Indian Journal of Experimental Biology
Vol. 43, April 2005, pp. 373-376

In vitro studies of *Bacopa monnieri*—An important medicinal plant with reference to its biochemical variations

H P Mohapatra & S P Rath*

Post Graduate Department of Botany, Utkal University, Vanivihar, Bhubaneswar 751 004, India

Received 6 April 2004; revised 22 November 2004

Micropropagation of *Bacopa monnieri* was achieved on MS and B5 medium supplemented with BAP and NAA using leaf explants and nodal segments. Best results were found on MS medium in both the explants with BAP (2.0 mg/l) showing higher percentage of regeneration. Besides that the biochemical parameters, like chlorophyll, carbohydrate, protein, of leaves both *in vivo* and *in vitro* have also been carried out in order to establish the sustainability of plants.

Keywords : *Bacopa monnieri*, Medicinal plant, Micropropagation, Multiple shoots.

Bacopa monnieri (L.) Wettst. syn. *Herpestis monnieri* (L.) H.B. and K. is an important medicinal plant, commonly known as brahmi. It is being used as a memory vitalizer in the indigenous systems of medicine. The plant is distributed throughout the country having its habitat in wet, damp and marshy places. The juice of brahmi leaves mixed in milk improves memory and adds to mental ability[1]. Brahmine is one of the major ingredients which helps for preparation of large number of Ayurvedic hair and message oils. The Ayurvedic practitioners also use this plant in nervous and urine disorders, convulsions, mental problems and in treatment of asthma[1]. The clinical data proved that it has an antianxiety with adaptogenic property[2]. The plant is an important constituent of Brahmighrita, a medicated ghee used in epilepsy, insanity, asthenia and other low dynamic conditions[1]. An ayurvedic drug namely Mentat containing Brahmi has shown improvement in children with behavioural problems and patients with various types of epilepsy[3]. Recent study has also reported anticancer activity of *Bacopa monnieri* using Sarcoma-180 cell culture[4]. The medicinal properties of the herb are attributed to the plant as saponin bacoside-A which is present in all parts of the plant[5-7]. A new drug Memory plus[8] has been released in the market has a result of the expectation of the natural population of plant has become endangered. This overexploitation has a positive threat for the plant to the extent of being extinct.

Hence, an immediate need for accessing the natural population leading to a rapid multiplication of this important drug yielding plant has become imperative. The characteristics of rapid vegetative growth, available morphological variation and short sexual life cycles has led to the possibilities of using *Bacopa monnieri* in the developmental studies relating to bioprospection[9].

The present study has been initiated for developing a micropropagation protocol after accessing the response of different explants (node and leaf explants) and to find out a suitable media with/without supplementation of phytohormones. The technique would facilitate rapid multiplication of this drug yielding plant, besides different biochemical parameters like chlorophyll, carbohydrate, protein of leaves have also been worked out in order to establish the sustainability of plants.

Materials and Methods

Young shoots were defoliated and the nodal segments (5-6 nodes; 7-8 cm in length) were collected from the garden of the institute. The explants were washed thoroughly under running tap water with teepol (5%) for 30 min, surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min, followed by thorough rinse with sterile distilled water before implantation and the single node was dissected out (1 cm) and aseptically inoculated on the media. Murashige and Skoog[10] medium and B5 medium[11] were tried for rapid propagation of explants but MS medium gave the best result. MS medium (Hi media) supplemented with sucrose (30 g/l) and 0.8% (w/v) agar (Hi media, India) was used with different concentrations of phytohoromones *viz*, 6-benzy-

*correspondent author: E-mail: shibaprasad2001@yahoo.co.uk

laminopurine (BAP; 1.0 mg/l-5.0 mg/l), α-naphthalene acetic acid (NAA; 0.05 mg/l) singly or in combination. The *p*H of the medium was adjusted to 5.8 prior to the addition of agar, the gelling agent. The aliquots of the medium 30 ml were dispensed into Erlenmeyer conical flasks (Borosil, India), capped with aluminium foil and autoclaved at 121°C for 20 min. All the flasks containing medium were stored in culture room. All the cultures were maintained at 25°±2°C with 55±5% RH in a culture room provided with 16/8 hr light/dark period [light intensity of 2000 lux, provided by cool white fluorescent tubes (Philips, India)].

The growth response of different explants was studied at weekly intervals in terms of initiation and distribution of shoot and root regeneration. The individual grown shoots were transferred into the culture tubes containing the rooting medium as indole-3-butyric acid (IBA; 0.5-2.0 mg/l) and α-naphthalene acetic acid (NAA; 0.5-2.0 mg/l) and placed in an inclined manner.

Each experiment was conducted in 5 replicates and repeated 3 times. The value of mean standard deviation was recorded and the profuse regenerating shoot from nodal explants were coded arbitarily by naked eye and given in terms of percentage of response of explants. For quantitative phytochemical evaluation for cholorophyll, sugar[12], reducing sugar[13] and protein[14], methods used were described elsewhere.

Results and Discussion

The leaf explants of *Bacopa monnieri* was tried with MS and B5 media supplemented with BAP for direct regeneration of shoots and roots (Table 1) and it was observed that the response at 2.0 mg/l with MS gave the best result having 80.5% success after 15 days (Fig. 1A). After 4 weeks of culture, multiple shoots were formed (Fig. 1B-C). Higher percentage of

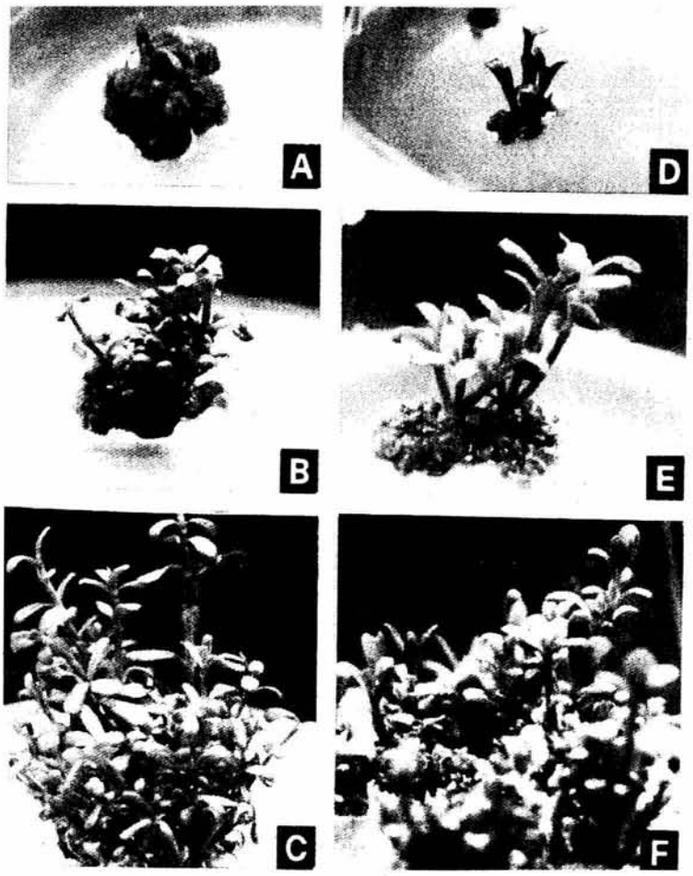

Fig. 1—Micropropagation of *Bacopa monnieri* (L.) Wettst. (A) Response of leaf explants on shoots initiation; (B) Development of multiple shoots from a single leaf; (C) Elongation and growth of multiple shoots after 30 days of culture; (D) shoots initiation from the nodal segments after 7 days; (E) Multiple shoots formed after 15 days; (F) Response of all nodal segments to multiple shoots formation

BAP proved to be inhibitory. The node explants were also directly exposed to MS and B5 media and trial was taken to that of leaf explants. The nodal explants also responded in a similar way however, the response was still higher at 2.0 mg/l BAP with 95% of success after 7 days of treatment (Table 2; Fig. 1D). The shoots became 4-5 cm long after 15 days of culture (Fig. 1E,F). IBA (0.5-2.0 mg/l) was used for promoting regeneration into roots from the base of shoots.

Several explants adopted to propagate plants *in vitro* and it was observed that the number of shoots that could regenerate roots were comparatively more prevalent in nodal explants than that of the leaf explants. Differentiation of abnormal plantlets from internode on Nitsch medium has been observed earlier with various levels of auxin and cytokinin[15], therefore, the same could not be tried in the present investigation, as there is no earlier report of plantlet regeneration.

In this connection, the statement of the authors[9] need to be reinvestigated as stated that, leaf and stem explants of *Bacopa monnieri* seems have self sufficiency of regeneration without addition of phytohormones to the growth medium. Further, they have

Table 1—Morphogenic response of cultured leaf (whole) explants of *Bacopa monnieri*
[Values are means of 5 replicates]

Plant growth regulators (mg/l)	Shooting response(%)		Mean number of shoots/explant	
BAP	MS	B5	MS	B5
0.0	47.8	—	2.5	—
1.0	52	30.2	3.2	2.2
2.0	80.5	35.1	4.3	1.8
3.0	61.6	—	3.0	—
5.0	4.1.1	—	1.5	—

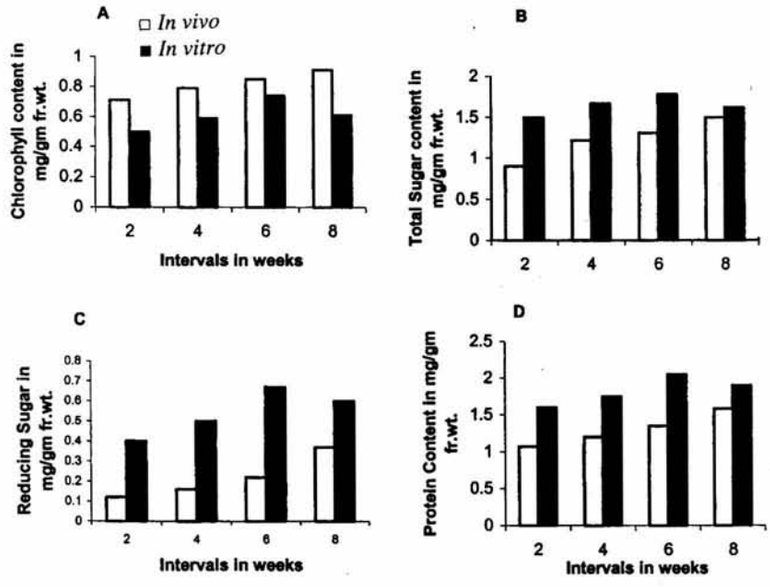

Fig. 2—Comparative studies of (A) chlorophyll; (B) sugar; (C) reducing sugar; D) protein between the *in vivo* and *in vitro* leaves of *Bacopa monnieri*

Table 2—Effect of growth regulators on multiple shoot formation from node explants of *Bacopa monnieri*
[Values are mean of 5 replicates]

Phytohormones		Shooting response (%)		Mean number of shoots/culture		Mean height of shoots in cm		Mean number of leaves/shoot	
BAP	NAA	MS	B5	MS	B5	MS	B5	MS	B5
0.0	0.0	60	—	3.5	—	0.5	—	2.5	—
1.0		65	—	4.6	—	0.84	—	4.2	—
2.0		95	30.5	9.4	2.1	2.06	0.5	8.2	1.2
3.0	0.05	55	25.0	3.0	2.0	0.48	0.3	3.4	1.0
5.0	0.05	30	—	2.0	—	0.3	—	2.0	—

stated that roots and shoots are found to regenerate from all types of explants derived from leaf and stems with varying frequency. The present study contradicted earlier views, as it was observed that supplementation of BAP was instrumental for enhancing high frequency of shoot regeneration in MS medium. On the other hand, plant morphogenetic studies carried out by earlier workers have revealed that an exogenous supply of growth regulators in growth medium is essential in plant growth and organogenesis. Of course the supplementation of BAP without any combination of auxin both in leaf explants (Tables 1, 2) enhanced good percentage of regeneration which suggested that there was a self-sufficiency in *Bacopa monnieri* in regenerating plantlets. Addition of phytohormones only enhanced the percentage of regeneration. This suggested that the plant material can be used as a model system of understanding the molecular component of self regeneratibility[9]. Apart of the conditions of regeneration of leaf, nodal explants can also be used for micropropagation of *Bacopa monnieri.*

The phytochemical analysis concerning chlorophyll (Fig. 2A), carbohydrate (Fig. 2B, C) and protein (Fig. 2D) did not suggest any clue for enhancement of percentage of regeneration as chlorophyll contents. However, there was a decrease in sugar contents and an increase in proteins due to use of phytohormones.

References

1 Kapoor L D, Handbook of Ayurvedic medicinal plants, (CRC Press, Boca Raton) 1990.

2 Srivastava P S, Ali G, Iqbal M, Narula A & Bharati N, *Micropropagation of Bacopa and effects of heavy metals on growth performance* (Gyanodaya Prakashan, Nainital, India) 2002, 325.

3 Sharan R, Khare R A, Clinical trial of Mentat in patients with various types of epilepsy, *Probe,* 33 (1994) 160.

4 Elangovan V, Govindaswamy S, Ramamoorthy N & Balasubramanian K, *In vitro* studies on the anticancer activity of *Bacopa monnieri, Fitoterapia,* LXVI- 3 (1995) 211.

5 Chatterjee N, Rastogi R P & Dhar M L, Chemical examination of *Bacopa monnieri* Wettst : Part I-Isolation of chemical constituents, *Indian J Chem,* 1 (1993) 211.

6 Kawai K I & Shibata S, Pseudojujubogenin, a new sapogenin from *Bacopa monnieri, Phytochemistry,* 2 (1978) 287.

7 Kulshreshtha D K & Rastogi R P, Bacogenin A, A novel dammarene triterpene sapogenin from *Bacopa monnieri, Phytochemistry,* 4 (1975) 887.

8 Annual Report XI, (Central Drug Research Institute, Lucknow) 1995.

9 Mathur S & Kumar S, Phytohormone self sufficiency for regeneration in the leaf and stem explants of *Bacopa monnieri, J Med Aroma Plant Sci,* 20 (1998) 1056.

10 Murashige T & Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant,* 15 (1962) 473.

11 Gamburg O L Miller R A & Ojima K, Plant tissue culture methods, *Exp Cell Res,* 50 (1968) 151.

12 Dreywood R, Analysis of Sugar, *Ind Eng Chem Anal,* 18 (1946) 499.

13 Nelson N, Analysis of Sugar, *J Bio Chem,* (1944) 153.

14 Lowry O H, Rose brough N J, Farr A L & Randall R J, Protein measurement with the Folin -Phenol reagent, *J Biol Chem,* 193 (1951) 265.

15 Thakur S, Ganapathy P S & Johri B M, Differentiation of abnormal plantlets in *Bacopa monnieri., Phytomorphology,* 26 (1977) 222.