

## PROPAGATION OF *BACOPA MONNIERI* (BRAHMI): IMPORTANT MEDICINAL PLANT

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### ABSTRACT

An experiments were conducted for standardization of *in-vitro* propagation technique of *Bacopa monnieri* (L.), a medicinally herbs of India. *Bacopa monnieri* (Brahmi) has been used in the various ancient traditional system of medicine for centuries including Ayurveda. Brahmi originate naturally in India, has a long history of use in number of disorders particularly anxiety, intellect and poor memory and used as anti-inflammatory, anticancer and antioxidant activities, analgesic, antipyretic. Quality planting material is major factor for any economically important medicinal plants and Micro-propagation technique may play an important role. Healthy nodal segments of the herb were used as explants with basic MS medium for shoot initiation and multiplication containing various combinations of different growth regulators. For the initiation of ex-plant BA (0.1 to 0.6 mg/l), IA (0.1 to 0.5 mg/l) and NA (0.1 and 0.5 mg/l) was used, while concentration of BA (0.5 to 1.5 mg/l), IA (0.5 mg/l) and NA (0.5 mg/l) was used for multiplication. MS full and 1/2MS were used for rooting of plantlets with 25 to 150 mg/l Activated Charcoal (AC). Maximum mean number of initiated plantlets  $1.8 \pm 0.42$  with mean length  $3.00 \pm 0.94$  were found in MS medium treated with lowest concentration of BA, while maximum mean number of multiplied plantlets  $10.00 \pm 2.58$  with mean length  $6.1 \pm 1.91$  were found in MS medium treated with 1mg/l BA and 0.5mg/l IA. Maximum mean number of roods  $12.4 \pm 1.074968$  with Mean length  $9.19 \pm 0.68$  was observed on 1/2MS medium with 100 mg/l activated charcoal. The rooted plantlets were successfully hardened in 1:1:1 ratio of sand: soil: vermicompost and successfully established in soil.

**Keywords:** Brahmi, *Bacopamonniera*, Micropropagation, Medicinal Plants, Plant Tissue Culture

### INTRODUCTION

Brahmi is considered as the main rejuvenating herb, which played a very important role in Ayurvedic therapies (Kapil and Sharma, 2014). "Brahmi" has been used in the Ayurvedic system of medicine for centuries. *Bacopa monnieri* (L.), commonly known as "Brahmi", is a member of the Family *Scrophulariaceae*, is placed second in the priority list of Indian medicinal plants (Anonymous, 1997). It is commonly found on the banks of rivers and lakes. It has been used for centuries in legends and traditional system of medicine as a memory enhancer (Bhattacharya *et al.*, 1998; Shankar and Singh, 2000), anti-inflammatory (Williams *et al.*, 2014), analgesic, Antidiarrhoeal, Cytotoxic activity (Afjalus *et al.*, 2012), antipyretic (Bammidi *et al.*, 2011), sedative and anti-epileptic agent (Srivastava *et al.*, 2009). In addition to its unique medicinal use, *Bacopa monnieri* has also been linked to phytoremediation programmes for the removal of heavy metals such as cadmium and chromium (Subashri and Pillai, 2014). Brahmi leaves are oblong, sessile and fleshy. In the Ayurvedic *Bacopa* has been recognized for its brain enhancement personality. *Bacopa monnieri* is a small, creeping herb with numerous branches, small oblong leaves and light purple flowers. In India and the tropics it grows naturally in wet soil, shallow water and marches of *Bacopa monnieri* (Brahmi) (Mohapatra and Rath, 2005). Family *Scrophulariaceae* is also known as *Madhyarasayana* in Ayurveda as it increase mental-clarity and brain stimulating action, it also possesses anti-inflammatory, analgesic, antipyretic, 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). Saponins such as bacosides A, B, C and D which are the active triterpenoid principles and known as "memory chemicals" (Rastogi *et al.*, 1994). Only a very limited research has been carried out on the plant, under the present study assumes singular significance and it is supposed to

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contribute a great deal to the existing literature. The micro propagation protocol of the medicinally important plant, *Bacopa monnieri* was standardized using nodal segments as explants. This review highlights mainly the recent development and achievements made for the multiple shoots and root regeneration of *Bacopa monnieri* (Brahmi). The present paper highlights the morphogenic response of various auxins and cytokinins on *Bacopa monnieri*.

## MATERIALS AND METHODS

### Collection of Explants

Branches of Brahmi were collected from healthy growing plants from medicinal garden of Prof. T.S. Murthi Science and Technology Station Obedullaganj, Raisen (M.P.)

### Sterilization of Explants

Nodal explants were cut and washed in running tap water to remove the superficial dust particles and mud adhering to its surface. Explants were washed with sevelon (5-10 drops/100ml) in a vial by gentle agitating conditions. The explants were thoroughly rinsed with distilled water for several times. Again these explants were dipped in to the 1% fungicide (Bavistin) treatment was given for 15 minutes and then washed with distilled water. For surface sterilization, Explants were transferred to sterile empty flasks under aseptic conditions and given a quick dip in 70% alcohol and subsequently they were washed in distilled water. After that, the explants were surface sterilized with different concentration of sterile ( $\text{HgCl}_2$ ) for different duration as per the treatment to find out the best treatment for sterilization of explants. To remove the traces of sterile explants were washed in sterilized distilled water at least 5-6 times. The procedure was carried out in the inoculation chamber under laminar air flow hood.

### Preparation of MS Medium

Culture media was prepared as per described method of Murashig and Skoog (1962) and different growth regulator was added as per requirement. For the initiation of ex-plant BA (0.1 to 0.6 mg/l), IA (0.1 to 0.5 mg/l) and NA (0.1 and 0.5 mg/l) was used, while concentration of BA (0.5 to 1.5 mg/l), IA (0.5 mg/l) and NA (0.5 mg/l) was used for multiplication. MS full and 1/2MS were used for rooting of plantlets with 25 to 150 mg/l Activated Charcoal (AC) combination adding 30 g/l sucrose and 5.7% agar. The hormones used for experiment were taken from stock solutions, which were previously prepared and kept under cold condition in refrigerator (Doods and Roberts, 1985). The pH of the medium was adjusted to 5.7 with 0.1 NaOH before autoclaving at 15 lbs and 121°C for 18 min.

### Aseptic Inoculations of Explants

Nodal segments about 0.5-0.8 cm were prepared aseptically and were implanted vertically on Surface disinfected nodal explants were inoculated onto full strength MS medium (Murashige and Skoog, 1962) fortified with specific concentrations of growth regulators. The cultures were incubated at a constant temperature of  $26 \pm 2^\circ\text{C}$  with  $16 \pm 1$  h photoperiod (3000 lux).

## RESULTS AND DISCUSSION

### Results

#### Surface Sterilization and Induction of Axillary Shoots

Treatment of explants with 0.1%  $\text{HgCl}_2$  for 3 minutes resulted 100% contamination-free viable cultures. Final observation after 3-4 weeks showed that MS media supplemented with 0.1 mg/l of BAP proved to be most capability in shoot induction. On this medium an average of  $1.8 \pm 0.42$  shoots with mean shoot length  $3 \pm 0.94$  cm were obtained (Table 1, Figure 1A).

#### Shoot Multiplication

Shoot multiplication is depend on different type of concentration. Sometimes BAP increasing is best for shoot or just opposite. Activated auxiliary shoots from the nodal explants and transfer to fresh medium containing 1.0 mg/l BAP and 0.5 IAA to establish a stock of shoots used for *in vitro* multiplication. When we look Results in the present study showed the essential of plant growth regulators for *in vitro* multiplication, as the shoots cultured on basal medium did not multiply and become dead. BAP and IAA at a concentration of 1.0 mg/l and 0.5 just gave an average of  $10.00 \pm 2.58$  shoots with mean shoot length

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6.01  $\pm$  1.91 cm after 3-4 weeks of culture (Figure 1B). Increasing the concentration of BAP to 3.0 mg/l, a Decrease in shoot multiplication rate was observed. Trials on effect of cytokinin: auxin ration on *in vitro* shoot multiplication of Brahmi showed that results were better than in medium with 3.0 mg/l BAP. However, comparative number, length and health of shoots on media with BAP + IAA/NAA were not as good as in media containing 1.0 mg/l BAP and 0.5 IAA (Table 2).

**Table 1: Effect of Plant Growth Regulators on *in Vitro* Axillary Shoot Induction in *B. Monnieri***

S. No	MS + PGR (mg/l)			Observations after 3- 4 Weeks		
	BAP	IAA	NAA	Mean Shoot Number	Mean Shoot Length (cm)	
Control	0.0	0.0	0.0			
1	0.1	0.0	0.0	1.8 $\pm$ 0.42	3.00 $\pm$ 0.94	
2	0.2	0.0	0.1	0.9 $\pm$ 0.99	1.7 $\pm$ 1.1	
3	0.3	0.5	0.0	0.7 $\pm$ 0.70	1.4 $\pm$ 1.17	
4	0.4	0.0	0.1	0.5 $\pm$ 0.70	1.6 $\pm$ 1.26	
5	0.5	0.0	0.0	0.4 $\pm$ 0.69	2.8 $\pm$ 1.13	
6	0.6	0.5	0.5	0.4 $\pm$ 0.51	1.3 $\pm$ 1.41	

**Note:** Each treatment consisted of 10 replications. Data (Mean  $\pm$ SE) were recorded after 20 days of culture.

**Table 2: Effect of Plant Growth Regulators on *in Vitro* Axillary Shoot Multiplication in *B. Monnieri***

S. No	MS + PGR (mg/l)			Observations after 3- 4 Weeks		
	BAP	IAA	NAA	Mean Shoot Number	Mean Shoot Length (cm)	
Control	0.0	0.0	0.0		0.00 $\pm$ 0.00	
1	0.5	0.5	0.0	3.5 $\pm$ 2.58	3.6 $\pm$ 1.89	
2	0.1	0.5	0.0	10.00 $\pm$ 2.58	6.1 $\pm$ 1.91	
3	1.5	0.0	0.5	4.7 $\pm$ 2.54	3.1 $\pm$ 1.52	
4	1.5	0.5	0.0	4.8 $\pm$ 2.61	3.22 $\pm$ 1.76	
5	2.0	0.0	0.0	4.4 $\pm$ 1.50	4.00 $\pm$ 2.05	

**Note:** Each treatment consisted of 10 replications. Data (Mean  $\pm$ SE) were recorded after 20 days of culture.

### *In vitro* Rooting

After 30 Days of growth, rooting growth is rarely increase day by day in best culture. The multiple-shoot clumps produced on this medium were transferred to solidified MS growth-regulator-free medium for shoot elongation and rooting. On opposite, shoots were also observed for rooting on full- or half-strength MS medium with Activated Charcol ensuing excellent response for root induction. Maximum rooting was recorded in medium containing 100 mg/l Activated Charcol Supplemented with ½ MS medium (Figure 1C). On this medium an average of 12.4  $\pm$  1.074968 roots with average root length 9.19 $\pm$ 0.6806 cm was observed after 3-4 weeks (Table 3).

### Hardening

After 5 weeks complete rooting medium, they were transfer to pots soil: sand: manure (1:1:1) and maintained in greenhouse or poly house. In poly house is carefully monitored for growth, development and water done as must required. Long and healthy plants transferred to the field condition open ground where 100% growth rate was measured.



**Figure 1A: Initiation of *Bacopa Monnieri***



**Figure 1B: Multiplication of *Bacopa Monnieri***



**Figure 1C: Rooting of Shoot *Bacopa Monnieri***

### **Discussion**

*Bacopa monnieri* is a medicinal plant used in Ayurvedic medicine for thousands of years to treatment for mental illness, asthma, anxiety, and age-related, Antioxidant, Stress, cough and cold etc. Mercuric chloride is highly antimicrobial against for both fungi and bacteria at low concentrations (upto 0.1 %) it is perhaps the most effective disinfective agent for soil-borne fungi (Nwokocha *et al.*, 2015). In this study, 100% contamination-free viable cultures were obtained by treatment of explants with 0.1% HgCl<sub>2</sub> for 3

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minutes. From the present studies MS media proved to be the best culture medium for the establishment of shoot culture in *B. monnieri* plant. This work was undertaken in order to learn the tissue culture technique for medicinally important plant *Bacopa* which is the mostly used for memory enhancing purposes. Final observation after 3-4 weeks showed that MS media supplemented with 0.1 mg/l of BAP proved to be most capability in shoot induction. On this medium an average of  $1.8 \pm 0.42$  shoots with mean shoot length  $3 \pm 0.94$  cm were obtained. Numerous reports of BAP as bud inducer at concentrations ranging from 1.0-5.0 mg/l have already published (Shrivastava et al., 1999; Binita et al., 2005; Ramesh et al., 2006; Sharma et al., 2010; Chandra et al., 2012; Kaur et al., 2013; Mohanta et al., 2014; Behera et al., 2015).

These present results are supported by the findings of other workers who have also observed and experimentally found the positive influence of MS medium for optimum shoot and root multiplication in different *Bacopa* species. Activated axillary shoots from the nodal explants and transfer to fresh medium containing 1.0 mg/l BAP and 0.5 IAA to establish a stock of shoots used for *in vitro* multiplication. This observation is supported by previous studies on *B. monnieri* (Chaplot et al., 2005; Ceasar et al., 2009; Vijayakumar et al., 2010; Kumari et al., 2010; Showkat et al., 2010; Yusuf et al., 2011; Mehta et al., 2012; Pandiyan and Selvaraj, 2012; Jain et al., 2013; Asha et al., 2013; Tanveer et al., 2010; Kaur et al., 2013; Behera et al., 2015). MS medium with Activated Charcol ensuing excellent response for root induction. Maximum rooting was recorded in medium containing 100 mg/l Activated Charcol Supplemented with  $\frac{1}{2}$  MS medium. Tissue culture raised plants are need acclimatization before field transfer. For this purpose *in-vitro* regenerated plantlets were shifted to pots and kept in Polyhouse for about a month. The protocol resulted in development of healthy plants without any need of intermediary hardening treatment. After 5 weeks complete rooting medium, they were transfer to pots soil: sand: manure (1:1:1) and maintained in greenhouse or polyhouse. So, this technology is effective as it produces thousands of plants in a short span of time.

**Table 3: Rooting Response of *in Vitro* Regeneration Excised Shoots (10 Repeats)**

S. No.	Medium	Activated Charcoal (mg/l)	No. of Roots/ Plantlets (Mean_SE)
1	MS	-	$6.6 \pm 1.264911$
2	$\frac{1}{2}$ MS	-	$6.9 \pm 0.994429$
3	$\frac{1}{2}$ MS	25	$7.7 \pm 0.823273$
4	$\frac{1}{2}$ MS	50	$9.2 \pm 1.032796$
5	$\frac{1}{2}$ MS	100	$12.4 \pm 1.074968$
6	$\frac{1}{2}$ MS	125	$12.3 \pm 1.251666$
7	$\frac{1}{2}$ MS	150	$9.3 \pm 1.766981$

**Note:** Each treatment consisted of 10 replications. Data (Mean  $\pm$ SE) were recorded after 20 days of culture.

## Conclusion

*Bacopa monnieri* has always been a topic of interest of researchers. From tissue culture point of view several studies have been performed to propagate the plant *in vitro*. The highlights of the study are Use of low concentration of plant growth regulators and minimization of time required for field transfer of tissue culture raised plantlets. Free plants produced open the scope for utilization of plant material for antimicrobial testing and suitable pharmaceutical preparations. Apart from this *in vitro* propagation of *Bacopa monnieri* showed a highest rate of multiplication which cannot be seen in naturally found species of *Bacopa monnieri*. The Bacoppa research will give a new insight of research in medicinal components of plants through various advance techniques.

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