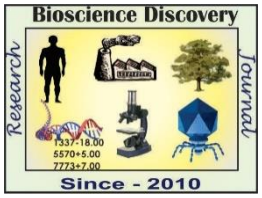
Bioscience Discovery, 11(3):146-150, July - 2020

Open Access, Peer Reviewed, Referred Research Journal

Print & Online available on https://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article



Micropropagation studies in *Bacopa monnieri* L.

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Article Info

Received: 10-5-2020
Revised: 22-06-2020
Accepted: 25-06-2020

Keywords: *Bacopa monnieri*, growth hormones, auxiliary shoot proliferation, transplantation, IAA, NAA & 2, 4-D

Abstract

Bacopa monnieri L. is used as a brain tonic to enhance memory development, learning and concentration and to provide relief to patients with anxiety or epileptic disorders. The plant has also been used in India and Pakistan as a cardiac tonic. Recent research has been focused primarily on Bacopa's cognitive-enhancing effects. Especially, memory, learning and concentration and results support the traditional ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome and gastric ulcers also supports the ayurvedic uses of Bacopa (Shakoor et al., 1994).The present investigations micropropagation studies have been carried out in highly medicinal plant *Bacopa monnieri* L. Effect of auxins (IAA, NAA & 2, 4-D) and cytokinins (BA, KIN & TDZ) on shoot induction and IAA and TDZ on root induction were assessed. Callus induction on Murashige and Skoog's medium (MS) supplemented with NAA, 2, 4-D and TDZ at various combinations/concentrations were also investigated.

INTRODUCTION

Bacopa monnieri L. is used as a brain tonic to enhance memory development, learning and concentration and to provide relief to patients with anxiety or epileptic disorders. The plant has also been used in India and Pakistan as a cardiac tonic. Recent research has been focused primarily on Bacopa's cognitive-enhancing effects. Especially, memory, learning and concentration and results support the traditional ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome and gastric ulcers also supports the ayurvedic uses of Bacopa (Shakoor *et al*., 1994).), *Taxus bacatal, T. wallichiana* (Himalayan Yew) and *Artemisia annua*. WHO estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs? The developed nations are also looking for eco-friendly treatment of various diseases through plant based source. In addition, many valuable herbal drugs have been discovered by knowing that particular plant was used by the ancient folk healers for the treatment of some kind of ailment (Ekka & Dixit, 2007).

It is considered in wide sense which comprises the various culture methods of plant organs, tissues which facilitates experimental approach with a large objective of developmental biology and crop modification. It provides new possibilities for in vitro propagation and manipulation of plants and also recognized as an efficient tool for rapid clonal propagation (Negrutiu *et al.*, 1984). Murashige and Skoog's medium is commonly used for plant tissue culture studies (Murashige & Skoog's, 1962). Hence, the present study is justifiably planned to propagate the valuable medicinal plant *Bacopa monnieri* L. in in vitro condition with various combinations / concentrations of plant growth regulators, and transplant the plants from laboratory in to field condition.

MATERIALS AND METHODS

Collection of plant material

Bacopa plant was collected from Botanical garden, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The collected plant parts were kept under green house condition for further study.

Murashige & Skoog's medium

Murashige and Skoog's medium was used for the cultivation of *B. monnieri* L. at in vitro condition. The MS medium was prepared by adding required amounts of stock solutions and final volume was made up with distilled water, the composition of the MS medium was given in Table 1. The pH of the medium was adjusted to 5.8 using 1 N NaOH/ KCl. About 50 ml of the medium was poured into sterile culture bottles. The culture bottles with MS medium was autoclaved at 121°C for 20 min. at 15 lbs pressure and transferred to the media storage room where they were kept under aseptic condition for further experimental study (Murashige & Skoog, 1962).

2.3. Selection of explants & sterilization

The explants were first washed with running tap water for 30 min. to remove the soil particles and other extraneous fine particles. The explants parts such as nodal segment, stem, leaf and root were cut from the healthy plant of *B. monnieri* L. and washed with tap water for 5-10 times, and they were soaked in 0.2-0.5% bavistin and 0.03% streptomycin aqueous solution for 10 min. It was gently washed twice in sterile double distilled water. The explants were immersed in aqueous solutions of savlon (1.5% v/v chlorohexidine gluconate solution & 3% w/v cetrimide) for 10 min. Then the explants were washed twice thoroughly with sterile double distilled water. After this treatment, the explants were surface sterilized with 0.01% HgCl2 aqueous solution for 1 min. and rinsed thoroughly with sterile double distilled water (Tiwari *et al.*, 2001).

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 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 (Anilkumar & Sajeevan, 2005).

Effect of plant growth regulators on shoot induction

The MS medium was supplemented with the plant growth regulators like auxin (IAA & NAA) and cytokinins (BA, KIN & TDZ) at 42 different combinations. The combinations and their concentrations were mentioned in Table 2. The explants were inoculated at appropriate condition in culture bottles, and the shoot induction rate was observed, triplicates were used for each treatment (Yadav & Padmaja, 2005; Anilkumar & Sajeevan, 2005).

Rooting of the shoots

The surface sterilized explants were cut into small pieces. For root induction, MS basal medium was supplemented with IAA 0.2 mg/l and TDZ 0.04 mg/l and the initial pH of the medium was adjusted to 5.8. The explants were implanted aseptically on the culture media. All the cultures were maintained in culture room at 25±2°C for 8-16 h of day and night break under the white fluorescent light (Mahendran & Sampath, 2005). After incubation the root induction rate was observed. Triplicates were maintained in each treatment.

Effect of growth hormones on root induction

Murashige and Skoog basal medium was supplemented with different concentrations of auxin (IAA) 0.1- 0.5 mg/l and TDZ 0.01-0.06 mg/l and pH of the medium was adjusted to 5.8. The explants were implanted on the culture media. All the cultures were maintained at culture room at 25±2°C for 8- 16 h of day and night break under the cool white fluorescent light and the root induction rate was observed (Thorat *et al.*, 2006).

Callus induction

Explants originated from leaf were taken from established cultures of *B. monnieri* L. for callus induction. The MS basal medium was supplemented with 0.5 mg/l NAA and 0.25 mg/l TDZ. After inoculation with established culture, the culture bottles were sealed properly, labeled and the triplicates were maintained. Then they were transferred to the incubation room and kept in appropriate condition. After two weeks, the callus induction rate was recorded (Nagaraja *et al.*, 2003).

Effect of plant growth regulators on callus induction

The PGR'S such as auxin and cytokinin were supplemented into MS medium at 25 different

combinations (Table 4) and callus induction rate was observed (Anilkumar *et al.,* 2005).

RESULTS AND DISCUSSION

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 micropropagation (Ignacimuthu, 1997). 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).

During the present investigations, to raise stock culture, nodal explants were taken from the field growing wild plants. The auxiliary bud was found initiated from both leaf and nodal explants on hormone free MS medium within 9 d. Shoot buds of *B. monnieri* L. were also initiated on the MS basal medium supplemented with 0.5 mg/I IAA and 0.5 mg/l KIN within 25-30 d from the nodal explants. Thejavathi *et al.* (2001) has also been used shoot tip and nodal explants for the micropropagation studies of *B. monnieri* L. Most of the other research studies for other medicinal plant species have shown the use of cytokinins alone or in combination with other different concentrations for plant culture initiation. For eg. 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).

Table No. 1: Effect of IAA, BAP and KIN on Multiplication in *Bacopa moneri* L. **using nodal segment as explants.**

Growth regulators Mg/l			**No. Of Shoots / Explants**	**(%) Shoot formation**
IAA	**BAP**	**KIN**		
0.5	0.5	-	2.69+0.184	25
	1.0	-	2.76+0.229	30
	1.5	-	2.83+0.167	60
	2.0	-	3.81+0.181	75
	2.5	**-**	**4.35+0.181**	**80**
	3.0	-	3.90+0.207	70
	-	0.5	1.67+0.174	20
	-	1.0	2.74+0.400	20
	-	1.5	3.14+0.567	40
	-	2.0	3.81+0.181	75
	-	**2.5**	**5.62 +0.203**	**95**
	-	3.0	2.83+0.167	70

*****After 35 days, mean ± SE of 5 replicates.

Effect of growth regulators on induction of shoot buds

The present study was investigated the effect of various plant growth regulators (PGR) on shoot induction in 42 different combinations of which better results was observed in MS medium containing the following combinations of PGRs such as 0.5 mg/l NAA, 1 mg/l TDZ, 1.5 mg/l BA, 0.5 mg/l IAA and 0.5 mg/l BA + 0.5 mg/l KIN + 0.5 mg/l NAA. Remarkably, the MS medium containing 1 mg/I BA + 0.4 mg/l KIN + 0.4 mg/l NAA was showed excellent shoot formation. In this combination, the shooting response was observed as 100% and maximum shoot length was recorded as 6.38±0.99 cm after 3 weeks. Similarly from leaf explants, excellent shoot formation was observed in 0.5 mg/l KIN containing MS medium and maximum shoot length was recorded as 3.00±0.61 cm when compared with other growth regulators combinations.

The moderate level of shoot formation was observed with the other growth regulators. Similar type of work has been reported by Kameri *et al.* (2005) for *Wedelia chinensis*. MS media containing different concentrations and combinations of growth regulators were found to promote multiple shoots from both nodal and shoot tip explants. Multiple shoots start arising from nodes after 18-22 d of inoculation. Growth regulator combinations of BAP (2 mg/l) + IBA (0.5 mg/l) was producing maximum number of shoots (5.2±1.55) and longer shoot length (5.2±1.12 cm) from nodal segments. Thus, it has been reported that the plant growth regulators greatly influence the shoot induction but it is varied depends upon the selected plant

Conclusion

In the present study, a fruitful protocol was set up for *B. monnieri* through multiple shoot induction. This protocol can be exploited for commercial propagation and conservation of potential endangered medicinal plant resources.

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Fig. 1 Micropropagation studies in Ba*copa monneri*

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How to cite this article

Shilpa Chavan, Pallavi Prashad and Narayan Pandhure, 2020. Micropropagation studies in *Bacopa Monnieri* L. *Bioscience Discovery*, **11**(3):146-150.

Google Scholar citation: https://scholar.google.co.in/citations?user=vPzEyC8AAAAJ&hl=en