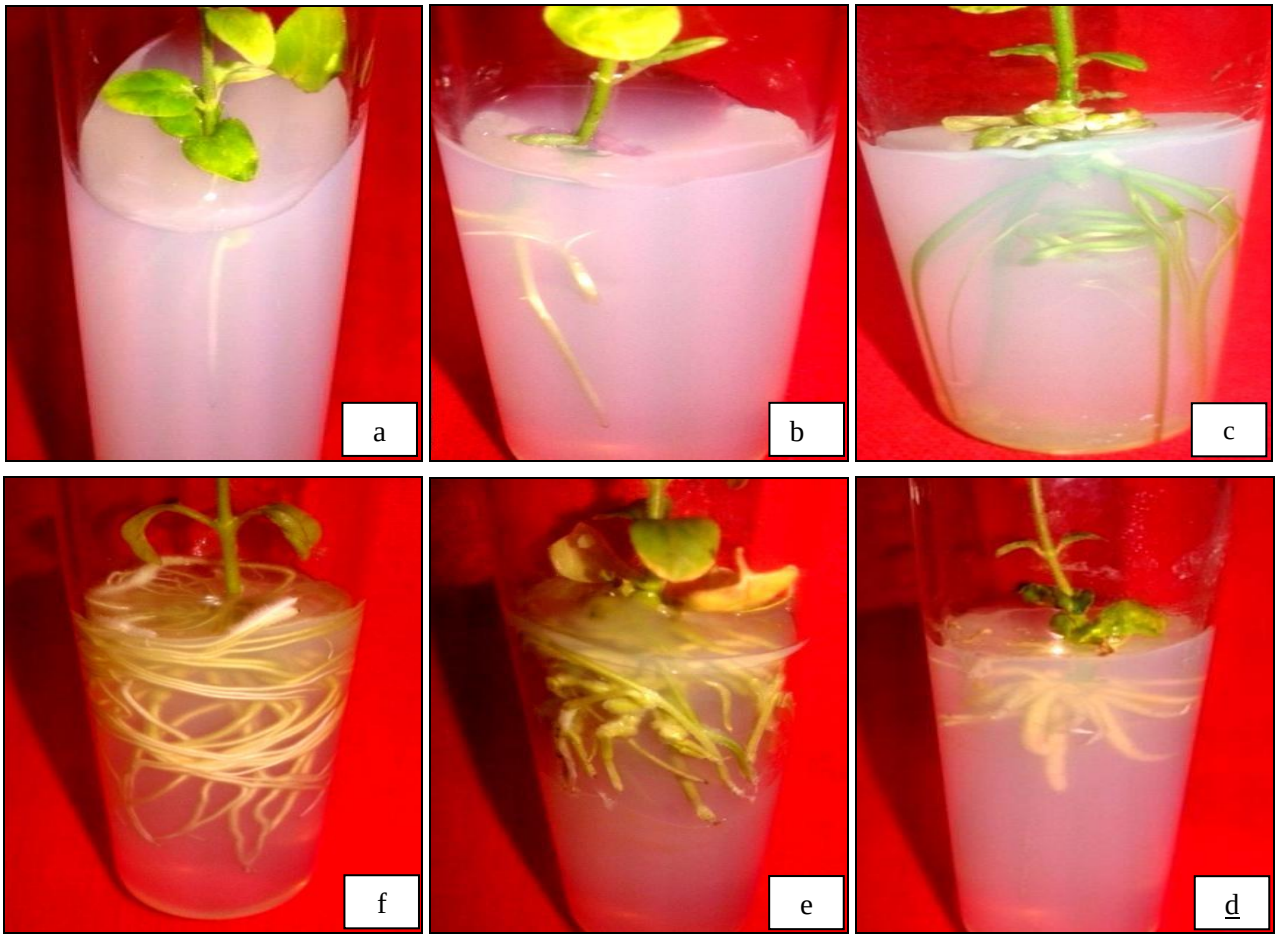

Fig. 4 Initiation of roots from basal end of regenerated shoot on MS medium. Fig. (a) and (b) Further elongation of roots after 2 weeks. Fig. (c) Formation of 5- 7 roots on MS medium. Fig. (d-e) Formation of thick branched roots on MS medium with BAP+IBA (1.0+2.0 mg/l) Fig. (f) Further growth and elongation of roots.

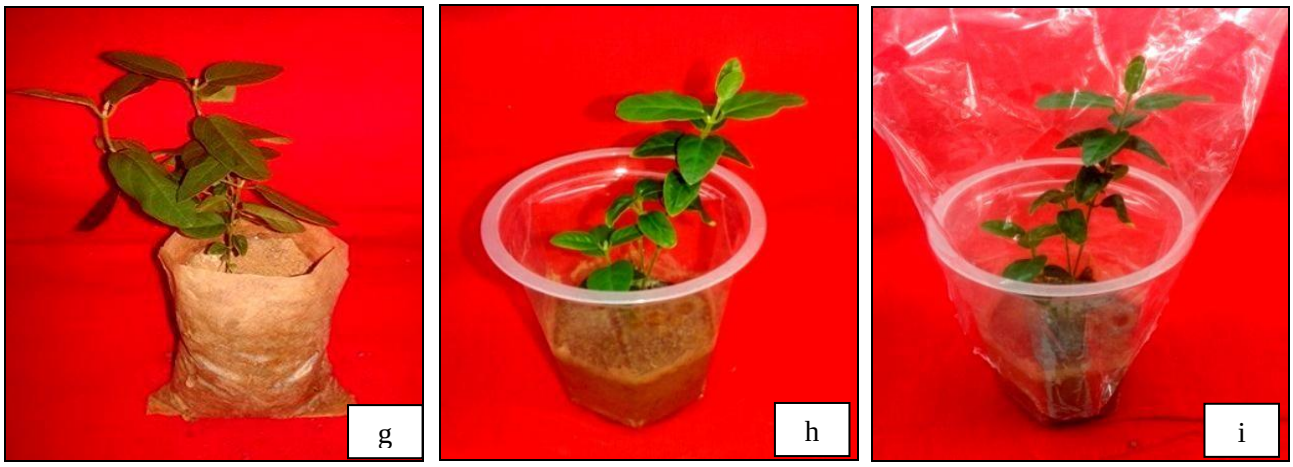

Fig 5: Plantlets in plastic cups containing potting mixture covered with perforated plastic bags. Fig. (g&h) Perforated plastic bag removed. Fig. (i) Plantlet transferred to poly bag and kept under growth room conditions

Discussion

The medicinal plant of *Tylophora indica* was accepted not in top of by means of an inspection to developed a copied of method designed in favor of its accumulation creation below *micropraparation* situation. Furthermore, proliferation means of relating to divide a part is to a certain extent not easy the same as plant stem divide a part not success appropriate the treated of roots by means of different type of hormones, (Chandrasekhar *et al*. 2006) [2, 3]. This is unfortunate velocity and seed germination as well find off it is big level proliferation (Thomas *et al*. 2005) [20]

Shoot organogenesis in addition to somatic embryogenesis were successfully tough (88-96%) as well as transferred to the field (Chaudhuri. K.N *et al*. 2004). Two weeks later of hardening, the plantlets were shifted to the green house anywhere they grew and established well showing a high velocity of survival (90%) (Manjula S *et al*. 2000) [11].

Efficient shoot multiplication using a combination of cytokinins from axillary bud explants has been reported earlier (Reddy *et al*. 1998; Sraswathy *et al*. 2002; Baskaran *et al*. 2007) [1, 14]. Superiority of BAP and Kinetin in combination has been found for micropropagation of other

woody perennials (Das *et al*. 2011; Komalavalli *et al.*1997) [6, 10]

The previous work report of IBA was the better then IAA and NAA for the induction of root in *Tylophora* through means of (Thomas *et al*. 2005) [20]. The good result of IBA was very efficient to induction of root is as well reported in to *Swainsona formosa* (Joy Thomps *et al.*1997) [9], *Acacia sinuata* (Lour.) Merr. (Shahzad *et al.* 2006) [17], *Melia azedarach* L. (Shahzad *et al.*2001) [16], *Mentha arvensis* L. (Shahzad *et al.* 2002) [18] and *Cunila galioides* Benth. (Fracaro *et al.* 2001) [7], Karoshi & Hedge have suggested 2500 ppm IBA treatment to improve the root in ability of apical shoot cuttings in *Tylophora indica*.

Conclusion

In conclusion the protocol reported *in vitro* propagation of *Tylophora indica. Tylophora indica* is very efficient for the production of a high frequency of adventitious shoot regeneration across a wide range of *Tylophora indica* genotype where the site of shoot differentiation is predictable and occurs in a short spon of 2 weeks. The present protocol describes on *in vitro* propagation from Nodal and internodal explants of *Tylophora indica.* High frequency regeneration of multiple adventitious shoots in over 90% of nodal and intermodal explants that are devoid of preexisting meristems. Such a generally applicable to biotechnological improvement of *Tylophora indica*, an important of the plants.

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