RESEARCH ARTICLE

Hormonal Control of Morphogenesis *in vitro* in Nodal Segments of *Tylophora indica*

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Received: 18 March 2017/Revised: 28 May 2017/Accepted: 23 June 2017

ABSTRACT- The present study describes the hormonal regulation of morphogenesis *in vitro* in nodal segments of *T. indica*. The nodal explants, sterilized with 0.1% $HgCl_2$, were cultured on Murashige and Skoog Medium enriched with various combinations and concentrations of plant hormones auxin and cytokinin to study the hormonal regulation in morphogenesis *in vitro* in *T. indica*. BAP at high concentration could not evoke any morphogenetic response in nodal explants. Calli formation at the basal part of nodal explants were noted on medium containing BAP (0.1 mg/L) and 2,4-D (5.0 mg/L). 0.1 mg/L BAP was found most effective in the shoot development of the *T. indica*. Rhizogenesis was observed on half-strength MS medium supplemented with 1.0 mg/l IAA and 0.1 mg/l NAA. The study may also be used in mass-propagation and conservation of this medicinal plant species.

Key-words- Plant growth regulators, Morphogenesis *in vitro*, *Tylophora indica,* Rhizogenesis

INTRODUCTION

T. indica (Burm. f.) Merrill, a member of Asclepiadaceae family, is an important medicinal plant species, which is used to cure various ailments such as bronchial asthma, bronchitis, rheumatism, inflammation, allergies and dermatitis [1-2]. The powdered leaves, stem and root contain several medicinally important alkaloids including tylophorine, tylophorinine [3]. Unfortunately, due to over exploitation for its bioactive constituents and lack of cultivation practices, the natural populations of this plant species has declined rapidly in the last few decades. Recent developments in biotechnology provide new methods for conservation of threatened flora, using *in vitro* culture techniques. Through these techniques, mass-propagation of desired plant may be achieved by culturing explants on nutrient medium under *in vitro* conditions.

Plant hormones regulate many aspects of plant growth and development. Both auxin and cytokinin have been known for a long time to act either synergistically or antagonistically to control several significant developmental processes, such as the formation and maintenance of meristem. Therefore, in the present study, efforts were carried to screen the effects of various plant growth regulators on morphogenesis *in vitro* in *T. indica*. This type of study is highly beneficial to understand the role of various plant growth regulators on plant growth and development.

Access this article online	
Quick Response Code	Website: www.ijlssr.com
	DOI: 10.21276/ijlssr.2017.3.4.25

MATERIALS AND METHODS

Plant material and surface sterilization: Nodal explants were collected during May 2011 to June 2011from healthy plants of *T. indica* growing in the plant nursery at the Jaipur National University, Jaipur (India). The excised nodal explants were washed thoroughly under running tap water to eliminate dust particles for 30 min and then sterilized in 0.1% $HgCl_2$ solution for 4–6 min followed by thorough washing with sterile double distilled water.

Culture media and growth conditions: To study the hormonal regulation of morphogenesis *in vitro*, the sterilized nodal explants were cultured on Murashige and Skoog medium (MS media) [4] supplemented with various concentrations and combinations of auxin and cytokinin. The pH of the media was maintained 5.8 before autoclaving at 121 ^{o}C for 20 min. Cultured flasks inoculation were incubated at 25±2 ^{o}C and 65–70% relative humidity with photoperiod of 16/8 h at 3000 lux intensity by florescent tubes.

RESULTS AND DISCUSSION

Plant growth regulators play fairly important roles in many aspects of plant growth and development. The interaction between auxin and cytokinin is particularly important to control morphogenesis in plants. In the present study, nodal explants of *T. indica* did not show any morphogenetic response on media supplemented with various concentrations of Kinetin (Fig. 1 A). Shoot development was observed on low concentration of BAP (0.5 mg/L) (Fig. 1 B). Higher concentration of 6-Benzylaminopurine (BAP) showed inhibitory effects on shoot development. Similar, the inhibitory effect of higher concentration of BAP on shoot development was observed in Gymemma sylvestre [5], Cunila galioides [6], Dictyospermum ovalifolium [7], and Codiaeum variegatum [8]. Auxins (2,4-D, IAA, IBA, NAA) alone, or in combination did not trigger any response in nodal explants of *T. indica*. The development of compact calli was noted on medium supplemented with BAP (0.1 mg/L) and 2,4-D (5.0 mg/L) (Fig. 1 C). Low concentration of BAP (0.1 mg/L) was found most effective in shoot development of the *T. indica* (Fig. 1 D).

Rhizogenesis followed by callus formation was noted on medium fortified with 0.5 mg/L BAP and 5.0 mg/NAA (Fig. 1 E). Rhizogenesis was observed when nodal explants were cultured in ½ MS medium containing 1.0 mg/l IAA (Fig. 1 F). Frequency of rhizogenesis was enhanced when NAA was incorporated along with IAA (Fig. 1 G). ½ MS medium containing 1.0 mg/l IAA and 0.1 mg/l NAA produced high frequency root induction in nodal explants of *T. indica*.

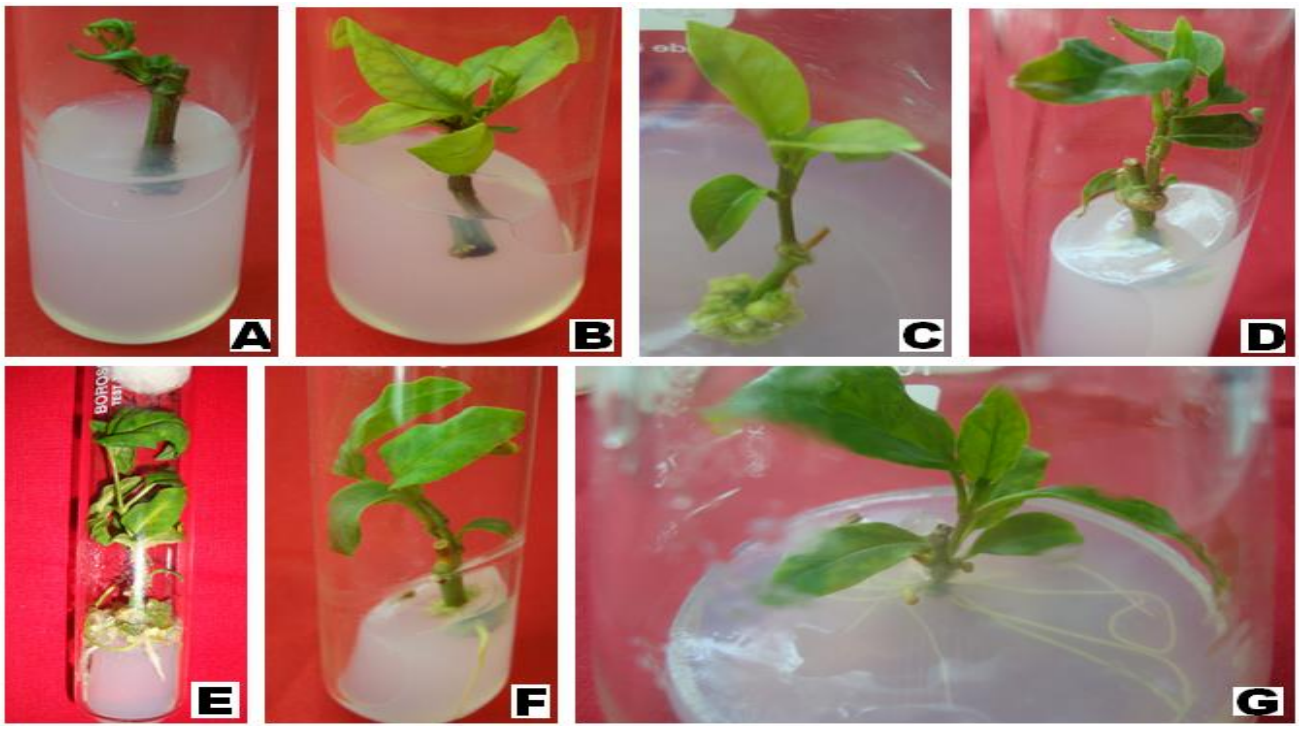

Fig. 1: Nodal explants of *T. indica* showing *in vitro* shoot development on MS medium containing 1.0 mg/L KIN (A), and 0.5 mg/L BAP (B); Rhizogenesis followed by shoot development on BAP (0.1 mg/L) and 2,4-D (5.0 mg/L) (C); Elongated shoot on 0.1 mg/L, BAP (D) And rhizogenesis (E-F)

CONCLUSIONS

The present study clearly demonstrates that BAP play an essential role in shoot development of the *T. indica* plant. Over-expression of BAP causes an inhibitory effect on shoot formation. IAA and NAA at low concentrations act synergistically to promote rhizogenesis *in vitro* in the *T. indica*. The study will be useful to understand the hormonal basis of morphogenesis and plant development.

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How to cite this article:
Soni V, Bhushan M: Hormonal Control of Morphogenesis *in vitro* in Nodal Segments of *Tylophora indica*. Int. J. Life Sci. Scienti. Res.,2017; 3(4):1250-1252. DOI:10.21276/ijlssr.2017.3.4.25
Source of Financial Support: Nil, **Conflict of interest:** Nil