Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Rapid Micropropagation of *Tylophora indica*

R.K.Nema[a*], K.G.Ramawat[b], G.D.Gupta[c], Y.S.Tanwar[a], Meeta Mathur[b]

[a]*Bhupal Nobles' College of Pharmacy, Udaipur*
[b]*Laboratory of Biomolecular Technology, College of Science, MLS University, Udaipur*
[c]*Sagar Institute of Pharmacy, Sagar*
**Corresponding Author-E mail: nema_pharmacy@yahoo.co.in*

ABSTRACT - *In vitro* raised shoot culture of *T. indica* was established on MS medium with 2.5 mg/l BA and 0.1mg/l IAA. An excellent rate of shoot multiplication was achieved. The tissue culture-generated shoots were rooted in the medium containing IAA for in vitro development of roots. A highly regenerative system has been developed by using leaf explants from mature plant. This system can be effectively used to multiply the plant material.

KEY WORDS: Micropropagation, *Tylophora asthmatica, Tylophora indica*.

INTRODUCTION

Tylophora indica (Burm.f.) Merrill (Asclepiadaceae) syn. *T. asthmatica* W. and A. (Shah and Kapoor, 1974) is commonly known as "Dama-Bel" which is a perennial branching climber which grows wild in the plain forests in India. *Tylophora* comprises of 50 species which are distributed in Africa, Asia, Australia and Oceanic Islands. Some of its species have reputation in folklore medicine. Both alkaloidal and non-alkaloidal constituents have been isolated and characterized from them(1).The powdered leaves, stem and root contains 0.2-0.3 percent (up to 0.42-0.46 %) of alkaloids. The alkaloidal content is not affected by seasonal variations (2, 3).

The plant is a small, slender, much branched, under shrub, or twining, pubescent herb, perennial twining climber with long and fleshy roots. Stems are elongate and glabrous but not much branched. Leaves are 5-10 cm by 2.5-5.7 cm, elliptic- oblong and acute at the top. Flowers are in umbel shape, penducle arises between the petioles. Ascending up to 1,260 m, rootstock 2.5-5 cm thick, roots stout, cord like, covered with light brown, corky bark, longitudinally fissured, leaves ovate or lanceolate, thick; flowers greenish yellow outside, purplish within, in many flowered umbels, follicles fusiform, divaricate, follicles ovoid lanceolate, glabrous, with thick pericarp, 5 cm long, seed broadly ovoid, flat (4,5).

Asthma is one of the most common chronic diseases in modern society and there is increasing evidence to suggest that its incidence and severity are increasing. This plant has been traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, bronchitis, rheumatism, and dermatitis. *T. indica* also produces antiallergic activity which is comparable to that of disodium cromoglycate on perfusion of sensitized rat lungs (6).

The present investigation deals with development of efficient and reproducible methods for rapid multiplication of Tylophora *indica* whose pharmaceutical value has been established. *T. indica* is rapidly disappearing and is now listed as one of the plant species in India vulnerable to extinction. Therefore, it is necessary to devise a method for the development of potentially large-scale multiplication protocol for commercial production of this endangered species.

MATERIALS AND METHODS

Plants of *T. indica* were collected from the different localities near Udaipur and maintained in botanical garden of College of Science, M. L. S. University, Udaipur. Leaves were washed with tap water for at least 30 minutes followed by soaking in 5% v/v teepol for 5 minutes then subsequently rinsed with distilled water and then with 70% alcohol. The leaf explants were disinfected by immersing in mercuric chloride solution (0.1% w/v) in sterilized distilled water for five minutes and subsequently rinsed thrice with sterilized distilled water on laminar air flow bench.

The Murashige and Skoog (1962) medium was gelled with 0.8% w/v Agar Type-I (Himedia) and pH of the medium was adjusted to 5.8 with 0.5 N HCl or NaOH after incorporation of all the ingredients and various concentrations of plant growth regulators(7). 100 ml medium was dispensed in each conical flasks (250 ml) and 20 ml medium in each culture tube (25x150mm). Flasks and tubes were plugged with nonabsorbent cotton and autoclaved for 20 minutes and 15 minutes respectively at 121^0 C (1.05 kg $cm^{-2)}$. Sterilized leaf

explants were cut into pieces of about 1cm^2 and then aseptically transferred on to each flask and tube.

All cultures were maintained in the culture room at 26.0 ± 0.5^{0}C with 16-hours light/8-hour dark photoperiods under white fluorescent light (Philips Cool TL 36 W, 220 V, light intensity 36 µmol. m^{-2}s^{-1} and 55-60% relative humidity).

Shoot bud differentiation

MS medium supplemented with various concentrations and combinations of cytokinins and auxins as shown in Table 1 and 2 was used for differentiation from nodular callus obtained from leaf explant. MS medium without growth regulators served as control. Ten replicates were used for each treatment and cultures were grown for successive passages on the same medium. All cultures were subcultured every 4 weeks. Data on explant response, number of shoot buds/ shoots formed per explant or subcultured callus and length of shoot formed were recorded after 4 weeks of growth.

Root formation

Shoots obtained from *in vitro* cultures (3-4 cm. long) were isolated from differentiating mass and cultured on MS medium supplemented with different concentrations of auxins (Table 3). Further, salt strength of MS medium was varied by incorporating either of IAA, NAA, or IBA. Plantlets obtained were transferred in the pots. Data were recorded on percentage of rooting, mean number of roots and length of roots after transferring onto rooting media.

The regenerated shoots from leaf derived callus were transferred to different rooting media and root formation was visible from basal cut and within 10 days of inoculation.

RESULTS AND DISCUSSION

A highly regenerative system has been developed in *T. Indica* using leaf explants from mature plant. This system can be effectively used to multiply the plant material. Leaf explants were cultured on MS medium supplemented with kinetin and IAA or BA. Initial growth of callus/ shoot buds formed directly were slow. In subsequent passages on the same medium nodular callus grew on MS medium supplemented with kinetin/BA and low concentration of IAA (0.1 mg/l). In absence of auxin, dire of shoot buds were formed from leaf explants, which on subsequent passage, proliferated producing differentiating mass of nodular callus when transferred on media containing a cytokinin and with or without an auxin.

Table 1 : Effects of MS medium supplemented with cytokinin on shoot formation

PGR (mg/l)		No. of	Mean shoot
Kinetin	BAP		
0.5	0.0	3±1.77	0.5±0.49
1.0	0.0	21±2.64	1.5±0.82
2.5	0.0	52±5.93	3.5±1.46
5.0	0.0	42±3.46	3.0±1.01
0.0	0.5	5±1.84	0.4±0.47
0.0	1.0	27±2.44	1.2±0.96
0.0	2.5	57±5.93	3.6±1.52
0.0	5.0	48±5.46	3.2±1.03

Table 2 : Effects on MS medium supplemented with low concentration of IAA along with moderate concentration of cytokinin on shoot bud differentiation.

PGR (mg/l)			No. of shoots	Mean shoot length (cm.)
BA	IAA	Kinetin		
1.0	0.1	0.0	32±1.62	2.0±0.94
2.5	0.1	0.0	62±1.84	4.5±1.32
0.0	0.1	1.0	24±1.38	1.7±0.92
0.0	0.1	2.5	54±1.76	4.2±1.24

Table 3 : Effects of plant growth regulators and salt concentration of MS medium on rooting of Tylophora indica

PGR (mg/l)	MS Full		MS Half		MS One fourth	
	No. of roots	Root length mm	No. of roots	Root length mm	No. of roots	Root length mm
IAA (1.0)	4.20 ± 1.2	2.74 ± 1.1	4.50 ± 1.1	25.13± 2.8	3.80 ± 1.3	5.68 ± 1.4
IAA (2.0)	3.00 ± 0.8	1.46 ± 0.4	3.30 ± 0.9	17.49 ± 1.4	1.70 ± 0.8	3.04 ± 0.8
NAA (1.0)	2.20 ± 0.8	2.05 ± 1.3	2.70 ± 1.3	15.22 ± 1.1	3.20 ± 1.1	5.26 ± 0.9
NAA (2.0)	1.70 ± 0.8	1.21 ± 0.5	2.50 ± 1.4	6.92 ± 0.8	1.80 ± 0.8	2.93 ± 1.1
IBA (1.0)	2.80 ± 1.5	2.45 ± 0.8	4.40 ± 1.2	5.24 ± 1.1	3.50 ± 1.3	2.20 ± 0.7
IBA (2.0)	1.80 ± 1.0	1.57 ± 0.6	2.10 ± 1.0	2.59 ± 0.5	1.90 ± 1.0	0.55 ± 0.3

Figure: 1.1 - *Compact globular callus with a few shoot buds on the medium containing BA (2.5 mg/l) and IAA (0.1mg/l).*

Fig 1.2 - Multiple shoots developed on MS-half salt medium containing BA (2.5 mg/l) and IAA (0.1mg/l).

Fig 1.3 Regeneration of large number of multiple shoots

Fig 1.4 Plantlet formation from shoots grown on rooting medium containing IAA (1.0 mg/l) in MS-half salt concentration.

Callus transferred on medium containing kinetin or BA alone produced shoot buds within 4 weeks. Number of shoot buds increased with increased cytokinin in the medium irrespective of the kinetin or BA. However, maximal concentration (5 mg/l) was slightly inhibitory to shoot bud formation and elongation. At lowest concentration, a few small shoot buds were formed at the end of growth period. The optimal concentration of BA and kinetin was 2.5 mg/l for shoot number and elongation (Table 1).

Using optimal and a lower concentration IAA (0.1 mg/l) was incorporated in the medium to invigorate the growth. Incorporation of IAA enhanced nodular growth with healthy shoot buds formation. On second passage, these cultures produced profuse buds and shoot elongation was observed (Table 2). These culture when maintained on the same medium produced large number of shoots per flask (250 ml). There was overall increase in growth. The maximum number of shoots was recorded on MS medium containing BA (2.5 mg/l) and IAA (0.1 mg/l). During organogenesis, nodular, compact and white green callus was also produced continuously in all the treatments containing an auxin.

This provided a source of continuous regenerating system. Shoot formation was observed in almost all treatments containing cytokinin and an auxin.

Through callus formation from leaf explants was observed at different concentrations and combinations of cytokinins and auxins, shoot formation required on cytokinin containing medium. Callus response to auxin/ cytokinin concentration and ratio was in accordance with that observed by Skoog and Miller (1957) and subsequent workers (8). Incorporation of auxin in low concentration with cytokinin was observed beneficial to invigorating the callus and shoot formation. This was slightly different from that observed in *T. indica* by Faisal and Anis (2003). This difference might be due to two reasons: i) They used NAA instead of IAA and ii) They used 0.1 and 0.5 mg/l concentrations. NAA can withstand autoclaving while IAA might have decomposed during autoclaving, resulting in further lower action by this auxin (9). The result observed by

Chaudhury and coworkers (2004) using root explants was in accordance with the results obtained in this investigation (10). Initially explants were cultured on a wide range of media containing different concentration and combination of growth regulators inducing phenoxy acids but no direct or indirect somatic embryogenesis was observed as reported by Faisal *et al*. (2003).

Maximum number of roots per shoot was produced on IAA supplemented medium as compared to media supplemented with NAA and IBA. Reduction is MS salt strength to half was observed beneficial in enhancing rooting in all the three auxins supplemented media. Further, reduction in salt strength of MS medium had an inhibitory effect. Root growth was also maximum on IAA supplemented medium irrespective of salt strength or concentration of auxin Figure 1.1, 1.2, 1.3 & 1.4.

CONCLUSION

It may be concluded from present investigation that the efficient method developed for rapid micropropagation of *T. indica* using leaf callus can be used for propagation of this endangered medicinal plant. The continuous regenerative system developed did not lose regenerative potential in cultures past few years. The tissue culture techniques developed can be useful for propagation and also for the conservation of the germplasm of this medicinally important plant which can enhance the rate of multiplication and can reduce the time period and cost of production.

ACKNOWLEDGEMENTS

One of the author (R.K.Nema) is thankful to the Head, Department of Molecular Biotechnology, Science College, M.L.S.University, Udaipur for providing necessary facilities to carry out this work.

REFERENCES

1. N.C. Shah, L.D. Kapoor, *Merrill*. **100**: 632-35(1974).
2. M. Ali, K.K Bhutani, *Phytochemistry* **28(12)**: 3513-17 (1989).
3. Anonymous, The wealth of India. Raw Materials, PID and CSIR, New Delhi. **10**: 398-99(1976).
4. R.P. Rastogi, B.N. Mehrotra, Compendium of Indian medicinal plants, CDRI Lucknow and PID and CSIR. New-Delhi. **2**: 232-33; 690-91(1993).
5. R.P. Rastogi, B.N. Mehrotra, Compendium of Indian Medicinal Plants, CDRI Lucknow and NISCOM. New- Delhi. **5**: 868-70(1998).
6. S.S. Nayampalli, U.K. Sheth, *Indian J. Pharmacol*. **11(3)**: 229-32(1979).
7. T. Murashige, F., Skoog *Physol. Plantarum*. **15**: 473-97(1962).
8. F. Skoog, C.O. Miller, *Exp. Biol*. **11**: 118-30(1957).
9. M. Faisal, M. Anis, *Plant Cell Tiss. Org. Cult*. **75**: 125-29(2003).
10. 10. K.N. Chaudhuri, B. Ghosh, S. Jha, *Plant Cell Rep*. **22(10)**: 731-40(2004).

MILESTONES AND ACHIEVEMENTS – PHCOG.NET - (2004 -2006)

Pharmacognosy Network Worldwide is a non-profit network dedicated to Natural Products Research in order to develop promising drugs.

Phcog.net was started on July 6, 2004

- Development and launch of Website – www.phcog.net
- Initiation of Discussion forum – http://groups.yahoo.com/group/phcog/
- Started a forum – www.phcog.net/forum.php
- Started a New Online peer reviewed magazine – **Pharmacognosy Magazine** (PHCOG MAG). Editorial team was finalized for the term of three years (2004-2007).
- Release of four issues in 2005.
- Project Phcog Refbase started in the month of May 2005.
- Release of 8th issue of Pharmacognosy Magazine in Oct 2006.
- Print version of Pharmacognosy Magazine
- Knowledge base section – http://www.phcog.net/knowledge
- Online web based manuscript handling system – http://www.phcogmag.com
- First issue of Phcog E -news – http://www.phcog.net/bulletin
- Phcog.Net Careers – http://www.phcog.net/careers
- Phcog.Net Global Activities – http://www.phcog.net/global