International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Effect Of Hormone On Callus Induction in *Boerhaavia Diffusa* L.

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ABSTRACT - *Boerhaavia diffusa* (Punarnava) is pungent, bitter, hot and laxative. It is a cooling stomachic antipyretic and cardiotonic. It stimulates the function of heart and kidney and has been found specific for diabetes, jaundice and general debility. It is used to cure sascites, anasarca, asthma and in scanty urine. In the present study callus induction in Boerhaavia diffusa has been developed in culture medium with hormone. Apical leaves were used as explants for callus induction on MS medium containing2, 4-D (2, 4-Dichlorophenoxi acetic acid) and BAP (6-benzylaminopurine). The callus formation was monitored every week. The calli in most of the cultures were green and friable in nature. In the future, to avoid contamination and produce better callus each steps have to be done more carefully.

Key words: *Boerhaavia diffusa, Punarnava, Explants ,Callus, Hormone.*

I. INTRODUCTION

Boerhaavia diffusa L., is an herbaceous weed of the family Nyctaginaceae commonly Known as 'Punarnava' and is widely distributed in the tropics and sub-tropics (Mahesh et al, 2012). It has a long history of indigenous uses by tribal people and in Ayurvedic or natural herbal medicines. The whole plant of *B. diffusa* is a very useful source of the drug Punarnava, which is documented in India. The active principle contained in this herb is an alkaloid, known as Punarnavine (Chopra, 1969). The roots and leaves with flowers have been found to be highly potent (CSIR, 1988). In ayurvedic medicine, different parts of this plant were reported to have various medicinal properties. It was used in renal ailments as diuretic (Anand, 1995) and to treat seminal weakness and blood pressure (Gaitonde *et. al*., 1974). It is also used in the treatment of stomach ache, anemia, cough, cold and a potent antidote for snake and rat bites (Chopra *et. al.*, 1956), in the treatment of nephritic syndrome (Singh and Udupa 1972), hepatitis, gall bladder abnormalities and urinary disorders (Mudgal, 1975).The flowers and seeds are used as contraceptive. Callus is defined as an unorganized tissue mass growing on solid substrate. Callus

formation is central to many investigative and applied tissue culture procedures. Callus can be multiplied and later used to clone numerous whole plants. Since extensive callus formation can be induced by elevated hormone levels, tissue culture media designed to produce callus contain pharmacological additions of cytokinins and auxins.

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II. MATERIALS AND METHODS

1. Explants source and preparation:

Actively growing young leaves B.diffusa were collected from the field grown plants. The surfaces of explants usually carry a wide range of microbial contaminants. Thus, the explants must be thoroughly surface sterilized before inoculating on culture medium to avoid infection. For callus initiation leaves of young vegetative stem with a size of 1.5-2.0 cm were excised from the ex-vivo plant and washed under running tap water for 30 min. and then with liquid detergent. The explants were then disinfected using 0.1 %(w/v) HgCl2 (Hi-media) for 2 min (Mrudul et al., 2001; Neeta et al., 2002; Chan and Tong, 2004; Yusuf et al., 2007; Sundram et al., 2012). There after the segments were washed 5-7 times with sterile distilled water.

2. Culture media preparation and explants implantation

Murashige and Skoog's (MS) medium supplemented with sucrose (3%) and agar (0.8%) were prepared with different concentrations of auxin and cytokinin. The pH of media was adjusted to 5.8 before gelling with 0.8% agar &was subjected for autoclaving. The media was then poured in Flask stored at room temprature. The prepared explants were implanted on the media and were maintained for data analysis. The experiment was set in triplicate.

3. Culture maintainance

Culture was maintained at 26±2ºC with a photoperiodism of 16h under illumination of 4000 lux under aseptic condition of culture room and was observed for callus.

III. RESULTS AND DISCUSSION

In our present investigation callus induction were directly initiated from Apical leaves explants of *Boerhaaviadiffusa* after successful sterilization. The Apical leaves explants were transferred to fresh MS medium supplemented with different concentration of auxins and cytokinins including (2, 4-D, BAP, Kn,IAA,IBA). On MS medium supplemented with 2, 4-D of the leaf explants showed callus initiation. MS basal medium supplemented with BAP+2, 4-D induced callus in different cultures after three – four weeks of inoculation as shown in (Table1). The optimum concentration of BAP (0.5mg/l) +2, 4-sD (5.0mg/l) in terms of maximum mean percentage of callusing was observed (Photo1). The efficacy of exogenous 2, 4-D found in this experiment, was also been reported with other medicinal plants by various authors. Results described by Rani et al. (2003), Thomas and Maseena (2006), Hassan et al. (2009) , Davallo et al. (2014) were also in agreement with our result for using this synthetic plant growth regulator in the culture medium for callus induction of Withania somnifera, Cardiospermum halicacabum Linn , Abrus precatorious, and Jasminum sambac respectively. Our finding in the experiment regarding type of explants responses contradict the finding of Hassan et al. (2009),found the best of calli responses only from apical leaves with same texture.

Figure: 1

TABLE- 1

MEDIUM	MS+Surcose(3.0/mg/l)+2,4-D(5.0mg/l)+BAP/Kn(0.5-2mg/l)	
INCUBATION	26±2ºC in 16h photoperiod for three-four weeks	
Cytokinins **0.5** **1.0** **2.0**	Respose C^{+++} C^{++} C^{++}	Remark Green,firable callus.
Kn **0.5** **1.0** **2.0**	 C^{-} C^{-} C^{+}	 Brown callus

C^{-} -No callusing
C^{+} -Slighit callusing
C^{++} -Moderate callusing
C^{+++} - Maximum callusing

IV. ACKNOWLEDGEMENTS

The author acknowledged to UGC project grants for the financial support and thanks to Director of Poddar College for providing necessary facilities and encouragement.

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