

DIRECT AXILLARY SHOOT REGENERATION WITH NODAL EXPLANTS OF *Bacopa monnieri* (L.) Pennell – A MULTI MEDICINAL HERB




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ABSTRACT. The aim of the present investigation is to develop an optimized culture medium for direct induction of adventitious multiple shoots from nodal explants of *Bacopa monnieri* an important medicinal herb. The nodal explants were excised from the *in vivo* *B. monnieri* and were inoculated over different media fortified with cytokinins BAP or KIN alone or in combination with IAA for *in vitro* response. The rooting was established with Murashige and Skoog media containing with auxin NAA. All the treatments are performed in a controlled aseptic chamber and maintained the cultures at temperature 22°C for 4 and 2 weeks respectively for shoot induction and rhizogenesis. The results are expressed in mean of three replicates with standard error. Adventitious multiple shoots proliferated from nodal explants cultured on all the tested media. The highest per cent shoot induction, shoot number per node and shoot length was observed on MS medium containing BAP(1.5mg/l) and IAA(0.5mg/l). The excised shootlets cultured on MS media fortified with various levels of NAA and identified 0.2mg/l NAA was best for maximum rhizogenesis. The rooted plants were successfully transferred to the field. Overall, the present study showed superior results for shoot number per node over previous reports. Therefore, it is hoped that this report will contribute to our knowledge about *in vitro* mass propagation of medicinal herb *B. monnieri*.

Keywords: BAP, Brahmi, KIN, IAA, NAA, Adventitious shoots

INTRODUCTION

The recent estimates of the World Health Organization, more than 3.5 billion people in the developing world rely on plants as components of their primary health care. The demands of the majority of the people in developing countries for medicinal plants have been met by indiscriminate harvesting of the spontaneous flora from the natural habitats and also rapid urbanization and industrialization limits the valuable medicinal plant flora. As a result, many plant species have become extinct and some are endangered. Therefore a systematic cultivation of medicinal plants is introduced in order to conserve biodiversity and protect rare, endangered and threatened plant species. *Bacopa monnieri* (L.) Pennell is an endangered multi-medicinal plant 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. The plant is distributed throughout the country having its habitat in wet, damp and marshy places. It is used for the treatment of various ailments, such as antipyretic, antiinflammatory, analgesic, asthma, epilepsy, insanity, anticancer, antioxidant activities, blood clearing, hoarseness, memory enhancement, water retention [1, 2, 3, 4, 5, 6]. It consists valuable pharmacological chemical compounds such as Alkaloids (Nicotine, Brahmine and

Herpentine); Terpenoids (Tetracyclic terpenoids (bacoside A, B, C and D); Stigmasterol; β -Sitosterol); Flavonoids (Luteolin, Apigenin; Quercetin) and Phenolic compounds (Tannins) [7, 8, 9, 10]. Currently *B. monnieri* plant under extinction risk due to the unsustainable collection of raw material from natural habitats hence, it is already placed under threatened category [11]. *In situ* conservation of plants may not be sufficient to protect them since its seeds have short viability and shows poor germination on the other hand, vegetative propagation through stem cuttings is a very slow process and depends on environmental, seasonal changes [12, 13, 14].

In vitro plant regeneration techniques have been established as a useful concept for *ex situ* conservation of rare, endangered and threatened plant species [15]. On the basis of its vast medicinal importance and present status of this herb an attempt was made to develop an efficient plant regeneration protocol for *in vitro* culture using nodal explants.

MATERIALS AND METHODS

Explant preparation

The nodal explants of *Bacopa monnieri* were collected from the Experimental Farm of Botany Department, Andhra University, Visakhapatnam, Andhra Pradesh, India. Then were surface sterilized with 0.1% HgCl_2 for 3 minutes followed by rinsing with sterile distilled water for 3 to 4 times. The surface sterilized explants were inoculated on various culture media for *in vitro* response.

Micropropagation

The basal MS medium [16] supplemented with different concentrations and combinations of 6-benzyl aminopurine (BAP; 0.5, 1.0, 1.5, 2.0 and 2.5mg/l), Kinetin (KIN; 0.5, 1.0, 1.5, 2.0 and 2.5mg/l), Indole-3-acetic acid (0.5mg/l) combined with BAP or KIN concentrations i.e. (0.5, 1.0, 1.5, 2.0 and 2.5mg/l) were tested for bud break and proliferation of multiple shoot induction and subcultured at every 15 days on the same media. The number of shoot buds and their length (cm) were recorded after third subculture. To test their rooting capacity, the shoot raised *in vitro* were excised and transferred on to MS media fortified with various concentrations of Naphthaleneacetic acid (NAA; 0.1; 0.2; 0.3 and 0.4mg/L). The efficiency of rhizogenesis i.e., frequency of rooting (%), number of roots per shoot and root length (cm) were recorded after 2 weeks of culture.

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 $22\pm 2^\circ\text{C}$ in the culture room with a 16 hour photoperiod under an illumination of $20\text{m mol m}^{-2}\text{s}^{-1}$ 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 and transplanted to clay pot containing sand and soil (1:1) and were maintained in green house conditions.

Statistical Analysis

All the experiments were repeated for 3 times and the data were expressed as Mean \pm Standard error using minitab statistical software.

RESULTS AND DISCUSSION

The present investigation was carried out to induce adventitious multiple shoots via direct organogenesis in nodal explants of *B. monnieri* using cytokinins (BAP and KIN) alone or in combination with IAA. The effect of different plant growth regulator combinations on per cent shoots regeneration, number of shoots per node and shoot length is presented in Table 1. Swelling and bud break followed by formation of multiple shoots and simultaneous shoot elongation from nodal explants in all the hormone treatments were noticed in 45 days of culture. In the present study, shoot multiplication and subsequent elongation of shoots was achieved in the same media and is a beneficial one since it eliminates the micro shoot elongation and also shortens the micropropagation duration. However, in many plant species, plant regeneration requires different media for propagation and shoot elongation which makes the micropropagation procedures very cumbersome and also uneconomical [17].

The result showed that the nodal explants cultured on MS basal medium without growth regulators failed to induce shoot proliferation. Among the plant growth regulators the effect of BAP alone or in combination with IAA was significantly superior over KIN alone or in a recipe with IAA for per cent shoot induction, shoot number per explant and shoot length. This was in agreement with the earlier reports of Balaraju *et al.* [18], Mehta *et al.* [19], Molsaghi *et al.* [20] in the medicinal plants *Vitex agnus-castu*, *B. monnieri* and *Aloe vera* respectively. Of the various concentrations of BAP tested 1.5mg/l proved to be potential for *in vitro* response and produced 78.2 shoots per explant with 70 per cent regenerated shoots and exhibited 5.3(cm) shoot length. While, MS media fortified with various levels of KIN tested 1.5mg/l gave good response, i.e., 65 per cent (regenerated shoots), 58 shoots per node and 5(cm) shoot length after 45 days of culture however, further increase in concentration more than 1.5mg/l of BAP or KIN induced stunted shoots. On the other hand application of BAP in combination with IAA or KIN blend with IAA resulted in high-frequency shoot regeneration and respectable shoot length. This might be due to the synergetic activity of cytokinin with auxin which leads to rapid cell division, forming a large number of relatively small and undifferentiated cells [21, 22]. Among the various combined media tested the MS+BAP(1.5mg/l)+IAA(0.5mg/l) was observed significantly superior over other media in terms of per cent shoot induction (95), shoot number per node (168) and shoot length (5.9cm) Fig. 1.

The shoots raised *in vitro* failed to develop roots in the basal MS medium, hence, the root initiation was achieved from the bases of excised shoots in the presence of auxin NAA after two weeks of culture. The per cent root induction, number of roots per shoot and root length were varied with the concentration of NAA (Table 2). Among the auxin concentrations tested, NAA (0.2mg/l) produced maximum rhizogenesis i.e., per cent root induction (100), root number per shoot (14.5) and root length (10.2 cm). These results agree with the previous findings of Zote *et al.* [23] with slight variations. As of now the current results show that 168 shoots per node, which can be considered as effective regeneration in comparison with earlier reports around the globe on *B. monnieri* nodal explants (presented in chronological order for no. of shoots/node) viz., 4.98 shoots [19],

5.75 shoots [24], 9.4 shoots [25], 17 shoots [26], 18 shoots [27], 18.35 shoots [28], 18.4 shoots [29], 18.7 shoots [30], 18.8 shoots [31], 20 shoots [32], 23 shoots [33], 25 shoots [34], 41 shoots [35], 42 shoots [36], 42.5 shoots [37], 43 shoots [38], 56 shoots [39], 90.7 shoots [40], 110 shoots [41] and 114.2 shoots [42]. Hence the obtained results are recommended for pharmaceutical laboratories to meet the adequate amount of raw material for isolation and characterization of herbal drugs available in *B. monnieri*. The well developed shoots with roots were transferred to pots containing sterile vermiculate and established in greenhouse conditions. The acclimatized plantlets showed 95% survival rate and didn't show any detectable variation in their growth and morphological characteristics, when compared to *in vivo* plants.



Fig.1. *In vitro* direct adventitious multiple shoot induction in *Bacopa monnieri*.
(a) Nodal explants showing bud break and micro shoot initials, (b) Clumped shoots,
(c) Profusely induced shoots with elongation, (d) Rooting of regenerated shoot,
(e) Hardened plant, (f) Biomass of Transplanted Plant.

Table 1. Morphogenetic response of nodal explants of *B. monnieri* at various concentrations of Cytokinin (BAP, KIN) and IAA

S.No.	Media	<i>In vitro</i> response		
		(%) Shoot regeneration	No. of shoots/node	Shoot length(cm)
1.	MS+BAP(0.5mg/l)	55.00	39.70±0.16	3.40±0.11
2.	MS+BAP(1.0mg/l)	63.00	52.50±0.22	4.80±0.27
3.	MS+BAP(1.5mg/l)	70.00	78.20±0.19	5.30±0.15
4.	MS+BAP(2.0mg/l)	59.00	61.60±0.26	3.80±0.30
5.	MS+BAP(2.5mg/l)	50.00	54.50±0.18	2.10±0.22
6.	MS+KIN(0.5mg/l)	48.00	33.50±0.20	3.20±0.10
8.	MS+KIN(1.0mg/l)	53.00	46.30±0.14	4.10±0.18
9.	MS+KIN(1.5mg/l)	65.00	58.00±0.25	5.00±0.09
10.	MS+KIN(2.0mg/l)	52.00	41.90±0.21	2.60±0.22
11.	MS+KIN(2.5mg/l)	45.00	32.60±0.24	1.70±0.19
12.	MS+BAP(0.5mg/l)+IAA(0.5mg/l)	68.00	84.40±0.30	4.90±0.13
13.	MS+BAP(1.0mg/l)+IAA(0.5mg/l)	80.00	110.30±0.14	5.40±0.20
14.	MS+BAP(1.5mg/l)+IAA(0.5mg/l)	95.00	168.00±0.23	5.90±0.30
15.	MS+BAP(2.0mg/l)+IAA(0.5mg/l)	76.00	106.20±0.33	4.20±0.24
16.	MS+BAP(2.5mg/l)+IAA(0.5mg/l)	64.00	77.30±0.22	3.60±0.17
17.	MS+KIN(0.5mg/l)+IAA(0.5mg/l)	56.00	60.60±0.28	4.80±0.10
18.	MS+KIN(1.0mg/l)+IAA(0.5mg/l)	67.00	93.50±0.15	5.10±0.20
19.	MS+KIN(1.5mg/l)+IAA(0.5mg/l)	80.00	126.50±0.23	5.25±0.28
20.	MS+KIN(2.0mg/l)+IAA(0.5mg/l)	62.00	81.60±0.19	2.80±0.16
21.	MS+KIN(2.5mg/l)+IAA(0.5mg/l)	50.00	64.30±0.10	1.90±0.25

Table 2. Effect of NAA on root induction from *in vitro* regenerated shoots of *B. monnieri*.

S.No.	Media	Rhizogenesis		
		(%)Root induction	Root no. /shoot	Root length (cm)
1.	MS+NAA(0.1mg/l)	100	9.80±0.20	5.60±0.26
2.	MS+NAA(0.2mg/l)	100	14.50±0.16	10.20±0.16
3.	MS+NAA(0.3mg/l)	100	12.70±0.19	7.80±0.17
4.	MS+NAA(0.4mg/l)	100	11.60±0.22	6.90±0.23

CONCLUSION

The protocol standardized through this study demonstrated the possibility of mass propagation of the medicinal plant *B. monnieri* using nodal explants. The present study

also highlights different concentration of cytokinins (BAP and KIN) alone or in combination with IAA on adventitious multiple shoots proliferation.

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