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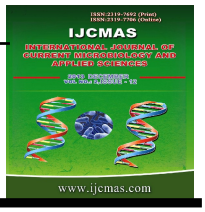

Original Research Article

In- vitro regeneration of *Bacopa monnieri* (L.): A highly valuable medicinal plant

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A B S T R A C T

Keywords

Bacopa monnieri (L.) Pennell; Medicinal plants; *in-vitro* propagation; Conservation; MS medium; plant growth regulators.

Bacopa monnieri is a highly vulnerable medicinal plant from the Scorphulariaceae family. It is used as medicinal purpose and main constituent in different pharmaceutical products, but it lost from their natural habitats due to indiscriminate collection for pay pharmaceutical demands. Here we described an efficient protocol for the *in-vitro* propagation of *Bacopa monnieri* (L.). Shoot initiation was induced by culturing nodal and inter-nodal explants on MS medium containing 3% (w/v) sucrose, 0.8% (w/v) agar with different combination and concentrations of plant growth regulators. The greatest percentage of shoot multiplication achieved when explants were cultured on MS medium supplemented with 1.0 (mg/l) BAP and 0.5 (mg/l) NAA with 85% of response. The micro-shoots were separated from the multiple shoots and sub-cultured on the rooting medium. The *in –vitro* grown plantlets were successfully established in the field with 70-80% survival. This protocol could be utilized for conservation and mass multiplication of this economically important and commercially exploited plant.

Introduction

India has great diversity of medicinal plants. These medicinal plants are used in Ayurvedic, Unani medicines and a number of pharmaceutical products. *Bacopa monnieri* (L.) is one of them. It belongs to the family Scorphulariaceae commonly known as 'Brahmi' or Nirbrahmi has originated from India. It is a genus of spreading herbs, commonly growing in damp and marshy places throughout India, ascending up to an altitude of 1320 m. It is a small creeping, glabrous, succulent, herb rooting at nodes. It is an ancient and renowned medicinal plant with a legendary reputation as memory vitalizer (Anonymous, 1998). There are many chemical components present in *Bacopa monnieri* like alkaloids (herpestine, nicotine and brahmine), saponins, sterols, flavonoids, glycosides, betulic acid and phytosterols (Jain & Kulshreshtha, 1993). It possesses anti-inflammatory, immunomodulatory, anti-oxidant, anti-cancer and anti-pyretic activity due to

many active chemical components (Satyavati *et al*., 1976; Jain *et al*., 1994; Elangovan *et al*., 1995; Tripathi *et al*., 1996; Vohora *et al*., 1997). It is used to treat asthma, insanity, epilepsy, and hoarseness, enlargement of spleen, snake bite, rheumatism, leprosy, eczema and ringworm. It is also used as a diuretic, appetitive and a cardio tonic (Basu and Walia, 1994).

B. monnieri placed second in a priority list of most important Indian medicinal plants on the basis of its medicinal properties, commercial value and potential for further research and development (Mohapatra and Rath, 2005; Sharma *et al*., 2007). It is important to conserve this medicinal plant through different techniques. Plant tissue culture remains one of the most basic biotechnological techniques with its varied and vast applications. The rapidity of multiplication of true-to-type plants and efficient transplantation of *B. monnieri* can be useful in conservation and propagation of elite plants for commercial exploitation.

Protocols for *in vitro* clonal propagation and conservation have also been conducted in *B. monnieri* by several workers (Shrivastava and Rajani, 1999; Tiwari *et al*., 2000; Tejavathi *et al*., 2001; Binita *et al*., 2005; Sharma *et al*., 2007; Banerjee and Srivastava, 2008; George *et al*., 2004 and Joshi *et al*., 2010). Thakur *et al.,* observed the role of benzyl amino purine on shoot bud regeneration from single epidermal cells of "Brahmi". The same group later reported the development of abnormal plantlets with alternate phyllotaxy, instead of opposite arrangement (Thakur *et. al.,* 1976). Here, we report a micropropagation protocol of *Bacopa monnieri* using nodal and internodal parts of the stem as explants.

Materials and Methods

Source of explants

Plants of *Bacopa monnieri* were obtained from Sanjeevani plant nursery, Bhopal (M.P.). After collection of plants they were maintained in pots in the greenhouse of our institute. The different parts of the plant were used as explants for *in- vitro* regeneration.

Selection of explants

Initial experiments were conducted by using the nodal, inter-nodal, shoot tip and leaves of *Bacopa monnieri* as explants material.

Sterilization of explants

The leaves and stem of *Bacopa monnieri* were thoroughly washed with running tap water followed by washing with surfactant (Tween-20: 2-3 drops/100 ml water). They were subsequently washed with 0.01% mercuric chloride ($HgCl_2$) for 5 min., and repeatedly washed with sterilized water (4-5 times) and cultured on MS medium (Murashige and Skoog, 1962) with different plant growth regulators at pH 5.8 supplemented with necessary macro-nutrients, micro-nutrients, an iron source, vitamins, 3% sucrose and 0.8% (w/v) agar (Himedia, India) as a gelling agent.

Inoculation of explants

For initiation, various explants as describe above were inoculated on agar based semi-solid MS medium (Himedia, India) among the different explants only nodal explants responded. Nodal portion was inoculated on MS medium supplemented with different concentration of BAP (0.05-2.0 mg/l) alone. For shoot multiplication,

initiated shoots were transferred into shoot multiplication media with the combination of BAP (0.25-1.50 mg/l) and NAA (0.5 mg/l). The regenerated shoots were sub-cultured every three weeks in the same media composition. Experiments were also carried out to check the effect of different plant growth regulators. For rooting, 3-4 micro-shoots of 2-3 cm. were cultured on *in-vitro* rooting medium supplemented with IBA (0.10-0.30 mg/l) alone and record the response of plant every week.

The experiments were performed in replicates of ten for each type of explants and all experiments repeated three times. The growth responses of explants were recorded every week.

Hardening

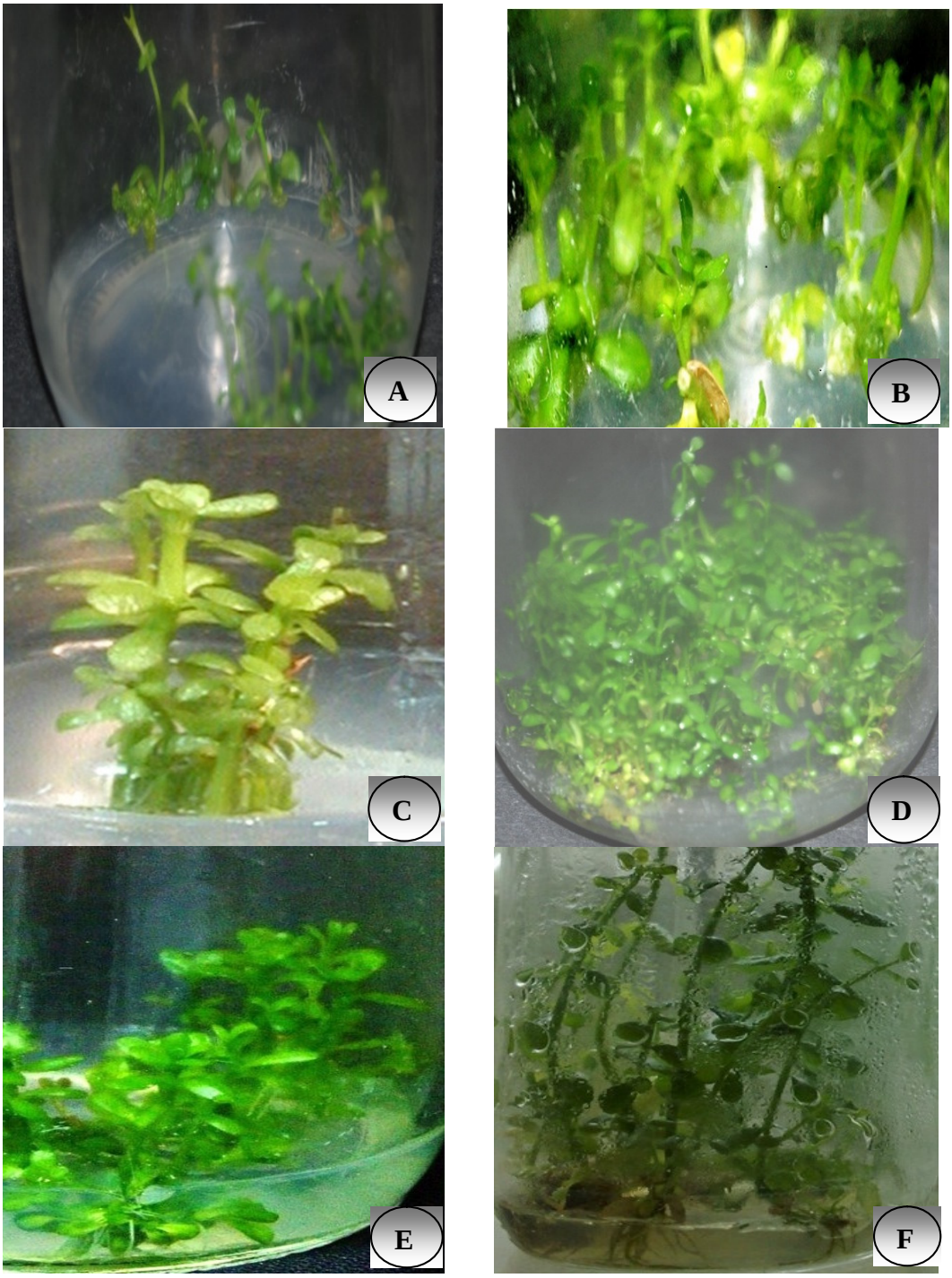
Rooted plantlets were removed from the medium, freed of agar by washing in running tap water and planted in sand: compost mixture (1:2) at about 80% relative humidity under the polyethylene bags in the greenhouse. The plantlets were hardened for 20 days and then transplanted in the field.

Results and Discussion

After 2-3 weeks of culture, explants showed the growth response in different culture medium. Different parts were used as explants material. But fast and good response was observed in nodal explants. These explants inoculated on MS medium containing different combination of growth hormones (Table-1). However, the ideal condition for shoot initiation was observed on the medium containing 0.50 mg/l BAP within 21 days of culture with an average shoot length of 2.6±0.31 with 87 % of response. When the initiated explants were implanted on shoot multiplication medium containing BAP with combination of NAA showed multiple shoots within two weeks of inoculation. Subculture was done after every four weeks. The best response of multiple shoots (42.5±0.76) are observed on MS medium supplemented with 1.0 BAP and 0.5 NAA with 85% of response and the average shoot length was 4.3±0.11 cm. (Table-2). When the explants were implanted on MS media for multiplication, maximum shoot length was found. These results are related with finding of other workers, who have also noted the effectiveness of MS medium for optimum shoot multiplication in different *Bacopa* species (Tejavanti *et al.,* 2001; Sharma, 2007; Banerjee and Shirivastava, 2008; George, 2004) and several workers have reported multiple shoot induction with cytokinins in the growth medium (Clog *et al*., 1990; Stamp *et al*., 1990) as we find in our investigation. Binita *et al.,* also have reported importance of auxin (0.2 mg/l IAA) and cytokinin (1.0 mg/l BA) for shoot multiplication in MS solidify and liquid media (Binita *et al.,* 2005).

In our experiment we use a propagule of 3-4 shoots with the length of 2-3 cm was cultured on MS medium supplemented with IBA. The best rooting response 86% was obtained on MS medium supplemented with 0.25 mg/l IBA with an average of 3.5±0.76 roots per shoot over a period of 4 weeks (Table-3), which is in agreement with the results reported by Torrey *et al.,* and Hu and Wang. The role of auxin in root development was established and reviewed by Torrey (1976). Hu and Wang reported that there is enough residual cytokinin present in

Figure.1 Photographic representation of *Bacopa monnieri* (L.)



(A, B): Shoot initiation in MS solidify medium (C, D): Shoot multiplication in MS solidify medium (E, F): Root formation in MS solidify medium

Table.1 Effect of cytokinins (BAP) on multiple shoot induction in MS medium from nodal explants after 21 days.

Treatments	MS medium+PGR (mg/l)	Shoot initiation response (%)	No. of shoot initiation per explants	Average shoot length (cm)
I_1	MS+0.05 BAP	48	1.2±0.11	1.5±0.51
I_2	MS+0.25 BAP	72	1.3±1.11	2.0±0.33
I_3	**MS+0.50 BAP**	**87**	**3.4±0.16**	**2.6±0.31**
I_4	MS+1.00 BAP	82	3.1±2.33	2.5±0.19
I_5	MS+2.00 BAP	68	2.3±0.81	1.9±1.31

Table.2 Effect of auxin (NAA) and cytokinin (BAP) on shoot multiplication in MS medium after 28 days.

Treatments	MS medium+PGR (mg/l)	Shoot multiplication response (%)	No. of shoots per culture explant	Average shoot length (cm)
M_1	MS+0.25 BAP+0.5 NAA	67	21.3±1.15	2.5±1.15
M_2	MS+0.5 BAP+0.5 NAA	72	25.3±1.52	2.3±0.16
M_3	**MS+1.0 BAP+0.5 NAA**	**85**	**42.5±0.76**	**4.3±0.11**
M_4	MS+1.25 BAP+0.5 NAA	78	35.2±1.85	3.4±0.19
M_5	MS+1.50 BAP+0.5 NAA	75	30.3±0.99	3.2±0.16

Table.3 Effect of auxin (IBA) on root induction in MS medium after 21 days.

Treatments	MS medium+PGR (mg/l)	Root induction response (%)	No. of roots per culture	Morphology of roots
R_1	MS+0.10 IBA	57	2.3±0.57	Thin, short
R_2	MS+0.15IBA	70	2.8±1.79	Thin, short
R_3	MS+0.20 IBA	82	3.1±0.61	Thin, short
R_4	**MS+0.25 IBA**	**86**	**3.5±0.76**	**Thin, long**
R_5	MS+0.30 IBA	75	2.5±0.88	Thin, long

Figure.2 Graphical representation of effect of cytokinins (BAP) on multiple shoot induction in MS medium from nodal explants after 21 days

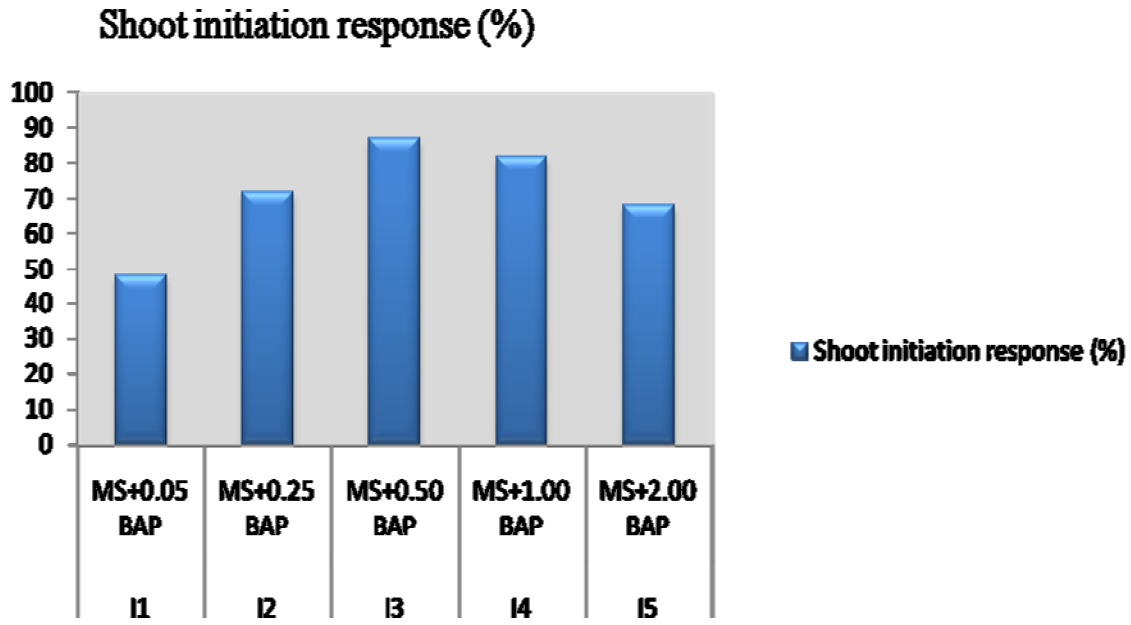


Figure.3 Graphical representation of effect of auxin (NAA) and cytokinin (BAP) on shoot multiplication in MS medium after 28 days.

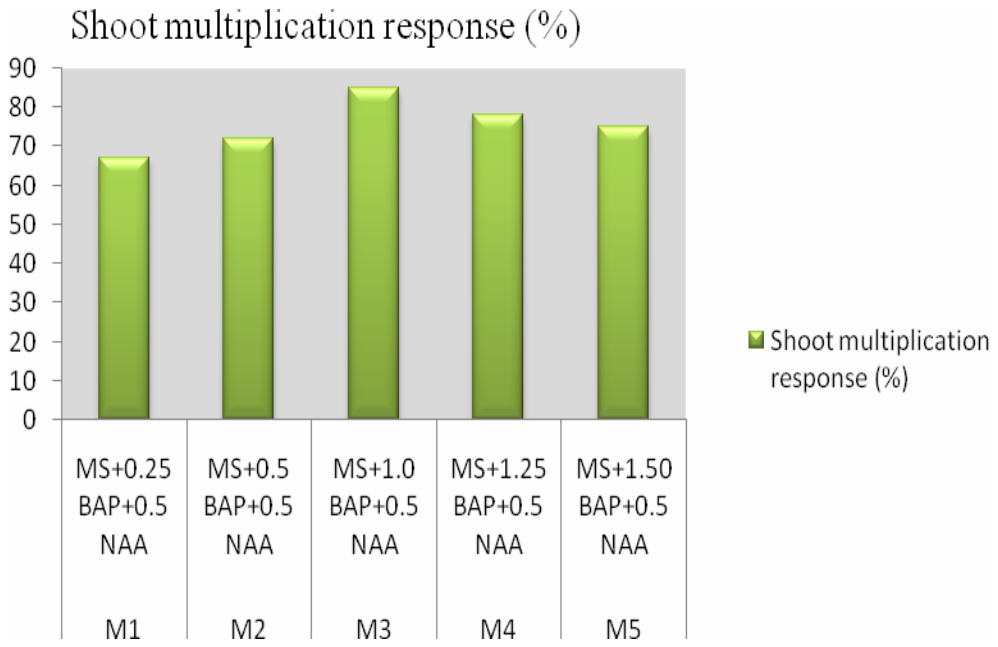
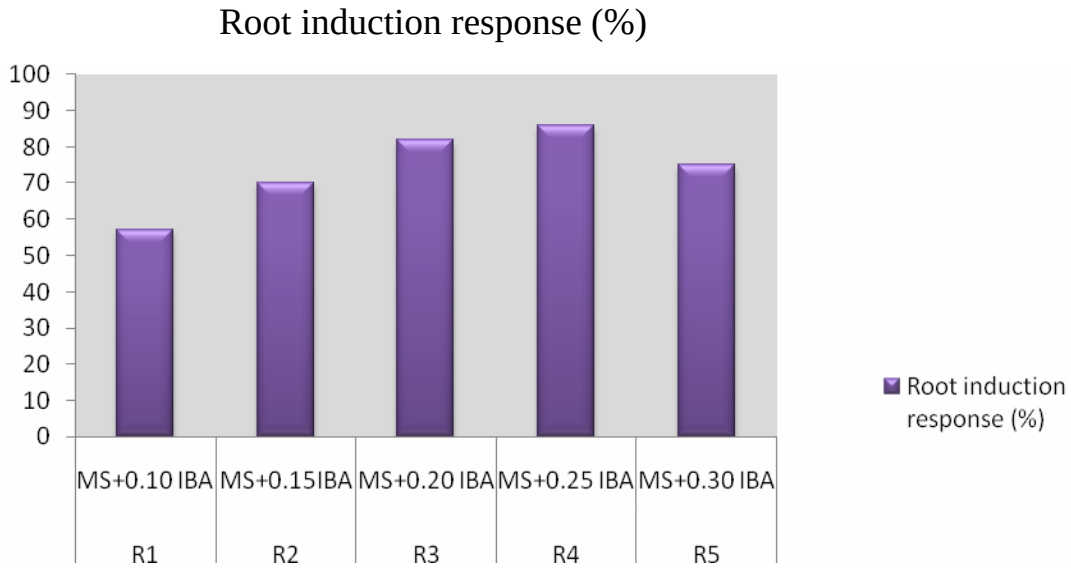


Figure.4 Graphical representation of effect of auxin (IBA) on root induction in MS medium after 21days.



shoots therefore, little or no cytokinin is required in rooting medium (Hu and Wang, 1983).

Rooted plantlets were transplanted *ex-vitro* and keeps in pots under greenhouse conditions for one month followed by their field transfer, approximate 70-80% plantlets survived. By the protocol describe here we can obtain 5-6 rooted plants from one explants in 90 days (in three stage growth) in rapidity of multiplication of true-to-type plants and efficient transplantation of *Bacopa monnieri* can be useful in conservation and propagation of elite plants for commercial exploitation.

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