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TISSUE CULTURE STUDY FOR MICROPROPAGATION OF *BOERHAAVIA DIFFUSA* (L), AN IMPORTANT MEDICINAL HERB.

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ABSTRACT:

Boerhaavia diffusa (L.) belongs to family *Nyctaginaceae*, commonly known as **Punarnava,** is an important medicinal herb. This medicinal herb is mostly found in disturbed areas. From its wild habitat, the herb is over exploited by the agents of traders, due to which the species has become vulnerable. In the present study, attempts have been made to propagate the species *in vitro* by culturing nodal and shoot apex explants in culture medium under aseptic conditions. Nodal explants were cultured in MS basal medium + six different (0.5-4.0 mg/l) concentrations of BAP and KN, separately + 3% sucrose and gelled with 0.8% agar. Here MS+1.5 mg/l BAP induced axillary bud production on 98.64% nodal explants and 93.24% in shoot apex explants. The maximum number of axillary buds on nodal explants was 11.75 while in shoot apex it was 6.91. The mean length of axillary shoot in nodal explant was 5.44 cm while in shoot bud it was 4.84 respectively. In the similar concentration of KN the percentage of response for axillary shoot bud formation was 88.74 in nodal explants and 84.35 in shoot apex explants. Similarly, the mean number of axillary buds on nodal explants was 3.83 and on shoot apex explants it was 2.48 only. The mean length of axillary shoots was 4.76 in nodal explants and 3.85 in shoot apex. Here, it was further observed that neither the lower concentration nor the higher concentrations of the cytokinins were more promising with respect to axillary shoot bud initiation either on nodal or shoot apex explants of *Boerhaavia diffusa*. Well grown plantlets wee used for rooting in half strength MS medium, supplemented with four different concentrations of auxins (IAA, NAA and IBA) alone and IBA+IAA and IBA+NAA at 1.0+0.5 and 1.0+1.0 mg/l concentrations. When ½ MS + 1.0 mg/l IAA alone was used the percentage response for rooting was 874.0, mean number of

root is 4.76 and mean length was 2.46 cm respectively. But in case of ½ MS+ 2.0 mg/l NAA, the percentage response for rooting was 64.0, mean root number was 2.28 and mean length 2.40cm. Similarly, in ½ MS+1.0 mg/l IBA, the percentage response was 76.0, mean root number 5.22 and mean length was 3.24 cm respectively. Best percentage of response for initiation of roots *in vitro* was in ½ MS+1.0 mg/l IBA+1.0 mg/l IAA, which was 92%, the mean root number 6.36 and mean length 4.32 cm, while in the similar concentration of ½ MS+IBA+NAA it was only 53.0% the mean number was 4.52 and length 2.44cm. Here, ½ MS+ 1.0 mg/l IBA + 0.5 mg/l NAA gave better response. The time taken for shoot bud initiation was minimum where the percentage response for above was maximum.

KEY WORDS:
Boerhaavia diffusa, Punarnava, Micropropagation, Nodal and Shoot Apex Explants, *In Vitro* Rooting, Axillary Buds, Vulnerable.

INTRODUCTION:

Boerhaavia diffusa commonly known as Punarnava, belongs to family *Nyctaginaceae*. This medicinal herb has a high demand by the local herbalist and Ayurvedic companies. It is among the 46 medicinal plant species in high trade sources mainly from its wild habitat and commonly in wastelands. (Ved and Goraya, 2007). Different parts such as leaves, stem and roots bear different phytochemicals. The plant parts are being used in the preparation of Ayurvedic medicines such as Abana (Heart care); Bonnisan, Diabecon (Glucose care), Geriforte (Urinary problem), Punarnava, Chyavanprash etc. (Chaudhary and Dantu, 2011). Murti *et al*; (2010) reported that extract of this plant is being used to cure diabetic. It is also used in the treatment of stomachache, anemia, cough and a potent antidote for snake and rat bites (Roy, 2008). It is also used as expectorant; its regular use stimulates the function of heart, and kidney. It also helps to reduce symptoms of breast cancer. (Ahmed-Belkacem *et al*; 2007). Parti and Satheesh, 2004; Nalamouli *et al*; 2004. Extract of leaf is used to cure jaundice. The vaidya prescribe these plant products to cure epilepsy, and to improve seminal weakness and blood pressure (Gaitonde *et al*; 1974). *Boerhaavia diffusa* is known for its pharmacological properties like antilymphoproliferative, antiasthmatic, antibacterial, anti-inflammatory, antileprosy, antidiabetic, immune-modulation and anti metastatic (Mehrotra *et al*; 2002). Root extracts are used as an adjuvant to treat pulmonary tuberculosis. It is a very important source of the alkaloid drug, Punarnavnine, which is documented as a diuretic in Indian Pharmacopoeia (Kant *et al*; 2001). Srivastava *et al*; (2015) reported that two alkaloids, Boeravinones G and H are isolated from the roots of this plant. They also reported that it contains a ribosome inactivating protein BDP-30. The glycoprotein isolated and purified also inhibited grown of several plant viruses (Awasthi and Menzel, 1866. Bharali *et al*; (2003) that the plant extracts revealed cancer chemopreventive property against skin papillomagenesis.

Tissue culture and micropropagation of medicinal plants have been reported by different workers. This technique is also being used for a mass multiplication and conservation of rate and endangered plant species. (Pandey *et al*; 2019). Another workers like Degn *et al*; (2011) reported in Ilex khasiana, Sadeq *et al*; (2013) in *Heliotropium indicum*, Deepak *et al*; (2016) *Salacia oblonga,* de Souza *et al*; (2019); Ritika Kumari (2019) in *Bacopa monnieri*. Tissue culture work in medicinal plants have also been done by Bhansali *et al*; (1978); Biswas *et al*; (2009). Ray and Bhattacharya (2008), Sudarshana *et al*; (2008); Jenifer *et al*; (2012) and several other workers. In the present work attempt has been done to produce multiple shoots form nodal and shoot apex explants of *Boerhaavia diffusa* (L.).

MATERIALS & METHODS:

Preparation of Culture Medium:

In the present study MS Medium (Murashige and Skoog, 1962) was used. For this all the chemicals (Hi-media) were arranged and stock solutions 20X for Macronutrients, 200X for micro, organic and Iron (I,II,III,IV) were prepared by weighting the required amounts of these ingredients. They were dissolved separately and stored in 1 L reagent bottles with proper labels. Now for the preparation of 1L Medium, required volumes from the stock solutions were taken in 500cc conical flask. The volume was made 500 by adding distilled water. 3 g sucrose was added. The pH was adjusted to 5.8. In another conical flask containing 500 ml distilled water, 8g agar powder was dissolved by heating. Then they were mixed. Volume became 1L. Desired amount of cytokinins were added separately to get different concentration. The medium was dispensed in 250 cc culture flask (30ml) and in 125 cc culture tubes (20 ml). After plugging and wrapping with Aluminium foil all the flaks and culture tubes were autoclaved at 15 lb pressure for 20 min. ½ strength MS medium with different concentrations of auxins was used for rooting. All the culture medium containing flaks and tubes were stored at low temperature for 3 days.

Preparation of Explants:

Healthy branches of *Boerhaavia diffusa* were collected from its wild habitat growing in the campus of University at Muzaffarpur. In the laboratory, healthy but young branches was separated. Leaves were removed and stem was cut into pieces, so that each piece had one node with dormant axillary buds. Similarly, shoot apex was separated. They were kept in a conical flask and washed under running tap water for 4-5 minutes. This was followed by washing with 5% Teepol (5% v/v), for 10 minutes. Explants were taken out and kept in 1% (w/v) Bavistin a systemic fungicide for 10 minutes. Then flask was shaken vigorously, so that there was uniform contact of the fungicide with the explant. The materials were rinsed thrice with distilled water and then treated with 70% ethanol (10-15 sec), followed by treatment with 0.1% (w/v) mercuric chloride solution for 2-3 minutes. Explants were

rinsed several times with pre-sterilized distilled water to remove even a trace of chemical attached on the surface of the explants.

Inoculation:

Explants were inoculated in the culture flask and tubes containing MS medium supplemented with different concentrations of cytokinins, separately. During inoculation both ends of the nodal explants were cut with sharp and pre-sterilized blade. While in shoot apex only basal end was cut. All inoculations were done under aseptic conditions of Laminar Air Flow Chamber. Inoculated culture flasks and tubes were incubated in culture room maintained at 26±1^{0}C temperature, 16/8 hours light and dark period with the help of white fluorescent tubes and 65-70% humidity. Observations were done on alternate day. Any contaminated culture if found was replaced and discarded after autoclaving. Observation was made for percentage response for bud initiation, days after which buds were initiated, number of buds per explant and mean length of the shoots etc. After 3 weeks sub cultures were done. Plantlets formed *in vitro* were excised after 6-7 weeks and used for root initiation in rooting medium. Here also percentage response, days after roots initiated, numbers of roots per explants, means lengths of roots were noted. All the experiments were done in triplicate and each time 15 culture were taken for each experiment. Mean of the data has been tabulated in table-1 and table-2 and used for results and discussion.

RESULTS AND DISCUSSION:

Nodal segments with axillary buds from healthy branches were used as primary explants. Similarly, shoot apex was used for primary explants. From the table-1, it may be noted that nodal and shoot apex explants inoculated in MS+ six different concentrations of BAP and Kinetin used separately, induced bud initiation but there were difference in percentage of response, number of shoots initiated its length etc. Percentage of response for bud initiation on nodal and shoot apex explants ranged between 51.37-98.64. In nodal explants from 0.5 to 4.0 mg/l concentrations of BAP, and from 50.42 to 93.24% in shoot apex explants. In case of nodal explants the highest percentage of response 98.64 was found in MS+1.5 mg/l BAP, while lowest percentage 51.37 was found in MS+ 4.0 mg/l BAP respectively. Similarly, highest percentage of response in shoot apex explant was noted in similar concentration of BAP that was 93.24, lowest 50.42% respectively. In case of MS+ six different concentration of KN, MS+1.5 mg/l KN induced highest buds that was 88.74% in nodal explants and 84.35% in shoot apex explant. Here also lowest percentage of response for bud initiation on nodal explants was 49.62 in MS+4.0 mg/l BAP and 46.28% in shoot apex explants.

From the table-1, it may be noted that number of axillary shoots were 11.75 on the nodal explants in MS+1.5 mg/l BAP and 6.91 in shoot apex explant. Similarly, the mean length was 5.92 cm and 4.84cm respectively. Here also lowest number of shoots and its size was obtained in MS+0.5 mg/l BP,

followed by MS+4.0 mg/l BAP respectively. In case of MS+1.5 mg/l KN the mean number of shoots on nodal explants was 5.64 on nodal explants and 2.55 on shoot apex explant respectively. This was also true for the number and mean length of the shoots. In case of MS+0.5 mg/l KN the mean number shoots on nodal explants was 2.26, on nodal explants while in shoot apex it was 1.63, while mean length in this was 3.65cm in case of nodal explants and 2.72 in shoot apex explants respectively. Lowest number of shoots and smaller lengths of shoots were also obtained in MS+4.0 mg/l KN.

6-7 week old plantlets were inoculated in half strength MS + four different concentrations (0.5, 1.0, 2.0, 3.0 mg/l) of IAA, NAA and IBA alone and two different concentration 1.5 and 1.0 mg/l of IAA and NAA along with 1.0 mg/l IBA respectively. Maximum percentage of response 79.0 was obtained in ½ MS + 1.0 mg/l IAA, where roots were initiated after 13-16 days of inoculation, and the mean numbers of roots were 4.76 while the mean length was 3.42 cm respectively. Highest percentage of response for rooting 64.0 was found in ½ MS+ 2.0 mg/l NAA, where the mean number of roots was 2.28, mean length 2.40 cm and initiation was after 20-22 days. ½ MS+ 3.0 mg/l NAA, had less percentage but number of roots was 3.30, mean length 2.12 and initiation after 18-20 days. In ½ MS + 1.0 mg/l IBA there percentage of response for rooting was 76.0; mean number of roots 5.22, mean length 3.18 cm and initiation was after 16-20 days of inoculation. In ½ MS + 1.0 mg/l IBA + 0.5 mg/l IAA the percentage of response was 85.0, mean number of roots 5.28 and mean length 3.24 cm, and initiation was after 12-16 days of inoculation. ½ MS + 1.0 mg/l IBA+ 1.0 mg/l IAA initiated rooting in 92.0% the mean number of roots 6.36, mean length 4.32cm respectively. At the similar concentrations ½ MS+ 1.0 mg/l IBA+ 0.5 mg/l NAA, ½ MS+ 1.0 mg/l IBA + 1.0 mg/l NAA the percentage of response was 60.0 and 53.0, the mean number or roots 5.24, and 4.52, the mean length 3.76 and 2.49 cm, and days after varies 13-17 to 16-20 days after inoculation.

DISCUSSION:

Nodal and shoot apex explants were inoculated in MS + six different concentrations of BAP and KN separately. Although shoot buds were initiated in all the culture conditions but highest percentage of response 98.64 and 93.24 in MS+ 1.5 mg/l BAP were obtained in nodal and shoot apex explants of *Boerhaavia diffusa* (L.) respectively. In this culture conditions there were highest mean number of axillary branches, their mean number and lengths respectively. Further, it may be noted that neither the lowest nor the highest concentrations of BAP was more promising with respect to axillary bud initiation. In case of KN, also highest percentage of response in both the nodal and shoot apex explants was in MS+ 1.5 mg/l KN that was 88.74 for nodal explants and 84.34 for shoot apex explants respectively. Increasing concentrations of both BAP and KN had no increasing percentage of response of mean number of shoots in both the explants. Findings of the present work are in agreements with

that of the findings of Das and Pal (2005); Sujatha and Ramyitha (2007); Rai *et al*; (2009) and Lal and Singh (2010); Sadeq *et al*; (2016); and Pandey *et al*; (2019).

Although rooting was observed in all the four concentration of three auxins IAA, NAA and IBA (0.5, 1.0, 2.0 and 3.0 mg/l) but best result was obtained in ½ MS + 1.0 mg/l IBA, when used alone or ½ MS + 1.0 mg/l IBA + 1.0 mg/l IAA respectively.

CONCLUSION:

Boerhaavia diffusa is an important medicinal herb having high traditional demand. Micropropagation may help in conservation of the species. As per the above finding MS + 1.5 mg/l BAP is most suitable culture medium, nodal segments are the best explants and ½ MS + 1.0 mg/l IBA + 1.0 mg/l IAA is the best culture conditions for rooting in the tissue culture raised plantlets.

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Table-1

Auxin mg/l	Concentration mg/l	% response for rooting	Days after inoculation	Mean number of roots/ Plantlets	Mean length of roots (cm)
½ MS	0	32.0	18-25	2.24	1.26
½ MS + IAA	0.5	63.0	16-18	4.16	2.18
	1.0	74.0	13-16	4.76	3.42
	2.0	69.0	14-17	3.40	2.46
	3.0	51.0	14-16	3.22	2.12
½ MS + NAA	0.5	43.0	20-24	2.82	2.10
	1.0	55.0	21-25	3.14	3.28
	2.0	64.0	20-22	2.28	2.40
	3.0	40.2	18-20	3.30	2.12
½ MS+ IBA	0.5	48.0	18-24	3.56	2.58
	1.0	76.0	16-20	5.22	3.18
	2.0	65.0	17-22	4.33	2.80
	3.0	72.0	17-22	3.15	2.12
½ MS+IBA+IAA	1.0+0.5	85.0	12-16	5.28	3.24
	1.0+1.0	92.0	13-15	6.36	4.32
½ MS+ IBA+ NAA	1.0+0.5	60.0	13-17	5.24	3.76
	1.0+1.0	53.0	16-20	4.62	2.44

Table-2

MS + Growth Hormones mg/l		Mean Percentage of shoot formation on explants		Mean number of shoots/ explant		Mean length of plantlets (cm)	
BAP	KN	Node	Shoot Apex	Node	Shoot Apex	Node	Shoot Apex
0.5	00	64.26	53.65	2.61	2.24	4.28	3.48
1.0	00	81.42	69.83	5.43	4.32	4.86	4.26

1.5	00	98.64	93.24	11.75	6.91	5.92	4.84
2.0	00	72.53	68.42	6.47	4.89	5.44	4.37
3.0	00	61.75	58.71	5.92	4.27	5.19	4.75
4.0	00	51.37	50.42	5.64	2.55	4.28	3.83
00	0.5	43.81	47.52	2.26	1.63	3.65	2.72
00	1.0	69.46	67.63	2.61	1.81	4.37	3.61
00	1.5	88.74	84.35	3.83	2.48	4.76	3.85
00	2.0	70.25	66.74	3.35	2.66	5.14	4.77
00	3.0	58.53	55.46	2.47	2.24	4.83	4.49
00	4.0	49.62	46.28	2.22	1.72	3.92	3.36

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