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In-vitro callus induction and shoot regeneration in *Boerhaavia diffusa* L.

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ABSTRACT

In the present study the protocol for callus induction and regeneration in Boerhaavia diffusa has been developed in culture medium. Young apical leaves, nodal region and roots was used as explants for callus induction on MS medium containing 2-4- D and Kinetin. Callus initiation was first recorded in the lamina of leaf and nodal region. The optimum % of calli was obtained from leaf lamina. The calli in most of the cultures were yellowish white and friable in nature. After callosogenesis the regeneration of shoot takes place.

Key words: *Boerhaavia diffuse*, Regeneration.

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 there has been much progress in this technology for some medicinal plants. 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 those plants, which are specially use for the food and medicine, is one of the cause of their rapid depletion from the natural habitats. Micropropogation offer a great potential for conservation and large scale multiplication of such useful species and subsequent exploitation.

Boerhaavia diffusa L., is an herbaceous 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, which is documented in India Pharmacopoeia as a diuretic; the active principle contained in this herb is an alkaloid, known as Punarnavine (Chopra, 1969). Pharmacological studies have demonstrated that punarnava possesses punarnavoside, which

exhibits a wide range of properties - diuretic (Gaitonde *et. al.*, 1974), anti-inflammatory (Bhalla *et. al.*, 1968), antifibrinolytic (Jain and Khanna, 1989), antibacterial (Olukoya *et. al.*, 1993), antihepatotoxic (Mishra, 1980), anthelmintic febrifuge, antileprosy, antiasthmatic, antiurethritis (Nadkarni, 1976) and antilymphoproliferative activity (Mehrotra *et. al.*, 2002). The roots of *B. diffusa* are a rich source of a basic protein or antiviral agent, which is used for inducing systemic resistance in many susceptible crops against commonly occurring viruses (Verma *et. al.*, 1979, Awasthi et al.1989). The purified glycoprotein from *B. diffusa* reduced infection and multiplication of tomato yellow leaf curl virus (Awasthi and Rizvi 1999), papaya ring spot virus (Awasthi, 2000) and cucumber green mottle mosaic virus (Awasthi et al. 2003). The aqueous crude extract from the dried roots was also found significantly active against a number of viruses - mung bean yellow mosaic virus (Awasthi, 2000), bean common mosaic virus (Singh and Awasthi, 2002) and water melon mosaic virus (Awasthi, 2002).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 (Chopra *et. al.*, 1956).

A large number of publications on the chemistry, pharmacology and several other aspects have been made, but here have been a few reports on *in vitro* regeneration of *Boerhaavia 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 study was undertaken to examine the potential of different explants of plant with different concentrations of hormone(s), alone or in combination for rapid initiation of callus and regeneration.

MATERIALS AND METHODS

Boerhaavia diffusa (L.) plants used in the present study were collected from the wild populations. Different explants were used for establishing callus including apical leaf, node and root explants. These were washed thoroughly under running tap water for 15 min. Subsequently sterilization was carried out in laminar airflow cabinet under aseptic conditions. Then explants were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, followed by 70% ethyl alcohol 2-3 min, then washed 3-4 times with sterile double-distilled water and inoculated on agar-solidified MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of 2,4-D, Kinetin and BAP, either alone or in combination. The pH of the medium is adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C with a photoperiod. Callus was subcultured after 12 days on the original callus-inducing medium. *In vitro* rooted plantlets were taken out from the conical flask and gently washed to free them from medium. Then they 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. After ten days polyethylene bags was removed and after two months the plants were planted in the open field.

RESULTS AND DISCUSSION

Initially explants from leaf, node and root, were cultured on MS medium with different concentration of 2, 4-d, Kinetin and BAP alone or in combination for callusing. The best result in term of percentage response of callus induction was obtained (96.66%) on 2, 4-D (5 mg/L) and kinetin (0.2 mg/L) in leaf explant and followed by node explant (76.66%) on the same hormonal concentration. Callus obtained from these explants were whitish friable and very soft in nature. Percentage of callus induction followed by leaf and node explants i.e., 66.66% and 50.00% respectively supplemented by Kinetin (5 mg/L) alone. Nature of callus in this case also found to be very soft and whitish friable. (Table 1 and Plate1). Root explant at all not responded for the callus induction on alone concentrations of 2,4-D, kinetin and BAP as well as not on combination of these hormones.

Table 1: Effect of different concentration of 2, 4- D with Kinetin and BAP on callus induction

Sr. No	Auxins (mg/l)	Cytokines (mg/l)		% of callus response			Nature of callus
	2,4-D	Kinetin	BAP	Leaf Explants	Node Explants	Root Explants	
1	1	-	-	36.66±1.92	23.33±3.84	NO	Y,W,F
2	3	-	-	50±0	36.66±1.92	NO	Y,W,F
3	**5**	**-**	**-**	**66.66±1.92**	**50±3.33**	NO	**W,F**
4	7	-	-	40±3.33	26.66±1.92	NO	Y,W,F
5	1	0.1	-	40±0	20±3.33	NO	Y,W,F
6	3	0.1	-	56.66±1.92	23.33±1.92	NO	Y,W,F
7	5	0.1	-	70±0	43.33±3.84	NO	Y,W,F
8	7	0.1	-	46.66±1.92	40±3.33	NO	Y,W,F
9	1	0.2	-	40±3.33	33.33±1.92	NO	Y,W,F
10	3	0.2	-	60±3.33	50±3.33	NO	Y,W,F
11	**5**	**0.2**	-	**96.66±1.92**	**76.66±1.92**	NO	**W,F**
12	7	0.2	-	66.66±1.92	60±3.33	NO	W,F
13	1	0.3	-	33.33±1.92	23.33±1.92	NO	Y,W,F
14	3	0.3	-	40±3.33	33.33±3.84	NO	Y,W,F
15	5	0.3	-	53.33±1.92	36.66±1.92	NO	Y,W,F
16	7	0.3	-	43.33±1.92	26.66±1.92	NO	Y,W,F
17	1	-	0.1	36.66±1.92	23.33±1.92	NO	Y,W,F
18	3	-	0.1	43.33±1.92	26.66±1.92	NO	Y,W,F
19	5	-	0.1	46.66±3.84	43.33±3.84	NO	Y,W,F
20	7	-	0.1	33.33±1.92	26.66±1.92	NO	Y,W,F
21	1	-	0.2	46.66±1.92	30±5.77	NO	Y,W,F
22	3	-	0.2	50±0	33.33±5.09	NO	Y,W,F
23	5	-	0.2	60±3.33	50±3.33	NO	Y,W,F
24	7	-	0.2	40±0	30±3.33	NO	Y,W,F
25	1	-	0.3	23.33±1.92	20±3.33	NO	Y,W,F
26	3	-	0.3	40±3.33	30±0	NO	Y,W,F
27	5	-	0.3	56.66±1.92	46.66±5.09	NO	Y,W,F
28	7	-	0.3	50±3.33	33.33±1.92	NO	Y,W,F

Media – MS +3% sucrose, NR = No Response, Y- Yellowish, W- White, F- Friable.

MS medium frequently used for micro propagation in large number of plants (Feyissa *et. al*., 2005). It was reported that the *Terminalia belerica* culture grew better on MS medium (Rathore *et. al.*, 2008). Similarly according to Crouch and Staden (1988) and Perica and Berljak (1996), the medium for in-vitro multiplication and callus induction of *Drosera* plant is MS medium. MS medium also reported as a superior medium for the micropropagation of *Coptis teeta* (Tandon, 2007). In addition of this several workers reported importance of auxins for callus induction (Bhat *et. al.*, 1992; Barna and Waklu, 1994: Bindahani *et. al.*, 2004). Use of explant from different plant parts viz. root, stem, leaf and inflorescence tip and parts of seedlings suggested that each and every parts of the plant has potential to regenerate a complete plant, it also proves totipotency aspect of cells. This aspect has been utilized by (Nagappa *et. al.*, 2008) for multiplication of plants. Result is supported by the work carried out for the callus induction in *Pterocarpus santhalinus* L. (Rajeshwari *et. al.*, 2008) and in *Terminalia bellerica* (Rathore *et. al.*, 2008) by inoculating node on MS medium supplemented with 2,4-D and Kinetin.

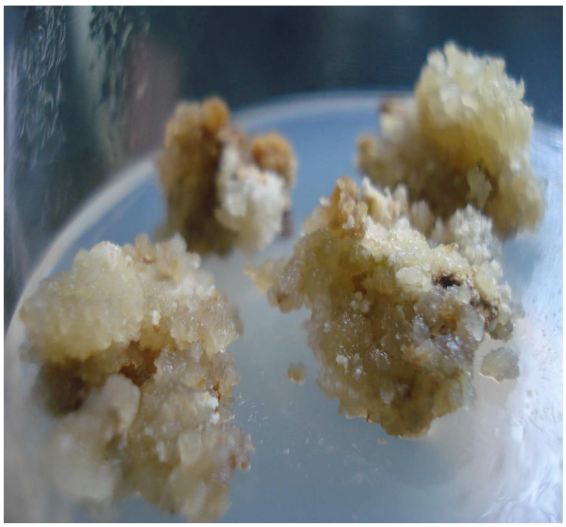
Table 2: Effect of different concentration of 2, 4-D with Kinetin and BAP on shoot induction

Sr. No	Auxin (mg/ml)	Cytokines (mg/ml		% of Shooting Response	Shoot Morphology
	2,4-D	Kinetin	BAP		
1	-	0.1		36.66±1.92	Thin, Short
2	-	0.2		66.66±1.92	Green, Short
3	-	0.3		40±0	Thin, Short
4	-		0.1	20±3.33	Thin, Short
5	-		0.2	33.33±1.92	Thin, Short
6	-		0.3	43.33±5.09	Thin, Short
7	0.25	0.1		46.66±1.92	Thin, Short
8	0.25	0.2		60±3.33	Green, Short
9	0.25	0.3		50±0	Thin, Short
10	0.5	0.1		63.33±3.84	Green, Short
11	**0.5**	**0.2**		**90±3.33**	**Green, Long**
12	**0.5**	**0.3**		**73.33±1.92**	**Green, Long**
13	0.25		0.1	53.33±5.09	Thin, Short
14	0.25		0.2	33.33±1.92	Thin, Short
15	0.25		0.3	16.66±1.92	Thin, Short
16	0.5		0.1	30±3.33	Thin, Short
17	0.5		0.2	53.33±1.92	Thin, Short
18	0.5		0.3	26.66±1.92	Thin, Short

Further study was carried out for shoot regeneration capacity. Leaf and node explants were cultured on MS supplemented with different concentrations of BAP, Kinetin and 2, 4-D alone or in various combinations for shoot regeneration. In both the leaf and nodal explants, only the node explant gave response. The highest percentage (90±3.33) of shoot induction was observed in MS + 0.5 mg/l 2.4-D + 0.2 mg/l kinetin (Table 2 and Plate 1). In this combination shoot morphology was found to be green and long. The medium containing Kinetin alone also produces shoots. On the medium containing BAP alone node explant responded well and produced more shoots. The results are supported by the work done by Handique and Bhattacharjee (2000) in which they found that the BAP and Kinetin favors shoot proliferation. Roy *et. al.*, (1995) observed similar response in case of medicinal plant *Rauvolfia serpentina*. More or less similar response was also observed Nagarajan e*t. al*., (2005) in *B. diffusa* and Ahmed *et. al.*, (2001) in *Holarrhena*

antidysenterica L. Rahman *et. al.,* (1999) also observed similar effects on *Emblica officinalis* culture.

Plate 1: Growth of *Boerhaavia diffusa* (L.) callus from apical leaf and node explants after two week in MS media supplemented with different hormone concentrations.



Leaf callus of *Boerhaavia diffusa*

In-vitro* regeneration of *Boerhaavia diffusa

In-vitro* rooting of *Boerhaavia diffusa

In-vitro* rooting of *Boerhaavia diffusa

CONCLUSION

From the above study, it is concluded that the callus induction in *Boerhaavia diffusa* were established from leaf and nodal region of the plant on MS medium supplemented with 2,4-D, kinetin and BAP. It is also concluded that multiple shoot cultures of *Boerhaavia diffusa* were established from nodal explants on MS medium supplemented with combination of hormones Kinetin (0.2mg/l) and 2,4-D (0.5 mg/l). This study aims to develop a standard protocol to initiate

multiple shoot culture and standardization of media and hormonal concentration of plant that may provide a good source of pharmacologically active plant constituents.

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