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Effect of Growth Regulators on *in vitro* Multiplication of *Bacopamonnieri*

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Abstract Experiments have been carried out to standardize the *in-vitro* propagation technique of *Bacopamonnieri* (L.), which is a valuable medicinal herb of India. The experiments, healthy nodal segments of *Bacopamonnieri* were used as explants with basic MS medium to shoot initiation and multiplication containing different combinations of diverse growth regulators. The optimum time period of sterilization with no contamination and 100% surviva for shoot tips is two minutes using 0.1% $HgCl_2$ for three times. Maximum regeneration in shoot tips and nodal segment was observed at minimum concentration (0.25 mg/l) of auxins i.e. IBA and NAA. The optimum concentration of IBA and NAA is 0.25 mg/l for each. BAP and Kinetin were used alone maximum numbers of shoots were formed at the concentration of 1.0 mg/l. Both the cytokinins (BAP and Kinetin) were used in combination the best results were obtained in BAP (1.0 mg/l) + Kinetin (0.2 mg/l) followed by BAP (0.2 mg/l) + Kinetin (1.0 mg/l). Lower concentrations (1.0 and 0.2 mg/l) of cytokinins (BAP and Kinetin) either used alone or in combination shows good results. The rooted plantlets were effectively set up/planted in soil by hardening in 1:1:1 ratio of sand:soil: vermicompost.

Keywords *Bacopamonnieri,* Brahmi, Medicinal plant, Micropropagation.

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Introduction

Brahmi is the main rejuvenating herb, which played a very important role in Ayurvedic therapies (Kapil and Sharma 2014). Experiments were conducted for the standardization of the *in vitro* propagation technique of *Bacopamonnieri* (L.), a medicinal herb from India. Brahmi originated naturally in India and it has long history of use in various diseases, especially anxiety, intellect disorder and fade memory. It is used as analgesic and antipyretic (Bammidi et al. 2011). For any commercially important medicinal plant, quality planting material is a prime factor in which the micropropagation technique can play a vital role. For shoot initiation and multiplication, containing various combination of different growth regulators, healthy nodule segments of the medicinal herb were used as explants with a basic MS medium, while carrying the experiments. The optimum period of contamination-free sterilization and 100% survival for shoot tips is two min-

utes using 0.1% $HgCl_2$. Zero contamination with maximum survival was obtained when the nodal segments were treated with 0.1% mercuric chloride for three minutes. Peak regeneration in shoot tipe and nodal segment was observed at a minimal concentration (0.25 mg/l) of auxins, namely IBA and NAA. Both cytokinins (BAP and Kinetin) were used in combination the best results were obtained in BAP (1.0 mg/l) + Kinetin (0.2 mg/l) followed by BAP (0.2 mg/l) + Kinetin (1.0 mg/l). Lower concentrations (1.0 and 0.2 mg/l) of cytokinins (BAP and Kinetin) used alone or in combination give good results. Contributing a great deal to the existing literature, the rooted seedlings were effectively cured in a 1:1:1 ratio of sand : soil: vermicompost and were successfully planted in the soil. The present paper explains the morphogenic response of different concentrations of cytokinins and auxins on *Bacopamonnieri.*

Materials and Methods

Collection of explants

Shoot tip (1 cm) and nodal segments were used as an explants and have been excised from the field grown plants.

Sterilization of explants

Nodal explants were cut and washed in running tap water to remove the surface dust particles and mud sticking to its surface. Excised explants were first washed with detergent (Teepol), followed by washing in running tap water. The explants were then dipped in to the 1% fungicide (Bavistin). This treatment was given for 15 minutes and then washed with distilled water. Explants were transferred to sterile, empty flasks under aseptic conditions, for surface sterilization and given a quick dip in 70% alcohol and then these were washed in distilled water. Thereafter, to find out the best treatment for sterilization of explants, the explants were surface sterilized with different concentration of sterile ($HgCl_2$) for different duration as per the treatment. Explants were washed in sterilized distilled water at least 5-6 times to remove the traces. This procedure was carried out in an inoculation chamber under laminar air flow hood.

Table 1. Effect of different media on *in vitro* establishment of shoot tips of *Bacopamonnieri.*

Media used (mg/l)	Regeneration percentage		
	7^{th} days	14^{th} day	21^{st} days
E0	20	20	20
E1	100	100	100
E2	40	60	60
E3	20	40	40
E4	20	30	30
E5	00	00	00
E6	100	100	100
E7	60	80	80
E8	80	80	80
E9	20	40	40
E10	00	00	00

Preparation of MS medium

The MS medium culture media was prepared as per explained method of Murashig and Skoog and diverse growth regulator was added as per obligation.For the establishment of ex-plant IBA (0.25 to 4.0 mg/l), NAA (0.25 to 4.0 mg/l), while concentration of BAP (1.0 to 4.0 mg/l), and KIN (1.0 to 4.0 mg/l) alone or in combination was used for multiplication.

Results and Discussion

Surface sterilization and induction of auxiliary shoots

Treatment of explants with 0.1% $HgCl_2$ for 3 minutes resulted 100% contamination-free viable cultures. Final observation after 3-4 weeks showed that MS media supplemented with 0.25 mg/l of IBA and 0.25 mg/l NAA proved to be most capability in shoot induction. It was observed that maximum regeneration (100%) was at E1 and E6 (0.25 mg/l IBA and 0.25 mg/l NAA) among the different media used. The rate of regenerated continuously decreased with after E1 and E6 i.e.by increasing concentration of IBA and NAA. The explants started to turn yellow at higher concentration of IBA and NAA there was almost zero regeneration at E5 and E10 (Table 1).

Shoot multiplication

Shoot multiplication is depend on different type of concentration. Sometimes BAP increasing is best for

Table 2. Effect of different media on *in vitro* multiplication of shoots from nodal segment of *Bacopamonnieri.*

Media MS	BAP	Kinetin	No of shootlets formed
M1	-	-	1.00
M2	1.00	-	6.20
M3	2.00	-	4.40
M4	3.00	-	2.80
M5	4.00	-	1.00
M6	-	1.00	7.80
M7	-	2.00	3.40
M8	-	3.00	2.20
M9	-	4.00	2.60
M10	1.00	0.20	8.40
M11	1.00	0.40	6.80
M12	1.00	0.60	5.40
M13	1.00	0.80	2.20
M14	1.00	1.00	2.60
M15	0.20	1.00	6.40
M16	0.40	1.00	4.40
M17	0.60	1.00	3.60
M18	0.80	1.00	3.80
M19	1.00	1.00	2.40

shoot or just opposite. Activated auxiliary shoots from the nodal explants and transfer to fresh medium containing 1.0 mg/l BAP and 1.0 mg/l KIN to establish a stock of shoots used for *in vitro* multiplication. When we came to the results of the present study, it shows the essentiality of plant growth regulators for *in vitro* multiplication. The shoots cultured on basal medium didn't multiply and become dead. When the BAP alone was used the maximum number of shoot formed were 10 at BAP 1.0 mg/l and number of shoots increased to 7.4 at Kinetin 1.0 mg/l. On using different combinations of Kinetin and BAP it was found that the maximum numbers of shoots (10) were formed on BAP 1.0 + Kinetin 0.2 mg/l followed by (16.2) BAP 0.2 + Kinetin 1.0 mg/l. Lower concentrations (1.0 and 0.2 mg/l) of cytokinins (BAP and Kinetin) either used alone or in combination shows good results. Increasing the concentration of BAP to 3.0 mg/l, a decrease in shoot multiplication rate was observed. Experiments conducted shows that different auxin and cytokinin ratio shows better results on *in vitro* shoot multiplication medium of brahmi as compared to 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). Lower concentrations (1.0 and 0.2 mg/l) of cytokinins (BAP and Kinetin) either used alone or in combination shows good results.

Bacopamonnieri is a very popular medicinal plant. It is used in treatment for mental illness, asthma, anxiety, and age -related, antioxidant, stress, cough and cold in ayurvedic medicines. Mercuric chloride act as antimicrobial agent and act highly antimicrobial an antifungal even at low concentrations (upto 0.1%). It is a good 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% $HgCl_2$ for 3 minutes. From the present studies MS media proved to be the best culture medium for the establishment of shoot culture in *B.monnieri* plant. Here the work was done to develop effective and efficient tissue culture protocol to raise *in vitro* plant of brahmi which is used preliminary for memory enhancing purposes. Final observation after 3-4 weeks showed that MS media supplemented with 0.25 mg/l of IBA and 0.25 mg/l of NAA proved to be most capability in shoot induction. Numerous reports of BAP and IBA as bud inducer at concentrations ranging from 1.0-5.0 mg/l have already published (Sharma et al. 2010, Chandra et al. 2012, Kaur et al. 2013, Mohanta and Sahoo 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 0.2 KIN to establish a stock of shoots used for *in vitro* multiplication. This observation is supported by previous studies on *B. monnieri* (Ceasar et al. 2009, Vijay et al. 2016, 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). MS medium with activated Charcoal ensuing excellent response for root induction. 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. So, this technology is effective as it produces thousands of plants in a short span of time. Inspite of this *in vitro* propagation of *Bacopamonnieri* results in highest rate of multiplication in comparision

to naturally found species on *Bacopamonnieri*. The brahmi research will provide a new side of research in medicinal components of plants by various techniques.

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