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In-vitro Conservation of *Bacopa monniera* (L.) Wettst.: A Memory Booster Plant

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Abstract
A mass *in-vitro* propagation system of *Bacopa monniera* (L.) Wettst., a traditional medicinal herb, has been developed. Shoot proliferation and growth were greatly influenced by the months of the year during which the explant were collected. High frequency bud break coupled with maximum number of shoots through nodal segment were found in the month of June and August. Of the different growth regulators tried, for *in vitro* shoot regeneration, MS medium with BAP (1.0 mg/l) induced maximum number of proliferative shoots (65.00±1.453). Multiplication and elongation of the shoots were obtained after regular sub culturing on the same medium after 15-21 days. Rooting from basal end of the shoots occurred on MS medium with the addition of IBA (0.5 mg/l). After a hardening phase of 4 weeks, almost 95% transplantation was success in the field.

Key words: *Bacopa monniera* (L.) Wettst., *in vitro* propagation, medicinal herb, growth regulators

Introduction
The potential of plant tissue culture technologies revolutionizing in the commercial agriculture sector by enabling mass propagation of elite, high yielding and disease free plants throughout the commercial micro-propagation industries is considered as relevant to agribusiness as tissue culture has the capability to break the plateau in crop productivity, which are not possible through conventional methods (Shimomura *et al*., 1978).

Consumption of herbal medicines has become widespread and medicinal plants are the most important source of lifesaving drugs for the majority of the world's population (Zhou and Wu, 2006; Chaturvedi *et al*., 2007).

Bacopa monniera (L.) Wettst. "Brahmi" is a memory booster. Human cognition can be divided into four main categories of functions reception/acquisition, memory/learning, thinking/ reasoning and expression/ action (Lezak *et al*., 2004). Thus, any compound that improves this faculty of the brain is called Brahmi. It has been used since time immemorial as a tonic for improving memory. In the gurukuls of ancient India there was the practice to regularly administer brahmi to young students to help them learn sacred hymns and to infant's babies in the belief that it will open the gateway of intelligence (Hackman, 1998). As early as 800 B.C., an ancient Indian scripture, Atharav-Ved-Samhita, extolled the values of Brahmi in aiding the memorization of vedic hymns by scholars. From the fifth to twelfth century, the memory boosting herb was used by Buddhist scholars at Nalanda

University, the world's first international university. *Bacopa monniera* is a natural sedative and mood stabilizer, which is used to relax the Central Nervous System (CNS) as well as to support healthy cognitive functions like focus of memory.

Bacopa meditate the GAB Aergic system**, if** nervous system (Shukia *et al*., 1987; Kumar, 2006) triterpenoid saponins and bacosides of *B. monniera* play key role in enhancing nerve impulse transmission. Bacosides support the repair of damaged neurons by enhancing kinase activity, neuronal synthesis, restoration and regeneration of synaptic activity resulting in nerve impulse transmission. These effects make it a wonderful nerve tonic or nerve nourishing agent as against the neuroleptic drugs that modulate the behaviour (Russo and Borrelli, 2005). On children, it showed significant improvement in exploratory drive, improved perceptual image of patterns and increased perceptual organization and reasoning ability. It is also used as an anti depressant, for alteration deflect disorder, for anxiety and blood pressure as antioxidant for antimicrobial activity for Alzheimer's disease, for Epilepsy, for stress, for anxilytic effect, as anti-inflammatory, anticancer activity, for bronchitis asthma and chronic coughs as diuretic, arthritis, blood cleanser for hair loss, digestive problems. It is also used as a anti depressant, for alteration deflect disorder, for anxiety and blood pressure as antioxidant for antimicrobial activity for Alzheimer's disease, for Epilepsy, for stress, for anxilytic effect, as anti-inflammatory, anticancer activity, for bronchitis asthma and chronic coughs as diuretic, arthritis, blood cleanser for hair loss, digestive problems (Dorababu *et al*., 2004), epileptic fits, depression including post natal depression, to improve circulation and strengthen capillaries, impotence, premature ejaculation, frigidity, irregular menstruation, mental and physical fatigue and overwork. Brahmi has been esteemed as a cardiac, nerve and brain tonic, for restlessness, insomnia and over active mind, forgetfulness, confused and cloudy thoughts, anxiety, stress, hysteria, nervous breakdowns, insanity (Nathan *et al*., 2001). Often taken for fevers and also used for contusions, boils, sprains and fractures. In India the plant is used for all sorts of skin problems-eczema, psoriasis, abscess, and ulcerations- it is said to stimulate the growth of skin, hair and nails. In Pakistan, Brahmi is a home remedy for skin problems, rheumatism, piles, inflammations swellings, fever, dysentery, children's bowel complaints, mental weakness and to improve memory.

Micro-propagation of Brahmi has been conducted by various workers (Mathur and Kumar 1998; Tiwari *et al*., 1998, 2000, 2006; Ali *et al*., 1999; Srivastava and Rajani 1999; Tejavathi and Shailaja 1999; Misra *et al*., 2003; George *et al*., 2004; Singh and Singh, 2009). It has become imperative to reduce the existing pressure on natural population and supply constant plant materials for pharmaceutical industries. So, the present research describes a simple, reliable and repeatable protocol for the *in vitro* regeneration of *Bacopa monniera* by using axillary meristem.

Materials and methods

In vitro nodal segments (0.5-1.5 cm long) having axillary buds were procured from 1-2 months old healthy mother plant growing in the Botanical garden, University of Rajasthan, Jaipur.

The procured nodal segments were sterilized by keeping under running tap water for about 15-20 min to remove soil particles and then rinsed with liquid detergent

(Rankleen, Rankem (1%; v/v) for 3-5 min. Then they were rinsed with sterile double distilled water at least thrice to get rid of Rankleen. Prior to inoculation, these explants were subsequently surface sterilized in the laminar air flow chamber with 0.1% aqueous mercuric chloride ($HgCl_2$; w/v) for 2 min followed by repeated rinsing with sterile double distilled water.

The surfaced sterilized explants were then aseptically inoculated on sterile MS medium (Murashige and Skoog, 1962) comprising 3% sucrose as carbon source and 0.8% agar as solidifying agent. The medium was also supplemented with various growth regulators [auxins-IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (Naphthalene acetic acid) 2, 4-D (2, 4-dichlorophenoxyacetic acid) etc. and cytokinins-BAP (6-benzylaminopurine), Kn (6-furfurylaminopurine)] and pH of the medium was adjusted to 5.8±0.02 before autoclaving at pressure 1.06 Kg/cm^{-3}. For multiple shoot induction, different concentrations (0.25-5.0 mg/l) of BAP and Kn alone or in combination were used. However, various auxins such as NAA; 2, 4-D; IBA; IAA at different concentrations (0.5-8.0 mg/l) were examined for *in vitro* root induction.

The cultures were incubated in growth chamber at 25±2°C with 16 hrs cool, white and fluorescent light of 2000-2500 lux provided by 40-W fluorescent lamps and relative humidity of about 55±5%. For each treatment, eight replicates were used and every experiment was repeated at least thrice. Data on multiple shoot induction; elongation and rooting were taken and statistically analyzed. Observations were recorded periodically.

The aseptic nodal segments were cultured on MS medium supplemented with 0.25-5.0 mg/l of BAP and Kn alone and/or in combination. Physiological conditions and number of shoots per explants were observed periodically.

In vitro raised shoots measured about 6-7 cm in length with 3-4 nodes were taken out from the culture vials and transferred to half strength MS medium with different concentration (0.5-8.0 mg/l) of auxins like IBA; IAA; 2,4-D; NAA for root induction. The plantlets were taken out from culture vessel without damaging the delicate root system and rinsed with distilled water to remove adhering agar and then transferred to polycups containing vermin compost and autoclaved soil (1:3). High humidity was provided by keeping polycups covered with inverted glass beakers and kept in culture chamber. They were gradually exposed from artificial environmental conditions to natural conditions for their acclimatization.

Result and discussion

Plant development by any explants in vitro is influenced directly by more than one hormone interactions. Many factors influenced the shoot proliferation and its growth like season, in which the explants were collected, physiological state of the plant from which the explants were procured, size and orientation of the explants on the medium. High frequency bud break (92%) was observed in the month of June (Tab. 1). However, picking up the explants from healthy and young mother plant proved to best for the success of the experiments. Where the explants procured were sterilized and inoculated vertically with 0.8-1.0 cm. long, they were found to be optimum.

Plant development is influenced directly by the type, concentration of auxins and cytokinins in the medium. They are basically the signalling molecules for the

Table 1. Effect of seasonal influence on shoot bud induction through axillary and apical meristem in *Bacopa monniera*(L.) Wettst.

Medium	MS+sucrose (3.0%)+BAP(1.0 mg/l
Inoculum	Nodal segments, Shoot tip
Inoculum	At 26±2°C under 16 hours period for four weeks

Month of explant collection	Percentage response	No. of shoot buds/explant *Mean±S.E. $t_{0.05}$	Contamination factor
January	49	20.000±2.000	+
February	49	20.000±2.000	+
March	62	26.000±3.606	+
April	85	47.667±7.371	+
May	88	58.333±2.082	+
June	92	63.667±1.528	++
July	90	59.333±2.082	+++

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