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Effect of cytokinins on *in vitro* propagation of *Petunia hybrida* Hort. (Vilm)

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ABSTRACT
An efficient *in vitro* propagation protocol has been developed in *Petunia hybrida* using nodes as explants. Both direct as well as indirect shoot regeneration was achieved. Direct shoot regeneration was obtained on MS basal medium after 7 days of inoculation with 50% culture response. Medium containing BAP 0.5 mg/l resulted in direct shoot regeneration after 13 days of inoculation with 50% culture response. Best results were obtained on MS medium supplemented with BAP 1 mg/l which resulted in indirect shoot regeneration in 70% cultures after 5 average number of days. Indirect shooting with 20% culture response was also obtained on MS medium containing BAP 2 mg/l after 10 days. However, medium containing Kn 1 mg/l resulted only in direct shoot regeneration after 5 days of inoculation with 60% culture response.

Keywords: *Petunia hybrida, Solanaceae, Nodal explant, MS medium, BAP, Kn*

INTRODUCTION
The genus *Petunia* belongs to the family Solanaceae and contains around 35 species from South America. The plant is geographically distributed in temperate and sub-tropical regions of Argentina, Bolivia, Brazil Uruguay and Paraguay. A British nurseryman, Atkins (1834) was the first to obtain garden *Petunia* by hybridization. The commercial *Petunia hybrida* is derived from crosses between a bee-pollinated *P. integrifolia* and white-flowered, moth-pollinated *P. axillaris* (Sink, 1984). There are 2 categories of *Petunia* hybrids, grandiflora (large-flowered and of trailing habit) multiflora (many-flowered and bushier). *P. hybrida* is an economically important ornamental plant species (Davies *et al*., 1994). It is greatly diversified and available in a range of colors (Christopher, 1994). Development of new technologies, such as tissue culture results in further growth and success (Geneve *et al*., 1997). Many ornamental plants of commercial use, are being propagated by *in vitro* culture techniques (Rout and Jain, 2004). In the present study, this technique has been extended to one such ornamental plant, *P. hybrida* using nodal explants.

Methodology
Nodal explants were collected from plants growning in KUBG. Explants were first thoroughly washed under running tap water in order to remove dirt and dust followed by washing with detergent labolene and surfactant tween-20. Detergent was removed by washing the explants with double distilled water. Explants were then treated under laminar air flow hood with chemical sterilant (2% sodium hypochlorite) for 5 min. This was followed by washing with autoclaved double distilled water and finally inoculation of explants on sterilized Murashige and Skoog's (MS, 1962) medium, the medium was gelled with 8% agar and supplemented with different concentrations of cytokinins (BAP and Kn). The pH of the media was adjusted to 5.8 before autoclaving at 121 °C and 15 lb. The cultures were incubated at 22±4 ^{0}C and *maintained under controlled growth conditions. The periodic observations were recorded for callus induction and shoot regeneration.*

Results

*D*uring the present work effect of cytokinins on *in vitro* response of *P.hybrida* was studied using nodal

explants. MS basal medium as well as medium supplemented with different concentrations of cytokinins (BAP, Kn) was used. Callus was produced on medium fortified with BAP 1 mg/l and BAP 2mg/l after 8 and 13 average number of days of inoculation. Direct shoot regeneration (Fig. a) was obtained on MS basal medium after 7 days of inoculation with 50% culture response. Direct shoot regeneration was also achieved on MS medium supplemented with BAP 0.5 mg/l (Fig. b) after 13 days of inoculation with 50% culture response. Indirect shoot regeneration (through callusing; Fig.c) was obtained on medium containing BAP 1mg/l after 5 average number of days of callusing, with 70% culture response. Indirect shooting with 20% culture response was also obtained on Medium containing BAP at a concentration of 2 mg/l after 10 days of callusing phase (Fig. d). However, only direct shoot regeneration with 60% culture response was obtained after 5 days of inoculation, when medium was supplemented with Kn 1 mg/l (Fig. e).

a **b** **c** **d** **e**

Fig. : *In vitro* response of nodal explant

a. Direct shoot regeneration on MS basal medium

b. Direct shoot regeneration on MS + BAP 0.5 mg/l

c. Indirect shoot regeneration on MS + BAP 1 mg/l

d. Indirect shoot regeneration on MS + BAP 2 mg/l

e. Direct shoot regeneration on MS + Kn 1 mg/l

Table 1: Effect of different concentration of cytokinins on callus production and shoot regeneration from nodal explants

Treatments	Explant used	Callus Production	No. of days taken for Callus Production	Shoot regeneration	No. of days Taken for direct shoot regeneration	Percent culture response
MS basal	Node	_	_	+	7 days	50 %
MS+0.5mg/l BAP	Node	_	_	+	13 days	50 %
MS+1mg/l BAP	Node	+	8 days	+	5 days	70%
MS+2mg/l BAP	Node	+	13 days	+	10 days	20%
MS+1mg/l Kn	Node	_	_	+	5 days	60%

- No response

+ Positive response

Discussion

The present study was carried out to investigate the effect of various cytokinins (BAP and Kn) on *in vitro* morphogenesis of *P. hybrid* using nodes as explants. The explants exhibited direct shooting and indirect shooting (via callusing) when inoculated on MS medium supplemented with the above mentioned cytokinins. BAP was found more efficient than other cytokinins with respect to initiation and subsequent proliferation of shoots. Our results are in accordance with that of Tiwari *et al*.,(2000) and Fatima and Anis (2012) who also found BAP to be more efficient in terms of shoot initiation and proliferation in *Bacopa monniera* and *Withania somnifera* respectively. In the present study maximum regeneration percentage was observed on MS medium supplemented with BAP 1.0mg/l. The study of Abu-Qaoud *et al*., (2010) in *P.hybrida ;* Jackson and Hobbs (1989) in *P. sativum* also support our results .However, our results are in contrast to the results of Rzepka-Plevnes and Kurek,(2001) who reported MS medium fortified with BAP (3mg/l) best for *in vitro* propagation of *Fiscus benjamina*.

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