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In vitro Shoot Regeneration of *Bacopa monnieri* (L.) Using Cyanobacterial Media- A Novel Approach and Effect of Phytoregulators on *in vitro* micropropagation

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ABSTRACT

This review highlights the recent development and novel achievements made for the multiple shoots regeneration of Bacopa monnieri in Cyanobacterial medium as well as in MS medium. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. In Cyanobacterial culture medium Bacopa monnieri survived for long period of time and multiple shoot initiation was observed in 2mg/l Kn. When Bacopa monnieri was inoculated in MS medium supplemented with different concentrations of PGR's, maximum no. of shoots were observed in 0.5 mg/l BAP + 2.0 mg/l Kn and 0.5 mg/l Kn+1.0 mg/l BAP.

Keywords: Cyanobacterial culture medium, Medicinal plant, Micropropagation, Brahmi.

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 long time ago listed *Bacopa monnieri* as a threatened species [4]. There is a demand for improvement in the tissue culture protocol for 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*.

Due to the above mentioned difficulties; tissue culture is the only alternative for rapid mass propagation of *Bacopa* plants. Synthetic growth regulators enhance and accelerate the production of *in vitro* plants with good agronomical traits. The approach of using cyanobacterial cultures would overcome many barrier of micropropagation where

costly synthetic chemicals are involved. Cyanobacteria or blue green algae are prokaryotic photosynthetic microorganism that produces a wide array of substances, including plant growth regulators. These include antibiotics, vitamins and plant growth regulators [5]. Among the growth regulators, gibberellins, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been detected in *cyanobacteria* [6, 7, 8]. To bridge the gap between production and demand of *Bacopa*, there has to be an important and alternate protocol than the generally used one.
This study is an effort in this direction where a cyanobacterial culture has been used as a substitute media to MS media for *in vitro* micropropagation. In this present study the reason for choosing standard cyanobacterial culture is that it is known to produce the growth regulators auxin, cytokinin, gibberellin and other bioactive chemicals which are documented. These bioactive components produce vitamins, minerals, polyunsaturated fatty acid, carotenes, and other pigments that have an antioxidant activity to receiving attention. In addition it has antioxidative capacities to attribute biliproteins called as phycocyanin, proteins (60%-70%), vitamins, essential amino acid, minerals and essential fatty acid such as palmitic acid and linoleic acid which are produced by the cyanobacterial culture. This protocol is very cost effective and entirely novel where liquid culture of cyanobacteria has been used for the efficient regeneration of *Bacopa monnieri*.

MATERIALS AND METHODS

Algal culture

Stock cultures (*Nostoc muscorum, Chroococcidiopsis, ,Spirulina sp, Tolypothix sp,*) were taken from the Culture Collection of Algae Research Laboratory and Department of Biotechnology, Vital Biotech Research Institute, Kota and grown in Zarrouk's medium. Isolation and purification of cultures were carried out using the techniques adopted by Venkataraman [9]. Identification of maintained axenic cyanobacteria was carried out according to Desikachary [10, 11]. The isolated organisms were batch cultured (100ml) and grown in Conical flask (250ml). Tissue culture was initiated with inocula of 7 days old algal cultures. The cultures were incubated at 25 ^{o}C ± 2 in light intensity of about 2500 to 3000 Lux. The algae were harvested in the early stationary phase by vigorous shaking with sterilized glass beads for 15 min.

Culture media

After shaking, the cyanobacterial cultures were used as an alternative media against MS [12] supplemented with only sucrose (3 %w/v and 0.8% agar). The pH of the medium was adjusted between 5.2- 6.2 before gelling with agar (0.6% w/v) and autoclaved at 121º C under 15 lb pressure for 15-20 min.

Plant material and In vitro culture

The shoots (about 5-6 cm) of *Bacopa monnieri* plants were collected from the Herbal Garden, Kota. The branches with node explants were washed in running tap water and then treated 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 2 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 organized aseptically and were inoculated vertically on Cyanobacterial medium as well as MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) individually 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. The *p*H of the media was adjusted to 5.2- 6.2 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 at 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 were established, the shoots produced *in vitro* were subcultured on fresh medium after 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) were observed on MS media fortified with 0.5 BAP+3.0 Kn. Maximum shoot length7.54±0.31cm was notified in medium supplemented with 0.5 BAP+3.0 Kn.

RESULTS AND DISCUSSION

When nodal explants *Bacopa monnieri* inoculated in cyanobacterial medium supplemented with cyatokinin (BAP, Kn) (Table 1), best response was observed in kn rather than BAP (Table 2, Fig.1-A, B, Fig.2). Maximum no. of shoots was initiated in MS medium+2mg/l Kn. (5.08±0.51).

Cyanobacterial culture medium

1000ml Cyanobacterial culture medium was prepared by taking 40 ml cyanobacteria with 8%agar and 30% sucrose and following composition

Table-1: Cyanobacterial culture medium

Constituents	g/l
NaCl	5
KNO_3	2
$NaHCO_3$	1
K_2SO_4	1
$[(NH_4)2SO_4]$ $[Ca_3PO_4]$	0 .1
$MgSO_4.7H_2O$	0.2
$NH_2.CO_2.NH_2$	0.02
$FeSO_4.7H_2O$	0.005
NPK	2

Table-2: Effect of Cytokinin (BAP and Kn) in Cyanobacterial Media 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)
1.0	-	70	3.28±0.36
2.0	-	76	4.92±0.51
3.0	-	65	3.31±0.33
-	1.0	65	2.28±0.36
-	2.0	80	5.08±0.51
-	3.0	60	1.85±0.27

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

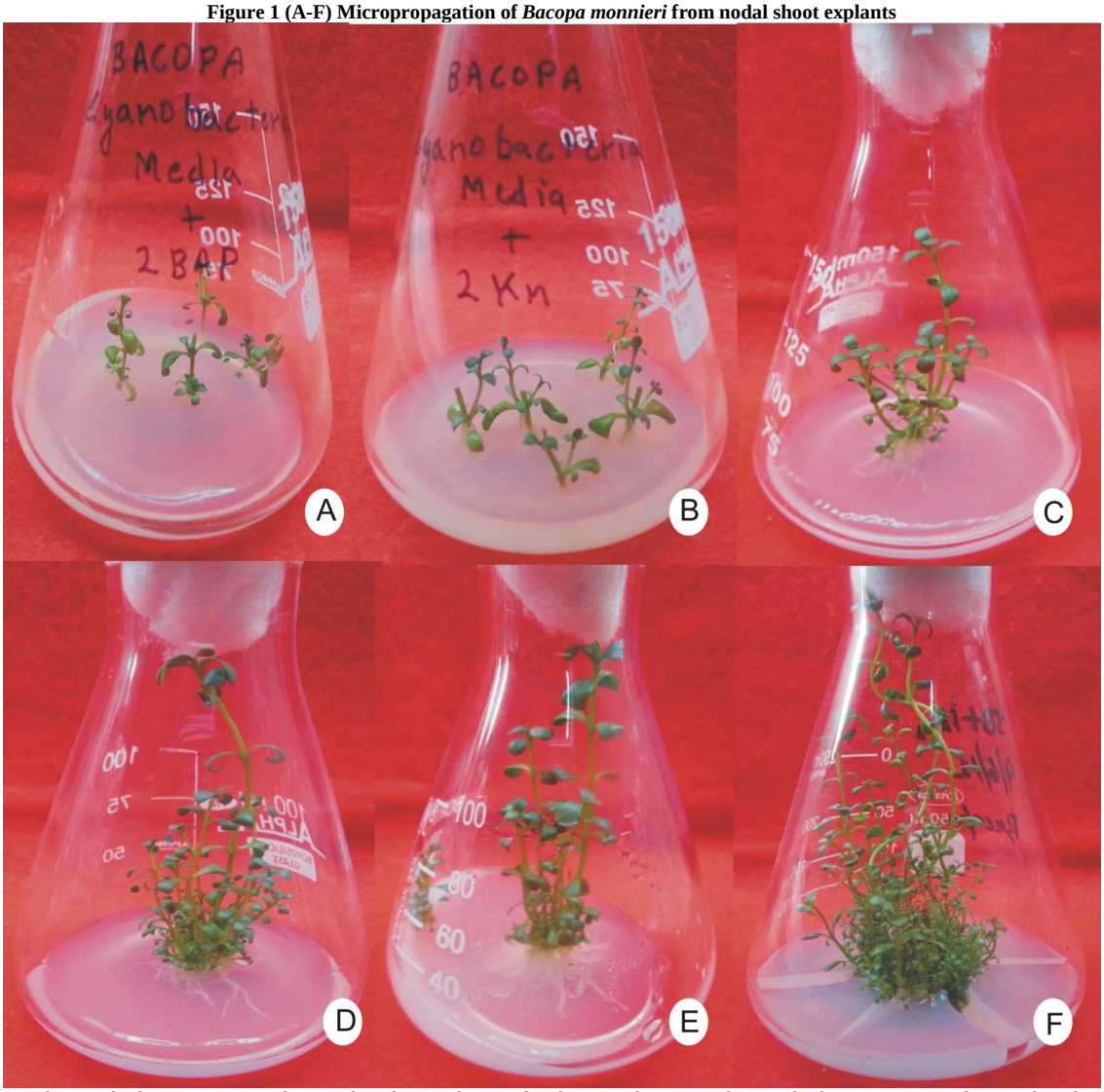
Hormone Con. (mg/ l) BAP	Hormone Con. (mg/ l) Kn	Response (%)	No. of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
1.0	-	80	3.42±0.58	6.51±0.76
2.0	-	70	2.28±0.71	6.56±0.84
3.0	-	65	2.71±0.56	7.62±0.53
4.0	-	55	3.28±0.36	5.08±0.51
5.0	-	40	2.85±0.51	3.31±0.33
-	1.0	55	2.28±0.36	6.47±0.29
-	2.0	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)

On the other hand, when explants was inoculated in MS medium containing BAP and Kn in the range 1.0-5.0 mg/l, (Table 3, Fig.1-C, 3), it showed enhanced shoot proliferation. BAP at 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. When BAP was used in combination with Kn a variety of responses were observed (Table 4, Fig. 1-

E, 4). 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 (Table 5, Fig.1-F, 5) 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).

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



***A.** Shoot multiplication on Cyanobacterial Media supplemented with 2.0 mg/l BAP, **B.** Shoot multiplication on Cyanobacterial Media supplemented with 2.0 mg/l Kn, **C.** Shoot multiplication on MS Media supplemented with 1.0 mg/l BAP, **D.** Shoot multiplication on MS Media supplemented with 2.0 mg/l Kn, **E.** Shoot multiplication on MS medium supplemented with 0.5 mg/l BAP+2.0 mg/l Kn, **F.** Shoot multiplication on MS medium supplemented with 0.5 mg/l Kn+1.0 mg/l BAP.*

Table-4: 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)

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

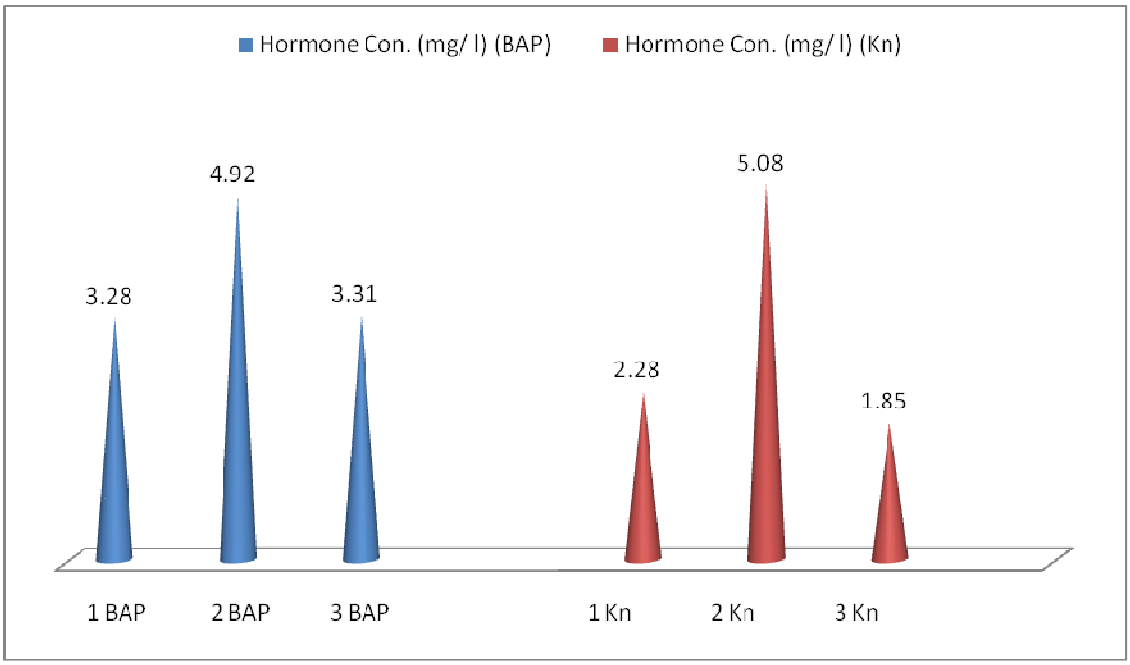


Figure-3: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot Explants of *Bacopa monnieri*

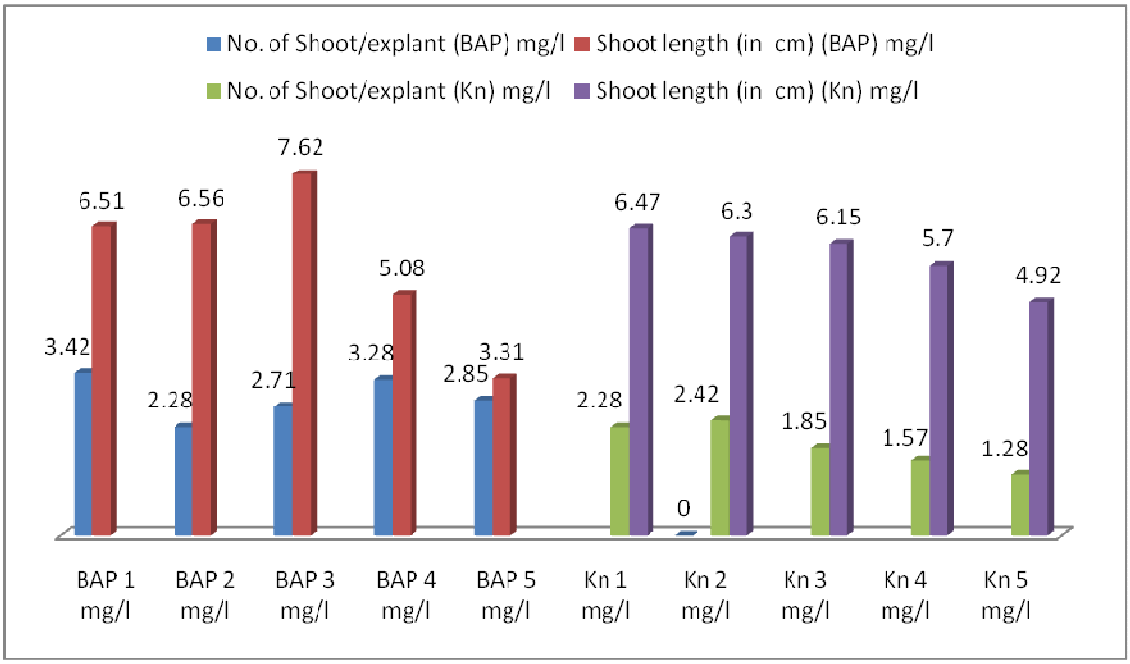


Figure-4: Interactive effect of cytokine (BAP + Kn) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

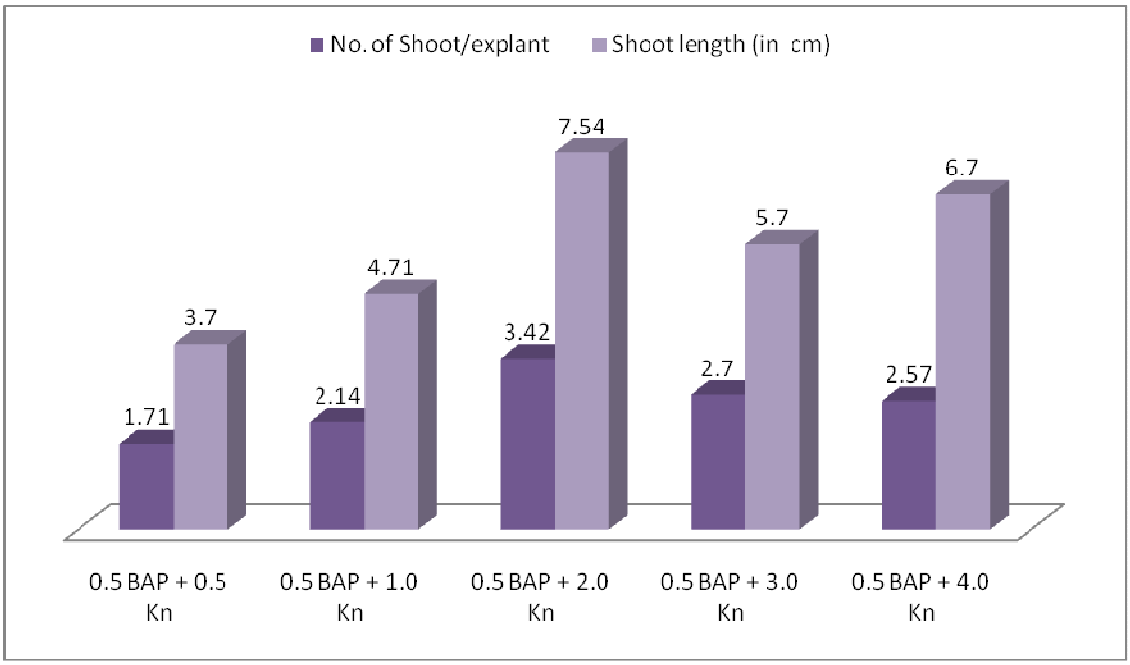


Figure-5: Interactive effect of cytokine (Kn + BAP) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

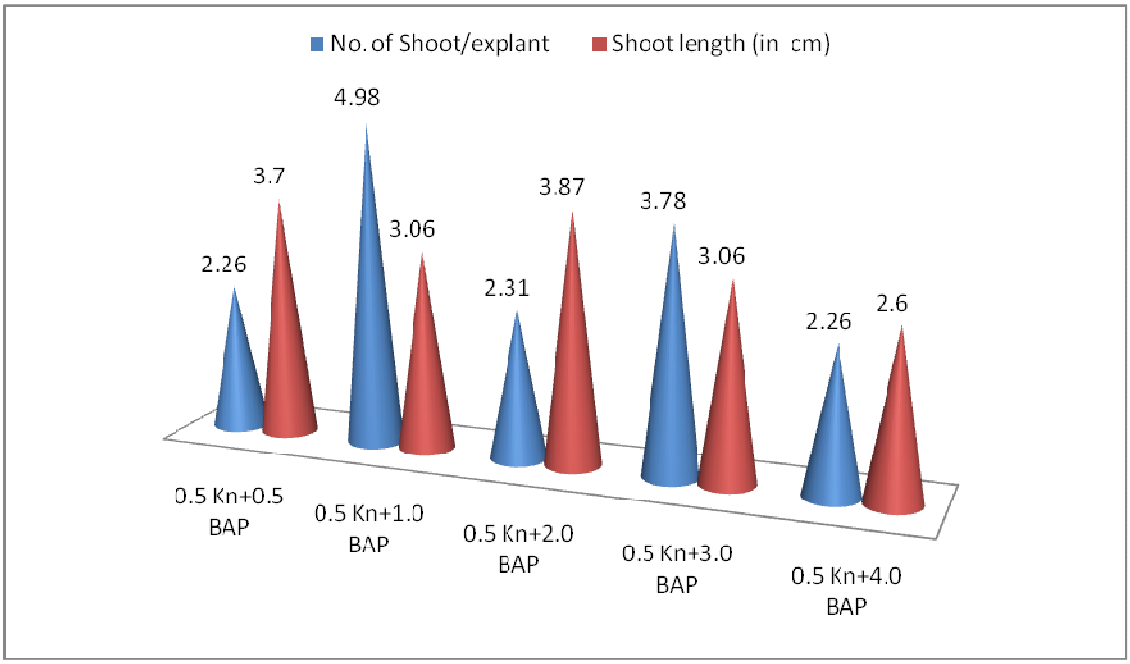


Table-5: Interactive effect of Cytokinin (Kn+ BAP) on shoot multiplication by Subculture of shoot clumps of *Bacopa monnieri*

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)

CONCLUSION

Cyanobacteria are known to excrete a large number of substances that stimulate shoot proliferation. Therefore we may say that the Cytokinin content of cyanobacteria used in the present study produces the observed increases in plant regeneration of *Bacopa*. In the present study cyanobacterial media was used against MS media, which is generally used in tissue culture. Cyanobacterial media showed significant promotive effect on initation of shoot primordia. This is a novel approach to regenerate *Bacopa* instead of use of synthetic growth regulators, cost effective and eco-friendly. Although further researches are going on in this direction this may be the first report of plant growth initiation on cyanobacterial culture medium alone without any additions.

So here in this article *Bacopa monnieri* was micropropagated with cyanobacterial medium. Best response was observed in medium supplemented with 2mg/l Kn (average no.of shoots 5.08±0.51).Whereas when *Bacopa monnieri* was inoculated in MS medium supplemented with different concentrations of PGR,s following results are evoked. The best growth of *Bacopa monnieri* was visualized on MS medium containing 1mg/l BAP. Beside this improved shoot generation with 0.5 mg/l BAP+ 2.0 mg/l Kn was recorded. (Average number of shoots 3.42±0.39, Average shoot length 7.54±0.31 cm).Maximum no. of shoot regeneration was observed in 2.0 mg/l Kn. Satisfactory result was notified 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).

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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] Metting B and Pyne J W, Biologically active compounds from microalgae. *Enzyme and Microbial Technology*. **1986,** 8: 386-394.
[6] Zaccaro Maria Cristina, *et al. Phytomorphology*. **2002,** 52 (4): 263-271.
[7] Zaccaro Maria Cristina, *et al. Elec.J.Biotech.* **2006,** 9 (3): 211-214.
[8] Molnár Z, Ördög V, *Acta Biol. Szeged*, **2005,** 49, (1-2):37-38.
[9] Molnár Z, Ördög V, *Acta Biol Szeged*, **2005,** 49, (1-2): 39-40.
[10] Desikachary TV Cyanophyta*:* Indian Council of Agricultural Research, New Delhi. **1959.**

[11] Whiteford L A and G J Schumacher, A manual of fresh- water algae. Published by Sparks Press. Releigh, N C. **1973.**
[12] Murashige T, Skoog F, *Physiol.Plant,* **1962,** 15: 473-497.
[13] Krishna Kumar H N, *Asian Journal of Plant Science and Research,* **2012,** 2 (2):173-179.
[14] Bhosale U P, *Asian Journal of Plant Science and Research,* **2011,** 1 (3):23-27.
[15] Mehta J, Sain M, Mathuriya BL, Naruka R, Kavia A, Sharma DR, *Asian Journal of Plant Science and Research,* **2012,** 2 (3): 364-368.
[16] Mehta J, Naruka R, Sain M, Dwivedi A, Sharma D, Mirza J, *Asian Journal of Plant Science and Research,* **2012,** 2 (4):518-523.
[17] Mehta J, Kaushal N, Sen P, Sharma D, Dhillon MD, Mathuriya BL, *European Journal of Experimental Biology,* **2012,** 2 (4):1278-1283.