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BIOCHEMICAL ASSESSMENT OF *IN VITRO* AND *IN VIVO* CULTURE OF *TYLOPHORA INDICA* (BURM. F.) MERR

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ABSTRACT
Tylophora indica (Burm. F.) Merr is an endangered plant which can be protected from extinction by its large scale production. Nodal segments of healthy plants are used as explants and cultured on MS Basal medium fortified with different growth regulators. Optimum shoot induction conditions from explants were established. *In vitro* and *in vivo* phytochemical test were done by using standard methods for chlorophyll, carbohydrates, proteins, lipids and starch. 3mg/l 2, 4 D showed maximum and success full callus production. Shoot initiation started in 7 days and best shoot regeneration reported with 3 mg/ml BAP in Basal medium. Combination of IBA and NAA in concentration 2 and 4 mg/l respectively proved to be best for root initiation. Concentration of chlorophyll, protein, lipid, carbohydrate, and starch *in vitro* and *in vivo* culture are investigated.

INTRODUCTION
Tylophora indica (Burm. F.) Merr (Indian ipecacuanha), a perennial branching climber of the family Asclepiadaceae, is medicinally important particularly for the treatment of asthma and bronchitis. It is traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis (Gupta *et al*., 1979). Apart from the above, it also seems to be a good remedy in traditional medicine as anti-psoriasis, seborrheic, anaphylactic, leucopenia and as an inhibitor of the Schultz-Dale reaction. The major constituent in this plant is alkaloid tylophorine that is responsible for a strong anti-inflammatory action. The other alkaloids include Tylophorinidine, Septicine and Isotylocrebrine.

Tylophora is believed as one of most important herbs and reported as an endangered species. In this communication an efficient and reproducible method of plant propagation through nodal explants of *T. Indica* is reported. This will provide an alternative for large scale production and protect the plant in its natural habitat. Keeping in view we carried out current work which can save the plant in its natural habitat and provide an alternative for large scale propagation.

MATERIALS AND METHODS
In vitro cultivation
Nodal segment from elite plants were used as an explants. Explants were washed thoroughly under running tap water with teepol (5%) for 20 minutes, surface sterilized with 0.1% (w/v) mercuric chloride for 5-10 minutes and finally washed for 3 to 5 times in distilled water (Dubey 1999) to remove the impurities and stubborn particles attached to the explants. Explants were placed vertically on MS basal media fortified with different growth regulators. Different concentrations of benzylaminopurine were used individually and in combination for shoot proliferation from the nodal

explants. Calluses were induced by using different concentrations of 2, 4-D. The culture were kept in conical flasks (100, 150 ml) covered with non absorbent cotton plugs under controlled conditions of temperature (25 ± 2^0C). The culture was kept under the light intensity of 3,000 Lux at the level of culture tubes using white fluorescent tube light. Photoperiod of 12 hours per day was maintained. The relative humidity of the room was maintained at 70%. Observations were recorded every 15 days intervals. Once culture conditions for optimum shoot induction from explants were established, the *in vitro* produced shoots were subcultured on fresh medium every 3 weeks.

Phytochemical investigation

Quantitative phytochemical evaluation of *in vivo* and *in vitro* plant parts were done by using following methods: chlorophyll (Arnon 1949), carbohydrate, protein Lowry *et al. (*1951) lipid Folch *et al.,(*1957) and starch McCready *et al.* (1950).

RESULTS AND DISCUSSION

The explants started swelling within 6-8 days after inoculation. The callus initiated from cut end of the explants. High percentages of callusing were obtained of green type in nature and were obtained in 2, 4-D (3mg/ml) (Table-1).

Table 1: Effect of 2, 4-D on *T. indica* and morphology of the callus

Explant	2, 4-D concentration (mg/l)	Successful callusing	Nature of callus
Nodal segment	1	+	Light green, hard
	2	+++	Light green, hard
	3	++++	Green, hard
	4	++	Light green, hard

++++ = 80%; +++ = 60%; ++ = 40%; + = 20%

Among the various concentrations of 2,4-D tested, 3mg/l 2,4-D showed the maximum callus formation. The percentage of regeneration decline with increase in concentration of 2,4-D. It was similar that of *Datura metel* where the same concentration of 2, 4-D was used by Arockiasvamy *et al*. (1999) and in *Hybanthu enneaspermus* by Natarajan *et al*. (1999). Among various hormones used 2, 4-D was the most successful.

Shoot regeneration from nodal culture

Nodal explants of *T. indica* were placed on MS medium supplemented with different concentrations of BAP resulted in shoot regeneration at varying frequency. Shoots were initiated from the nodal within 7 days. The best shoot regeneration was obtained at (3mg/ml) BAP (Table-2).

The elongated shoot were harvested and transferred to rooting medium. The explants were sub cultured on MS medium with the same concentration for mass production.

Table 2: Effect of BAP on shoot formation of nodal explants

Hormone concentration mg/l	% of shoot proliferation
1	55 %
2	60 %
3	80 %
4	60 %

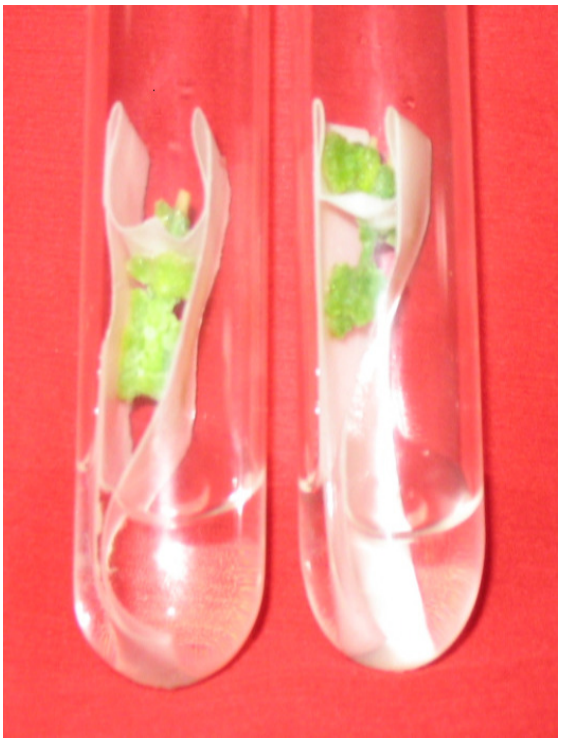

Fig. 1 Callus induction

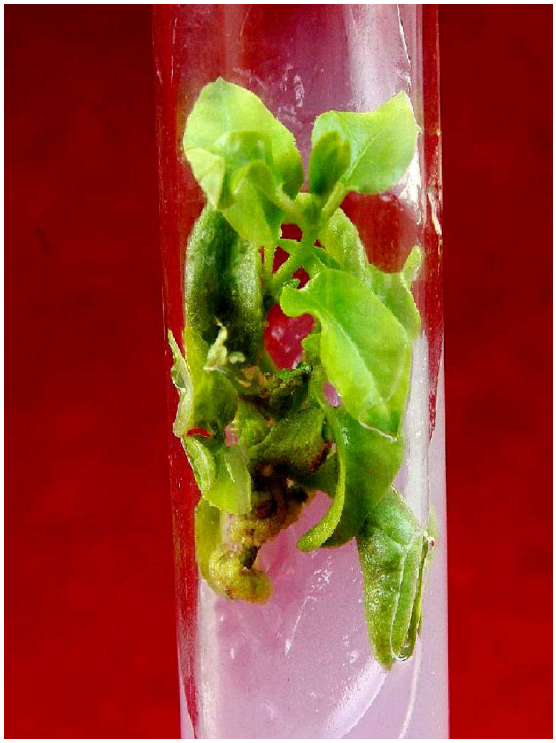

Fig. 2 Shoot proliferation

Root regeneration from nodal culture

Nodal explants of *T. indica* were transferred on MS Medium supplemented with different concentration of IBA (1-4mg/ml) and combination of IBA+NAA (2mg+1-4mg/ml). Among various concentrations tested, IBA(4mg/l) produced good results while combination of IBA+NAA proved to be best for root induction at 2+4 mg/l concentration. Complete Plantlets with elongated shoot and root system ready to be transferred to the soil.

Table 3: Effect of IBA on root formation of nodal culture

Explant	IBA concentration (mg/l)	Root regeneration (%)
Nodal segment	1	20
	2	40
	3	60
	4	70
Nodal segment	IBA + NAA (mg/l)	
	2+1	55
	2+2	60
	2+3	70
	2+4	90

Table 4: Mean Chlorophyll, Protein, Lipid, Carbohydrates and Starch in Stem and Leaf of *Tylophora indica*

Parameters	*In vivo*		*In vitro*	
Photochemical parameters	Leaf (mg/g fresh wt.)	Stem (mg/g fresh wt.)	Leaf (mg/g fresh wt.)	Stem (mg/g fresh wt.)
Chlorophyll a	0.07520	0.06275	0.066	0.0643
Chlorophyll b	0.04950	0.04913	0.0481	0.0312
Total Chlorophyll	0.1247	0.11188	0.1141	0.0955
Protein	0.80±0.036	0.440±0.37	0.690±0.03	0.500±0.011
Lipid	0.212±0.00	0.843±0.00	0.110±0.00	0.730±0.00
Carbohydrate	0.620±0.0026	0.870±0.005	0.830±0.001	0.890±0.002
Starch	0.737±0.003	1.096±0.015	0.867±0.001	1.050±0.019

The phytochemical analysis concerning chlorophyll, carbohydrate, protein and lipid did not suggest any clue for enhancement of percentage of regeneration as chlorophyll contents (Table: 4). However, there was a decrease in protein and lipid content and an increase in carbohydrate and starch due to effect of phytohormones.

In conclusion, the present study reveals an effective regeneration and multiplication protocol for *in vitro* propagation endangered *T. indica* which can be successfully used for large scale multiplication and propagation of this medicinally important and endangered species.

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