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Interactive effects of kinetin and IBA on shoot proliferation and bacoside production in *Bacopa monnieri L. Wettst*

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Abstract
Interactive effects of different concentrations of Kinetin and IBA were studied on shoot proliferation as well as Bacoside content of Brahmi (*Baccopa monnieri L. Wettst*) under *in vitro* conditions. Murashige and Skoogs medium was supplemented with two levels Kinetin (1.0 and 2.0 mg/l) with different levels of IBA (0.0, 0.15, 0.30 and 0.45 mg/l). Present investigation showed positive effects of interaction of kinetin and Indole 3 Butyric Acid on shoot proliferation, biomass production and bacoside production in Brahmi.

Keywords: *Bacopa monnieri*, *Kinetin*, Indole 3 Butyric Acid**,** Shoot proliferation, Bacoside.

Introduction
Bacopa monnieri L. Wettest, commonly known as ‘‘Brahmi’’, is a member of the Family *Plantagineace* is placed second in the priority list of Indian medicinal plants. 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.
It is used in traditional Indian medicine, the Ayurveda, for treatment of anxiety, in improving memory for several centuries. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to posses anti-inflammatory, analgesic, antipyretic and sedative, free radical scavenging and anti-lipid peroxidative activities. The pharmacological properties of brahmi are mainly due to the presence of saponins called ‘bacosides’ which are complex mixture of structurally closely related compounds, glycosides of either jujubogenin or pseudojujubogenin. Bacoside A is a major chemical entity shown to be responsible for memory-facilitating action of brahmi [1]. Brahmi 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. Kinetin is a 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. Combination effects of cytokinins and auxin were studied by earlier researchers and showed enhancing effects on shoot proliferation as well as bacoside cotent in Brahmi [2-4].

Materials and methods
Experiment was conducted in plant tissue culture laboratory of MGM Plants of Brahmi (*Bacopa monnieri* L. Wettst*)* were collected from Paithan, Aurangabad, Maharashtra. Disease

free, young and healthy plant was selected for carrying out the experiment. The 3rd and 4thnodal segment was selected as explants [2]. Explants were cut and wash under tap water for 15-20 min in order to wash off the external dust/contaminants. Then explants were washed with 2% Tween 80 solution for 10 min followed by 20 minutes running tap water washing. For further sterilization these explants were transferred to the laminar air flow. After that explants sterilized with 70% (v/v) ethanol for few seconds followed by 2-3 washing with sterilized double distilled water. Further the explants were treated with 0.01% (w/v) $HgCl_2$ for 10 min. Finally the explants were washed with sterilized double distilled water for 3-4 times and placed in sterilized double distilled water. After sterilization explants were trimmed and inoculated on MS media supplemented with different combinations of Kinetin and IBA. Media also composed of sucrose 3% and 0.65% Agar. The pH of media was adjusted to 5.6 - 5.8 and autoclaved the media 121ºc for 20 min [5]. Cultures were incubated in culture room at 25 ± 2ºC temperature with 16 hours photoperiod. The cut ends of explants were kept in such a way so as to have maximum contact with the medium [6]. Present experiment was laid in factorial randomised block design with total eight combinations and three replications each. Two levels of Kinetin (1.0 and 2.0 mg/l) in combination with four levels of IBA (0.0, 0.15, 0.30 and 0.45 mg/l) were used in this experiment.

Regenerated plant samples were observed for biometric observations after three weeks incubation and whole samples were collected and separately investigated for bacoside content. Saples were oven dried at 60^0C for 12 hrs and macerated in 180 ml of 95% ethanol for 3 day at room temperature and the resulting extract was filtered through filter paper (Whatman no. 1) the residue from the filtration was extracted again twice using the same procedure, then obtained filtrates were evaporated [7]. Ethanolic extracts were used for quantitative detection of Bacoside content in Brahmi. Ethanolic extract 40µl were diluted by using 95% ethanol and made up to 4 ml final volume and compared with standard Bacoside concentration procured from Sigma Aldrich. Analysis was carried out by using UV Spectrophotometer at 278 nm [8]. Data obtained from various biometric and biochemical observation was analyzed by "Analysis of variance" method by using randomised block design [9].

Results and Discussion

Various growth parameters of Brahmi were influenced by different combinations of kinetin and IBA under *in vitro.*

Shoot proliferation

Shoot proliferation was observed in nodal segments of *Bacopa monnieri* showed that the shoot bud regeneration was occurs in second week after inoculation (Plate 1a). Multiple shooting was observed in couple of weeks after inoculation (Plate 1c). Surprisingly callus formation was observed in couple of weeks after inoculation (Plate 1c), that might be the interactive effect of cytokinin with auxins under *in vitro* condition. Diverse shoot proliferation was observed after 21 DAI (Plate 1b) in different combination levels of Kinetin with IBA.

Data on mean number of shoots per explants were grown at 21 DAI are presented in Table 1 and 2.

Table 1: Effects of IBA and KN on shoot proliferation in Brahmi.

IBA (mg/l)	No. of shoots	Kinetin (mg/l)	No. of shoots
00	1.833	1.0	2.666
0.15	2.500	2.0	2.916
0.30	2.595	S.E.± 1.093	C.D. at 1% 0.328
0.45	2.500		

S.E.± 0.151, C.D. at 1% 0.453

Table 2: Interactive effect of Kinetin and IBA on Shoot proliferation.

Growth regulator levels		IBA (mg/l)			
		0.0	**0.15**	**0.30**	**0.45**
Kinetin (mg/l)	1.0	2.000	2.000	3.666	3.000
	2.0	1.666	3.000	5.000	2.000

S.E. ± 0.215, C.D. at 1 % 0.646

Independently (Table 1), Kinetin at 2.0 mg/l produced highest number of shoots i.e 2.916 followed by 1.0 mg/l (2.666 shoots/explant), were both at par with each other. The IBA at 0.30 mg/l found significantly superior over rest of the IBA treatments and produced 2.595 shoots per explant.

Whereas the combinations of growth regulators showed interactive effects on shoot proliferation, the level of Kinetin (2.0 mg/l) with IBA (0.30 mg/l) produced maximum shoots per explants i.e. five shoots per explants (Table 2). Earlier researchers were also reported the similar type of results in Brahmi such as Binita *et. al*. (2005), Naik *et. al.* (2009) and Kharde *et. al*. (2010) [2-4].

Biomass production

Fresh weight

Data on mean fresh weight per explants at 21 DAI are presented in Table 3 and 4.

Table 3: Effects of Kinetin and IBA on fresh weight of Brahmi.

IBA (mg/l)	Fresh weight (gm)	Kinetin (mg/l)	Fresh weight (gm)
00	0.146	1.0	0.166
0.15	0.187	2.0	0.168
0.30	0.179	S.E.± 0.0063	C.D. at 1% 0.019
0.45	0.150		

S.E.± 0.007, C.D. at 1% 0.023

Table 4: Interactive effect of Kinetin and IBA on Fresh weight of Brahmi.

Growth regulator levels		IBA (mg/l)			
		0.0	**0.15**	**0.30**	**0.45**
Kinetin (mg/l)	1.0	0.136	0.173	0.193	0.156
	2.0	0.156	0.196	0.166	0.143

S.E. ± 0.0126, C.D. at 1 % 0.038

Biomass production was significantly influenced due to the combination effect of Kinetin (2.0 mg/l) with IBA (0.15 mg/l) recorded highest fresh weight (0.196 gm) significantly superior over rest all treatments (Table 4). While independently Kinetin (2.0 mg/l) produces maximum fresh weight 0.168 gm per explant and IBA (0.15 mg/l) showed 0.187 gm per explant fresh weight in Brahmi (Table 3).

Dry weight

Data on mean dry weight of explants recorded at 21 DAI of growth are presented in Table 5 and 6.

Table 5: Effects of Kinetin and IBA on dry weight of Brahmi.

IBA (mg/l)	Dry weight (gm)	Kinetin (mg/l)	Dry weight (gm)
00	0.021	1.0	0.021
0.15	0.023	2.0	0.020
0.30	0.033	S.E.± 0.0016	C.D. at 1% N.S.
0.45	0.024		

S.E.± 0.0016, C.D. at 1% 0.005

Table 6: Interactive effect of Kinetin and IBA on dry weight of Brahmi.

Growth regulator levels		IBA (mg/l)			
		0.0	**0.15**	**0.30**	**0.45**
Kinetin (mg/l)	**1.0**	0.003	0.020	0.031	0.028
	2.0	0.028	0.027	0.034	0.021

S.E. ± 0.003, C.D. at 1 % 0.01

Dry weight accumulation was significantly influenced due to the combination effect of growth regulators, Kinetin (2.0 mg/l) with IBA (0.30 mg/l) recorded highest dry weight (0.34 gm) (Table 6). While independently both levels of Kinetin were at par with each other (Table 5). While IBA produces highest dry weight (0.33 gm) at 0.30 mg/l (Table 5) in Brahmi with in 21 DAI.

Bacoside production

Data on mean Bacoside production was recorded at 21 DAI of growth are presented in Table 7 and 8.

Table 7: Effects of Kinetin and IBA on Bacoside production Brahmi.

IBA (mg/l)	Dry weight (gm)	Kinetin (mg/l)	Dry weight (gm)
00	1.59	1.0	1.79
0.15	1.38	2.0	1.75
0.30	2.37	S.E.± 0.0076	C.D. at 1% 0.23
0.45	1.75		

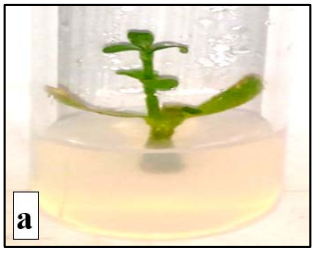
S.E.± 0.106, C.D. at 1% 0.318

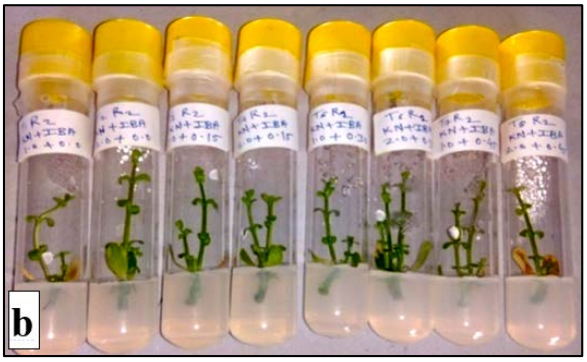
Table 8: Interactive effect of Kinetin and IBA on Bacoside production in Brahmi.

Growth regulator levels		IBA (mg/l)			
		0.0	**0.15**	**0.30**	**0.45**
Kinetin (mg/l)	1.0	1.70	1.31	2.26	1.87
	2.0	1.47	1.43	2.46	1.63

S.E. ± 0.149, C.D. at 1 % 0.448

Bacoside production was significantly influenced due to the combination effect of growth regulators within 21 DAI, Kinetin (2.0 mg/l) with IBA (0.30 mg/l) and produces maximum Bacoside content i.e. 2.46 was significantly superior over the rest of the treatments (Table 8). While independently both levels of Kinetin were at par with each other (Table 7), whereas the IBA maximum Bacoside content i.e. 2.37 and found superior over the rest of the IBA treatments (Table 7) in Brahmi with in 21 DAI.





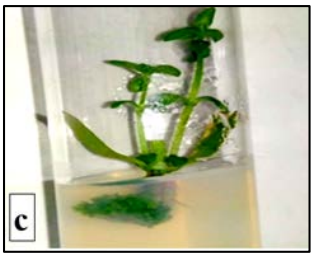


Plate 1: Effect of growth regulators on shoot proliferation of *Bacopa monnieri* 21 DAI, a) Shoot regeneration from nodal explant, b) variation in shoot proliferation in different combinations of Kinetin and IBA and c) Shoot multiplication as well as occurrence of callus at the base of intermodal segment immersed in media.

Conclusion

Present investigation concludes that the combination of cytokinin and auxins showed positive interaction on plant growth and secondary metabolite production in *Bacopa monneri* L. Wettst.

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