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Effect of different concentrations of BA on shoot proliferation of brahmi (*Bacopa monnieri*)

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ABSTRACT

Effect of different concentrations of BA in combination with IAA was observed on shoot bud proliferation from nodal explants, obtained from aseptic cultures of Brahmi (*Baccopa monnieri* Linn.) and effective protocol is described for rapid and large scale *in vitro* propagation of the valuable medicinal herb, *Bacopa monnieri* (L.) Pennell. This was achieved on MS solid medium supplemented with BA (1µM) showed multiple shoot (25) within 15 days of inoculation, when IAA (0.2 µM) was added shows 40 shoots per each explant. Elongation of shoots and subsequent root induction were achieved on the same proliferation medium only. On an average, within a period of three subcultures single nodal explant will produces 64000 shoots, respectively thereby favoring the economics of the cost of the materials and time factors. The regenerated plants resembled the mother plants in general habit without any morphological variation. A very simple one-step procedure for *in vitro* propagation of *Bacopa monnieri* has been established. This protocol can be used to generate foundation stocks of elite planting material for large scale cultivation.

Key words : *Bacopa monnieri* Linn., Growth regulators, Shoot Proliferation, *In vitro* multiplication.

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et al.*, 1997). Among the World's 25 best selling pharmaceutical medicines, 12 are plant derived (O'Neill and Lewis, 1993). *Bacopa monnieri* (Linn.) Pennell belonging to the family Scrophulariaceae is an amphibious plant of the tropics and normally found growing on the banks of rivers and lakes. It is commonly known in India as *brahmi* or *jala-brahmi*. It is a small creeping, glabrous and succulent herb with thick, soft, ascending branches and sessile, obovate-oblong or spatulate leaves; flowers are whitish blue with purple veins on long pedicels. It has a great market demand due to its high medicinal values. Moreover, because of the heavy demand and short supply, it is the most adulterated species in *Ayurvedic* formulations. So there is a need to mass-propagate the selected clones. Furthermore, their natural regeneration is hampered by death at two leaf stage and specific habitat requirement. The submerged shoots of *B. monnieri* can hardly ramify to attain the required growth and multiplication. Therefore, it is necessary to develop and standardize the large-scale multiplication through micropropagation. *Brahmi* is also known as "Medhya Rasayana" in Ayurveda as it increases mental clarity and brain stimulating action (Bhattacharya and Ghosal, 1998). It also possesses anti-inflammatory, analgesic, antipytretic, epilepsy, insanity, anticancer and antioxidant activities (Satyavati *et al.*, 1976; Jain *et al.*, 1994; Elangovan *et al.*, 1995; Tripathi *et al.*, 1996; Vohora *et al.*, 1997). It is also used in the treatment of asthma, hoarseness, water retention and blood cleaning. Moreover, leaf juice of brahmi is given to children for relief in bronchitis and diarrhoea.

In India the plant is used for all sorts of skin problems-eczema, psoriasis, abscess, Ulcerations - it is said to stimulate the growth of skin, hair and nails. In Pakistan, the herbal drug, Brahmi-buti, is used to treat skin diseases, leprosy, epilepsy, eczema, asthma, hoarseness of the voice, and diseases of the nervous system (Shakoor *et al.*, 1994). The medicinal properties of *Bacopa monnieri* responsible for improving memory-related functions have been attributed to the presence of different types of saponins such as bacosides A, B, C and D which are the active triterpenoid principles and known as "memory chemicals" (Rastogi *et al.*, 1994). These compounds are attributed with the capability to enhance the transmission efficiency of nerve impulses, thereby strengthening memory and cognition (Singh *et al.*, 1997).

MATERIALS AND METHODS

Nodal explants were obtained from three-month-old aseptic culture of *Bacopa monnieri* established on MS basal medium in "Plant Tissue Culture Laboratory of Department of Plant Biotechnology, MGM College of Agricultural Biotechnology, Aurangabad" during monsoon session of 2009-10. The aseptically growned healthy plants were selected from the aseptic culture of Brahmi. The plants were trimmed for unwanted/ damaged parts like dead/ yellow leaves, extra growth of leaves and roots.

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The nodal explants were isolated and inoculated on the agar based semi-solid medium. MS medium with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar was used. The pH of all media was adjusted to 5.8 before autoclaving at 121^{0}C (15 min). MS medium supplemented with different concentrations of BA (0.5, 1.0, 1.5 and 2.0 µM) in combination with IAA 0.2 µM was used for present investigation. The experiment was performed in replicates of ten for each type of media combination. The cultures were incubated in a culture room at 25 ± 1^{0}C under 16 h photoperiod provided by cool white fluorescent tubes. The growth responses of the explants was studied at weekly intervals in terms of the initiation and proliferation of shoots, callus induction and root regeneration. The regenerated shoots were subcultured every three weeks in the same medium. For acclimation, the regenerated plantlets were transferred to small plastic cups and then transferred to earthen pots containing sand, soil and farmyard manure in the ratio of 1: 1: 1. Initially, high humidity was maintained with water spray at regular intervals (Jasrai *et al.*, 1999) and then transferred in the "Poly House" for further growth.

RESULTS AND DISCUSSION

A one-step medium with low concentrations of cytokinin and auxin were found suitable for a rapid and large scale multiplication of Brahmi from nodal explants. However, earlier reports available on *Bacopa monnieri* demonstrated plant regeneration through axillary nodes, internodes and young leaves on media with high concentrations of cytokinin (Tiwari *et al.*, 2000; Shrivastava and Rajni, 1999). The nodal segments isolated from 3 months old aseptic cultures of Brahmi implanted on MS medium supplemented with only BA (1.1 µM) showed multiple shoots (25) within 15 days of incubation, similar results were reported by Mathur and Kumar (1998) and Tiwari *et al.* (1998). Addition of IAA (0.2 µM) with BA (1.1 µM) enhanced the number of shoots (40) from single node. The above results of shoot multiplication potential of IAA are in agreement with *Binita et al.*, 2005. Proliferation of shoot bud and elongation growth of shoots observed in the solid medium within 20 days. After 20 days of inoculation explants showed rooting without adding any rooting hormones. The nodal segment of Brahmi implanted on MS medium supplemented with BA (0.5µM) + IAA (0.2 µM) showed multiple shoot (25) within 15 day of inoculation. In case of concentration of BA(1.5µM) +IAA(0.2µM) and BA(2µM) +IAA(0.2µM) the nodal segment of Brahmi implanted on this medium showed callus formation and further organ formation (caulogenesis) within 20 days of inoculation. Approximately 5 - 6 cm long shoots with 3 - 4 cm roots were transferred to trays containing sand, soil and farmyard manure in the ratio of 1 : 1 : 1 and kept under shade for hardening. All plants regenerated from different explants were hardened in plastic cups and then in earthen pots. Initially high humidity was maintained by five sprays of water a day at 5 - 6 h interval. Finally these hardened plantlets transferred to the beds in polyhouse with 100 and 98% survival rate for nodal explants, respectively. No morphological variation of any nature was observed among the in vitro raised plants when compared with the mother stock. Research is said to be more successful if it is cost effective. The number of shoots (40 shoots) proliferated from single node per subculture was standardized from the commercial point of view, within a period of three subcultures, 64,000 number of shoots from single explant can be produced.

Table 1. Effect of BA on shoot proliferation of *Bacopa monnieri.*

Sr. No.	Concentration of BA (µM)	Concentration of IAA (µM)
1.	Control	Control
2.	1.0	0.0
3.	0.5	0.2
4.	1.0	0.2
5.	1.5	0.2
6.	2.0	0.2

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