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## *In vitro* Studies in *Tylophora asthmatica* (Burm.F) Merill

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### Abstract

*Tylophora asthmatica* (Burm. F) Merill has recently been included as one of the important drug from natural source for the treatment of respiratory diseases. Traditionally, *Tylophora asthmatica* has been used in treatment of asthma, dermatitis and rheumatism. The plant has been described as bronchodilator, emetic, expectorant and diaphoretic. Clinical studies have shown effectiveness of the drug in bronchial asthma and thus modern research withstands the ancient claims of traditional medicine. The leaf extract of *Tylophora 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. Looking towards this possibility efforts have been made to propagate this novel plant by Micropropagation.

**Keywords:** *Tylophora asthmatica*, Tylophorine, Bronchial asthma.

### Introduction

*Tylophora indica* is indigenous to India where it grows wild in the southern and eastern regions and has a longstanding reputation in the treatment of asthma (Biswas and Ghosh, 1973). 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 Apocyanaceae. The leaves and roots of *Tylophora indica* have been included in Bengal Pharmacopoeia since 1884 (Nadkarni, 1976). In Ayurveda, *Tylophora asthmatica* is known as *antamool*. The drug is official in Bengal pharmacopoeia (Kirtikar and Basu 2001). 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).

*Tylophora asthmatica* yields 0.2-0.3 % of alkaloids i.e. Tylophorine which is important alkaloids encountered and the percentage is not affected by seasonal variations. The extract of *Tylophora 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). Plant rose through the seeds shows tremendous genetic variation which is not suitable for commercial cultivation. Vegetative propagation is difficult in *Tylophora* due to low seed viability and germination rate (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. Thus, large-scale demand necessitates rapid multiplication of *Tylophora*. Plant tissue culture has been extensively utilized for the improvement of many medicinal plants. During the present investigations efforts have been made to multiple shoots using various explants.

### Materials and Methods

Plant was grown in nursery in botanical garden; Dr. Babasaheb Ambedkar Marathwada University and explants viz. shoot, leaves of *Tylophora asthmatica* were collected from the elite plant. Juvenile twigs were surface sterilized with HgCl<sub>2</sub> solution (0.1% w/v)

for four - five min followed by three washes with sterile distilled water. Nodal segments of twigs were cut (0.5 cm) and cultured on 8% (w/v) agar solidified MS supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kin) at different concentrations and combinations. Subculturing was done at an interval of 14 - 20 days. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at  $25 \pm 2^\circ\text{C}$  under 16/8 hr photoperiod. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimation.

## Results and Discussion

Within fifteen days of culture, callus was induced at the cut end of nodal explants. Explants like leaf, stem segment, grown on MS supplemented with 1, 2, 3 and 5 mg/l BAP and Kin either alone or in combination with 0.1 - 1.0 mg/l NAA, IAA and IBA (Table 1). Maximum (80%) callus was induced on MS fortified with 5.0 mg/l BAP with 0.5 mg/l NAA after two successive subcultures. In this combination light

yellowish green and nodular callus developed. Callus was also induced in BAP and Kin supplemented medium (Plate a, b). However, KIN was found to be more effective than BAP for induction of multiple shoots (Table 1). Highest number of multiple shoots formed was 14 using 5 mg/lit KIN and alone (Plate c,d). This was followed by 10 shoots using 4 mg/lit KIN alone. 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.

There were significant differences in regeneration frequencies of callus and multiplication of shoots. As stated by Martin (2002) the high morphogenic efficiency of node segments derived callus 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 culture elongated. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days.

**Table 1.** Effect of different concentrations of growth regulators on Explants

| Growth regulators(mg/l)      |     |     |     |     | % explants<br>producing<br>callus | No of shoots<br>produced |
|------------------------------|-----|-----|-----|-----|-----------------------------------|--------------------------|
| BAP                          | Kin | NAA | IAA | IBA |                                   |                          |
| Growth regulator Free Medium |     |     |     |     | -                                 | -                        |
| 1                            |     |     |     |     | 20 $\pm$ 3.84                     | 2 $\pm$ 1.52ab           |
| 2                            |     |     |     |     | 51 $\pm$ 1.22                     | 3 $\pm$ 1.52ab           |
| 5                            |     |     |     |     | 40 $\pm$ 1.21                     | 4 $\pm$ 2.08b            |
|                              | 1   |     |     |     | 18 $\pm$ 2.22                     | 3 $\pm$ 3.21ab           |
|                              | 2   |     |     |     | 31 $\pm$ 4.44                     | 10 $\pm$ 3.03b           |
|                              | 5   |     |     |     | 20 $\pm$ 1.21                     | 14 $\pm$ 3.46b           |
| 5                            |     | 0.1 |     |     | 40 $\pm$ 3.84                     | 2 $\pm$ 1.0ab            |
| 5                            |     | 0.5 |     |     | 82 $\pm$ 2.22                     | 2 $\pm$ 1.15ab           |
| 5                            |     | 1.0 |     |     | 55 $\pm$ 0.92                     | Nil                      |
| 5                            |     |     | 0.1 |     | 20 $\pm$ 3.84                     | Nil                      |
| 5                            |     |     | 0.5 |     | 44 $\pm$ 4.44                     | Nil                      |
| 5                            |     |     | 1.0 |     | 27 $\pm$ 1.13                     | Nil                      |
| 5                            |     |     |     | 0.1 | 31 $\pm$ 5.87                     | 2 $\pm$ 3.51ab           |
| 5                            |     |     |     | 0.5 | 40 $\pm$ 7.69                     | 2 $\pm$ 2,0ab            |
| 5                            |     |     |     | 1.0 | 20 $\pm$ 1.21                     | 3 $\pm$ 3.05a            |

a-Callus b-Shoots

Mean values within columns followed by the same letter are not significantly different at 5% level.

In *Tylophora indica* many tissue culture studies have reported for successful regeneration protocols. Somatic embryogenesis has been reported from mature leaves of *Tylophora indica* (Das and Rout,

2005, Choudhary et.al. 2005). Another protocol has been developed for high-frequency shoot regeneration and plant establishment of *Tylophora* from petiole-derived callus (Faisal and Anis, 2005).



a. Callus from stem as an explant



b. Callus from leaf as an explant



c. Multiple shoots



d. Multiple shoots

## Conclusion

Indian Ayurveda is a boon for us. Thousands of plants have been exploited during Vedic period. *Tylophora asthmatica* is one of the divine medicines used in treatment of asthma, dermatitis and rheumatism. The plant has been described for bronchodilator, emetic, expectorant and diaphoretic. Clinical studies have also shown effectiveness of the drug in bronchial asthma and thus modern research withstands the ancient claims of traditional medicine. Plant rose through the seeds shows tremendous genetic variation which is not suitable for commercial cultivation. Vegetative propagation is difficult in *Tylophora* due to low seed viability and germination rate (Thomas and Philip, 2005). Therefore present studies will be helpful for mass propagation of this plant.

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