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OPTIMIZATION OF MS MEDIA FOR MICROPROPAGATION OF AN MEDICINAL PLANT-*BACOPA MONNIERI* (L.)

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ABSTRACT

Bacopa Monnieri L. popularly called as *Brahmi* is an important medicinal plant, traditionally used as tonic to enhance memory development, learning, concentration and to provide relief to patients with anxiety or epileptic disorders (Debnath *et al*, *2006*). Environmental stress severely restricts the distribution and productivity of plants. In particular, salinity and drought are two major abiotic factors that limit crop productivity (Mahajan *et al* 2005). In order to overcome these problems of abiotic stress, tissue culture was used in the present study. Shoot apex and nodal explants of 1-1.2 cm were collected from young plants. They were watered twice a day. Explants were washed with running water for 20 min, immersed in Tween 20 solution for 10 min, washed thrice with doubled distilled water, sterilized using 0.1% (w/v) $HgCl_2$ for 5 min and then rinsed three times with sterile distilled water. *Bacopa monnieri* L. explants were inoculated on MS medium supplemented with 5mg/l BAP. The ph of MS medium was adjusted to 5.8 prior to autoclaving. The cultures were maintained at a temperature of 25+2^{o}C and 16 h photo period. The shoot and nodal explants after 15-20 days showed callogenesis as well as morphogenesis. The proliferating shoots were separated and re-inoculated on MS medium supplemented with 5 mg/l BAP.The growth of *Bacopa* for more than 45 days in tissue culture is a clear indicator that this medicinally important plant can be grown in adverse soil conditions.

KEYWORDS: Nodal explants, MS medium and Shoots proliferation.

INTRODUCTION

Bacopa monnieri L. also referred as, *Herpestis monnieri, water hyssop* and "*Brahmi*", has been used in Ayurvedic system of medicine for centuries (Debnath *et al, 2006*). Traditionally it was used as brain tonic. Bacopa's antioxidant properties may offer protection from free radical, damage in cardiovascular disease and certain types of cancer. (Mukherji and Dey, 1966).

Several industries and nurseries have standardized protocols for the multiplication of ornamentals plants like orchids genera, carnation, roses, chrysanthemum and plantation crop like banana and cardamom. The compounds responsible for the memory enhancing effects of *Bacopa monniera* are triterpenoid saponins called bacosides. The distribution and productivity of plants is restricted by environmental stress. The salinity and drought are two major factors that also limit productivity. Tissue culture is an efficient method to study the effects of abiotic stress on the cell metabolism. Cellular acclimatization to unfavourable environment can facilitate plants for continued survival and growth (Debnath *et al, 2008*). Metabolites that serve as "compatible solutes" differ among plant species and include polyhydroxylated sugar alcohol, (e.g. mannitol, sorbitol, trehalose) free amino acids (notably praline), and quaternary ammonium compounds (e.g. glycinebetaine, prolinebetaine and choline-O-sulfate) and tertiary salfonium compound 3- dimethyl- sulphoniopropionate. These compounds are reported to play pivotal role in celluar osmotic adjustment in response to osmotic and salt stresses. *Bacopa Monnieri* L. is commercially produced due to its immense benefits. *Herpestis monniera* was used as brain tonic to enhance memory development, learning, and concentration and to provide relief to patients with anxiety or epileptic disorders. The plant has also been used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of bronchoconstriction. Bacopa's antioxidant properties may prevent the damage in cardiovascular disease and certain types of cancer caused by free radicals.

The global context briefly suggests several tremendous opportunities for India, a country unrivalled in terms of diversity of systems and practices, in addition to being a major storehouse of biological diversity, with 4 of the 34-mega biodiversity areas of the world located within its borders. The global market would appear to be more receptive than ever to the mounting of a concentrated Indian effort at supplying it with medical materials and know-

how. Such an effort would also appear to be increasingly remunerative for the country. India is of course already an active participant in the global medicinal plants market having been for some time the world's largest supplier of raw materials (though an insignificant supplier of finished products). Moreover, medicinal plants are one of the most important components of the non-wood forest products sector, which supplies over 80% of India's net forest annual export earnings (http://web.idrc.ca/en/ev-21250-201-1-DO_TOPIC.html).

The agro climatic conditions of the country provides an ideal habitat for natural growth of variety of plants and herbs. Plants were the first medicines, and even as modern humans have developed sophisticated pharmaceutical chemicals to treat illness, medicinal plants remain an important tool for treating illness in most cultures. Human beings have been utilizing plants for basic preventive and curative health care. According to a survey carried out by WHO, 80% population of developing countries still rely on traditional medicines, mostly plant-based drugs (Anonymous, 1998). A rich heritage of knowledge on preventive and curative medicines was even available in Atharva Veda, Charkha, Sushruta etc. Moreover 25000 effective plant based formulation available under indigenous medicine. Over one and a half million practitioners of Indian system of Medicine use medicinal plants in preventive, primitive and curative applications. Since the dawn of history man has been in search of ways to find cure and relief from mental and physical illness. Although synthetic organic compounds have contributed in pharmaceutical applications, satisfactory therapy is available only for about one third of all human ailments known at present and several diseases like cancer: AIDS, autoimmune disease continue to evade reasonable solution. The main reason behind the revival of interest in plant based Ayurvedic drugs in recent years in the developed nations and developing countries is the side effects and high rising prices of the Allopathic medicines. Herbal remedies have attained popularity among the common people, due to increasing awareness of personal health maintenance through natural products. The developed nations are also looking for eco-friendly means for treatment of various diseases through plant source.

In India the plant is used for all sorts of skin problems- eczema, psoriasis, boil, ulcerations- it is thought to motivate the growth of skin, hair and nails. Indian Pennywort is also used for chronic rheumatism often as an ointment. In Pakistan, the herbal drug, Brahmi-buti, is used to treat skin diseases, leprosy, epilepsy, eczema, asthma, hoarseness of the voice, and diseases of the nervous system (Shakoor *et al*, 1994).

MATERIALS AND METHODS

Bacopa monnieri L. plant was obtained from the shade house of ethnobotanic garden, Department of Botany, North Lakhimpur College, Assam. They were watered twice a day. The shoot apex and nodal explants of 01-1.5 cm were collected from young plant grown in green house. Explants were washed with running tap water for 20 min, immersed in T-20 solution for 10 min after that washed with doubled distilled water, sterilised using 0.1%(w/v) HgCl2 for 5 min and then rinsed three times with sterilised distilled water. Nodal segments of B. monnieri were disinfected in 70% ethanol for 30s and then in a solution containing 25% sodium hypochlorite: 0.01% Tween-20 for 25 min. After that the segments were rinsed three times with distilled sterile water. Bacopa explants were inoculated on MS medium (Murashige and Skoog,1962) supplemented with 5 mg/L BAP, Agar 30 gm/L was added and the pH of MS medium was adjusted to 5.8 prior to autoclaving. The shoot and nodal explants after 20-30 days showed collogenesis as well as morphogenesis. The proliferating shoots were separated and re-inoculation on MS medium supplemented with 5 gm/L BAP.

RESULTS

In the present study the explants responded to the MS medium supplemented with 5 mg/L BAP. It was found that the nodal explants from 25-30 multiple shoot proliferation along with some callus formation with 15-20 days after sub culturing on the same medium. This shoot started to elongate after 20-25 days. The culture maintained in MS with 5 mg/L BAP and varying concentrations of mannitol also should distinct Morphogenic response such as multiple shoots proliferation with some callusing. Morphological differentiation shifted from shoot organogenesis to root formation with an increase in mannitol concentration in the medium without mannitol 20-25 visible shoot differentiated from the green callus whereas at 2% (w/v) mannitol. Shoots with 2 elongated with 1 cm, 4% (w/v) mannitol. Shoot with 2-3 stouter roots, at 6% (w/v) mannitol shoots with 3-4 roots at 8%(w/v) mannitol 4-5 shoot buds with 3 roots of length 2-3.5 and 10% (w/v) mannitol shoot of 4-5 roots of length 5 cm but no significant increased in shoot growth even after 30 days.

Shoot initiation and established from *Bacopa monnieri* L. nodal explants cultured on MS medium with various combinations of growth regulator i.e BAP alone and also in combination with NAA and Kn is described in Table 1. In the present experiment it was observed that out of the different hormonal combinations, maximum shoot regeneration was obtained with MS+0.5mg/l BAP+10mg/l 2,4,D and MS+10mg/l BAP+1.0mg/l 2,4,D. Future,

both shoots and roots were regenerated in the same medium combination. Therefore, no separate rooting medium was applied for rooting. In addition, it was noted that in the length of the roots were more compared to that of shoot.

Table 1:- Response of Brahmi nodal explants in different combination of media.

Medium concentration	Medium code	Response
MS basal medium (no horman)	M0	+
MS+1mg/LBAP+1mg/L IAA	C1	++
MS+0.5mg/LBAP+0.5mg/L IAA	C2	+++
MS+0.5mg/LBAP+0.5mg/L 2,4-D	C3	+++
MS+0.5mg/LBAP+1mg/L 2,4-D	C4	++++
MS+1mg/LBAP+1mg/L 2,4-D	C5	++++
MS+0.5mg/L 2,4-D	C6	++
MS+1mg/L2,4-D	C7	++
MS+2mg/L2,4-D	C8	++
MS+0.5mg/LNAA	C9	+++
MS+1mg/ LNAA	C10	++
MS+2mg/ LNAA	C11	++

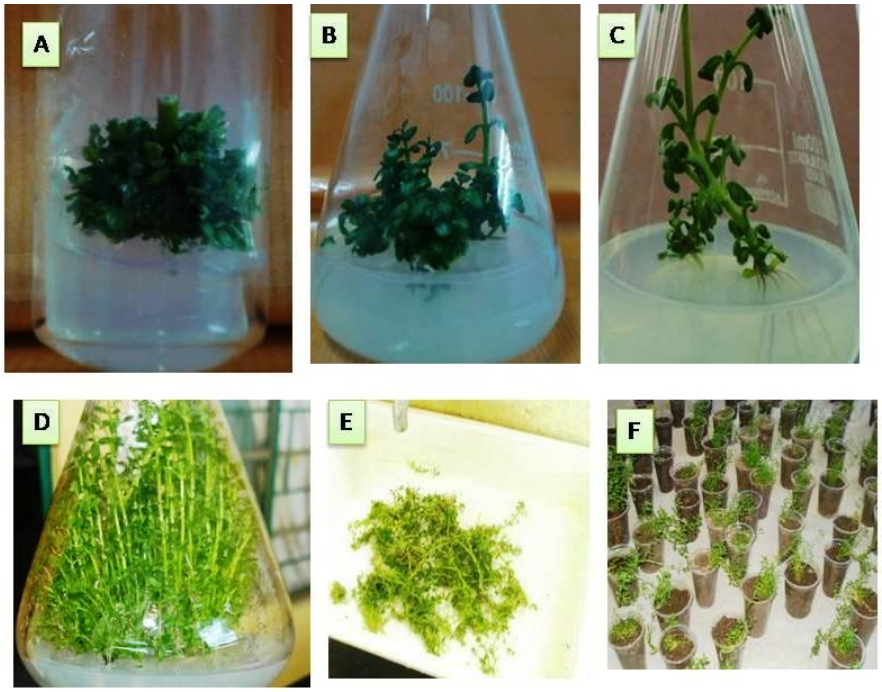

Fig:1: (A) Initiation of Culture (B) Subculture of plantlets (C) Subculture of plantlets (D) Plantlets elongation (E) Washing of regenerated plants (F) Transfer of Brahmi into pots.

DISCUSION

For shoot proliferation, growth regulators especially cytokinins (Lane 1979, Stolz 1979, Bhojwani 1980, Garland & Stolz 1981) are one of the most important factors affecting the

response. A range of cytokinins (Kinetin and BA) has been used in micropropagation work (Bhojwani and Razdan 1993).But a wider survey of the existing literature suggests that BA is the most reliable and useful cytokinin. A number of plants has been successfully multiplied on medium containing BA viz. in white clover (Bhojwani 1981) and hybrid willow (Bhojwani 1980), chickpea (Barna & Wakhlu 1994; Nair *et al*,1979), and *Iresine lindenii* (Sebastin & Barna 2003) BA is the most effective cytokinin for the shoot tip, meristem and bud culture because it overcomes the effect of the terminal (apical) bud and the axillary buds to grow. Abrie and Staden (2001), K.D.Mudoi and M. Borthakur(1998) also reported use of BA in shoot proliferation of bamboo plant. At higher levels cytokinins tends to induce adventitious bud formation (McComb, 1978; Zimmerman and Broome, 1980). But it was observed that the shoot proliferation rate decrease marginally, i.e, 30% response in BA (5.0 mg/L) which is half of the total response of BA (1.0 mg/L). In the present study also recorded shoot proliferation mostly occurred in the presence of cytokinin. Among the cytokinins tested, BA proved to be more effective. About 60% of shoot proliferation was found in medium with BA (1.0 mg/L) with constant light. The least response was shown in Kn (5.0 mg/L) kept under constant dark. Whereas 40% of shoot proliferation was got in medium Kn (1.0 & 2.0 mg/L). Keeping in mind the cost factor of agar, liquid medium containing also used for the shoot proliferation in *Bacopa monnieri.* About 45% of Shoot proliferation was observed in solid medium in compare to the 25% of the shoot proliferation in liquid medium. But use of liquid medium considerably reduces the cost of producing plants for the commercial purposes. During the project often appearance of brown and black colours in the culture mediums was due to delay in sub culturing (gestation period of 25-30 days). Leaf lamina were found to be inconspicuous and condensed which was due to supra optimal concentration of the hormones which are toxic. All the laboratory measure to ensure microbes' free area to let the least infection to be caused during inoculation. Even the explants were dipped in alcohol for few seconds before inoculation. In order to avoid the fungal attack the explants was treated with Oxtetracyc line injection by Martin & Brown Pharmaceuticals.

CONCLUSION

Bacopa monnieri L. is an important medicinal plant of human civilization, especially for the East Asian countries. Once called as poor man's timber is longer so due to mismanagement and monocarpic nature of flowering. But its propagation rate is very slow to meet commercial demand of high quality planting material for its commercial cultivation. So keeping this thing

in mind, micro propagation work is carried out on this plant. The objectives of the present study was to standardize optimum conditions for establishment of standard culture for shoot proliferation, rooting of micro shoots, hardening and transfer of plants to soil. But due to constant infection by endogenous factors and sheer lack of time we were able to complete only first portion of the work, i.e., shoot regeneration.

The conclusions Drawn from this study are,

1. Surface sterilization with mixed $HgCl_2$ (0.1% for 5-minutes) and 70% alcohol dip was best for the surface sterilization of the explants.
2. For the initiation of the culture, MS medium with BA 0.0-5.0 mg/L with Kn 0.0-5.0 mg//L was used.
3. Best shoot proliferation was achieved on MS medium containingBA1.0mg/L with Kn2.0 & 3.0 mg/L.
4. Solid medium with same composition was found to be better than liquid medium for shoot proliferation.
5. Twenty percent shoot showed rooting response on hormone –freemedium.
6. In liquid medium with constant darkness response was found to very poor.
7. Browning can be prevented by following the gestation period.
8. Shrinking of leaf lamina is avoided by giving proper amount of Phytohormones.
9. Anti fungal medicines can be used against fungal infection.
10. There exist certain endogenous factors that cause infections.

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