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SHORT COMMUNICATION

IN VITRO CULTURE PROTOCOL OF *TYLOPHORA ASTHMATICA*, AN ANTI-ASTHMATIC MEDICINAL HERB

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SUMMARY

***Tylophora asthamatica*, a perennial climbing plant and member of Asclepiadaceae, has tremendous medicinal value in Ayurvedic system of medicine. It is generally used for anti-asthamatic and anti-allergic treatment. Besides being effective in the treatment of bronchial asthma, bronchitis, hay fever, rheumatism and dermatitis, its major constituent alkaloid tylophorine also has anti-inflammatory action. Over-exploitation of T*ylophora* for its above mentioned medicinal properties has prompted to undertake its conservation strategies through plant tissue culture technique. For regenerating shoots of *Tylophora asthamatica* nodal segments were inoculated on to MS half strength and MS full strength media containing different concentrations and combinations of auxins (IAA and IBA) and cytokinins (Kinetin and BAP). The MS half strength medium fortified with BAP (1.5 mg/l), BAP + IBA (1.0 mg/l each), Kn + IBA (0.2 mg/l each) and Kn + IBA (0.5 mg/l each) favoured best response in terms of multiple shooting, leaf number, leaf length and rooting, respectively, whereas shoot elongation was best in case of full strength of MS medium fortified with BAP + kinetin (0.5 mg/l each) after 45 days of inoculation of explants. The plantlets thus developed were hardened and acclimatized in mixture of garden soil and sand (1:1).**

Key words*: Cytokinin, *in vitro* culture, medicinal plant, *Tylophora asthmatica

Anant Mole (*Tylophora asthmatica*), one of the well known medicinal plants, has been used traditionally for years in the Indian tradition of ayurvedic medicine to cure lung and breathing problems. It is used for the treatment of bronchial asthma, bronchitis, rheumatism and dermatitis in certain regions of India. *Tylophora* plays supportive role in the treatment and management of bronchial asthma (Shivpuri *et al.* 1972, Gupta *et al*. 1979, Gore *et al*. 1980). These effects are due to *Tylophora*'s ability to suppress the immune responses that can trigger asthma. The major constituent of *Tylophora* is the alkaloid tylophorine. Laboratory-based research has shown that this isolated plant extract exerts a strong anti-inflammatory action (Gopalkrishnan *et al*. 1979). These actions seem to support *Tylophora*'s traditional use as an anti-asthmatic and anti-allergic medication by Ayurvedic practitioners. One clinical trial with asthma sufferers found that *Tylophora* leaf (150 mg of leaf by weight) chewed and swallowed daily in the early morning for six days led to moderate to complete relief of their asthma symptoms (Shivpuri *et al*. 1969). Over-exploitation of this highly prized medicinal

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plant by local people and commercial establishments has adversely reduced its availability in the natural habitat. Owing to its medicinal importance, a study was undertaken to develop rapid and efficient micropropagation protocol.

Tylophora asthmatica, growing in the Department of Dravya-Guna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University was used as the source material. Fresh shoots measuring 1.0-1.5 cm were initially washed under tap water for 30 min followed by surface sterilization using 0.1% Bavistin for 5-6 min and 0.1% $HgCl_2$ for 3-4 min under Laminar air flow cabinet. The explants were then washed several times in sterile double distilled water to remove the traces of sterilants. The sterilized explants were inoculated on MS medium (Murashige and Skoog 1962) fortified with different concentrations (0.2, 0.5, 1.0 & 1.5 mg/l) and combinations of growth regulators such as BAP, Kinetin, BAP + Kinetin, BAP + IBA, IBA + Kinetin, and IAA + Kinetin to study their response on explant's multiplication potential. Culture was incubated in a culture room at 25±2ÚC and 50-60% relative humidity under a 16/8h (light/dark) photoperiod with light supplied by cool white fluorescent tubes of 2000-3000 lux. The shoots formed *in vitro* were isolated and sub-cultured in medium having different combination of growth regulators used for multiplication and rooting. The plantlets thus obtained were transferred to pots containing sterilized sand: soil (1:1) for hardening and acclimatization. The best culture response is reported 45 days after inoculation for each treatment of growth regulators. The experimental data represented mean ± S.D. of four replicates.

The explants of *Tylophora asthmatica* were cultured on to MS half strength and MS full strength medium in the presence of various concentrations and combinations of cytokinin and auxin. The cultures showed initiation of shoot primordium after 2-8 days of inoculation. The effect of growth regulators used in the medium, either singly or in combination, on shoot proliferation *in vitro* was investigated.

In MS half strength medium, highest number of shoots was observed (4.0±0.8) in the presence of BAP (1.5 mg/l) and maximum shoot length (1.30±0.1) in Kinetin alone (1.5 mg/l) (Table 1; Fig. 1). However, the combination of IBA and BAP (1mg/l each) as well as BAP and Kinetin (1.5 mg/l each) showed highest number of shoots (1.60±0.3). The maximum shoot length (2.60±0.3) was observed in the combination of IBA and BAP (0.5 mg/l each). This result is in conformity with another finding in *Oroxylum indicum* (Boro *et al*. 2007), where use of BAP produced better results: when BAP (0.5 mg/l) and IBA (0.2 mg/l) were combined showed significant increase in number of shoot, whereas BAP (0.5 mg/l) and IAA (0.1 mg/l) used together showed maximum shoot length. In the present study, highest number of leaves (2.80±0.4) and maximum leaf length (2.50±0.9) was observed when BAP (1.0 mg/l) was used singly. The combination of IBA and BAP (1.0 mg/l each) showed highest number of leaf (4.0±0.9), whereas IBA and Kinetin in combination (0.2 mg/l each) produced maximum leaf length (3.0±1.4). The multiple shoots (maximum four in number in some replicates) were observed in the MS half strength medium fortified with 1.5 mg/l BAP, while best rooting was obtained (2.20 ± 0.2) in the presence of IBA and Kinetin (0.5 mg/l each; data not shown). The initiation of roots was observed 45 DAI with average length of 3.5 cm. There was 100% rooting of microshoots observed. In *Oroxylum indicum* also, maximum number of root was favoured in presence of IBA (Boro *et al*. 2007). The root growth in the present study was best in half strength medium similar to that of *Aloe vera* culture (Dharmapal *et al*. 2007).

In MS full strength medium, the highest number of shoots (1.60±0.2) and shoot length (2.40±0.1) was observed in the presence of Kinetin alone at concentration 0.2 mg/l and 1.0 mg/l, respectively 45 days after inoculation (Table 2; Fig. 1). While the combination of IBA+BAP at concentration 1.0 mg/l each showed highest number of shoot (2.0±0.6), BAP+Kin at 0.5 mg/l concentration each produced maximum shoot length (3.60±0.7). Use of BAP favoured shoot proliferation in case of *Aloe vera* also where MS full strength medium supplemented with BAP (2.0 mg/l) and IBA (0.2 mg/l) showed highest number of shoots while highest shoot length was observed with 1.5 mg/l BAP and 0.2 mg/l IBA. In the present study, Kinetin alone produced highest number of leaves (2.30±0.5) at 0.5 mg/l while

Table 1. Effect of half strength MS medium on *in vitro* shoot proliferation and multiplication.

Growth Regulators (mg/l)	Response DAI	NOS			LOS			NOL			LOL		
		15D	30D	45D	15D	30D	45D	15D	30D	45D	15D	30D	45D
BAP													
0.2	8	1.0±0.5	1.30±0.5	1.30±0.5	0.20±0.1	0.50±0.1	0.70±0.3	NR	NR	1.60±0.6	NR	NR	0.90±0.1
0.5	5	1.0±0.5	2.0±1.0	2.20±1.0	0.40±0.1	0.60±0.1	0.70±0.3	NR	NR	1.0±0.5	NR	NR	0.70±0.3
1.0	5	1.0±0.5	2.30±0.1	2.60±0.8	0.60±0.2	0.80±0.2	0.80±0.3	1.0±0.4	2.30±1.2	2.80±0.4	0.40±0.1	2.30±0.8	2.50±0.9
1.5	7	1.0±0.5	3.0±0.9	4.0±0.8	0.80±0.1	0.90±0.2	0.80±0.1	NR	NR	1.30±0.5	NR	NR	0.80±0.2
Kinetin													
0.2	5	0.60±0.1	1.0±0.3	1.50±0.4	0.40±0.1	0.70±0.1	0.90±0.3	0.60±0.1	0.80±0.1	0.80±0.1	0.30±0.1	0.30±0.1	0.50±0.1
0.5	6	0.80±0.1	1.0±0.2	1.20±0.3	0.50±0.1	0.90±0.1	1.0±0.2	0.60±0.2	0.90±0.1	1.0±0.2	0.40±0.1	0.40±0.1	0.80±0.2
1.0	8	0.90±0.2	1.20±0.2	1.20±0.1	0.50±0.01	0.90±0.1	1.10±0.1	0.80±0.1	0.90±0.2	1.20±0.1	0.50±0.1	0.60±0.1	0.90±0.2
1.5	6	1.0±0.1	1.20±0.1	1.30±0.1	0.70±0.1	1.0±0.1	1.30±0.1	0.70±0.1	0.90±0.1	1.0±0.2	0.40±0.1	0.80±0.2	1.20±0.1
BAP+Kn													
0.2	4	1.25±0.5	1.25±0.5	1.25±0.5	0.73±0.5	0.80±0.3	1.05±0.2	2.30±1.0	2.30±0.5	3.0±0.8	0.70±0.2	0.80±0.1	0.90±0.1
0.5	4	1.25±0.2	1.30±0.3	1.30±0.1	0.50±0.1	0.70±0.1	0.80±0.1	2.0±0.5	3.30±1.0	3.70±0.58	0.50±0.1	1.20±0.3	1.30±0.2
1.0	3	1.40±0.2	1.30±0.1	1.30±0.1	0.70±0.2	0.70±0.1	0.90±0.2	1.70±0.8	2.0±0.8	2.20±0.6	1.10±0.5	1.20±0.2	1.20±0.1
1.5	4	1.40±0.1	1.60±0.5	1.60±0.3	0.20±0.1	0.30±0.1	0.40±0.1	0.30±0.58	1.33±0.6	2.0±0.9	0.40±0.6	0.70±0.2	1.50±0.6
IBA+BAP													
0.2	8	1.0±0.5	1.20±0.3	1.20±0.1	0.30±0.1	1.20±0.2	1.90±0.6	1.0±1.1*	3.0±1.2	3.0±0.8	0.38±0.4*	0.60±0.2	0.90±0.2
0.5	7	1.0±0.5	1.0±0.5	1.0±0.5	2.50±0.7	2.60±0.4	2.60±0.3	NR	1.0±0.1	2.50±0.7	NR	0.20±0.2*	0.70±0.1
1.0	4	0.90±0.3	1.0±0.4	1.50±0.7	1.70±0.3	1.80±0.1	1.80±0.1	2.50±3.5*	3.50±2.1	4.0±0.9	0.60±0.2	0.90±0.5	0.80±0.2
1.5	7	0.50±0.6	1.0±0.5	1.20±0.3	0.30±0.1	0.40±0.1	0.60±0.1	0.50±0.2	1.20±0.5	2.0±0.7	0.40±0.5	0.50±0.2	0.90±0.2
IBA+Kn													
0.2	5	1.0±0.4	1.30±0.3	1.30±0.2	0.40±0.1	0.50±.0.1	0.80±0.1	1.0±1.5*	2.50±1.0	2.50±0.8	0.50±0.2	1.20±0.3	3.0±1.4
0.5	4	1.0±0.4	1.0±0.1	1.20±0.1	0.30±0.1	0.40±0.1	0.80±0.1	2.30±0.5	2.70±0.7	2.8±0.6	0.80±0.3	1.2±0.2	1.30±0.2
1.0	7	1.20±0.3	1.20±0.2	1.20±0.1	0.20±0.1	0.30±0.1	0.50±0.1	0.80±0.3	2.50±0.8	2.80±1.0	0.90±0.3	1.0±0.4	1.20±0.2
1.5	4	1.30±0.3	1.30±0.1	1.50±0.4	0.20±0.1	0.50±0.1	0.60±0.1	1.30±0.5	1.30±0.5	1.50±0.6	0.90±0.1	1.0±0.1	1.20±0.3
IAA+Kn													
0.2	4	1.30±0.5	1.30±0.4	1.33±0.2	0.30±0.1	0.50±0.1	0.50±0.1	0.30±0.5*	0.80±0.5	1.30±0.6	0.80±0.3	0.80±0.1	0.90±0.1
0.5	3	0.70±0.2	1.0±0.4	1.10±0.4	0.50±0.2	1.80±0.6	1.90±0.5	0.70±0.2	1.30±0.5	1.30±0.3	0.80±0.2	1.10±0.3	1.10±0.2
1.0	4	0.50±0.1	0.70±0.1	0.70±0.1	0.60±0.1	0.80±0.2	0.80±0.1	NR	0.30±0.7*	0.80±0.2	NR	0.60±0.1	0.60±0.2
1.5	5	0.70±0.3	0.80±0.2	0.80±0.1	0.80±0.1	0.90±0.1	0.80±0.1	NR	0.30±0.1	1.30±0.1	NR	0.70±0.1	0.90±0.2

DAI – Days after inoculation, NOS – Number of Shoots, LOS – Length of Shoots (cm), NOL – Number of Leaves, LOL – Length of Leaves (cm), X-Contamination, NR – No Response, D – Days * In certain combination of normal concentration the response of culture varied to a great extent within replicates so the standard deviation in such cases is quite high, as compared to the mean.

Table 2. Effect of full strength MS medium on *in vitro* shoot proliferation and multiplication.

Growth Regulators (mg/l)	Response DAI	NOS			LOS			NOL			LOL		
		15D	30D	45D	15D	30D	45D	15D	30D	45D	15D	30D	45D
BAP													
0.2	3	1.0±0.4	1.0±0.2	1.30±0.2	0.30±0.1	0.70±0.3	0.80±0.3	0.50±0.1	1.30±0.2	2.0±0.9	0.20±0.4*	0.30±0.1	0.50±0.1
0.5	3	1.0±0.2	1.30±0.1	1.50±0.2	0.40±0.1	0.70±0.2	0.70±0.1	0.50±0.1	0.80±0.1	0.80±0.5	0.30±0.1	0.30±0.1	0.50±0.2
1.0	3	1.0±0.5	1.20±0.5	1.20±0.2	0.40±0.1	0.80±0.3	0.80±0.1	NR	1.30±0.6	1.60±0.3	NR	0.70±0.1	0.80±0.2
1.5	2	1.20±0.6	1.20±0.4	1.40±0.2	0.20±0.1	0.40±0.1	0.60±0.1	0.50±0.1	1.30±0.2	1.40±0.2	0.40±0.2	0.80±0.3	0.80±0.3
Kinetin													
0.2	3	1.30±0.5	1.30±0.4	1.60±0.2	0.90±0.6	1.0±0.5	1.20±0.5	0.80±0.3	1.30±0.5	1.80±0.6	0.70±0.2	0.90±0.3	0.90±0.1
0.5	2	1.0±0.2	1.40±0.4	1.50±0.3	0.90±0.5	1.30±0.5	1.60±0.3	0.80±0.3	1.30±0.4	2.30±0.5	0.40±0.2	0.40±0.1	0.80±0.4
1.0	2	1.0±0.5	1.0±0.2	1.30±0.1	1.40±0.2	2.40±0.2	2.40±0.1	1.70±0.5	1.70±0.1	1.80±0.2	0.70±0.2	0.80±0.1	1.0±0.1
1.5	2	1.0±0.5	1.0±0.3	1.40±0.4	0.70±0.2	1.20±0.4	1.30±0.3	1.30±0.2	1.30±0.1	1.50±0.2	0.80±0.2	0.80±0.1	0.90±0.1
BAP+Kn													
0.2	4	1.0±0.5	1.30±0.3	1.20±0.2	0.90±0.2	1.20±0.1	2.0±0.8	NR	1.20±0.3	2.0±1.0	NR	0.30±0.1	0.70±0.3
0.5	4	1.3±0.5	1.30±0.2	1.60±0.2	3.30±1.1	3.30±0.1	3.60±0.7	0.70±0.2	2.0±1.0	2.10±0.90	0.80±0.3	0.90±0.3	0.90±0.1
1.0	3	0.80±0.1	1.0±0.2	1.30±0.3	1.0±0.2	1.10±0.1	1.10±0.1	0.80±0.2	1.0±0.1	1.30±0.2	0.80±0.2	1.0±0.1	1.20±0.2
1.5	3	0.50±0.1	1.0±0.1	1.50±0.5	0.30±0.4*	0.50±0.1	0.70±0.1	0.80±0.1	0.80±0.2	0.90±0.1	0.90±0.1	0.90±0.1	1.10±0.1
IBA+BAP													
0.2	2	1.0±0.2	1.30±0.3	1.30±0.2	0.80±0.2	2.10±0.3	2.20±0.2	0.50±0.1	1.60±0.5	1.70±0.2	0.30±0.1	0.40±0.1	0.50±0.2
0.5	4	1.0±0.1	1.50±0.5	1.60±0.3	0.90±0.2	1.80±0.1	2.0±0.3	NR	NR	1.0±0.4	NR	NR	0.20±0.1
1.0	5	1.20±0.1	1.40±0.2	2.0±0.6	0.20±0.1	0.50±0.1	0.50±0.1	NR	NR	0.80±.2	NR	NR	0.70±0.3
1.5	5	1.0±0.3	1.20±0.2	2.0±0.6	0.50±0.1	0.60±0.1	0.80±0.2	NR	NR	0.80±0.1	NR	NR	0.90±0.2
IBA+Kn													
0.2	6	1.0±0.5	1.0±0.3	1.20±0.1	0.20±0.5*	0.60±.3	0.60±0.1	NR	1.80±0.5	2.50±0.5	NR	0.30±0.1	0.40±0.1
0.5	6	1.0±0.2	1.0±0.4	1.20±0.3	0.30±0.1	0.60±0.2	0.80±0.1	0.5±1.0*	1.50±0.9	2.30±0.3	0.20±0.1	0.40±0.1	0.80±0.2
1.0	6	1.0±0.1	1.0±0.4	1.20±0.3	0.50±0.2	0.60±0.2	0.60±0.1	NR	2.0±0.8	2.80±0.5	NR	0.60±0.2	0.80±0.1
1.5		X	X	X	X	X	X	X	X	X	X	X	X
IAA+Kn													
0.2	5	1.10±0.5	1.10±0.3	1.30±0.2	0.30±0.1	0.50±0.1	0.70±0.3	0.30±0.2	1.70±0.3	3.0±0.8	0.30±0.2	0.50±0.1	0.80±0.1
0.5	5	1.0±0.3	1.0±0.2	1.50±0.3	0.40±0.1	1.80±0.9	2.30±1.4	1.40±0.2	4.5±1.3	4.5±0.9	0.60±0.1	0.70±0.1	0.900.0
1.0	5	NR	0.50±0.1	1.0±0.4	NR	0.80±0.2	0.90±0.1	NR	0.90±0.1	2.0±0.6	NR	0.80±0.1	0.90±0.1
1.5		X	X	X	X	X	X	X	X	X	X	X	X

DAI – Days after inoculation, NOS – Number of Shoots, LOS – Length of Shoots (cm), NOL – Number of Leaves, LOL – Length of Leaves (cm), X-Contamination, NR – No Response, D – Days * In certain combination of normal concentration the response of culture varied to a great extent within replicates so the standard deviation in such cases is quite high, as compared to the mean

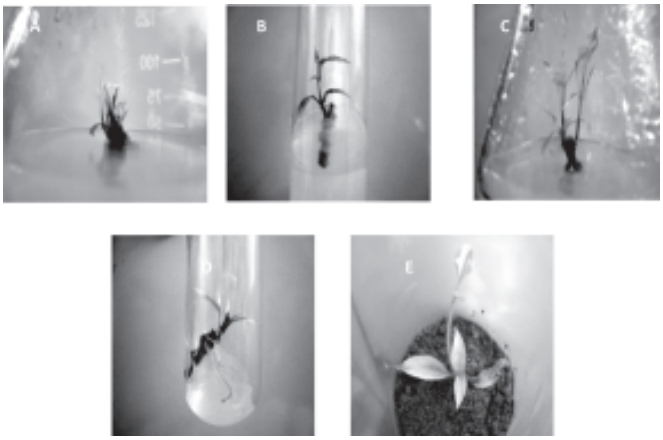

Fig.1. *In vitro* shoot and root proliferation in *Tylophora asthmatica*
A. ½MS + BAP (0.5 mg/l); B. MS+BAP+IBA (0.5 mg/l each); C. MS+BAP (1.5 mg/l); D. root formation in ½MS+IBA+Kn (0.5 mg/l each); E. a hardened *in vitro* raised plantlet.

maximum leaf length (1.0±0.1) at 1.0 mg/l concentration. However, when Kn combines with IAA (0.5 mg/l each) showed highest number of leaves (4.5±0.9), whereas combination of BAP and Kinetin (1.0 mg/l each) showed maximum leaf length (1.20±0.2). In the current study, the multiple shoots (in some replicates maximum of three in number) were observed in presence of BAP (0.5 mg/l). These findings are consistent with reports in *Stevia rebaudiana* (anti-diabetic medicinal plant), where maximum number of shoots was obtained in MS medium supplemented with only BAP (Sivaram and Mukundan 2003) while presence of IAA or IBA (0.1-0.25 mg/l) in MS medium favoured maximum root formation in *Picrorhiza kurroa* (Bist *et al*. 2007).

Overall, MS half strength medium favoured best response in terms of multiple shooting, leaf number, leaf length and rooting, whereas, shoot elongation was best in case of full strength of MS medium. This micropropagation protocol may be suitably used for mass scale production of *Tylophora asthmatica in vitro.*

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