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Clonal propagation of *Boerhaavia diffusa* Linn. – A Valuable Medicinal Plant

Sumita Dasgupta[1]* and MN Reddy[2]

[1] Assistant Professor, Bhagwan Mahavir College of M. Sc.Biotechnology, Surat - 395017, Gujarat India
[2] Professor, Department of Biosciences, Veer Narmad South Gujarat University, Surat - 395007, Gujarat, India

ABSTRACT

Boerhaavia diffusa Linn. is a valuable medicinal plant belonging to the family Nyctaginaceae. The whole plant is medicinally very valuable. Different parts of the plant are used in renal ailments as diuretic and to treat jaundice, liver problems, stomach ache, anemia, cough, cold. Tissue culture technology has now much been used in the propagation, improvement and conservation of genetic resources of medicinal plants. In the present investigation an efficient protocol was developed for inducing multiplication of the elite clones of *Boerhaavia diffusa*. Highest percentage (90%) of bud breaking and shoot initiation was achieved from the nodal explants on Basal Murashige and Skoog's (M.S.) media supplemented with 2.0mg/L of Benzylaminopurine (BAP) and 1.0 mg/L Napthylacetic acid (NAA). Large scale shoot multiplication was observed in presence of Adenine sulphate (Ads) in combination with cytokinins in the shoot proliferation media. Maximum number of shoots (17.30 $\pm$ 0.2) was obtained at 2.0mg/L Kn and 25.0mg/L of Ads. Rooting was best observed on MS medium supplemented with 0.5 mg/L Indole aceticacid (IAA). The regenerated plantlets were acclimated and were successfully established in soil.

Key words: *Boerhaavia diffusa,* nodal explants, clonal propagation, mass multiplication, adenine sulphate.

Corresponding author

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INTRODUCTION

Boerhaavia diffusa Linn. commonly known to the world as "Spreading hogweed" is a wild perennial creeping herb belonging to the family Nyctaginaceae. It is widely distributed over the tropical, subtropical and temperate regions of the world. The plant is medicinally very valuable consisting of a large number of phytoconstituents, namely, alkaloid, flavanoids, steroids, triterpenoids, lipids, and even high quantity of amino acids [1]. The major active principle of the plant is the alkaloid "punarnavine" [2]. It was discovered that these multiple constituents are responsible for the therapeutic properties of the plant [3]. Different parts of this plant are used in the treatment of renal problems [4], odema, dropsical condition, cardiac disorders, urinary and kidney problems [5]. It is also used in the treatment of stomach ache, anemia, cough, cold and a potent antidote for snake and rat bites [6]. The rural communities use this plant for the cure of jaundice as there is no medicine available against the disease in allopathy [7]. This plant is therefore under tremendous threat for extinction due to indiscriminate use by the rural people and pharmaceutical companies. In the present scenario of indiscriminate commercial exploitation of *Boerhaavia diffusa*, ex-*situ* conservation of its elite clones is very much essential [8]. Gamborg and Phillip [9] proposed micropropagtion as an effective technique of multiplication for those species in which clonal propagation is needed. And since in conventional system, this species is mostly propagated by seed [10], microproapagtion is the most suitable method for propagation of its healthy clones.

The objective of the present study was to generate a protocol for rapid and large scale multiplication of the elite clones of this species *via* direct organogenesis. Direct organogenesis is more reliable approach as compared to callus mediated organogenesis for producing genetically uniform planting material [11]. Because of the non uniform nature of callus tissue [12], genetic mutations are more frequent in shoots regenerated from callus, [13]. The present communication reports an efficient reproducible system for clonal propagation of *Boerhaavia diffusa*.

MATERIAL AND METHOD

Plant Material

Young and healthy branches were collected from one-year-old plant of *Boerhaavia diffusa*, grown and maintained by the Bappalal Vaidya Botanical Research Centre, Dept. of Biosciences of Veer Narmad South Gujarat University. The green young nodes (3^{rd} – 7^{th} from the apex), internodes, green young leaves with petioles were used as explants. The explants were excised, washed with running water for 30 min. Disinfecting the explants with 70% alcohol for 30-60 sec prior to surface sterilization in 0.1% $HgCl_2$ for 3 min was found to be very effective in reducing contamination. The surfaced sterilized explants were rinsed for 4-5 times in sterile distilled water, trimmed and inoculated on culture media.

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Medium and Culture conditions

The shoot induction media composed of Basal Murashige and Skoog's (M.S.) culture media with 30.0 gm /L sucrose, solidified with 0.8%Agar and supplemented with various combinations and concentrations of growth regulators viz. 1.0 -3.0 mg/L of benzylaminopurine(BAP), Kinetin (Kn) alone or combined with 0.5-2.0mg/L napthylacetic acid (NAA) , indole aceticacid (IAA). The pH of the medium was adjusted to5.7- 5.8 using 0.1 N HCl or 0.1 N NaOH before autoclaving. The media was dispensed in 25x150 mm culture tube, plugged with non-absorbent cotton and sterilized at a pressure of 15 lbs/inch^2 (121°C) for 20 min. All the cultures were maintained at 25 $\pm$ 2^o C with 16-h light/ 8-h dark photoperiods.

After shoot bud initiation, the explants were transferred to the shoot proliferation medium supplemented with 1.0- 3.0 mg/L BAP or Kn combined 15.0- 35.0 mg/L adenine sulphate (Ads).

Each treatment had ten replicates and all experiments were repeated thrice. The regenerated healthy shoots were separated and transferred to the rooting medium consisting of MS medium supplemented with 0.1-1.0 mg/L NAA, IAA or indole butyric acid (IBA). Experiments were repeated twice. The rooted shoots were thoroughly washed to remove the adhering gel and were transferred to thermocol cups containing sterile soil, sand and cow dung manure (1:1:1). The transferred plantlets were covered with polythene cover for 10 days to check excessive transpiration and were nourished regularly with MS salts. The acclimatized plantlets were then removed to the green house in earthen pots containing garden soil sand and cow dung manure. At the green house they were kept for 3weeks and were watered regularly. The plantlets were then transferred to fields.

RESULTS AND DISCUSSION

Direct shoot induction *via* axillary proliferation was achieved from the nodal explants of *Boerhaavia diffusa*. Leaf, petiole and internodal explants failed to show response for direct shoot induction. In all the treatments where BAP or Kn was used alone, the response of the explants was found to be very low with an average of 2-3 shoots per explants. Induction media supplemented with different concentrations and combinations of BAP and NAA was found to the most suitable for shoot initiation. At 2.0 mg/L BAP + 1.0 mg/L NAA, 90% of explants showed shoot bud formation within 4-5days (Table 1). Maximum number of shoot bud/shoot produced was 4.48 $\pm$ 0.3 (Fig. 1a). In order to improve the number of shoots, Ads, was used in shoot proliferation media along with cytokinins. After the shoot buds were induced, the explants were transferred from the induction media to the proliferation media supplemented with Ads in combination with BAP or Kn. Significant increase in the number of shoot buds was observed on media supplemented with Ads as compared to the media where it was absent. Highest number [17.30 $\pm$ 0.2] of shoots was obtained at MS + 2.0mg/L Kn + 25.0 mg/L Ads after three weeks of transfer [Fig.1b] from the induction media containing 2.0 mg/L BAP and 1.0 mg/L NAA. 100% of explants showed response for proliferation at this composition. It was also observed that Ads promoted shoot multiplication only after shoot buds were induced. It however failed

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to trigger bud breaking and initiate shooting. Maximum shoot proliferation was obtained at 25.0mg/L and 35.0 mg/L of Ads. Lower or higher level of Ads than this range did not produce satisfactory result. Of the two cytokinins used, Kn and Ads combination was found to be more efficient in shoot proliferation than BAP combination [Table 2].

TABLE -1 Effect of BA, Kn in combination with NAA on shoot induction from the nodal explants of *Boerhavia diffusa.*

Growth regulators (mg /L).	% regeneration (Mean ± S.E)	No. of shoots/explant (Mean ± S.E.)
MS + 2.0 BA + 0.5 NAA	30.0 ± 0.7	2.08 ± 0.2
MS + 3.0 BA + 0.5 NAA	50.0 ± 0.3	2.13 ± 0.1
MS + 2.0 BA + 1.0 NAA	**90.0 ± 0.0**	**4.48 ± 0.3**
MS + 3.0 BA + 1.0 NAA	80.0 ± 0.7	2.72 ± 0.3
MS + 2.0 BA + 2.0 NAA	60.0 ± 0.3	1.88 ± 0.2
MS + 3.0 BA + 2.0 NAA	50.0 ± 0.5	2.03 ± 0.2
MS + 2.0 Kn + 0.5 NAA	60.0 ± 0.0	4.50 ± 0.2
MS + 3.0 Kn + 0.5 NAA	60.0 ± 0.3	3.50± 0.3
MS + 2.0 Kn + 1.0 NAA	60.0 ± 0.3	3.23 ± 0.3
MS + 3.0 Kn + 1.0 NAA	70.0 ± 0.7	2.55 ± 0.3
MS + 2.0 Kn + 2.0 NAA	90.0 + 0.0	2.77 ± 0.1
MS + 3.0 Kn + 2.0 NAA	60.0 ± 0.5	2.20 ± 0.1

Values represent means ± standard error of 10 replicates per treatment in three repeated experiments.

Table-2 Effect of Ads in combination with BA, Kn on shoot multiplication of *B. diffusa* (after 3 weeks of transfer).

Growth regulators (mg /L)	% regeneration (Mean±S.E.)	No.ofshoots/ explants (Mean ± S.E.)
MS + 2.0 BA + 15.0 Ads	50.0 ± 1.3	4.56 ± 1.0
MS + 3.0 BA + 15.0 Ads	60.0 ± 0.0	4.73 ± 1.1
MS + 2.0 BA + 25.0 Ads	80.0 ± 0.7	9.50 ± 1.6
MS + 3.0 BA + 25.0 Ads	70.0 ± 0.5	9.00 ± 0.1
MS + 2.0 BA + 35.0 Ads	60.0 ± 0.3	7.91 ± 0.4
MS + 3.0 BA + 35.0 Ads	40.0 ± 0.0	7.16 ± 0.4
MS + 2.0 Kn + 15.0 Ads	60.0± 0.3	6.49 ± 0.9
MS + 3.0 Kn + 15.0 Ads	60.0 ± 0.6	6.83 ± 0.1
MS + 2.0 Kn + 25.0 Ads	**100.0 ± 0.0**	**17.33 ± 0.2**
MS + 3.0 Kn + 25.0 Ads	80.0 ± 0.3	12.67 ± 0.6
MS + 2.0 Kn + 35.0 Ads	50.0 ± 0.8	13.66 ± 0.8
MS + 3.0 Kn + 35.0 Ads	50.0 ± 1.7	11.00 ± 2.0

Values represent means ± standard error of 10 replicates per treatment in three repeated experiments.

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TABLE-3 Effects of IAA, IBA on root induction from *in vitro* raised shoots of *Boerhavia diffusa* (Data scored after 3 weeks).

Growth regulators (mg /L)	% rooting (Mean ± S.E.)	Mean No. of root ± S.E.	Mean root length (cm) ± S.E.
M.S. + 0.1 IAA	60.0 ± 0.0	3.40 ± 0.5	9.60 ± 0.6
M.S. + 0.5 IAA	**90.0 ± 0.0**	**8.22 ± 0.6**	**3.41 ± 0.3**
M.S. + 1.0 IAA	45.0 ± 5.0	3.25 ± 0.8	3.37 ± 0.2
M.S. + 0.1 IBA	20.0 ± 10.0	1.50 ± 0.5	3.20 ± 0.8
M.S. + 0.5 IBA	55.0 ± 5.0	5.40 ± 0.9	4.06 ± 0.7
M.S. + 1.0 IBA	50.0 ± 10.0	2.66 ± 0.3	3.50 ± 0.8

Values represent means ± standard error of 10 replicates per treatment in two repeated experiments.

For root induction, highest percentage (90%) of root induction with a maximum number of 8.22 ± 0.6 roots [Table-3] was recorded after 3 weeks of transfer on medium supplemented with 0.5mg/L IAA [Fig. 1c]. NAA did not show any root induction.

Direct organogenesis for shoot multiplication is a reliable technique for clonal propagation as it prevents somaclonal variations in the cultures [14]. Many previous workers have also achieved direct shoot induction in *Boerhaavia diffusa.* Bhansali et. al. [15] obtained adventitious shoots from stem explant on M.S. media containing BAP along with Kn and NAA. The number of shoots and rate of regeneration is however not mentioned in the report. Roy [10] achieved direct multiple shoot induction from nodal explants on media supplemented with BAP, NAA and 10% Coconut water. Synergistic effect of combination of BAP and NAA in multiple shoot induction, as that has been observed in the present study, was also reported by Nagarajan[16] and Biswas et.al. [17]. There is no report of adventitious shoot regeneration from leaf and petiole explants of this species. Direct root regeneration from leaf explants was reported by Shrivastava and Padhya[18] on media supplemented with 0.5 µm/L IAA . There is no previous report of using Ads for shoot multiplication in this plant. However in other plants like *Trifolium repens*, M.S. media supplemented with Adenine sulphate (40.0 mg/L) in combination with cytokinin was found to be the best for superior differentiation [19]. The induction medium supplemented with 5.0 mg/LBAP and 20.0 or 40.0 mg/L Ads resulted in the higher average of shoots formation in *Phaseolus vulgaris* L [20]. The effectiveness of Ads in shoot proliferation was reported in previous works with *Plumbago indica* [21] *Jatropha curcas* [22]. Ads, in plant tissue culture play an important role in division of cells, their elongation and also stimulate production of chlorophylls in cells [19]. These characteristics of Ads are exploited in the present study to maximize the number of shoots within a time period of about 4 weeks of inoculation.

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Fig 1 a Fig. 1 b Fig 1 c

Legends to Figure 1- Plantlet regeneration of *Boerhaavia diffusa*.
Fig1a- Shoot induction from the nodes at M.S.+ 2.0 mg/L BAP + 1.0 mg/L NAA.
Fig.1b- Large scale shoot proliferation at MS+2.0mg/L Kn +25.0mg/L Ads.
Fig.1c- Root induction at MS + 0.5mg/L IAA.

Fig. 2a Fig. 2b Fig. 2c

Legends to the figure 2-Acclimatization of *in vitro* regenerated plants
Fig.2a- Hardened plantlets transferred to green house.
Fig-2b- Plantlets acclimatized under green house conditions.
Fig.2c- Plant growing under *ex vitro* conditions after 6 months of transfer.

85% of the plants survived during hardening procedure [Fig 2a] and were transferred to green house in small earthen pots. These were kept at the green house for 3 weeks [Fig. 2b] and then were transplanted to field where they are showing health growth under *ex vitro* condition [Fig 2c].

CONCLUSION

To conclude, it can be said that large scale multiplication of elite clone of *Boerhaavia diffusa* was achieved from the young and healthy nodes. Rapid shoot induction was obtained on induction media supplemented with BAP and NAA. The shoot buds showed maximum multiplication on proliferation media supplemented with combination of Ads and Cytokinins. IAA was the most suitable root inducing hormone producing long and healthy roots from the *in*

vitro raised shoots. The rooted plantlets took 4-5weeks for hardening. This protocol will be helpful in rapid regeneration of healthy clones of *Boerhaavia diffusa* for *ex situ* conservation.

REFERENCES

[1] Sahu AN, Damiki L , Nilanjan G ,Dubey S. Pharmacog Rev 2008;2: 14-22.
[2] Agarwal RR, Dutt SS. Chemical Abstract 1936; 30:3585.
[3] Agarwal B,Das S, Pandey A. Asian J App Sci 2011; 4(7): 663-684.
[4] Anand RK. Flora and Fauna 1995; l6:167–170.
[5] Singh RH, Udupa KN. J. Res. Indian Med 1972; 7:28–33.
[6] Chopra RN, Nayar SL, Chopra IC, Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research (CSIR), New Delhi, India 1956; p. 39.
[7] Singh A. Curr Sci 2007; 93:446.
[8] Pandey, SK, Shukla RP. Trop Ecol 2001**;** (1): 137-140
[9] Gamborg OL, Phillips GC. Fundamental methods of plant cell, tissue and organ culture. Springer, Berlin, New York, 1995; pp. 3-20.
[10] Roy PK. Plant Tissue Cult Biotech 2008; 18: 49-56.
[11] Saini HK, Gill MS, Gill MIS. Indian J Biotechnol 2010; 9: 419-425.
[12] Thorpe TA. Plant Cell and Tissue Culture. Kluwer Academic Publishers, Dordrecht, 1994; pp: 21-30.
[13] D'Amato F. Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Springer, Berlin 1977.
[14] Hedayat M, Abdi GH, Khosh-Khui M. American-Eurasian J. Agric Environ Sci. 2009; 6(1): 81-87.
[15] Bhansali RR, Kumar A, Arya HC. Curr Sci 1978; 47**:** 551-552.
[16] Nagarajan SM, Suresh T, Rajasekaran S, Kannan TMS , Kulothungan S. Geobios-Jodhpur 2005; 32: 169-172.
[17] BiswasA, Bari MA, Roy M, Bhadra SK. Tissue Cult Biotech 2009; 19: 53-59.
[18] Shrivastava N, Padhya MA. Curr Sci 1995; 68:653–656.
[19] Vicas G. Analele Universitatii din Oradea, Fascicula Protectia Mediului 2011;17: 203-210.
[20] Gatica Arias AM, Valverde JM,Fonseca PR,Melara MV. Electronic J Biotechnol 2010;13 fulltext-7: 1-8.
[21] Chetia S, Handique PJ. Curr Sci 2000; 78: 1187-1188.
[22] Datta MM, Mukherjee P, Ghosh B , Jha T. Curr Sci 2007; 93: 1438-1442.
[23] Sharma A, Saxena S, Garima Z, Sardana J , Batra A. Libyan Agric Res Center J Int 2010; 5: 274-278.