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TISSUE CULTURE STUDIES ON BACOPA MONNIERI (L) PENNELL –A THREATENED MEDICINAL HERB

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ABSTRACT

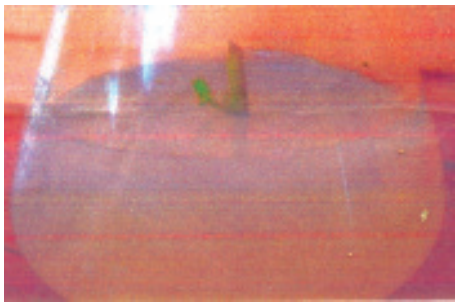
Bacopa monnieri L. Penn. commonly known as "Brahmi" is an important medicinal herb of the family Scrophulariaceae. It is the foremost brain tonic herb of the Indian System of Medicine and other traditional systems, used primarily as a nerve tonic, to treat insomnia and nervous tension. In the present work, protocol for the in vitro regeneration of Bacopa monnieri has been initiated by using nodal culture.Caulogenesis was induced from these nodal segments on MS medium supplemented with BAP (6-Benzyl Amino Purine) 0.5µM and NAA (á Naphthalene Acetic Acid) 0.5µM. Rhizogenesis of these shoots occurred when transferred to the same medium devoid of phytohormones. In vitro regenerated plantlets were healthy with dark green leaves in about 8 to 9 weeks.

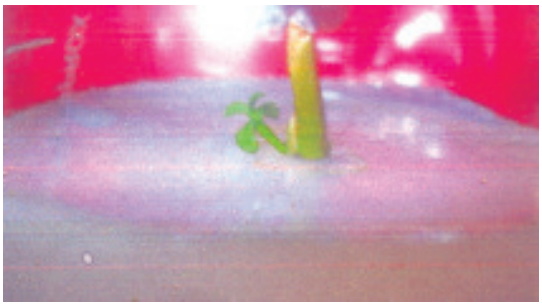
Key words: Nodal culture, *Bacopa monnieri*

Introduction:

Bacopa monnieri (L) belongs to the family Scrophulariaceae is an amphibious plant of tropics and normally found

growing on the banks of the rivers and lakes. It is commonly called as brahmi or jal brahmi in India. It has a great market demand due to its high medicinal uses. Brahmi is considered as the main rejuvenating herb for nerve and brain cells and therefore has played a very important role in Ayurvedic therapies for the treatment of cognitive disorders of aging (Anon, 2004; Russo and Borrelli, 2005; Ernst, 2006). It also has anti-inflammatory, analgesic, antipyretic, epilepsy, insanity, anticancer and antioxidant activities (Jain et al, 1994; Tripathi et al, 1996; Bafna and Balaraman, 2005; Sinha and Saxena, 2006). It is also used for the treatment of asthma It contains different types of saponions like bacosides A, B, C and D





which are active triterpenoid principles and known as "memory chemicals" (Rastogi et al, 2004; 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; Chakravarty et al, 2003). 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 *Bacopa monnieri* 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 is increasing rapidly. In view of the wider market demand, there is a need to conserve this highly endangered medicinal herb.

Materials and Methods:

Explant Collection and surface sterilization—The plantlets were directly obtained from Narkhede Farms, Jalgaon India. The plantlets were kept under ambient conditions. Nodal explants were used for the present study. The explants were washed under running tap water for 1 to 2 minutes and surface sterilization was achieved by 70 % (v/v) ethanol for 30 seconds and 0.1% (w/v) mercuric chloride for 60 seconds. Finally the explants were washed with sterile distilled water.

Culture media and conditions—These explants were inoculated on Basal MS medium fortified with 3% (w/v) sucrose. Medium was gelled with 0.5 to 1.8% (w/v) agar agar type-I (Hi media, Mumbai) and the pH was adjusted to 5.5 to 5.8. The media was autoclaved at 1.05 kg cm^{-2} for 20 minutes, which was further used as a culture medium. After autoclaving thermolabile ingradients like vitamins and phytohormones were added. For initiation of the culture nodal segments about 1.5 to 2 cms were inoculated on MS medium fortified with various concentrations and combinations of 6-Benzyl Aminopurine (BAP) and a Naphthalene

acetic acid (NAA). Each hormonal combination was tried in replicates in 10 test tubes. The cultures were maintained under controlled conditions of temperature of 25°C with humidity level of 60% and photoperiod of 16/8. The white light was provided by white flouroscent tubes (Crompton Greaves, India) at 1000-lux intensity.

The *in vitro* regenerated plantlets were transferred to root trainer containing a mixture of sterile soil and sand (3:1) for hardening. They were irrigated every 24 hours with a solution containing MS salts at half strength. High humidity level was maintained by covering the pots with polythene bags. Progressive puncturing of polythene bags decreased the humidity level. Simultaneously they were exposed to sunlight. After about 8 weeks, the hardened plants were transferred to nursery.

Results and Discussion:—The few earlier reports available on *Bacopa* demonstrated plant regeneration through axillary node, internode and young leaves on media with very high concentrations of cytokinins.(Shrivastava,1999) In this paper we have studied the effect of different concentrations specifically lower concentration of cytokinin on caulogenesis on *Bacopa* . Direct regeneration of caulogenesis occurred in mature explants (nodal segments) on MS – basal medium containing BAP (0.5 µM). These cultures are allowed to grow for four weeks when shoots attain an average height of 80 mm in 65% cultures. The shoots continue to grow till they attain a length of 100 mm in about seven weeks. (Fig-A).Caulogenesis was followed by rhizogenesis on same medium without phytohormones. (Fig-B).The in vitro regenerated 8-10 weeks old plantlets obtained from nodal cultures were hardened and transferred to the field after 15-20 days with survival frequency of 75+ 5 %. (Fig-C and D). Earlier *in vitro* regeneration through somatic embryogenesis (Tiwari et al, 1998), stem and leaf explants (Rajani and Shrivastava, 1999) callus cultures (Neetu Sharma, 2005), internodal segments (Banerjee and Shrivastava, 2008) have been reported. Our results suggest that this protocol can be used for large scale propagation of *Bacopa monnieri* without any seasonal constraints.

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