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# Development of a profused *In vitro* shoot multiplication using leaf explants of *Bacopa monnieri* (L.) Pennell

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## ABSTRACT

*Bacopa monnieri* (L.) Pennell is an important medicinal plant used for the preparation of medhyarasayan (rasayana). Leaf explants of field grown young plants of *B. monnieri* was used to establish an efficient regeneration protocol with cytokinin (BAP) and auxin (IAA). The highest multiplication, i.e. (220 shoots/leaf, a cumulative of 2200 shoots from 10 explants) were noticed after 45 days of culture in MS medium supplemented with BAP(1.5mg/L) and IAA(0.5mg/L). The optimum concentration of growth regulator for shoot elongation and rooting was recorded in MS+GA<sub>3</sub> (0.25mg/L) and MS+IBA (1.5mg/L) respectively. The rooted plantlets were successfully established in green house conditions.

**KEYWORDS:** Brahmi, Leaf, Medhyarasayan, Multiple shoots

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## INTRODUCTION

*Bacopa monnieri* (L.) Pennell belongs to the family Scrophulariaceae, in India it is popularly known as 'Brahmi' and is used in Ayurveda for the preparation of medhyarasayan a drug used to improve intelligence and memory. It is the second most important medicinal plant among the list of the most important Indian medicinal plants assessed on the basis of their medicinal importance, commercial value and potential for future research and development [1]. It has a great commercial market value due to its high medicinal importance and shows a majority of pharmacological activities viz., antipyretic, antiinflammatory, analgesic, asthma, epilepsy, insanity, anticancer, antioxidant activities, blood clearing, hoarseness, memory enhancement, water retention etc., have been documented by many researchers [2,3,4]. It contains different types of terpenoid secondary metabolites i.e. bacosides A, B, C and D which are popularly known as "memory enhancers" [5,6]. It has been enlisted among 178 species of medicinal plants of India with high trade requirement ( $\geq 100$  million tonnes/year). Unfortunately, the requirement of this plant material is fulfilled mainly by collection from the wastelands [7]. Due to the unsustainable collection of raw material from natural habitats it is already placed under threatened category [8]. The propagation of *B. monnieri* is generally through seeds and vegetative cuttings, but both the routes are not suitable for pilot scale production of *Bacopa* biomass due to its seeds have short viability and shows poor germination [9,10]. On

the other hand the vegetative propagation through stem cuttings is a very slow process and depends on environmental, seasonal changes [11,12]. Hence, the conventional method of propagation is inadequate to meet the demand of raw material of *B. monnieri*. By over viewing the present status of drastic depletion of *B. monnieri* many laboratories are being given special attention to adopt micropropagation systems for rapid multiplication of this threatened medicinal plant species. The application of plant tissue culture in ex-situ conservation of medicinal plants is emphasized in many reports [13-16]. Keeping in mind the present status and pharmaceutical importance of the *Bacopa*, the present study was aimed to develop a rapid and efficient *in vitro* regeneration protocol from leaf explants of *Bacopa*, further, which can be useful in conservation and propagation of elite plants for commercial exploitation of this important medicinal plant.

## MATERIALS AND METHODS

### Explant Preparation

The leaf explants of *B. monnieri* were collected from the Botany Experimental Farm, Andhra University, Visakhapatnam, Andhra Pradesh, India. Then was surface sterilized with 0.1% HgCl<sub>2</sub> and repeatedly washed in sterile distilled water and were transferred on to the various culture media for *in vitro* response.

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## Micropropagation

The basal MS medium [17] was used with different concentrations of cytokinin BAP (6-benzyl aminopurine) and auxin IAA (indole-3-acetic acid) and subculture at every 15 days on the same media. The number of shoot buds were recorded after third subculture, then the shoot buds was elongated on MS media with various levels of GA<sub>3</sub> (0.10-0.30mg/L) after two weeks of culture. To test their rooting capacity, the *in vitro* elongated shoots were excised and transferred on to MS media supplemented with various concentrations of IBA (0.5-2.5mg/L). The efficiency of rhizogenesis i.e., frequency of rooting (%), root length (cm) per shoot and number of roots per shoot were recorded after two weeks of culture. Each experiment consisted of 10 replicates.

## *In vitro* Conditions

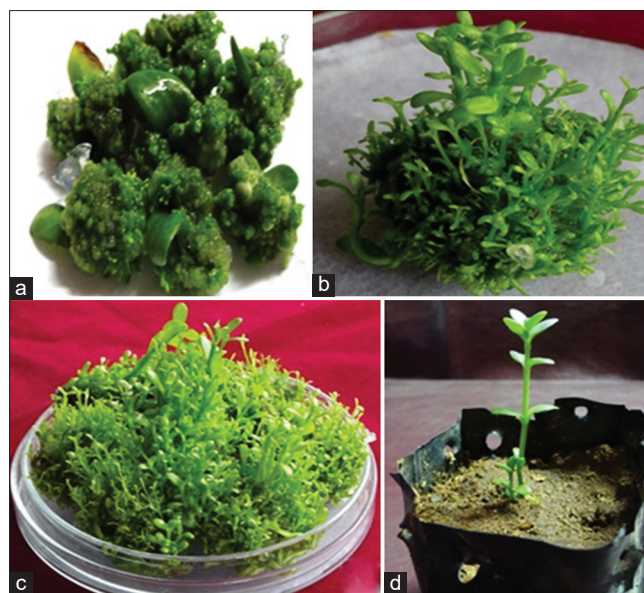
All media were supplemented with 3% (W/V) sucrose and 0.8% agar, the pH of the media was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving. The cultures were maintained at temperature 25 ± 2°C in the culture room with a 16 hours photoperiod under an illumination of 20 m mol m<sup>-2</sup>s<sup>-1</sup> photosynthetic photon flux density, provided by cool-white fluorescent light.

## Acclimatization

The rooted plants were removed from the culture medium, washed in running tap water to remove the remains of agar and transferred to plastic pots containing sterilized vermiculate. They were hardened for four weeks at temperature 25 ± 2°C under low humidity and transplanted to clay pot containing sand and soil (1:1) and were maintained in green house conditions.

## RESULTS AND DISCUSSION

*In vitro* propagation has been advocated as one of the most viable biotechnological tools for ex-situ conservation of rare, endangered and medicinally important plant species for future perspectives [18-20]. In the present study, we established an efficient regeneration system in leaf explants of *B. monnieri*. The leaf explants has started growth and producing multiple shoot initiation directly from the cut ends after two weeks of culture on MS medium supplemented with various levels of cytokinin BAP (0.5-3.0mg/L) in combination with auxin IAA (0.5mg/L or 1.0mg/L). The results revealed that all the tested media were effective in inducing direct regeneration. Hence, all the respondent leaf cultures were maintained in the same media for three consecutive subcultures. Produced a total of 2200 shoots per ten leaf explants and a mean of 220.16 ± 0.18 shoots per leaf explants with 100 per cent shooting was recorded in the medium MS+BAP (1.5mg/L)+IAA(0.5mg/L) while, 200 shoots per ten explants and an average of 28.12 ± 0.15 shoots per explant was noticed in the medium MS+BAP (3.0mg/L)+IAA(1.0mg/L) after 45 days of culture (Table 1 and Fig. 1). To my knowledge, i have established the protocol for rapid and superior production of multiple shoots to reproduce *B. monnieri* using leaf explants



**Figure 1:** *In vitro* regeneration of *Bacopa monnieri* using leaf explants: a) leaf explants showing multiple shoot initiation; b) Proliferation of multiple shoots; c) profuse multiple shoot clusters developed after 45 days of culture; d) acclimatized plant

**Table 1:** Multiple shoot induction from leaf explants of *Bacopa monnieri* on different levels of BAP and IAA with MS medium

S. No.	BAP(mg/L)	IAA(mg/L)	Response	
			% of Shooting*	Shoot no./Leaf*
1.	0.0	0.0	00.00±0.00	00.00±0.00
2.	0.5	0.5	76.80±0.19	98.00±0.14
3.	1.0	0.5	88.50±0.12	148.12±0.20
4.	1.5	0.5	100.00±0.00	220.16±0.18
5.	2.0	0.5	80.30±0.20	165.13±0.11
6.	2.5	0.5	75.80±0.20	85.18±0.09
7.	3.0	0.5	66.50±0.10	48.20±0.16
8.	0.5	1.0	28.20±0.22	30.14±0.23
9.	1.0	1.0	52.50±0.15	48.19±0.17
10.	1.5	1.0	68.10±0.23	75.14±0.10
11.	2.0	1.0	62.40±0.18	92.21±0.22
12.	2.5	1.0	55.30±0.25	86.12±0.18
13.	3.0	1.0	38.80±0.24	28.12±0.15

\*Mean±SE of 10 replicates

over the earlier reports on *Bacopa* leaf explants, they reported the shoot number ranging from 3.4 to 138.9 per explant [21-32]. An overview of all the results in the present study, noticed that maximum induction of multiple shoots took place on MS medium fortified with various concentrations of BAP with low concentration of IAA. Similar findings also observed by a few researchers [33-36]. This may be due to the synergistic effect of cytokinin (BAP) and auxin (IAA) has been demonstrated in many medicinal plants, for example *Santolina canescens* [37], *Bupleurum fruticosum* [38] and turmeric [39] who noticed that the low concentrations of an auxin in combination with a cytokinin influence the frequency of shoot induction and growth effectively on the other hand the high concentration of cytokinin induced the formation of stunted shoots has been observed [40,41]. The microshoots with an average length at 1.0-1.5cm were excised from the multiple shoot cluster

**Table 2:** *In vitro* shoot elongation of *Bacopa monnieri* on various concentrations of GA<sub>3</sub> with MS medium

S.No.	GA <sub>3</sub> (mg/L)	Shoot length (cm)*
1.	0.0	2.0±0.08
2.	0.10	2.8±0.14
3.	0.15	3.0±0.09
4.	0.20	5.2±0.18
5.	0.25	8.3±0.15
6.	0.30	4.5±0.20

\*Mean±SE of 10 replicates

**Table 3:** *In vitro* rooting of *Bacopa monnieri* on different concentrations of auxin IBA with MS medium

S.No.	IBA (mg/L)	Response		
		Root induction (%)*	Root no. /shoot*	Root length (cm)*
01.	0.0	00.0±0.00	00.0±0.00	0.0±0.00
02.	0.5	65.10±0.13	10.20±0.11	6.20±0.09
03.	1.0	77.30±0.18	12.18±0.14	8.00±0.17
04.	1.5	100.00±0.00	15.22±0.10	9.50±0.09
05.	2.0	69.20±0.15	9.20±0.21	7.10±0.18
06.	2.5	58.24±0.22	6.40±0.18	5.20±0.22

\*Mean±SE of 10 replicates

and transferred individually on MS medium fortifying with different levels of GA<sub>3</sub> (0.1 to 0.3mg/L) for elongation (Table 2). The higher shoot elongation (i.e., 8.3±0.15cm per shoot) was noticed on MS medium containing GA<sub>3</sub> (0.25mg/L). This may be due to the cell elongation roll of GA<sub>3</sub> [42]. The elongated shoots raised *in vitro* failed to develop roots in MS basal medium. However, root initiation was achieved from the bases of excised shoots in the presence of various concentrations of IBA two weeks of transfer. Rooting response have been varied with the concentrations of IBA used (Table 3). Among these concentrations of IBA, MS medium containing IBA (1.5mg/L) produced maximum response i.e. rooting frequency (100%), root number (15.22±0.10 per shoot) and root length (9.50±0.09cm per shoot). These results are agreed with the previous findings of Jain et al. [43]. Rooted plants were transplanted to green house condition after hardening showed 96% of survival. No detectable variations i.e., (growth and morphological characteristics) weren't observed in the acclimatized plants.

## CONCLUSIONS

The output of the research meets the objectives of the present study and reports a rapid and efficient multiple shoot regeneration of leaf explants of *B. monnieri* with cytokinin (BAP) and auxin (IAA). Further, the results of the study can be helpful in future production of true to the type clonally multiplied plants for pilot scale pharmacological use.

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