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RESEARCH ARTICLE

EFFECT OF GROWTH HORMONES ON *IN-VITRO* PROPAGATION OF THREATENED MEDICINAL SPECIES *BACOPA MONNIERI* L.

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ABSTRACT

An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species, *Bacopa monnieri* L. is in among these. We studied the effects of various treatment of growth regulators with MS medium on shoot and root induction from auxiliary bud of *Bacopa monnieri* L., collected from Forest Research Institute and Biotechnology Nursery, Prem Nagar, Dehradun. Growth hormones at different combinations brought out remarkable variations in shoot and root induction, respective length and their survivability. Maximum shoot length, % survivability and minimum days for bud induction were observed in BAP 0.40mg/l, with MS medium, whereas maximum root length % survivability and minimum days taken for root induction were observed in 0.4 mg/l IAA with MS medium.

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INTRODUCTION

Bacopa monnieri L. *(*Family Scrophulariaceae) also referred to as *Herpestis monnieri*, water hyssop, and "Brahmi" has been used in the ayurvedic system of medicine for centuries Debnath *et al*. (2008). Traditionally, it was used as a brain tonic to enhance memory development, learning, concentrate and to provide relief to patients with anxiety or epileptic disorders. This plant has also been used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of broncho-constriction. *Bacopa*'s antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer Mukherjee and Dey (1966). Environmental stress severely restricts the distribution and productivity of plants. In particular, salinity and drought are two major abiotic factors that limit crop productivity Abdelbe *et al.* (2003); Misra *et al.* (1990, 2002). Tissue culture is also an efficient means to study the effect of abiotic stress on the cell metabolism Das *et al*. (1990, 1992), Misra *et al.* (1990, 2002). Why tissue culture of *Bacopa monnieri*- the seed production is difficult in *Bacopa monnieri* and many times seeds do not show proper germination and seedling growth. Micro propagation is mainly used in individual plants having elite characters and protects them against segregation or mutation. In many medicinal plants including *Bacopa monnieri* planting material is becoming endangered so necessary to develop micro propagation protocols to preserve germplasm and for distribution during cultivation in new areas. Production of medicinal plant seedlings can be carried continuously without seasonal variation and environmental factors. Asexual multiplication using tissue culture techniques can be achieved by three approaches, enhancing auxiliary bud break, Production of adventitious buds Somatic embryogenesis.

An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species, *Bacopa monnieri* L is in among these. We studied the effects of various treatment of Growth regulators with MS medium on shoot and root induction from auxiliary bud of *Bacopa monnieri L,* collected from Forest Research Institute and Biotechnology nursery, Prem Nagar, Dehradun. Growth hormones at different combinations brought out remarkable variations in shoot and root induction, respective length and their survivability.

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MATERIALS AND METHODS

Collection of plant material

Bacopa planting material was collected from Forest research institute Dehradun and Biotechnology nursery, Prem Nagar, Dehradun.

Media preparation

Murashige and skoog's media was used for *in-vitro* micro propagation of *Bacopa monnieri. L*. MS medium was prepared by adding required amounts of stock solutions and final volume was made up with distilled water. The pH of the medium was adjusted to 5.8 using 1 N NaOH/KCl. The culture bottles with 15 ml MS medium was autoclaved at 121°C for 20 min. at 15 lbs pressure and transferred to the storage room.
Explants selection and sterilization

The disease free, young and healthy nodal explants were selected for carrying out study as young cells are supposed to have retained their totipotency. The leaves were removed from the explants and then washed under running tap water for 30 minutes in order to wash off the external dust/contaminants. In the next step explants were soaked in an aqueous solution containing 0.2% bavistin for 10 minutes. This was followed by gentle wash in sterile double distilled water for 5 minutes for two cycles. Then the explants were immersed in aqueous solutions of tepole (1.5% v/v) for 10 minutes and were shacked regularly. Then the explants were washed thoroughly with sterile double distilled water for 5 minutes (two cycles).

Initiation of cultures

There is a high risk of contamination of the MS medium at the time of transfer of the explants into the culture medium. Therefore, surface sterilized explants were transferred aseptically to sterile glass plate. Then undesirable and dead portions of both basal and the top portion of the explants were removed. The nodal explants were placed in an erect position in the culture bottle containing MS medium with various concentration of IAA and BAP as given in Table-1 with the help of sterile forceps. Then lid was closed carefully and sealed with Klin film.

The same procedure was used for all the explants. The culture bottles were kept in the growth room at 25±2°C, with a photoperiod of 16 h daylight and 8 h night breaks under the cool white fluorescent light (Kumar and Sajeevan, 2005).

RESULTS AND DISCUSSION

Present study on Micro propagation of Bacopa was based on research studies reported by Mathur and Kumar (1998), Tiwari *et al.* (1998) and Tiwari *et al.* (2001). To initiate the study, nodal explants were taken from field-established plants. The sterilization procedure includes soaking of explants in an aqueous solution containing 0.2 % bavistin followed by treatment with 0.1% mercuric chloride aqueous solution with intermediate steps of sterile water washing. Shrivastva and Rajani (1999) has described sterilization treatment of Bacopa, which includes use of 0.1% Mercuric chloride (w/v) for 2 minutes followed by rinsing thoroughly with sterile distilled water. Mathur and Kumar (1998) reported different sterilization treatment in which leaves and stem explants were shaken for 10 minutes in tween-20 and savlon (0.3%) v/v chlorohexidine gluconate in water for 10 minutes, rinsed in running water for 30 minutes, treated with 0.1% Mercuric chloride for 3-4 minutes and washed several times with sterile water. However it was also found that duration of treatment for mercuric chloride is very critical due to soft and herbaceous nature of explants. During surface sterilization treatment it was found that treatment with 0.1% mercuric chloride as referred by Shrivastva and Rajani (1999) and Mathur and Kumar (1998), leads to blackening of the explants. Hence limited treatment of 0.1% Mercuric chloride was given to the plants. The surface sterilization procedure was optimized and this helped in preventing blackening of tissues and establishment of clean cultures. The sterilization procedure initially followed.

Plant tissue culture techniques for ornamental as well as herbaceous plants have been well established. *In-vitro* propagation technique is a powerful tool for plant germplasm conservation hence tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm via micro propagation (Ignacimuthu, 1997).

Table 1. Effect of different hormonal concentration on shoot induction

S.No.	Growth regulator	Treatment	Shoot length in (cm)	% of survivability	Duration in bud induction
1.	BAP-0.1	1	1.550	10.0	6.00
2.	BAP-0.2	2	2.10	10.0	5.67
3.	BAP-0.3	3	1.475	15.0	6.50
4.	BAP-0.4	4	3.000	40.0	3.00
5.	BAP0.5	5	2.545	20.0	7.50
6.	BAP-0.5/IAA-0.1	6	1.785	40.0	5.00
7.	BAP-0.5/IAA0.2	7	1.670	40.0	5.50
8.	BAP-0.5/IAA-0.3	8	1.815	40.0	5.50
9.	BAP-0.5/IAA-0.4	9	2.950	30.0	6.50
10.	BAP-0.5/IAA-0.5	10	2.150	40.0	6.50
11.	BAP-0.1/IAA-0.5	11	1.800	20.0	4.00
12.	BAP-0.2/IAA-0.5	12	1.980	40.0	5.00
13.	BAP-0.3/IAA-0.5	13	2.900	40.0	5.00
14.	BAP-0.4/IAA-0.5	14	3.000	20.0	6.00
15.	BAP-0.5/IAA-0.5	15	2.600	40.0	7.00
	MEAN		2.169	27.4	5.93
	MAXIMUM		3.000	40.0	7.00
	MINIMUM		1.475	10.0	3.00
	CV%		14.5%	37.2%	49.7%
	LSD		0.74	24.01	6.93
	S.E		0.3149	10.21	2.946

Plant biotechnology is considered in a wide sense which comprises the various culture methods of plant organs and explants to facilitate experimental approaches with a large objective of developmental biology in grain legumes for crop modification (Ramawat, 2003).

In the present study shoot induction was observed on MS medium supplemented with BAP (Fig. 1) and combinations of BAP and IAA (Fig.2) in various concentrations, the auxiliary bud initiated from nodal explants on MS Medium supplemented with hormone BAP (0.1-0.5 mg/l).

The shoot length (1.50-3.00cm) with standard error (0.3149) and mean shoot length was observed (2.169 cm) among the regulator treatments. The maximum shoot length (3.00 cm) was found with 0.4 mg/l BAP and combination of BAP 0.4/IAA 0.5mg/l in MS medium and minimum shoot length (1.475 cm) was in 0.3mg/l BAP. Significant results were observed with (14.5%) coefficient variance and least significant difference was 0.74. The shoot length survivability was observed (10-40%) with standard error (10.21) with mean value of 27.4% among the treatments.

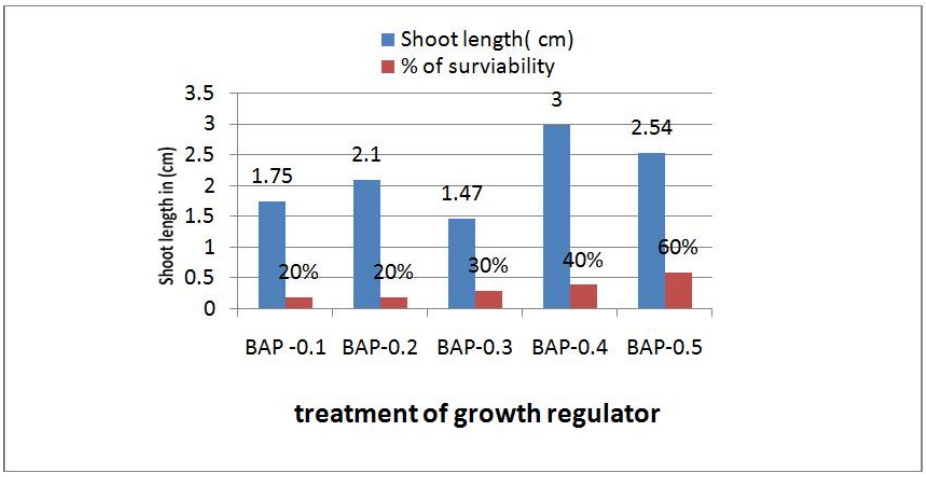

Fig. 1. Effect of BAP on shooting

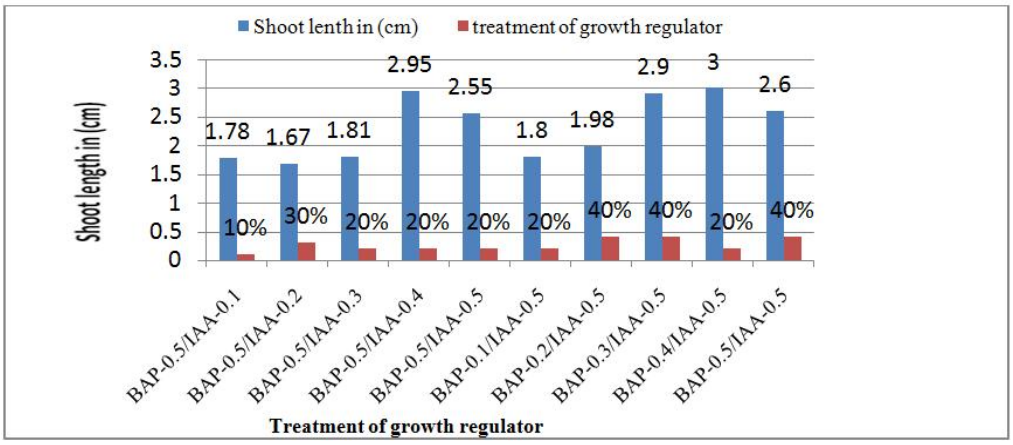

Fig. 2. Effect of combination of BAP and IAA on shooting

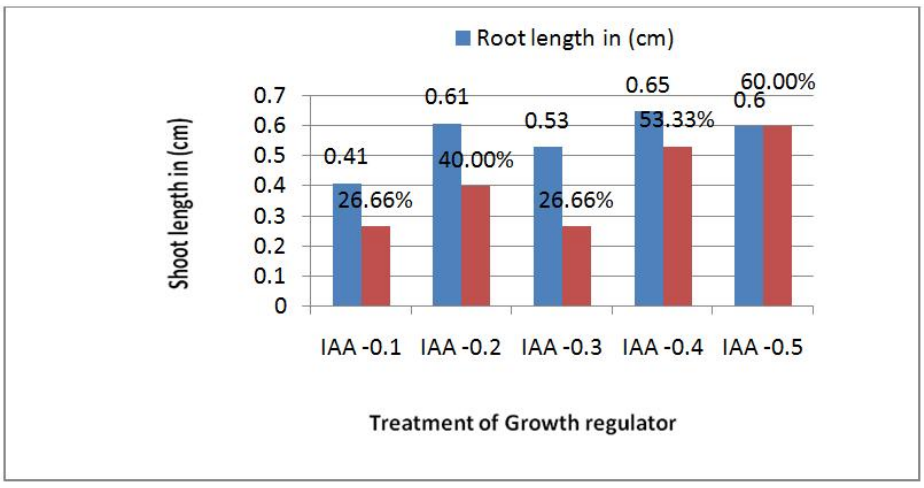

Fig. 3. Effect of IAA on rooting

Table 2. Effect of IAA on root induction

S.No.	Growth regulator	Treatment	Root length in (cm)	% of surviability	Duration in bud induction
1	IAA-0.1	1	0.415	10	7.25
2	IAA-0.2	2	0.612	18	7.8
3	IAA-0.3	3	0.5367	13.3	7
4	IAA-0.4	4	0.65	40	4
5	IAA-0.5	5	0.608	24	6
	MEAN		0.5616	19.5	6.68
	MAXIMUM		0.6500	40.0	7.80
	MINIMUM		0.4150	10.0	4.0
	CV%		2.5%	35.4%	30.9%
	LSD		0.024	12.18	3.64
	S.E.		0.01396	6.90	2.062

The maximum shoot length survivability (40%) was observed with 0.4 mg/l BAP in MS medium and minimum (10%) was in 0.1 mg/l BAP. Results were significant with 37.2 coefficients of variance and 24.01 least significant differences. The time taken was observed (3-8 days) with standard error 2.94 with the mean time (6 days) with various concentrations of BAP alone and combinations of BAP and IAA in MS medium. The maximum time taken for the bud induction was 8 days with 0.5 mg/l BAP and minimum was 3 days in 0.4mg/l BAP with MS medium. The significant results were observed with 49.7% coefficient variance and least significance of difference was 6.93 (Table1).

Root induction was observed on MS medium supplemented with IAA (Fig.3) in various concentrations, the auxiliary root initiated from nodal explants on MS Medium supplemented with hormone IAA (0.1-0.5 mg/l). The root length (0.4150-0.6500 cm) with standard error (0.01396) and mean length root was (0.5616) among the regulators treatment. The maximum root length (0.650cm) was observed with 0.4 mg/l IAA in MS medium and minimum root length (0.4150cm) was in 0.1mg/l IAA. The significant results were observed with (2.5%) coefficient variance and least significant difference was 0.24.

The root length survivability was observed (10-40%) with standard error (6.90) with mean value of 19.5% among the treatments. The maximum root length survivability (40%) was observed with 0.4 mg/l IAA in MS medium and minimum (10%) was in 0.1 mg/l IAA. Results were significant with 35.4% coefficient of variance and 12.18 least significant differences. The time taken was observed (4-8 days) with standard error 2.062 with the mean time (6 days) with various concentrations of IAA alone in MS medium. The maximum time taken for the root induction was 8 days with 0.2 mg/l IAA and minimum was 4 days in 0.4mg/l IAA with MS medium. The significant results were observed with 30.9 % coefficient variance and difference was 3.64 (Table 2). Ahmed *et al*. (2007) has also been reported that the maximum root induction (97.66%) was observed in MS medium fortified with 0.1 mg/1 of IAA. The root induction of sweetener plant Stevia (*Rebaudiana bertoni*) gradually decreased with increasing concentration of auxin except 0.1, 0.2, 0.5 mg/l IAA and 0.1 mg/l IBA. The rooting was not obtained on auxin omitted medium. Same study was also performed by Vijayakumar *et al*. (2010). Correspondingly, Thejavathi *et al.* (2001) has also been used shoot tip and nodal explants for the micro propagation studies of B. *monnieri* L. Most of the other research studies for other medicinal plant species have shown the use of cytokine alone or in combination with other different concentrations for plant culture initiation. In *Paederia foetida* and *Centella asiatica* multiple shoots were obtained in MS medium supplemented with BAP 1.0 mg/l (Singh *et al.,* 1999) and *Rauwolfia serpentina* on MS medium supplemented with benzyladenine and NAA (Sehrawat *et al.,* 2001).

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