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Effect of Different Concentrations of Iba in Combination with Ba on Biomass Yield 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 Biomass production 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 μ M) with constant BA (1.1 μ M) MS basal medium on nodal segment of Brahmi. Among IBA 0.30 μ M with constant BA (1.1 μ M) showed significantly higher Biomass production. 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.

Keyword: *Bacopa monnieri*, Biomass, 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 bestselling 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.

Brahmi is also known as "Medhya rasayanas" in *ayurveda* as it increases mental clarity and brain stimulating action (Bhattacharya and Ghosal 1998).

The medicinal properties of *Bacopa monnieri* responsible for improving memory-related function have been attributed to the presence of different types of saponins such as Bacosides A, B, C, and D called the “memory chemicals” (Rastogi 1994). *Bacopa* also contains variety of medically active substances *i.e.* stigma sterol, sapogenins and flavonoids. Other compounds are D-mannitol, betulic acid, beta-sisterol, octacosane, nicotine and amino acid. It also possesses anti-inflammatory, analgesic, antipretic, epilepsy, insanity, anticancer and antioxidant activities (Satyavati., 1976; Jain., 1994; Elanngovan 1995; Tripathi., 1996). It also used for the treatment of asthma, water retention and blood clearing. Leaf juice of brahmi is given to children for relief in bronchitis and diarrhoea. In Pakistan, the herbal drugs, Brahmi-buti, is used to treat skin diseases, leprosy, epilepsy, eczeme, asthma, hoarseness of the voice, and diseases of the nervous system (Shakoor 1994).

It has a great market demand due to its high medicinal value. Moreover, because of the heavy demand and short supply, it is the most adulterated species in *Ayurvedic* formulations. So there is need have to mass-propagation of selected clones.

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 used in the same

manner as IAA and is accepted around the world as a propagating and rooting hormone for ornamental and fruit graftings and cuttings. It is especially effective for initiating roots of both stems and leaves (Annonymus).

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 T₀ (0.00), T₁ (0.25), T₂ (0.30), T₃ (0.35), T₄ (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) HgCl₂ for 5 min. Finally the explants were washed with sterilized double distilled water and placed in sterilized double distilled water. After sterilization 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 T₂ (Figure 1). However in treatment T₂ (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).

Number of Nodes

Data on mean no of nodes per explants as influenced various treatment of IBA concentration were given in table no 2. Mean node no per explant at 35 DAI was 55.50.

Data presented in Table 2 revealed that mean node number of explant of *Bacopa monnieri* was influenced significantly due to different concentrations of IBA at 35 DAI. IBA concentration of 0.30 μM with BA 1.1 μM recorded highest node number of per explant and found significantly superior over rest of the IBA concentrations as well as control.

IBA concentration of 0.25 μM and 0.35 μM were on at par with each other and recorded significantly higher node number over IBA concentration of 0.40 μM and control. Similarly IBA concentration of 0.40 μM proved significantly superior over control.

This was due to symbiotic effect of auxin and cytokinin on growth of tissue cell expansion and cell division by the presence of Mg and Ca ion. similar result also reported by Butenko (1963) and Nishitha *et.al* (2006) with regard to higher node number.

Number of Leaves

Data on mean number of functional leaves per explant as influenced by various treatments of IBA concentration are given in Table 1.

The mean number of functional leaves per shoot of explant at 35 DAI was 111.2

Data presented in Table 1 revealed that mean number of functional leaves per explant of *Bacopa monnieri* were influenced significantly due to different concentration of IBA at 35 DAI. IBA

concentration 0.30 μM recorded highest number of functional leaves per explant and found significantly superior over rest of Treatments.

IBA concentration of 0.25 μM and 0.35 μM did not differ significantly with leaves per explants regards to mean no of explants but both were found significantly superior over 0.40 μM and control. The control treatment proved significantly inferior as compare to rest of the treatments in regarding no. of leaves per explants. This was due to more no. of nodes per explants in 0.30 μM of IBA in combination with 1.1 μM BA as no of nodes has a positive correlation with number of leaves per explants.

Table 1
Mean number of nodes, number of leaves, shoot length of explant as influenced by various concentration of IBA.

Treatments	Number of nodes	Number of Shoot leaves	Shoot length (cm)
T ₀ (0.00 mM)	41.00	82.50	5.90
T ₁ (0.25 mM)	59.00	118	9.15
T ₂ (0.30 mM)	72.00	143	15.00
T ₃ (0.35 mM)	57.30	116	21.00
T ₄ (0.40 mM)	47.25	95	11.13
Mean	55.50	111.2	12.6
S.E \pm	1.39	2.5	0.8
C.D 4.1	10.0	3.6	

Shoot Length

Data on mean shoot length (cm) of main shoot as influenced by various treatments of IBA concentration are given in Table number 1. The mean shoot length of explant at 35 DAI was at 12.6.

Data presented in Table 2 revealed that mean length (cm) of main shoot was influenced significantly due to different treatments at 35 DAI. IBA concentration of 0.35 μM recorded maximum length of main shoot and found significantly superior over rest of other treatment.

Similarly IBA concentration of 0.30 μM also recorded higher length of main shoot over 0.25 μM and 0.40 μM and control IBA concentration 0.25 μM did not differ significantly but both showed higher length of main shoot over control (BA 1.1 μM).

It indicated that positive response of IBA treatment was upto 0.35 μM due to main shoot was recorded significantly due to 0.40 μM . this might be due to production of gibberellin in plant which enhanced inter nodal distance.

Fresh Weight and Dry Matter of Plant

Data on mean fresh weight and dry matter of explants recorded at 35 DAI at various stages of growth are presented in Table 2.

Mean fresh and dry weight of explants recorded at 35 DAI was 4.3 gms and 0.41 gms respectively.

Data presented in Table 2 would indicated that the fresh and dry matter of the explant was influenced significantly due to different concentrations of IBA. Treatment T_2 (0.30 μM) recorded significantly higher fresh and dry weight of explants over rest of treatments. Similarly treatment T_3 (0.35 μM) proved significantly superior over T_1 , T_4 and control. However IBA concentration of 0.25 μM and 0.40 μM were at par and both recorded significantly higher fresh and dry weight over control. It indicated that the response of IBA concentration was up to 0.30 μM for increasing fresh and dry weight of explants of *Bacopa monnieri*.

This is due to higher no. of shoots and leaf no. in the treatment T_2 (0.30 μM). As number of shoots as well as number of leaves has positive correlation with total dry matter accumulation. This was due to synergistic effect of use of auxin and cytokinin on growth of tissue, cell expansion and cell division by the presence of Mg and Ca by increasing number of shoot per explant and leaf number which resulted in higher explants of *Bacopa monnieri*. Similar result also reported by Butenko (1973) and Nishita *et. al.* (2006).

Table 2
Mean number of fresh weight and dry weight of explant as influenced by various concentration of IBA.

Treatments	Fresh weight (gm)	Dry weight (gm)	Per cent of dry wt.
T_0 (0.00 μM)	3.10	0.29	9.35
T_1 (0.25 μM)	4.20	0.40	9.50
T_2 (0.30 μM)	5.38	0.77	14.58
T_3 (0.35 μM)	5.28	0.59	11.17
T_4 (0.40 μM)	3.70	0.40	10.81
S.E \pm	0.24	0.025	
C.D	1.33	0.10	

CONCLUSIONS

Based on various observation of present investigation following conclusion are drawn:

1. The optimum concentration of IBA 0.30 μM in combination with BA 1.1 μM required for maximum Biomass yield in combination with constant BA (1.1 μM).
2. The optimum concentration of IBA produce higher biomass.

REFERENCES

- Anonymous (1972), Plant Physiology Book F. C. Steward.
- Anonymous (2005), Plant Physiology Book S.K. Singh.
- Anonymous (2006), Plant Physiology Book by Sinha and Pandey
- Anonymous (2008), www.nmpb.nic.in
- Bhattacharya, S.K. and Ghosal, S. (1998), Anxiolytic activity of *Bacopa monnieri* An experimental study. *Phytomed*, **5**: 77-82.
- Binita, B.C.; Dave, M.A. and Jasraj, Y.T. (2005), *B. monnieri*: rapid efficient and cost effective micropropagation. *Plant tissue culture and Biotech*, **15**(2): 167-175.
- Chand, S.; Sahrawat, A.K. and Prakash, D.V. (1997), In vitro culture of *pimpinella anisum* L.(anise) J. P.J. *Biochem Biotech*, **6**: 1-5.

- Elangovan, V.; Govindasamy, S.; Ramamoorthy, N. and Balasubramanian, K. (1995). In vitro studies on the anticancer activity of *Bacopa monnieri*. *Fitoterapia* **66**(3): 211-215.
- Jain, P. and Kulshreshtha. (1993), Bacoside A1 a minor saponin from *Bacopa monniera*. *Phytochemistry*, **33**: pp. 449-451.
- Morel, G.M. (1960), Producing virus free Cymbidiom. *American Orchid Society Buletin* **29**: 495-497 .
- Murishige, T. and Skoog, F. (1962), A revised medium for rapid growth and biosassay with tobacco tissue cultures. *Plant Physiology*, **15**: 473-497.
- Murishige, T. (1974), Plant propagation through tissue culture. *Plant Physiology*, **25**: 135- 166.
- Nishitha, I. K.; Martin, P. K.; Ligimol; Shahanaz Beegum, A.; Madhusoodan, P. V. (2006), Micropropagation and encapsulation of medicinally important *Chonemorpha grandiflora*. *In vitro* cellular and developmental biology-Plant, **42**: 385-388.
- Panse, V.G. and Sukhathme, P.V. (1967), *Statistical Methods for Agricultural Workers*, ICAR Publication, New Delhi.
- O Neil, M. and Lewis, A. (1993), Human medicinal agent from plants in kinghorn AD balandrin MF. *Acs Symposium series* 534, Washington, DC. PP. 48.
- Patni, S.; Yaseer, Z.; Suhail, A. and Shamsuddin, J. (2010), *In vitro* Propagation and Callus Formation of *Bacopa monnieri* (L.) Penn. *Plant Tissue Cult. and Biotech* **20**(2): 119-125.
- Rastogi, S.; Mehrotra, B.N. and kulshreshtaha, D.K. (1994), proceeding 4th international congress of ethnobiology deep publication, New Delhi, pp. 93.
- Sattayavati, G.V.; Raina, M. K. and Sharma, M. (1976), *Indian medicinal plants vol-1* Indian council of medical research , New delhi, pp. 20-35.
- Shakoor, Abdul.; Akram,Mahmood.; Asharaf, C.M. and Siddiqui, M.R. (1994), Pharmagonistic study and chemical/pharmacological evaluation of Brahmi, **37**(3): pp. 92-109.
- Tiwari, V.; Tiwari, K.N. and Singh, B.D. (2000), Suitability of Liquid cultures for *in vitro* multiplication of *Bacopa monniera* Linn. *Wettst. Phytomorphology* **50**(3&4):
- Tripathi, Y.B.; Chaurasia, S.; Tripathi, E.; Upadhyay, A. and Dubey, G.P. (1996), *Bacopa monnieri* L. as an antioxidant mechanism of action. *Indian Journal of Experimental Biology* **4**(6): pp. 523-526.