Journal of Agricultural Technology 2012 Vol. 8(3): 1099-1108
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

# *In vitro* multiplication of *Bacopa monnieri* (L.) Pennell from shoot tip and nodal explants

**P. Pandiyan[1] and T. Selvaraj[2*]**

[1]PG and Research Department of Botany and Microbiology, AVVM Sri Pushpam College (Autonomous), Poondi, Thanjavur district, Tamil Nadu, India, [2]Department of Plant Sciences and Horticulture, College of Agriculture and Veterinary Sciences, Ambo University, Post Box No.19, Ambo, Ethiopia, East Africa

P. Pandiyan and T. Selvaraj (2012) *In vitro* multiplication of *Bacopa monnieri* (L.) Pennell from shoot tip and nodal explants. Journal of Agricultural Technology 8(3): 1099-1108.

*Bacopa monnieri* (L.) Pennell (Scrophulariaceae), popularly known as 'Brahmi' or 'Jal-brahmi' in India, is one of the sources of the medhya rasayan drugs (that counteract stress and improve intelligence and memory) of Ayurveda. It is prescribed for a variety of therapeutic indications including antipyretic, anti-inflammatory, analgesic, epilepsy, insanity, anticancer, antioxidant activities and memory enhancement. Therefore, the present study was to determine the *in vitro* mass multiplication of B. monnieri by using shoot tip and nodal explants which were inoculated in the Murashige and Skoog's (MS) medium fortified with various growth regulators such as 6 - benzyl aminopurine (BAP), Indole-3-butric acid ( IBA ), α-Napthalene acetic acid (NAA), Kinetin (KN) and Gibberellic acid ($GA_3$). Nodal explants responded better than the shoot tip explants and gave maximum shoots on BAP + KN + NAA (0.5 to 2.0 mg/l) supplemented medium. The regenerated shoots were rooted on MS medium with NAA 0.5 mg/l and IBA 1 mg/l gave good results within ten days. Almost 96% of the rooted shoots survived hardening under glass house and transferred to the field. The regenerated plants did not show any morphological change and variation in levels of secondary metabolites when compared with the mother stock.

**Key words:** Explants, Shoot tip, Nodal, in vitro Propagation, *Bacopa monnieri.*

## Introduction

Plants are the most important source of medicines and play a key role in world health (Kala, 2005). Collection of medicinal plants on a mass scale from the natural habitats leads to depletion of plant resources. Today's medicinal plants are important to the global economy, as approximately 80% of traditional medicine preparations involve the use of plants or plant extracts (Vieira and Skorupa, 1993; Dhyani and Kala, 2005). The increasing demand for herbal

* Corresponding author: T. Selvaraj; e-mail: tselvaraj_1956@yahoo.com

medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. Micro propagation is of special use for the conservation of these valuable genotypes (Abhyankar and Chinchanikar, 1996) with shoot culture, which is often utilized to maintain clonal fidelity, would be of special advantage. The *in vitro* propagated medicinal plants furnish a ready source of biochemical characterization and identification of active constituents (Banerjee and Shrivastava, 2006).

*Bacopa monnieri* (L.)Pennell (Scrophulariaceae), popularly known as 'Brahmi' or 'Jal-brahmi' in India, is one of the sources of the medhya rasayan drugs (that counteract stress and improve intelligence and memory) of Ayurveda. It has a great market demand due to its high medicinal values. It is prescribed for a variety of therapeutic indications including antipyretic, anti-inflammatory, analgesic, epilepsy, insanity, anticancer, antioxidant activities and memory enhancement (Satyavati *et al*. 1976; Jain and Kulshreshtha, 1993; Sinha and Saxena, 2006). It is also used in the treatment of asthma, hoarseness, water retention and blood cleaning. It contains different types of saponins such as bacosides A, B, C and D which are the active triterpenoid principles and known as "memory chemicals" (Rastogi *et al*. 1994; Sivaramakrishna *et al*. 2005). Two new dammarane - type jujubogegin bisdesmosides, bacosaponins E and F of biological interest have also been isolated from this herb (Mahato *et al*. 2000; Chakravarthy *et al*. 2003). The drug is included in several Ayurvedic formulations such as Brahmighratam and Sarasvataristham (Anonymous,1978; Sivarajan and Balachandran, 1994) and found to be effective in case of anxiety neurosis (Singh *et al*. 1979). In a recent study conducted in Indian medicinal plants (Anonymous,1997), *B. monnieri* was placed second in a priority list of the most important Indian medicinal plants evaluated on the basis of their medicinal importance, commercial value and potential for further research and development.

With an increasing word-wide demand for plant derived medicines and formulations (Parnhan, 1996), there has been a concomitant increase in the demand for raw material. Hence, there is a need to develop approaches for ensuring the availability of raw material of a consistent quality from regular and viable sources. The present study reports an efficient micro propagation system for regenerating a large number of plants directly from shoot tip and nodal explants of *B. monnieri* which would form a strategy in the conservation of this important medicinal plant.

## Materials and methods

The methods of plant tissue culture were the standard method as described in Plant Cell, Tissue and Organ Culture Fundamental Methods ( Gamborg and Phillips, 2004). The explants were selected from 3 month old matured plants of *B.monnieri* growing in the Botanical Garden of AVVM Sri Pushpam College, Poondi, Thanjavur district, Tamil Nadu, India. Nodal and shoot tip explants were used for direct regeneration on MS medium (Murashige and Skoog,1962). The explants were first washed with tap water for about half an hour, followed by 2-4 drops of liquid soap for 10-20 min. After rinsing with tap water thoroughly, the explants were surface sterilized with 0.1% mercuric chloride solution for 2-3 min. This was followed by washing with sterile distilled water 3-4 times to remove the traces of $HgCl_2$ solution. The shoot tip and nodal explants were inoculated by inserting their cut ends in the MS medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of BAP or KN individually or along with NAA to include multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.6 before gelling with agar and autoclaved at 121$^{o}$C at 15 lb pressure for 20 min. The cultures were maintained at 25 ± 2$^{o}$C under the light intensity of 3000 lux provided by cool white fluorescent lamps.

Shoots initiated from both the explants were excised after 30 days and cultured on MS medium, supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of $GA_3$, for shoot proliferation and elongation. The shoots (5-6 cm long) bearing at least 4.5 internodes were excised from the mass of proliferated shoots and transferred to the rooting medium containing 0.5, 1.0, 1.5 and 2.0 mg/l of either IBA or NAA. Rooted plantlets were transferred to polycups and PVC pots containing sterile soil and perlite (1:1) and covered with plastic bags to maintain 85 – 92% humidity. Subsequently, the plantlets were transferred to glass house after one month. The plantlets were planted in the soil after one month period of hardening. Experiments were set up in completely randomized block design. Ten cultures were raised for each treatment and all experiments were conducted thrice. Data on number of shoots,shoot length and number of roots and root length were determined. The data are statistically analyzed and the means were compared using students t-test at $\alpha$= 0.01 and 0.05. For preliminary qualitative phytochemical evaluation by thin layer chromatography (TLC), shoots from both the field- grown plants and the 5-6 week old shoot cultures were dried in an oven at 55$^{o}$C and passed through a 40 mesh sieve. The powder that resulted was extracted in 50% methanol for 24h; the extract was concentrated under vacuum and then evaporated to dryness. The residue was dissolved in a solvent system of ethyl acetate : methanol : water (16:2.5:1.6),

spotted on pre coated silica gel G TLC plates (E .Merck) and developed in the same solvent system. The spots were visualized by spraying the plates with anisaldehyde reagent (Wagner *et al*. 1984).

**Results and discussions**

MS medium supplemented with different concentrations of BAP/KN in combination with NAA resulted in initiation of callus and shoots from shoot tip and nodal explants (Table 1; Figs. 1a, 1b, 1c & 1d). Maximum number of multiple shoots were induced in MS medium supplemented with 1.5 mg/l BAP (Fig. 2b) when compared to other and higher concentrations used. Hence it is suggested that this optimum concentration of BAP promotes multiple shoot induction. Similar reports were also obtained with the cultures of *Phyllanthus amarus* (Ghanti *et al*. 2004), *Celastrus paniculatus* (Nair and Seeni, 2001) and *Withania somnifera* (Chandran *et al*. 2007). The higher concentrations of BAP inhibited the formation of shoots, and even when the shoots so formed were short and thick (Fig.2a). Such thick rosette type of shoot formation was recorded when higher concentrations of BAP was used in the case of *Melissa officinalis* (Tavares *et al*. 1996) and *Withania somnifera* (Chandran *et al*. 2007). Multiple shoots were also induced from shoot tip and nodal explants on MS medium supplemented with different concentrations of KN (0.5 – 2.0 MG/1).The number of shoot length were higher on the medium containing 2.0 mg/1. The higher concentration of KN inhibited the shoot formation from the shoot explants (Fig.3). Two cytokinins namely BAP and KN were used with auxin (NAA) on the medium, which induced maximum number of shoot initiation. Combinations of BAP, KN and NAA (each 1mg/1) gave maximum response in the induction of more number (Fig.4) of shoots than the individual cytokinins. Similar findings had also been reported by Vadawale *et al*. (2006) in *Vitex negundo* and in *Withania somnifera* by Chandran *et al*. (2007).

The dwarf shoots sub cultured on MS medium supplemented with $GA_3$ (3mg/1) showed maximum elongation. For root induction, plantlets were transferred to MS medium supplemented with different concentrations of IBA and NAA (Table 2). Number of roots per explant and root length were more on the medium containing IBA (1mg/1) and NAA (0.5 mg/1) (Fig.5 a, b & c). The number of roots and root length decreased when the concentrations of IBA and NAA were increased. IBA proved slightly superior to NAA in terms of root induction .The influence of IBA on enhanced root formation had also been reported in the case of *Phyllanthus amarus* (Nair and Seeni, 2001), *Centella asiatica* (Banerjee, 1999), *Phyllanthus caroliniensis* (Catapan *et al*. 2000) and *Withania somnifera* (Chandran *et al*. 2007).

Rooted plantlets when transferred to poly cups and PVC pots containing sterile soil and perlite (1:1) (Fig.6 a & b) got well acclimatized and exhibited 92% survivability when transferred to glass house. Qualitative TLC studies of the regenerated shoots from shoot tip and nodal explants revealed a phyto chemical profile similar to that of the field grown plants. The quantification of bacosides and TLC fingerprinting studies of such shoot cultures are underway.

**Table 1.** Efficacy of MS medium fortified with different growth regulators on shoot tip and nodal explants of *B. monnieri* after 4 weeks of culture

| Growth regulators(mg/1) | % of response | Mean number of shoots from shoot tip | Mean number of shoots from nodal explants |
|---|---|---|---|
| BAP | | | |
| 0.5 | 60 | 4.6±0.3 | 5.4±0.2 |
| 1.0 | 80 | 5.4±0.5 | 6.4±0.4 |
| 1.5 | 100 | 6.8±0.2 | 7.4±0.4 |
| 2.0 | 100 | 5.6±0.4 | 5.4±0.4 |
| KN | | | |
| 0.5 | 70 | 3.6±0.2 | 3.2±0.2 |
| 1.0 | 90 | 5.2±0.4 | 5.4±0.4 |
| 1.5 | 100 | 6.4±0.4 | 7.6±0.6 |
| 2.0 | 100 | 8.6±0.6 | 9.2±0.8 |
| BAP+KN(each 1mg./1) | 100 | 12.4±0.6 | 12.6±0.6 |
| BAP+NAA(each 1mg./1) | 100 | 14.2±0.6 | 14.6±0.4 |
| BAP+KN+NAA(each 1mg./1) | 100 | 16.8±0.8 | 18.4±0.8 |

Note: Values represent mean ±standard deviation of 10 replicates per treatment in three repeated experiments.

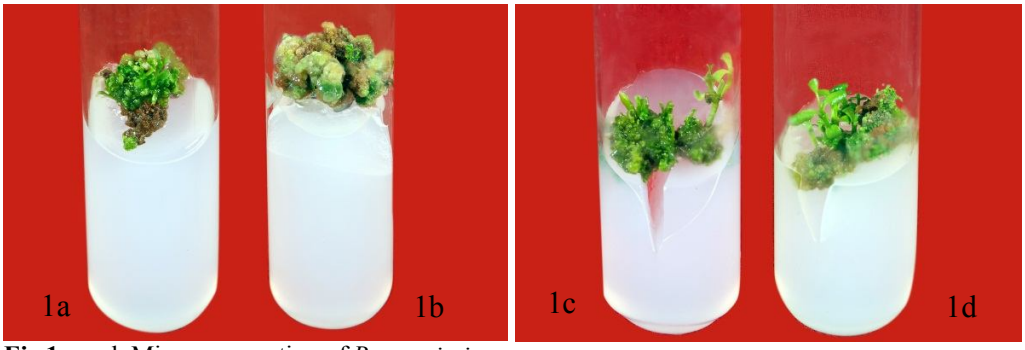


**Fig.1.** a - d. Micropropagation of *B.monnieri.*
Callus formation from shoot tip explant culture on MS medium with BAP+NAA+KN (each 1mg/1 after one weak)
Callus formation from nodal explant culture on MS medium with BAP+NAA+KN (each

1mg/l) after one weak
Multiple shoot regeneration was formed from shoot tip explant culture on MS medium with BAP+NAA+KN (each 1 mg/1) after two weeks.
Multiple shoot regeneration was formed from nodal explant culture on MS medium containing BAP+NAA+KN (each 1 mg/1) after two weeks

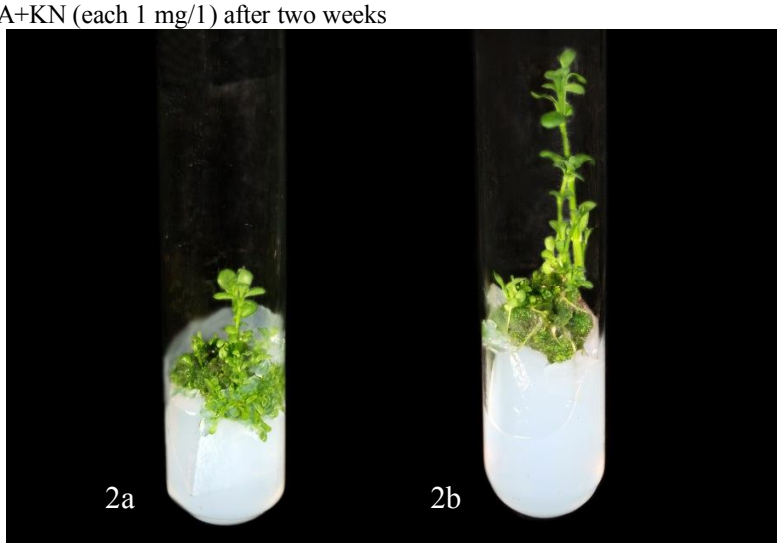


**Fig. 2.** a Shoot initiation from the shoot tip explant on MS medium containing 2mg/1 BAP after two weeks, 2b Long shoots formation from shoot tip explant culture on MS medium containing 1.5 mg/1 BAP after three weeks

**Fig. 3.** Nodal explant cuture on MS medium supplemented with 2mg/1 KN after two weeks.

**Fig. 4.** Four weeks old culture showing emergence of multiple shoots from nodal explant on MS medium supplemented with BAP+NAA+KN (each 1mg/1)

**Table 2.** Effect of auxins on root induction from in vitro raised shoot of *B.monnieri* after 4 weeks of culture

| Growth regulators(mg/1) | % of response | Mean number of roots/shoot | Mean root length (cm) |
|---|---|---|---|
| IBA(0.5)+NAA(0.5) | 60 | 8.62±0.8 | 6.82±0.8 |
| IBA(1.0)+NAA(0.5) | 100 | 12.40±0.6 | 10.72±0.8 |
| IBA(1.5)+NAA(0.5) | 80 | 10.62±0.8 | 8.28±0.6 |
| IBA(2.5)+NAA(0.5) | 60 | 7.69±0.6 | 4.24±0.4 |

Note: Values represents meant standard deviation of 10 replicates per treatment in three repeated experiments.

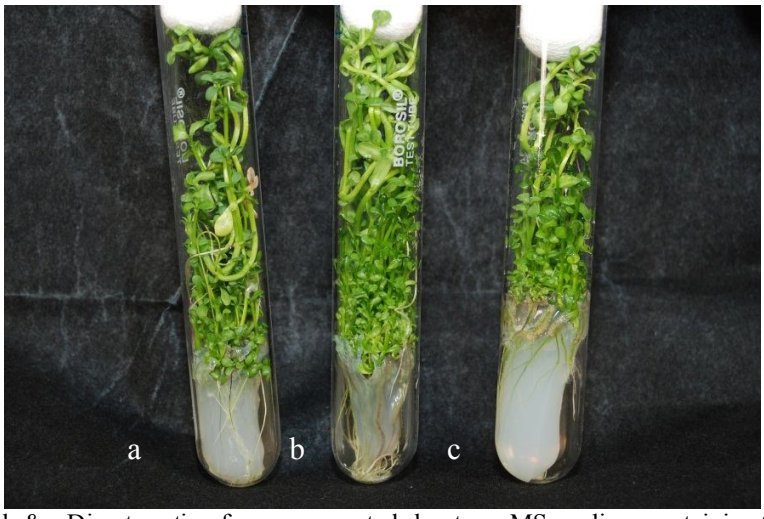


**Fig.5.** a, b &c. Direct rooting from regenerated shoots on MS medium containing 0.5 mg/1 NAA and 1.0 mg /1 IBA after 6 weeks of culture.

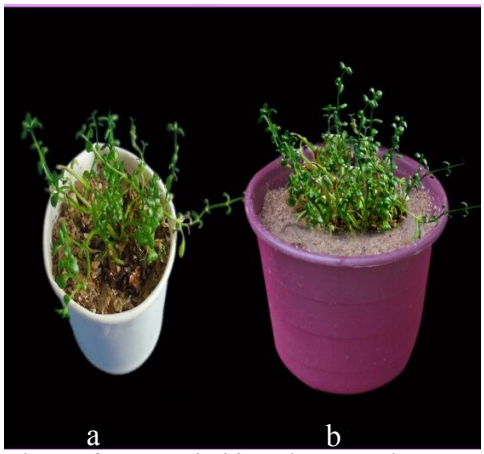


**Fig. 6.** a & b Hardened plants of B. monnieri in poly cup and PVC pot containing sterile soil and Perlite (1:1)

## Conclusion

Contrary to earlier reports of the use and need of very high concentrations of cytokinins for Brahmi growth, the present work has deciphered methods of improving *in vitro* propagation by developing a novel improved protocol highlighting efficient reproducible and reliable techniques for mass multiplication of a medicinally and economically important herb *B. monnieri*. *B. monnieri* has a high morphogenic potential, and the explants readily responded to cytokinins in the culture medium and formed multiple shoot buds. Of the three growth regulators tried, we found BAP to be more suitable than KN as the former resulted in a quicker and better response than the latter. Nodal explants responded better than the shoot tip explants and gave maximum shoots on BAP + KN + NAA (0.5 to 2.0 mg/1) with supplemented medium and number of roots per explant (12.40) and root length (10.72)were more on the medium containing IBA (1 mg/1) and NAA (0.5mg/1).

Thus this proves that this present protocol could successfully be used for large scale clonal propagation without any seasonal constraint and also for conservation and commercial propagation of this medicinal plant in the Indian sub continent. Moreover shoot tip and nodal explants were able to give rise to 16.8 and 18.4 shoots per explant respectively. Such superior shoot culture could be a better source for getting bacosides compounds and can also be used for genetic transformation studies through Agrobacterium. The present study is a stepping stone for *in vitro* production of required active principles of *B. monnieri*. This protocol is novel because of its minimal requirements and cost effectiveness for propagation.

## References


Anonymous, (1978). The Ayurvedic formulary of India part I, 1st edition, Government of India press, Faridabad, India. Pp.386.

Anonymous, (1997). Indian medicinal plants: a sector study. Occasional paper No 54, Export Import Bank of India,Quest publication, Bombay, India, 2, pp.140.

Banerjee, S. (1999). In vitro multiplication of *Centella asiatica*: a medicinal herb from leaf explant, Current Science 76: 147-148.

Banerjee, M and Shrivastava, S. (2006). In vitro regeneration of Jatropha curcas (Ratanjyot): propects for biofuel production and medicines. Ind.J.Botanical Research, 2, 195-200.

Catapan, E. Otuki,M.K and Viana,A.M. (2000). In vitro culture of *Phyllanthus caroliniensis*, Plant Cell, Tissue and Organ Culture 62: 195 – 202.

Chakravarty, A.K, Garai,S and Masuda,K. (2003). Bacopasides III - V : three new triterpenoid glycosides from *Bacopa monnieri*, Chemistry and Pharamaceutical Bulletin, Tokyo, 51: 215-217.

Chandran C., Karthikeyan K and Kulothungan, S (2007). In vitro propagation of *Withania somnifera* (L.) Dunal. from shoot tip and nodal explants, Journal of Scientific Transactions in Environment and Technovation, 1(1): 15-18.

Dhyani, P.P and Kala, C.P. (2005). Current research on medicinal plants: five lesser known but valuable aspects, Current Science 88: 335.

Gamborg, O.L; and Phillips, G.C. (2004). Plant Cell, Tissue and Organ culture Fundamental methods, Narosa publishing House, New Delhi, India, pp.420.

Ghanti, K.S; Govindaraju,B; venugopal,R.B; Rao,S.R; Kaviraj,C.P and Jabeen,F.T.Z, (2004). High frequently shoot regeneration from *Phyllanthus amarus* Schum & Thonn, Indian Journal of Boitechnology 3:103 – 107.

Jain, P and Kulshreshtha, D, (1993). Bacoside A1, a minor saponin from *Bacopa monnieri*, Phytochemistry 33:449-451.

Kala, C.P. ( 2005). Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of the Indian Himalayas, Conservation Biology 19: 368-378.

Mahato, S.B; Gari,S and Chakravarty,A.K, (2000). Bacosaponins E and F: two jujubogenin bisdesmosides from *Bacopa monnieri*, Phytochemistry 53:711-714.

Murashige, T; and Skoog,F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures, Physiology and plants 15: 472-497.

Nair, L.G and Seeni, S (2001). Rapid in vitro multiplication and restoration of *Celastrus paniculatus* Willd.Subsps. paniculatus(Celastraceae), a medicinal woody climber, Journal of Experimental Biology 39:697-704.

Rastogi, S; Mehrotra,B.N and Kulshreshtha,D.K, (1994). In: Deep publications (ed) proceedings of IV international congress of Ethnobiology, New Delhi, India, pp. 93.

Satyavati, G.V, Raina,M.K and Sharma, M. (1976). Indian medicinal plants, Vol. 1. Indian Council of Medical Research, New Delhi, India, pp. 20-35.

Sinha, S. and Saxena, R. (2006). Effect of iron on lipid peroxidation and enzymatic and non enzymatic antioxidant and bacosides - a content in medicinal plant *Bacopa monnier* L., Chemosphere 62:1340-50.

Singh, R.H; Singh,R.L and Seni,P.O.(1979). Studies on the anti-anxiety effect of the medhya rasayana drug brahmi (*Bacopa monnieri*).Part-II Experimental studies. J.Resident Indian Medicine Yoga and Homeopathy 14: 1-6

Sivarajan, V.V and Balachandran, I. (1994). Ayurvedic drugs and their plant sources, Oxford and IBH publication, New Delhi, India, pp. 235.

Tavares, A.C; Pimenta, M.C and Goncaves, M.T. (1996). Micropropagation of *Melissa officinalis* through proliferation of axillary shoots, Plant cell and Reproduction 15: 441-444.

Vadawale, A.V; Barve,D.M and Dave,A.M. (2006). In vitro flowering and rapid propagation of *Vitex negundo* L. a medicinal plant, Indian Journal of Biotechnology 5: 112-116.

Wagner, H; Bladt,H and Zgainski, E.M. (1984). Plant drug analysis - a thin layer chromatography Atlas, Springer, Berlin Heidelberg, New York, USA, pp: 285.

(Published in May 2012)