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An effective method for high frequency Multiple shoots regeneration and callus induction of *Bacopa monnieri* (L.) Pennel.: An important medicinal plant

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ABSTRACT

This review highlights the recent development and achievements made for the multiple shoots regeneration and callus induction of Bacopa monnieri. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments were highest in MS medium supplemented with 0.5 mg/l BAP + 2.0 mg/l Kn and 0.5 mg/l Kn+1.0 mg/l BAP. For rooting different concentration of IBA were used and highest rooting was recorded on MS medium supplemented with 2.0 mg/l IBA. The rooted Plantlets were hardened initially in culture room conditions and then transferred to misthouse. Leaf petiole explants were used for the purpose of callus induction. Best growth was observed in MS medium supplemented with 0.25 mg/l 2, 4-D+ 0.5 mg/l Kn and 0.25 mg/l 2,4-D+ 0.1mg/l BAP.

Keywords: Shoot multiplication, Brahmi, Medicinal plant, Micropropagation, Callus induction.

INTRODUCTION

Bacopa monnieri (L.), commonly known as ‘‘Brahmi’’, is a member of the Family Scrophulariaceae, is placed second in the priority list of Indian medicinal plants [1]. It is commonly found on the banks of rivers and lakes. It has been used for centuries in folklore and traditional system of medicine as a memory enhancer, anti-inflammatory, analgesic, antipyretic, sedative and anti-epileptic agent. The memory enhancing effects of *Bacopa monnieri* have been attributed to the active constituent bacosides A and B [2]. In addition to its unique medicinal use, *Bacopa monnieri* has also been linked to phytoremediation programmes for the removal of heavy metals such as cadmium and chromium.

In 1990, the annual requirement of this plant was 12.7 × 106 kg of dry biomass at a value of $34 million [3]. With increasing demand for herbal drugs, the natural populations of *Bacopa monnieri* are threatened with overexploitation. So The International Union for Conservation of Natural and National Resources has a long time ago listed *Bacopa monnieri* as a threatened species [4]. There is a demand for further improvement in the tissue culture protocol for the mass multiplication of *Bacopa monnieri*, both for commercial farming system and later, if required for replanting in the natural habitat when the plant population declines. We have developed an innovative micropropagation protocol that has not been attempted so far in *Bacopa monnieri*.

The aim of the present study was to develop high frequency multiple shoots regeneration of *Bacopa monnieri* utilizing the least number and various concentrations of PGRs. This protocol also offers the rapid callus formation from the leaf petiole. For both purposes Auxin (2, 4-D) and cytokine (kn., BAP) are used.

MATERIALS AND METHODS

The branches (about 5-6 cm) of shoots of *Bacopa monnieri* plants were collected from the Herbal Garden, Kota. The branches with node explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing them five times with double distilled water inside the Laminar Air flow chamber. Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same experiments were repeated for shoot multiplication. The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were subcultured on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations and combination of BAP and Kn. Among these, the maximum number of shoots (3.42±0.39) was developed on MS media fortified with 0.5 BAP+3.0 Kn. Maximum shoot length was observed as 7.54±0.31cm.of a medium supplemented with 0.5 BAP+3.0 Kn. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (0.5-2.5 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose. The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with Soilrite composition (soil: sand: peat moss) and irrigated with 1/4 MS salt solution. These bottles were kept in controlled environmental conditions of culture room. After 3 wk of growth, the plantlets were transferred to misthouse for further growth.

RESULTS AND DISCUSSION

The nodal explants, when inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 1.0 mg/l concentration evoked best response. Shoots after their initial proliferation on medium containing 1.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than Kn and the maximum number of shoots were obtained on its 1.0 mg/l concentration (Table 1, Fig. 1- A, B, Fig. 2). When BAP was used in combination with Kn a variety of responses were observed (Table 2, 3 Fig. 1-C, D, Fig. 3). But best response was observed on medium containing 0.5 mg/l BAP + 2.0 mg/l Kn (Average number of shoots 3.42±0.39, shoot length 7.54±0.31 cm) and in another combination of Kn and BAP, best response was observed on medium containing 0.5 mg/l Kn+1.0 mg/l BAP (Average number of shoots 4.98±0.74, shoot length 3.06±0.22 cm). The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins.

Auxins in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 2.0mg/l IBA, where roots measuring 1.68±0.32 cm (average) were formed (Table 4, Fig. 1-E, Fig. 5). *In vitro* rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly (Fig. G). Leaf petiole explants were used for the purpose of callus induction. Highest diameter of callus was observed on MS Medium fortified with 0.25 mg/l 2,4-D+ 0.5 mg/l Kn (callus diameter 3.6 cm) and 0.25 mg/l 2,4-D+ 0.1mg/l BAP (callus diameter 3.2 cm), (Table 5, Fig. H-I).

Table-1: Effect of Cytokinin (BAP and Kn) on shoot proliferation from Nodal shoot explant of *Bacopa monnieri*

Hormone Con. (mg/ l) BAP	Hormone Con. (mg/ l) Kn	Response (%)	No. of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
1.0	-	80	3.42±0.58	6.51±0.76
2.0	-	70	2.28±0.71	6.56±0.84
3.0	-	65	2.71±0.56	7.62±0.53
4.0	-	55	3.28±0.36	5.08±0.51
5.0	-	40	2.85±0.51	3.31±0.33
-	1.0	55	2.28±0.36	6.47±0.29
-	2.0	75	2.42±0.39	6.30±0.26
-	3.0	60	1.85±0.27	6.15±0.24
-	4.0	40	1.57±0.40	5.70±0.41
-	5.0	30	1.28±0.36	4.92±0.51

Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-2: Interactive effect of Cytokinin (BAP+ Kn) on shoot multiplication by Subculture of shoot clumps of *Bacopa monnieri*

Hormone Con. (mg/ l)	No. of Shoot/explant	Shoot length (in cm)	Shooting Response (%)
0.5 BAP + 0.5 Kn	1.71±0.38	3.70±0.28	70
0.5 BAP + 1.0 Kn	2.14±0.51	4.71±0.29	80
0.5 BAP + 2.0 Kn	3.42±0.39	7.54±0.31	90
0.5 BAP + 3.0 Kn	2.70±0.36	5.70±0.41	85
0.5 BAP + 4.0 Kn	2.57±0.40	6.70± 0.39	82

Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-3: Interactive effect of Cytokinin (Kn+ BAP) on shoot multiplication by Subculture of shoot clumps of *Bacopa monnieri*

Hormone Con. (mg/ l)	No. of Shoot/explant	Shoot length (in cm)	Shooting Response (%)
0.5 Kn+0.5 BAP	2.26±0.24	3.70±0.28	70
0.5 Kn+1.0 BAP	4.98±0.74	3.06±0.22	95
0.5 Kn+2.0 BAP	2.31±0.48	3.87±0.39	80
0.5 Kn+3.0 BAP	3.78±0.57	3.06±0.22	85
0.5 Kn+4.0 BAP	2.26±0.24	2.60±0.51	83

Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-4: Effect of Auxin (IBA) on root induction from isolated shoot of *Bacopa monnieri*

Hormone Con. (mg/ l)	No. of roots/explants	Root length (in cm)	Rooting Response (%)
0.5 IBA	1.08±0.19	0.43±0.33	78
1.0 IBA	1.38±0.37	0.92±0.10	80
1.5 IBA	1.40±0.52	1.06±0.08	85
2.0 IBA	3.60±0.51	1.68±0.32	95
2.5 IBA	2.80±0.73	0.51±0.05	73

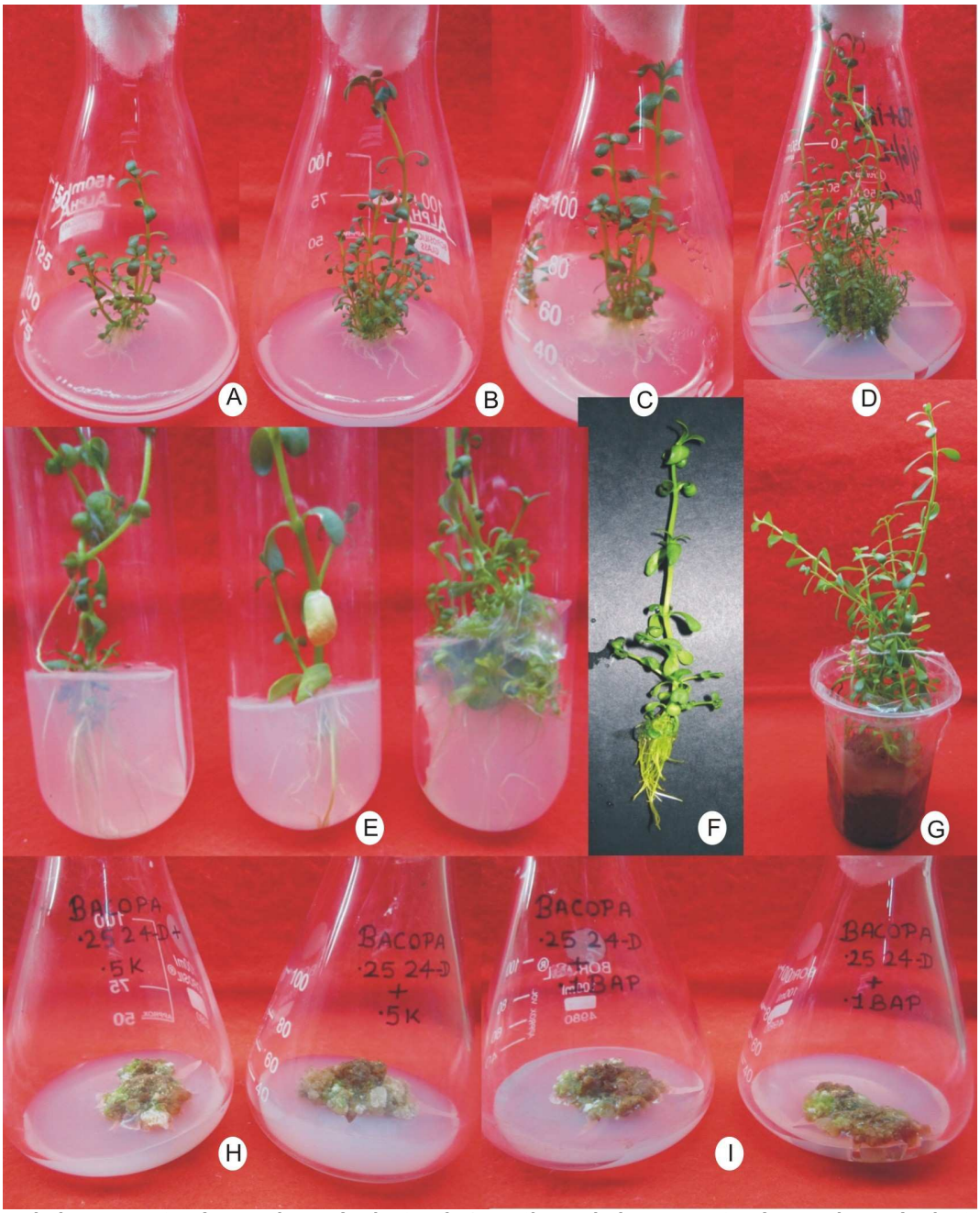
Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-5: Effect of different Hormones on Callus proliferation and Morphology

Hormone Conc. (mg/l)	Callus diameter after 7 weeks subculture (cm)	Callus proliferation Scoring	Color of callus	Morphology of callus
2,4-D + Kn				
0.25 + 0.3	1.9	++	Brownish	Friable
0.25 + 0.4	2.4	+++	Whitish brown	Nodular
0.25 + 0.5	3.6	++++	Brownish green	Compact
2,4-D + BAP				
0.25 + 0.1	3.2	++++	Dark Brownish green	Trodden
0.25 + 0.2	2.4	++	Whitish green	Friable
0.25 + 0.3	2.6	+++	Brownish green	Nodular

'++++' *Intense*, '+++' *Moderate*, '++' *Meager*

Figure 1 (A-G) Micropropagation of *Bacopa monnieri* from nodal shoot explants



***A.** Shoot multiplication on MS medium supplemented with 1.0 mg/l BAP, **B.** Shoot multiplication on MS medium supplemented with 2.0 mg/l Kn, **C.** Shoot multiplication on MS medium supplemented with 0.5 mg/l BAP+2.0 mg/l Kn, **D.** Shoot multiplication on MS medium supplemented with 0.5 mg/l Kn+1.0 mg/l BAP, **E.** In vitro root induction on ¼ of MS medium supplemented with 2.0 mg/l IBA, **F.** 4 weeks old rooted plant for hardening, **G.** hardened plant growing on soilrite moistened with basal medium, **H**. Callus induction from leaf petiole on MS medium supplemented with 0.25 mg/l 2,4-D +0.5 mg/l Kn, **I.** Callus induction from leaf petiole on MS medium supplemented with 0.25 mg/l 2,4-D +0.1 mg/l BAP.*

Figure-2: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Bacopa monnieri*

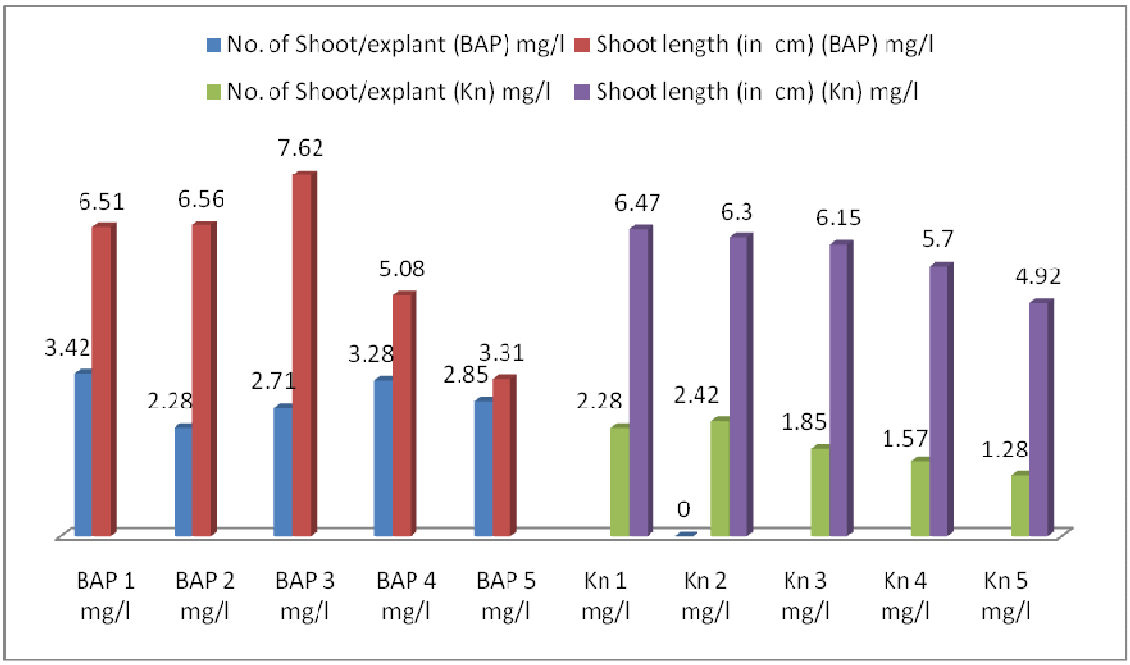


Figure-3: Interactive effect of cytokine (BAP + Kn) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

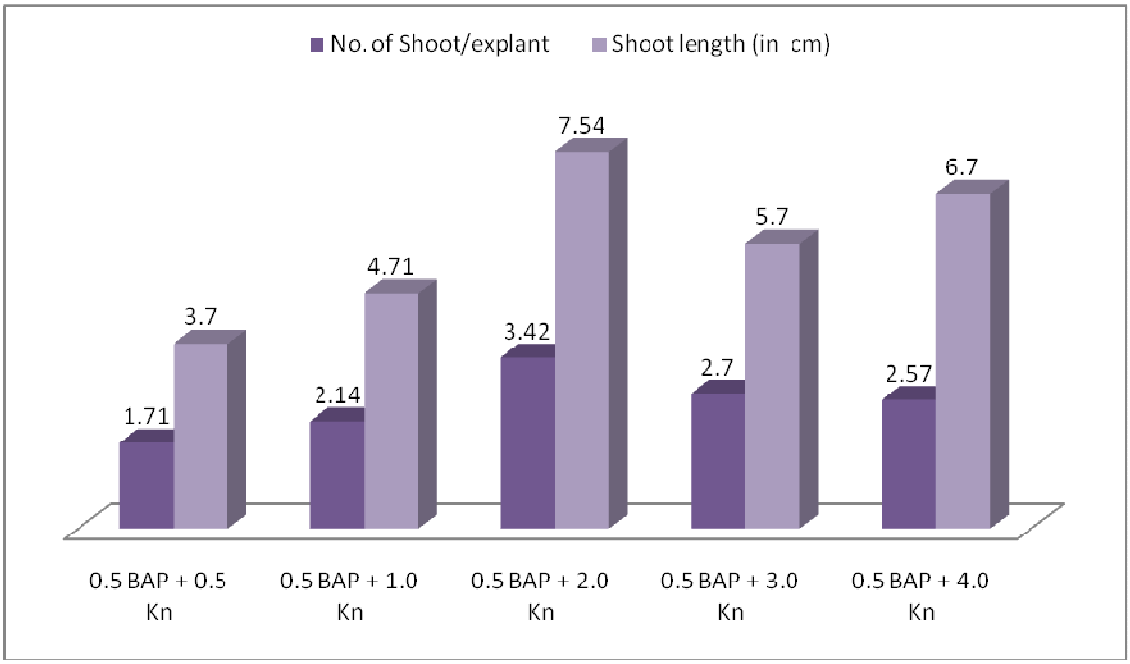


Figure-4: Interactive effect of cytokine (Kn + BAP) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

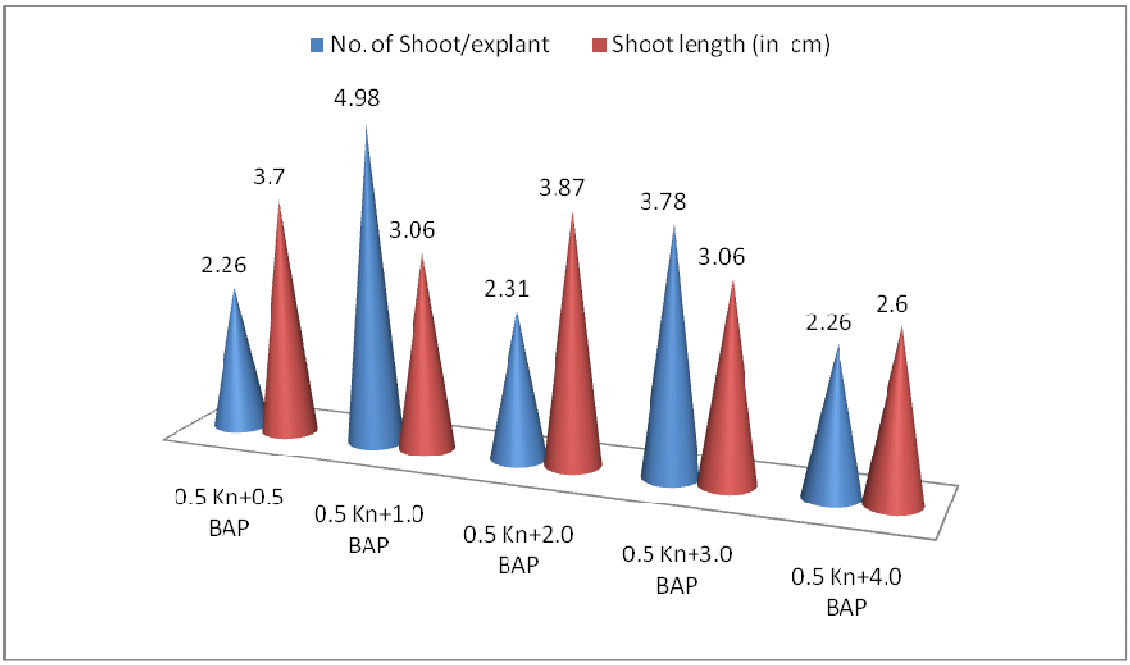
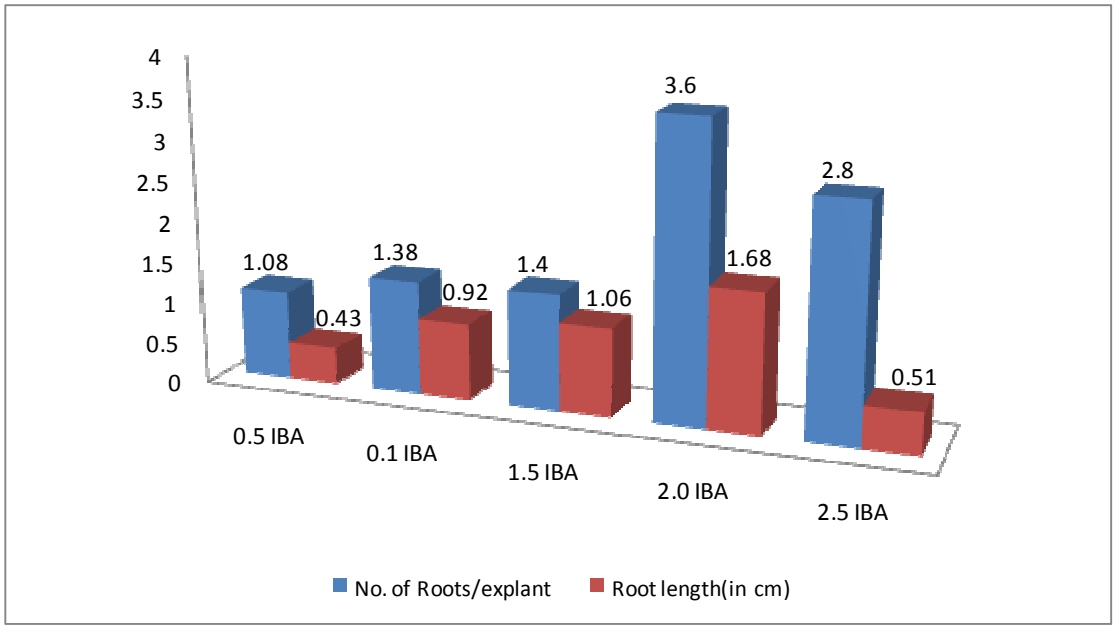


Figure-5: Effect of Auxin (IBA) on root induction from isolated shoots of *Bacopa monnieri*



CONCLUSION

The seedlings derived from explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Bacopa monnieri*, MS medium containing 1.0 mg/l BAP was the best for culture initiation. We have found that *Bacopa monnieri* culture grew better on MS medium in comparison to other media. In *Bacopa monnieri* 1.0 mg/l BAP was most suitable for shoot multiplication. We also observed improvement in shoot multiplication by different concentrations of Kn. (0.5-4.0 mg/l) in medium along with BAP (0.5 mg/l). Best shooting response was observed on media containing 0.5 mg/l BAP+ 2.0 mg/l Kn (Average number of shoots 3.42±0.39,

Average shoot length 7.54±0.31 cm). In different concentrations of Kn. (1.0-5.0 mg/l) *Bacopa monnieri* give best shooting response in 2.0 mg/l Kn. We also observed improvement in shoot multiplication by different concentrations of BAP (0.5-4.0 mg/l) in medium along with Kn. (0.5 mg/l). Best shooting response was observed on media containing 0.5 mg/l KN+1.0 mg/l BAP (Average number of shoots 4.98±0.74, shoot length 3.06±0.22 cm).

IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Bacopa monnieri*, 2.0mg/l IBA proved to be best for *in vitro* rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to misthouse where they exhibited growth with 90% survival. Most responsive callus induction was observed on MS medium supplemented with 0.25 mg/l 2,4-D +0.5 mg/l Kn and 0.25 mg/l 2,4-D +0.1 mg/l BAP.

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REFERENCES

[1] Anonymous, Indian Medicinal Plants: a sector study. Occasional paper No. 54. Export-Import Bank of India. Quest Publication, Bombay, India, **1997.**
[2] Singh RH, Singh L, *J Res Ayurveda and Siddha*, **1980,** 1:33–148.
[3] Ahmad R.U. Medicinal plants used in ISM – their procurement, cultivation, regeneration and import/export aspects: a report. In: Govil J.N., Singh V.K., Hashmi S. (eds) *Medicinal Plants: New Vistas of Research* (Part 1) Today & Tomorrow Printers and Publishers, New Delhi, pp. **1993,** 221–225.
[4] Pandey N.K., Tiwari K.C., Tiwari R.N., Joshi G.C., Pandey V.N., Pandey G. Medicinal plants of Kumaon Himalaya, strategies for conservation. In: Dhar U. (ed) *Himalayan Biodiversity Conservation Strategies.* Himaliya Publication, Nanital, pp. **1993,** 3:293–302.
[5] Shrivastava N, Rajani M, *Plant Cell Rep*, **1999,** 18: 919–923.
[6] Rai D, Bhatia G, Palit G, Pal R, Singh S, Singh HK, *Pharmacol Biochem Behav*, **2003,** 75:823–830.
[7] Rajani M, Bacopa monnieri, a nootropic drug. In: Ramawat KG, Merillon JM (eds) Bioactive molecules and medicinal plants. Springer, Berlin, pp, **2008,** 175–195.
[8] Tripathi YB, Chaurasia S, Tripathi E, Upadhyaya A, Dubey GP, *Indian J Exp Biol*, **1996,** 34:523–526.
[9] Krishna Kumar H N, *Asian Journal of Plant Science and Research*, **2012,** 2 (2):173-179.
[10] Verma O P, *Advances in Applied Science Research*, **2012,** 3 (3):1449-1453.
[11] Tanwer B S, *Advances in Applied Science Research*, **2011,** 2 (1): 208-213.
[12] Bhosale U P, *Asian Journal of Plant Science and Research*, **2011,** 1 (3):23-27.
[13] Mehta J, Sain M, Mathuriya BL, Naruka R, Kavia A, Sharma DR, *Asian Journal of Plant Science and Research*, **2012,** 2 (3): 364-368.
[14] Mehta J, Naruka R, Sain M, Dwivedi A, Sharma D, Mirza J, *Asian Journal of Plant Science and Research*, **2012,** 2 (4):518-523.
[15] Mehta J, Kaushal N, Sen P, Sharma D, Dhillon MD, Mathuriya BL, *European Journal of Experimental Biology*, **2012,** 2 (4):1278-1283.