



Effect of Cytokinine on *Bacopa monnieri* (L.) Pennel - An Important Medicinal Plant

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ABSTRACT

This review highlights the recent development and effect of Cytokinine made for the multiple shoots regeneration 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.

Key words: 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 plants¹. 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 B². 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 12.7×10^6 kg of dry biomass at a value of \$34 million³. 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 species⁴. 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.

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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.

MATERIAL 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 pH 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 ($25 \pm 2^\circ\text{C}$), 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 ± 0.31 cm. of a medium supplemented with 0.5 BAP+3.0 Kn.

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.

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

Hormone Con. (mg/ l)	Hormone Con. (mg/ l)	Response (%)	No. of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
BAP	Kn			
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 Column 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 Column 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 Column do not different significantly at P< 0.05 (Tukey's test)



Fig. 1: (A-D) Effect of Cytokinin on *Bacopa monnieri* (L.) Pennel

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

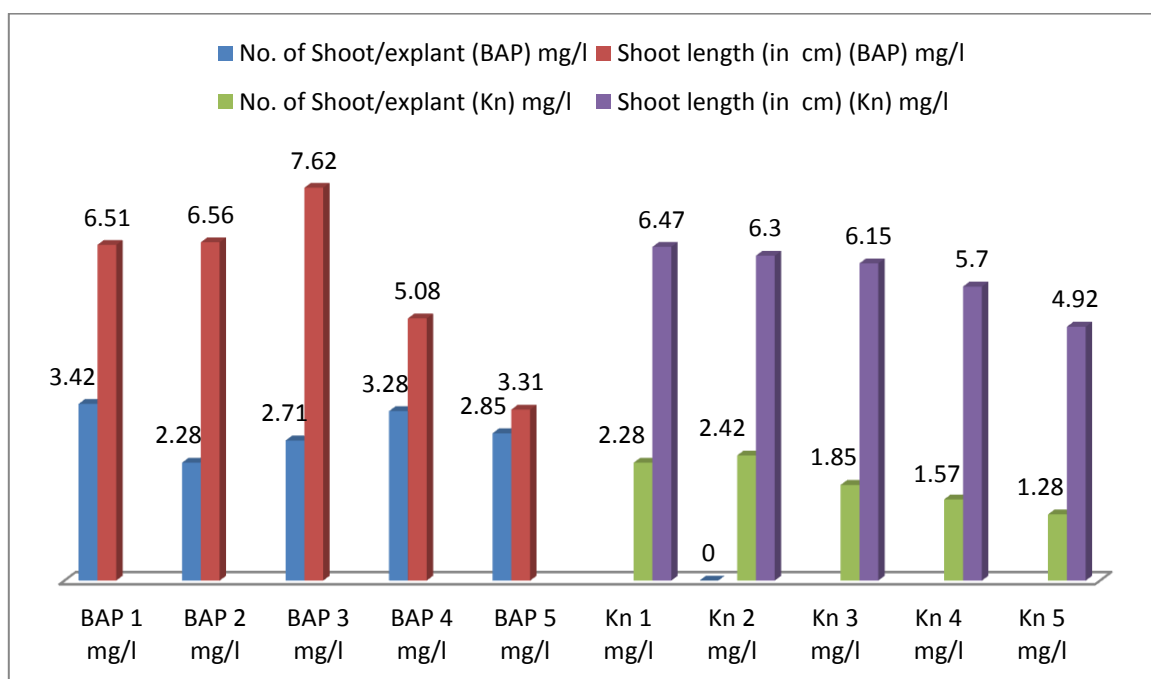


Fig. 2: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Bacopa monnieri*

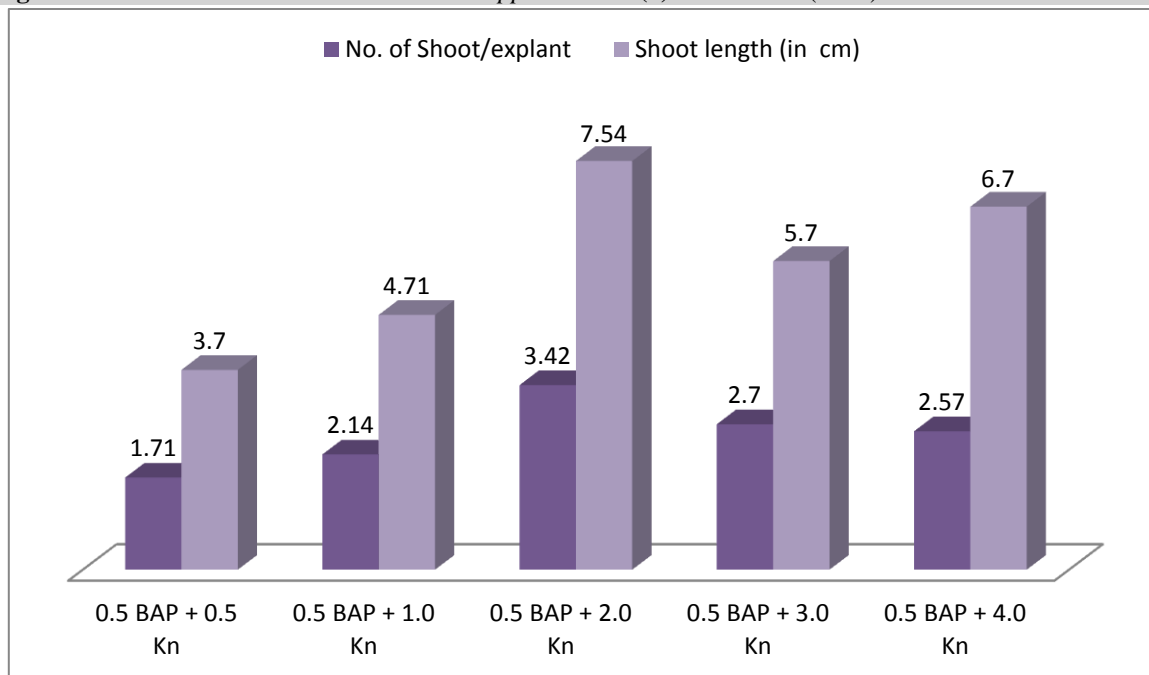


Fig. 3: Interactive effect of cytokine (BAP + Kn) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

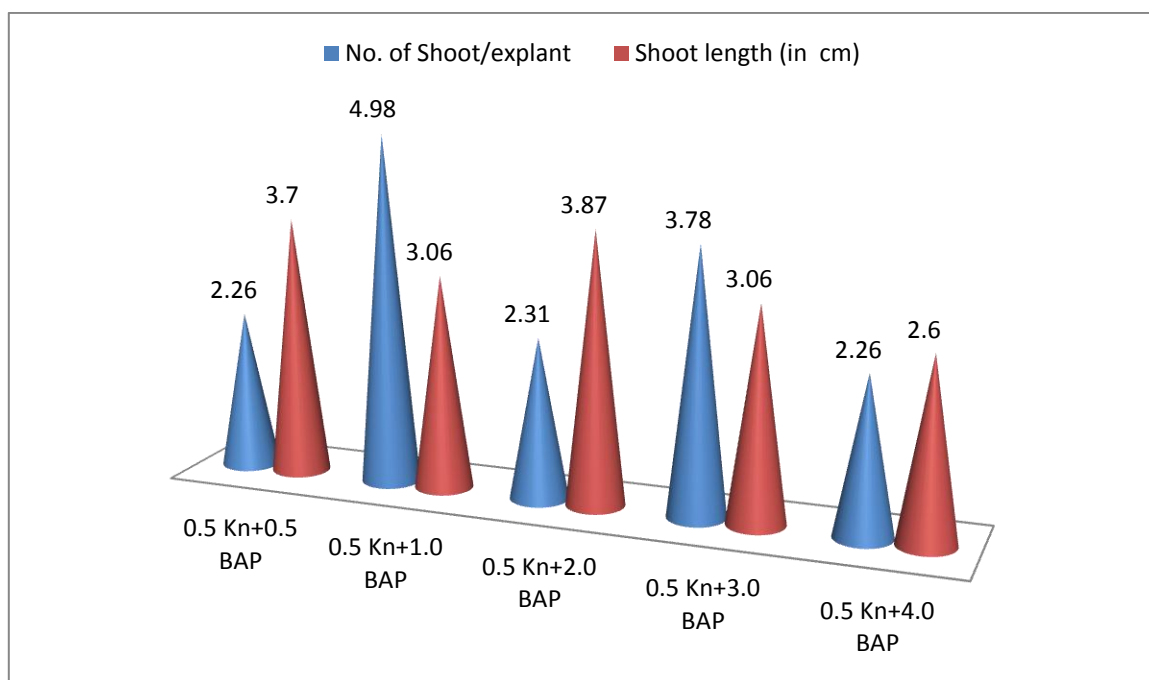


Fig. 4: Interactive effect of cytokine (Kn + BAP) on shoot multiplication by subculture of shoot clumps of *Bacopa monnieri*

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).

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