Optimizing *In Vitro* Propagation of *Schizostachyum dullooa (Gamble) R.B. Majumdar* Using Plant Growth Regulators and Sterilization Protocols

Shailendra Singh Yadav*[1] Anya Shrivastava[2] Manisha Rathi[2,] Sandeep Fellows[1]Pradeep Vasudeva[1]
[1]*Biotechnology Division, State Forest Research Institute, Jabalpur, Madhya Pradesh, India*
[2]*Department of Microbiology, Govt. M. H. College of Home Science and Science for Women Autonomous, Jabalpur, Madhya Pradesh, India*

Abstract- *Schizostachyum dullooa* (Gamble) R.B. Majumdar (family: Poaceae), locally known as *dolu*, is a high-priority bamboo species of significant economic, cultural, and ecological importance across North East India and adjoining countries. Conventional propagation of this species is severely restricted by infrequent gregarious flowering, high seedling mortality (80–90%), and the limitations of vegetative propagation methods. The present study was undertaken to optimization an efficient in vitro micropropagation protocol for *S. dullooa* through axillary bud culture of nodal explants, with particular emphasis on optimizing surface sterilization and plant growth regulator (PGR) regimes. Nodal segments (2–3 cm) collected from healthy mother plants maintained at the State Forest Research Institute (SFRI) Bambusetum, Jabalpur, Madhya Pradesh, were subjected to sequential surface sterilization treatments comprising 70% ethanol, Bavistin (carbendazim, 50% WP) at concentrations of 1–5% (w/v) for 10–30 minutes, and mercuric chloride ($HgCl_2$) at 0.1–0.5% (w/v) for 2–6 minutes. Sterilized explants were cultured on full-strength Murashige and Skoog (MS) medium (1962) supplemented with 6-Benzylaminopurine (BAP 1.0–4.0 mg L^{-1}) in combination with α-Naphthaleneacetic acid (NAA 1.0–4.0 mg L^{-1}). Growth parameters bud break percentage, number of shoots, shoot length, and number of leaves were recorded at 7-day intervals over a 28-day culture period and this process repeat in four month. Among the Bavistin treatments, 2% Bavistin for 25 minutes (T_4) proved most effective, reducing contamination to 2% (98% survival). For $HgCl_2$ treatments, 0.2% $HgCl_2$ applied for 5 minutes (T_4) was optimal, yielding a contamination rate of 3% (97% survival). PGR evaluation revealed that MS medium supplemented with 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_4) consistently produced the best results across all developmental stages, achieving 100% bud break by Day 7, a maximum of 4 shoots per explants by Day 14, maximum shoot length of 2.60 cm by Day 21, and 4 leaves per explants by Day 28. Higher PGR concentrations (4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA) inhibited all growth parameters, indicating phytotoxic effects at supraoptimal concentrations. The sequential sterilization protocol (70% ethanol → 2% Bavistin, 25 min → 0.2% $HgCl_2$, 5 min) combined with MS medium supplemented with 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA is proposed as a reliable, reproducible protocol for large-scale in vitro propagation of *S. dullooa*, with important implications for commercial production, forest restoration, and germplasm conservation of this priority bamboo species.

Keywords- *Schizostachyum dullooa*; axillary bud culture; micropropagation; Murashige and Skoog medium; BAP; NAA; Bavistin; mercuric chloride; tissue culture; contamination bamboo conservation.

I. INTRODUCTION

Bamboo, belonging to the family Poaceae, subfamily Bambusoideae, and tribe Bambuseae, represents one of the most diverse and economically significant groups of grasses in the world. With approximately 120 genera and around 1,640 species distributed across tropical, subtropical, and temperate regions, bamboo covers nearly 3% of the world's forest area (Chaitali et al., 2020). The majority of bamboo diversity approximately 1,000 species is concentrated in Asian countries including China, India, Nepal, Vietnam, Laos, Thailand, Bhutan, Bangladesh, and Myanmar. Owing to its remarkable versatility, rapid growth, and wide-ranging utility, bamboo has earned the epithet "The Green Gold" across several Asian nations.

India holds the distinction of being the second richest country in bamboo genetic resources after China, harboring 23 genera and 136 species across its diverse forest ecosystems (Chaitali et al., 2020). The country accounts for approximately 10% of the world's bamboo forest area. Notable bamboo genera found in India *include Bambusa, Dendrocalamus, Ochlandra, Oxytenanthera, Phyllostachys, Pseudoxytenanthera, Schizostachyum*, and several others (Tiwari et al., 1992). Bamboo is a fast-growing perennial plant with woody, ringed stems (culms) capable of growing up to 30 cm per day, attaining heights exceeding 40 meters. Its culms arise from branching clusters of thick rhizomes and exhibit remarkable structural adaptability, thriving on steep hillsides, degraded lands, gullies, and along water bodies. Most bamboo species flower only after 12 years and require a minimum annual rainfall of 100 cm with high atmospheric humidity for optimal growth (Dhavala et al., 2015).

The multifaceted utility of bamboo has long been recognized across cultures, earning it such evocative appellations as "Poor Man's Timber," Friend of the People," and "Wood of a Thousand Uses" (Tewari et al., 1992; Sawarkar et al., 2020; Singh et al., 2021; Ajoy et al., 2022). Its unique combination of strength, lightness, straightness, abundance, ease of propagation, and short time to maturity makes it an indispensable resource in construction, handicrafts, pulp and paper industries, food, and traditional medicine. Beyond its direct economic value, bamboo plays a critical ecological role in carbon sequestration, soil stabilization, and watershed protection, contributing significantly to sustainable land management and environmental conservation.

Among the commercially and ecologically important bamboo species, *Schizostachyum dullooa* (Gamble) R.B. Majumdar locally known as dolu or doloo occupies a position of considerable significance. First described as *Teinostachyum dullooa* by J.S. Gamble in 1896 from a Myanmar specimen, the species was subsequently reclassified under the genus *Schizostachyum* by R.B. Majumdar (family: Poaceae). It is a medium-sized, evergreen, clump-forming bamboo reaching 6–9 meters in height with culm diameters of 5–8 cm and exceptionally long internodes of 40–75 cm. The species is semelparous, flowering gregariously once in its lifetime approximately every 15 years followed by mass die-off. Seeds though produced rapidly post-flowering, exhibit very high seedling mortality (80–90%) within four months, posing a severe constraint on natural regeneration.

Schizostachyum dullooa is listed among the 38 high-priority bamboo species identified by the International Network for Bamboo and Rattan (INBAR) and the International Plant Genetic Resources Institute (Rao and Rao, 1998), and is also recognized among the 19 priority species under India's National Mission on Bamboo Application (Haridasan and Tiwari, 2008). Its natural distribution encompasses North East India particularly Nagaland, Manipur, Mizoram, Tripura, Arunachal Pradesh, and Assam as well as Bangladesh, Nepal, Bhutan, and Myanmar. The species thrives at elevations of 300–1,500 m in warm, humid climates with 700–1,500 mm annual rainfall, preferring well-drained, fertile soils with slightly acidic to neutral pH. It finds applications in house construction, fencing, craft-making, kite-making (particularly for festive kite-making in Western India, owing to its lightness, flexibility, and long internodes), traditional food preparation, incense stick manufacture, mat weaving, and soil stabilization. Economically, a bundle of green culms fetches INR 170–200, while processed flattened bamboo ("dap") commands INR 200–250 in local markets.

Despite its considerable economic and ecological importance, large-scale cultivation of *Schizostachyum dullooa* remains constrained by the limitations of conventional propagation methods. Traditional approaches such as culm cuttings, rhizome division, and offset cutting are hampered by low multiplication rates, strong seasonality, and difficulties in handling and transportation of planting material. Seed propagation is seldom viable due to the species' infrequent, gregarious flowering and high post-germination mortality. These inherent limitations critically impede the sustained commercial supply of quality planting material and hinder conservation efforts for this priority species.

Plant tissue culture offers a scientifically robust and commercially viable alternative for the rapid, season-independent, large-scale multiplication of bamboo (Bharat et al., 2019). Micropropagation exploits the

principle of cellular totipotency the ability of a single plant cell to regenerate into a complete organism enabling the production of genetically uniform, disease-free planting material in large quantities throughout the year. Among the various micropropagation approaches, axillary bud culture is particularly favored for bamboo owing to its ability to maintain clonal fidelity of elite genotypes (Murashige, 1974). Successful tissue culture protocols have been developed for several bamboo species including *Gigantochloa atriviolaceae, Dendrocalamus giganteus, Bambusa tulda, Melocanna baccifera, Drepanostachyum falcatum, Bambusa bambos, Bambusa balcooa, Dendrocalamus strictus, Pseudoxytenanthera stocksii, and Schizostachyum dullooa*. However, surface sterilization the critical first step in establishing aseptic explants cultures remains a major challenge, particularly for woody, field-collected bamboo nodal explants that harbor a high load of surface contaminants.

In the context of the growing demand for quality planting material of *Schizostachyum dullooa* and the need for its conservation and sustainable management, the present investigation was undertaken with two primary objectives: (i) to standardize an efficient protocol for in vitro propagation of S. dullooa, and (ii) to evaluate the effect of surface sterilization treatments using Bavistin (carbendazim-based fungicide) and Mercuric Chloride ($HgCl_2$) on contamination control and explants survival. The findings of this study are expected to contribute a reliable and reproducible micropropagation protocol that can serve as the foundation for large-scale commercial production and conservation of this priority bamboo species.

II. MATERIALS AND METHODS

2.1 Plant Material and Explants Collection

Nodal explants were collected from phenotypically healthy mother plants of *Schizostachyum dullooa* (Gamble) R.B. Majumdar maintained at the Bambusetum of the State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh, India. Young, actively growing nodal segments were selected to ensure high regenerative potential. Explants were excised using sterile secateurs and immediately transported to the laboratory to prevent desiccation. Nodal segments of 2–3 cm length with an intact axillary bud were used as the primary explants. All collections were performed in the early morning hours to minimize exposure to direct sunlight and to reduce the initial microbial load. Explants were processed and inoculated on the same day of collection to maximize viability.

2.3 Culture Medium Preparation

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) was used as the standard nutrient medium for all experiments. The full-strength MS medium was prepared from four concentrated stock solutions: Stock I (macronutrients), Stock II (micronutrients), Stock III and Stock IV (vitamins and organic supplements). Sucrose (3%, w/v; 30 g L^{-1}) was used as the carbon source, and Agar-Agar (0.8%, w/v; 8 g L^{-1}) served as the solidifying agent. The medium pH was adjusted to 5.7 ± 0.1 using 0.1 N NaOH or 0.1 N HCl prior to agar addition. PGRs were incorporated at specified concentrations as required for each experimental treatment. Following agar dissolution by microwave heating, the medium was dispensed into culture vessels and autoclaved at 121°C and 15 psi for 20–30 minutes.

2.4 Surface Sterilization of Explants

A sequential chemical surface sterilization protocol was adopted for decontamination of nodal explants. The leaf sheaths surrounding the nodal segments were carefully removed, and the exposed surface was wiped with 70% ethanol-moistened cotton. Explants were then rinsed under running tap water for 10–15 minutes, followed by 3–4 washes with sterile double-distilled water (SDDW). All subsequent sterilization steps were performed under gentle agitation on an orbital shaker at 170 rpm to ensure uniform surface exposure to the sterilizing agents.

2.5 Ethanol Treatment

Explants were immersed in 70% (v/v) ethanol for 2 minutes as a primary surface-disinfection step. Following treatment, explants were rinsed 3–4 times with SDDW to remove residual ethanol.

2.6 Effect of Bavistin on Explants Decontamination

To evaluate the fungicidal efficacy of Bavistin (carbendazim, 50% WP) on contamination control and explants survival, five concentrations of Bavistin (1–

5%, w/v) were tested with inversely varied exposure durations. A 2% (w/v) working solution was prepared by dissolving 2 g of Bavistin powder in 100 mL SDDW. Each concentration was tested on a minimum of 20 explants, and the experiment was conducted in three independent replicates. Following each treatment, explants were rinsed with SDDW (3–4 times) before proceeding to the next step.

2.7 Effect of Mercuric Chloride ($HgCl_2$) on Explants Decontamination

The effect of mercuric chloride ($HgCl_2$) as a secondary disinfectant was assessed at five concentrations (0.1–0.5%, w/v) with decreasing exposure durations. A 0.1% (w/v) stock solution was prepared by dissolving 0.1 g $HgCl_2$ in 100 mL SDDW. Following $HgCl_2$ treatment, explants were subjected to 4–5 rigorous rinses with SDDW to completely eliminate mercuric ion residues before inoculation. The experiment was conducted in three independent replicates with a minimum of 20 explants per treatment.

2.8 Inoculation and Culture Establishment

Sterilized explants were inoculated aseptically onto solidified MS medium under the laminar airflow cabinet. The basal end of each nodal segment was trimmed with a sterile scalpel at a slight oblique angle to increase the surface area available for nutrient uptake. Explants were inserted individually into culture tubes or bottles containing the appropriate medium. Culture vessels were sealed with screw caps and secured with Parafilm to maintain aseptic conditions. All culture vessels were labelled with the explants identity, medium composition, and date of inoculation before transfer to the culture room.

2.9 Effect of Plant Growth Regulators on In Vitro Development

To investigate the influence of exogenous plant growth regulators (PGRs) on different stages of in vitro development bud break, shoot proliferation, shoot elongation, and rooting MS medium was supplemented with varying concentrations of 6-Benzylaminopurine (BAP; cytokinin) alone and in combination with α-Naphthaleneacetic acid (NAA; auxin). BAP was dissolved in a minimum volume of 1 N NaOH and NAA in 95% ethanol; stock solutions (1 mg mL^{-1}) were prepared and stored at 4°C until use. The PGR treatment combinations employed in the study are presented in Table 4. Cultures were evaluated at 7-day intervals and growth parameters were recorded over the experimental period.

2.10 Sub-culturing

Shoot clusters and elongated shoots were sub-cultured at intervals of 3–4 weeks to fresh MS medium supplemented with the appropriate PGR combination. For sub-culturing, explants were aseptically removed from culture vessels using sterile forceps, transferred to a sterile Petri dish, and necrotic or hyperhydric tissue was excised with a sterile scalpel. Individual shoots or shoot clusters were re-inoculated into fresh medium, sealed, labelled, and returned to the culture room under standard incubation conditions.

2.11 Culture Room Conditions

All cultures were maintained in a dedicated culture room at a controlled temperature of 25 ± 2°C under a 16-hour photoperiod provided by white fluorescent LED lamps at an irradiance of 3,000–8,000 lux, with 8 hours of darkness. Relative humidity was maintained between 60–80% to prevent excessive condensation that may promote contamination.

2.12 Observations and Data Recording

Cultures were examined every 7 days for contamination, explant browning, bud break, shoot initiation, proliferation, and elongation. The percentage of contaminated explants, percentage of surviving explants, and percentage of responsive explants exhibiting bud break were calculated for each surface sterilization treatment. Data were recorded over the complete experimental period, and mean values were calculated from three independent replicates.

III. RESULTS AND DISCUSSION

The present study investigated the in vitro propagation of *Schizostachyum dullooa* (Gamble) R.B. Majumdar using nodal explants obtained from healthy mother plants maintained at the Bambusetum of the State Forest Research Institute (SFRI), Jabalpur, Madhya Pradesh. Three sets of experiments were conducted: (i) evaluation of different concentrations and combinations of plant growth regulators (PGRs) BAP and NAA on key in vitro developmental parameters;

(ii) assessment of Bavistin (carbendazim) as a fungicidal surface sterilant at varying concentrations and exposure durations; and (iii) evaluation of mercuric chloride ($HgCl_2$) as a chemical disinfectant for contamination control. Results from all three experiments are presented and discussed below.

3.1 Effect of Plant Growth Regulators on In Vitro Development

Nodal explants of *S. dullooa* were cultured on MS medium supplemented with BAP (1.0–4.0 mg L^{-1}) alone and in combination with NAA (1.0–4.0 mg L^{-1}) across six treatment combinations (T_1–T_6). Growth responses including bud break percentage (Day 7), number of shoots (Day 14), shoot length (Day 21), and number of leaves (Day 28) were recorded at 7-day intervals. The results are summarized in Table 1 and develop of different stages of plant growth shown in figure no. 1.

3.2 Bud Break Response

Bud break, the earliest indicator of in vitro responsiveness, was observed across all six treatments including the hormone-free control (T_1). The highest bud break response of 100% was recorded in T_4 (3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA) and T_5 (4.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA) by Day 7 of inoculation. The control treatment (T_1, 0 mg L^{-1} PGR) and T_2 (1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA) both recorded 80% bud break, suggesting a baseline endogenous hormonal activity. The lowest bud break (75%) was observed in T_6 (4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA), indicating that supraoptimal concentrations of both BAP and NAA may exert an inhibitory effect on bud break induction. These findings are consistent with earlier reports that moderate cytokinin concentrations, in synergy with low auxin levels, are conducive to axillary bud release in bamboo (Murashige, 1974; Bharat et al., 2019).

3.4 Shoot Proliferation

Shoot proliferation was evaluated at Day 14. The maximum number of shoots (4 shoots per explant) was produced in T_4 (3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA), followed by T_3 (3 shoots; 2.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA) and T_5 (3 shoots; 4.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA). No shoot proliferation was observed in T_1 (control), confirming the absolute requirement for exogenous cytokinins to promote axillary shoot multiplication in *S. dullooa*. A marked decline in shoot number was observed in T_6 (1 shoot; 4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA), reinforcing the view that excessive auxin supplementation antagonizes cytokinin-driven proliferation by promoting callus induction rather than organogenesis. The relationship between BAP concentration and shoot number showed a clear optimum at 3.0 mg L^{-1}, beyond which proliferation efficiency declined. Similar trends have been reported for *Bambusa balcooa* and *Dendrocalamus strictus* where BAP at 3.0–4.0 mg L^{-1} yielded the highest shoot numbers (Bharat et al., 2019).

3.5 Shoot Elongation

Shoot elongation was measured at Day 21. The maximum shoot length of 2.60 cm was recorded in T_4 (3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA), followed closely by T_2 (2.59 cm; 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA) and T_1 control (2.48 cm). Interestingly, the control produced a comparatively elongated shoot despite the absence of exogenous PGRs, which may be attributed to the utilization of endogenous growth hormones under low-competition conditions. The shortest shoots were recorded in T_6 (1.40 cm; 4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA) and T_3 (1.48 cm; 2.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA). The inhibition of shoot elongation at higher combined PGR concentrations is likely attributable to hormonal imbalance favouring cell division over cell elongation, a phenomenon commonly observed in tissue culture systems subjected to high cytokinin-to-auxin ratios (Murashige, 1974).

3.6 Leaf Development

The number of leaves an indicator of overall shoot maturity and vigor was counted at Day 28 indicates in figure 1. A maximum of 4 leaves was observed in T_4 (3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA), mirroring the trend in shoot number and shoot length, further confirming T_4 as the optimal PGR combination for *S. dullooa* in vitro development. The control (T_1) and T_6 each produced no leaves and 1 leaf respectively, consistent with impaired shoot development in the absence of optimized PGR supplementation.

Treatment	BAP (mg L^{-1})	NAA (mg L^{-1})	Bud Break (%) Day 7	No. of Shoots Day 14	Shoot Length (cm) Day 21	No. of Leaves Day 28
T_1 (Control)	0	0	80	0	2.48	0
T_2	1.0	1.0	80	2	2.59	2
T_3	2.0	2.0	93	3	1.48	3
T_4*	3.0	2.0	100	4	2.60	4
T_5	4.0	3.0	100	3	2.17	3
T_6	4.0	4.0	75	1	1.40	1

Table 1. Effect of different concentrations and combinations of BAP and NAA on in vitro development of Schizostachyum dullooa nodal explants on MS medium (indicates optimal treatment).*

Values are means of three independent replicates (n = 20 explants per treatment). BAP = 6-Benzylaminopurine; NAA = α-Naphthaleneacetic acid.

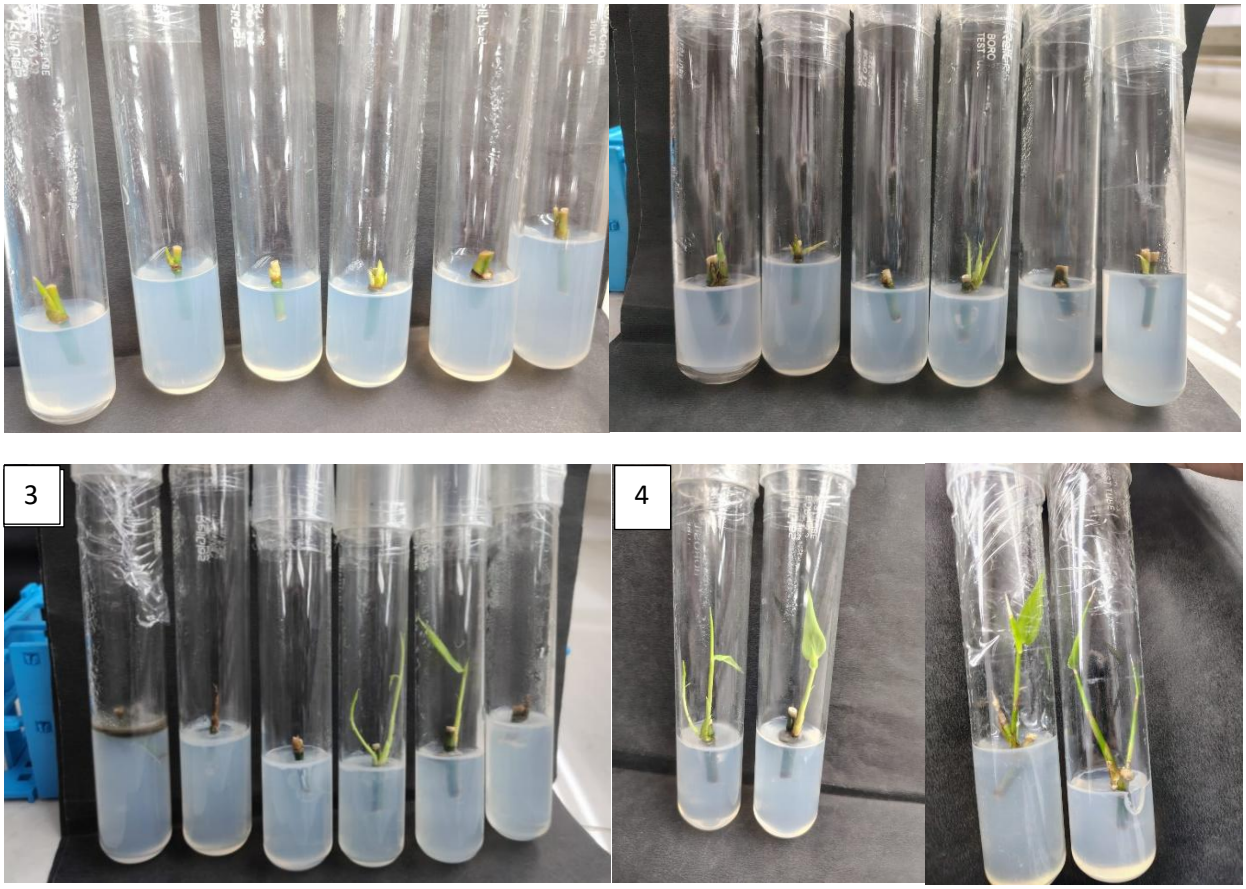

Figure No. 1: - 1. Bud break in 7 days 2. No. of Shoots in 14 days 3. Shoot Length in 21 days 4. No. of leaves in 28 days in *Schizostachyum dullooa* explant.

3.7 Effect of Bavistin (Carbendazim) on Contamination Control

Fungal and bacterial contamination of explants is a major impediment to successful in vitro culture establishment, particularly for field-collected bamboo nodal segments that carry a substantial epiphytic microbial load. Bavistin (carbendazim 50% WP), a broad-spectrum systemic benzimidazole fungicide, was evaluated as a pre-inoculation surface sterilant for

nodal explants of *S. dullooa* at five concentrations (1–5%, w/v) with inversely varying exposure durations (10–30 min). Contamination rates were assessed 15 days after inoculation and are presented in Table 2.

The results demonstrated a clear, non-linear relationship between Bavistin concentration, exposure duration, and contamination rate. Treatment T_4 (2% Bavistin, 25 min) was the most effective, yielding the lowest contamination rate of 2%, corresponding to a survival efficiency of 98%. This outcome significantly outperformed the untreated control (T_0), which recorded a contamination rate of 95%, indicating the near-complete inability of unsterilized field explants to establish aseptic cultures. A progressive decrease in contamination was observed from T_1 (5% Bavistin, 10 min; 30% contamination) through T_2 (4%, 15 min; 20%) and T_4 (2%, 25 min; 2%), followed by a sharp increase in contamination at T_3 (3%, 20 min; 50%) and T_5 (1%, 30 min; 60%). The elevated contamination at T_3 and T_5 suggests that neither the intermediate concentration (3%) with moderate exposure, nor the lowest concentration (1%) with extended exposure, was sufficient to achieve adequate microbial elimination. The superior performance of T_4 indicates that a moderate Bavistin concentration coupled with an appropriately extended exposure time achieves the optimum balance between fungicidal efficacy and explant phytotoxicity. Higher concentrations (4–5%) with shorter durations, while reducing contamination compared to control, may not ensure complete surface decontamination, while lower concentrations (1%) appear inadequate regardless of duration. The efficacy of carbendazim-based fungicides at 1–2% for pre-inoculation treatment of bamboo explants has been corroborated by earlier studies on *Bambusa bambos* and *Dendrocalamus strictus* (Bharat et al., 2019; Singh et al., 2021).

Treatment	Bavistin Conc. (%)	Exposure Duration (min)	Contamination Rate (%)
T_0 (Control)	–	0	95
T_1	5	10	30
T_2	4	15	20
T_3	3	20	50
T_4*	2	25	2
T_5	1	30	60

Table 2. Effect of Bavistin (carbendazim) concentration and exposure duration on contamination rate of Schizostachyum dullooa nodal explants after 15 days of in vitro culture (* indicates optimal treatment).

Values represent contamination percentage recorded at Day 15 post-inoculation (n = 20 explants per treatment; three replicates). T_0 = untreated control.

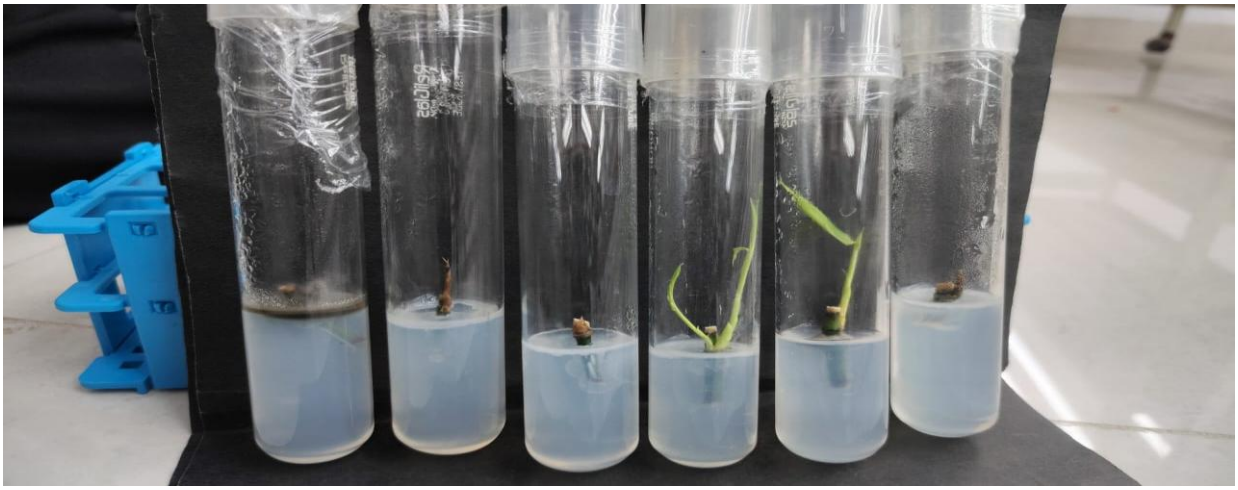

Figure No.2: - Contamination express in different inoculants

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

Mercuric chloride ($HgCl_2$), a potent broad-spectrum chemical disinfectant, was evaluated as a secondary sterilizing agent following ethanol and Bavistin pre-treatment of *S. dullooa* nodal explants. Five concentrations of $HgCl_2$ (0.1–0.5%, w/v) with decreasing exposure durations (2–6 min) were tested, and contamination rates were recorded 15 days post-inoculation (Table 3). $HgCl_2$ acts by denaturing microbial proteins and disrupting cell membranes; however, its high phytotoxicity necessitates careful optimization of both concentration and exposure duration to preserve explants viability.

Treatment T_4 (0.2% $HgCl_2$ for 5 min) yielded the minimum contamination rate of 3%, representing a success rate of 97% the most effective treatment in this experiment. The untreated control (T_0) recorded a contamination rate of 98%, confirming the critical necessity of chemical sterilization for establishing aseptic cultures from field material. Among the tested concentrations, contamination rates declined from T_1 (0.5%, 2 min; 40%) to T_3 (0.3%, 4 min; 20%) and reached a minimum at T_4 (0.2%, 5 min; 3%), before rising sharply to 70% at T_5 (0.1%, 6 min), indicating that the lowest concentration was insufficient for adequate decontamination despite the longest exposure. Interestingly, T_2 (0.4%, 3 min; 50%) showed higher contamination than both T_1 and T_3, suggesting that at intermediate concentrations, a 3-minute exposure may be inadequate while being sufficiently phytotoxic to compromise explants defense responses. The optimality of 0.2% $HgCl_2$ for 5 minutes is consistent with findings reported for other bamboo species. Patel et al. (2000) and Bharat et al. (2019) documented 0.1–0.2% $HgCl_2$ for 5–10 min as effective sterilization protocols for *Dendrocalamus giganteus* and *Bambusa balcooa* respectively, corroborating the present findings.

Treatment	$HgCl_2$ Conc. (%)	Exposure Duration (min)	Contamination Rate (%)
T_0 (Control)	–	0	98
T_1	0.5	2	40
T_2	0.4	3	50
T_3	0.3	4	20
T_4*	0.2	5	3
T_5	0.1	6	70

Table 3. Effect of mercuric chloride ($HgCl_2$) concentration and exposure duration on contamination rate of *Schizostachyum dullooa* nodal explants after 15 days of in vitro culture (* indicates optimal treatment).

Values represent contamination percentage recorded at Day 15 post-inoculation (n = 20 explants per treatment; three replicates). T_0 = untreated control. $HgCl_2$ = mercuric chloride.

3.9 Comparative Assessment and Implications

Taken collectively, the results of the present study establish a coherent and reproducible in vitro propagation protocol for *S. dullooa*. The sequential sterilization regimen — 70% ethanol (2 min) → 2% Bavistin (25 min) → 0.2% $HgCl_2$ (5 min), with intermediate SDDW rinses achieved near-complete decontamination while maintaining explants viability, reducing contamination to 2–3% compared to 95–98% in untreated controls. For in vitro shoot development, MS medium supplemented with 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (T_4) was identified as the optimal PGR combination, supporting maximum bud break (100%), shoot number (4), shoot length (2.60 cm), and leaf number (4) over a 28-day culture period.

The superiority of the T_4 PGR combination (3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA) over higher concentration treatments highlights the importance of hormonal balance over absolute concentration in regulating organogenesis. The pronounced inhibitory effect of supraoptimal PGR concentrations (T_6: 4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA) on all measured growth parameters — from bud break (75%) to leaf number (1) — underscores the phytotoxic potential of excess growth regulators and their role in redirecting tissue towards unorganized callus rather than structured shoot

formation. These outcomes are in broad agreement with published protocols for economically important bamboo species (Bharat et al., 2019; Ajoy et al., 2022) and validate the utility of axillary bud culture as a reliable approach for clonal propagation of *S. dullooa*. Future studies may focus on optimizing rooting induction using IBA supplementation and acclimatization protocols to improve ex vitro establishment rates.

No conflict of interest

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