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In Vitro Regeneration of Tylophora asthmatica (L. F.) Wight & Arn

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Abstract: Protocol for in vitropropagation of Tylophora asthamatica was successfully established by taking nodal segment and leaf segment as an explants. These explants were transferredon MS medium supplemented with various concentrations of growth regulators viz. BAP,2-4, D andIAA. Best resultswere obtained within 4- 5 weeks of incubation. Induction of callus as well as multiple shoot formation was achieved using leaf and nodal explants.MS medium fortified with 2.0 mg/l, 3.0mg/land 4.0mg/1 BAP in combination or either alonewith IAAtaking concentration0.2 mg/l, 0.4mg/l and 0.6mg/lhas shown maximum rate of multiplication.Traditionally, Tylophora asthmatica has been used in treatment of asthma, dermatitis and rheumatism. The plant has been described as bronchodilator, emetic, expectorant and diaphoretic (Shah and Kapoor, 1976). Present protocol forin vitro conservation of important medicinal plantT. asthamaticais important for high frequency regeneration and conservation.

Key Words: In vitro,Tylophora asthamatica, Micro propagation, Callus induction,Explant.

I. INTRODUCTION

Tylophora asthamatica is important medicinal plant belonging to the family Apocynaceae.It is commonly known as Indian ipecac and Marathi Pitta Mari. *Tylophora* is perennial, climber herbaceous plant native to south east India.*T.asthamatica* contain 0.2 to 0.3 % alkaloids i.e. tylophorine and it is useful against the asthma and rheumatism disease. *Tylophora asthmatica* is a perennial plant native to south and east India. This name has been derived from two ancient Greek words. 'Tylos' meaning "knot" and 'phoros' meaning "bearing". It was earlier placed in Asclepiadaceae which has now been sunk into Apocyanaceace. *T.indica* is indigenous to India where it grows wild in the southern and eastern regions and has a long standing reputation in the treatment of asthma (Biswas and Ghosh, 1973). The leaves and roots of *T.indica* have been included in Bengal Pharmacopoeia since 1884 (Nadkarni, 1976). In Ayurveda, *T. asthmatica* is known as antamool. The drug is official in Bengal pharmacopoeia (Kirtikar and Basu 2001). *Tylophora. asthmatica* contains 0.2-0.3 % of alkaloids. Tylophorine and tylophornine are important alkaloids encountered and the percentage is not affected by seasonal variations. The extract of *T. asthmatica* marketed by pharmaceutical companies is standardized to contain 0.1% of the total alkaloids. Recent studies have confirmed the anti-inflammatory activity of Tylophorine (Gupta et. al 2010). Thus, large-scale production could be achieved through rapid *in vitro*multiplication of Tylophora. Plant tissue culture has been extensively utilized for the improvement of many medicinal plants, hence efforts has been made to propagate this plant *in vitro*.

II. MATERIAL AND METHODS

During the present investigations explantsof *T. asthmatica* was taken from elite plant growing in botanical garden of Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad.This plant material used forpreliminary experimental material wasJuvenile twigs,leaves, shoots and nodal segments. These explants were surface sterilized with 70% ethanol for one minute followed by $HgCl_2$ solution (0.2% w/v) and three subsequent washes with sterile distilled water. Affected end of explants with HgCl2 were cut and inoculated on Murashige and Skoog (1962) medium fortified with 0.3% (w/v) clerigelas solidified agent. MS was also supplemented with various growth regulators viz. BAPIAA, and 2,4-D at different concentrations and combinations. The pH of the medium adjustedwas 5.8 before autoclaving. All cultures were maintained at 25 ± 2°C under 16/8 hr. photoperiod.

III. RESULTS AND DISCUSSION

During present studies shoot tip, leaf and nodal explants were aseptically inoculated on MS medium fortified with various proportions of auxin and cytokines for testing their capabilities for callus and shoot proliferation. Shoot and nodal segment of *T. asthamatica* as an explant werecapable for shoot proliferation. Proliferation of callus at the cut end of both explants was observed within fifteen days of culture.

Explants viz. leaf and stem segments were inoculated on MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 2.5, 2-4 D and 2.0 mg/1 BAP(Table1). Maximumresponse for regeneration of callus (80%)was achieved on MS medium fortified with 2, 4-D at 2.0 mg/1aloneand withBAPat 2.0 mg/1 after 20 days.Highest rate of callus induction (75%) was

from stem explant.Callus induced was light yellowish, greenishin colour and nodular. Callus was also noticed in cultures with BAP alone and with IAA (Plate a, b).The response of callus regeneration was less effective as compared to the 2, 4-D with MS medium (Table 1).

When apical shootswere used an explants and inoculated on MS medium which was fortified with different combinations of auxins and cytokininrate of regeneration was higher. Maximum response for callus and shoot regeneration were recorded on MS + 2.0 mg/l BAP + 0.5 mg/l IAA from shoot tip as an explant. Highest multiple shoots were regenerated on MS +2.5 mg/lit BAP with low concentration of Auxins (Table 2.5).According to Faisal and Anis (2005), callus forms frequently at the basal cut ends of nodal explants on cytokinin enriched medium in species exhibiting strong apical dominance by using MS medium plus 2,4,5-T. During the present investigation significant differences in regeneration frequencies were recorded using various concentration and combination of growth regulators viz. 2, 4-D, BAP, and IAA.

The callus derived from nodal and leaf segment has potential to raise shoot and callus. Similar observationswere also reported by Sadguna et. al.(2013). Low concentration of BAP and 2, 4-D was responsible for induction of greenish callus after 16 day from leaf disc explant in *T. indica*. Therefore nodal and leaf segment explants were shown higher capacity of caulogenesis in *T. asthamatica*.Manjula S. et al.(2000), induced somatic embryo from leaf derived callus on MS media supplemented with 1-2 mg/l BAP + 0.1-1.0 mg/l IAA in *T. indica*. High morphogenic efficiency of callusderived from nodal segment may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds were also developed from callus cultures. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days.

Table 1.Effect of phyto-hormones on callusinduction and shoot proliferation

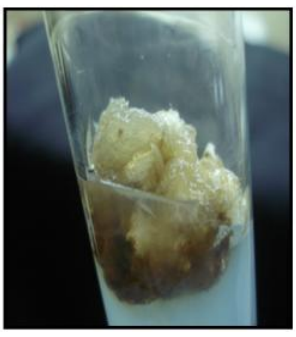
Explant	**Growth regulator Mg/l 2, 4-D+BAP**	**Callus induction frequency**	**Nature of callus**	**Proliferation of shoot %**
Leaf	0.0 + 0.0	-	-	-
	0.5 + 2.0	+++	Greenish	-
	1.0 2.0	++	Greenish	-
	1.5 + 2.0	+++	Fibril	-
	2.0 + 2.0	++	Yellowish	-
Stem node	0.5 + 2.0	+++	whitish	10
	2.0 2.0	+++	Greenish	15
	1.5 + 2.0	+++	Greenish	12
	2.0 + 2.0	++	Yellowish	5
Shoot	0.5 + 2.0	+++	Pale Yellowish	15
	3.0 2.0	+++	Greenish	12
	1.5 + 2.0	++	Greenish	10
	2.0 + 2.0	++	Greenish	10

*** + Poor callus, ++, Moderate callus, +++ massive callus induction**

Table 2. Effect of BAP on shoot regeneration of *T. Asthamatica*

Explant	BAP + IAA	Induction of C/S	Length of shoot (µ±SE)
Shoot tip	0.0 + 0.0	-	-
	0.5 + 0.2	S	3.1±0.245
	1.0 + 0.2	S	3.0±0.240
	1.5 + 0.2	S+C	3.5±0.212
	2.0 + 0.2	S+C	3.8±0.195
	2.5 + 0.2	S+C	3.4± 0.208
	3.0 + 0.2	S+C	3.2± 2.32

***Mean ± SE=0.005**



1. Callus from leaf 2. Shoot induction along with callus


3. Callus and shoots induced from shoot tip 4. Multiple shoot formation

IV. CONCLUSION

In case of *Tylophora asthamatica* it was observed that plant rose through the seeds shows tremendous genetic variation which is not suitable for commercial cultivation. Propagation is also difficult in Tylophora due to low seed viability and low rate of germination (Thomas and Philip, 2005). In addition, the destruction caused by harvesting the roots as a source of drug has threatened the survival of the plant. *In vitro*studies onregeneration of valuable medicinal plants are proving useful now a days. This piece of work will be useful to restore the germplasm of this important plant species.

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