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Original Article

INVITRO REGENERATION OF *BACOPA MONNIERI (L.)* PENNEL.-A MULTIPURPOSE MEDICINAL PLANT

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ABSTRACT

Objective: The aim of the present study is to develop a protocol for the multiple shoots and root regeneration through callus induction of *Bacopa monnieri.*

Methods: In the present investigation, multiple shoot regeneration was done with MS medium supplemented with various plant growth regulators. Then half strength MS medium with free plant growth regulator was used for rooting purpose.

Results: Shootlets were regenerated from internodal part of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments were highest in MS medium supplemented with 1.0 mg/l BA,1.0mg/l TDZ and 4.92mg/l 2ip. For rooting, PGR free half strength MS medium was used. The rooted plantlets were hardened initially in culture room conditions and then transferred to mist house.

Conclusion: A perfect protocol was standardized for shoot and root regeneration. Best growth was observed in MS medium supplemented with 1.0 mg/l BA, 1.0mg/l TDZ and 4.92mg/l 2ip.

Keywords: Shoot regeneration, *Bacopa monnieri*, Callus induction, MS medium.

INTRODUCTION

Bacopa monnieri is a well-known medicinal herb in Indian system of medicine as brahmi (Sanskrit) and Indian water hyssop, it belongs to the family of scrophulariaceae. The plant is commonly found in wet, damp and marshy areas. In 1990, the annual requirement of this plant was 12.7 × 106 kg of dry biomass at a value of $34 million [1]. Indian material medical cites the uses of this plant as a brain tonic, which is effective in maintaining the vigor and intellect [2]. It is a reputed nervine tonic used for its ability to enhance memory, improve intellectual and cognitive functions, anti-inflammatory [4], analgesic [5], antipyretic [6], sedative [7] and as antiepileptic agent [8]. It is also known to improve the working and reference memory by restoring the alterations in cellular oxidants and antioxidant enzymes. *Bacopa monnieri* has also been linked to phytoremediation programs for the removal of heavy metals such as cadmium and chromium. The active principle constituents, reported in *Bacopa monnieri* are alkaloids; Brahmine, Herpestine and saponins. The memory- enhancing effects have been attributed to the presence of saponins, Bacoside A and Bacoside B. Pharmacological activities of Bacopa monnieri are attributed to saponin compounds present in the alcoholic extract of the plant. Due to progressively increasing demand, more than 90% of plant species used by industry are collected from the wild source of which 70% involves unorganized harvesting, leading to extinction of the plant. So the International Union for Conservation of Natural and National Resources as a long time ago listed *Bacopa monnieri* as a threatened species. There is a demand for further improvement in tissue culture protocol for mass multiplication of *Bacopa monnieri*, for both commercial farming system and later, if required for replanting in the natural habitat when the plant population declines.

MATERIALS AND METHODS

Plant Collection

Explants used for *invitro* regeneration was collected from Thanjavur district, Tamilnadu. The plant materials were identified and authenticated by Sidha Research Institute, Chennai, Tamilnadu, India.

Explants Sterilization

The plant material was washed under running tap water for 10 minutes and they were rinsed with 70% alcohol for 1 minute. The final step of sterilization was carried out in a horizontal laminar air flow chamber by rinsing the plant material twice in sterile distilled water, followed by 0.01% mercuric chloride solution for 3 minutes.

Micro Propagation

The internodal segments were excised with sterile scalpel blade, then it were inoculated on Murashige and Skoog medium [9] supplemented with plant growth regulators(PGR) such as BA,TDZ, 2ip at specific concentration. Same experiment was repeated for shoot multiplication. The pH of the media was adjusted to 5.9±0.02 with 1N NaOH or 1N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121^{o}C and 15 psi for 15-20 mins. The cultures were incubated under controlled conditions of temperature (25±2^{o}C), light (2000-2500 lux for 16 hr/day provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after 45 days. The shoots produced through *in vitro* were sub cultured on fresh medium on every 3 week. The nodal and shoot tip explants were inoculated in various concentrations of BA, TDZ, and 2ip among these the maximum number of shoots (4.00 ± 1.0) with BA, (3.33±1.1) with TDZ, (41. 0 ± 2.0) with 2ip was developed on MS media [10]. Maximum shoot length (3.90±0.10) of a medium supplemented with 4.92 2ip. Rooting of elongated shoots was attempted under *in vitro* conditions.

Rooting and Hardening

The *invitro* rooted plantlets were transferred to culture bottles 1/4th filled with soil rite composition (soil: sand: peat moss) and irrigated with ¼ MS salt solution. These bottles were kept in controlled

environmental conditions of culture room. After 3week of growth, the plantlets were transferred to mist house for further growth [11].

RESULT AND DISCUSSION

The internodal explants, when inoculated on MS medium containing BA and TDZ in the concentration of 1.0 mg/l, and for 2ip in the concentration of 4.92 mg/l showed enhanced shoot proliferation. Shoots after their initial proliferation on medium containing 1.0mg/ l BA were sub cultured on same fresh medium after every 21 days. Incorporation of BA, TDZ, 2ip into MS medium supported multiplication of shoots in culture, 2ip proved to be a better choice than BA and TDZ and the maximum number of shoots were obtained on its 4.92mg/l concentration (Table 1). The half strength MS medium without PGR also induces rooting of regenerated shoots (Table 2). *Invitro* rooted plantlets were initially hardened in culture room condition were leaves expanded after 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly. Maximum length of shoots was observed on MS medium fortified with 2ip.

Table 1: Effect of different concentration of cytokinins BA, TDZ and 2iP on *in vitro* shoot multiplication from *Bacopa monnieri*

BA (mg/l)	Shoot Induction (%)	Number of Shoots Per explant	Shoot Length (cm)
0.25	29.3 ± 2.9	1.67 ± 0.5	1.50 ± 0.10
0.5	40.0 ± 5.0	3.00 ± 0.0	2.20 ± 0.20
1.0	42.3 ± 5.7	4.00 ± 1.0	2.43 ± 0.15
1.5	24.3 ± 5.8	2.33 ± 1.2	2.33 ± 0.05
2.0	-	-	-

TDZ (mg/l)	Shoot Induction (%)	Number of Shoots Per explants	Shoot Length (cm)
0.25	23.7 ± 2.8	1.67 ± 0.6	1.50 ± 0.10
0.5	35.3 ± 5.7	3.00 ± 1.0	2.50 ± 0.20
1.0	46.6 ± 2.8	3.33 ± 1.1	1.90 ± 0.10
1.5	32.7 ± 2.8	2.00 ± 0.0	1.73 ± 0.12
2.0	45.0 ± 8.6	2.67 ± 1.1	1.40 ± 0.10

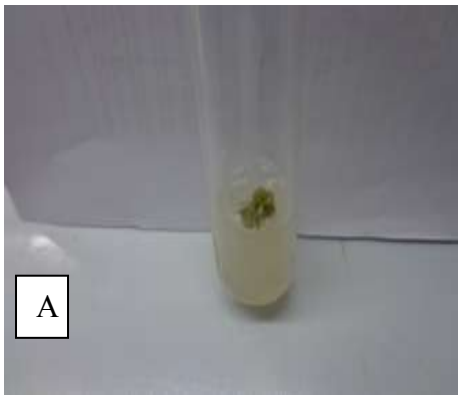
2ip (mg/l)	Shoot Induction (%)	Number of Shoots Per explants	Shoot Length (cm)
0.492	44.0 ± 8.6	2.67 ± 1.2	3.16 ± 0.11
2.46	50.0 ± 5.0	8.00 ± 1.0	3.30 ± 0.20
4.92	75.0 ± 5.0	41. 0 ± 2.0	3.90 ± 0.10
7.38	35.0 ± 5.0	2.33 ± 0.5	2.57 ± 0.21
9.85	38.3 ± 7.6	3.00 ± 1.0	2.40 ± 0.26

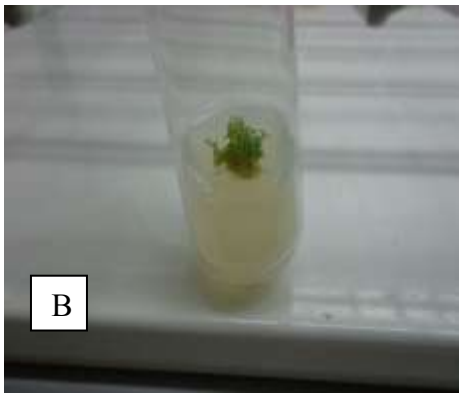
Results represent mean ± SD of three replicated experiments and data were recorded after 45 days of culture.

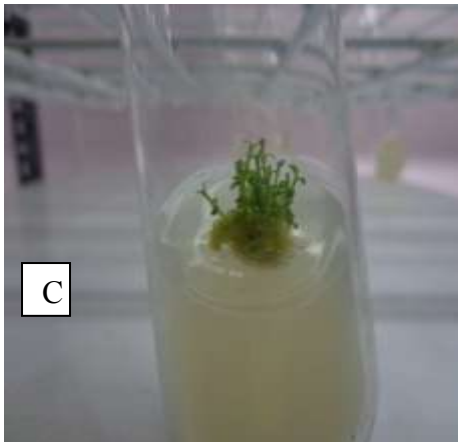
Table 2: Rooting response on *in vitro* shoots of *Bacopa monnieri* on growth regulator free half strength MS medium.

Medium	% Response	Number of Roots/shoots	Root Length (cm)
½ MS	100 ± 0.0	7.3 ± 0.25	5.4 ± 0.2

Data were recorded after 30 days of culture. Results represent mean ± SD of six replicated experiments.



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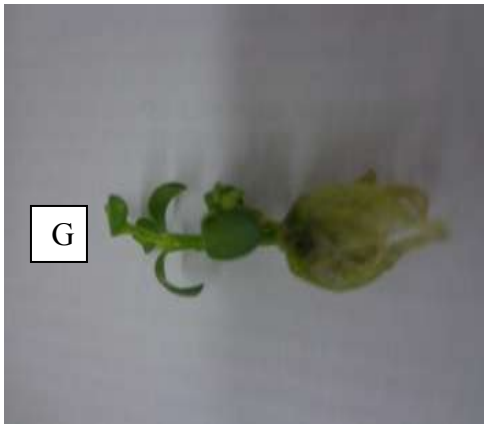
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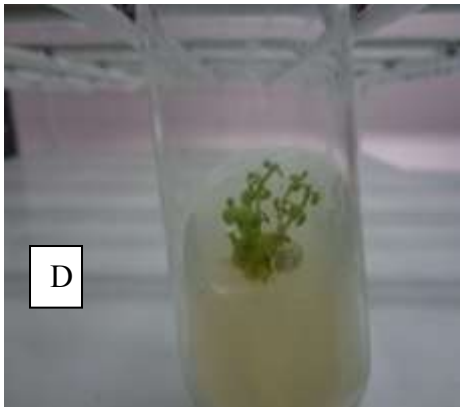
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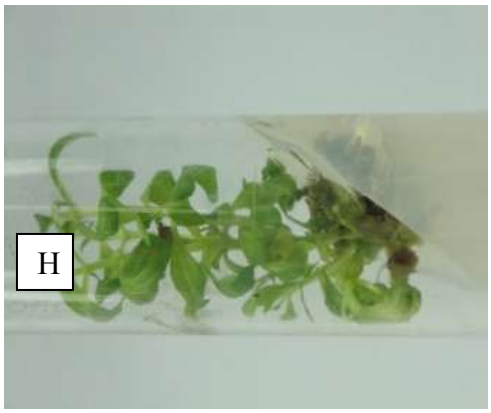
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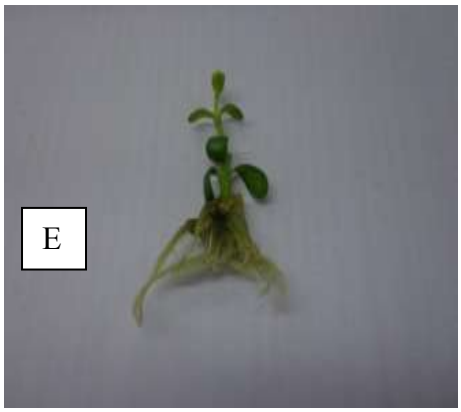
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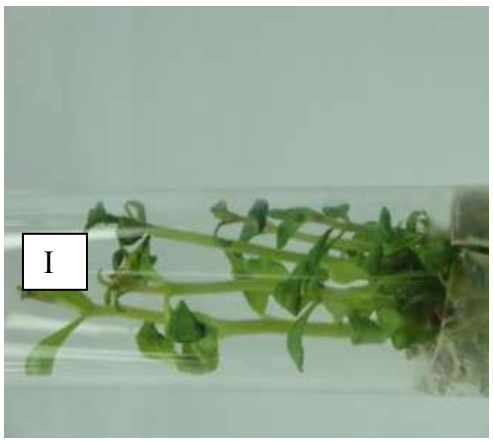
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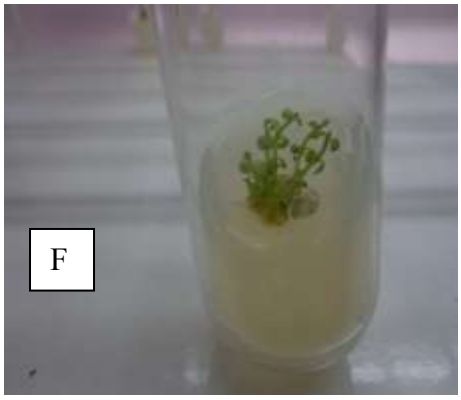
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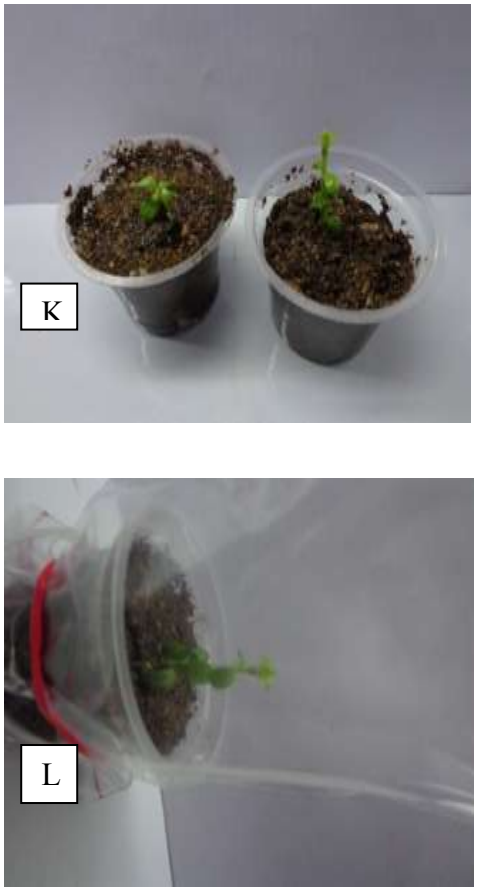

Fig. 1: Callus initiation to Regeneration of *Bacopa monnieri* (A, B): Callus initiation in MS solidify medium, (C,D,E): Shoot multiplication in MS solidify medium, (F, G): Root formation in MS solidify medium, (H,I):Regeneration of plant on solidify medium, (J,K,L) :Hardening of *Baccopa monnieri* plant

REFERENCES

1. Ahmad R.U. Medicinal plants used in ISM – their procurement, cultivation, regeneration and import/export aspects: a report. In: Govil J.N., Singh V.K., Hashmi S. (eds) Medicinal Plants: New Vistas of Research (Part 1) Today & Tomorrow Printers and Publishers, New Delhi.1993: 221–225.
2. Sharath Rajashekharappaa, V.Krishna. Antibacterial Activity of Bacoside-A- an Active Constituent Isolated of Bacopa Monnieri (L.) Wettest. Pharmacologyonline.2008; 2: 517-528.
3. C. Stough, J. Lloyd, J. Clarke, L. Downey, C. Hutchison, T. Rodgers, P. Nathan. The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology.2001;156(4):481-484.
4. Shabana Channa, Ahsana Dar, Shazia Anjum, Muhammad Yaqoob, Atta-ur-Rahman. Anti-inflammatory activity of *Bacopa monnieri* in rodents. Journal of Ethnopharmacology. 2006; 104(1-2):286-289.
5. Hemayet Hossain, Md.Sariful Islam Howlader, Shubhra Kanti Dey, Arpona Hira and Arif Ahmed. Evaluation of Analgesic, Antidiarrhoeal and Cytotoxic Activities of Ethanolic Extract of Bacopa monnieri (L). British Journal of Pharmaceutical Research. 2012;2(3):188-196.
6. Srinivasa Rao Bammidi, Sharan Suresh Volluri, Seema Chaitanya Chippada, Sumanjali Avanigadda, Meena Vangalapati. A Review on Pharmacological Studies of Bacopa monniera. J. Chem. Bio. Phy. Sci. 2011; 1(2),250 – 259.
7. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. Bacopa monniera Linn. as an Antioxidant: Mechanism of action.Indian Journal of Experimental Biology.1996; 34(6):523-526.
8. Priyaverma, Poonamsingh, Behrose S. Gandhi. Prophylactic efficacy of Bacopa monnieri on Decabromodiphenyl Ether (pbde-209)-induced alterations in oxidative status and spatial memory in mice. Asian J Pharm Clin Res.2013;6(3):242-247.
9. Murashige, T., and Skoog, F.A. Revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol.Plant.1962;15:473-497.
10. Subashri B, Justin Koilpillai Y. High Frequency Regeneration of Bacopa monnieri plant callus derived from Internode. Int J Pharma and Bioscience.2012;4(1):263-266
11. Jaspreet Kaur, Kalpana Nautiyal and Manu Pant. In vitro propagation of Bacopa monnieri (L.) Wettst A medicinally priced herb. Int.J.Curr.Microbiol.App.Sci.2013;2(8): 131-138.