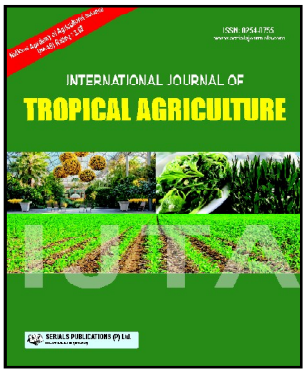

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Effect of Different Concentrations of Iba in Combination With Ba on Shoot Proliferation of *Bacopa Monnieri* (Brahmi)

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Abstract: A lab experiment was carried out during summer season of 2011-12 at Dept. of Plant Biotechnology, MGM College of Agricultural Biotechnology, Aurangabad to study effect of different concentrations of IBA in combination with BA on shoot proliferation of *Bacopa monnieri (*Brahmi) under in *vitro* condition. The experiment was laid out in completely randomized block design with five treatments of IBA (0.00, 0.25, 0.30, 0.35, 0.40 µM) with constant BA (1.1µM) MS basal medium on nodal segment of Brahmi. Among IBA 0.30µM with constant BA (1.1µM) showed significantly higher shoot proliferation. On an average within a period of 3 subcultures more than 39000 shoots can be produced from single nodal segment. Thus present protocol can be used to generate foundation stock of elite planting material for large scale cultivation.

Keywords: *Bacopa monneri, shoot proliferation, BA, IBA,MS (media).*

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most the drug industries depends on part of plants for the production of pharmaceutical compounds (Chand., 1997). Among the world's 25 best selling pharmaceutical medicines, 12 are plant derived (O'Neill and Lewis 1993). *Bacopa monnieri* [L.] Pennell is one of the most important medicinal plant belonging to the family Scrophulariaceae originated from India and Srilanka. It is an amphibious plant of the tropics and normally found growing on the banks of rivers and lakes. It is commonly known as Bramhi or Jala-bramhi in India. It is small creeping, glabrous and succulent herb with thick, soft, ascending branches and sessile, obovate ablong or spatulate leaves; whitish blue flowers with purple veins on long pedicles.

This plant is known to grow under varying soil and climatic conditions. It grows exceptionally well in poorly drained soils and waterlogged areas under sub-tropical conditions. The plants grow faster at high temperatures (33-40°C) and (65-80%) humidity and should be cultivated in summer-rainy season. In India Bramhi is cultivated in the area of Uttar Pradesh, Punjab, Haryana, Bihar, Bengal, Tamil Nadu, Kerala, Karnataka, Foot hills of Himachal Pradesh and Uttaranchal.

More than 90% of plant species used by the industry are however collected from the wild source of which 70% involves unorganized harvesting. This factor poses a serious threat to the genetic stock and the biodiversity of medicinal plant. Natural regeneration is also hampered by death of plant at two leaf stage and specific habitat requirement. The submerged shoots of Bacopa can hardly attain the required growth and multiplication. The role of extinction of medicinally important plant species is further accelerated by habitat degradation, illegal trade practices, and loss of regeneration potential of degraded forests, policies and regulations. Recent reports of National Medicinal Plant Board (NMPB), 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 (http://www.nmpb.nic.in/prioritisedmedicinalplants.htm). Hence there is need to conserve Bramhi and provide uniform, disease free seedlings to the farmer. Micropropogation provide uniform, disease free seedlings throughout the year.

Plant growth regulators play an important role in micro propagation. Cytokinins and auxins are the group of plant growth hormones and the ratios of these two groups of plant hormones affect most major growth periods during a plant's lifetime. Cytokinin influence cell division and shoot formation and also responsible for mediating auxin transport throughout the plant. They have a highly synergistic effect in concern with auxin. Auxin influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins. BA (6-Benzylaminopurine) is a first-generation synthetic cytokinin which elicits plant growth and development responses by stimulating cell division and induces shoots when incorporated in tissue culture media. IBA (Indole-3-butyric acid) is 0used in the same manner as IAA and is accepted around the world as a propagating and rooting hormone for ornamental and fruit grafting and cuttings. It is especially effective for initiating roots of both stems and leaves (Anonymous).

Considering the above points an experiment entitled "Effect of different concentrations of IBA in combination with BA on shoot proliferation of Bacopa monnieri (Brahmi)" was planned during the November 2011 to April 2012 in MGM College of Agricultural Biotechnology, Aurangabad with the following objectives.

1. To study the effect of Auxin on shoot proliferation in combination with constant cytokinines under *in vitro* condition.
2. To find out optimum concentration of IBA in combination with BA for shoot proliferation in *in vitro* condition.

MATERIALS AND METHODS

The details of various material used and experimental methods adopted during the course of present investigation are narrated in this chapter under suitable sub-heads.

Media Requirements

Nutritional requirement for optimal growth of a tissue in *vitro* may vary with the species. As such, no single medium can be suggested as being entirely satisfactory for all types of plant tissues and organs. When starting with a new system it is essential to work out a medium that would fulfill the requirement of that tissue. In order to formulate a suitable medium

for a new system it would be better to start with a well known medium such as MS. By making minor changes, through a series of experiments, a new medium may be evolved to accommodate specific requirements of the plant material in question. The concentrated stocks of the major salts, minor salts and growth regulators were prepared and stored under refrigeration. Auxins were prepared by dissolving in 1N KOH and cytokinins in 1 N HCl before making up the final volume with distilled water.

Auxins are generally used in plant cell culture at a concentration range of 0.01-10.0 mg/l. When added in appropriate concentrations they may regulate cell elongation, tissue swelling, cell division, formation of adventitious roots and inhibition of adventitious and axillary shoot formation, callus initiation and growth, and induction of embryogenesis (S. K. Singh, 2005)

Cytokinins are generally used in plant cell culture at a concentration range of 0.1-10.0 mg/l. When added in appropriate concentrations they may regulate cell division, stimulate auxiliary and adventitious shoot proliferation, regulate differentiation, inhibit root formation, activate RNA synthesis, and stimulate protein and enzyme activity (S. K. Singh, 2005).

Media Preparation

The medium was prepared by adding required amounts of stock solutions and final volume was made up with distilled water. The pH is adjusted to 5.8 and agar was used for solidifying the medium. 20 ml media was poured into test tubes. They were then autoclaved at 121°C for 20 minutes at 15 psi pressure and transferred to the media storage room where they were kept under aseptic conditions till their further use.

Media

Media was prepared by using MS basal medium + BA (1.1 µM) + IBA (Variable Conc. 0.00, 0.25, 0.30, 0.35 and 0.40, µM) + Sucrose 3% + 0.65% Agar. The pH of media was adjusted to 5.6 - 5.8.

Experimental Details

The plants of *Bacopa monnieri* were collected from Shirdi, Dist. Ahmednagar (M.S.) area and planted in nursery of MGM College of Agricultural Biotechnology Aurangabad Experiment was conducted with Completely Randomized Design (CRD) with five treatment i.e IBA concentration T_0 (0.00), T_1 (0.25), T_2 (0.30), T_3 (0.35), T_4 (0.40) with four replication in plant tissue culture laboratory of MGM College of Agricultural Biotechnology, Aurangabad.

Explant Selection and Sterilization

The disease free, young and healthy nodal explants were selected for carrying out study as young cells are supposed to have retained their totipotency.

The healthy, disease free, young nodal explants were selected for experimentation. Each explant contain two nodes. Explants were cut and washed under tap water for 5 min in order to wash off the external dust/contaminants. Then explants was washed with 2% Tween-20 solution for 15 min followed by 20 min tap water washing followed by repeated rinsing with distilled water for 5 min. After that explant was transferred to laminar air flow for further sterilization with 70% (v/v) ethanol for few seconds followed by 5 min washing with sterilized double distilled water. The explants were treated with 0.2% sodium hypochloride solution and washed with sterilized double distilled water for 5 times. Then explants were treated with 0.2% Bavistin for 5 min followed by 5 sterilized double distilled water rinsing. Further sterilization was done with 0.01% (w/v) $HgCl_2$ for 5 min. Finally the explants were washed with sterilized double distilled water and placed in sterilized double distilled water. After sterilised explants were trimmed and inoculated on MS media supplemented with different combination of IBA and 1.1 µM BA and incubated in culture room at

25 ± 2°C temperature with 16 hours photoperiod (Patni *et. al.,* 2010).

Explants were observed weekly interval in respect to callus initiation at the base of explants and number of shoot bud initiated from callus, etc.

RESULTS AND DISCUSSION

The various growth aspects of *Bacopa monnieri* as influenced by different concentrations of auxin in combination with cytokinins under *invitro* conditions have been studied in detail and the results of these findings are presented in this chapter.

Callus Formation

Callus initiation was observed at the base of nodal segment after 14 days of inoculation in all treatments except treatment T_2 (Figure 1). However in treatment T_2 (IBA 0.30 µM) callus initiation was observed 3 days earlier as compared to other treatments. This indicated that concentration of IBA 0.30 µM with BA 1.1 µM produced ethylene earlier than other treatments. Ethylene enhances radial cell expansion leading to callus formation (S. K. Singh, 2005).

Shoot Proliferation

Data on mean number of shoots per explants at various stages of growth are presented in Table.

Mean number of shoots per explant at 21, 28 and 35 DAI was 33.2, 52.6 and 77.4 respectively.

Data presented in Table indicated that shoot proliferation was not influenced significantly due to various concentrations of IBA at 21 DAI. However IBA concentration of 0.30 µM recorded numerically higher shoot number as compared to other treatments. Non- significant differences among the different treatments in relation to number of shoots per explants might be due to slow initiation of shoots from explants, *i.e.* due to lag phase.

The no. of shoots per explant was influenced significantly due to different concentration of IBA at 28 and 35 DAI.

Table 1
Mean number of shoots per explants as influenced by different concentration of IBA

Treatments	*21 DAI*	*28 DAI*	*35 DAI*
T_0 (0.00 µM)	5.50	9.00	18.75
T_1 (0.25 µM)	8.50	14.00	29.50
T_2 (0.30 µM)	12.00	17.25	34.50
T_3 (0.35 µM)	8.00	13.25	28.00
T_4 (0.40 µM)	7.50	12.25	23.50
Mean	8.3	13.15	19.35
S.E±	7.21	0.94	0.99
C.D.	NS	2.80	2.98

The IBA concentration of 0.30 µM recorded maximum shoot number (17.25) and found significantly superior over rest treatments. IBA concentration of 0.25 µM, 0.35 µM and 0.40 µM did not differed significantly in respect to no. of shoots per explant and found significantly superior over control. It indicated that that significant response of IBA concentration on shoot proliferation was upto T_2 (0.30 µM) and the shoot number declined their after. This might be due to optimum ratio of BA and IBA concentration helped for enhancing shoot number of explants of Bacopa monieri.

F.C. Steward, 1972 also reported the beneficial effects of Cytokinin and Auxin (optimum ratio) on shoot proliferation of plant. At 35 DAI IBA concentration (0.30 µM) recorded maximum shoots per explant (34.50) and found significantly superior over rest of the treatments. The IBA concentration of 0.25 µM and 0.35 µM were at par and both recorded significantly higher number of shoots per explants over IBA concentration of 0.40 µM and control. Similarly IBA concentration of 0.40 µM recorded significantly higher number of shoot per explant over control, *i.e.* no IBA.

Higher number of shoots per explant in treatment T_2 (0.30 µM) indicated that the inter action effect of BA (1.1 µM) and IBA (0.30 µM) respectively was found beneficial. This was due to synergistic

effect of Auxin and cytokinin on growth of tissue fevered cell expansion in "Pre aged tissue and cell division by the presence of Ca and Mg ions. Butenko (1996) also reported beneficial effects in combination with cytokinins on shoot proliferation in Helianthus tubrasum.

Nishita *et. al* 2006 also reported higher number of shoots per explant of *Chonemorpha grandiflora* when treated with BA and IBA in optimum ratio as compare to separate use of BA as well as IBA.

CONCLUSIONS

Present investigation showed IBA (0.30 µM) in combination with BA 1.1 µM recorded highest result in no. of shoots as compared to control as well as other concentration of IBA. That's why the optimum concentration of IBA 0.30 µM in combination with BA1.1 µM required for maximum shoot induction in combination with constant BA (1.1 µM).

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