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Optimization of Surface Sterilization and Plant Growth Regulator Regimes for *In vitro* Micropropagation of *Dendrocalamus stocksii* (Munro) Benth.ex Gamble via Nodal Segment Culture

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ABSTRACT:
Dendrocalamus stocksii (Munro) Benth.ex Gamble is an ecologically and industrially important sympodial bamboo species distributed across the Western Ghats of India, valued for its structural strength and diverse commercial applications. Conventional propagation of this species is severely constrained by the gregarious flowering habit, low seed viability, and limited efficiency of vegetative methods, necessitating the development of reliable *in vitro* propagation protocols. The present study was undertaken to optimize surface sterilization procedures and plant growth regulator (PGR) regimes for the micropropagation of D. stocksii using nodal segment explants collected from the Bamboosetum, State Forest Research Institute, Jabalpur, Madhya Pradesh, India. Surface sterilization experiments demonstrated that sequential treatment with 1.0% Bavistin (carbendazim) for 30 minutes followed by 0.10% mercuric chloride (HgCl2) for 10 minutes yielded the minimum contamination rate of 3% and maximum explant survival of 90%. For shoot proliferation, nodal segments were cultured on Murashige and Skoog (MS) basal medium supplemented with varying concentrations of 6-Benzylaminopurine (BAP; 0.0–4.0 mg/L) in combination with Naphthalene Acetic Acid (NAA; 0.0–0.5 mg/L). The treatment comprising 3.0 mg/L BAP + 0.5 mg/L NAA proved optimal, producing the highest shoot initiation (85–90%), proliferation (90–95%), and elongation (90%) at 7, 14, and 21 days post-inoculation, respectively, with well-developed, healthy plantlets recorded at 28 days. Supraoptimal BAP concentrations (4.0 mg/L) resulted in marked growth inhibition and callus formation. The protocols established in this study provide a reproducible scientific foundation for the large-scale clonal propagation and conservation of D. stocksii.

KEY WORDS: *Dendrocalamus Stocksii*, Micropropagation, Nodal Segment Culture, BAP, NAA, Surface Sterilization, Shoot Proliferation, Plant Tissue Culture.

INTRODUCTION

Bamboo, a member of the family Poaceae and subfamily Bambusoideae, represents one of the most ecologically and economically significant plant groups in the world. Comprising over 1,500 species distributed across approximately 50 genera, bamboo occupies diverse ecological niches spanning tropical, subtropical, and temperate regions of Asia, Africa, and the Americas [26,12]. Its remarkable biological attributes including an exceptionally rapid growth rate, high biomass productivity, and a renewable harvest cycle have earned it the designation of "green gold" among plant resources [22] Beyond its economic utility, bamboo fulfils critical ecological functions: it sequesters carbon at rates comparable to fast-growing tree species, stabilises degraded soils, regulates local hydrology, and supports diverse faunal communities within bamboo-dominant ecosystems[15,9].

Dendrocalamus stocksii (Munro) Benth.ex Gamble is a clump-forming, sympodial bamboo species of considerable ecological and industrial importance. Naturally distributed across the tropical and sub-tropical forests of the Central and Western Ghats of India encompassing the states of Maharashtra, Goa, Karnataka, and Kerala it is known regionally by vernacular names including Marihal, Manga, Men, and Chiva [23]. The species is characterised by its large, straight, thick-walled culms attaining heights of 10–20 metres and diameters of 5–10 cm, properties that render it particularly well-suited for construction, scaffolding, furniture fabrication, and handicraft industries [24]. Its adaptability to diverse soil types, high biomass yield, and structural integrity position D. stocksii as a priority species for commercial cultivation, agroforestry integration, and ecological restoration programmes in peninsular India.

Despite its multifaceted significance, the large-scale deployment of *Dendrocalamus stocksii* in plantation and conservation programmes is severely constrained by the inherent limitations of conventional propagation methods. Seed-based propagation is fundamentally unreliable owing to the species' gregarious, mast-seeding reproductive strategy a phenomenon wherein bamboo populations flower synchronously, set seed, and subsequently die at intervals of several decades [10,7]. The infrequency and unpredictability of flowering events, combined with the characteristically low viability and rapid loss of germinability of bamboo seed, render seed propagation practically unfeasible for sustained nursery production [20]. Vegetative propagation techniques, including rhizome division and branch or culm cuttings, are constrained by the limited availability of suitable propagules, the low rooting efficiency of mature tissue, the labour and resource costs associated with large-scale operations, and the inherent risk of pathogen transmission from source plants to propagated material [2].

Plant tissue culture and specifically micropropagation offers a scientifically robust and practically viable alternative for the rapid, year-round, large-scale clonal multiplication of elite bamboo genotypes under aseptic laboratory conditions. Micropropagation bypasses the limitations of conventional methods by enabling the regeneration of genetically uniform, pathogen-free plantlets from meristematic explant sources, independent of seasonal constraints [13,3]. The nodal segment, bearing an axillary meristem of established organogenic competence, has been established as the preferred explant type for shoot multiplication in recalcitrant woody monocots, including numerous bamboo species [19,1]. The successful deployment of micropropagation protocols for bamboo species, however, is contingent upon the careful optimization of two rate-limiting parameters: the elimination of microbial contamination through effective explant surface sterilization, and the identification of appropriate plant growth regulator (PGR) concentrations and combinations that reliably promote shoot proliferation and subsequent root induction.

Plant growth regulators occupy a central position in the regulation of morphogenesis in plant tissue culture systems. Cytokinins, and particularly 6-Benzylaminopurine (BAP), are the most widely employed class of PGRs for the stimulation of axillary bud break and multiple shoot proliferation in bamboo micropropagation, functioning by overcoming apical dominance and activating latent axillary meristems [25,21. The concentration of BAP must be empirically optimized for each species, as both sub-optimal and supra-optimal concentrations impair shoot induction, with excessive cytokinin levels frequently associated with vitrification, abnormal morphology, and callus formation at the expense of organised shoot development [14]. Auxins, including Naphthalene Acetic Acid (NAA) and Indole-3-butyric acid (IBA), complement cytokinin activity by promoting root initiation in regenerated shoots and maintaining cellular polarity during organogenesis [27]. The judicious combination and sequential application of cytokinins and auxins across distinct culture stages constitutes the methodological cornerstone of successful bamboo micropropagation protocols.

Successful micropropagation protocols have previously been established for a number of commercially important bamboo species, including Bambusa bambos [1], Dendrocalamus asper [17], Bambus abalcooa [4], and Dendrocalamus giganteus [16], confirming the general applicability of cytokinin-auxin-based tissue culture systems to this plant group. However, species-specific variation in explant responsiveness, optimal PGR concentrations, and rooting behaviour necessitates independent protocol development for each target species. To date, no systematically optimized micropropagation protocol has been reported for *Dendrocalamus stocksii*, representing a significant gap in the literature that limits the conservation and commercial exploitation of this priority species.

The present investigation was therefore designed to develop and optimize an *in vitro* propagation protocol for *Dendrocalamus stocksii* using nodal segment explants sourced from the Bamboosetum maintained at the State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India. The study was specifically directed towards achieving the following objectives:

1. To evaluate the effect of different plant growth regulators (PGRs), with emphasis on varying concentrations of BAP in combination with NAA, on axillary bud break and shoot proliferation in *Dendrocalamus stocksii* under *in vitro* conditions.
2. To optimize surface sterilization treatments by assessing the efficacy of Bavistin (carbendazim) and mercuric chloride (HgCl2) in minimizing microbial contamination while preserving explant viability.

The findings of this study was expected to contribute to the growing body of knowledge on bamboo tissue culture and provide a reproducible, evidence-based foundation for the large-scale clonal propagation of D. stocksii in support of plantation forestry, agroforestry, and conservation programmes in India.

MATERIALS AND METHODS

Plant Material and Explant Collection: Healthy, phenotypically superior plants of *Dendrocalamus stocksii* (Munro) Benth.ex Gamble were selected as the source of explant material from the Bamboosetum maintained under the Biotechnology Division, State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India. Nodal segments bearing intact axillary buds were chosen as the explant type on account of their established high regenerative and morphogenic competence in monocotyledonous tissue culture systems (Ramanayake et al., 2001). Explants of uniform size (approximately 2–3 cm in length) were excised from actively growing, young shoots using sterile sharp secateurs and immediately transported to the laboratory in sealed polythene bags lined with moistened sterile filter paper to minimize desiccation stress and preserve tissue viability. To ensure optimal explant responsiveness, collection and inoculation were performed on the same day, thus minimizing the interval between excision and culture establishment.

Laboratory Infrastructure and Equipment: All experimental procedures were carried out in the dedicated Plant Tissue Culture Laboratory, Biotechnology Division, State Forest Research Institute, Jabalpur. Standard laboratory glassware including conical flasks, beakers, volumetric flasks, measuring cylinders, pipettes, Petri dishes, culture tubes, and test tube stands were also employed throughout. Sterile stainless-steel forceps, scalpels fitted with size 22 surgical blades, and scissors were used for all explant manipulation and trimming procedures under aseptic conditions.

2.3 Surface Sterilization of Explants: Effective surface sterilization, balancing the elimination of microbial contaminants with the preservation of explant viability, is the foundational prerequisite for successful *in vitro* culture establishment [21]. A sequential, multi-agent sterilization protocol was developed and optimized for nodal segment explants of D. stocksii as detailed below.

Pre-wash: Freshly collected nodal segments were washed thoroughly under running tap water for 20–30 minutes to remove gross surface particulates, dust, and loosely adhering epiphytic microorganisms.

Detergent treatment: Explants were immersed in a dilute commercial detergent solution (Extran, 2–3 drops per 100 mL sterile distilled water) for 10 minutes with gentle agitation, followed by rinsing with sterile double-distilled water (DDW) three to four times to remove detergent residues.

Table 2.2: Sequential surface sterilization protocol for nodal segment explants of *Dendrocalamus stocksii*

Step	Agent	Concentration	Duration	Purpose
1	Tap water wash	—	20–30 min	Removal of gross surface contaminants
2	Detergent (Extran)	Dilute (2–3 drops/100 mL)	10 min	Reduction of surface microbial load
3	Sterile DDW rinse	—	3–4 rinses	Removal of detergent residue
4	Bavistin (carbendazim)	0.0–1.0% (w/v)	25–30 min	Antifungal surface treatment
5	Sterile DDW rinse	—	3–4 rinses	Removal of Bavistin residue
6	Ethanol	70% (v/v)	30–60 sec	Broad surface disinfection
7	Mercuric chloride ($HgCl_2$)	0.01–0.10% (w/v)	5–10 min	Broad-spectrum surface sterilization
8	Sterile DDW rinse	—	4–5 rinses	Complete removal of $HgCl_2$

Fungicide treatment (Bavistin): To reduce surface fungal load, explants were treated with aqueous solutions of Bavistin (carbendazim; BASF, India) at varying concentrations (0.0%, 0.1%, 0.2%, 0.6%, 0.8%, and 1.0% w/v) for exposure durations of 25–30 minutes on an orbital shaker. Solutions were prepared by dissolving the requisite quantity of Bavistin in sterile double-distilled water. Following treatment, explants were rinsed with sterile DDW three to four times.

Mercuric chloride ($HgCl_2$) treatment: Explants were subsequently surface-sterilized by immersion in aqueous solutions of mercuric chloride ($HgCl_2$) at concentrations of 0.01%, 0.02%, 0.05%, and 0.10% (w/v) for 5–10 minutes under continuous agitation. Mercuric chloride functions as a potent broad-spectrum biocide effective against both bacterial and fungal contaminants through disruption of cell membrane integrity and enzyme inactivation [11].

Final rinse: Following $HgCl_2$ treatment, all explants were rinsed exhaustively four to five times with sterile double-distilled water inside the laminar air flow cabinet to ensure complete removal of residual sterilant prior to inoculation.The complete sterilization protocol is summarized in Table 2.2.

Table 2.4: Stock solution compositions for MS medium preparation

Stock	Components	Concentration
Stock I — Macronutrients (20×)	NH_4NO_3, KNO_3, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4	20× concentrated
Stock II — Micronutrients (200×)	KI, H_3BO_3, $MnSO_4$, $ZnSO_4$, Na_2MoO_4, $CuSO_4$, $CoCl_2$	200× concentrated
Stock III — Iron (200×)	$FeSO_4 \cdot 7H_2O$, $Na_2EDTA \cdot 2H_2O$	200× concentrated
Stock IV — Vitamins (200×)	myo-Inositol, Nicotinic acid, Pyridoxine, Thiamine, Glycine	200× concentrated

Culture Medium Composition and Preparation: Murashige and Skoog (MS) basal medium [11] was used as the standard nutrient formulation for all *in vitro* propagation experiments, as it provides a comprehensive supply of macronutrients, micronutrients, vitamins, and organic supplements required for optimal *in vitro* growth of plant tissues.

Preparation of Stock Solutions: To facilitate accurate and efficient medium preparation, concentrated stock solutions were prepared for macronutrients (Stock I, 20×), micronutrients (Stock II, 200×), iron (Stock III, 200×), and vitamins (Stock IV, 200×) as detailed in Table 2.4. Stock solutions were stored at 4°C in amber glass bottles and used within four weeks of preparation.

Medium Preparation Procedure: For the preparation of one litre of MS medium, 500 mL of double-distilled water was taken in a conical flask and 30 g of sucrose was added and dissolved completely with stirring. Stock solutions were added sequentially: 50 mL of Stock I, 5 mL each of Stocks II, III, and IV. The required volume of plant growth regulator (PGR) stock solution was then incorporated. The volume was made up to 1,000 mL with sterile DDW and the pH adjusted to 5.7 ± 0.1 using 1N HCl or 1N NaOH as required, verified with a calibrated digital pH meter. Agar (8 g/L) was then added and dissolved by heating in a microwave oven until a clear, homogeneous solution was obtained. The medium was dispensed (approximately 15–20 mL) into pre-cleaned borosilicate culture tubes or conical flasks, sealed with cotton plugs or polypropylene caps, and sterilized by autoclaving at 121°C and 15 psi for 20–30 minutes (moist heat sterilization). Heat-sensitive PGRs such as BAP and NAA were filter-sterilized through 0.22 µm membrane filters and added aseptically to the autoclaved, cooled medium (approximately 50°C) prior to dispensing. Sterilized media were allowed to solidify at room temperature and stored at 4°C until use.

Plant Growth Regulators (PGRs): Plant growth regulators were incorporated into the MS basal medium to investigate their effects on shoot proliferation and root induction. The cytokinins and auxins employed, along with their concentration and different ranges.

Stock solutions of BAP and NAA were prepared by dissolving the required quantity in a minimum volume of 1N NaOH, followed by dilution to the target concentration with double-distilled water. NAA stock solutions were prepared by dissolving in a minimum volume of 95% ethanol before diluting with DDW. All PGR stock solutions were stored at 4°C and filter-sterilized prior to addition to autoclaved medium.

Inoculation and Culture Conditions: All inoculation procedures were performed within the laminar air flow (LAF) cabinet, which was decontaminated by exposure to UV irradiation for 20 minutes prior to each session and wiped thoroughly with 70% ethanol. Forceps, scalpels, and scissors were sterilized by immersion in 70% ethanol and flaming between operations on different explants to prevent cross-contamination. Surface-sterilized nodal segments were trimmed at both cut ends using a sterile scalpel to remove damaged or discoloured tissue, and inoculated vertically into culture tubes with the basal end embedded 5–8 mm into the solidified medium. Culture vessels were sealed with Parafilm

or polypropylene caps and labelled with treatment details and date of inoculation.

Inoculated cultures were transferred immediately to a controlled-environment culture room maintained at 25 ± 2°C under a 16-hour photoperiod (2,000–3,000 lux light intensity provided by cool-white fluorescent lamps) and 8 hours of darkness, with relative humidity maintained at 60–70%. Cultures were examined at regular intervals for evidence of microbial contamination, bud break, callus formation, shoot initiation, and root development.

Subculturing: Subculturing was performed at intervals of three to four weeks to sustain shoot proliferation and transfer developing shoots to fresh medium. Prior to each subculture session, the LAF cabinet was UV-irradiated for 20 minutes and wiped with 70% ethanol. Instruments were re-sterilized using a hot-bead sterilizer (250°C, 15 seconds) between transfers. Shoots were excised at the nodal region using sterile scalpels and transferred individually to freshly prepared medium of the appropriate composition. Growth parameters including shoot initiation percentage, shoot proliferation percentage, shoot elongation (cm), and rooting response were recorded at intervals of 7, 14, 21, and 28 days post-inoculation.

Hardening and Acclimatization: Well-developed, rooted plantlets were carefully removed from culture vessels and the adhering agar was washed off completely under running water to prevent fungal proliferation in the substrate. Plantlets were transferred to a soil mixture composed of garden soil, cocopeat, and vermiculite (1:1:1, v/v/v) in plastic pots. Pots were maintained initially in a humidity chamber under controlled conditions (high humidity, 25 ± 2°C, diffuse light) to minimize transplant shock, and relative humidity was progressively reduced over a period of two to three weeks to acclimatize the plantlets to ambient ex vitro conditions. The survival percentage and growth vigour of hardened plantlets were recorded at weekly intervals.

Experimental Design and Statistical Analysis: All experiments were conducted in a completely randomized design (CRD) with a minimum of three replicates per treatment, each replicate comprising five culture tubes. Data are expressed as mean ± standard error (SE). One-way Analysis of Variance (ANOVA) was applied to assess the statistical significance of differences among treatment groups, followed by Duncan's Multiple Range Test (DMRT) for post-hoc pairwise comparisons at a significance level of $p \leq 0.05$. All statistical analyses were performed using SPSS software (version 26.0, IBM Corp.).

RESULTS AND DISCUSSION

The present investigation evaluated the effect of varying concentrations and combinations of plant growth regulators (PGRs) on *in vitro* shoot initiation, proliferation, elongation, and plantlet development in *Dendrocalamus stocksii*, alongside the optimization of surface sterilization protocols using Bavistin (carbendazim) and mercuric chloride (HgCl2). Observations were recorded at regular intervals of 7, 14, 21, and 28 days post-inoculation. The results are presented and discussed under the following sub-sections.

3.1 Experiment I: Optimization of Surface Sterilization Protocols

Effect of Bavistin (Carbendazim) Concentration and Exposure Duration on Contamination Rate: Surface sterilization constitutes the most critical preliminary step in the establishment of axenic plant tissue cultures, as field-collected explants of woody monocots such as *Dendrocalamus stocksii* invariably harbour a substantial load of fungal endophytes, surface bacteria, and airborne spores that are refractory to simple washing procedures (Bhojwani and Razdan, 1996). In the present study, nodal segment explants were treated with Bavistin (carbendazim) solutions across a concentration gradient of 0.0–1.0% (w/v) for exposure durations of 5–35 minutes. The resultant contamination rates are presented in Table 3.1.

Table 3.1: Effect of Bavistin concentration and exposure duration on contamination rate in nodal segment explants of *Dendrocalamus stocksii*

Treatment	Bavistin Concentration (% w/v)	Duration (min)	Contamination Rate (%)
T0	0.0 (Control)	5	90
T1	0.1	10	70
T2	0.2	15	50
T3	0.6	20	30
T4	1.0	30	3
T5	0.8	35	15

A pronounced concentration- and duration-dependent reduction in contamination rate was observed across the Bavistin treatment series (Table 3.1). The untreated control (T0; 0.0% Bavistin, 5 minutes) exhibited the highest contamination rate of 90%, confirming that surface washing alone is inadequate for the elimination of fungal and bacterial contaminants from bamboo nodal explants. Contamination rates declined progressively with increasing Bavistin concentration and exposure duration, from 70% in T1 (0.1%, 10 min) to 50% in T2 (0.2%, 15 min) and 30% in T3 (0.6%, 20 min). The minimum contamination rate of 3% was recorded in T4 (1.0% Bavistin, 30 min), establishing this as the most effective fungicidal treatment among those evaluated.

Notably, T5 (0.8% Bavistin, 35 min) yielded a higher contamination rate (15%) than T4, despite its longer exposure duration. This apparent anomaly may be attributable to the

sub-optimal fungicidal concentration of 0.8% relative to 1.0%, confirming that concentration is the primary determinant of Bavistin efficacy over exposure time within the ranges tested. Carbendazim, the active systemic benzimidazole component of Bavistin, exerts its antifungal action through inhibition of fungal tubulin polymerization, thereby disrupting mitosis and cell division in sensitive fungal species [5]. Its systemic mode of action renders it particularly effective against endophytic fungi that are inaccessible to surface-acting agents. Comparable findings have been reported by [1] in Bambusa bambos and by [20] in Bambusa vulgaris, wherein Bavistin treatment at concentrations of 0.5–1.0% significantly reduced fungal contamination in bamboo nodal cultures. The treatment T4 (1.0% Bavistin, 30 minutes) is therefore recommended as the optimal fungicidal sterilization step for D. stocksii nodal explants prior to HgCl2 treatment.

Effect of Mercuric Chloride ($HgCl_2$) Concentration and Exposure Duration on Explant Survival: Following Bavistin pre-treatment, explants were subjected to mercuric chloride (HgCl2) solutions at concentrations of 0.01–0.10% (w/v) for durations of 2–10 minutes to achieve complete broad-spectrum surface decontamination. Explant survival rates under each treatment are presented in Table 3.2.

Table 3.2: Effect of HgCl2 concentration and exposure duration on explant survival in *Dendrocalamus stocksii* nodal segments

Treatment	$HgCl_2$ Concentration (% w/v)	Duration (min)	Survival (%)	Observation
T1	0.01	2	60	High contamination
T2	0.02	3	70	Moderate sterilization
T3	0.05	5	50	Tissue damage observed
T4	0.10	10	90	Best sterilization, maximum survival

The results demonstrated a clear relationship between $HgCl_2$ concentration, exposure duration, and explant survival (Table 3.2). At the lowest concentration of 0.01% $HgCl_2$ for 2 minutes (T1), a survival rate of only 60% was recorded, accompanied by persistent high contamination, indicating insufficient sterilization efficacy. Moderate sterilization was achieved at 0.02% for 3 minutes (T2), yielding 70% survival. Treatment T3 (0.05%, 5 minutes) produced an unexpectedly lower survival rate of 50%, likely attributable to the onset of phytotoxic tissue damage at this concentration-duration combination, consistent with the known cytotoxic properties of mercuric ions at intermediate exposure thresholds [11].

The highest explant survival of 90% with minimum contamination was recorded in T4 (0.10% $HgCl_2$, 10 minutes), establishing this treatment as the optimal $HgCl_2$ sterilization condition for D. stocksii nodal segments. Mercuric chloride exerts its biocidal action through the non-specific precipitation of cellular proteins and inactivation of sulfhydryl-containing enzymes in microbial cells, making it one of the most potent broad-spectrum surface sterilants available for plant tissue culture applications [8]. The efficacy of 0.10% HgCl2 at 5–10 minutes in bamboo tissue culture has similarly been reported by [15] in Bambusa balcooa and by [15] in Dendrocalamus giganteus, lending strong comparative support to the present findings. The superior outcome obtained at T4 relative to T3 further demonstrates that, within species-specific tolerance thresholds, a higher concentration of HgCl2 combined with an adequate exposure period achieves decontamination without proportionally increasing phytotoxic injury, provided that thorough post-treatment rinsing with sterile distilled water is performed.

The combined sequential protocol of 1.0% Bavistin for 30 minutes followed by 0.10% $HgCl_2$ for 10 minutes, with exhaustive rinsing after each step, is thus recommended as the optimized surface sterilization protocol for nodal segment explants of D. stocksii.

3.2 Experiment II: Effect of BAP and NAA on *In Vitro* Shoot Initiation, Proliferation, Elongation, and Plantlet Development *In vitro*-raised nodal segments of D. stocksii were cultured on MS basal medium supplemented with varying concentrations of BAP (0.0–4.0 mg/L) in combination with NAA (0.0–0.5 mg/L). Growth responses were recorded at 7, 14, 21, and 28 days post-inoculation to capture the successive stages of shoot initiation, proliferation, elongation, and final plantlet development, respectively. The consolidated data are presented in Table 3.3.

Effect on Shoot Initiation (7 Days): Shoot initiation, defined as the visible emergence and elongation of an axillary bud from the nodal explant, was recorded at 7 days post-inoculation. A well-defined concentration-dependent pattern of response was observed across the BAP–NAA treatment series (Table 3.3). In the hormone-free control (T0), a markedly low shoot initiation of 20–30% was recorded, confirming that endogenous hormonal levels within the explant tissue were insufficient to overcome axillary bud dormancy under *in vitro* conditions. The poor initiation response in the absence of exogenous PGRs is

consistent with the reported high cytokinin requirement for activation of dormant axillary meristems in bamboo species [14,21,1].

Shoot initiation improved progressively with increasing BAP concentration, from 45% in T1 (1.0 mg/L BAP + 0.2 mg/L NAA) to 65% in T2 (2.0 mg/L BAP + 0.3 mg/L NAA). The maximum shoot initiation of 85–90% was recorded in T3 (3.0 mg/L BAP + 0.5 mg/L NAA), demonstrating that this PGR combination most effectively released axillary buds from dormancy. The stimulatory effect of BAP on bud break is attributable to its role in promoting cytokinin-regulated transcription of cell cycle genes, specifically those governing the G1-to-S phase transition, thereby activating meristematic cells that are arrested under apical dominance [25]; [27]. The supplementary presence of NAA at 0.5 mg/L likely contributed to initiation by modulating auxin-cytokinin crosstalk and facilitating vascular differentiation at the base of emerging shoots.

Table 3.3: Effect of BAP and NAA concentrations on in vitro growth parameters of *Dendrocalamus stocksii* at successive observation intervals

Treatment	BAP (mg/L)	NAA (mg/L)	7 Days Initiation (%)	14Days Proliferation (%)	21 Days Elongation (%)	28 Days Final Status
T0	0.0	0.0	20–30	25–35	30–40	Very poor growth
T1	1.0	0.2	45	55–60	60–65	Weak shooting
T2	2.0	0.3	65	75–80	85–90	Moderate shooting
T3	3.0	0.5	85–90	90–95	90	Excellent shooting; healthy plantlets
T4	4.0	0.5	50–60	55–65	60	Callus formation; poor growth

At the supraoptimal concentration of 4.0 mg/L BAP (T4), shoot initiation declined substantially to 50–60%, consistent with the well-established phenomenon of cytokinin toxicity at elevated concentrations, wherein excessive BAP disrupts hormonal homeostasis, induces oxidative stress, and suppresses normal organogenesis in favour of unorganized callus proliferation [14]. Similar inhibitory responses to high BAP concentrations have been reported in Dendrocalamus asper [17]and Bambusa bambos [1], strongly corroborating the present findings.

Effect on Shoot Proliferation (14 Days): Shoot proliferation, assessed as the percentage of explants producing multiple shoots per nodal segment, was recorded at 14 days post-inoculation. The proliferation data broadly paralleled the initiation response, with a pronounced concentration-dependent optimum at 3.0 mg/L BAP (Table 3.3). The control (T0) recorded a very low proliferation of 25–35%, confirming the essential role of exogenous cytokinins in driving cell division and multiple shoot formation under *in vitro* conditions. Proliferation increased substantially from 55–60% in T1 (1.0 mg/L BAP) to 75–80% in T2 (2.0 mg/L BAP), with the maximum proliferation of 90–95% achieved in T3 (3.0 mg/L BAP + 0.5 mg/L NAA). This treatment consistently produced multiple shoots per explant, with vigorous axillary bud activation and compact, well-organized shoot clusters.

The superior performance of T3 reflects the synergistic interaction between BAP-mediated cytokinin signalling and NAA-mediated auxin activity: while BAP drives cell division and lateral bud release, NAA at low concentrations facilitates shoot vascularization and maintains meristematic cell competence without suppressing shoot organogenesis [6]. Comparable proliferation rates at 2.5–3.0 mg/L BAP have been reported in Bambusa balcooa by [15], in Dendrocalamus strictus by Pattnaik and Chand (1996), and in Dendrocalamus giganteus by [15], confirming that 3.0 mg/L BAP represents a broadly optimal cytokinin concentration for multiple shoot induction in tropical bamboo species.

At T4 (4.0 mg/L BAP), proliferation declined markedly to 55–65% and was accompanied by the formation of abnormal, hyper-hydric callus tissue at the base of explants, indicative of the transition from organised organogenesis to unorganized somatic cell proliferation under conditions of hormonal excess [14]. This inhibitory response underscores the critical importance of maintaining BAP within species-specific optimal thresholds during the proliferation stage.

Effect on Shoot Elongation (21 Days): Shoot elongation, recorded at 21 days post-inoculation, represents a critical developmental transition from compact shoot clusters to individualized, elongated shoots of sufficient length for subsequent rooting or subculture. In the control (T0), elongation was very poor (30–40%), and shoots remained stunted and physiologically weak, consistent with the absence of exogenous growth hormone supplementation. Moderate elongation (60–65%) was observed at T1 (1.0 mg/L BAP + 0.2 mg/L NAA), which improved to 85–90% in T2 (2.0 mg/L BAP + 0.3 mg/L NAA). The highest elongation response of approximately 90% was recorded in T3 (3.0 mg/L BAP + 0.5 mg/L NAA), wherein shoots were well-elongated, turgid, and displayed normal leaf expansion.

The positive effect of low-concentration NAA on shoot elongation in this study is consistent with the established role of auxins in promoting cell elongation through the acid growth mechanism, wherein auxin-induced proton pumping loosens the cell wall and facilitates turgor-driven cellular expansion (Taiz and Zeiger, 2010). The combination of cytokinin-driven cell division with auxin-promoted elongation in T3 thus produced the most morphologically advanced shoots at 21 days. At T4 (4.0 mg/L BAP), elongation declined to 60%, likely reflecting the known antagonistic effect of supraoptimal cytokinin concentrations on internode elongation, mediated through interference with the gibberellin signalling pathway responsible for cell elongation in shoot internodes (14 27].

Effect on Final Plantlet Development and Shooting (28 Days): At 28 days post-inoculation, the overall status of plantlet development encompassing shoot organisation, root initiation, and general physiological vigour was assessed for each treatment. In the hormone-free control (T0), no proper shooting or root development was observed and cultures exhibited minimal growth, confirming that auxin supplementation is essential for rhizogenesis and that endogenous auxin levels are insufficient to sustain organised plantlet development under *in vitro* conditions.

Weak, poorly rooted shoots were observed in T1 (1.0 mg/L BAP + 0.2 mg/L NAA), and moderate shooting with limited rooting was recorded in T2 (2.0 mg/L BAP + 0.3 mg/L NAA). The most favourable outcome was achieved in T3 (3.0 mg/L BAP + 0.5 mg/L NAA), which produced well-developed plantlets with healthy, well-organised shoot systems and initial root primordia, indicating readiness for transfer to dedicated rooting medium and subsequent hardening. These plantlets exhibited normal morphology, robust leaf development, and uniform growth vigour, confirming the suitability of this PGR regime for the complete *in vitro* establishment of D. stocksii. At the highest PGR concentration (T4: 4.0 mg/L BAP + 0.5 mg/L NAA), plantlet quality was markedly compromised, with callus formation at the nodal base, poor shoot organisation, and negligible root initiation. Excessive cytokinin concentrations are known to promote unorganized callus proliferation at the expense of organised shoot and root development, and may also induce epigenetic changes that reduce the regenerative competence of cultured tissue over successive subcultures [14, 8]. This result strongly argues against the use of BAP concentrations exceeding 3.0 mg/L in D. stocksii micropropagation. Effect of BAP and NAA Concentrations on *In vitro* Shoot Initiation, Proliferation, Elongation, and Plantlet Development in *Dendrocalamus stocksii* results shown in bellow mention table no 3.4.

Table 3.4: Effect of BAP and NAA Concentrations on In Vitro Shoot Initiation, Proliferation, Elongation, and Plantlet Development in Dendrocalamus stocksii at Successive Observation Intervals (7, 14, 21, and 28 Days Post-Inoculation) on MS Basal

BAP (mg/L)	NAA (mg/L)	7 Days Initiation (%)	14 Days Proliferation (%)	21 Days Elongation (%)	28 Days Shooting / Final Status
0.0	0.0	20–30%	25–35%	30–40%	Very poor growth
1.0	0.2	45%	55–60%	60–65%	Weak shooting
2.0	0.3	65%	75–80%	85–90%	Moderate shooting
3.0	0.5	85–90%	90–95%	90%	Excellent shooting, healthy plantlets □
4.0	0.5	50–60%	55–65%	60%	Callus formation, poor growth

□Optimal treatment combination. BAP = 6-Benzylaminopurine; NAA = Naphthaleneacetic acid. Values represent percentage response of explants under each treatment.

The findings the present studies were in concordance with a substantial body of literature on bamboo micropropagation. [20] reported optimal shoot multiplication at 2.0–3.0 mg/L BAP in Bambusa vulgaris; [4] identified 3.0 mg/L BAP as the optimal concentration in Bambusa balcooa; and [11] similarly reported maximum proliferation at 3.0 mg/L BAP in Dendrocalamus giganteus. The convergence of these findings with the present results confirms that 3.0 mg/L BAP represents a broadly applicable optimum for cytokinin-mediated axillary shoot proliferation in tropical sympodial bamboo species, and that the inclusion of a low-concentration auxin complement (0.5 mg/L NAA) significantly enhances both elongation and overall plantlet quality.

CONCLUSION

The present investigation successfully established an optimized *in vitro* micropropagation protocol for *Dendrocalamus stocksii*, encompassing both surface sterilization and plant growth regulator management across all critical developmental stages. With respect to surface sterilization, a sequential treatment protocol comprising 1.0% Bavistin (carbendazim) for 30

minutes followed by 0.10% $HgCl_2$ for 10 minutes, with exhaustive rinsing after each step, proved most effective. This combination reduced contamination to a minimum of 3% while maintaining maximum explant survival of 90%, and is hereby recommended as the standard sterilization protocol for nodal segment explants of D. stocksii.

For *in vitro* growth and development, MS basal medium supplemented with 3.0 mg/L BAP in combination with 0.5 mg/L NAA (Treatment T3) consistently yielded the most favourable outcomes across all four observation intervals. This treatment achieved maximum shoot initiation (85–90%) at 7 days, highest proliferation (90–95%) at 14 days, optimal elongation (approximately 90%) at 21 days, and produced well-organized, healthy plantlets with incipient root primordia at 28 days. Both sub-optimal PGR concentrations (T1, T2) and supraoptimal concentrations (T4) resulted in significantly inferior growth responses, with T4 additionally inducing undesirable callus formation and morphological abnormalities indicative of cytokinin toxicity.

The convergence of the present findings with published data from related bamboo species including Bambusa vulgaris, Bambusa balcooa, Dendrocalamus strictus, and Dendrocalamus giganteus strongly validates the reproducibility and broader applicability of this protocol within tropical sympodial bamboos. The synergistic interaction between BAP-mediated cytokinin signalling and low-concentration NAA-mediated auxin activity observed in T3 represents the critical hormonal balance underlying successful *in vitro* establishment of this species.

These results collectively provide a reliable, reproducible, and scalable micropropagation protocol for D. stocksii that can be adopted for large-scale clonal propagation, germplasm conservation, and commercial nursery production of this economically and ecologically significant bamboo species.

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