Biosciences, Biotechnology Research Asia Vol. **4**(1), 307-311 (2007)

In vitro tissue culture study (*Somatic embryogenesis*) on *Bacopa monniera* (A medicinal herb)

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(Received: March 02, 2007; Accepted: May 10, 2007)

ABSTRACT

Somatic embryogenesis of *Bacopa monniera* (a small creeping herb) Family Scrophulariaceae was achieved by culturing excised apical and axillary buds on MS (Murashiage and Skoog) basal media supplemented with various combinations of growth hormones Viz. Cytokinin and Auxin, the Cytokinin that is BAP (6-Benzyl amino purine) and Kinitin along with the combination of Auxin. Culture was incubated under white florescent light (2000-3000 lux) intensity for 16 hours photoperiod & 8 hours dark at temperature of 25 ± 3ºC & relative humidity of 50 to 60%. The pH of the medium was adjusted between 5.5-5.8 by using 0.1N NaOH prior to the experiment. The *in vitro* developed plantlets were subjected to a hardening schedule. The developing plantlets were transferred to soil. The plantlets were gradually exposed to the natural environment and irrigated regularly with sterile distilled water.

Key words: Medicinal Plant, *Bacopa monniera*, Brahmi, Embryogenesis,

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds, Chand *et al.* (1997). Among the World's 25 best selling pharmaceutical medicines, 12 are plant derived, O'Neill and Lewis (1993).

Bacopa monnieri (Scrophulariaceae) is a perennial, creeping herb whose habitat includes wetlands and muddy shores. Common names include Water Hyssop and brahmi (brahmi is also the Ayurvedic name given to *Centella asiatica* and other herbs).Famed in Ayurvedic medicine, brahmi has antioxidant properties. It has been reported to reduce oxidation of fats in the blood stream, which is a risk factor for cardiovascular diseases.It has been used for centuries to help benefit epilepsy, memory capacity, increase concentration, and reduce stress-induced anxiety.It is listed as a nootropic, a drug that enhances cognitive ability, Rastogi *et al.* (1994), Das Gupta and Khanjoj (1996). In India, this plant has also been used traditionally to consecrate new born babies in the belief that it will open the gateway of intelligence. Recent studies suggest bacopa may improve intellectual activity, *Stough et al.(2001) and Roodenrys et al.(2002).*The plant was placed second in priority list of the most important Indian medicinal plants evaluated on the basis of their medicinal value and potential for further research and development, Shrivastav and Rajni (1999). The medicinal properties of *B. monniera* have been attributed to the presence of different types of saponins, e.g. bacosides A, B, C and D, Rastogi *et al.* (1994). The plant also contains several alkaloids, such as nicotine, brahmine, herpestine; several other chemicals like stigmasterol, b-sitosterol, Basu and Walia (1944), Basu *et al.* (1967). *B. monniera* has been recognized as the major priority species on the basis of their medicinal importance, commercial value and potential for further research and development, Karki and Williams (1999). Shoot regeneration from different explants of *B. monniera were* studied by Tiwari *et al.* (1998 & 2000).

The tissue culture importance of the plant lies in its medicinal values as large scale production is necessary to cover the deficit that occurs during manufacturing the drugs, hence there is need to develop micro propagation protocol for ensuring the availability of raw material of a constant quality from regular and viable sources. In view of the above, this work was designed for in-vitro high level micro propagation and conservation of *Bacopa monniera* from somatic embryogenesis procedure through internodes of the plant.

MATERIALS AND METHODS

The plant *Bacopa monniera* was collected from local garden of medicinal plants in Bhopal and the excised shoots were washed thoroughly with running tap water to remove the soil, followed by drops of liquid soap for ten minutes. To remove soap solution explants were rinsed with tap water and finally with distilled water for several minutes. After washing cleaned explant was subjected to chemical sterilization in the hood of laminar airflow with 0.1% W/V with $HgCl_2$ solution for five minutes. Explants were washed with sterile double distilled water to remove the traces of $HgCl_2$.

The explant was inoculated under aseptic condition. The Murashige and Skoog (MS) medium (1962) was used as basal medium throughout the experiment. The medium was supplemented with various combinations of phytohormones viz. cytokinin and auxin, the cytokinin i.e. BAP and kinitin along with the combination of auxin, NAA in different concentration was used for the experiment. The pH of the medium was adjusted between 5.5-5.8 by using 0.1N NaOH prior to the experiment. The culture was incubated under white florescent light (2000-3000 lux intensity) for 16 hrs photoperiod and 8 hrs dark at the temperature of 25 ±3 ^{O}C and relative humidity of 50-60 %. The *in vitro* developed plantlets were subjected to a hardening schedule and then were transferred to soil. The plantlets were gradually exposed to the natural environment and irrigated regularly with sterile distilled water.

Table - 1: Effect of phytohormones on tissue culture of *Bacopa monniera*

M.S medium + Auxin/ Cytokinin Concentration (mg/l)	Culture withMultiplication Of Shoot (%)	Average length of Shoot (cm)	Callusing
BAP			
0.2	40%	6-7	+
0.5	45%	7-8	+
1.0	45%	6-8	+
Kinitin			
0.2	40%	3-4	++
0.5	35%	2-4	++
1.0	45%	3-5	+++
BAP+Kinetin			
0.5+0.1	60%	2-3	+
1.0+0.5	60%	2-4	+
2.0+0.5	65%	4-5	++
BAP+NAA			
0.2+0.1	70%	6-7	+
0.5+0.5	70%	6-8	+
0.5+1.0	80%	6-9	++
1.0+1.0	80%	8-9	++
2.0+1.0	70%	8-9	++
2.0+0.5	60%	6-7	+

+ = Less callusing. +++ = High callusing.

RESULTS AND DISCUSSION

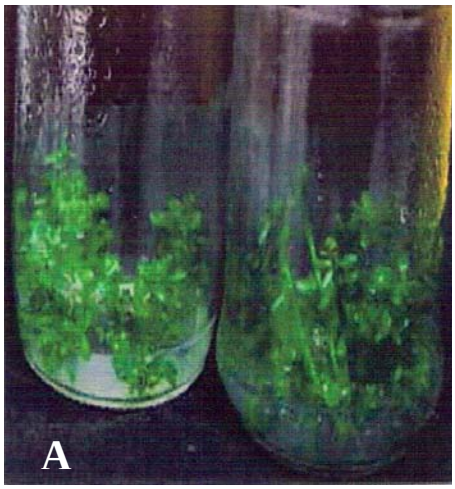
Interests in *in vitro* clonal propagation of plants originated from the success of Morel (1960) with orchids. Bonnet and Torrey (1965) succeeded in micropropagation of *Convolvulus arvensis* when they developed plantlets through shoot buds. Chaplot Binita *et al.* (2005) also studied the micropropagation on *Bacopa monniera.*

In the present study Initiation of growth from lower end of the stem (Fig: A) was investigated in all the media tried with the combination of different phytohormones and responses were scored under their influence (Table: 1). The formation of shoots with callus was induced after 15-30 days (Fig: B & C). The age of the explant was found to play an important role in the induction of somatic embryogenesis.

The effect of BAP (0.5-1.0mg/l) with NAA (1.0mg/l) was found to be highest for multiplication of shoots (up to 80%) with average length of shoot as 8-9cm in comparison to the concentration of BAP (2.0mg/l) and kinitin (0.5mg/l) which resulted in 65% of shoot multiplication and 4-5cm average shoot length. The kinitin and BAP resulted minimum shoot multiplication i.e. 45% with average shoot length of 3-5cm in kinitin (1.0 mg/l) and 45% shoot multiplication in BAP (0.5mg/l) with average length of shoot as 7-8cm when these were used alone. The maximum micropropagation of *Bacopa monniera* was reported in 15 days (Fig: B) Kamili *et al.* (2003) developed a reproducible protocol for micropropagation of *Cichorium intubus* through in vitro culture on MS (Half salt strength) medium supplemented with various phytohormonal regimes.

Most of the plants require both auxin and cytokinin in growth medium for callus initiation, Dowd *et al.* (1998) observed that a callus grown on Auxin reduces the yield of secondary metabolites, while callus grown on combination of Auxin and Cytokinin showed maximum production of secondary metabolites. Mehra and Cheema (1980) also reported callus induction from *Populus citiate* stem on MS medium containing BAP and NAA.

A number of scientists are engaged in micropropagation and in vitro preservation of plants



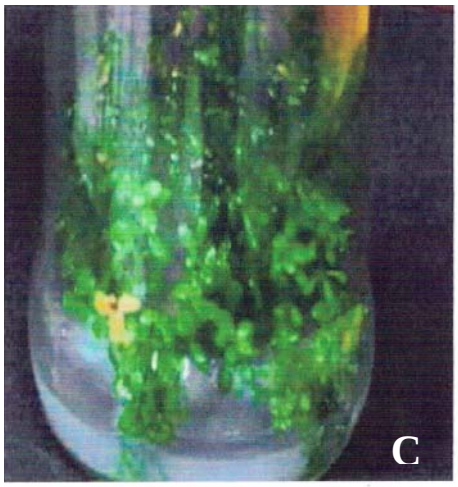


Fig. -1:
A. Multiplication from lower end of shoot.
B. Multplication after 15 days & root formation.
C. Multiple shoot formation after 25 days.

Table - 2: Effect of medium (MS) for the multiplication and root initiation

M.S. medium	Multiplication Response(%age)	No. of roots
M.S. Basal Media	80%	10–15
M.S. (half Salt strength)	70%	9–10
M.S liquid (full Salt strength)	80–85%	10–15

known to have therapeutic properties. Commercialization of tissue culture of medicinal plants, however has received a poor response compared to ornamental plants. It is estimated that world wide approximately only 5% of total propagation of medicinal plants is through tissue culture. Regenerating through somatic embryogenesis has been reported in many medicinal plants. Krishnan and Seeni (1994) recorded the highest multiplication in *Woodfordia fruiticosa,* when callus initiation medium was supplemented with a combination of BAP and NAA followed by subculture in media containing BAP alone. The shoot proliferation was observed to be much faster at the reduced concentration of cytokinin. The decrease in the strength of the basal salts to half strength in the culture medium showed profuse rooting of shoots (Table: 2). The MS media in full salt strength was found to be more responsive (80-85%) producing 10-15 roots (Fig: C).

Bacopa monniera an important medicinal herb is one such plant, which is exploited at an alarming rate for curing various diseases by tribal and local people. In order to save it from becoming endangered in near future, present studies were taken with an aim to develop protocol for its propagation and conservation.

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ABBREVIATIONS

BAP: 6-benzylaminopurine
NAA: 1-naphthalene acetic acid
MS: Murashige and Skoog (1962) medium.