

PLANT REGENERATION FROM LEAF EXPLANTS OF *TYLOPHORA INDICA* (BURM.F.) MERRILL.: AN IMPORTANT MEDICINAL PLANT

MANU PANT AND PRABHA BISHT

Tissue Culture Discipline, Botany Division, FRI, Dehradun (Uttarakhand).

Introduction

Tylophora indica (Burm.F.) Merrill, a member of Asclepiaceae, commonly known as 'Antamul' grows as a wild climber native to the plain and hill forests of eastern and southern India up to an altitude of 900m (Anon., 1976). The roots and leaves of this indigenous medicinal plant have been traditionally used for the treatment of asthma, bronchitis, whooping cough, dysentery, diarrhoea, rheumatism, gouty pains and hydrophobia. They are also known to possess stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic properties (Shivpuri *et al.*, 1968). Pharmacological investigations have confirmed the anti-asthmatic effects of its leaf extracts (Shivpuri *et al.*, 1972). The major alkaloid tylophorine – has been reported to have immunosuppressive, anti-inflammatory (Gopalakrishnan *et al.*, 1980) and anti-tumour (Donaldson *et al.*, 1968) properties. The leaf and stem extracts as well as the minor alkaloid present-tylophorinidine-account for anti-leukemia properties (Mulchandani *et al.*, 1971, Gellert, 1982).

Over exploitation and lack of organized cultivation has led to rapid declination in wild populations of *T. indica*. Attempts to allow the replenishment or commercial plantations of this medicinally important herb are neither sufficient nor efficient. Consequently, the species is rapidly disappearing and is now listed as one of the plant species in India vulnerable to extinction (Faisal and Anis, 2003). With the conventional methods like vegetative propagation and seed propagation offering a number of constraints, (Jayanti and Mandal 2001) concerted efforts need to be made to conserve this species *ex-situ* through *in-vitro* methods.

In vitro propagation via callus culture would be a potent tool in ensuring mass multiplication of this important medicinal plant. Regeneration studies on callus and protoplast cultures of *T. indica* have previously been reported by Rao *et al.* (1970); Rao and Narayanswamy (1972); Mhatre *et al.* (1984); Faisal and Anis (2003; 2005); Dennis *et al.* (2005). The present investigation was undertaken to optimize a rapid and reproducible *in-vitro* regeneration system from field-derived leaf explants of *T. indica*.

Material and Methods

Plant material and sterilization

Leaves collected from 9-year-old mature plant of *T. indica* growing in nursery, were cut into appropriate sizes and washed under running tap water to remove the dust particles adhering to the surface. Thereafter; explants were washed with liquid detergent (Teepol, 5-10 drops/100 ml.) followed by washing with surfactant Tween-20 (2 drops /100 ml solution) in gentle agitating conditions for about 5 minutes and then washed under running tap water. Fungicide treatment [1% Bavistin (50% carbendazim WP)] was then given for 20 minutes. The explants were then surface sterilized with mercuric chloride solution (0.1% w/v) for 10 minutes followed by 4 to 5 rinses in sterilized distilled water to remove the traces of sterilant. Leaf bits were excised to appropriate sizes and incisions were made to promote callusing.

Media and culture conditions

The basal medium comprised the mineral salts and organic nutrients of the MS medium (Murashige and Skoog, 1962), fortified with 3% sucrose (w/v) and 0.7% bacteriological agar (w/v). The pH of medium was adjusted to 5.8 by using 1N NaOH or 1N HCl prior to addition of agar. Medium was sterilized by autoclaving at 1.0 kg/cm² (121°C) pressure for 15 min. All the cultures were incubated in a culture room at 25°C ± 2°C for 16 hours in light (illuminated by 40 watt cool white fluorescent tubes, 1200 lux) and 8 hours in dark.

Callus induction

BAP (4.44 - 13.32 µM), 2, 4-D (0.90 - 2.26 µM) and thidiazuron (TDZ) (0.23 - 0.68 µM), alone and in combination, were tested for their effect on callus formation from leaf explant. MS medium without any plant growth regulator was used as control. In all experiments, MS medium was also supplemented with 50 mg/l amoxicillin to prevent bacterial contamination.

Data on callus induction was evaluated visually by assigning degree of response ranging from + (less callusing) to ++++ (large amount of callus).

Shoot differentiation and multiplication

Resultant callus was transferred on to MS medium supplemented with varying concentrations and combinations of 6, Benzylaminopurine (BAP) (4.44-13.32

μM) alone and in combination with Indole-3-acetic acid (IAA) (1.14-2.85 μM) and/or GA_3 (0.58 μM) for differentiation of shoots. *In-vitro* regenerated shoots were subcultured onto fresh medium of same composition for further multiplication. Observations pertaining to average number of shoots and average shoot length were recorded after a period of 4 weeks.

***In vitro* Rooting**

Regenerated shoots having 1 or 2 nodes were excised and transferred on to half-strength MS medium supplemented with 2.46 μM indole-3-butyric acid (IBA) for *in vitro* rooting of shoots.

Hardening and Acclimatization

Once rooted, the regenerated plantlets were carefully extracted from tissue culture vessels and washed gently in water to remove excess agar medium and sucrose traces to discourage infection by fungal contaminants. The plantlets were blotted lightly on filter paper and then planted into polybags containing a mixture of soil: sand: manure (1:1:1) and maintained in mist chamber.

The plants were kept covered under polybags for 2 weeks to ensure high humidity so as to prevent excessive water loss during "hardening-off" period. The bags were periodically removed and after 3 weeks the plants were transferred to net house to acclimatize plants to field conditions.

Data analysis

Experiments were repeated thrice and data represent the mean of three experiments. Each treatment consisted of minimum 24 replicates. Mean and standard error was calculated from the data obtained.

Results and Discussion

The removal of all microorganisms with a minimum damage to the plant system to be cultured is the major objective of surface decontamination (Dodds and Roberts, 1985). In the present study, a 10 minutes decontamination period with 0.1% HgCl_2 was determined to be optimal for leaf explants. The medium used for callus induction was supplemented with 50 mg/l amoxicillin in order to minimize bacterial contamination that was observed in all experiments where the antibiotic was not used.

In our study, no callusing occurred on MS medium lacking PGRs. While evaluating the PGR treatments for callus production, leaf explants exhibited callus initiation after 2 weeks of culture. Wound treatment increased callusing only when the cut regions were in direct contact with the medium. Though all combinations showed

callusing, degree of callusing varied with different levels of PGRs. BAP was comparatively less efficiently in callus induction as compared to 2,4-D or TDZ. Optimal amount of highly proliferating greenish-white compact callus was obtained on full strength MS medium supplemented with 2, 4 dichlorophen oxyacetic acid (2, 4-D) (1.35 μM) and TDZ (0.68 μM) (Table 1; Figure A, B). 2, 4 -D is known to be the most common auxin used to initiate callus cultures (George, 2008). The efficiency of a combination of 2, 4-D and TDZ in inducing callus from leaf explants has been reported in *T. indica* (Thomas and Philip, 2005), *Hemerocallis* (Li *et al.*, 2010). Contrastingly, Faisal and Anis (2003) obtained optimal callusing on MS + 10 μM 2, 4-T from leaf explants of *Tylophora indica*.

In some cases, the callus may remain undifferentiated though showing prolific growth regardless of hormones and metabolites to which they are exposed. Such observations have been made in *Holarrhena antidysenterica* (Heble *et al.*, 1971), *Citrus grandis* (Chaturvedi *et al.*, 1974 a,b), *Ceropegia sahyadrica* (Nikam and Savant, 2007). Similar observations were made in the present study where shoot bud differentiation did not occur on callus-induction medium. Henceforth, after 5 weeks of culture, regenerated callus was shifted on to MS medium supplemented with BAP and /or IAA where callus began to produce organized tissue in the form of bud-like structures. Adventitious shoot bud differentiation became abundant within 4 weeks of transfer. Shoots originated from original bud-like structures were separated from one another and cultured on to fresh medium of same composition for further multiplication. Observations after 4 weeks revealed that MS medium supplemented with BAP (8.88 μM) and IAA (1.14 μM) gave a maximum number of 8.41 ± 0.30 shoots with shoot length 1.1 ± 0.05 . The synergistic effect of BAP and IAA in efficient shoot multiplication has been observed in *Leptadenia reticulata* (Arya *et al.*, 2003), *Vanasushava pedata* (Karuppusamy *et al.*, 2006), *Cardiospermum halicacabum* (Thomas and Maseena, 2006), *Tylophora indica* (Nema *et al.*, 2007), *Ruta graveolens* (Bohidar *et al.*, 2008).

In the present study, the differentiated buds on BAP + IAA supplemented medium did not elongate further and were in form of rosette clumps. Shoot elongation along with leaf formation was observed when GA_3 at a concentration of 0.58 μM was incorporated into the medium. On medium fortified with BAP (8.88 μM), IAA (1.14 μM) and GA_3 (0.58 μM) optimal amount of shoot multiplication with an average of 13.66 ± 0.42 shoots and mean shoot length 1.4 ± 0.08 cm was achieved after 4 weeks of culture (Table 2; Figure C,D).

Table 1

Effect of Plant Growth Regulators on Callus Induction from Leaf Explant of *Tylophora Indica*.

S.No.	MS Medium + Plant Growth Regulators Concentrations (μ M)			Degree of callusing
	BAP	2,4-D	TDZ	
Control	0.0	0.0	0.0	-
1.	4.44	0.0	0.0	+
2.	8.88	0.0	0.0	+
3.	13.32	0.0	0.0	+
4.	0.0	0.90	0.0	+
5.	0.0	1.35	0.0	++
6.	0.0	2.26	0.0	++
7.	0.0	0.0	0.23	++
8.	0.0	0.0	0.45	++
9.	0.0	0.0	0.68	++
10.	0.0	0.90	0.23	++
11.	0.0	0.90	0.45	++
12.	0.0	0.90	0.68	++
13.	0.0	1.35	0.23	++
14.	0.0	1.35	0.45	+++
15.	0.0	1.35	0.68	++++
16.	0.0	2.26	0.23	+++
17.	0.0	2.26	0.45	++
18.	0.0	2.26	0.68	++

Note: Number of '+' signs indicate the degree of callusing

Table 2

Effect of plant growth regulators on shoot regeneration from callus in *Tylophora Indica*.

S. NO.	MS medium + Plant Growth Regulators concentrations (μ M)			Number of Shoots (mean $\pm$ S.E.)	Shoot length in cm (mean $\pm$ S.E.)
	BAP	IAA	GA ₃		
Control	-	-	-	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
1.	4.44	0.00	0.00	1.70 $\pm$ 0.11	0.60 $\pm$ 0.04
2.	8.88	0.00	0.00	2.87 $\pm$ 0.20	0.60 $\pm$ 0.06
3.	13.32	0.00	0.00	2.58 $\pm$ 0.22	0.70 $\pm$ 0.05
4.	4.44	1.14	0.00	6.83 $\pm$ 0.31	1.0 $\pm$ 0.05
5.	8.88	1.14	0.00	8.41 $\pm$ 0.30	1.1 $\pm$ 0.05
6.	13.32	1.14	0.00	7.04 $\pm$ 0.24	1.0 $\pm$ 0.06
7.	4.44	2.85	0.00	4.79 $\pm$ 0.21	0.8 $\pm$ 0.04
8.	8.88	2.85	0.00	6.08 $\pm$ 0.23	1.0 $\pm$ 0.07
9.	13.32	2.85	0.00	6.16 $\pm$ 0.29	1.0 $\pm$ 0.05
10.	4.44	1.14	0.58	13.66 $\pm$ 0.42	1.4 $\pm$ 0.08
11.	8.88	1.14	0.58	14.25$\pm$0.62	1.9$\pm$0.06
12.	13.32	1.14	0.58	12.58 $\pm$ 0.56	1.72 $\pm$ 0.04

GA₃ is reported to be required for adventitious shoot development from callus once the meristemoids are formed (Jarret and Hasegawa, 1981). This observation is in confirmation with previous reports which indicate that GA₃ is conducive for *in vitro* differentiation of shoot buds and promotes shoot elongation shoot regeneration in works on *Rubus caesius* (Babic and Neskoic, 1984),

Simmondsia chinensis (Jacoboni and Standardi, 1987), *Saussurea lappa* (Arora and Bhojwani, 1989), *Ficus benjamina* (Delamomarco and Picazo, 1994), *Acacia sinuata* (Vengadesan *et al.*, 2000, 2003). *Isodon wightii* (Thirugnanasampandan *et al.*, 2010). For long-term maintenance of cultures routine subculturing was done on full strength MS medium supplemented with 1.0 mg/l

BAP after periodic intervals of 4 weeks.

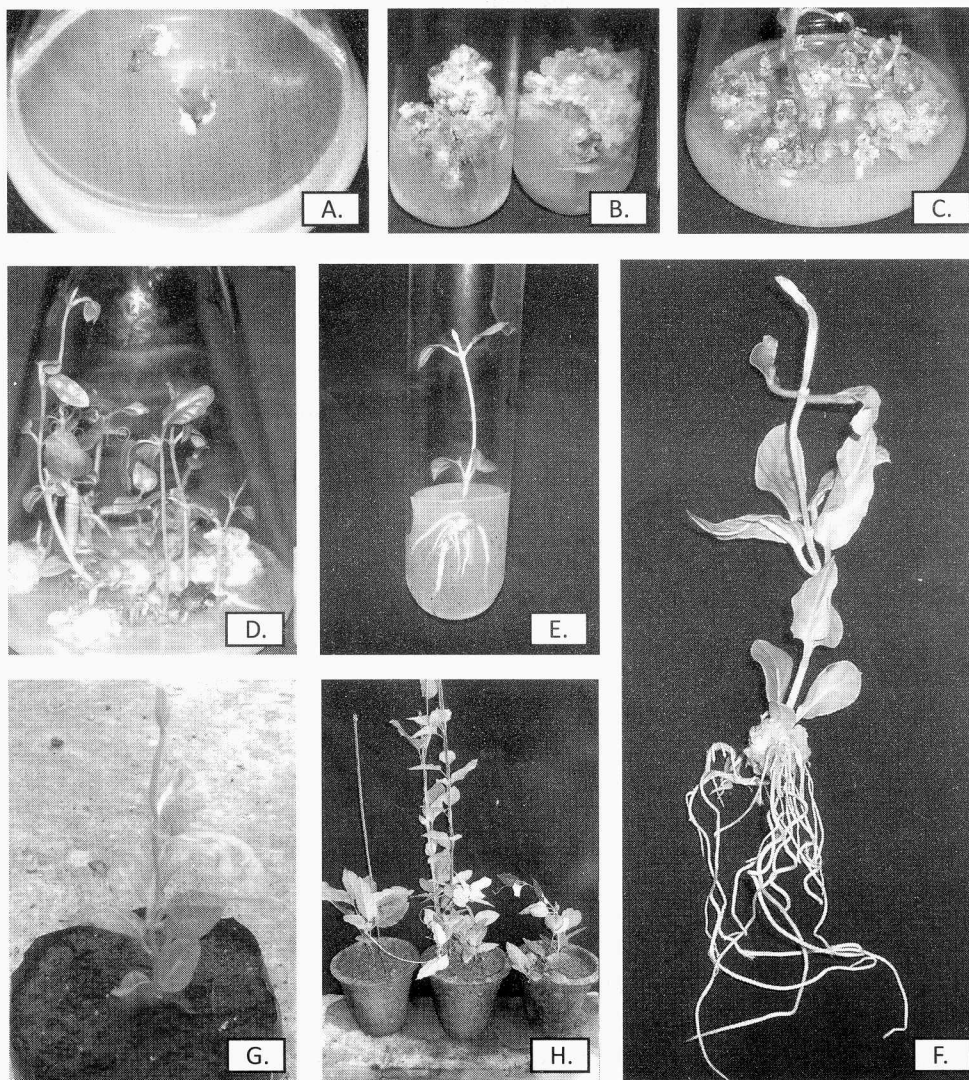
In our previous study on *in vitro* regeneration of *T. indica* through axillary bud culture, $\frac{1}{2}$ strength MS supplemented with $2.46\mu\text{M}$ IBA proved to be most efficient for *in vitro* rooting of shoots (Bisht *et al.*, 2009). Same combination was employed for rooting of *in vitro* raised shoots in the present study and 90% rooting was achieved (Figure E,F).

In vitro raised plants are heterotrophic in their mode of nutrition and cannot withstand the environmental conditions without proper hardening and acclimatization. In the present study hardening and acclimatization was carried out for 2 months before field transfer. The five week old tissue culture raised plantlets were transferred to polybags containing a mixture of soil: sand : manure (1:1:1), covered with perforated

polythene bags and kept in mist chamber for 3 weeks. Thereafter, they were shifted to open shade house conditions for acclimatization. The polythene bags were initially withdrawn periodically and later completely removed on emergence of new leaves (Figure G). In shade house the plants were further transferred to earthen pots and shifted to field conditions where they gave a survival rate of over 90% (Figure H).

The present communication connotes a simple and affordable *in vitro* protocol for large scale propagation of *Tylophora indica* through a dedifferentiated callus state. The method would be useful not only in providing a continuous supply of explants even at locations where these plants are not available but also for use in further genetic improvement and biochemical studies.

Figure (A- H)



Regeneration of plants from leaf explant of *Tylophora indica* (Burm. F.) Merrill.

A: Callus induced from leaf explant, B. Callus multiplication, C. Initiation of shoots, D. Multiple shoot formation, E. In-vitro rooting, F. Rooted plantlet ready for transfer to polybags, G. Hardened plantlet, H. Potted plants.

SUMMARY

A procedure for the regeneration of complete plantlets of *Tylophora indica* from cultured leaf callus is described. Callus was obtained from leaf explants on MS medium supplemented with varying concentrations and combinations of BAP, 2,4-D and TDZ. 2, 4-D (1.35 μ M) in combination with TDZ (0.68 μ M) proved to be most effective for callus induction. Adventitious shoots were regenerated from surface of callus after transferring onto shoot induction medium, the optimal hormone combination being BAP (8.88 μ M), IAA (1.14 μ M) and GA₃ (0.58 μ M) giving an average of 14.25 $\pm$ 0.62 shoots with mean shoot length 1.9 $\pm$ 0.06 cm after 4 weeks. Individual elongated shoots were rooted on half-strength MS medium containing 2.46 μ M IBA. The *in-vitro* raised plantlets with well developed shoot and roots were acclimatized successfully and shifted to field conditions with a survival rate of over 90%.

Key words: *Tylophora indica*, *in-vitro* regeneration, leaf explant, callus culture.

टायलोफोरा इण्डिका (बर्म वत्स) मेरिल की पत्तियों से लिए उपपौधों से पादप

पुनर्जनन कराना- महत्वपूर्ण औषध पादप

मनु पन्त व प्रभा बिष्ट

सारांश

संवर्ध - प्राप्त पर्णकिण से टायलोफोरा इण्डिका से पूरे उपपौधे पुनर्जनित कराने की विधि का वर्णन दिया गया है। पर्ण से काटे अंश से किण एमएस माध्यम द्वारा प्राप्त किया गया जिसमें विभिन्न संकेन्द्रेणों और संयोगों में बीएपी, 2, 4-डी और टीडीजैड मिलाया गया था। 2, 4-डी (1.35 μ M) जिसमें टीडीजैड (0.68 μ M) मिलाया गया था। किणप्रेरण के लिए सबसे अधिक प्रभावकारी पाया गया। आगन्तुक प्ररोह किण के तल से निकले जिन्हें प्ररोह प्रेरक माध्यम से स्थानान्तरित कर दिया था, इष्टतम हार्मोन संयोग बीएपी (8.88 μ M), इण्डोल एसेटिक अम्ल (1.14 μ M) और गिबबेरेलिक अम्ल (0.58 μ M) का रहा जिससे 14.25 $\pm$ 0.62 प्ररोह निकले जिनका माध्य लंबाई 1.9 $\pm$ 0.06 सेमी. लगाने के 4 सप्ताह बाद रहती पाई गई एकल लम्बे-बड़े प्ररोहों से आधी शक्ति वाले एम.एस.माध्यम में रखकर जड़े निकलवाई गई जिसमें 2.46 μ इण्डोल ब्यूटिरिक अम्ल मिलाया गया था। कांच में उगाए उप पौधे जिनमें सुविकसित प्ररोह और जड़े निकल आई थी सफलतापूर्वक ऋतु प्रभावित किए गए, जिन्हें फिर क्षेत्र दशा में बदल दिया गया। इनकी अतिजीवितता दर 90% सेमी ज्यादा रही।

References

- Anon. (1976). The wealth of India, Raw Materials. Vol. X: Publications and Informations Directorate. CSIR, New Delhi, India.
- Arora, R. and S.S. Bhojwani (1989). *In vitro* propagation and low temperature storage of *Saussurea lappa* C B Clarke, an endangered medicinal plant. *Plant Cell Rep.*, **8**: 44-47.
- Arya, V., N.S. Shekhawat and R.P. Singh (2003). Micropropagation of *Leptadenia reticulata* - a medicinal plant. *In Vitro Cell Dev. Biol.-Plant*, **39**: 180-185.
- Babic, V. and M. Neskovic (1984). Propagation of three blackberry cultivars from small apical buds *in vitro*. *J. Hort. Sci.*, **59**: 183-185.
- Bisht, P., M. Pant and J.M.S. Chauhan (2009). *In-vitro* clonal propagation through axillary bud-culture of *Tylophora indica* (Burm.F.) Merrill: a multipurpose medicinal plant. *Indian Forester*, **135** (11): 1505-1510.
- Bohidar, S., M. Thirunavoukkrasu and T.V. Rao (2008). Effect of plant growth regulators on *in vitro* micropropagation of 'Garden Rue' (*Ruta graveolens* L.). *Int. J. Integrative Biol.*, **3** (1): 36-43.
- Chaturvedi, H.C., A.R. Chowdhury and G.C. Mitra (1974b). Morphogenesis in stem callus tissue of *Citrus grandis* in long-term cultures - a biochemical analysis. *Curr. Sci.*, **43**: 139-142.
- Chaturvedi, H.C., A.R. Chowdhury, and G.C. Mitra (1974a). Shoot bud differentiation in stem- callus tissue of *Citrus grandis* and correlated changes in its free amino acid contents. *Curr. Sci.*, **43**: 139-142.
- Delamomarcro, J.B. and I. Picazo (1994). Effect of growth regulators on *in vitro* propagation of *Ficus benjamina* cv. Exotica. *Biologia Plantarum*, **36**: 167-173.
- Dennis, Thomas, J. and P. Boban (2005). Thidiazuron-induced high-frequency shoot organogenesis from leaf-derived callus of a medicinal climber *Tylophora indica* (Burm. F.) Merrill. *In Vitro Cell Dev. Biol. Plant*, **41**(2): 124-128.
- Dodds, J.H. and L.W. Roberts (1985). *Experiments in plant tissue culture*, Second edition. Cambridge University Press, Cambridge.
- Donaldson, G.R., M.R. Atkinson and A.W. Murray (1968). Inhibition of protein synthesis in Ehrlich ascites-tumor cells by the phenanthrene alkaloids tylophorine, tylocrine and cryptopleurine. *Biochem. Biophys. Res. Commun.*, **31**: 104-109.
- Faisal, M. and M. Anis (2003). Rapid mass propagation of *Tylophora indica* Merrill. via leaf callus culture. *Plant Cell Tiss Org Cult*, **75**: 125-129.
- Faisal, M. and M. Anis (2005). An efficient *in vitro* method for mass propagation of *Tylophora indica*. *Biologia Plantarum*, **49**(2): 257-260.
- Gellert, E. (1982). The indolizidine alkaloids. *J. Nat. Prod.*, **45**: 50-73.
- George, E. F. (2008). Plant Tissue Culture Procedure- Background. In: *Plant Propagation by Tissue Culture*, 3rd edition, Vol. I. (George, E.F., Hall, M.A. and Deklerk, G.J. Eds). The Background. Springer, Dordrecht, The Netherlands, pp. 1-28.

- Gopalakrishnan, C., D. Shankaranarayan and S.K. Nazimuddin (1980). Effect of tylophrine, a major alkaloid *Tylophora indica*, on immunopathological and inflammatory reactions. *IJMR*, **71**: 940-948
- Heble, H.R., S. Narayanaswamy and M.S. Chadha (1971). 2-4 methylenecholesterol in tissue cultures of *Holoarrhena antidysenterica*. *Z. Naturf.*, **26b**: 1382.
- Jacoboni, A. and A. Standardi (1987). Tissue culture of jojoba (*Simmondsia chinensis* Link.). *Acta Hort.*, **212**: 557-560.
- Jarret R.L. and P.M. Hasegawa (1981). An analysis of the effect of gibberellic acid on adventitious shoots formation and development from tuber discs of potato. *Env. Exp. Bot.* **21**: 436
- Jayanthi, M. and P.K. Mandal (2001). Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plants in *Tylophora indica* (Burm. f. Merrill.). *In Vitro Cell. Dev. Biol. Plant*, **37**: 576-580
- Karuppusamy, S., C. Kiranmai, V. Aruna, and T. Pullaiah (2006). Micropropagation of *Vanasushava pedata* – an endangered medicinal plant of South India. *Plant Tissue Cult. and Biotech.*, **16**(2): 85-94.
- Li, Z., K. Mize and F. Campbell (2010). Regeneration of daylily (*Heemerocallis*) from young leaf segments. *Plant Cell Tiss. Org. Cult.*, **102**(2): 199-204.
- Mhatre, M., V.A. Bapat and P.S. Rao (1984). Plant regeneration in protoplast cultures of *Tylophora indica*. *J. Plant Physiol.*, **115**: 231-235.
- Mulchandani, N.B., S.S. Iyer and L.P., Badheka (1971). Structure of Tylophorinidine, a new potential antitumor alkaloid from *Tylophora indica*. *Chem. India.*, **19**: 505-506.
- Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, **15**: 473-497.
- Nema, R.K., K.G. Ramawat, G.D. Gupta, Y.S. Tanwar and M. Mathur (2007). Rapid micropropagation of *Tylophora indica*. *Pharmacognocy Magazine*, **3**: 52-55.
- Nikam, T.D. and R.S. Savant (2007). Callus culture and micropropagation of *Ceropegia sahyadrica* and Kulk.: an edible starchy tuberous rare asclepiad. *Indian J. Plant Physiol.*, **12**(2): 108-114.
- Rao, P.S. and S. Narayanswamy (1972). Morphological investigations in callus cultures of *Tylophora indica*. *Physiol. Plant*, **27**: 271-276.
- Rao, P.S., S. Narayanswamy, and B.D. Benjamin (1970). Differentiation *ex ovulo* of embryos and plantlets in stem tissue cultures of *Tylophora indica*. *Physiol. Plant*, **23**: 140-144.
- Shivpuri, D.N., M.P.S. Menon and D. Prakash (1968). Preliminary studies in *Tylophora indica* in the treatment of asthma and allergic rhinitis. *J Assoc. Phys. India*, **16** (1): 9-15.
- Shivpuri, D.N., S.C. Singhal and D. Prakash (1972). Treatments of asthma with an alcoholic extract of *Tylophora indica*: a crossover, double-blind study. *Ann. Allergy*, **30**: 407-412.
- Thirugnanasampandan, R., G. Mahendran and V.N. Bai (2010). High frequency *in vitro* propagation of *Isodon wightii* (Benth) H. Hara. *Acta Physiol. Plant*, **32**: 405-409.
- Thomas, D.T. and B. Philip (2005). Thidiazuron induced high frequency shoot organogenesis from leaf derived callus of a medicinal climber: *Tylophora indica* (Burm.f.) Merrill. *In Vitro Cell Dev. Biol.-Plant*, **41**: 124-128.
- Thomas, T.D. and E.A. Maseena (2006). Callus induction and plant regeneration in *Cardispermum halicacabum* Linn. An important medicinal plant. *Scientia Horticulturae*, **108**(3): 332-336.
- Vengadesan, G., A. Ganapathi, R.P. Anand and V.R. Anbazhagan (2000). *In vitro* organogenesis and plant formation in *Acacia sinuata*. *Plant Cell Tiss. Org. Cult.*, **61**: 23-28.
- Vengadesan, G., A. Ganapathi, S. Amuth and N. Selvaraj (2003). High - frequency plant regeneration from cotyledon callus of *Acacia sinuata* (Lour.) Merr. *In Vitro Cell Dev. Biol.-Plant*, **39**: 28-33.
-