

Cost Effective Protocol for Micropropagation of *Bacopa Monnieri* Using Leaf Explants

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Abstract: Owing to the resurgence in herbal based therapies, plants which serve as raw materials are required in large quantities to meet their increasing demand. Additional efforts are employed through various conventional and non-conventional ways for propagation and conservation of medicinal plants. *Bacopa monnieri* (L.) Wettst (Family: Scrophulariaceae) is one such medicinal plant used in Indian traditional systems of cure for various diseases and thus it becomes necessary to conserve this plant species. An efficient, cost effective protocol has been standardized using leaves as explants for in vitro propagation of *B. monnieri*. Leaves of five different accessions of *B. monnieri* collected from various regions of North India were cultured on agar gelled Murashige & Skoog (MS) & Gamborg's (B₅) media without the addition of any expensive nutritional supplements (plant growth regulators, growth additives). De novo shoot initiation from leaf explants was observed after 20 days of inoculation accompanied by rooting in the form of a single tap root in all the accessions in both the media tested. Maximum shoots/explant (5.5±0.65), leaves/explant (17.9±0.75) and roots/explant (6.3±0.65) were obtained for leaves of Accession BM003 cultured on MS media. Through this study we conclude that MS media is superior over B₅ media for in vitro shoot multiplication and plantlet regeneration of *B. monnieri*. The protocol can cut down the cost of micropropagation of this endangered herb and also minimize the excessive and reckless use of plant propagation explants (shoot tips, nodes).

Keywords: Brahmi, bacosides, leaf regeneration, shoot multiplication, medicinal plants

1. Introduction

“The thinking person’s herb” or “Brahmi” are common names for the plant *Bacopa monnieri* (L.)Wettst (Family Scrophulariaceae) which is a small perennial succulent creeping herb found throughout the Indian subcontinent commonly found growing in wet, damp, marshy areas [1]. This plant species has been used for almost 3000 years in India by Ayurvedic practitioners and is classified as Medhyarasayna - a drug to improve memory [2]. The medicinal properties of the herb are attributed to the presence of triterpenoid saponins especially bacosides, which are known for their cognitive enhancing effects specifically for memory, learning and concentration. There has been a rapid expansion of biopharmaceutical industries during the last few years which have lead to depletion of several herbal plants from their natural habitats. *Bacopa monnieri* has also been enlisted as a threatened species a long time ago by International Union for Conservation of Natural and National Resources [3]. It has been the focus of many research groups to develop strategies for its conservation [4], *in vitro* regeneration [5], micropropagation and shoot regeneration [6] using different explants like leaves, internodal segments and shoot tips. All these studies have employed the use of a number of additives e.g. plant growth regulators, nutritional supplements which make the whole process expensive. The present study demonstrates the regeneration ability of a single leaf in 5 different accessions of *B. monnieri* without the addition of any plant growth regulators which can be used as a cost effective strategy for the conservation of this medicinally important plant species.

2. Materials and Method

2.1 Plant Material

Small shoots (1.5 - 2 cm) bearing leaves (leaf size:0.7 -0.9 cm length, 0.3 - 0.5 cm width) were collected from healthy vegetatively propagated net house grown plants from five different accessions of *Bacopa monnieri* (Table 1) habituated in the Herbal Garden of SMVDU, Katra, Jammu and Kashmir (Latitude = 28°66' North, Longitude = 77°21' East and Altitude = 754m). The leaves were washed thoroughly under tap water to remove solid dirt and a 2% (w/v) Tween 20 (Himedia, India) solution and then treated with 1% (v/v) Sodium hypochlorite for 3 minutes and kept under running tap water for 60 minutes. The explants were aseptically surface sterilized by treatment with 0.2% (w/v) HgCl₂ solution for 2 minutes followed by washing 3-4 times with sterile distilled water to remove traces of sterilants.

2.2 Aseptic Culture Conditions

The entire leaf was used as an explant to establish cultures on Murashige & Skoog (MS) [7] and Gamborg's (B₅) [8] media sterilized by autoclaving (121°C under 15-20 psi for 15min). Throughout the experiment the media pH was maintained at 5.8 and gelled with 0.7% (w/v) agar. The explants were inoculated on to the media contained in culture tubes (15 ml) and incubated in the culture room maintained at 25 ± 2°C temperature with 16 hour photoperiod provided by cool white fluorescent CFL bulbs (3000 lux).

Five replicates were used for each treatment for different accessions of *Bacopa monnieri* and monitored for 8 weeks with a subculture period of 4 weeks. Data was recorded every week and the experiment was repeated two times.

Table 1: Geographical data on collection of different accessions of *Bacopa monnieri*

Sample code	Location of Collection	Latitude	Longitude	Altitude
BM001	Indian Institute of Integrative Medicine, Jammu, J&K	32°50'N	74°24'E	305m
BM002	Rajinder Agriculture University, Pusa, Samastipur, Bihar	25°55'N	85°5'E	173m
BM003	Forest Research Institute, Dehradun, Uttarakhand	30°34'N	77°99'E	440m
BM004	Institute of Himalayan Bio-resource Technology, Palampur, Himachal Pradesh	32°7'N	76°31'E	1472m
BM005	Wild collection, Jhajarkotli, Reasi, J&K	25°53'N	74°58'E	1732m

3. Results and Discussion

De novo shoot initiation from leaf explants on both MS and B₅ media was observed after 20 days of inoculation accompanied by rooting in the form of single tap root followed by shoot multiplication from the 6th week onwards and leaf regeneration from 7th week. These studies exploit the fact that both MS and B₅ media are suitable for *in vitro* shoot regeneration of *Bacopa monnieri* without using any additional plant growth regulator from a single leaf explant. Although the regeneration pattern was similar for all the five accessions studied, however, the regeneration process was found to be different. Maximum number of leaves (17.9±0.75), roots (6.3±0.65) and shoots (5.5±0.65) were obtained for Accession BM003 raised in MS media however, the minimum number of leaves (2.6±0.42), roots (0.7±0.15) and shoots (0.9±0.17) were recorded for Accession BM004 raised on B₅ media (Table 2; Fig 1, 2). Table 2 depicts the data obtained after 8 weeks for mean number of leaves, roots and shoots raised from single leaf on MS and B₅ media for all the five accessions studied. The data obtained emphasizes the fact that amongst all the accessions studied BM003 showed higher regeneration ability than the other accessions and the fact that MS media is superior over B₅ media for *in vitro* regeneration in *Bacopa monnieri*. Earlier studies have reported *in vitro* regeneration and shoot multiplication of *B. monnieri* using leaf as an explant on MS media, however, at the expense of plant growth regulators (PGRs) [6, 9]. The protocol employed in our study can be used for mass micropropagation to cut down the cost of PGR's and reduce the number of different explants used from a single plant for its conservation. The results indicate that the large scale propagation and conservation of *B. monnieri* by tissue culture is feasible, cost effective in this endangered and medicinally important plant with simplicity.

Table 2: Effect of different plant growth media on number of leaves, roots, and shoots obtained in five accessions:

Accession	Treatment					
	MS Media			B ₅ Media		
	Mean no. of Leaves ± SE	Mean no. of Roots ± SE	Mean no. of Shoots ± SE	Mean no. of Leaves ± SE	Mean no. of Roots ± SE	Mean no. of Shoots ± SE
BM001	16.5±0.67	4.9±0.27	4.8±0.38	9.8±0.20	3.9±0.23	3.0±0.14
BM002	11.9±0.45	2.4±0.30	3.7±0.26	8.7±0.21	1.4±0.16	2.5±0.16
BM003	17.9±0.75	6.3±0.65	5.5±0.65	10.9±0.27	4.6±0.43	3.2±0.20
BM004	5.3±0.42	1.1±0.10	1.1±0.10	2.6±0.42	0.7±0.15	0.9±0.17
BM005	10.3±0.52	1.7±0.15	2.8±0.33	6.6±0.31	1.5±0.16	2.1±0.10

(*Values represent mean of 10 replicates and SE is the Standard error for 2 experiments.)

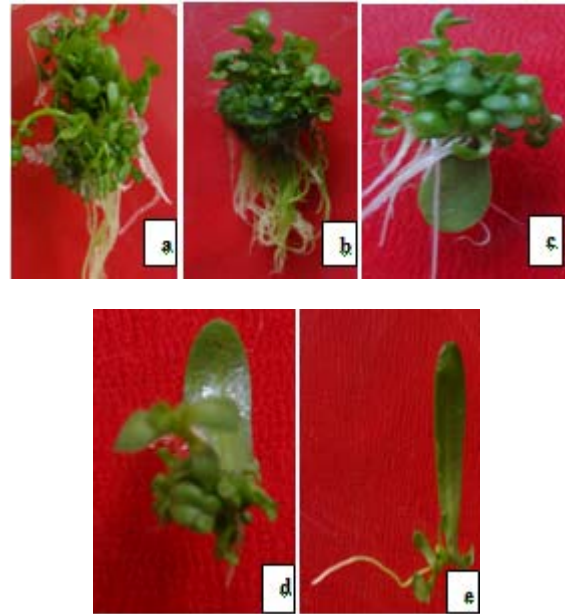


Figure 1: *In vitro* plantlet regeneration using single leaf explants after 8 weeks of inoculation in MS media for five accessions of *Bacopa monnieri*. a- BM003, b- BM001, c- BM002, d- BM005, e- BM004.

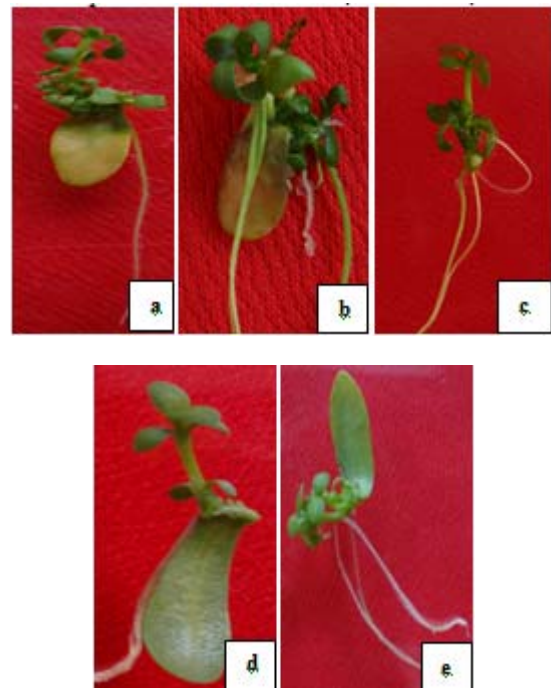


Figure 2: *In vitro* plantlet regeneration using single leaf explants after 8 weeks of inoculation in B₅ media for five accessions of *Bacopa monnieri*. a- BM003, b- BM001, c- BM002, d- BM005, e- BM004.

4. Conclusion

Through this protocol we conclude that in *Bacopa monnieri* a plantlet can be regenerated *in vitro* through a single leaf raised in MS/B₅ media without addition of any additional plant growth regulator and amongst the two media tested MS media was found to be better for maximum shoot and leaf multiplication. Among all the five accessions studied it

was found that maximum yield was obtained for accession BM003 followed by BM001>BM002>BM005>BM004. This study provides an insight into the fact that using cost effective alternatives in tissue culture, we can propagate and conserve endangered and elite plant species. Future efforts are in progress to evaluate the phytochemical ability of the regenerants and determination of their bacoside content.

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