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High frequency regeneration in *Tylophora asthmatica* (L. f.) Wight & Arn.

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Abstract
Present investigation deals with establishment of standard protocol for high frequency regeneration in medicinal plant *Tylophora asthmatica* (L. f.) Wight & Arn. Regeneration of plant has achieved on MS-media in combination with various concentrations of BAP and KIN along with IAA. The most suitable concentration was recorded at 3.0 mg/L of BAP along with IAA for high frequency of regeneration. KIN along with IAA facilitates multiple shooting along with somatic embryogenesis. Rhizogenesis were achieved using IAA and NAA and plants hardening protocol was followed and regeneration of plants was achieved.

Keywords: IAA, KIN, BAP and *Tylophora asthmatica* (L. f.) Wight & Arn.

1. Introduction
The plant *Tylophora asthmatica* (L. f.) Wight & Arn. belong to Asclepiadaceae and commonly known as Indian Ipecse or Antmool. It is commonly found in hill forests. It is twining under shrubs with thin creeping rootstock, grows up to 1.5 meters, leaves opposite rounded at base, acute or apiculate, glaucous and glabrous (Naik, 1987) [6] and roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers minute, 1-1.5 cm across, in 2-3 flowered fascicles in axillary umbellate cymes. Fruit a follicle up to 7 × 1cm ovoid to lanceolate, tapering at apex forming fine mucro, finally striate, glabrous, Seeds 0.6-0.8 × 0.3-0.4cm long (Gupta, 2003) [3]

The phytochemical studies revealed that the plant contains alkaloids such as Tylophorine, tylophorinine, tylophorinidine, septicine, isotylocrebrine, tylophorinicine and sterols, flavanoids, wax, resins, tannins etc. (Govindhari *et al*., 1975) [2]. Due to presence of above secondary metabolites the plant has been utilized traditionally for the curing bronchial asthma, jaundice and inflammation (Kirtikar and Basu, 1991) [5]. Ayurveda revealed that the plant has been used in making formulation for asthma, dermatitis and rheumatism (Chopra *et al*., 1986) [1] and showing antitumor, immunomodulatory, antioxidant, anti-asthmatic, smooth muscle relaxant, antihistaminic, hypotensive, anti-rheumatic activities. As the plant species is categorized as threatened species under IUCN red data list. Considering this fact the present study is aimed to develop standard protocol for high frequency regeneration for rapid propagation and enhance secondary metabolite.

2. Material and Method
The Plant *Tylophora asthmatica* (L. f.) Wight & Arn. was grown in the greenhouse located in Botanical Garden, Dr. Babasaheb Ambedkar Marathwada University Aurangabad and used as experimental material. The temperature in the greenhouse varied from 28 to 32^{0}C and 60% of humidity was maintained with sprinkler. Different explants were used for establishing cultures from leaves and nodal segment and it was taken from 1 year-old plants and inoculated after surface sterilization on MS fortified with different growth regulators.

3. Surface sterilization and Inoculation
Explant such as leaves and nodal segments were taken from juvenile plantlets and washed twice with tap water. Leaves were surface-sterilized with 0.01% (w/v) mercuric chloride for 3 min, followed by 3–4 times washed with sterile double distilled water and inoculated on agar-solidified MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of BAP and KIN in combination with IAA. The pH of the medium was adjusted to 5.8 before sterilization. Cultures were maintained at 25±1ºC with a 16-h photoperiod. Callus was sub-cultured after 25 days on the original callus-inducing medium. Established callus were transfer on medium containing different concentration of IAA along with BAP and KIN for high frequency regeneration. Observation were noted at regular Interval and recorded in Table.

4. Result and Discussion
Observation recorded revealed that induction of callus was achieved on MS-Media supplemented with various concentrations of BAP and KIN along with IAA using leaves and nodal segment explant. In case of leaf explant poor to moderate type of callus induction was achieved using 0.5-2.0 mg/L of IAA and BAP. Most suitable concentration was recorded at 0.5 mg/L of IAA along with BAP, which revealed moderate type of callus with 66.67 % of Callus induction frequency. Whereas, massive type of callus were induced using nodal segment explant with 86.66 followed by 73.33 percentage of callus induction frequency at 0.5 mg/L and 1.0 mg/L of BAP along with IAA. IAA in combination with KIN was also tested for induction of callus. Observation revealed that poor to moderate callus induction frequency. Most suitable concentration recorded that 1.5 mg/L and 0.5 mg/L of KIN along with IAA with 53.33 and 66.67 percent of callus induction using leaves and

nodal segment explant, respectively. Callus induced using KIN after 2 weeks facilities development of somatic embryo.

Table 1: Effect of IAA along with BAP and KIN on different explant

Growth regulators mg/L			Induction of Callus/Shoot %	
			Source of explant	
IAA	**BAP**	**KIN**	**Leaf**	**Nodal Segment**
0.5	0.5		66.67 mC	86.66 MC/S
1.0	1.0	--	60.00 mC	73.33 MC
1.5	1.5		53.33 PC	60.00
2.0	2.0		40.00 PC	46.66
0.5		0.5	33.33 PC	66.67mC/SE
1.0	--	1.0	40.00 PC/SE	53.33 PC
1.5		1.5	53.33 PC/SE	46.67 PC
2.0		2.0	33.33 PC	40.00 PC
High frequency regeneration				
			No. of Shoot	**Regeneration %**
0.5	1.0		5.13±0.98	66.67
	2.0		7.02±1.17	73.33
	3.0		8.73±1.22	80.00
	4.0		8.00±1.33	73.33
		1.0	4.06±0.93	60.00
		2.0	4.33±0.89	66.67
		3.0	5.53±0.91	73.33
		4.0	4.66±0.83	73.33

PC=Poor Callus, mC=Moderate Callus, MC=Massive Callus, MC/S= Massive Callus with shoot, SE= Somatic embryogenesis.

After 25 days callus were sub-cultured on MS-Medium incorporated with various concentration of BAP and KIN ranging from 1-4 mg/L along with 0.5 mg/L IAA. Maximum number of shoots was recorded at 3.0 mg/L of BAP in combination with IAA, which was 8.73±1.22 number of shoots with 80.00 % of shoot regeneration. In case of KIN along with IAA highest numbers of shoots recorded at 3.0 mg/L KIN shows 73.33 % of shoot regeneration with 5.53±0.91 number of shoot per explant.

In vitro propagation protocol of *Tylophora asthmatica* (L. f.) Wight & Arn. Were developed successfully earlier by Sulekha Rani, and Rana, J. S. (2010) [7]; Kaushik *et al*., (2010) [4]. Sulekha Rani and Rana (2010) [7] developed standard protocol for regeneration of *Tylophora* on MS medium supplemented with 2.0mg/L BAP whereas, kaushik *et al*., reported the most suitable concentration 3.0 mg/L of BAP, similarly kind of result were recorded in present investigation, with high frequency regeneration. Faisal *et al*., (2005) reported high frequency regeneration using TDZ and 2, 4-D whereas, in present investigation, it was using 3.0 mg/L BAP and KIN along with IAA was achieved.

Multiple shoots

Multiple shoots

5. Conclusion

Tylophora asthmatica (L. f.) Wight & Arn. is threatened species and it is used in Ayurveda to cure various diseases, hence the conservation of plant is in urgency. For conservation, new alternate method must be developed. Present investigation provides rapid and high frequency regeneration for the conservation of the plant species.

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