ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 07, Issue, 10, pp.15726-15730, October, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

EFFICIENT PROTOCOL FOR MICROPROPAGATION OF *TYLOPHORA INDICA* (BURM F.) MERILL AN ENDANGERED MEDICINAL PLANT THROUGH NODAL EXPLANTS

***Prashanti, M. and Rajeev, L.**

Department of Botany, Osmania University, Hyderabad, Telangana – 500007

ARTICLE INFO

Article History:
Received 16th July, 2017
Received in revised form
20th August, 2017
Accepted 27th September, 2017
Published online 10th October, 2017

Keywords:

Tylophora indica (Burm f.),
Micropropagation, Nodal explants,
Adenine sulphate, Shoot multiplication,
Root induction.

****Corresponding author***

ABSTRACT

The present study deals with an efficient *in vitro* propagation of an endangered medicinally important *Tylophora indica* (Burm f.) Merill well known in traditional medicine for the treatment of asthma, bronchitis, whooping cough and dysentery. Micropropagation of *T.indica* was carried out using various combinations and concentrations of growth hormones among which best result was observed with Adenine sulphate (2.0mg/l) in combination with BAP(1.0mg/l).A callus free multiple shoot formation was observed with the above combination. Rooting from the *in vitro* grown shoots were induced from ½ strength MS medium supplemented with IBA (1.0mg/l).The plantlets obtained were successfully acclimatized and transferred under field condition. The present protocol employed in this study can be used for mass propagation of *T.indica* to meet the pharmaceutical demands for commercial purpose.

Citation: Prashanti, M. and Rajeev, L. 2017. "Efficient protocol for micropropagation of tylophora indica (burm f.) merill an endangered medicinal plant through nodal explants, *International Journal of Development Research*, 7, (10), 15726-15730.

INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. *Tylophora indica* (Burm f.) Merrill. commonly known as "Antmool" is a perennial plant belongs to family Asclepiadaceae found mostly in south and east India. The plant contains several phenanthroindolizidine alkaloids (Gellert 1982) among which tylophorine being the major alkaloid possessing immunosuppressive, anti-inflammatory (Gopalakrishnan *et al.,* 1980) and anti-tumor (Donaldson *et al.,* 1968) properties, it also possess anticancer activity because of the presence of tylophorinidine isolated from the roots (Mulchandani *et al.,* 1971). They also show stimulant and diaphoretic properties and also used for the treatment of asthma (Shivpuri *et al.,* 1968) and rheumatic gouty pains (Anonymous, 1976). Due to poor vegetative propagation, low seed viability, low germination rate and over exploitation from natural strands an effective micropropagation protocol needs to be developed to meet the pharmaceutical needs. Micropropagation of *T. indica* were earlier reported using various growth regulators and explants by (Sharma and Chandel 1992; Faisal *et al.,* 2007) and callus-mediated somatic embryogenesis from leaf (Jayanthi and Mandal 2001; Chandrasekhar *et al.,* 2006; Sahai *et al.* 2010), inter nodal (Thomas 2006). Though much work was reported earlier, no work using adenine sulphate in combination with BAP has been reported. Hence the present study was taken up by using this combination to achieve a higher frequency of regeneration from nodal explants of *T.indica.*

MATERIALS AND METHODS

Plant material and sterilization

Healthy explants (Leaf, node and internode) were collected from the Herbal garden, Department of Botany, Osmania University. They were thoroughly washed under running tap water for 20 minutes, followed by intermittent shaking in few

drops of Tween 20 (2 drops in 100ml of water) for 1 min and rinsed with distilled water. The explants were again sterilized with 0.1% Hgcl for 6-8 min and finally rinsed with sterile distilled water for 15-20 min. The surface sterilized explants were cultured on MS medium (Murashige and Skoog 1962) containing various concentrations of growth regulators [6-benzylaminopurine (BAP), Kinetin (KN), Adenine sulphate (AS)] with varied concentrations either alone or in combination. The pH of the medium was adjusted to 5.8 and medium was autoclaved at 121° C at 15 psi pressure for 20 min. Medium was dispensed into 20 ml culture tubes and explants were inoculated into the culture medium under aseptic conditions and maintained at 25±2° C under 16 h photoperiod.

Multiple shoot formation

Shoot initiation was observed on MS culture medium supplemented with various plant growth regulators at different concentrations (0.1-3.0mg/l) either alone and in combination. Subculturing was done every 4 weeks from *in vitro* grown shoots having 2-3 nodes for shoot elongation. The results of number of shoots per explant and the shoot length were recorded after 8 weeks of culture.

Root induction

In vitro elongated shoots (5-6cm length) were cultured onto full strength and half strength MS medium supplemented with different concentrations of IAA and IBA (0.1-3.0 mg/l) and

Table 1. Effect of 6'benzyladenine purine (BAP) on shoot regeneration from nodal explants of *T.indica* after 8 weeks of culture

Sl.no	Concentration of growth regulator (mg/l)	Regeneration (%) of nodal explants	Mean Shoot number per explant (Mean ± SE*)	Mean Shoot length(cm) (Mean ± SE*)
1	0.1	45	1.88±0.08	1.05±0.05
2	0.5	58	2.65±0.19	2.76±0.11
3	1.0	74	5.4±0.25	4.08±0.26
4	1.5	70	4.25±0.36	2.98±0.16
5	2.0	65	3.83±0.09	268±0.10
6	2.5	56	3.05±0.04	1.13±0.07
7	3.0	50	1.95±0.14	1.08±0.07

* All the Values are mean±SE of three independent. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test (P ≤0.05).

Table 2. Effect of Adenine sulphate (AS) on shoot regeneration from nodal explants of *T.indica* after 8 weeks of culture

Sl.No	Concentration of growth regulator (mg/l)	Regeneration (%) of nodal explants	No. of shoots per explant (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
1	0.1	45	1.16±0.098	1.07±0.05
2	0.5	60	2.58±0.17	2.16±0.080
3	1.0	75	3.55±0.16	3.13±0.08
4	1.5	80	4.63±0.14	4.12±0.10
5	2.0	92	6.83±0.19	5.13±0.12
6	2.5	80	4.767±0.14	3.95±0.076
7	3.0	75	4.16±0.16	3.13±0.11

*All the Values are mean±SE of three independent. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test (P ≤0.05).

Table 3. Effect of Kinetin (Kn) on shoot regeneration from nodal explants of *T.indica* after 8 weeks of culture

Sl.No	Concentration of growth regulator (mg/l)	Regeneration (%) of nodal explants	No. of shoots per explant (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
1	0.1	30	0.95±0.07	0.96±0.08
2	0.5	40	2.6±0.16	2.2±0.09
3	1.0	60	3.4±0.21	3.1±0.096
4	1.5	85	4.8± 0.20	4.0±0.09
5	2.0	75	3.48±0.17	3.6±0.16
6	2.5	65	2.76±0.23	2.9±0.06
7	3.0	45	1.15±0.076	2.1±0.067

*All the Values are mean±SE of three independent. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test (P ≤0.05).

Table 4. Effect of 6'benzyladenine purine (BAP) + Adenine sulphate (AS) on shoot regeneration from nodal explants of *T.indica* after 8 weeks of culture

Growth Regulator concentration (mg/l)	Regeneration (%) of nodal explants	No. of shoots per explant (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
1.0 + 0.1	46	2.41±0.11	3.03±0.12
1.0 + 0.5	51	4.23±0.24	4.25±0.20
1.0 + 1.0	78	7.55±0.26	6.15±0.21
1.0 + 2.0	84	10.82±0.39	8.28±0.33
1.0 + 3.0	72	7.53±0.20	5.06±0.16
1.0 + 4.0	59	5.18±0.13	4.38±0.35

*All of the experiments were conducted with a minimum of 20 replicates per treatment. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test at 0.05 level of significance.

Table 5. Effect of IBA on root regeneration from *in vitro* developed shoots of *T.indica* in MS medium after six weeks of culture

Sl.no	Concentration of IBA(mg/l)	Regeneration (%)	No.of roots per shoot (Mean ± SE*)	Root length(cm) (Mean ± SE*)
1	0.1	40	3.08±0.09	4.68±0.070
2	0.5	55	4.03±0.12	7.78±0.087
3	1.0	79	8.73±0.12	10.05±0.084
4	1.5	69	5.18±0.09	7.36±0.14
5	2.0	50	3.36±0.13	5.1±0.089
6	3.0	45	2.01±0.047	3.63±0.12

*All the Values are mean±SE of three independent. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test (P ≤0.05).

Table 6. Effect of IAA on root regeneration from *in vitro* developed of *T.indica* in MS medium after six weeks of culture

Sl.No.	Concentration of IAA(mg/l)	Regeneration(%)	No.of roots per shoot (Mean ± SE*)	Root length(cm) (Mean ± SE*)
1	0.1	45	2.91±0.09	2.2±0.08
2	0.5	57	3.48±0.11	3.43±0.12
3	1.0	60	4.08±0.07	3.8±0.08
4	1.5	56	3.0±0.070	3.1±0.16
5	2.0	52	2.18±0.06	2.81±0.07
6	3.0	45	1.96±0.06	2.35±0.11

*All the Values are mean±SE of three independent. The data were analyzed using one-way analysis of variance (*ANOVA*) and means were compared using Tukey's test (P ≤0.05).

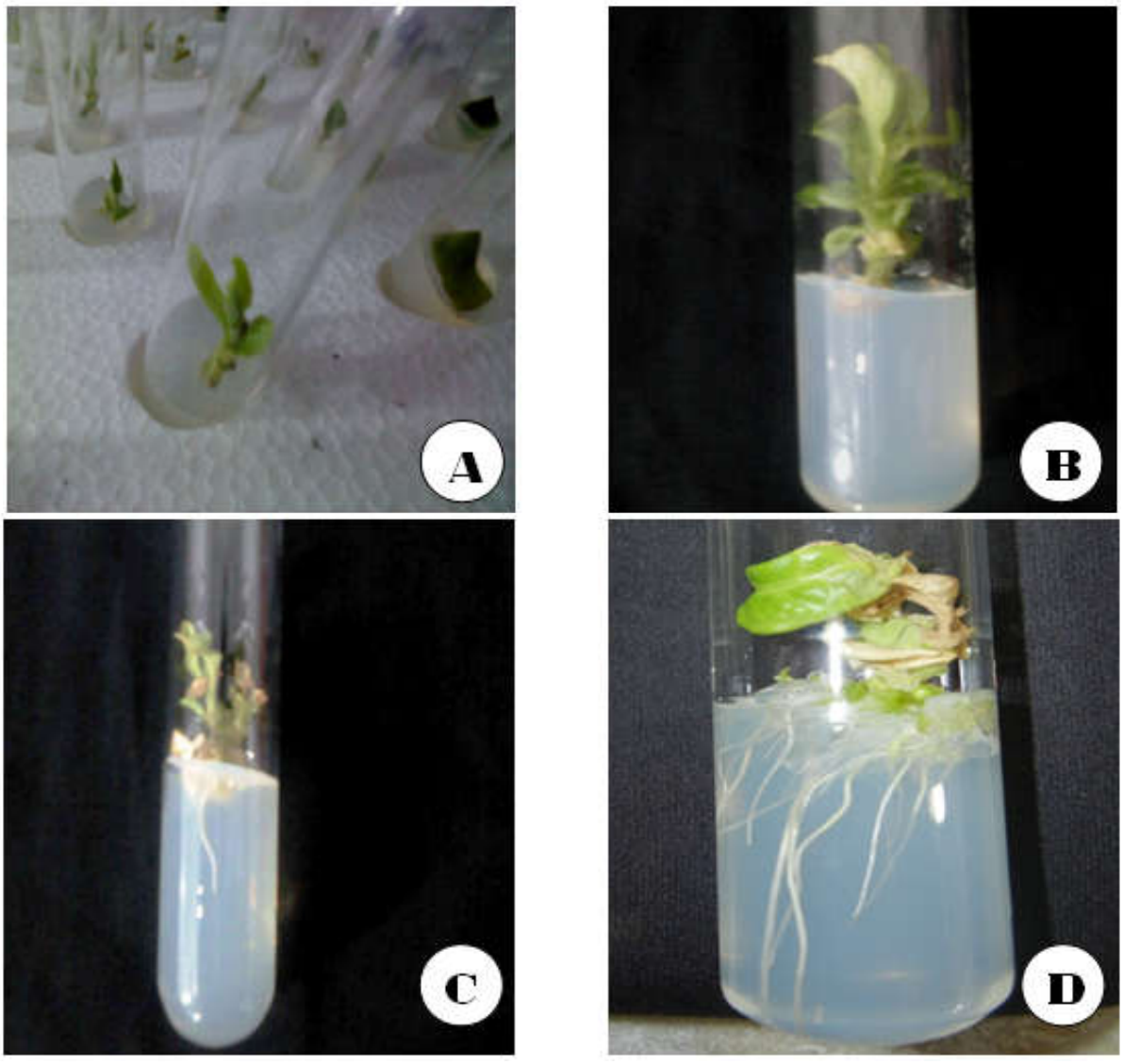

Fig1 (A-D). Various stages of i*n vitro* propagation of *T.indica*: (A): Shoot initiation from nodal explant (B) Multiple shoot regeneration on MS+AS (2.0mg/l) + BAP (1.0mg/l) after 8 weeks of inoculation, (C): Root initiation from *in vitro* raised shoot, (D):Multiple root formation on ½ MS+IBA (1.0 mg/l)

data was recorded on percentage of rooting, mean number and length of the roots after 8 weeks. Completely i*n vitro* developed plantlets were thoroughly washed to remove agar traces and transferred to plastic cups containing sterilized sand and soil (1:2) and acclimatized in the green house.

RESULTS AND DISCUSSION

Among different explants cultured on MS medium (leaf, internode and node), nodal explants responded well and fast by shoot bud initiation within a week. Callus formation was observed with leaf explants cultured on MS medium supplemented with all the concentrations and combinations of growth regulators while internode explants showed poor response. Nodal explants did not respond on MS medium without growth regulators. The percentage of response, shoot number and shoot length varied with various concentrations of growth hormones. Though all the concentrations of BAP, AS and Kn alone facilitated the shoot initiation, better response was seen in combination of Adenine sulphate and BAP (Table 1, 2, 3). The morphological response of nodal explants to various concentrations of AS and BAP was summarized in Table 4. Though nodal explants on MS medium responded moderately with AS and BAP alone, highest multiple shoots was recorded with Adenine sulphate (2.0mg/l) in combination with BAP (1.0mg/l) with mean number of shoots (10.82±0.39) and shoot length (8.28±0.33)(Fig1 A&B). Lower concentration of AS below the optimal concentration (0.1mg/l -2.0mg/l) produced less number of shoots (Table 4). When the concentration of AS was increased above 2.0mg/l a decreased number of shoot formation and shoot length were observed. Our results of AS shows similar results with Shamy (2002) in Bougainvillea plant, Hassan (2011) in *Balanites aegyptiaca L.* which proved that adenine sulphate alone did not showed much significance in producing more number of shoots.

BAP in combination with an auxin has been demonstrated in many medicinal plants from the Asclepiadaceae family, such as *Gymnema sylvestre* (Reddy *et al.,* 1998), *Holostemma annulare* (Sudha *et al.,* 1998), *Hemidesmus indicus* (Sree kumar *et al.,* 2000), *Holostemma ada-kodien* (Martin 2002), *Leptadenia reticulate* (Arya *et al.,* 2003), and *Ceropegia candelabrum* (Beena *et al.,* 2003). Kn proved to be the best in *Phyllanthus urinaria* producing maximum shoot proliferation, where as it showed contrast results in our study producing less number of shoots when compared with AS and BAP. The best rooting was observed with half strength MS medium compared to full strength MS medium. Of the different concentrations of IAA and IBA tested, best response was obtained with (1.0 mg/l) IBA with (8.73±0.12) number of roots and root length (10.05±0.084)(Table 5&6)(Fig1 C&D). Lower concentration of IBA facilitated better root formation than the higher concentration. Superiority of IBA over IAA and NAA in root formation has also been reported in by (Fracro 2001) in *Cunila galoides* and (Shahzad 2007) in *Clitoria ternatea*. Similar responses were observed in different plant species (Sahoo and Chand 1998; Komalavalli and Rao 2000; Sivakumar, and Krishnamurthy 2000).

Conclusion

In conclusion, the present study is an efficient protocol of multiple shoot formation from nodal explants that can be used for large scale mass propagation and conservation of *T.indica* to overcome problems in conventional methods of propagation. This protocol imparts highly repeatable, successful and rapid technique that can be utilized for the commercial propagation and *ex situ* conservation of this medicinal plant.

Acknowledgements

The author(s) are thankful to Department of Botany, Osmania University for providing funding and facilities to carry out this research work.

REFERENCES

Anonymous. 1976. The wealth of India. Raw Materials, Vol. X: Publication and Information Directorate. CSIR, New Delhi, India.

Arya, V., Shekhawat, N.S. and Singh, R.P. 2003. Micropropagation of *Leptadenia reticulata*—a medicinal plant. *In vitro Cell Dev Biol Plant,* 39:180–185

Beena, M.R., Martin, K.P., Kirti, P.B. and Hariharan, M. 2003. Rapid *in vitro* propagation of medicinally important *Ceropegia candelabrum*. *Plant Cell Tiss Org Cult,* 72:285–289

Chandrasekhar, T., Hussian, M.T., Gopal, G.R. and Rao, J.V.S. 2006. Somatic embryogenesis of *Tylophora indica* (Burm. f.) Merril. An important medicinal plant. *International Journal of Applied Sciences & Engineering* 4: 33–40.

Donaldson, G.R., Atkinson, M.R. and Murray, A.W. 1968. Inhibition of protein synthesis in Ehrlich ascites-tumor cells by the phenanthrene alkaloids tylophorine, tylocrebrine and cryptopleurine. *Biochem Biophys Res Commun*, 1: 104–109.

El-Shamy, H.A.A., 2002. *In vitro* culture studies on Bougainvillea plant, Ph. D. Thesis, Fac.of Agric., Zagazig Univ.

Faisal M. Ahmed Naseem and Mohammad Anis, 2007. An efficient micropropagation system for *Tylophora indica*: an endangered, medicinally important plant. Plant Biotechnology Reports, 1 (3), 55-161.

Fracro, F. and Echeverrigaray, S. 2001. Micropagation of *Cunila galoides*, a popular medicinal plant of South Brazil. *Plant Cell Tiss Org Cult.* 64: 1-4.

Gellert, E. 1982. *Journal of Natural Products,* 45, 50–73.

Gopalakrishnan, C., Shankaranarayan, D., Nazimudeen, S.K. and Kameswaran, L. 1980. Effect of tylophorine, a major alkaloid of *Tylophora indica*, on immunopathological and inflammatory reactions. *Indian Journal of Medical Research,* 71: 940–948.

Hassan, H.M.S. 2011.*In vitro* micropropagation of *Balanites aegyptiaca*, L plants. Ph. D. Thesis, Fac. of Environ. Agric. Sci. Suez Canal Univ.

Jayanthi, M. and Mandal, P.K. 2001. Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plantlets in *Tylophora indica* (Burm. f.) Merrill. *In Vitro* Cellular & Developmental Biology- Plant 37:576–580.

Komalavalli, N. and Rao, M.V. 2000. In vitro Micropropagation of *Gymnema sylvestre* –A Multipurpose Medicinal Plant. *Plant Cell Tiss. Org. Cult.,* 61, 97-105.

Martin, K.P. 2002. Rapid propagation of *Holostemma ada-kodien Schult*., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep* 21:112–117

Mulchandani, N.B., Iyer, S.S. and Badheka, L.P. 1971. Structure of tylophorinidine: a new potential antitumor alkaloid from *Tylophora indica*. *Chem Ind,* 19:505–506.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue Natural Products., 54 (4): 986-992.

Reddy, P.S., Gopal, G.R. and Sita, G.L. 1998. *In vitro* multiplication of *Gymnema sylvestre* R. Br. an important medicinal plant. *Curr Sci,* 75:843–845

Sahai, A., Shahzhad, A. and Anis, M. 2010. High frequency plant production via shoot organogenesis and somatic embryogenesis from callus in *Tylophora indica*. Turkish journal of Botany.

Sahoo, Y. and Chand, P.K. 1998. Micropropagation of *Vitex negundo* L., a Woody Aromatic Medicinal Shrub, Through High Frequency Axillary Shoot Proliferation. *Plant Cell Rep.,* 18, 301-307.

Shahzad, A., Faisal, M. and Anis, M. 2007. Micro propagation through excised root culture of *Clitoria ternatea* and comparison between *in vitro* regenerated plants and seedlings. *Ann Appl Biol.,* 150: 341-349.

Sharma, N. and Chandel, K.P.S. 1992. Effects of ascorbic acid on axillary shoot induction in *Tylophora indica* (Burm. F) Merrill. Plant Cell, *Tissue & Organ Culture,* 29: 109-113.

Shivpuri, D.N., Menon, M.P. and Prakash, D. 1968. Preliminary studies in *Tylophora indica* in the treatment of asthma and allergic rhinitis. *J. Assoc. Phys. Ind.* 16(1): 9-15.

Sivakumar, G. and Krishnamurthy, K.V. 2000. Micropropagation of *Gloriosa superba* L. an Endangered Species of Asia and Africa. Current Science, 78(1), 30–32.

Sreekumar, S., Seeni, S. and Pushpangadan, P. 2000. Micropropagation of *Hemidesmus indicus* for cultivation and production of 2-hydroxy 4-methyl benzaldehyde. *Plant Cell Tiss Org Cult,* 62:211–218

Sudha, G.C., Krishnan, P.N. and Pushpangadan, P. 1998. *In vitro* propagation of *Holostemma annulare* (Roxb.) K. Schum. A rare medicinal plant. *In vitro Cell Dev Biol Plant,* 33:57–63.

Thomas, T.D. 2006. Effect of sugars, gibberellic acid and abscisic acid on somatic embryogenesis in *Tylophora indica* (Burm. F.) Merrill. *Chinese Journal of Biotechnology,* 22(3): 465-47

****