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Research Article

In Vitro Studies In Medicinal Plant *Bacopa monnieri* L.

Pramod Fuke, Sangeeta Ahuja[1] and Narayan Pandhure

[1]Dept of Botany, Sir Sayyed College, Aurangabad
Tissue Culture Laboratory, Department of Botany,
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004

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Abstract

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 callus 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. The auxiliary buds formed from both leaf and nodal explants on hormone free MS medium within 9 d. Growth hormones at different combinations brought out remarkable variations in shoot, root and callus induction.

INTRODUCTION

WHO estimated that 80% of the population of developing countries rely 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). Technology Information forecasting and assessment council (TIFAC) has recommended 45 medicinal plant species of which 7 plants were recommended specifically for immediate attention during this decade, they are as follows: Aloe vera (Ghrita Kumari), *Bacopa monnieri* L. (Brahmi), *Centella asiatica* (Mandukparni, Gotukola), *Rawolfia serpenrtina* (Sarpagandha), *Catharanthus roseus* (Periwinkle), *Taxus bacatal T. wallichiana* (Himalayan Yew) and *Artemisia annua*. Traditionally, *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).

MATERIALS AND METHODS

Plant Tissue Culture at a glance

Plant tissue culture is the process of small pieces of living tissues (explants) isolated from a plant and grown aseptically for indefinite periods on a semi defined or defined nutrient medium (Ignacimuthu, 1997). 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 monnieri* L. plant was collected from Shervarayan Hills, Western Ghats of India at Salem district of Tamil Nadu. 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).

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).

Establishment of cultures:

The explants with bud proliferation cultures were transferred to culture tubes containing fresh MS medium. After 21-25 d of incubation the initiated plants were taken out from the culture bottle and transferred into fresh semi-solid MS media. Then the bottles were kept in culture room at 25±2°C for 8-16 h of day and night under the low temperature with white fluorescent light (Murashige & Skoog, 1962). After experimental days, the full matured culture was obtained and they were further subcultured in MS medium supplemented with different plant growth hormones at different concentrations for regeneration of shoots.

Callus induction:

Leaf explants 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). The combinations and their concentrations were mentioned in Table1.

Effect of plant growth regulators on callus induction:

The plant growth regulators such as auxin and cytokinin were supplemented into MS medium at 25 different combinations 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).

Table No. 1: Effect of different concentrations of 2, 4-D and NAA in MS on callus induction of *Bacopa moneri* L.

Source of explants	Conc. of 2,4 D (mg/L)	Conc. of NAA (mg/L)	Frequency: of callus Induction	Texture of callus	Response / color of Callus	Induction of callus
Leaf	0.5	-	-	--	Swelling of explants	No callus
	1.0	-	++	Friable	Yellowgreen	Callus
	1.5	**-**	**+++**	**Friable**	**Greenish**	**Callus**
	2.0	-	++	Friable	Yellowgreen	Callus
	2.5	-	++	Friable	Yellowgreen	Callus
	3.0	-	+	Friable	Yellowish	Callus
	-	0.5	-	-	-	No callus
	-	1.0	+	Friable	Yellowish	Callus
	-	1.5	++	Friable	Yellowish	Callus
	-	**2.0**	**+++**	**Friable**	**Yellowish**	**Callus**
	-	2,5	++	Friable	Yellowish	Callus
	-	3.0	+	Friable	Yellowish	Callus
Nodal segment	0.5	-	+	Compact	Greenish	Callus
	1.0	-	+	Compact	Greenish	Callus
	1.5	-	+	Compact	Yellowish	Callus
	2.0	-	+++	**Compact**	**Yellowish**	**Callus**
	2.5	-	++	Compact	Yellowgreen	Callus
	3.0	-	+	Compact	Yellowgreen	Callus
	-	0.5	--	-	Swelling of explants	No Callus
	-	1.0	+	Compact	Yellowgreen	Callus
	-	1.5	++	Compact	Yellowgreen	Callus
	-	2.0	++	Compact	Yellowgreen	Callus
	-	**2.5**	**+++**	**Compact**	**Yellowgreen**	**Callus**
	-	3.0	++	Compact	Yellowgreen	Callus

--No Callus, +Poor Callus, ++Moderate Callus, +++Massive Callus

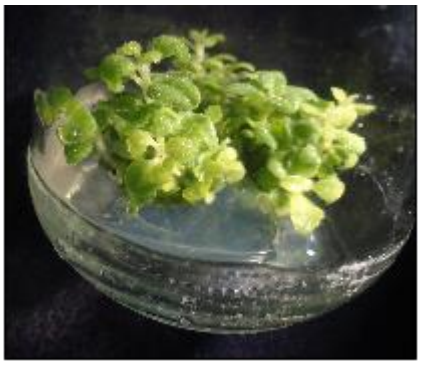
IAA = Indole acetic acid; NAA = α-Naphthalene acetic acid;KIN = kinetin; TDZ = thiodizuron; BA = Benzyl adenine; 2-4-dichlorophenoxyacetic acid

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 study, 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. Correspondingly, 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).

Conclusion

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

Callus induction from explant in *Bacopa moneri* L



Shoot induction from explant in *Bacopa moneri* L

Fig. 1: Effect of different concentrations of 2, 4-D and NAA in MS on callus induction of *Bacopa moneri* L.

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