UKaaZ

Annals of Phytomedicine 1(1): 110-117, 2012

ANNALS OF
PHYTOMEDICINE
An International Journal

Efficient plant regeneration from leaf explants of *Bacopa monniera* (L.) Wettst. : A threatened medicinal herb

Srinath Rao, P. Rajkumar, C. Kaviraj and P. Asra Parveen

Plant Tissue Culture and Genetic Engineering Laboratory, Department of Botany, Gulbarga University, Gulbarga-585 106, Karnataka, India.

Received for publication January 1, 2012; accepted February 30, 2012

Abstract

An efficient plant regeneration protocol was developed from leaf and internode (stem) explants of *Bacopa monniera* L. on MS basal medium, supplemented with Cytokinins like BAP, KN, and TDZ used singly. The leaf cultures expressed maximum plant regeneration potential with 138.9 ± 0.50 shoots per explants on MS + 2.0 mg/l BAP. The *in vitro* regenerated dwarf shoots were further elongated on MS medium supplemented with GA_3 (0.5 mg/l). *In vitro* shoots about 3-4cm was then excised from shoot clumps and transferred to rooting medium containing 2.0mg/l IBA. Green and robust shoots developed and successfully rooted within 15 days of culture on rooting medium. The rooted plantlets were hardened on half strength MS basal liquid medium and subsequently transferred to polycups containing vermiculate, sand and soil in the ratio of 1: 2: 2. *In vitro* plantlets were finally transferred to a green house where 90% plants survived.

Key words: Auxins, *Bacopa monniera*, cytokinins, callus, organogenesis

Introduction

Plants have been an important source of medicine for thousands of years. According to an estimate by World Health Organization, even today up to 80% of people still rely mainly on traditional remedies such as herbs for the their medicines. Plants are also the source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain active ingredients obtained from plant substances.*Bacopa monniera* (L.)Wettst. (Syn. *Herpestis monniera* L) belongs to family, Scrophulariaceae. It is commonly known in India as 'Brahmi' or 'Nirbrahmi,' is an ancient and renowned medicinal plants with legendary reputation as a memory vitalizer (Anonymous, 1988). In the traditional system of Indian medicine (Ayurveda), Brahmi is classified as medhyarasayana, *i.e*., a drug that is supposed to counteract the effects of mental stress and improve intelligence and memory function. Brahmi is found to be effective in case of anxiety and neurosis (Singh *et al*., 1979). It possesses anti-inflammatory, analgesic, antipyretic, anticancer and antioxidant activities (Satyavati *et al.*, 1976; Agrawal, 1993; Jain *et al.*, 1994; Tripathi *et al.*, 1996; Vohora *et al.*, 1997; Bafna and Balaraman, 2005). It is observed to cure asthama, epilepsy and insanity (Basu and Walia, 1944; Tiwari *et al.*, 1999). In a recent study, *Bacopa monniera* was placed second in a priority list of the most important Indian medicinal plants evaluated on the basis of medicinal importance, commercial value and potential for further research and development (Anonymous, 1997).

Bacopa monniera contains several alkaloids such as nicotine, brahmine, herpestine and saponins such as hersaponin, bacosides A, B, C, D and several other chemicals like stigmastanol, β-sitosterol and stigmasterol (Basu and Walia, 1944; Basu *et al*., 1967). These bacosides are active triterpenoid principles known as "Memory chemicals"

Author for correspondence: Dr. Srinath Rao
Professor, Department of Botany, Gulbarga University, Gulbarga-585 106, Karnataka, India.
E-mail: srinathraom@rediffmail.com
Tel.: +91-09986110109

(Rastogi *et al.,* 1994; Sing and Dhawan, 1997; Sivaramakrishna *et al.,* 2005). According to Ahmad (1993), the estimated annual requirement of *Bacopa monniera* is around 12,700 tons of dry biomass valued at Rs. 1.5 billion and is met solely from the natural populations, leading to their gradual depletion.

Seeds of *B. monniera* are poor propagules due to their short viability (two months) and frequent seedling death at the two-leveled stage, which makes it difficult to raise plants from seeds. Vegetative propagation is slow and is further hampered by specific habitat requirement and poor performance of propagules. Recently in a report by the National Medicinal Plant Board (NMPB) and Government of India and Technology Information Forecasting and Assessment Council (TIFAC) has recommended immediate attention to few medicinal plants, among which *B. monniera* prominently features, which makes this plant in the category of highly endangered plants in India. According to NMPB, the popularity of the Bacopa-based drugs are increasing rapidly.

In vitro propagation of plants hold tremendous potential for the production of highly-quality plant based medicines (Murch *et al.,* 2000). This can be achieved through different methods including micropropagation. Numerous factors are reported to influence the success of *in vitro* propagation of different medicinal plants (Hussey, 1980). The development of a rapid clonal multiplication of this medicinally important herb has become imperative in order to reduce the existing pressure on natural populations and supply constant plant materials for pharmaceutical industry. The present paper deals with comparative study of growth hormones on induction of multiple shoots by using different explants to ensure reproducible protocol for this economically important and threatened medicinal herb.

Materials and Methods

Plant materials

Bacopa monniera (L.) plants were obtained from Botanical garden, Gulbarga University, Gulbarga. Leaf and internodal explants were excised from healthy plants and were pretreated with Teepol (5%) for 5 min; surface sterilized with 0.1% mercuric chloride for 2-3 min and finally rinsed 3 times with sterile distilled water to remove traces of mercuric chloride.

Culture medium and conditions

Internodal explants were inoculated by inserting their cut ends and leaf explant with adaxial side touching on two media that of B_5 (Gamborg *et al.,* 1968) and MS (Murashige and Skoog's, 1962) medium supplemented with different Cytokinins *viz.*, BAP, TDZ, and Kin alone, were used for induction of organogenesis. The medium contained 3% sucrose (w/v). The pH of the medium was adjusted to 5.8 and solidified with 0.8% agar before autoclaving at a pressure of 1.06kg cm^2 for 20 min. The cultures were maintained at a temperature of 25 ± 2^0C under 16/8 hrs (light/ dark) under 42 µM m^{-2} s^{-1} light intensity provided by white cool fluorescent light

Elongation and rooting of shoots

The *in vitro* initiated individual shoots about 4-5cm long were separated and transferred to MS basal medium containing different concentration of GA_3 for elongation of shoots. These elongated shoots bearing atleast 4-5 internodes were excised from the mass of proliferated shoots and were transferred to rooting media, supplemented with different concentration of NAA or IBA (Indole Butynic Acid). The rooted plants were taken out from the culture tubes, washed with sterile distilled water to remove adhering agar and transferred to MS basal liquid medium for two weeks for hardening. After hardening, these plants were subsequently transferred to plastic cups with sterile vermiculate, sand and soil (1: 2: 2). The plantlets were kept at 80% relative humidity and temperature 25 ± 2^0C under a 16 h photoperiod for acclimatization. The plants were flooded with 1/8^{th} MS macronutrients biweekly. Established plants were transplanted to soil under natural condition and the survival rate was recorded

Statistical analysis

The experimental design was random and factorial with cytokinins as independent variables. The data pertaining to the number of shoots, shoot length and roots were subjected to analysis of variance (ANOVA) test and mean were determined by Duncan's New Multiple Range Test (DNMRT). Two replicates were maintained for each treatment, each replicate had 20 plants and all the experiments were repeated twice.

Results and Discussion

Both explants responded to organogenesis with varying degrees, leaf explants was found to be best source followed by intermodal explants. Shoot buds started to appear between 8-14 days after incubation depending on the type of cytokinins and media used. Best response was noticed on MS medium supplemented with cytokins (Table 1). Our results are in agreement with those of Sharma *et al*. (2010), hence, for further studies, only MS medium was used. All the cytokinins used at all the concentrations used, responded well in terms of frequency and number of shoots formed per explant though with varying frequencies among the two explants leaf, proved superior than internodal explant. A similar trend was reported by Tiwari *et al*. (2001) and Srivastava and Rajani (2000) in this species.

All three cytokinins (0.5 mg-3mg/l) used, induced shoot initiation though, with various frequencies (Table 2).The frequency of shoot induction was about 2% on MS medium devoid of growth regulators and about 2-3 shoots per explants were noticed, however, the frequency of shoot induction was 100% on 2mg/l BAP supplemented media, followed by Kin, (90%). The frequency of shoot induction was between 40-68% on media supplemented with TDZ. The number of shoots per explants was highest on medium supplemented with 2.0mg/l BAP followed by Kin and TDZ, thus, demonstrating that BAP was most essential for shoot induction in this species.

Superiority of BAP over Kinetin has been reported in *B. monniera* (Tiwari *et al.*, 1999, 2001; Banerjee and Shrivatava, 2008). However, Praveen *et al.* (2009) reported that Kn at 2.5 mg/l was found better than BAP in inducing multiple shoots in this species. Contrary to our observations, Sharma *et al*. (2010) reported that very low concentrations of BAP (0.2 mg/l) favored multiple shoot induction from nodal explants in this species. Increasing the concentration of all the cytokinins from 0.5to 2 mg/l, resulted in gradual increase in number of shoots per culture. However, higher concentration (2.5 and 3.0mg/l) resulted in decreased number of shoots supporting the view expressed earlier by Tiwari *et al.* (2001). They further reported that response of cytokinins was dependent on the type of cytokinins used *i.e.*, leaves were more responsive to TDZ than BAP, however, they stated that though the number of shoots was more on TDZ containing medium they were stunted when compared to shoots growing on BAP containing medium. Same was reported by Praven *et al*. (2009) also. In the present investigation, we didn't find any differential requirement amongst the explant for a particular cytokinin. There is varying reports with regard to number of shoot buds induced by BAP. We observed highest number (104.8 ± 2.31) of multiple shoots from leaf explant at 2 mg/l BAP in agreement to reports of (Tiwari *et al.*, 2001). However, Banerjee and Shrivasthava (2008) and Tejvathi and Shailaja (1999) reported only 8-14 shoots per nodal explant at same concentration of BAP. Shoot regeneration from Trimethoprim and Bavistin is reported by Tiwari *et al*. (2006) and further it is proved to be better than BAP in enhancing number of multiple shoots in this species. The variations reported in terms of type and concentrations of growth hormones and differential responses of explants reported may be attributed to lot of genetic variation among South Indian *Bacopa monnieri* as reported by Karthikeyan *et al.* (2011).

Elongation of shoots

The *in vitro,* dwarf shoots were separated from the shoots clumps and transferred on MS medium, supplemented with different concentration of GA_3 (0.5-1.5mg/l) for elongation of shoots. The highest shoot length (10.5±0.33cm) was observed in shoots derived from leaf explants (Table 4; Plate 2, a&b).Tiwari *et al.* (2001) reported that shoot elongation was observed in *Bacopa* resulted upon transfer to hormone free medium.

Rhizogenesis

For induction of roots, *in vitro* developed plantlets were transferred to rooting media consisting of ½ strength MS salts along with auxins NAA or IBA. Both NAA and IBA induced root within two week of culture in all the treatment. Among the two auxins tested, 2.0mg/l IBA induced maximum number (57.0 ± 0.75) of roots per shoot (Plate 3, Figure b) as compared to other concentration of IBA and NAA (Tabe 5). Better response of IBA to NAA and IAA induced rooting has been reported in this species (Tiwari *et al.*, 2001). The roots formed on IBA supplemented medium were thick, long and dark coloured as compared to those on NAA supplemented medium. Earlier reports in this species suggests IBA is best for root induction and growth (Tiwari, *et al*., 1999; Sharma, *et al*., 2010).

After four weeks in the rooting medium, the rooted shoots were transformed to MS basal liquid medium for two weeks. None of the plantlets survived when directly transformed from rooting medium to the pots under natural condition. About 75-80% of the transplanted plants survived, if the plants in the culture tubes were kept in normal room temperature for seven-eight days before transplantation in pots and reared for three weeks (Plate 3, Figure d). The plants were reared under semi-controlled temperature (32 ± 2^0C) and light (2000lux) in growth chamber with 80% humidity. During this period, the acclimatized shoots elongated, leaves expanded and turned deep green in color and plantlets looked healthier.

After three weeks, these plants were transferred to an open place and gradually acclimated to outdoor condition, where 90% of plants survived and they produced flower and seeds. The technique described here appears to be readily adaptable for large scale clonal propagation and the genetic transformation studies in *B. monniera*.

In conclusion, it can be said that cytokins specially BAP play a major role in tissue culture of *B. monnieri* and it has very high morphogenic potential. However, lot of variation occur in *B. monnieri* with respect to tissue culture response, different explant respond to different type and concentrations of cytokinins and the same explant may show differential response to a particular cytokinin. However, BAP seems to be better cytokinin option compared to Kn and TDZ. This variation may be due to lot of genetic variation among South Indian cultivars of *B. monnieri* as revealed by RAPD analysis reported by Karthikeyan *et al*. (2011).

Table 1. Frequency and days taken for initiation of shoots from different explants of Brahmi (*Bacopa monniera* L.) on MS medium

Explants	Initiation of shoots (Days)		Frequency of regeneration (%)	
	MS	B_5	MS	B_5
Leaf	9-10[b]	10-11[b]	100[a]	100[a]
Internode	8-9[a]	11-12[c]	100[a]	90[b]
Node	11-12[d]	13-14[d]	80[b]	60[d]

Data represents average of two replicates; each replicate consists of 25 cultures.
Mean ± Standard error.
Mean followed by the different superscript in columns is significantly different from each other according to ANNOVA and DMRT. P = 0.05 levels.

Table 2. Frequency, number of shoot and shoot length obtained from leaf and Internode explants of Brahmi (*Bacopa monniera* L.) supplemented with different concentrations of cytokinins on MS medium

Concentrations of growth regulators (mg/l)	Leaf			Internode		
	Frequency (%)	No. shoots/ culture	Shoot length/ culture (cm)	Frequency (%)	No. shoots/ culture	Shoot length/ culture (cm)
BAP						
0.5	100	31.6 ± 0.90[b]	3.8 ± 0.10[g]	100	28.0 ± 0.50[d]	2.9 ± 0.75[d]
1.0	100	53.6 ± 0.28[c]	5.8 ± 0.27[d]	100	46.4 ± 0.75[d]	4.2 ± 0.66[d]
1.5	100	89.2 ± 0.28[b]	8.2 ± 0.25[b]	100	72.0 ± 0.68[b]	6.6 ± 0.33[b]
2.0	100	104.8 ± 2.31[a]	9.2 ± 0.54[a]	100	89.9 ± 0.43[a]	8.2 ± 0.50[a]
2.5	100	61.2 ± 0.66[d]	4.8 ± 0.50[f]	90	34.8 ± 0.65[e]	3.8 ± 0.33[c]
3.0	80	22.5 ± 0.75[e]	3.0 ± 0.50[g]	90	20.5 ± 0.98[c]	3.4 ± 0.10[c]
Kn						
0.5	90	25.1 ± 0.17[f]	3.6 ± 0.66[g]	100	20.8 ± 0.24[c]	3.8 ± 0.75[c]
1.0	90	40.6 ± 0.35[g]	5.8 ± 0.33[d]	80	40.8 ± 0.55[d]	5.4 ± 0.33[c]
1.5	90	72.8 ± 2.80	8.4 ± 0.80 [b]	80	64.6 ± 0.46[c]	6.8 ± 0.86[a]
2.0	90	82.6 ± 3.20[c]	7.6 ± 0.78[c]	78	60.4 ± 0.86[c]	6.8 ± 1.20[a]
2.5	86	54.8 ± 2,40[c]	4.0 ± 0.06	80	60.8 ± 0,98[c]	4.5 ± 0.98[d]
3.0	68	28.0 ± 1.50[h]	2.2 ± 0.10[g]	80	26.8 ± 0.80[h]	2.5. ± 0.65[d]
TDZ						
0.5	90	22.8 ± 0.98[e]	2.6 ± 0.88[g]			
1.0	100	28.6 ± 0.35[c]	2.2 ± 0.75[g]	90	11.2 ± 0.20[e]	2.1 ± 0.75[d]
1.5	100	40.8 ± 0.90[d]	5.2 ± 0.00[f]	80	16.4 ± 0.25[g]	5.7 ± 0.75[e]
2.0	80	78.3 ± 0.45[c]	6.8 ± 0.45[f]	80	12.6 ± 0.50[e]	6.4 ± 0.25[b]
2.5	78	0.2 ± 1.20	2.8 ± 0.20[g]	70	32.6 ± 1.20[e]	2.8 ± 0.36[d]
3.0	62	18.6 ±	2.4 ± 0.20[g]	70	14.8 ±0.86[g]	2.4 ± 0.08[d]

Data represents average of two replicates; each replicate consists of 25 cultures.
Mean ± Standard error.
Mean followed by the different superscript in a column are not significantly different from each other. P = d" 0.05 levels according to ANNOVA and DMRT.

Table 3. Effect of GA_3 on *in vitro* shoots elongation in Brahmi (*Bacopa monniera* L.) on M medium

Concentration of growth regulator (mg/1)				
	Leaf		**Internode**	
	Frequency (%)	**Shoot length/ culture (cm)**	**Frequency (%)**	**Shoot length/ culture (cm)**
GA_3				
0.5	100	10.5 ± 0.33^a	100	10.3 ± 0.34^a
1.0	100	9.0 ± 0.33^b	100	8.4 ± 0.29^b
1.5	100	8.3 ± 0.50^c	100	8.8 ± 0.75^c

Data represents average of two replicates; each replicates consist of 25 cultures.

Mean ± Standard error.

Mean followed by the different superscript in a column are significantly different from each other according to ANNOVA and DMRT. P = 0.05

Table 4. Frequency, number of root obtained in vitro on Brahmi (*Bacopa monniera* L.) supplemented with different concentration of NAA and IBA on MS medium

Concentrations of growth regulator (mg/1)				
	Leaf		Internode	
	Frequency (%)	No. roots/culture	Frequency (%)	No. roots/ culture
NAA				
1.0	100	28.7 ± 0.67^e	100	34.3 ± 0.45^c
2.0	100	34.4 ± 0.50^d	100	41.3 ± 0.32^b
3.0	100	44.9 ± 0.00^c	100	48.7 ± 0.54^b
IBA				
1.0	100	49.9 ± 0.66^b	100	32.3 ± 0.65^c
2.0	100	57.0 ± 0.75^a	100	56.8 ± 0.75^a
3.0	100	42.7 ± 0.33^c	100	39.3 ± 0.86^c

Data represents average of three replicates; each replicate consists of 25 cultures.

Mean ± Standard error.

Mean followed by the different superscript in a column are significantly different from each other according to ANNOVA and DMRT. P = 0.05

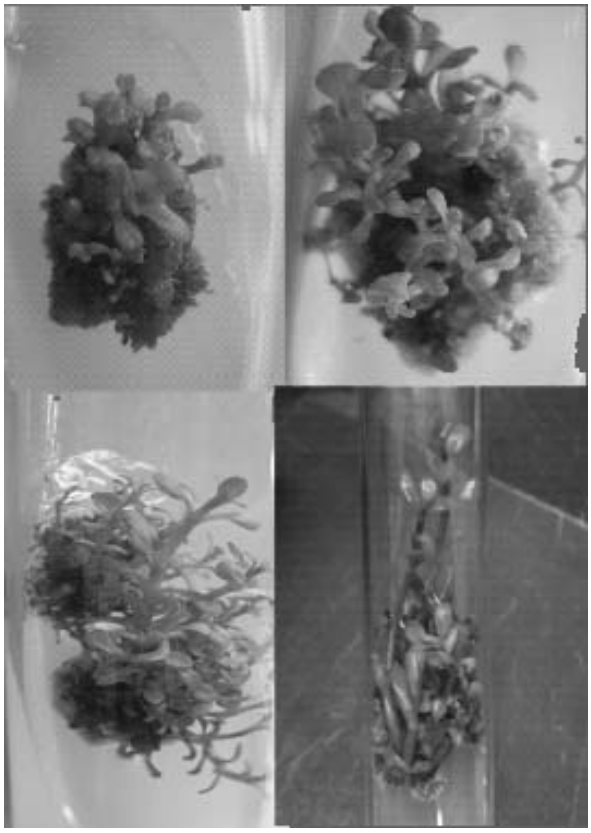

Plate 1. Organogenesis in *B. monneri*

a. Initiation of shoots on TDZ supplemented medium(stunted shoots)

b. Initiation of shoots on BAP supplemented medium(elongated shoots)

c. Initiation of shoots on Kn supplemented medium

d. Long, green and healthy shoots on BAP supplemented medium

Plate 2. Shoot elongation on GA_3 medium

a & b. shoot elongation on medium supplemented with 0.5 and 1mg/l GA_3, respectively

Plate 3. Rhizogenesis in *B. monnieri*

a. Rooting on NAA supplemented medium(weak roots)

b. Rooting on IBA supplemented medium(thick roots)

c. A potted plantlet.

Reference

Agrawal, A. (1993). A comparative study of psychotropic drugs and bio-feedback therapy in the prevention and management of psychosomatic disorders. A Ph.D. Thesis submitted to Banaras Hindu University), Varanasi, India.

Ahmad, R.U. (1993). Medicinal plants used in ISM- their procurement, cultivation,regeneration and import/export aspects: A report. *In*: Govil J N, Singh V K, Hashmi S (eds.) medicinal plants: New vistas of research (Part-1). Today and Tomorrow Printers and Publisher, New Delhi, pp 221-225.

Anonymous. (1988). The Wealth of India: Raw Materials. Council of Scientific and Industrial Research, New Delhi, **2**: 2-3.

Anonymous. (1997). Indian Medicinal Plants: A sector study. Occasional paper No. 54. Export/ import bank of India, Quest Publication, Bombay, India.

Banerjee, M. and Shrivastava S. (2008). An improved protocol for *in vitro* multiplication of *Bacopa monnieri* (L.) World J. Microbiol. Biotechnol., **24**:1355–1359

Basu, N.K. and Walia, J.S. (1944). The chemical investigation of the leaves of *Herpestis monniera*. Indian J. Pharm., **4**: 84-91.

Basu, N.K.; Rastogi, R.P. and Dhar, M.L. (1967). Chemical examination of *Bacopa monniera* Wettst: part III, Bacoside B. Indian J. Chem., **5**: 84-86.

Gamborg, O.L.; Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Expt. Cell Res., **50**: 151-158.

Hussey, G **.** (1980). *In vitro* propagation. In: Ingram D S, Helgeson J P (eds.) Tissue culture for plant pathologists. Oxford: Blackwell Scientific, 51-61.

Jain, P.; Khanna, N.K.; Trehan, N.; Pendse, V.K. and Odhwani, J.L. (1994). Anti-inflammatory effects of an Ayurvedic, Brahmi Rasayana, in rodents. Indian J. Exp. Biol., **32**:633-636.

Karthikeyan, A.; Madahanraj, A.; Pandian, S.K. and Manikandan, R. (2011). Genetic variation among highly endangered *Bacopa monnieri* (L.) Pennel from South India as detected using RAPD analysis. Genet, Resour, Crop Evol., **58**: 769-782

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, **15**: 437-497.

Murch, S.J.; KrishnaRaj, S. and Saxena, P.K. (2000). Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated St John's wort (*Hypericum perforatum* L. cv Anthos) plants. Plant Cell Rept., **19**: 698-704.

Praveen, N.; Naik, P.M.; Manohar, S.H.; Nayeem, A. and Murthy, H.N. (2009). *In vitro* regeneration of Brahmi shoots using semi solid and liquid cultures and quantitative analysis of Bacoside-A. Acta Physiol. Plantarum.

Rastogi, S.; Pal, R. and Kulshreshtha, D.K. (1994). Bacoside A_3-A triterpenoid saponin from *Bacopa monniera*. Phytochemistry, **36**: 133-137.

Shivaramakrisha, C.; Rao, C.V.; Trimurtulu, G.; Vanisree, M. and Subbaraju, G.V. (2005). Triterpenoid glycosides from *Bacopa monniera*. Phytochemistry, **66**: 2719-2728.

Singh, R.H.; Singh, R.L. and Seni, P.O. (1979). Studies on the anti-anxiety effect of the medha rasayana drug Brahmi (*Bacopa monniera*), Part-II experimental studies. Journal Res. Indian Med. Yoga, Homeopath, **14**: 1-6

Singh, H.K. and Dhawan, B.N. (1997). Neuropsycho phamacological effect of the Ayurvedic no tropic *Bacopa monnieri* Linn. (Brahmi). Ind. J Pharmaco., **29**: 359-365.

Srivastava, N. and Rajani, M. (2000). Multiple shoot regeneration and tissue culturestudies on *Bacopa monnieri* (L.) Pennell. Plant Cell Rep., **18**:919–923

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

Tejvathi, D.H. and Shailaja.K.S. (1999). Regeneration of plants from the cultures of *Bacopa monnieri* (L.) Pennel.

Tejavathi, D.H.; Sowmya, R. and Shailaja, K.S. (2001). Micropropagation of *Bacopa monniera* using shoot tip and nodal explants. Journal Trop. Med. Plants., **2(1)**: 39-45.24.

Tiwari, V.; Singh, B.D. and Nath, T.B. (1999). Shoot regeneration and somatic embryogenesis from different explants of Brahmi (*Bacopa monniera* L.). Plant Cell Rept., **17**: 538-543.

Tiwari, V.; Tiwari, K.N. and Singh, B.D. (2000). Suitability of liquid culture for *in vitro* multiplication of *Bacopa monniera* (L.) Wettst. Phytomorphology, **50**:337–342

Tiwari, V.; Tiwari, K. N. and Singh, B. D. (2000). Shoot bud regeneration from different explants of *Bacopa monniera* (L.) Wettst. by trimethoprim and bavistin Plant Cell Rep., **25**: 629–635

Tiwari, V.; Kavindra, N.T. and Singh, B.D. (2001). Comparative studies of cytokinins on *in vitro* propagation of *Bacopa monniera*. Plant Cell Tissue Organ Cult., **66**: 9-16

Tripathi,Y.B.; Chaurasia, S.; Tripathi, E.; Upadhyay, A. and Dubey, G.P. (1996). *Bacopa monniera* L. as an antioxidant: mechanism of action. Indian J. Exp. Biol., **34**:523-526.

Vohora, S.B.; Khanna, T. and Athar, M. (1997). Analgesic activity of bacosine, a new triterpene isolated from *Bacopa monniera*. Fitoterapia, **68**: 361-365.