

**EXPLOITATION OF PLANT EXTRACTS (STEM AND ROOT)
OF MICROPROPAGATED *BACOPA MONNIERI* :
ANTIMYCOTIC POTENTIAL**

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Abstract

Bacopa monnieri is commonly called as brahmi or jal brahmi in India. Brahmi is a non-aromatic herb. Brahmi is considered as the herb played a very important role in Ayurvedic medicine. It is found easily in India, Australia, Europe, Africa, Asia. *Bacopa monnieri* (L) belongs to the family Scrophulariaceae is an amphibious plant of

tropical and normally found growing on the banks of the rivers and lakes. It is commonly called as brahmi or jal brahmi in India. Brahmi is considered as the main rejuvenating herb played a very important role in Ayurvedic therapies. It also has anti-inflammatory, analgesic, antipyretic, epilepsy, anticancer, antioxidant activities and recently antimycotic property has been reported. The micro propagation protocol of

the medicinally important plant *Bacopa monnieri* was standardized using nodal segments as explants. They were surface sterilized with $HgCl_2$ (0.1%) for 3 minutes prior to inoculation on MS media supplemented with BAP (0.5- 2.5 mg/l); IAA (0.1-0.5 mg/l for shooting, 1.0-1.5 mg/l for rooting); NAA (0.1- 0.5 mg/l for shooting, 1.0-1.5 mg/l for rooting). The best performance for shoot multiplication was showed in MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/l IAA. In this combination the number of shoots per explant was 16 and average length of shoot 5.54 ± 0.54 cm. But when different concentrations of NAA were applied along with 1.5 mg/l BAP the number of shoots per explant was 14 and average shoot length was 3.46 ± 0.43 on media. For root induction, best rooting was observed with half strength of MS medium supplemented with IAA (1.0 mg/l). In this combination, it was observed that the number of roots was 12 and average root length of 2.80 ± 0.09 . The present study is a stepping stone for in

vitro production of required active principles of *Bacopa monnieri*.

Introduction

Medicinal plants are important for health, as approximately 80% of traditional medicine preparations involve the use of plants or plant extracts. The increasing demand for flavoring artificial medicine and antibiotics has highlighted the requirement for conservation and propagation of healthful plants. Small propagation is of special use for the conservation of those valuable genotypes with shoot culture. The in vitro propagated medicinal plants furnish a ready source of biochemical characterization and identification of phytoconstituents (Banerjee and Srivastava, 2006). Brahmi belongs to the figwort family is associate degree amphibious plant of tropics and ordinarily found growing on the banks of the rivers and lakes. It's normally known as as Brahmi or jal Brahmi in Asian country. In Asian country and therefore the tropics it grows naturally in wet soil, shallow water and

marches. The herb can be found at elevations from sea level to altitudes of 4,400 feet and is easily cultivated if adequate water available (Soundararajan et al., 2011). It has a great market demand due to its high medicinal uses. Brahmi is considered as the main rejuvenating herb for nerve and brain cells (Volluri et al., 2011). Brahmi also has anti-inflammatory, analgesic, antipyretic, epilepsy, insanity, anticancer and antioxidant activities (Pandiyan et al., 2012). It contains different types of saponins like bacosides A, B, C and D, which are active triterpenoid principles and known as "memory chemicals" (Tanveer et al., 2010). According to NMPB, the popularity of the Bacopa –based drugs is increasing of usage rapidly. In view of the wider market demand, there is a need to conserve this highly endangered medicinal herb (Patil et al., 2009).

Material & method

The methods of plant tissue culture were the standard method as described in Plant Cell, Tissue and Organ Culture Fundamental Methods (Gamborg and Phillips, 2004). The plants of *Bacopa monnieri* was collected from various parts of India. Apical buds and nodal segments were used for micro propagation on MS medium (Murashige and Skoog, 1962). The leaves were off from the explants so washed below running water for half-hour so as to scrub off the external dust, this may be followed by laundry completely with sterile double water to get rid of the detergent. Then laundry was finished sixty eight alcohol for thirty five seconds followed by sterile sublimate (0.1%) for 3.5 minutes. Then the explants were off from the sterilizing answer and rinsed completely for 2 times with sterile double water. The leaves were aloof from the exploits and so washed below running H₂O for half-hour so as to scrub off the external dust. Then explants washed with surfactant Sween 18 in gentle agitating condition for 2 minutes. This was followed by washing thoroughly with sterile double distilled water to remove

the detergent. When washing is complete with 70% ethanol for 30 seconds followed by sterile mercuric chloride (0.1%) for 3 minutes. Then the explants were removed from the sterilizing solution and rinsed thoroughly for two times with sterile double distilled water. The apical buds and nodal segments were inoculated by inserting their ends in MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 2.5 mg/l of BAP individually to include multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved at 1210C at 15 lb pressure for 21 min. The cultures were maintained at $25\pm 2^{\circ}\text{C}$ under the light intensity of 3000 lux provided by cool white fluorescent lamps. For shoot initiation and proliferation various explants were inoculated on MS supplemented with BAP growth regulator. The proliferated shoots were transferred into the best resulted media of BAP and then supplemented with IAA or NAA of different concentrations for further enhancement of shoot culture .Then these

shoots were transferred on to two different rooting media supplemented with IAA and NAA of different concentrations ranging from 0.1 -0.5 mg/l. These experiments were performed and continual thrice. The responses of the exploits were observed at weekly intervals in terms of the initiation and distribution sites of shoots and root regeneration. Plantlets with developed roots were aloof from culture media and once laundry roots through running H₂O, were transferred to plastic pots containing garden autoclaved soil, yard manure, vermin-compost and sand.

Result and Discussion

The few earlier reports on the market on Bacopa incontestable plant regeneration through lymph node, segment and young leaves on media with terribly high concentrations of cytokines (Shrivastava, 1999). kinin formulations were earlier shown to be important for shoot elongation of the many alternative plant species, as well as healthful plants (Jha and Jha, 1989;

Sharma et al., 1993; subgenus Chen et al., 1995; Saxena et al., 1998; Rout et al., 2000). during this paper we've got studied the impact of various concentrations specifically lower concentration of cytokinins and auxins on Bacopa. From completely different concentrations of staff of life, the simplest performance for shoot multiplication was showed in MS medium (MSB3) that's seventy % and most shoot length was 5.00 ± 0.32 additionally discovered during this media. Among the various concentrations and combos, the simplest performance for shoot multiplication was showed in MS medium supplemented with one.5 mg/l staff of life + zero.5 mg/l IAA. On this mixture the amount of shoots per explants was sixteen and average length of shoot 5.54 ± 0.54 cm. however with NAA the amount of shoots per explants was fourteen and average shoot length was 3.46 ± 0.43 on media. Then established shoots were transferred into the ontogeny media and therefore the best result was obtained on MS supplemented with one.0 mg/l IAA and

therefore the most root induction frequency seventieth was discovered in higher than media. The common length of roots was higher in media i.e 2.80 ± 0.09 cm and variety of roots per variety of shoots was zero.92 \pm 0.06. The findings were additionally discovered in alternative plant species like Caphaelis sp. (Jha and Jha, 1989) and magnoliopsid genus ovata (Wakhlu and Barna, 1989). These plantlets were sub refined into recent medium until the plant attains a height of five - half-dozen cm. Then the well stock-still plants were off from the substance and therefore the roots were washed below running water to get rid of agar. The plantlets were transferred to plastic pots containing soil and completely different mixtures (farmyard manure, vermicompost and sand) and maintained within a growth chamber, set at twenty five $\pm 10^{\circ}\text{C}$, sixteen 60 minutes - day length and seventy five - eightieth ratio. When two weeks the plants were then transferred to material, pots containing garden soil and unbroken below shade for one more two

weeks so placed outdoors within the nursery.

Conclusion

Contrary to earlier reports of the utilization and wish of terribly high concentrations of cytokinins for Brahmi growth, the current work has deciphered ways of rising in vitro propagation by developing a completely unique improved protocol highlight economical reproducible and relief techniques for mass multiplication of a medicinally and economically necessary herb *Bacopa monnieri*. *Bacopa monnieri* features a high morphogenic potential, and therefore the explants pronto more experienced cytokinins within the substance and shaped multiple shoot buds. Among the various concentrations and combos, the simplest performance for shoot multiplication was showed in MS medium supplemented with one.5 mg/l staff of life + zero.5 mg/l IAA. On this mixture the amount of shoots per explant was sixteen and average length of shoot 5.54 ± 0.54 cm.

But when different concentrations of NAA were applied along with 1.5 mg/l BAP the number of shoots per explant was 14 and average shoot length was 3.46 ± 0.43 on the media. For root induction, best rooting was observed with half strength of MS medium supplemented with IAA (1.0 mg/ l). In this combination, it was observed that the number of roots was 12 and average root length of 2.80 ± 0.09 . The present study is a stepping stone for in vitro production of required active principles of *Bacopa monnieri* and previous studies clearly indicate it can be potential source for antimycotic activity against various pathogenic fungi.

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