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In Vitro Shoot Initiation and Multiplication of Bambusa Balcooa Using Cytokinin Combinations

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Abstract: *The present study was undertaken to develop and standardize an efficient in vitro micropropagation protocol for Bambusa balcooa, an economically and ecologically important bamboo species. Healthy nodal segments collected from disease free plants were used as explants for in vitro culture. A systematic methodology involving explant selection, surface sterilization, media preparation, incubation under controlled conditions, shoot initiation, and shoot multiplication was followed. Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of cytokinins, namely 6-benzylaminopurine (BAP) and kinetin (KIN), was used to evaluate their effect on shoot initiation and multiplication.*

Shoot initiation was successfully achieved on all treatment combinations. Among them, MS medium supplemented with 1.5 mg L^{-1} BAP and 2.0 mg L^{-1} KIN proved to be the most effective for shoot initiation, producing an average of 2.75 shoots per explant with a mean shoot length of 3.22 cm after three weeks of culture. For shoot multiplication, regenerated shoots were subcultured on MS medium containing the same growth regulator combinations. The highest multiplication response was recorded on MS medium fortified with 1.5 mg L^{-1} BAP and 1.5 mg L^{-1} KIN, yielding an average of 8.08 shoots per explant with a mean shoot length of 4.07 cm after one month.

The results of the study demonstrate the synergistic effect of BAP and kinetin on shoot regeneration and proliferation in Bambusa balcooa. The standardized protocol developed in this study offers a reliable method for rapid clonal propagation and has significant potential for large-scale production, commercial cultivation, and conservation of this important bamboo species.

Keywords: Bambusa balcooa, BAP, kinetin, MS medium, shoot multiplication

I. INTRODUCTION

Bamboo is recognized as one of the fastest growing and most versatile plants on Earth (Akinlabi et al., 2017). It represents a unique group of tall perennial grasses characterized by woody, jointed stems known as culms. Taxonomically, bamboo belongs to the subfamily Bambusoideae under the grass family Poaceae (Gramineae) (Ohrnberger, 1999). Approximately 123 genera and more than 1500 species of bamboo plant have been identified across the world Ahmad et al. 2021. *Bambusa balcooa* Roxb. is an indigenous and widely distributed species of North East India (Das et al., 2012).

In India, the growing demand for quality bamboo planting material has resulted in a significant gap between supply and requirement, particularly for economically important species such as *Bambusa balcooa* (Shirin et al., 2026). At the same time, the need to shift from wood to sustainable alternative raw materials has highlighted bamboo as a promising substitute due to its rapid growth and wide utility. Efficient *in vitro* propagation, supported by the selection of superior genotypes and optimized combinations of plant growth regulators, enables large-scale multiplication of high-quality planting material, improves shoot multiplication and rooting, and ensures successful acclimatization, thereby supporting commercial plantations while reducing pressure on forest resources (Nurhayani et al., 2018).

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II. MATERIALS AND METHODS

Selection of Explants: Healthy, vigorous, and disease free plants of *Bambusa balcooa* growing on the campus of the College of Agriculture, Pune, were selected as the source of explants. Young shoots and nodal segments were excised from the plants chosen and used as explants, as these tissues are reported to exhibit higher responsiveness and regeneration potential under *in vitro* conditions in bamboo species.

Methods: In the present study, a systematic methodology was followed to standardize an in vitro propagation protocol for *Bambusa balcooa*. The experimental procedure involved sequential steps including explant collection, surface sterilization, preparation of culture media, inoculation of explants, incubation under controlled environmental conditions, and periodic observation of growth and morphogenic responses.

Preparation of Culture Media: MS medium (Murashige and Skoog, 1962) was used as the basal medium, as it is widely reported to be effective for the micropropagation of ***Bambusa balcooa***. The composition and preparation procedure for one liter of MS medium were followed according to Illi et al., 2016). Stock solutions of macro and micronutrients were prepared by dissolving the required quantities of salts separately and thoroughly mixed using a vortex mixer to ensure complete dissolution. Subsequently, 30 g of sucrose and 100 mg of myo-inositol were dissolved in approximately 200 ml of double-distilled water. Plant growth regulators (PGRs) were incorporated into the medium at varying concentrations to optimize shoot initiation and regeneration responses in the modified MS medium. The pH of the medium was adjusted to 5.6–5.8 using 1 N NaOH or 0.1 N HCl, and the volume was made up to 400 ml with double-distilled water. For solidification, 7.5 g of agar was added to the medium and heated at 60°C until completely dissolved. The final volume was then adjusted to 1000 ml with double-distilled water. The prepared medium was dispensed into culture bottles at a volume of approximately 25–30 ml per bottle, sealed with caps, and sterilized by autoclaving at 121°C under 15 psi pressure for 15–20 minutes. After autoclaving, the culture media were allowed to cool and solidify at room temperature and stored in a cool, dry place until further use.

Table 1: Different cytokinin concentrations used in the culture medium.

Sr. No.	Treatment name	Hormone combinations (mg/L)
1	T0	M.S. (Control)
2	T1	M.S. + BAP 1.5 + Kinetin 0.5
3	T2	M.S. + BAP 1.5 + Kinetin 1.0
4	T3	M.S. + BAP 1.5 + Kinetin 1.5
5	T4	M.S. + BAP 1.5 + Kinetin 2.0

Sterilization of Glassware and Instruments: Heat-resistant borosilicate glassware, including beakers, test tubes, culture bottles, pipettes, measuring cylinders, Petri plates, and conical flasks, were used for the present investigation. All glassware was initially cleaned by soaking in liquid detergent overnight, followed by thorough washing with tap water. The cleaned glassware was then dried and sterilized in a hot air oven at 120°C for 3-4 hours.

Metal instruments such as forceps, spatulas, blade holders, and scalpels, along with distilled water, were sterilized by autoclaving at 121°C under a pressure of 15 lb/in² for 20 minutes. Culture tubes and conical flasks containing media were tightly capped or plugged with non-absorbent cotton prior to autoclaving to prevent contamination.

Surface Sterilization of Explants The collected nodal segments were initially washed thoroughly under running tap water to remove surface-adhered dirt and debris. To control fungal contamination, the explants were treated with 0.2% Bavistin solution for 10 minutes, followed by rinsing with sterile distilled water.

Surface sterilization was carried out by immersing the explants in 15% sodium hypochlorite solution for 5 minutes, followed by several washes with sterile distilled water. The explants were then briefly treated with 70% ethanol for 1 minute and subsequently rinsed again with sterile distilled water.

For complete disinfection, the explants were further treated with 0.1% mercuric chloride ($HgCl_2$) solution for 5 minutes under aseptic conditions. After each sterilization step, the explants were washed 2-3 times with sterile distilled water to remove residual traces of sterilizing agents before inoculation.

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Inoculation of Explants: Following surface sterilization, the explants were transferred to a sterile glass plate using sterile forceps under strict aseptic conditions inside a laminar airflow cabinet. The explants were trimmed into small segments of approximately 2–3 cm in length using a sterile scalpel.

During inoculation, the explants were carefully positioned to ensure proper contact with the culture medium. The explants were gently pressed into the medium with sterile forceps to secure their placement. After vertical inoculation of the explants into the culture bottles, the mouths of the bottles were briefly flamed, tightly capped, and sealed with parafilm to prevent external contamination. Each culture bottle was properly labeled with details such as medium composition, bottle number, and date of inoculation before being transferred to the culture room.

The inoculated cultures were incubated at a temperature of 25 ± 2°C under a photoperiod of 16 hours light and 8 hours darkness. Illumination was provided by cool white fluorescent lamps with a light intensity of approximately 8,000–10,000 lux in the culture room.

Shoot Multiplication: Shoot multiplication was performed using healthy and vigorous shoots measuring 4–5 cm in length obtained from the initiation stage. The selected shoots were transferred to (MS) medium supplemented with 6-benzylaminopurine (BAP) at a concentration of 1.5 mg L^{-1} in combination with different concentrations of kinetin (KIN) (0.5, 1.0, 1.5, and 2.0 mg L^{-1}) to induce and enhance shoot proliferation.

The cultures were maintained under controlled environmental conditions at 25 ± 2°C with a photoperiod of 16 hours light and 8 hours darkness. Observations on shoot multiplication parameters, including the number of shoots per explant and mean shoot length, were recorded after three weeks of culture.

III. RESULTS AND DISCUSSION

Shoot Initiation of *Bambusa balcooa*

Nodal segments of *Bambusa balcooa* were cultured on MSmedium supplemented with 6-benzylaminopurine (BAP) at 1.5 mg L^{-1} in combination with four different concentrations of kinetin (KIN) (0.5, 1.0, 1.5, and 2.0 mg L^{-1}) to evaluate their effect on shoot initiation. Observations recorded after three weeks of culture revealed that shoot induction occurred in all treatment combinations; however, variations were observed in both the number of shoots formed and shoot length.

Table.2: Effect of BAP and Kinetin on Shoot Initiation in *Bambusa balcooa*

Sr. No.	Medium + Hormone (mg/L)	Average shoot length (in cm)	Average no. of Shoots /explant	Shooting after Inoculation(days)
1	MS0 (Control)	1.2	1.05	12-13
2	MS + 1.5mg BAP + 0.5mg KIN	2.05	1.25	10-12
3	MS+ 1.5mg BAP + 1.0mg KIN	2.73	1.65	10-12
4	MS+ 1.5mg BAP + 1.5mg KIN	3.07	2.0	8-10
5	MS+ 1.5mg BAP + 2.0mg KIN	3.22	2.75	8-10

Shoot Multiplication of *Bambusa balcooa*

Following successful shoot initiation, the regenerated shoots of *Bambusa balcooa* were subcultured onto MS medium containing the same combinations of growth regulators to assess the shoot multiplication response. Observations recorded after one month of culture showed a significant increase in both the number and length of shoots across all treatment combinations.

The maximum shoot multiplication response was observed on MS medium supplemented with 1.5 mg L^{-1} BAP in combination with 1.5 mg L^{-1} kinetin, which produced an average of 8.08 shoots per explant with a mean shoot length of 4.07 cm table no.3. In contrast, the minimum response was recorded on MS medium containing 1.5 mg L^{-1} BAP + 0.5 mg L^{-1} kinetin, resulting in an average of 2.50 shoots per explant and a comparatively shorter mean shoot length of 3.25 cm.

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Table No. 3: Effect of different concentrations of BAP and KIN on Shoot Multiplication in *Bambusa balcooa*

Treatment No.	Medium + Hormone (mg/L)	Average shoot length (in cm)	Average no. of Shoots /explant
T0	MS0 (Control)	2.3	0.7
T1	MS + 1.5mg BAP + 0.5mg KIN	2.5	3.25
T2	MS+ 1.5mg BAP + 1.0mg KIN	2.73	5.65
T3	MS+ 1.5mg BAP + 1.5mg KIN	4.07	8.08
T4	MS+ 1.5mg BAP + 2.0mg KIN	6.22	4.75

Fig. 1: In vitro shoot Multiplication of *Bambusa balcooa* After 1 Month under optimized BAP and Kinetin Concentration.

IV. CONCLUSION

The present study entitled *"In vitro Micropropagation of Bamboo (Bambusa balcooa)"* successfully established a standardized and efficient protocol for the large-scale propagation of *Bambusa balcooa*. The effects of different concentrations of cytokinins, namely 6-benzylaminopurine (BAP) and kinetin (KIN), in MS medium were evaluated for shoot initiation and multiplication.

The results revealed that MS medium supplemented with 1.5 mg L^{-1} BAP in combination with 2.0 mg L^{-1} kinetin was most effective for shoot initiation, producing the highest shoot regeneration response with an average of 2.75 shoots per explant and a mean shoot length of 3.22 cm table no.2 . For shoot multiplication, the optimum response was obtained on MS medium fortified with 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} kinetin, which resulted in the maximum multiplication rate of 8.08 shoots per explant with an average shoot length of 4.07 cm table No. 2.

The findings of the present investigation contribute significantly to the improvement of micropropagation techniques for *Bambusa balcooa* and demonstrate the potential of this optimized protocol for rapid clonal multiplication. The established protocol can be effectively utilized for mass propagation, commercial cultivation, and conservation of this economically important bamboo species and may serve as a basis for future research in bamboo biotechnology.

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