In Vitro Micropropagation of *Bambusa vulgaris* (Green) Schrad. ex J.C. Wendl.: Optimization of Surface Sterilization Protocols and Plant Growth Regulator-Mediated Shoot Morphogenesis

Shailendra Singh Yadav[1], Akshaya Tomar[2], Sudhir Chandel[3], Sandeep Fellows[4], Pradeep Vasudeva[5]
[1,4,5] *Biotechnology Division, State Forest Research Institute, Jabalpur Madhya Pradesh, India*
[2,3] *Department of Microbiology, Govt. M.H. College of Home Science and Science for Woman Autonomous, Jabalpur, Madhya Pradesh, India*

***Abstract*—*Bambusa vulgaris* Schrad. ex J.C. Wendl. (Family: Poaceae) is one of the most economically and ecologically important bamboo species in tropical Asia, yet its near-complete dependence on vegetative propagation and the inherent limitations of conventional multiplication methods severely restrict large-scale planting material production. The present study was undertaken to standardize a reliable in vitro micropropagation protocol for B. vulgaris using nodal explants bearing axillary buds collected during the active growing season (January–April) from healthy mother plants maintained at the State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India. A sequential surface sterilization protocol was optimized using Bavistin® (carbendazim) and mercuric chloride ($HgCl_2$) at varying concentrations and exposure durations. Treatment with 0.5% Bavistin® for 30 minutes effectively reduced fungal contamination to 20% while maintaining explant viability, whereas 0.03% $HgCl_2$ for 8 minutes was identified as the optimal bactericidal treatment, achieving contamination rates of 15–25% without phytotoxic injury to the nodal meristem. Explants were cultured on Murashige and Skoog (MS) basal medium supplemented with varying concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). Among all hormonal treatments evaluated, the combination of 3.0 mg L^{-1} BAP and 0.6 mg L^{-1} NAA (T_3) elicited the highest morphogenetic response, recording 65–70% shoot initiation at Day 7, 75–80% proliferation at Day 14, 90% elongation at Day 21, and excellent overall shoot development at Day 28. Both sub-optimal (0.1 mg L^{-1} BAP) and supra-optimal (4.0 mg L^{-1} BAP) concentrations produced inferior outcomes, with the latter inducing undesirable callus formation, consistent with the classical Skoog–Miller cytokinin–auxin balance model. The integrated protocol described here provides a reproducible and scalable pathway for mass propagation of *B. vulgaris*, with direct applications in agroforestry, industrial bamboo cultivation, and biodiversity conservation programs.**

***Index Terms*—*Bambusa vulgaris*, micropropagation, nodal explant, BAP, NAA, surface sterilization, mercuric chloride, carbendazim, shoot organogenesis, plant tissue culture**

I. INTRODUCTION

Bamboo, a member of the subfamily Bambusoideae within the family Poaceae, is one of the most ecologically and economically significant groups of plants in the world. With over 1,500 species distributed across tropical, subtropical, and temperate regions, bamboo has long served as a critical natural resource for billions of people, particularly across Asia, Africa, and Latin America (Lobovikov et al., 2007). Among the numerous bamboo species, *Bambusa vulgaris* Schrad. ex J.C. Wendl. (Family: Poaceae) stands out as one of the most widely cultivated and utilized species globally. Commonly known as common bamboo, giant bamboo, or green bamboo, it is a clumping, non-thorny, sympodial bamboo native to Southeast Asia and widely naturalized across tropical and subtropical regions of the world (McClure, 1966; Dransfield & Widjaja, 1995).

The species is characterized by its dense tufted culms reaching heights of 10–20 m and diameters of 4–10

cm, with internodal lengths of 20–45 cm. The culms are initially dark green, becoming yellow-green with age, and the species is notable for its robust growth, adaptability to diverse soil types, and remarkable vegetative propagation capacity (Stapleton, 1994). Unlike many bamboo species, *B. vulgaris* rarely flowers, and when gregarious flowering does occur typically once every 30–50 years seed viability is extremely low due to irregular meiosis and poor pollen fertility (Nadgauda et al., 1990; Ramanayake & Yakandawala, 1996). This reproductive constraint poses a fundamental challenge for large-scale, genetically uniform multiplication of the species.

India is among the world's largest bamboo-growing nations, with approximately 13.96 million hectares under bamboo cover, accounting for roughly 12.8% of the country's total forest area (FSI, 2019). Madhya Pradesh alone harbors approximately 1.84 million hectares of bamboo forests the largest state-wise bamboo area in India followed by Arunachal Pradesh (1.57 million ha), Maharashtra (1.35 million ha), and Odisha (1.12 million ha) (NITI Aayog, 2017). *Bambusa vulgaris* is extensively distributed across Madhya Pradesh, Chhattisgarh, Odisha, West Bengal, and the northeastern hill states, where it thrives along riverbanks and moist forest margins up to 1,500 m elevation (Tewari, 1992).

1.2 Economic and Ecological Importance of *Bambusa vulgaris*

Bambusa vulgaris occupies a position of immense socio-economic importance. Its culms are widely used in construction (scaffolding, roofing, wall paneling), furniture manufacturing, and as raw material in the paper and pulp industry (Liese, 1987; Scurlock et al., 2000). The edible young shoots are a nutritional staple and commercial delicacy in South and Southeast Asian cuisines. Artisanal industries rely on bamboo for weaving baskets, mats, and household implements, providing rural livelihoods to millions of people (Lobovikov et al., 2007).

Ecologically, *B. vulgaris* plays a significant role in soil erosion control, particularly on sloped terrains and riverbanks in tropical regions. Its extensive rhizome network binds soil effectively and contributes to watershed conservation (Nath et al., 2009). Additionally, bamboo forests are recognized as efficient carbon sinks; **B. vulgaris** exhibits high biomass productivity and has been explored for carbon sequestration programs and phytoremediation of wastewater owing to its rapid nutrient uptake capacity (Kleinhenz & Midmore, 2001; Nath et al., 2015). Its ornamental value also makes it widely planted in parks, gardens, and landscape designs across tropical regions.

1.3 Conventional Propagation and Its Limitations

Conventional propagation methods for *B. vulgaris* include rhizome division, culm cuttings, branch cuttings, and offset transplantation. While these vegetative techniques are routinely employed, they are associated with several significant limitations. These methods are seasonal, labor-intensive, and inefficient for large-scale nursery production. Moreover, the planting material produced is bulky, difficult to transport, and subject to high mortality rates during establishment (Banik, 1995; Anand et al., 2000).

The near-absence of reliable seed-based reproduction in *B. vulgaris* a consequence of the species' infrequent and gregarious flowering cycle and inherently low pollen viability renders seed propagation practically non-viable at a commercial scale (Ramanayake & Yakandawala, 1996). Consequently, meeting the growing industrial and agroforestry demand for genetically uniform, high-quality bamboo planting material necessitates the development of alternative propagation systems. Plant tissue culture has emerged as the most promising solution to overcome these bottlenecks (Pattnaik & Chand, 1996).

1.4 Plant Tissue Culture: Concept and Significance

Plant tissue culture refers to the in vitro aseptic cultivation of cells, tissues, or organs on chemically defined nutrient media under controlled physical conditions of light, temperature, and humidity (Murashige, 1974). The theoretical foundation of tissue culture rests on the principle of totipotency, first proposed by Haberlandt (1902), which postulates that every living plant cell retains the complete genetic information necessary to regenerate an entire organism (Thorpe, 2007).

In practice, tissue culture protocols for bamboo involve the sequential steps of explant selection, surface sterilization, inoculation on nutrient media (most commonly Murashige & Skoog, 1962 basal medium), callus induction, shoot organogenesis or

somatic embryogenesis, root induction, and ex vitro hardening of regenerated plantlets (Murashige & Skoog, 1962; George, 1993). The judicious selection of plant growth regulators (PGRs), particularly cytokinins (e.g., 6-benzylaminopurine [BAP], kinetin) and auxins (e.g., naphthaleneacetic acid [NAA], indole-3-butyric acid [IBA]), is critical in governing the morphogenetic pathway of the explant (George et al., 2008).

1.5 Status of Tissue Culture Research in Bamboo

Tissue culture-mediated micropropagation of bamboo has been studied in several species over the past four decades. Early pioneering work by Nadgauda et al. (1990) demonstrated successful in vitro regeneration of *Bambusa arundinacea*, laying the groundwork for subsequent protocols in related species. Node explants containing axillary buds have been consistently identified as the most responsive and contamination-resistant explant source for bamboo micropropagation, as they retain meristematic competence and show rapid bud break in culture (Pattnaik & Chand, 1996; Goyal et al., 2012).

For *Bambusa vulgaris* specifically, several studies have reported successful shoot proliferation and plantlet regeneration using nodal explants cultured on MS medium supplemented with varying concentrations of BAP and kinetin (Anand et al., 2000; Islam et al., 2010). Cytokinin type and concentration have been shown to critically influence shoot multiplication rates, with BAP generally outperforming kinetin at equimolar concentrations in bamboo species. However, standardization of an optimized, reproducible micropropagation protocol specifically for *B. vulgaris* remains a research priority, as contamination rates, browning of explants due to phenolic exudation, and difficulties in ex vitro acclimatization continue to limit large-scale adoption (Ravindra & Bhojwani, 2010; Goyal et al., 2012).

Surface sterilization, a critical prerequisite for successful in vitro culture, determines the balance between microbial contamination and explant viability. The use of mercuric chloride (HgCl2), sodium hypochlorite (NaOCl), and ethanol at varied concentrations and exposure durations has been reported across bamboo tissue culture studies, with species-specific and seasonal variability necessitating optimization for each system (Pattnaik & Chand, 1996; Islam et al., 2010).

1.6 Objectives of the Present Study

Given the enormous industrial, ecological, and socio-economic significance of *Bambusa vulgaris* and the inherent limitations of conventional propagation methods, there is an urgent need to develop a reliable, efficient, and scalable in vitro micropropagation protocol for this species. Despite existing literature on bamboo tissue culture, a standardized, reproducible protocol for mass propagation of *B. vulgaris* with optimized sterilization and PGR regimes has not been fully established in the context of the central Indian germplasm. In light of the above, the present investigation was undertaken with the following objectives:

(i) To develop an effective surface sterilization protocol to minimize microbial contamination and maximize explant survival.

(ii) To determine suitable concentrations and combinations of plant growth regulators, particularly cytokinins (BAP and kinetin), for optimum shoot induction and proliferation from nodal explants of Bambusa vulgaris.

II. MATERIALS AND METHODS

2.1 Plant Material and Explant Collection

The present investigation was conducted using *Bambusa vulgaris* Schrad. ex J.C. Wendl. as the experimental plant material. Healthy, disease-free mother plants of B. vulgaris were identified and selected from the Bamboosetium, under Biotechnology Division, State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India (23.1815°N, 79.9864°E). Explant collection was carried out during the active growing season (January–April), when young bamboo culms are in the vegetative growth phase and meristematic activity is at its peak (Pattnaik & Chand, 1996).

Nodal segments bearing axillary buds were selected as the primary explant source, as nodal explants from bamboo species have been reported to exhibit superior morphogenetic responsiveness compared to other explant types such as leaf segments or root tips (Goyal et al., 2012). Inter-nodal stem segments of approximately 3–5 cm in length were excised from young, actively growing culm branches of phenotypically superior mother plants using sterilized pruning shears. The outer culm surface was first wiped with 70% ethanol-moistened cotton to remove

adhering dust, wax, and surface debris prior to excision. Collected explants were immediately transported to the laboratory in sterile ziplock bags containing a few drops of sterile water to prevent desiccation.

2.2 Sterilization of Glassware and Laboratory Equipment

All glassware (culture tubes, conical flasks, beakers, measuring cylinders, and Petri dishes) and metallic instruments (forceps, scalpels, and scissors) were thoroughly cleaned with laboratory-grade detergent, rinsed under running tap water, and dried prior to sterilization. Glassware was autoclaved at 121°C at a pressure of 15 psi (103.4 kPa) for 15–20 minutes in an upright autoclave. Post-autoclaving, the glassware was subjected to an additional wash with 70% ethanol for 10 minutes, rinsed thoroughly with double-distilled water (DDW), and re-autoclaved under identical conditions to ensure complete elimination of any residual contamination (George, 1993). Metallic instruments were flame-sterilized by dipping in 95% ethanol and passing through a blue Bunsen flame for 2–3 seconds before each use. The laminar airflow cabinet (LAF) was decontaminated by UV irradiation for 30 minutes prior to each work session and wiped with 70% ethanol immediately before use.

Key sterilization parameters for glassware and media preparation were as follows:

Table 2.1: Standard sterilization parameters employed during the study

Parameter	Value
Autoclave temperature	121°C
Pressure	15 psi (103.4 kPa)
Duration (glassware)	15–20 minutes
Duration (culture media)	20–30 minutes
Ethanol concentration	70% (v/v)
UV irradiation (LAF bench)	30 minutes before use

2.3 Preparation of Murashige and Skoog (MS) Basal Medium

The Murashige and Skoog (1962) basal salt medium was used throughout the study for all culture stages (Murashige & Skoog, 1962). The medium was prepared from four concentrated stock solutions to ensure accuracy and reproducibility in large-scale preparation. The chemical composition of the MS basal medium and the method of stock solution preparation

2.3.1 Step-by-Step Protocol for Media Preparation (per 1 litre)

The medium was prepared using the following sequential procedure:

1. Approximately 500 mL of double-distilled water (DDW) was taken in a one-litre borosilicate glass conical flask.
2. 30 g of sucrose (analytical grade) was added and dissolved completely by stirring on a magnetic stirrer.
3. 50 mL of Stock A (macronutrients, 20×) was added and mixed thoroughly.
4. 5 mL each of Stock B (micronutrients, 200×), Stock C (iron chelate, 200×), and Stock D (vitamins, 200×) were added sequentially with continuous stirring.
5. The required volume of plant growth regulator (PGR) stock solution was added as per the experimental treatment design (see Section 3.5).
6. The final volume was made up to exactly 1,000 mL with DDW.
7. The pH of the medium was adjusted to 5.7–5.8 using 0.1 N NaOH or 0.1 N HCl, as measured with a calibrated digital pH meter. Accurate pH adjustment is critical, as a pH below 5.5 impairs agar gelation and a pH above 6.0 promotes nutrient precipitation (George et al., 2008).
8. Agar–Agar (8 g L^{-1}) was added to the solution and dissolved by heating in a microwave oven with intermittent stirring until the solution turned clear and transparent.
9. The hot medium was dispensed into borosilicate glass culture tubes (approximately 15–20 mL per tube) while still in a liquid state.
10. The culture tubes were sealed with non-absorbent cotton plugs wrapped in aluminium foil and autoclaved at 121°C, 15 psi for 20–30 minutes.

11. The sterilized medium was allowed to cool and solidify at room temperature on a level surface before use.

2.4 Surface Sterilization of Explants

Effective surface sterilization of bamboo explants is a critical and technically demanding step, as the dense culm surface harbours diverse epiphytic microorganisms including bacteria and fungi, while excessive sterilant concentration or exposure duration may cause irreversible tissue damage (Pattnaik & Chand, 1996; Islam et al., 2010). A multi-step sequential sterilization protocol was standardized for Bambusa vulgaris nodal explants in the present study, as described in Table 2.2. All steps from Extran® washing onward were conducted under the laminar airflow cabinet to maintain aseptic conditions.

Table 2.2: Sequential surface sterilization protocol for Bambusa vulgaris nodal explants

Step	Treatment Agent	Concentration / Duration	Purpose
1	Running tap water	10 min	Remove surface dust and debris
2	Extran® (detergent solution)	1% / 5 min	Reduce surface microbial load
3	Sterile double-distilled water (SDDW)	3–4 rinses	Remove detergent residue
4	Carbendazim (Bavistin®)	1% / 30 min	Eliminate fungal contamination
5	SDDW	3–4 rinses	Remove fungicide residue
6	Ethanol	70% / 1 min	Surface disinfection
7	SDDW	2–3 rinses	Remove ethanol
8	Mercuric chloride ($HgCl_2$)*	0.1% / 10 min	Eliminate bacterial contamination
9	SDDW (under LAF)	4–5 rinses	Thorough removal of $HgCl_2$ residue

*Note: Mercuric chloride ($HgCl_2$) is a hazardous heavy-metal disinfectant. All $HgCl_2$ solutions and wash water were collected in labelled waste containers and disposed of as per institutional biohazard protocols in compliance with the Hazardous Waste Management Rules (MoEFCC, 2016).

2.5 Inoculation of Explants

All inoculation procedures were performed aseptically inside the laminar airflow (LAF) bench (Murashige, 1974). The operator's hands and forearms were thoroughly scrubbed with 70% ethanol before commencing work. The sterilized explants were placed in sterile Petri dishes lined with sterile filter paper. Using flame-sterilized and air-cooled forceps, each nodal explant was trimmed to a length of approximately 1.5–2.0 cm, retaining the intact axillary bud. The cut surfaces were further swabbed with 70% ethanol to minimize cut-end contamination. The cotton-plugged culture tubes were individually uncapped, and the opening was briefly flamed over a spirit lamp for 1–2 seconds to create a positive heat barrier against airborne contamination. Each explant was placed vertically into the solidified medium using sterile forceps, with the proximal end (nodal end bearing the axillary bud) oriented upward. The tube opening was re-flamed before replacing the cotton plug. Each tube was sealed with Parafilm® and labelled with the treatment code, date of inoculation, and replicate number.

2.6 Culture Maintenance and Incubation Conditions

Following inoculation, all culture tubes were transferred to a controlled-environment culture room maintained under the conditions specified in Table 2.3. Cultures were inspected visually at two-day intervals during the first two weeks to monitor contamination and explant response. Contaminated cultures were immediately removed and autoclaved before disposal. Responding cultures were subcultured onto fresh medium of the same composition every four weeks to prevent nutrient depletion and phenolic accumulation.

Table 2.3: Physical conditions maintained in the culture room during incubation

Parameter	Condition
Temperature	25 ± 2°C
Photoperiod	16 h light / 8 h dark
Light intensity	1000–3000 lux (cool white fluorescent)
Relative humidity	50–60%
Culture vessel	Borosilicate glass test tubes (25 × 150 mm)
Subculture interval	Every 4 weeks

2.7 Parameters Recorded and Statistical Analysis

2.7.1 Parameters Recorded

The following response parameters were recorded during the in vitro phase of the study:

- Days to bud break: Number of days from inoculation to visible bud emergence from the axillary node.
- Percentage of explant response (%): Number of explants showing bud break or shoot initiation expressed as a percentage of the total number of explants inoculated per treatment.
- Number of shoots per explant: Total number of shoots (≥ 0.5 cm) produced per explant at four and six weeks after inoculation.
- Shoot length (cm): Length of the longest shoot measured from the nodal base to the apical tip at four and six weeks.
- Number of nodes per shoot: Count of nodes on the primary shoot at six weeks.
- Percentage of rooting: Proportion of shoots producing visible roots after transfer to rooting medium.
- Number of roots per explant: Count of roots per individual plantlet.
- Root length (cm): Length of the longest root per plantlet measured at the time of hardening.
- Percentage of hardening survival (%): Proportion of transferred plantlets surviving to six weeks post-transfer.

2.8.2 Statistical Analysis

The experimental data were statistically analyzed using one-way Analysis of Variance (ANOVA) and two-way ANOVA, as appropriate, to evaluate the effect of PGR type and concentration on the recorded response parameters. All experiments were conducted in a Completely Randomized Design (CRD) with five replications per treatment. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at the 5% level of significance ($p \leq 0.05$) (Duncan, 1955). Contamination rate was calculated as the percentage of contaminated cultures out of the total inoculated. All calculations and statistical analyses were performed using SPSS v. 22.0 (IBM Corp.) and Microsoft Excel 2019. Results are presented as mean ± standard error (SE) of the mean.

III. RESULTS AND DISCUSSION

3.1 Experimental Results

The present study was undertaken to standardize plant tissue culture techniques for the mass propagation of *Bambusa vulgaris* Schrad. ex J.C. Wendl., with particular emphasis on (i) optimizing surface sterilization protocols using Bavistin® (carbendazim) and mercuric chloride ($HgCl_2$), and (ii) evaluating the effect of plant growth regulators specifically 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) on in vitro shoot induction, proliferation, and elongation. Results are presented and discussed under three sub-sections.

3.2 Effect of BAP and NAA on In Vitro Shoot Development of *Bambusa vulgaris*

The influence of varying concentrations of BAP and NAA on shoot initiation (Day 7), proliferation (Day 14), elongation (Day 21), and overall developmental status (Day 28) in Bambusa vulgaris nodal explants cultured on MS basal medium is presented in Table 3.1.

Table 3.1: Effect of BAP and NAA concentrations on in vitro shoot initiation, proliferation, elongation, and final developmental status of Bambusa vulgaris at weekly intervals

Treatment	BAP (mg L^{-1})	NAA (mg L^{-1})	Day 7 – Initiation (%)	Day 14 – Proliferation (%)	Day 21 – Elongation (%)	Day 28 – Final Status
T_0	0.0	0.0	10–15%	15–20%	20–25%	Very poor growth (control)
T_1	0.1	0.3	30–40%	45–50%	55–60%	Weak shooting
T_2	2.0	0.4	60%	70–80%	80–85%	Moderate shooting
T_3	3.0	0.6	65–70%	75–80%	90%	Excellent growth ✓
T_4	4.0	0.5	50–60%	55–60%	60%	Callus formation; weak growth

Note: All treatments were applied on MS basal medium (pH 5.7–5.8) supplemented with 3% sucrose and 0.8% agar. Values represent mean percentage response (n = 5 replicates per treatment). ✓ denotes the optimal treatment.

3.2.1 Control Treatment [T_0]

In the hormone-free control medium (T_0), explants exhibited extremely low morphogenetic responsiveness throughout the observation period. Shoot initiation was recorded at a mere 10–15% at Day 7, with marginal improvement to 20–25% elongation by Day 21 and a final status of very poor growth at Day 28. The observed basal-level response may be attributed to endogenous phytohormones inherently present within the nodal tissue, which are insufficient to sustain organized growth in vitro in the absence of exogenous PGR supplementation (Skoog & Miller, 1957). These findings are consistent with reports by Anand et al. (2000), who similarly documented negligible shoot proliferation in Bambusa vulgaris on hormone-free MS medium. The control data unequivocally confirm that exogenous plant growth regulators are indispensable for in vitro propagation of this species.

3.2.2 Low Concentration Treatment [T_1]

Treatment T_1 produced a measurable improvement over the control, with shoot initiation rising to 30–40% by Day 7 and proliferation reaching 45–50% by Day 14. However, the final 28-day status was described as weak shooting, suggesting that while this concentration successfully activates axillary bud break, it lacks the cytokinin stimulus required to sustain continued proliferation and elongation. Cytokinin insufficiency at low concentrations fails to override the apical dominance mechanism, resulting in reduced lateral bud activation (George et al., 2008). The relatively higher NAA:BAP ratio in this treatment may also have shifted the hormonal balance marginally toward auxin-driven responses, partially restricting shoot differentiation.

3.2.3 Moderate Concentration Treatment [T_2]

A marked improvement in all growth parameters was recorded at T_2, with initiation reaching 60%, proliferation 70–80%, and elongation 80–85% by Day 21. The final response was classified as moderate shooting. These results indicate that 2.0 mg L^{-1} BAP provides a sufficient cytokinin signal to effectively suppress apical dominance and stimulate multiple axillary bud activation (Murashige, 1974). The accompanying 0.4 mg L^{-1} NAA presumably augments cell division at the basal node zone without suppressing shoot differentiation, resulting in a productive auxin–cytokinin balance. Similar results were reported by Islam et al. (2010) for B. vulgaris, where 2.0 mg L^{-1} BAP was identified as an effective concentration for axillary bud proliferation.

3.2.4 Optimal Treatment [T_3]

The highest morphogenetic response across all parameters was recorded in T_3 (3.0 mg L^{-1} BAP + 0.6 mg L^{-1} NAA). Shoot initiation reached 65–70% by Day 7, proliferation was 75–80% by Day 14, and elongation attained a peak of 90% by Day 21, with the final status described as excellent growth at Day 28. This treatment is therefore identified as the optimal PGR combination for in vitro shoot propagation of *Bambusa vulgaris* in the present study.

The superior performance of T_3 can be attributed to the high cytokinin:auxin ratio (5:1 approximation on a molar basis), which strongly promotes shoot organogenesis over callogenesis, in accordance with the classical Skoog–Miller (1957) hormonal model

(Skoog & Miller, 1957). BAP at 3.0 mg L^{-1} is a potent cytokinin that effectively stimulates adenine-based signalling pathways leading to rapid cell division in the meristematic region, while 0.6 mg L^{-1} NAA provides the complementary auxin stimulus required for basipetal nutrient transport and cell elongation. These findings corroborate those of Ramanayake & Yakandawala (1996), who reported that BAP at 2.5–3.5 mg L^{-1} was most effective for shoot proliferation in bamboo species, and are further supported by Pattnaik & Chand (1996), who noted the importance of an optimal BAP concentration window for maximum bud break in sympodial bamboos.

3.2.5 Supra-Optimal Treatment [T_4]

Contrary to expectations, increasing the BAP concentration to 4.0 mg L^{-1} (T_4) resulted in a significant decline in the overall growth response compared to T_3. While some initiation (50–60%) and proliferation (55–60%) were recorded, the 28-day final status showed callus formation with weak shoot development. This phenomenon wherein excessive cytokinin concentration promotes undifferentiated callus rather than organized shoot meristems is well-documented in bamboo tissue culture literature and is attributed to disruption of the intracellular auxin–cytokinin homeostasis at high exogenous cytokinin levels (George et al., 2008). Cytokinin excess has been shown to upregulate wound-response genes and trigger disorganized parenchymatous proliferation at the nodal interface, resulting in callus formation at the expense of axillary bud activation (Goyal et al., 2012). These findings confirm the existence of a dose-dependent optimum window for BAP in B. vulgaris, beyond which the growth response deteriorates.

3.3 Effect of Bavistin® (Carbendazim) on Contamination Control in Bambusa vulgaris Explants

Fungal contamination is among the most critical constraints limiting in vitro culture success in bamboo species, as the dense and microscopically rough surface of bamboo culms harbours abundant epiphytic and endophytic fungi (Pattnaik & Chand, 1996). The effect of Bavistin® (carbendazim, a systemic benzimidazole fungicide) at varying concentrations (0.0–0.6% w/v) and exposure durations (2–35 min) on contamination rate in *B. vulgaris* nodal explants is summarized in Table 3.2.

Table 3.2: Effect of Bavistin® (carbendazim) concentration and exposure duration on contamination rate in *Bambusa vulgaris* nodal explant cultures

Treatment	Bavistin® (% w/v)	Exposure (min)	Contamination Rate (%)	Remark
T_0	0.0%	2	100%	No sterilization; complete contamination
T_1	0.1%	5	80%	Insufficient; high contamination
T_2	0.2%	10	60%	Partial control; moderate contamination
T_3	0.3%	15	50%	Moderate control; 50% contamination
T_4	0.4%	20	30%	Good control; acceptable contamination
T_5	0.5%	30	20%	Very good control; low contamination
T_6	0.6%	35	10%	Excellent; minimal contamination ✓

Note: Contamination rate expressed as percentage of total inoculated explants showing microbial growth (bacteria and/or fungi) within 14 days of culture. n = 20 explants per treatment. ✓ denotes recommended treatment.

In the untreated control (T_0), 100% contamination was recorded within 7 days, confirming the absolute necessity of pre-sterilization treatment. The progressive increase in Bavistin® concentration and exposure time resulted in a consistent and statistically significant ($p \leq 0.05$) reduction in contamination rate across treatments T_1 through T_6. At T_1 (0.1% / 5 min), contamination remained high at 80%, indicating that neither the concentration nor the duration was sufficient to penetrate the thick outer culm surface or eliminate endophytic fungal propagules.

The most practically effective treatment was T_5 (0.5% Bavistin® for 30 minutes), which achieved a contamination rate of 20% while maintaining acceptable explant viability, and T_6 (0.6% / 35 min) which reduced contamination further to 10%. However, at T_6, a slight reduction in explant greenness and metabolic vigour was visually noted

upon inoculation, suggesting the onset of phytotoxicity due to prolonged fungicide exposure. Carbendazim at high concentrations has been reported to interfere with microtubule assembly in plant cells, potentially affecting meristematic activity (George, 1993). Based on these results, a Bavistin® treatment of 0.5% for 30 minutes is recommended as part of the pre-sterilization sequence prior to the LAF-based final sterilization steps, as it provides a favourable balance between contamination reduction and explant viability.

3.4 Effect of Mercuric Chloride ($HgCl_2$) on Surface Sterilization of *Bambusa vulgaris* Explants

Mercuric chloride is a powerful broad-spectrum surface disinfectant widely employed in plant tissue culture for its potent bactericidal and fungicidal properties, operating through mercuric ion-mediated denaturation of microbial protein and enzyme systems (George et al., 2008). However, Hg^{2+} ions are phytotoxic above threshold concentrations and exposure durations, requiring precise optimization for each plant species and explant type. The results of $HgCl_2$ sterilization treatment on contamination in *B. vulgaris* nodal explants are presented in Table 3.3.

Table 3.3: Effect of mercuric chloride ($HgCl_2$) concentration and exposure duration on contamination rate and explant response in Bambusa vulgaris nodal explant cultures

Treatment	$HgCl_2$ (% w/v)	Exposure (min)	Contamination Rate (%)	Remark
T_0	0.00%	2	100%	No sterilization; complete contamination
T_1	0.05%	3	70–80%	Poor sterilization; heavy contamination
T_2	0.01%	5	50–60%	Moderate effect; partial reduction
T_3	0.02%	6	30–40%	Good sterilization; significant reduction
T_4	0.03%	8	15–25%	Very good result; minimal contamination ✓
T_5	0.04%	10	5–10%	Excellent sterilization; slight tissue damage begins
T_6	0.05%	15	0–5%	Complete sterilization; pronounced phytotoxicity

Note: Contamination rate expressed as percentage of total inoculated explants (n = 20 per treatment) showing microbial growth within 14 days. Phytotoxicity was assessed as percentage of surviving, metabolically responsive explants at Day 7 post-inoculation. ✓ denotes recommended treatment.

A highly significant dose-dependent relationship was observed between $HgCl_2$ concentration/exposure time and contamination reduction. The control (T_0: 0.00% / 2 min) showed 100% contamination. At T_1 (0.05% / 3 min), contamination was still unacceptably high at 70–80%, indicating that brief exposure to this concentration is inadequate for bamboo explant decontamination. T_2 (0.01% / 5 min) and T_3 (0.02% / 6 min) showed moderate improvement, reducing contamination to 50–60% and 30–40%, respectively.

The optimal treatment in the present study was identified as T_4 (0.03% $HgCl_2$ for 8 minutes), which achieved contamination rates of 15–25% while maintaining explant viability and morphogenetic competence. Explants from this treatment showed normal bud break and healthy shoot development upon subsequent culture on PGR-supplemented MS medium. T_5 (0.04% / 10 min) further reduced contamination to 5–10%, but visual inspection of explants revealed early signs of tissue browning at the cut ends indicative of oxidative stress induced by heavy metal toxicity (Pattnaik & Chand, 1996). At T_6 (0.05% / 15 min), while contamination was virtually eliminated (0–5%), the explants exhibited pronounced phytotoxicity including severe necrosis of the nodal region and failure to initiate axillary buds rendering this treatment biologically unacceptable despite its superior sterilization efficacy.

These findings are in agreement with those of Islam et al. (2010), who reported that 0.1% $HgCl_2$ for 10 minutes was effective for B. vulgaris explants, and Goyal et al. (2012), who recommended 0.1–0.2% $HgCl_2$ for 5–10 minutes for *Dendrocalamus hamiltonii*. The lower optimal concentration (0.03%) observed in the present study may be attributed to the use of young, actively growing internodal segments, which are more physiologically sensitive to chemical sterilants than mature tissues.

IV. CONCLUSION

The present investigation systematically evaluated the critical parameters governing successful in vitro propagation of *Bambusa vulgaris* Green one of the most economically and ecologically significant bamboo species of tropical Asia with a focus on surface sterilization optimization and plant growth regulator (PGR) mediated shoot morphogenesis. The principal findings of the study may be summarized as follows:

- Surface sterilization with Bavistin® (carbendazim): A concentration of 0.5% w/v applied for 30 minutes provided the most effective fungal contamination control (contamination rate: 20%) while preserving explant viability. Higher concentrations (0.6% / 35 min), though more effective in reducing contamination to 10%, showed signs of phytotoxicity and are not recommended for routine use.
- Surface sterilization with $HgCl_2$ (mercuric chloride): Treatment with 0.03% $HgCl_2$ for 8 minutes was identified as the optimal sterilization regime, achieving contamination rates of 15–25% without significant phytotoxic damage to the nodal meristem. Concentrations above 0.04% induced visible tissue necrosis and suppressed axillary bud activation, confirming the existence of a narrow safe-effective window for $HgCl_2$ in B. vulgaris.
- PGR optimization for shoot development: Among all tested hormonal combinations on MS basal medium, 3.0 mg L^{-1} BAP combined with 0.6 mg L^{-1} NAA (T_3) elicited the highest morphogenetic response 65–70% initiation at Day 7, 75–80% proliferation at Day 14, 90% elongation at Day 21, and excellent overall shoot development at Day 28. This treatment is therefore recommended as the standard PGR protocol for micropropagation of *B. vulgaris*.
- Dose-dependent hormonal effects: Both sub-optimal (T_1) and supra-optimal (T_4) BAP concentrations resulted in inferior responses the former due to insufficient cytokinin stimulus for bud break, and the latter due to excessive cytokinin-induced dedifferentiation and callus formation. These results confirm the classical Skoog–Miller model in the context of bamboo tissue culture.
- Integrated protocol efficacy: Regenerated plantlets derived from the combined optimized sterilization and hormonal protocol exhibited healthy morphology, vigorous shoot and root development, and satisfactory survival rates during ex vitro acclimatization, validating the overall effectiveness of the standardized protocol.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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