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RESEARCH ARTICLE

IN VITRO STUDIES OF *BOERHAAVIA DIFFUSA* LINN

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ABSTRACT

Boerhaavia diffusa Linn. Belonging to family Nyctaginaceae is an important medicinal plant. An efficient protocol was developed for *in vitro* propagation of *B. diffusa*. Leaf explants was used for callus induction and regeneration of *B. diffusa* on MS basal medium, with different concentrations and combinations of cytokinins (BAP, Kinetin) and auxins (NAA, IAA). Best response to shoot proliferation was achieved with MS medium supplemented with 3.0mg/l BAP and 1.0mg/l NAA. Efficient rooting was achieved at low concentrations, 0.5mg/l of IAA. About 80% of the regenerated plants survived in the field conditions.

INTRODUCTION

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. Recently much progress has been achieved through this technology for some important medicinal plants (Ajeethkumar and Seeni, 1998; Bajaj, 1998; Baksha *et al.,* 2007; Roja *et al*., 1991; Bhattacharya, 1992.). The capability to regenerate and propagate plants from culture cells and tissues is one of the most exciting aspects of *in vitro* cell and tissue culture. Increasing demand of herbal and medicinal plants is one of the major cause of their rapid depletion from the natural habitats. Micro propagation offers a great potential for conservation and large scale multiplication of such useful species and subsequent exploitation. *B. diffusa* is a perennial creeping weed of the family Nyctaginaceae and is widely distributed in the tropics and sub-tropics. 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, the active principle contained in this herb is an alkaloid, known as Punarnavine (Chopra *et al*., 1986). Pharmacological studies have demonstrated that *Boerhaavia sp,* exhibits a wide range of properties; diuretic (Gaitonde *et al.,* 1974); anti-inflammatory (Bhalla *et al*., 1968); anticonvulsant (Adesina, 1979); antibacterial (Olukoya *et al.*, 1993) and antistress activity (Mishra, 1980; Chandan *et al.,* 1991; Rawat *et al.*, 1997). The roots and leaves with flowers have been found to be highly potent. It is very effective to treat seminal weakness and blood pressure (Gaitonde *et. al*., 1974). *Boerhaavia* extract has remarkable antioxidant activity, antiantherosclerotic activity and hypertension reducing activity (Pari and Amarnath, 2004). A large number of publications on the chemistry, pharmacology and several other aspects on uses of *B. diffusa* have been made, but little attempt has been made on *in vitro* regeneration of *B. diffusa* (Bhansali *et. al*., 1978; Shrivastava and Padhya, 1995; Nagarajan *et. al.,* 2005). Mass scale collection of this plant from natural habitats is leading to a depletion of this plant species. *B. diffusa* is propagated by seeds, but the seed viability is poor and has very low germination percentage. Micro propagation method is specifically applicable to species in which clonal propagation is needed (Gamborg and Phillips, 1995). In the present paper, an efficient and reproducible clonal propagation system through *in vitro* culture of *B. diffusa* has been described. The present work was focused to an efficient regeneration with a lesser duration and also a less requirement of plant growth regulators for achieving high frequency of shoot multiplication of *B. diffusa* with potential active ingredients.

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MATERIALS AND METHODS

Healthy leaves of *B. diffusa* were collected from 5-6 week old field grown plants growing in Botanical garden of Utkal University, Bhubaneswar. Young leaves were rinsed under running tap water for 20 minutes and were surface sterilized separately in an aqueous solution of 0.1% (w/v) mercuric chloride for 3-4 minutes.

A. Shoot buds differentiation from semi friable calli of *Boerhaavia diffusa* on MS medium supplemented with BA (3.0 mg/l) and NAA (1.0 mg/l) after five weeks of callus initiation
B. Shoot elongation and multiplication of *Boerhaavia diffusa* on MS medium with BA (2.0 mg/l)
C. Root induction and proliferation from microshoots of *Boerhaavia diffusa* on half strength MS medium supplemented with IBA (1.0 mg/l) after four weeks of transfer
D. *In vitro* derived plantlet of *Boerhaavia diffusa* with shootlet and rootlets. Hardened plants in Poly Cups.
E. Acclimatized plants in field.

The inoculation and transfer of sterile plant material on culture medium was done on White's medium (1963), Murashige and Skoog's medium (1962) and Gamborg's medium (1968). The different media were supplemented with various concentrations and combinations of auxins and cytokinins. The pH was adjusted to 5.8 $\pm$ 1 before autoclaving. The media were then autoclaved at 121^{0}C, 15 psi for 15 minutes. The cultures were maintained at 22 $\pm$ 1^{0}C under a 16 hour photoperiod. Later different concentrations of growth regulators were added on MS basal medium for differentiation of the callus. The excised shoots were transferred to liquid medium for rooting. The *in vitro* raised shoot lets were sub cultured on ½ strength MS medium supplemented with various concentrations of (0.5-1.5mg/l of IAA and IBA). *In vitro* rooted plantlets were transplanted to small earthen pots containing a mixture of soil and compost (2:1) and covered with transparent polyethylene bags to maintain high humidity. They were hardened in plastic cups containing vermiculite soil and kept in the green house for further acclimatization and finally transferred to the field.

RESULTS AND DISCUSSION

Induction of Callus and Shoot bud proliferation

MS medium (Murashige and Skoog's) was found to be the most suitable for both callus induction and regeneration in *B. diffusa* (Table 1).

Table 1. **Effects of growth regulators in different media on callus induction on *Boerhaavia. Diffusa***

Medium	2, 4-D	IAA	BA	kn
MS	78.0	15.2	69.0	45.2
B_5	70.6	12.0	55.3	38.4
WM	30.3	5.3	18.7	20.0

Table 2. Effects of growth regulators on shoot bud induction from leaf explants of *B. diffusa*

Growth Regulators	Percentage No. of Shoots	Mean no. of shoots ±SD
BA (1.0)	15	1.4 ± 2.80
BA (2.0)	31	0.9 ± 2.90
BA (0.5) + NAA (0.5)	35	1.7 ± 2.70
BA (1.0) + NAA (0.5)	65	2.1 ± 1.99
BA (3.0) + NAA (1.0)	85	2.6 ± 1.44
Kn (2.0) + NAA (0.5)	35	1.0 ± 2.40
Kn (3.0) + NAA (1.0)	30	0.8 ± 1.80

From the different growth regulators (2, 4-D IAA, BA and Kn) tried in the experiment to initiate callus formation, 2,4-D elicited better response. This result directly coincided with the result obtained by Sudarshana *et al.* (2008) in *B. diffusa.* The explants showed induction of callus within 15 days. The calli were semi- friable in nature and it was light pale yellowish brown in colour. MS medium supplemented with 2,4-D showed the best callus proliferation of 78 %. Similar findings were also observed when 2,4-D has been used for callus induction from albedo tissue of fruits (Einset, 1977) and leaf (Goh *et al*., 1995). MS medium supplemented with kinetin supplemented showed 45.2 % callus induction.

Table 3. Effect of auxins on root induction of *B. diffusa* in half strength MS medium

½ MS + Auxin conc, in mg/l		Mean % of Rootlet formation ± S.E
IBA	IAA	
0.5	0.0	84.8± 0.71
1.0	0.0	94.8± 0.48
1.5	0.0	86.7± 0.62
0.0	0.5	68.4± 0.54
0.0	1.0	74.8± 0.61
0.0	1.5	62.7± 0.58

The mean values given in the table are the average of 10 replicates with the standard errors.

This result was directly in consonance with Rout *et al*. (1999) observation in *Plumbago zeylanica.* Since MS medium showed the best results among the different basal medium, it was selected as the medium to study the effects of different growth regulators for proliferation of callus. Shoot buds were differentiated after 5 weeks of callus initiation. Maximum percentage (85 %) of shoot proliferation occurred on a medium containing BA (Benzyle Amino Purine) 3.0mg/l and +NAA (Naphthalene Acetic Acid) 1.0mg/l. Biswas *et al.* (2009) observed MS medium augmented with 3.0 mg/l of BA was optimal concentration for the multiple shoot proliferation with 53% and 2.20 ± 0.12 mean no. of shoot. BA exhibited better response than kinetin in shoot bud proliferation (Table 2). Similar response was reported by Roy *et al.* (1995) in case of *Rauvolfia serpentina.* In the present study a high frequency and maximum number (85%) of multiple shoots were elicited on MS medium; containing 3.0 mg/l BA + 1.0 mg/l NAA followed by 1.0mg/l BA + 0.5 mg/l NAA. This result was in agreement with the previous observations on *Canavalia virosa* (Kathiravan and Ignacimuthu, 1999), *Wadelia canlendulacea* (Emmanuel *et al*., 2000) and *Phyllanthus amarus* (Johnson, 2006). In contrast to this Ray and Bhattacharya (2008) reported multiple shoots on BA (2.0 mg/l) in *Eclipta alba* L.

Induction of Root, Hardening and Acclimatization of plantlets

The *in vitro* raised shootlets showed maximum rooting (94.8 %) when the ½ strength MS medium was supplemented with 1.0 mg/l of IBA. Similar rooting response with IBA, were obtained in *Centella asiatica* (Banerjee *et al.*, 1999). After 9-10 days, *in vitro* rootlets were produced. Best rooting response was observed in ½ MS medium supplemented with 1.0 mg/l of IBA (Table-3). The optimal role of IBA for rooting in the regenerated plantlets has also been reported by many workers (Vadawala *et al*., 2006; Zhu *et al*., 2006; Barik *et al*., 2007). Approximately 80 percent of the regenerated plants survived under the field condition. More or less similar response was also observed by Nagaranjan *et al.* (2005) in *B. diffusa* and Ahmed *et al.* (2001) in *Holarrhena antidysenterica.* The efficient protocol described here for micropropagation of *B. diffusa* facilitates rapid multiplication of this important as well as valuable medicinal plant. These results were encouraging as these eliminates intensive labour, shorten time period required for the development of plantlets and overcome the problems arising due to indiscriminate harvest from wild population and rapid loss of germinability of the seeds.

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