EFFECT OF DIFFERENT CYTOKININ CONCENTRATIONS ON IN VITRO SHOOT MULTIPLICATION OF *BAMBUSA TULDA* ROXB. FROM NODAL CUTTINGS

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Abstract: *Bambusa tulda* Roxb. is an economically important bamboo species that belongs to the order Poales, family Poaceae and sub-family Bambusoideae. It is a sympodial species that grows well in tropical and sub-tropical regions of India. *Bambusa tulda* is tall, sturdy and quickly growing bamboo species having succulent shoots that are rich in phytosterols. The species is overexploited due to its economic importance. Large scale production is not sufficient by the conventional method of propagation due to their low multiplication rate, time consuming, high cost and labor intensive. For the conservation and mass propagation of different bamboo species, *in vitro* culture is providing to be a promising process. So, in the present study, an experiment was conducted to achieve a protocol for an effective concentration of cytokinin on the shoot proliferation and multiplication of *Bambusa tulda* Roxb. using nodal cuttings. The nodal explants were used to induce multiple shoots proliferation using various concentration of BAP and Kn on MS medium. The MS medium supplemented with BAP (3.0mg/l) supported the maximum *in vitro* shoot multiplication. The result of the experiment also showed that, 3 mg/l BAP concentration was found to be more effective for shoot proliferation of *Bambusa tulda*.

Keywords: *Bambusa tulda*; Cytokinins; Nodal cuttings; Shoot multiplication

1. Introduction:

Bamboo is regarded as the "Green Gold" of the world because of its strong and versatile nature for providing ecological, economic and livelihood security [1,2]. Bamboo culms has a high potential in agricultural part to assist to a sustainable development in minimizing the risk of erosion [3]. Furthermore, bamboo has the capacity to hold a high quantity of carbon content, making it beneficial for carbon sequestration and lowering global warming [4,5]. Of about 1,439 species of bamboo occurring all over the world [6], 136 species have been found in India, forming the richest bamboo genetic resources [1]. *Bambusa tulda* Roxb. is an economically important bamboo species found in North East India [7]. Shoots of *B. tulda* are rich in phytosterols [8]. *B. tulda* is also used for building houses, baskets and containers, fishing equipment, brooms, decorative items and stools [9]. This species mainly occurs in moist alluvial flat land and grows in an altitude of 1500 m above the sea level. High reserves of organic matter, nitrogen, potassium, calcium and phosphorus are contained under the soil where this species grows [10]. The flowering cycle of *B. tulda* vary from 30 to 60 years [11]. Due to its multiple uses, the species is overexploited, conventional method of propagation is not suitable for large scale production, tissue culture is the only convenient method [12] for overcoming this problem. Extensive research regarding the micropropagation of bamboos has been done in the recent years [13-18]. Studies have shown that cytokinin have the ability to enhance shoot initiation [19]. As a result, an effective and secure protocol for shoot proliferation and multiplication has been developed for this economically important plant using nodal cuttings and various concentrations of cytokinins, with the aim of developing large-scale propagation in the immediate future.

2. Materials and methods

Sources of plant materials

Young juvenile shoots from healthy mother plant of *B. tulda* were collected from Pathsala, Bajali district of

Assam, as explants during 2017 and were stored in refrigerator at 4 °C, wrapped in plastic bags, to maintain its viability.

Explant sterilization

Healthy nodal segments about 2 cm in length were excised from young lateral branches of the main culm of *Bambusa tulda*. Leaf sheath were removed from the nodal segments, sized and washed with tap water and then kept in tap water for about an hour. This helped in getting rid of the brownish exudation from the explants during the culture. The explants were surface sterilized by washing with soap water and savlon followed by dipping in 1% Tween 20 (1 ml Tween 20 in 100 ml Double distilled water) for 30 minutes. During this period frequent agitation is done to physically remove most micro-organisms and some debris. After that, the sterilizing solution is decanted and washed with distilled water several times to remove the traces of Tween 20. After Tween 20, an antifungal treatment with 0.5% Bavistin (0.5g in 100ml Double distilled water) for 30 minutes was carried out with gentle shaking. To eliminate all residues of Bavistin, the explants were washed several times with double distilled water. Prior to inoculation, the explants were then treated with 0.1% $HgCl_2$ for 7 minutes under sterile conditions in the laminar air flow cabinet. After decanting the sterilized solution, the explants were washed repeatedly with autoclaved distilled water for three times to remove traces of $HgCl_2$. The explants were then inoculated on the culture tubes having autoclavable lids and conical flasks containing 20ml, 30ml and 50ml media respectively under aseptic conditions.

Media and Culture condition

The explants were cultured on MS medium with non- absorbent cotton plugged into the culture tubes. In the present study, MS medium was prepared using the stock solutions along with meso-inositol (100mg/l), 3% sucrose and different concentrations of Cytokinins- 6-Benzylaminopurine (BAP) and Kinetin (Kn). Prior to inserting 7.0 g/L agar and autoclaving at 121 °C for 20 min at 15 psi, the pH of the medium was changed to 5.7 with 0.1 N NaOH or HCl. The cultures were held at a constant temperature of 25 ± 2 °C and were kept on a 16/8 h (light/dark) photoperiod with a light intensity of 45 mol $m^{-2}S^{-1}$ (approx).

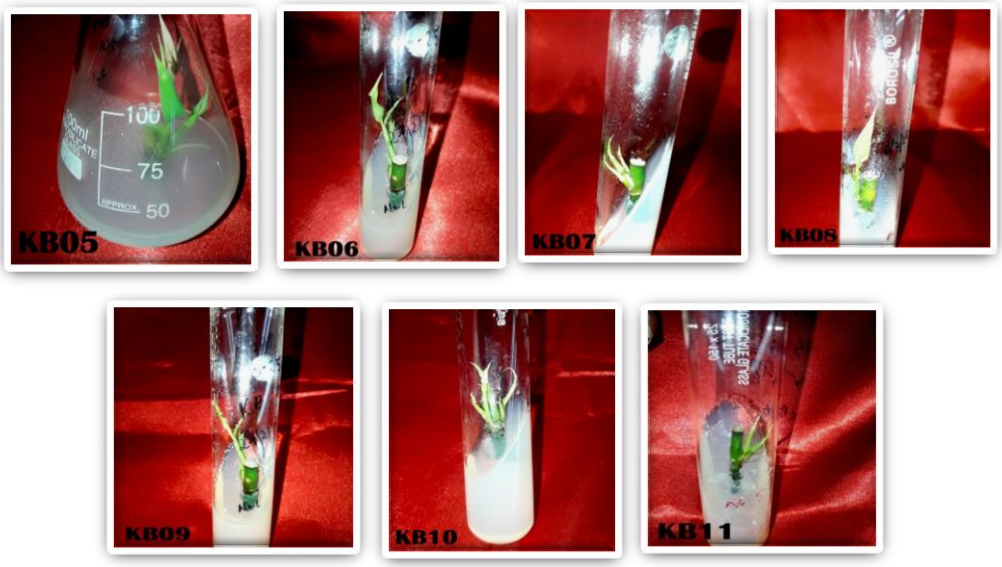
Shoot bud initiation and multiplication

After 4 weeks, the observations were recorded. The proliferated shoots were taken from the mother plant and sub- cultured on MS medium with various Cytokinin concentrations. Again, after about 4 weeks, the observations were recorded.

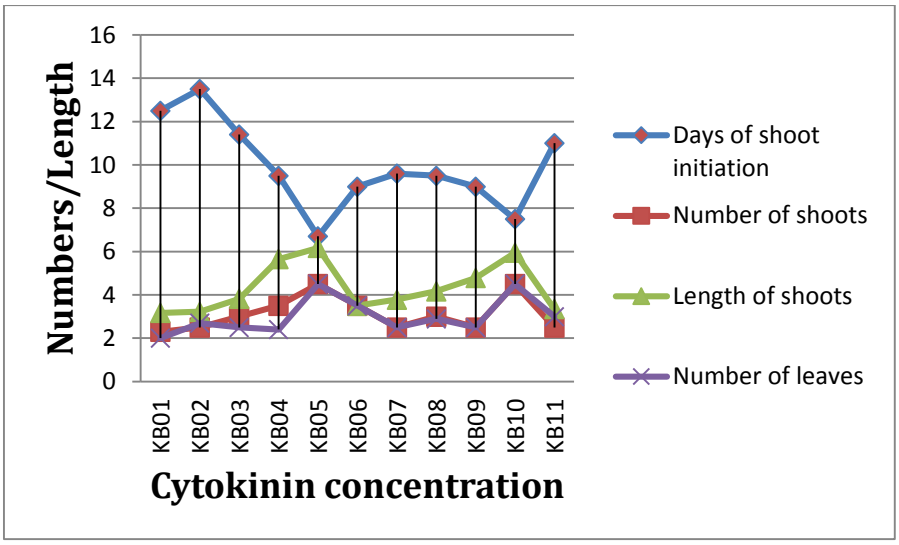
3. Result and discussion

Shoot bud proliferation

After proliferation, the elongated shoots were excised and transferred to fresh MS medium containing different concentrations of cytokinin. Different cytokinin concentrations affected the percentage of culture responses, the number of shoots initiated, the shoot length, and number of bud break duration (Figure1). This variation could be due to size, age and other conditions of the explants. Of the various cytokinin concentrations, MS medium with 3.0mg/l BAP showed the highest frequency of bud break. The days of shoot initiation, number of shoots, shoot length and numbers of leaves are shown in table 1



Note: KB01= control (without PGR); KB02= 0.5 mg/l BAP; KB03=1.0 mg/l BAP; KB04=2.0 mg/l BAP; KB05= 3.0 mg/l BAP; KB06= 4.0 mg/l BAP; KB07=0.5 mg/l KN; KB08= 1.0 mg/l KN; KB09=2.0 mg/l KN; KB10=3.0 mg/l KN; KB11= 4.0 mg/l KN



Note: KB01= Hormone free (control); KB02=MS+0.5 mg/l BAP; KB03=MS+1.0 mg/l BAP; KB04=MS+2.0 mg/l BAP; KB05=MS+3.0 mg/l BAP; KB06=MS+4.0 mg/l BAP; KB07=MS+0.5 mg/l KN; KB08=MS+1.0 mg/l KN; KB09=MS+2.0 mg/l KN; KB10=MS+3.0 mg/l KN; KB11=MS+4.0 mg/l KN.

Figure 1: Effect of BAP and KN on shoot proliferation of *B. tulda* after 25 days.

Table 1: Effect of BAP and KN concentration on in vitro shoot proliferation of *B. tulda* in MS medium.

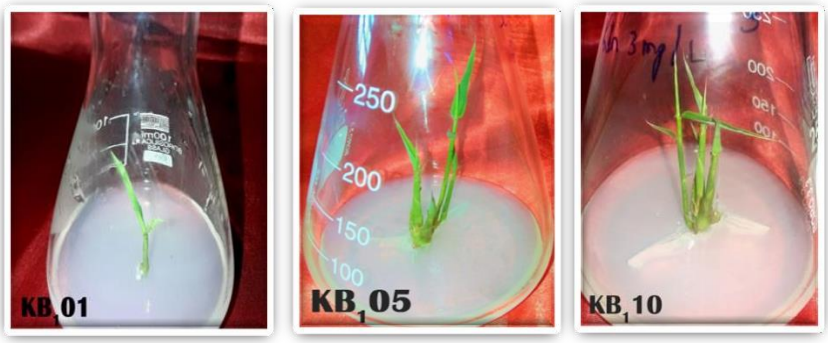
Treatment code	Concentration of cytokinins (mg/l)		Days of shoot initiation	No of shoots	Length of shoots (cm)	No of leaves
	BAP	KN				
KB01	0	0	12.5±0.7	2.3±0.48	3.17±0.46	2.0±1.41
KB02	0.5	0	13.5±2.12	2.5±0.70	3.22±0.89	2.7±0.48
KB03	1.0	0	11.4±1.71	3.0±0.47	3.82±0.49	2.5±0.70
KB04	2.0	0	9.5±3.53	3.5±0.71	5.65±0.57	2.5±0.70
KB05	3.0	0	6.7±0.48	4.5±0.70	6.17±1.10	4.5±0.70

KB06	4.0	0	9.0±1.41	3.5±0.71	3.5±0.68	3.5±0.71
KB07	0	0.5	9.6±0.84	2.5±0.70	3.78±0.25	2.5±0.71
KB08	0	1.0	9.5±0.0	3.0±1.4	4.17±0.46	2.9±0.32
KB09	0	2.0	9.0±0.71	3.5±0.71	4.78±0.70	2.5±0.70
KB10	0	3.0	7.5±2.12	4.5±0.71	5.95±0.45	4.5±0.70
KB11	0	4.0	11.0±1.41	2.5±0.71	3.35±0.59	3.0±1.40

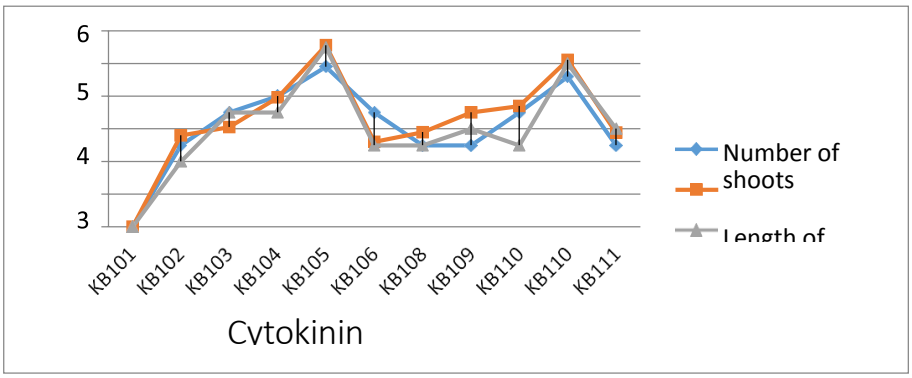
Note: ±=Standard Deviation

Shoot bud multiplication

Shoot multiplication is also found to be highest in MS medium incorporated with 3mg/l BAP. The average number of shoots was observed to be highest at 3.0 mg/l BAP (4.9±0.32) with average shoot length (5.56±1.83) followed by 3.0 mg/l KN with average number of shoots (4.6±0.52) and average shoot length (5.11±1.43). Treatments such as MS+0.5 mg/l, MS+1.0 mg/l, MS+ 2.0 mg/l and MS+4.0mg/l BAP showed more mean number of shoots compared to MS+0.5 mg/l, MS+1.0 mg/l, MS+2.0 mg/l and MS+4.0 mg/l KN treatments. The average superiority of BAP over KN in shoot induction may be due to production of natural hormone such as zeatin within the tissues than other synthetic cytokinins [20]. Accordingly, plant tissues have the ability to metabolize natural hormones faster than the artificial growth hormones. The shortest shoot length was recorded 4.0 mg/l BAP (2.6±0.72) followed by 4.0 mg/l KN (2.88±0.73) and 0.5 mg/l BAP (2.8±1.11). The highest number of leaves were also recorded at 3.0 mg/l BAP (5.5±0.71) followed by 3.0 mg/l KN (5.0±1.41). The average number of leaves observed at different concentrations of cytokinins (Figure 2) is shown in table (Table2).



Note: $KB_1$01=Control (without PGRs); $KB_1$05=3.0 mg/l BAP; $KB_1$10=3.0 mg/l KN



Note: $KB_1$01=Hormone free (control); $KB_1$02=MS+0.5 mg/l BAP; $KB_1$03=MS+1.0 mg/l BAP; $KB_1$04=MS+2.0 mg/l BAP; $KB_1$05=MS+3.0 mg/l BAP; $KB_1$06=MS+4.0 mg/l BAP; $KB_1$07=MS+0.5 mg/l KN; $KB_1$08=MS+1.0 mg/l KN; $KB_1$09=MS+2.0 mg/l KN; $KB_1$10=MS+3.0 mg/l KN; $KB_1$11=MS+4.0 mg/l KN.

Figure 2: Effect of BAP and KN on shoot multiplication after 25 days.

Table 2: Effect of BAP and KN concentration in MS medium on in vitro shoot multiplication of *B. tulda*.

Treatment code	Concentration of cytokinins (mg/l)		No of shoots	Length of shoots (cm)	No of leaves
	BAP	KN			
KB101	0	0	0	0	0
KB102	0.5	0	2.5±0.71	2.8±1.11	2.0±0.0
KB103	1.0	0	3.5±0.70	3.05±0.63	3.5±0.71
KB104	2.0	0	4.0±1.41	3.96±1.32	3.5±0.71
KB105	3.0	0	4.9±0.32	5.56±1.83	5.5±0.71
KB106	4.0	0	3.5±0.71	2.6±0.72	2.5±0.70
KB107	0	0.5	2.5±0.70	2.9±0.20	2.5±0.70
KB108	0	1.0	2.5±0.70	3.5±0.90	3.0±1.41
KB109	0	2.0	3.5±0.70	3.7±0.75	2.5±0.70
KB110	0	3.0	4.6±0.52	5.11±1.43	5.0±1.41
$KB_1$11	0	4.0	2.5±0.70	2.88±0.73	3.0±1.41

4. Conclusion

The current research contributes to the conception of a regular protocol in which 3 mg/l BAP was found to be beneficial for Bambusa tuda bud break and shoot multiplication. As a result, the research offers a viable method for micropropagating B. tulda from nodal segments of a field-grown culm.

Acknowledgement

Our sincere thanks to University of Science and Technology, Meghalaya for providing necessary facilities for conducting the experiments.

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