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HIGH FREQUENCY REGENERATION OF *BACOPA MONNIERI* PLANT CALLUS DERIVED FROM INTERNODE

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ABSTRACT

Bacopa Monnieri is an important medicinal plant belonging to the family Scrophulariaceae. Plant regeneration was achieved from internode. High frequency regeneration of callus was achieved from internodes inoculated on MS medium supplemented with Kinetin and 2, 4, D at 7µM concentration each. Primary shoot was induced from this callus in the medium containing. 0.1 kinetin and 1mg/l 2, 4, D. In vitro plantlets were successfully established with ex vitro.

KEYWORDS: **Bacopa Monnieri, Plant regeneration, internode, invitro, Callus**

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INTRODUCTION

Bacopa Monnieri (Scrophulariaceae), commonly known as Brahmi is an important medicinal herb. It is bitter in taste; has been used in the Ayurvedic system of medicine for centuries. Brahmi which is available in abundance in India and has a long history of use in the tradition Ayurvedic medicine for the treatment of a number of disorders[1,2,3], particularly those involving anxiety, intellect and poor memory. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration. Compounds responsible for the pharmacological effects of Bacopa Monnieri include alkaloids, saponins, and sterols. Many alkaloids like Brahmine and herpestine, saponins[4], d-mannitol and hersaponin, and monnierin were isolated in India over 40 years ago. Other active constituents identified include betulic acid, stigmastarol, beta-sitosterol, as well as numerous bacosides[5] and bacopasaponins. The constituents responsible for Bacopa's cognitive effects are bacoside A and bacoside B. Earlier invitro propagation through shoot tips, auxiliary buds[6] and somatic embryogenies[11] was reported in this species. In the present investigation we saw successful regeneration of Bacopa monnieri callus derived from internode on MS basal medium.

MATERIALS AND METHODS

Collection of Plant Material and Surface Sterilization

Bacopa monnieri plants were directly obtained from Laurel Higher Secondary School of Pattukkottai, Thanjavur District. The explants which is required for our present study, internode were excised, kept in running tap water for 15-20 minutes and treated with1% twin 80 for 10minutes followed by repeated rinsing with autoclaved distilled water. Further sterilization was done under aseptic conditions in Laminar Air flow chamber. Explants were surface sterilized with 0.1% $HgCl_2$ (w/v) for 3-4 minutes and later thoroughly washed with distilled water to remove the traces of Mercuric Chloride.

Callus Induction

These sterilized explants were cut into suitable size and cultured on MS medium[7] in conical Flask with different concentration and combinations of kinetin and 2, 4, D. For this experiment MS medium was supplemented with 2% (w/v) sucrose and gelled with 0.9%(w/v) agar. Its pH was adjusted to 5.8 with 1N HCl prior to autoclaving. The cultures were incubated in a culture room at 25+2 ˚C with a photoperiod of 16hours at 1000-lux intensity provided by white fluorescent light.

RESULTS AND DISCUSSION

For callus induction and plant regeneration, the internode explants[8] were cultured on MS medium with different concentration of auxin and cytokine. After 10-15 days of inoculation, callus formation was observed (Figure 1) .The highest mass of callus induction was achieved in the combination of kinetin and 2,4,D at 7µM each. The higher concentration of MS supplemented with 0.5 mg/l 2, 4-D was previously found to promoted callus formation from the leaf explants in Bacopa monnieri[9]. The internode derived calli was subcultured on MS medium supplemented again with 6 different concentrations and combinations of kinetin and 2, 4,D. Shoot initiation appeared from these calli within 12 days of subculture at 0.1 kinetin and 1mg/l 2,4,D.Thus 0.1 kinetin and 1mg/l 2,4,D were found to be an ideal treatment for root induction as well as elongation. After 35 days a well shooted plantlets were obtained (Figure 2). Subsequently the plantlets were removed from the agar medium and planted in pat containing sterile soil and sand. After proper acclimatization, the plantlets were transferred in the natural condition with 80% survival[10]. The Percentage of shoot response (Table 1) was

recorded at an interval of six days and finally after 7 weeks.

Table 1
Effect of growth regulators from internodal explants of Bacopa monnieri

Ms medium+Growth regulator		Percentage of shoot response
Kinetin	2,4,D	
0	0	0
0.05	0.5	22
0.1	1	78
0.2	2	18
0.3	3	52
0.4	4	68

MS media +Growth Regulator Concentration (mg/l) Root induction rate %

Figure 1
Good Callus Proliferation

Figure 2
Regeneration of shoot from intermodal explants

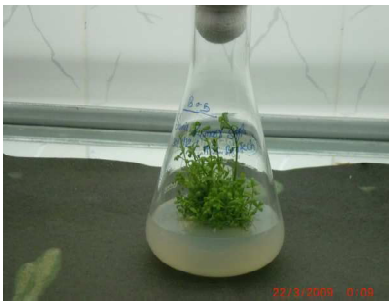

Figure 3
Regeneration of Plant

CONCLUSION

In the present study an efficient protocol has been developed for the high frequency regeneration of *Bacopa monnieri* plant callus derived from internode.

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