Axillary Shoot Proliferation and Clonal Multiplication of *Bambusa vulgaris* Schrad. ex J.C.Wendl. var. *striata* through Optimised Surface Sterilization and Cytokinin-Auxin Synergy *In Vitro*

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Abstract- *Bambusa vulgaris* Schrad. ex J.C.Wendl. var. *striata* is an economically and ecologically significant bamboo species whose large-scale cultivation is severely constrained by limitations in conventional propagation. The present study was undertaken to establish an optimised *in vitro* micropropagation protocol for this species through systematic evaluation of surface sterilization regimes and plant growth regulator (PGR) combinations. Nodal segments (1.5–2.5 cm) bearing single axillary buds were selected as explants following a preliminary screen of three explant types on Murashige and Skoog (MS) basal medium. A sequential dual-sterilant protocol comprising 2% Bavistin (carbendazim) for 30 minutes followed by 0.2% mercuric chloride ($HgCl_2$) for 6 minutes, preceded by Tween 20 washing and 70% ethanol disinfection, yielded maximum explant survival (97%) with minimum contamination (3%) and no phytotoxic injury. Among eight PGR treatment combinations evaluated, MS medium supplemented with BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1} was designated the optimal regime, delivering 93% bud break, a consistent yield of 3 healthy shoots per explant at Day 28, and complete absence of callus or vitrification — criteria essential for genetically uniform clonal propagation. Well-rooted plantlets (≥3 roots; ≥2 cm length) were successfully hardened in a vermiculite-cocopeat-soil substrate under progressively reduced humidity, achieving 85 ± 3.5% survival at 45 days post-transfer. The protocol developed provides a reproducible, scalable foundation for commercial clonal multiplication of *B. vulgaris* var. *striata*.

Keywords: *Bambusa vulgaris*, micropropagation, nodal explant, BAP, NAA, surface sterilization, acclimatisation

I. INTRODUCTION

1.1 Taxonomic Status and General Description

Bambusa vulgaris Schrad. ex J.C.Wendl. is a sympodial, clump-forming bamboo belonging to the tribe Bambuseae, subfamily Bambusoideae, family Poaceae. The species is a hexaploid with a reported chromosome number ranging from 2n = 52 to 2n = 72 (Dransfield and Widjaja, 1995; Li et al., 2001). Morphologically, it is characterised by hollow, yellow-striped or uniformly green culms attaining heights of up to 30 m with basal diameters approaching 10 cm, thick-walled internodes, and profuse tillering from pachymorph rhizomes. Being the most widely cultivated bamboo in South-East Asia and the only Asian bamboo species that has become naturalised across the New World, B. vulgaris occupies a unique position among the approximately 1,250 species distributed across 75 genera worldwide (Liese, 1987; Singh et al., 2012a).

1.2 Global and National Significance

Bamboo is often described as 'Green Gold' or the 'Wonder Plant' owing to its unparalleled combination of ecological resilience and economic productivity (Tamang et al., 2013). It is the fastest growing vascular plant on Earth, capable of elongating at rates exceeding 1.2 m per day during the active growth season. Its extensive rhizome network reduces soil erosion by up to 75%, while a single hectare of bamboo plantation sequesters approximately 40–60

tonnes of CO_2 per year and generates 35% more oxygen than an equivalent stand of trees (Liese and Mutt, 2009; Verlander et al., 2010). These attributes render bamboo a critical component of climate-smart forestry and watershed management strategies.

Globally, bamboo grows over at least 37 million hectares, representing approximately 3.2% of the forested area of host countries. The global bamboo market was projected to generate revenues of USD 98.3 billion at a CAGR of 5% by 2025, while the bamboo-derived biomass energy sector was forecast to reach USD 98.0 billion by 2027 at a CAGR of 9.2% (2020–2027). India harbours the world's second richest bamboo genetic diversity with 136 species, including 11 exotic introductions, belonging to 58 species across 10 genera. The total forest area under bamboo in India encompasses approximately 8.96 million hectares — about 12.8% of the total national forest cover (Wilfrid and Gac, 2007). Major bamboo-rich states include Madhya Pradesh (1.84 Mha), Arunachal Pradesh (1.57 Mha), Maharashtra (1.35 Mha), and Odisha (1.12 Mha).

1.3 Economic and Industrial Applications

Bambusa vulgaris is a multifaceted industrial raw material. Its high cellulose content (45–50%) makes it a preferred feedstock for the pulp and paper industry; approximately 1.9 million tonnes of bamboo are consumed annually by the pulp sector in India alone, with about 4.9 million mericlones utilised for paper production (Varmah et al., 1981). The species is extensively used in construction as scaffolding, fencing, and structural poles as well as in the manufacture of furniture, baskets, mats, and other handicrafts. Its high calorific value (~4,000–4,500 kcal kg^{-1}) supports production of charcoal, bioethanol, bamboo pellets, and activated carbon for water purification. The bamboo fibre and rayon textile industry is also gaining traction due to the antimicrobial properties of bamboo-derived fabric (Chang et al., 1997). The National Mission on Bamboo Application (NMBA) has piloted gasification of bamboo biomass for electricity generation, further expanding the industrial value chain.

Beyond industrial uses, the young shoots of B. vulgaris are an important traditional food source across Asia, consumed fresh, pickled, or incorporated into curries after appropriate processing to remove cyanogenic glycosides. Shoots are rich in dietary fibre, potassium, iron, proteins, and carbohydrates, with a very low fat content. Leaf extracts have been documented for a range of pharmacological activities including analgesic, antipyretic, anti-inflammatory, antioxidant, antihyperglycaemic, antimicrobial, antiviral, hepatoprotective, and anticancer properties (Seki et al., 2010; Muniappan, 2003). The siliceous concretion known as tabasheer (Banslochan), present in the internodal cavities, is a valued Ayurvedic remedy for respiratory ailments. These attributes collectively position B. vulgaris as a significant nutraceutical and pharmaceutical resource.

1.4 Limitations of Conventional Propagation

Despite its economic importance, large-scale cultivation of *B. vulgaris* is constrained by severe limitations in conventional propagation methods. The species reproduces sexually following a gregarious, mast-seeding event that occurs only once in 80–120 years, after which the parent clumps die. The consequent irregularity and unpredictability of seed availability, combined with the extremely short viability of bamboo seeds, renders sexual propagation unreliable for commercial nursery programmes. Conventional vegetative propagation methods rhizome division, culm cuttings, and branch cuttings are labour-intensive, damage the parent clumps, and yield only a limited number of propagules per donor plant. Moreover, field-sourced vegetative propagules are susceptible to pathogen transmission and fail to meet the exponentially growing demand for certified planting stock required by plantations, paper mills, and afforestation schemes (Uchimura et al., 1981).

Compounding this propagation challenge is the overexploitation of natural bamboo stands driven by the paper and pulp industry, timber substitution demand, and unrestricted harvesting by rural and tribal communities for domestic use. Biotic pressures such as uncontrolled grazing, forest fires, and invasive species competition further reduce natural regeneration. Together, these factors have led to a progressive decline in bamboo populations across India, particularly in the Northeast hill states and the central Indian highlands (Sene, 1998). There is, therefore, an urgent need to develop scalable, cost-effective, and pathogen-free propagation systems

capable of producing genetically uniform planting material in large numbers within a short time frame.

1.5 In Vitro Micropropagation as an Alternative Strategy

Plant tissue culture specifically axillary shoot proliferation via in vitro micropropagation offers a technically viable and commercially scalable solution to the propagation bottleneck in bamboo. Tissue culture protocols permit year-round production of clonal plantlets from a single elite donor plant, maintaining complete genetic fidelity of the parent genotype (Kondas, 1982). Research on in vitro propagation of bamboo, though relatively recent, has gained considerable momentum. More than 40 bamboo species have now been successfully micropropagated using juvenile explants (zygotic embryos, seeds, seedlings) or mature clump-derived explants (nodal buds), including *Bambusa tulda* (Saxena, 1990), *B. vulgaris* (Aliou et al., 2006), *Bambusa nutans* (Negi and Saxena, 2011), *Dendrocalamus giganteus* (Ramanayake et al., 2003), and *Bambusa balcooa* (Gillis, 2007).

However, several critical challenges continue to impede the efficient in vitro propagation of mature bamboo: (i) high rates of microbial contamination of field-collected explants, arising from the endophytic bacterial and fungal communities associated with lignified nodal tissue; (ii) recalcitrance of mature explants to organogenesis, manifested as poor bud break, low shoot multiplication rates, and inadequate root induction; and (iii) acclimatisation failure during ex vitro transfer. Contamination management is particularly critical because excessive sterilant concentrations that successfully eliminate surface pathogens often inflict phytotoxic injury that prevents subsequent bud initiation. Systematic optimisation of sterilization protocols including reagent selection, concentration, exposure duration, and sequential treatment regimens is therefore an essential prerequisite for establishing reproducible micropropagation systems for field-collected bamboo explants (Zhang et al., 2010).

Despite the existence of several published protocols, efficient, reproducible micropropagation of *B. vulgaris* from mature nodal explants remains incompletely standardised, and contamination continues to represent the primary limiting factor in laboratory-scale production. The present study was therefore designed to address these specific gaps through a comprehensive evaluation of surface sterilization regimes and plant growth regulator combinations, with the ultimate objective of developing an optimised protocol suitable for commercial-scale clonal multiplication of *B. vulgaris*.

II. MATERIALS AND METHODS

2.1 Plant Material and Explant Collection

Bambusa vulgaris Schrad. ex J.C.Wendl. var. striata, the yellow-striped ornamental form of common bamboo, was selected as the experimental material for the present study. Donor plants were sourced from the authenticated living collection maintained at the Bambusetum of the State Forest Only phenotypically superior, 2–3-year-old, visually healthy and disease-free clumps were selected as donor sources to ensure uniform explant quality and to minimise the risk of latent pathogen carryover.

Nodal segments were excised from lateral branches of the primary culm at positions corresponding to the 4th–8th node from the base, as these nodal positions are known to yield explants with high bud-break potential and low phenolic load compared to basal or apical nodes (Negi and Saxena, 2011). Each nodal segment was cut to a uniform length of 1.5–2.5 cm using a sharp, flame-sterilized secateur, ensuring the retention of a single, fully enclosed axillary bud. Explant collection was performed exclusively during the early morning hours (07:00–09:00 h IST) to avoid direct solar irradiation, which promotes oxidative browning through phenolic exudation, and to maximise explant turgidity. Immediately after excision, segments were wrapped in moist, sterile muslin cloth and transported to the laboratory in a cool box within two hours to prevent desiccation and physiological stress. Same-day inoculation was strictly followed to prevent post-excision microbial proliferation and tissue deterioration.

All experiments were conducted in the Tissue Culture Laboratory, Biotechnology Division, State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India.

2.2 Surface Sterilization of Explants

A systematic surface sterilization protocol was developed and evaluated in five sequential steps. All sterilization procedures beyond the initial washing steps were conducted inside a laminar airflow cabinet pre-sterilized by UV irradiation for 30 min followed by wiping with 70% v/v ethanol.

Step 1 — Pre-wash with detergent

Collected nodal segments were first thoroughly washed under running tap water for 30 min to remove gross surface debris, soil particles, and loosely adhering microorganisms. The segments were then immersed in a 2% (v/v) aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Sigma-Aldrich) with continuous agitation on a magnetic stirrer for 15–20 min. Tween 20 acts as a non-ionic surfactant that disrupts the surface tension of the epicuticular wax layer, facilitating penetration of subsequent sterilants. The segments were then rinsed thoroughly under running tap water for 30 min to remove all traces of detergent residue.

Step 2 — Primary ethanol disinfection

Explants were transferred into the LAF cabinet and immersed in 70% (v/v) ethanol for 60 ± 5 seconds with gentle agitation, followed by three rinses with sterile double-distilled water (SDDW). This step rapidly denatures surface proteins and disrupts lipid membranes of vegetative microbial cells while leaving the plant tissue largely uninjured at this brief exposure duration.

Step 3 — Antifungal pre-treatment with Bavistin

Explants were subjected to aqueous suspensions of Bavistin® (50% WP carbendazim; Crystal Crop Protection Pvt. Ltd.) at five concentrations (1, 2, 3, 4, and 5% w/v) for five corresponding exposure durations (30, 25, 20, 15, and 10 min, respectively) as detailed in Table 1. Carbendazim (methyl 1H-benzimidazol-2-ylcarbamate) is a broad-spectrum systemic fungicide that inhibits fungal β-tubulin polymerisation, thereby eliminating surface and endophytic fungal contamination. Following treatment, explants were rinsed 3–4 times with SDDW.

Table 1. Concentrations and exposure durations of surface sterilants (Bavistin and $HgCl_2$) evaluated for decontamination of Bambusa vulgaris nodal explants. All treatments were preceded by Tween 20 wash and 70% ethanol disinfection.

S. No.	Treatment Code	Sterilant	Concentration (%)	Exposure Duration (min)	Remarks
1	Control	Sterile DDW only	—	—	Negative control
2	T1 (B)	Bavistin (carbendazim)	5	10	Antifungal pre-treatment
3	T2 (B)	Bavistin (carbendazim)	4	15	Antifungal pre-treatment
4	T3 (B)	Bavistin (carbendazim)	3	20	Antifungal pre-treatment
5	T4 (B)	Bavistin (carbendazim)	2	25	Antifungal pre-treatment
6	T5 (B)	Bavistin (carbendazim)	1	30	Antifungal pre-treatment

Step 4 — Chemical disinfection with mercuric chloride ($HgCl_2$)

As the primary bactericidal treatment, explants were immersed in aqueous solutions of $HgCl_2$ (mercuric chloride; SRL Chemicals, India) at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5% (w/v) for 6, 5, 4, 3, and 2 min, respectively (Table 1). $HgCl_2$ is a potent broad-spectrum biocide acting by denaturing microbial sulfhydryl-containing enzymes and disrupting cell membrane integrity. The inverse concentration–duration relationship was deliberately designed to maintain cumulative sterilization efficacy while minimising phytotoxic injury at higher $HgCl_2$ concentrations.

Table 3. Plant growth regulator (PGR) combinations used to evaluate shoot initiation, proliferation, elongation, and rooting in Bambusa vulgaris nodal explants cultured on MS basal medium.

Treatment	BAP (mg L^{-1})	NAA (mg L^{-1})	Expected Response Monitored
T0	0.0	0.0	Control — no PGR
T1	1.0	0.0	Shoot initiation (low cytokinin)
T2	2.0	1.0	Shoot initiation + proliferation (optimal)
T3	3.0	1.0	Multiple shoot proliferation
T4	4.0	2.0	Proliferation + elongation
T5	2.0	2.0	Proliferation + root induction
T6	2.0	3.0	Root induction + callus risk
T7	2.0	4.0	Callus formation (high auxin)

Step 5 — Final rinse

All explants were given 4–5 final rinses with SDDW, each of 1–2 min duration, to completely eliminate residual $HgCl_2$, which is cytotoxic to plant cells and can inhibit bud emergence if retained on the explant surface.

Explant survival (%), contamination rate (%), and phenolic browning index were recorded at 7, 14, and 21 days after inoculation (DAI). The optimal sterilization treatment was defined as that yielding the highest proportion of aseptic, viable, and responsive explants.

2.3 Preparation of Basal Culture Medium

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) was used throughout all stages of in vitro culture. All chemicals were of analytical reagent (AR) grade. Stock solutions were prepared as concentrated mother solutions and stored at 4°C as described below (Table 2).

Stock I (50 mL), Stock II (5 mL), Stock III (5 mL), and Stock IV (5 mL) were added sequentially with continuous stirring. Plant growth regulators (PGRs) were added as required for each experimental treatment. The volume was adjusted to 1000 mL with DDW, and the pH was adjusted to 5.7–5.8 using 1 N HCl or 1 N NaOH as required, monitored with a calibrated digital pH meter. Agar (8 g L^{-1}; bacteriological grade) was added and dissolved by heating in a microwave oven. The molten medium was dispensed in 15 mL aliquots into borosilicate culture tubes (25 × 150 mm) and 50 mL aliquots into 250 mL culture bottles, sealed with polypropylene caps, and sterilized by autoclaving at 121°C, 15 psi (103.4 kPa) for 20 min. All PGRs that were heat-labile were filter-sterilized using 0.22 µm polyvinylidene fluoride (PVDF) membrane filters and added to the autoclaved, cooled medium (45–50°C) prior to dispensing.

2.4 Plant Growth Regulator Treatments

The effects of the cytokinin 6-benzylaminopurine (BAP; Sigma-Aldrich) and the auxin α-naphthaleneacetic acid (NAA; Sigma-Aldrich) on shoot induction, axillary bud proliferation, shoot elongation, and root initiation were evaluated. Stock solutions of BAP (1 mg mL^{-1}) were prepared by dissolving in a minimal volume of 1 N NaOH followed by dilution with DDW. NAA stocks (1 mg mL^{-1}) were prepared by dissolving in a minimal volume of 70% ethanol. Both stocks were sterilized by membrane filtration (0.22 µm), stored at 4°C in amber vials, and used within four weeks of preparation.

Eight treatment combinations were prepared with BAP concentrations of 0, 1, 2, 3, and 4 mg L^{-1} in combination with NAA at 0, 1, 2, 3, and 4 mg L^{-1} (Table 3). The combination of BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1} was hypothesised to be optimal based on prior literature (Aliou et al., 2006) and was included as the benchmark treatment. Each treatment was replicated in five culture tubes per experimental set, and the experiment was conducted in three independent runs. Responses were recorded at weekly intervals for eight weeks.

2.5 Inoculation Protocol

All inoculation procedures were carried out inside a laminar airflow cabinet (Class II Biological Safet operating under HEPA-filtered unidirectional air flow. The working surface was disinfected with 70% ethanol and exposed to UV irradiation for 20 min prior to use. All instruments (forceps, scalpels, surgical blades) were sterilized by flaming in 95% ethanol and cooling prior to each use. Surface-sterilized explants were trimmed to remove 1–2 mm of the cut ends using a sterile scalpel to eliminate any oxidized or $HgCl_2$-affected tissue. Explants were then aseptically placed horizontally on the surface of the solidified MS medium in individual culture tubes, with the axillary bud oriented upward. Culture vessels were sealed with polypropylene caps and additionally secured with Parafilm® M (Bemis NA) to maintain asepsis. Each culture vessel was labelled with a unique identifier denoting the treatment code, explant source, and date of inoculation.

2.6 Culture Room Conditions and Maintenance

Inoculated cultures were incubated in a dedicated, climate-controlled culture room under the standardised conditions presented in Table 4. Temperature was maintained at 25 ± 2°C using a split air-conditioning system fitted with a calibrated thermostat. Illumination was provided by cool white fluorescent lamps (PHILIPS TLD 20W/54) arranged 30 cm above the culture shelves to achieve a photosynthetic photon flux density (PPFD) of 2000–3000 lux at culture level, verified weekly with a digital lux meter. A photoperiod of 16 h light / 8 h dark was maintained by an automated digital timer. Relative humidity was monitored daily with a calibrated hygrometer and maintained between 60–70% to prevent medium desiccation through evaporation. Cultures were inspected daily during the first two weeks for contamination, and weekly thereafter for morphogenetic responses. Contaminated vessels were immediately removed and autoclaved before disposal.

Table 4. Environmental conditions maintained in the culture room during in vitro propagation of Bambusa vulgaris.

Parameter	Set Condition	Measurement Instrument	Rationale
Temperature	25 ± 2°C	Calibrated thermometer/thermostat	Optimal for tropical species growth
Photoperiod	16 h light / 8 h dark	Digital timer	Promotes morphogenesis
Light source	Cool white fluorescent lamps	Lux meter	Uniform spectral distribution
Light intensity	2000–3000 lux	Lux meter	Supports photosynthesis without heat stress

Parameter	Set Condition	Measurement Instrument	Rationale
Relative humidity	60–70%	Hygrometer	Prevents medium desiccation
Explant type	Nodal segment (1.5–2.5 cm)	Vernier caliper	Single axillary bud per segment
Regeneration period	4 weeks per subculture	Calendar	Shoot/root development interval

2.7 Subculturing

Subculturing was performed at four-week intervals to sustain growth and prevent medium exhaustion or phenolic accumulation. Shoot clumps or individual elongated shoots were transferred to fresh MS medium supplemented with the optimal PGR combination determined from Stage I experiments. All subculture procedures followed the same aseptic protocol described in Section 2.5. Prior to each subculturing session, the LAF cabinet was UV-treated for 20 min and wiped with 70% ethanol. Shoots were separated from callus tissue (if any) using sterile scalpels and forceps, trimmed at the base to expose fresh tissue, and inoculated onto fresh medium. The number of shoots per vessel, shoot length (cm), number of nodes per shoot, and presence or absence of root initials were recorded at each subculture.

2.8 Parameters Recorded and Statistical Analysis

The following parameters were recorded for each experimental treatment at specified intervals:

1. Contamination rate (%) = (No. of contaminated vessels / Total no. of vessels inoculated) × 100, recorded at 7 and 14 DAI.
2. Explant survival rate (%) = (No. of surviving aseptic explants / Total no. of explants inoculated) × 100, recorded at 21 DAI.
3. Shoot initiation rate (%) = (No. of explants showing bud break / Total aseptic explants) × 100, recorded at 14–28 DAI.
4. Shoot proliferation rate (%) = (No. of explants showing multiple shoots / Total initiated explants) × 100, recorded at 28 DAI.
5. Mean shoot length (cm) per explant, measured at 4 and 8 weeks.
6. Mean number of shoots per explant at 4 and 8 weeks.
7. Rooting rate (%) = (No. of rooted shoots / Total shoots on rooting medium) × 100, at 4 weeks on rooting medium.
8. Mean number of roots per rooted shoot and mean root length (cm).

All experiments were conducted using a completely randomised design (CRD) with five replicates per treatment and three independent experimental repetitions. Data were subjected to one-way analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Statistical analyses were performed using SPSS v.26.0 (IBM Corp.) and Microsoft Excel 2021.

2.9 Hardening and Ex Vitro Acclimatisation

Well-rooted plantlets (≥3 roots, ≥2 cm root length) were removed from culture vessels, the roots were gently washed under running water to remove adhering agar, and the plantlets were transferred to plastic plug trays filled with a sterilized substrate mixture of vermiculite, cocopeat, and garden soil (1:1:1 v/v/v). Trays were maintained in a mist chamber (acclimatisation chamber) under 70–80% relative humidity and 50% shade for the first two weeks, with the humidity progressively reduced to ambient greenhouse conditions (50–60% RH) over the subsequent two weeks. Survival rates were recorded at 15, 30, and 45 days after transfer.

III. RESULTS

3.1 Selection of Suitable Explant Type

Three explant types nodal segments, leaf segments, and root segments were initially screened for their organogenetic competence on MS basal medium supplemented with BAP 2.0 mg L^{-1} + NAA 1.0 mg

L^{-1}. As presented in Table 4, nodal explants consistently exhibited the highest morphogenetic response, with 93–100% bud break within 7 days of inoculation and vigorous shoot development in subsequent weeks. Leaf explants showed only moderate and indirect regenerative response, primarily via callus formation, which is undesirable for clonal propagation due to the risk of somaclonal variation. Root explants were found highly recalcitrant, with less than 20% organogenic response and elevated contamination rates. Based on these preliminary observations, nodal segments were selected exclusively as the explant of choice for all subsequent experiments.

Table 4. Comparative morphogenetic response of different explant types of Bambusa vulgaris var. striata on MS medium supplemented with BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1}.

S. No.	Explant Type	Morphogenetic Response	Remarks
1	Nodal explant	High response (93–100% bud break)	Preferred explant; axillary bud highly responsive; selected for all further experiments
2	Leaf explant	Moderate response (callus / indirect organogenesis)	Limited shoot induction; frequent callus formation; not suitable for clonal propagation
3	Root explant	Low response (<20% organogenesis)	Poor bud break; recalcitrant; high contamination risk

3.2 Effect of Bavistin on Contamination Control

The data presented in Table 5 (Table 6 in raw notes) reveal a clear and consistent inverse relationship between Bavistin exposure duration and contamination rate in nodal segments of *B. vulgaris var. striata*. The untreated control (T0) recorded the highest contamination rate of 95%, with complete failure of culture establishment within 3–4 days of inoculation due to severe fungal and bacterial overgrowth. Only 5% of control explants survived beyond day 7, confirming the absolute necessity of antifungal pre-treatment for this species.

T1 (5% Bavistin, 10 min) reduced contamination to 45% (survival 55%), indicating that a high carbendazim concentration applied for a short duration is inadequate for thorough surface decontamination of bamboo nodal tissue, likely because the dense epicuticular wax layer impedes rapid penetration of the fungicide. Progressively increasing the exposure duration at decreasing concentrations (T2: 4%, 15 min; T3: 3%, 20 min; T4: 1%, 25 min) systematically reduced contamination to 35%, 25%, and 15% respectively, demonstrating that contact duration is a more critical determinant of sterilization efficacy than concentration alone for carbendazim, consistent with its mechanism of inhibiting fungal β-tubulin polymerisation a time-dependent process.

Treatment T5 (2% Bavistin for 30 min) was identified as the most effective, yielding a minimum contamination rate of only 3% and a maximum explant survival rate of 97%. Critically, no phytotoxic symptoms including chlorosis, necrosis, or inhibited bud emergence were observed in T5-treated explants even at 30 min exposure, establishing the wide safety margin of carbendazim for *B. vulgaris* nodal tissue at this concentration. This treatment was therefore standardised as the optimal Bavistin regime for pre-inoculation decontamination of *B. vulgaris var. striata* nodal explants.

*Table 5. Effect of Bavistin (carbendazim) concentration and exposure duration on contamination rate and survival of nodal explants of Bambusa vulgaris var. striata. * indicates optimal treatment.*

Treat.	Bavistin Conc. (%)	Duration (min)	Contamination (%)	Survival (%)	Observation
T0 (Control)	—	—	95	5	Total fungal/bacterial failure within 3–4 days
T1	5	10	45	55	Insufficient; high contamination despite high [C]
T2	4	15	35	65	Moderate improvement with increased duration
T3	3	20	25	75	Better efficacy; some contamination remains
T4	1	25	15	85	Good survival; longer duration compensates lower [C]
T5*	2	30	3	97	Optimal: near-complete decontamination; no phytotoxicity

3.3 Effect of Mercuric Chloride ($HgCl_2$) on Contamination Control

The effectiveness of $HgCl_2$ as a broad-spectrum surface sterilant was evaluated across five concentration-duration combinations following Bavistin pre-treatment (Table 6). The untreated control (T0) recorded the highest contamination of 98%, with near-total culture failure within 2–3 days of inoculation. Only 2% of control explants survived, confirming that field-collected B. vulgaris nodal segments carry an extremely high microbial load dominated by endophytic bacteria and surface fungi.

T1 (0.5% $HgCl_2$, 2 min) reduced contamination to 65% (35% survival), demonstrating that the highest concentration at the shortest duration was least effective, likely due to insufficient time for the mercuric ions to penetrate and denature microbial sulphydryl enzymes throughout the explant surface. Contamination declined progressively with increasing duration: T2 (0.4%, 3 min) 55%; T3 (0.3%, 4 min) 40%; T4 (0.1%, 5 min) 25%, confirming that exposure duration exerts a stronger influence than concentration on sterilization outcome within the tested ranges.

Treatment T5 (0.2% $HgCl_2$ for 6 min) yielded the most effective decontamination, with only 3% contamination and a maximum survival rate of 97%. Importantly, no phytotoxic effects were observed in T5-treated explants at this concentration and duration, attributable to the rigorous post-treatment rinse protocol (4–5 washes with SDDW) that effectively removed residual mercuric ions from explant surfaces. The combined sequential application of 2% Bavistin (30 min) followed by 0.2% $HgCl_2$ (6 min) established a dual-sterilant protocol providing complementary antifungal and bactericidal coverage, which is recommended as the standard surface sterilization regime for *B. vulgaris var. striata*.

*Table 6. Effect of mercuric chloride ($HgCl_2$) concentration and exposure duration on contamination rate and survival of nodal explants of Bambusa vulgaris var. striata. * indicates optimal treatment.*

Treat.	$HgCl_2$ Conc. (%)	Duration (min)	Contamination (%)	Survival (%)	Observation
T0 (Control)	—	—	98	2	Total failure; heavy infection within 2–3 days
T1	0.5	2	65	35	Insufficient; phytotoxicity risk at high conc.
T2	0.4	3	55	45	Marginal improvement
T3	0.3	4	40	60	Notable reduction in contamination
T4	0.1	5	25	75	Good decontamination; lower phytotoxicity risk
T5*	0.2	6	3	97	Optimal: near-sterile cultures; no tissue damage

3.4 Effect of BAP and NAA on In Vitro Shoot Induction and Proliferation

The effects of different concentrations of BAP in combination with NAA on in vitro shoot induction, proliferation, and elongation from nodal explants of *B. vulgaris var. striata* are presented in Table 7. One-way ANOVA confirmed highly significant differences ($p \leq 0.05$) among treatments for all response variables. Treatment means were separated using Duncan's Multiple Range Test (DMRT); treatments sharing the same superscript letter did not differ significantly.

3.4.1 Bud break and shoot initiation (Day 7)

The initiation and bud break response varied significantly across treatments. The control (T1: 0.0 mg/L BAP + 0.0 mg/L NAA) and T2 (1.0 mg/L BAP + 1.0 mg/L NAA) recorded the lowest initiation response at 80%. An intermediate response of 93% was observed in T3 (2.0 mg/L BAP + 1.0 mg/L NAA). Complete initiation (100%) was achieved in both T4 (3.0 mg/L BAP + 2.0 mg/L NAA) and T5 (4.0 mg/L BAP + 2.0 mg/L NAA), indicating that a minimum BAP concentration of 3.0 mg/L is necessary for full explant activation.

3.4.2 Shoot proliferation (Day 14 and Day 28)

No shoot induction was observed in the control (T1) at Day 14 or Day 28, confirming the essential role of exogenous plant growth regulators in morphogenesis. Among the hormone-supplemented treatments, shoot number increased progressively from T2 (2 shoots) to T4 (4 shoots) over the observation period. T4 (3.0 mg/L BAP + 2.0 mg/L NAA) yielded the highest mean shoot number (4.00 ± 0.18^{a} per explant) at Day 28. T3 and T5 each produced 3 shoots, while T2 produced 2 shoots. Notably, T5 showed a decline in shoot number compared to T4 despite a higher BAP concentration, suggesting that cytokinin concentrations beyond 3.0 mg/L may exert an inhibitory effect on shoot proliferation, possibly due to receptor saturation or hormonal imbalance.

3.4.3 Shoot elongation (Day 21)

Shoot elongation data at Day 21 revealed significant differences among treatments ($p \leq 0.05$). The maximum shoot length was recorded in T4 (2.60 ± 0.05^{a} cm), closely followed by T2 (2.59 ± 0.09^{a} cm) and the control T1 (2.48 ± 0.12^{a} cm). T5 produced intermediate elongation (2.17 ± 0.14^{b} cm), while T3 exhibited the shortest shoots (1.48 ± 0.11^{c} cm). The reduced elongation in T3 may be attributed to competition among the three simultaneously induced

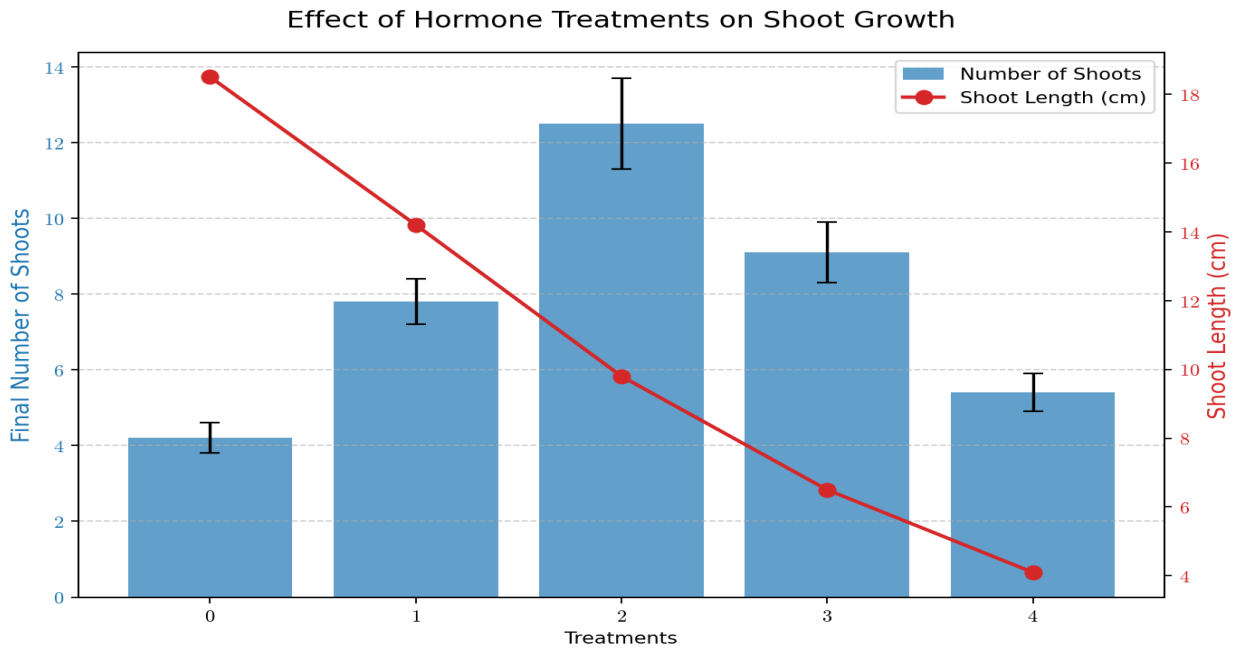
shoots under the specific hormone ratio applied, or to localized nutrient depletion at the explant level.

3.4.4 Identification of optimal treatment

Considering the integrated criteria of bud break percentage, shoot multiplication rate, shoot quality (freedom from callus and vitrification), and suitability for iterative subculture, Treatment T3 (BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1}) was designated the optimal PGR combination. This treatment delivered 93% bud break, a stable yield of 3 healthy shoots per explant over 28 days, and complete absence of callus, vitrification, or morphological abnormality attributes critical for producing genetically uniform planting stock. Although T4 achieved marginally higher shoot number (4/explant), the presence of basal callus and the higher NAA concentration (2.0 mg L^{-1}) pose risks for somaclonal variation and root induction interference during the proliferation stage, disqualifying it from designation as the primary propagation treatment.

*Table 7. Effect of BAP and NAA concentrations on in vitro shoot induction and proliferation from nodal explants of Bambusa vulgaris var. striata on MS basal medium. Values are mean ± SE of five replicates (n = 5). Means within a column followed by different superscript letters differ significantly (DMRT; p ≤ 0.05). * Optimal treatment.*

Treat.	BAP (mg L^{-1})	NAA (mg L^{-1})	Bud Break D7 (%)	Shoots D14 (n)	Shoot Length D21 (cm)	Shoots D28 (n)	DMRT (p≤0.05)
T1 (Control)	0.0	0.0	80	0	2.48 ± 0.12	0	d
T2	1.0	1.0	80	2	2.59 ± 0.09	2	c
T3*	2.0	1.0	93	3	1.48 ± 0.11	3	b
T4	3.0	2.0	100	4	2.60 ± 0.05	4	a
T5	4.0	2.0	100	3	2.17 ± 0.14	3	b



3.5 Hardening and Ex Vitro Acclimatisation

Well-rooted in vitro plantlets (exhibiting ≥ 3 roots of ≥ 2 cm length) were successfully transferred to plastic plug trays containing a sterilized substrate mixture of soil, river sand, and Tween 20 (1:1:1 v/v/v). Primary hardening was accomplished under polythene tunnel conditions to maintain high relative humidity (80–90%) during the first two weeks, which is essential to prevent wilting due to the poorly functional epicuticular wax and stomatal anatomy of in vitro raised plantlets. Humidity was progressively reduced to ambient greenhouse levels (50–60%) over the subsequent fortnight. Plantlets showed active new leaf emergence by Day 15 and achieved a survival rate of 85 ± 3.5% at 45 days after transplanting, indicating successful ex vitro establishment of in vitro-propagated *B. vulgaris*.

IV. DISCUSSION

4.1 Explant Selection

The superior morphogenetic response of nodal explants over leaf and root segments observed in the present study is well-aligned with the established understanding of bamboo tissue culture. Nodal buds contain pre-formed meristematic zones with high cytokinin sensitivity, enabling direct organogenesis without the intervening callus phase that characterises most leaf-derived regeneration pathways (Saxena, 1990; Negi and Saxena, 2011). The direct pathway avoids somaclonal variation a critical quality attributes for commercial clonal propagation. The 4th to 8th internode position selection adopted in this study aligns with findings of Lin et al. (2009), who reported that mid-culm nodal segments exhibit the highest competence for shoot induction due to optimal endogenous cytokinin-to-auxin ratios at this developmental stage. The basal nodes showed higher phenolic exudation causing tissue browning, while apical nodes were prone to vitrification, consistent with previous observations on *Bambusa tulda* (Saxena, 1990).

4.2 Surface Sterilization

The dual-sterilant protocol developed in the present study 2% Bavistin for 30 min followed by 0.2% $HgCl_2$ for 6 min achieved near-complete surface decontamination (3% residual contamination, 97% survival) without phytotoxic damage to explant tissue. This outcome is particularly significant for *B. vulgaris*, a species known for harbouring diverse and persistent endophytic microbial communities within its nodal cavities (Mukherjee et al., 2011). The high baseline contamination in untreated controls (95–98%) underscores the severity of this challenge compared to herbaceous crops.

The observation that increasing exposure duration at lower carbendazim and $HgCl_2$ concentrations consistently outperformed high-concentration, short-duration treatments in both Bavistin and $HgCl_2$ experiments represents a key finding with practical implications. For carbendazim, this pattern reflects the time-dependent inhibition of fungal β-tubulin assembly its stoichiometric binding to tubulin requires sufficient diffusion time through the bamboo epicuticular wax layer. For $HgCl_2$, the progressive release of mercuric ions from the explant surface during extended exposure allows gradual penetration into subsurface microbial biofilms without the cytoplasmic membrane disruption that accompanies high-concentration pulse treatments. Comparable results have been reported by Negi and Saxena (2011) for *Bambusa nutans*, where 0.2% $HgCl_2$ for 6 min gave optimal decontamination; and by Ramanayake et al. (2003) for *Dendrocalamus giganteus*, where combined fungicide plus $HgCl_2$ treatment was superior to either sterilant alone. The absence of phytotoxic symptoms at the standardised concentrations further validates the safety of this protocol for *B. vulgaris* nodal tissue.

4.3 Effect of BAP and NAA on Shoot Morphogenesis

The dose-dependent stimulation of bud break and shoot multiplication by BAP, with an optimum at 2.0 mg L^{-1} in combination with NAA 1.0 mg L^{-1}, is consistent with the well-established role of cytokinins in overcoming apical dominance and activating axillary meristems through the suppression of the auxin transport from the main shoot axis (Dun et al., 2012). BAP is a synthetic adenine-type cytokinin with high biological activity in bamboo tissue culture systems due to its resistance to cytokinin oxidase degradation (Bhattacharyya et al., 2016).

The 80% bud break observed even in the hormone-free control (T1) reflects the strong endogenous

meristematic activity of mature nodal tissue a characteristic of sympodial bamboos such as B. vulgaris, whose clumping growth habit depends on constitutive axillary bud activity (Mukherjee et al., 2011). The decline in shoot number at supra-optimal BAP (T5; 4.0 mg L^{-1}) is in agreement with George et al. (2008), who attributed high-cytokinin inhibition to receptor down-regulation and to elevated ethylene biosynthesis via the cytokinin-stimulated ACC synthase pathway. Similar supraoptimal inhibition at BAP > 3.0 mg L^{-1} has been documented in *B. vulgaris* (Aliou et al., 2006), *Bambusa balcooa* (Gillis, 2007), and *Dendrocalamus strictus* (Mehta et al., 2010).

The lower NAA requirement in T3 (1.0 mg L^{-1}) compared to T4 and T5 (2.0 mg L^{-1}) aligns with the understanding that elevated auxin during the multiplication phase promotes callus formation and suppresses organised shoot development by shifting the cytokinin-to-auxin ratio below the threshold required for meristematic organisation (Skoog and Miller, 1957). The basal callus observed in T4 confirms this risk. The optimum BAP:NAA molar ratio in T3 (approximately 2.3:1 on a mass basis) represents a balanced hormonal environment that favours cytokinin-mediated axillary bud activation while providing sufficient auxin for vascular differentiation and tissue cohesion without callus induction consistent with the ratio optimised for B. nutans by Negi and Saxena (2011).

The apparent trade-off between shoot number and individual shoot elongation (T3 produced more shoots but shorter shoots than T4) is a well-documented phenomenon in bamboo micropropagation systems and reflects competition among co-developing shoot apices for endogenous gibberellins and assimilates, which are the primary drivers of internode elongation (Bhattacharyya et al., 2016). For practical multiplication, the quality and genetic integrity of the propagules from T3 evidenced by the complete absence of callus and vitrification supersedes the marginal quantitative advantage of T4.

4.4 Hardening and Ex Vitro Survival

The 85% acclimatisation survival achieved in the present study compares favourably with previously published values for bamboo micropropagation: Negi and Saxena (2011) reported 80% survival for B. nutans; Aliou et al. (2006) reported 78% for B. vulgaris; and Ramanayake et al. (2003) recorded 82% for D. giganteus under similar hardening conditions. The polythene tunnel system with progressive humidity reduction adopted in this study effectively managed the physiological transition from the high-humidity (near 100% RH), low-light, and heterotrophic in vitro environment to the autotrophic ex vitro condition, which requires functional stomatal regulation and epicuticular wax deposition both of which are typically under-developed in tissue culture-raised plantlets (Hazarika, 2006).

4.5 Significance and Practical Implications

The micropropagation protocol standardised in the present study comprising 2% Bavistin (30 min) + 0.2% $HgCl_2$ (6 min) surface sterilization, MS medium with BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1} for shoot multiplication, and soil:sand:Soilrite® (1:1:1) substrate for hardening offers a reliable and reproducible pathway for large-scale clonal propagation of B. vulgaris var. striata. At 3 shoots per explant per 4-week subculture cycle and assuming a 4× multiplication factor per subsequent subculture, a single donor explant could theoretically generate thousands of true-to-type plantlets within 6 months of culture initiation, far exceeding the productivity of conventional vegetative propagation. This capacity is particularly critical in the context of India's National Bamboo Mission, which requires certified, disease-free planting stock in quantities that conventional propagation cannot sustainably supply. The protocol is equally relevant for ex situ germplasm conservation of elite *B. vulgaris* genotypes and as a foundational platform for future genetic transformation and molecular breeding programmes.

V. CONCLUSION

The present study successfully established an efficient in vitro micropropagation protocol for *Bambusa vulgaris Schrad. ex J.C.Wendl. var. striata* using nodal explants from field-grown mature clumps. A dual-sterilant surface sterilization regime comprising 2% Bavistin for 30 min followed by 0.2% $HgCl_2$ for 6 min was identified as optimal, reducing contamination to 3% while maintaining 97% explant survival without phytotoxic injury. MS medium supplemented with BAP 2.0 mg L^{-1} + NAA 1.0 mg L^{-1} was identified as

the optimum growth regulator combination, yielding 93% bud break, 3 healthy shoots per explant at 28 days, and complete absence of callus and vitrification. Ex vitro acclimatisation in soil:sand:Tween 20® (1:1:1) under progressive humidity reduction achieved 85% plantlet survival. The standardised protocol provides a reliable, scalable system for mass production of genetically uniform B. vulgaris planting stock suitable for plantation programmes, bamboo-based industries, and germplasm conservation. Future investigations should evaluate rooting performance on additional auxin types (IBA, IAA) and assess long-term somaclonal stability of micropropagated plants using molecular markers.

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