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In vitro organogenesis techniques in black bamboo (*Phyllostachys nigra*)

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Abstract
In vitro organogenesis protocol for *Phyllostachys nigra* (Black bamboo) was developed, a commercially valuable and ornamental bamboo species. Due to limitations associated with traditional propagation methods such as unpredictable flowering behaviour, low seed viability and high contamination, micropropagation through tissue culture has emerged as a promising solution for large-scale propagation of this species. This study, conducted at the Plant Biotechnology Centre, DBSKKV, Dapoli during 2024-2025, focused on standardizing multiple aspects of *in vitro* culture, including explant selection, sterilization techniques, media composition, shoot multiplication, rooting and hardening. Among the various sterilization treatments treatments of axillary nodal explants, the effective treatment with Carbendazim (0.2%), Streptomycin (0.2%), Sodium hypochlorite (5%), Hydrogen peroxide (0.2%), Mercuric chloride (0.15%) and (70%) ethanol recorded the highest aseptic culture establishment (45.00%) with a (50.00%) survival rate. Axillary nodal explants, showing a sprouting rate of (84.00%) and a survival rate of (76.66%). Explants measuring 3.0 cm in length and 2.5 mm in diameter demonstrated the highest shoot induction (9.67 shoots/explant). Murashige and Skoog (MS) medium supplemented with 4.0 mg/l BAP was most effective for shoot initiation (86.50%), while 4.5 mg/l BAP promoted maximum shoot multiplication (9.00 shoots/explant). For root induction, MS medium supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA recorded the best rooting response (87.50%), resulting in an average of (9.66 /propagule) within 12 days. During hardening, a potting mixture of soil, vermicompost, and cocopeat in a 1:1:1 ratio ensured the highest plantlet survival rate (83.73%). The standardized protocol established through this study provides a sustainable method for the mass multiplication of *Phyllostachys nigra*, contributing to its conservation and commercial cultivation.

Keywords: *Phyllostachys nigra*, Black bamboo, *In vitro* organogenesis, Micropropagation, Nodal explant, BAP (6-Benzylaminopurine), Shoot induction, Rooting, Tissue culture, Plant growth regulators, Aseptic culture, Hardening

Introduction
Bamboo, a fast-growing and eco-friendly member of the family *Poaceae* and subfamily *Bambusoideae*, has long been valued as a sustainable and versatile natural resource. Globally, it encompasses about 75 genera and approximately 1,500 species distributed across tropical, subtropical, and temperate regions, except in Europe and West Asia (Tewari, 1992) [25]. Among these, India ranks second only to China in bamboo diversity, possessing 136 species across 23 genera, which occupy about 16 million hectares of land (Rai and Chauhan, 1998). Due to its high biomass production, rapid growth and multiple applications, bamboo is often referred to as "green gold" and "poor man's timber" (Tewari, 1992) [25].

Phyllostachys nigra (Black Bamboo) is an exotic species native to Taiwan and one of the most ornamental and commercially valued bamboos. Its young culms, initially light green, gradually develop into a distinctive purple-black coloration due to anthocyanin accumulation (Bae *et al.*, 2006) [3]. The species attains a height of 3-5 m with culm diameters ranging from 2-4 cm (Li *et al.*, 2011) [13]. It is widely used as a construction material in regions of Indonesia and as an ornamental species across Asia and beyond. However, despite its ecological and economic importance, large-scale cultivation of *P. nigra* remains limited. The demand for bamboo-based products in India has increased significantly from 2.2 million tons in 1980 to 13.48 million tons in 2003 driven by construction, paper, furniture and handicraft industries (Varmah and Bahadur, 1980; Planning Commission, 2003) [26]. Consequently, natural bamboo reserves are under pressure, and propagation efforts have not kept pace with exploitation. Conventional propagation methods such as rhizome division, branch cuttings and culm rooting are inadequate for large-scale production due to slow multiplication rates, difficulty in transport and high labor costs (Kumar, 1991; Banik, 1994) [12, 5]. Additionally, most woody bamboos, including *Phyllostachys* species, flower gregariously and die post-flowering, leading to scarcity of viable seeds (Banik, 2015) [4].

In vitro propagation techniques offer a promising alternative for the rapid, large-scale production of true-to-type and disease-free planting material. Micropropagation through nodal explants, axillary shoot proliferation,and somatic embryogenesis has shown successful outcomes in several bamboo species such as *Gigantochloa atroviolacea* (Bisht *et al*., 2010) [6], *Dendrocalamus giganteus* (Devi *et al*., 2012) [8], *Bambusa tulda*, *Melocanna baccifera* (Waikhom and Louis, 2014), *Drepanostachyum falcatum* (Saini *et al*., 2016) [21], *Bambusa bambos* (Raju and Roy, 2016) [18], *Bambusa balcooa* (Ansari *et al*., 2017) [1] and *Dendrocalamus strictus* (Rajput *et al*., 2019) [17]. However, limited research exists on standardized micropropagation protocols for *P. nigra* (Hassan and Debergh, 1987; Huang *et al*., 2002) [10, 11]. Tissue culture-based regeneration can overcome seed shortages, seasonal dependency and propagation inefficiency associated with traditional methods, thereby supporting the establishment of commercial plantations. Given the increasing industrial demand, ecological benefits and propagation challenges, the present study aims to develop an efficient *in vitro* organogenesis protocol for *Phyllostachys nigra* using nodal explants. This work contributes to the long-term objective of ensuring sustainable bamboo resource development and providing a reliable supply of quality planting material for both industrial and ecological applications.

Materials and Methods

Plant Material and Experimental Site

The present investigation entitled *"In vitro organogenesis technique in Black Bamboo (Phyllostachys nigra)"* was carried out during the academic year 2024-2025 at the Plant Biotechnology Centre, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.), India. Actively growing shoots of *Phyllostachys nigra* were collected from naturally growing clumps located at the Biodiversity Park, College of Forestry, Dapoli. Juvenile shoots containing apical and axillary buds were selected as explant sources for *in vitro* culture initiation.

Culture Media and Chemicals

Murashige and Skoog (MS) (1962) basal medium was used throughout the study. The medium was supplemented with 40 gm/L sucrose as a carbon source, 100 mg/L myo-inositol and solidified with 1 gm/l gelrite + 3 gm/l agar. The pH of the medium was adjusted to 5.8 ± 0.1 by using 0.1N NaOH or 0.1N HCl by using 0.1N NaOH or 0.1N HCl before adding gelrite and further autoclaving at 121 °C and 15 psi for 20 minutes. Analytical reagent (AR) grade chemicals of HiMedia Company were used for media preparation. The growth regulators employed included cytokinins 6-benzylaminopurine (BAP) and kinetin (Kin) and auxins naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA). Ascorbic acid (20 mg/L), Polyvinylpyrrolidone (PVP) (50 mg/L), Plantomycin (50 mg/L), Bavistin (200 mg/L) and sodium hypochlorite (300 µL/L) were incorporated as antioxidant and antimicrobial supplements to minimize phenolic browning and contamination.

Sterilization of Glassware and Instruments

All glassware, including culture bottles, flasks and pipettes, were thoroughly cleaned with detergent solution containing 0.1% Tween-20, rinsed in double-distilled water and oven-dried at 105 °C. Forceps, scalpels and surgical blades were wrapped in aluminum foil and sterilized by autoclaving at 121 °C and 15 psi for 20 minutes. The laminar airflow cabinet was wiped with 70% ethanol and exposed to UV light for 20 minutes before inoculation. All inoculations were performed aseptically near a flame to maintain sterility.

Surface Sterilization of Explants

Nodal and apical segments were collected from mature mother plants by using sterile secateurs, (2-4 cm in length) were excised from current-year culms bearing axillary or apical buds. The leaf sheath was removed and the explants were washed under running tap water for 30 minutes. The excised explants were transported to the laboratory and kept in distilled water for 45 min. Initial surface sterilization involved wiping the explants with 70% alcohol using sterilized cotton rinsing 2 times with distilled water and then treated with 0.2% Tween-20 detergent, followed by rinsing with sterile distilled water. Pre-sterilization treatment included immersion in a solution of 0.2% Bavistin and 0.2% Streptomycin for 1-2 hours. Surface sterilization was performed inside the laminar airflow chamber using combinations of 70% ethanol for 60 sec, followed by one rinse for 5 min with distilled water. After washing, the explants were sterilized with 5% sodium hypochlorite for 12 min (do not give any wash), then treat with 0.2% hydrogen peroxide (H_2O_2) for 5 min followed by rinsing with sterile distilled water for two times and 0.15% mercuric chloride ($HgCl_2$) for 6 min, followed by several rinses with sterile distilled water to remove the $HgCl_2$ traces. Twelve sterilization treatments were tested to determine the optimal combination for achieving maximum aseptic culture and survival percentage.

Culture Conditions

All cultures were incubated at 25 ± 2 °C with a 16-hour photoperiod (light intensity: 1600 lux) and 8 hours of darkness, under a relative humidity of 90%. Subculturing was carried out at intervals of 2-3 weeks. Observations were made at regular intervals and recorded the responses of explants at every step.

Shoot Initiation and Establishment

Surface-sterilized nodal explants were inoculated on MS basal medium supplemented with different concentrations of BAP (2.0-6.0 mg/L) for shoot initiation. Observations were recorded for days required for bud break, percentage of explant response, number of shoots per explant and mean shoot length after 30 days of culture. The best-performing treatment was used for subsequent proliferation studies.

Shoot Multiplication

Established shoots were subcultured on MS medium containing different concentrations and combinations of BAP (0.5-3.0 mg/L) and kinetin (0.5-3.0 mg/L) to induce multiple shoot proliferation. The effect of varying shoot clump sizes (1-10 shoots per clump) on shoot multiplication efficiency was also evaluated. Data were recorded for the number of new shoots per explant, mean shoot length and percentage proliferation after eight weeks of culture.

Root Induction

Elongated shoots were transferred to half-strength MS medium supplemented with different concentrations of IBA (0.5-2.0 mg/L) and NAA (0.5-1.5 mg/L) individually or in combination for root induction. The number of roots per shoot, root length and percentage of rooting were recorded after four weeks of incubation.

Hardening and Acclimatization

Rooted plantlets were carefully removed from culture vessels and washed thoroughly under running tap water to remove traces of the culture medium. The plantlets were transferred to protrays containing various potting mixtures red soil, cocopeat and vermicompost in different proportions. The mixture of red soil:cocopeat:vermicompost (1:1:1) provided the best results in terms of survival and growth. Plantlets were covered with transparent polybags to maintain humidity, which was gradually reduced over a period of 10-12 days. After four weeks, the hardened plantlets were transferred to polybags and maintained under greenhouse conditions.

Experimental Design and Statistical Analysis

The experiment was conducted in a Completely Randomized Design (CRD) with three replications per treatment and four explants per replication. The data were statistically analyzed using OPSTAT software, following the methods described by Panse and Sukhatme (1995). The significance of treatment differences was evaluated at a 1% level of probability (CD at 1%).

Results

Selection of Explant types

Healthy and disease-free explants were selected for organogenesis. Two types of explants e.g. nodal and apical segments were tested on MS medium supplemented with ascorbic acid (20 mg/L), PVP (50 mg/L), Plantomycin (50 mg/L), sodium hypochlorite (300 µL), carbendazim (200 mg/L) and varying concentrations of BAP (2.5-6.0 mg/L). After two weeks of inoculation, the nodal explants exhibited superior establishment and regeneration compared to apical explants. Within 14 days, nodal explants showed an average survival rate of (76.66%) and a sprouting rate of (84.00%). In contrast, apical explants turned brownish to black within a week and failed to survive. Hence, nodal explants were identified as the most suitable explant type for *in vitro* organogenesis in *Phyllostachys nigra*.

Selection of Explant Size

Among the different sizes of nodal segments tested, explants of 3.0 cm length × 2.5 mm diameter (ES-5) showed the highest sprouting (86.33%) and produced 9.67 shoots per explant within 14 days. Explants of 3.0 cm × 3.0 mm (ES-6) also responded well with (83.66%) sprouting and 8.33 shoots per explant. However, smaller explants (2 cm × 1.5 mm) showed poor response, while larger segments (4 cm × 3 mm; ES-8) sprouted after 15 days with only 5.33 shoots. Hence, ES-5 was identified as the optimal explant size for culture establishment (Table 1, Plate 1).

Table 1: Selection of explant size and effect on bud sprouting

Tr. No.	Tr. Code	Explant size		Sprouting%	Avg. No. of shoots per explant	Days required for sprouting
		Length (cm)	Diameter (mm)			
1	ES-1	2	1.5	59.66 (50.55)	1.33	22
2	ES-2	2	2	64.33 (53.31)	2.66	22
3	ES-3	2	2.5	73.66 (59.14)	6.33	20
4	ES-4	3	2	82.33 (65.12)	8.00	16
5	ES-5	3	2.5	86.33 (68.27)	9.67	14
6	ES-6	3	3	83.66 (66.15)	8.33	15
7	ES-7	4	2.5	78.66 (62.47)	7.67	18
8	ES-8	4	3	77.33(61.54)	5.33	15
9	ES-9	4	3.5	75.66 (60.42)	6.33	16
10	ES-10	4	4	68.33 (55.73)	4.00	17
SE (m)				0.99	0.39	
CD (at 1%)				2.93	1.72	

Note: ES - Explant Size (Figures in parentheses indicate arcsine values)

Surface sterilization of explants

The effectiveness of twelve sterilization treatments was assessed for aseptic culture establishment in nodal explants of *Phyllostachys nigra* (Table 2). Among these, the treatment SS-12 (0.2% carbendazim for 60 min + 0.2% streptomycin for 60 min + 5% sodium hypochlorite for 12 min + 0.2% H_2O_2 for 5 min + 0.15% $HgCl_2$ for 6 min + 70% ethanol for 1 min) provided the best results, recording (45.00%) aseptic culture establishment and (50.00%) survival. This was followed by SS-11, which achieved (44.66%) aseptic culture establishment and (51.51%) survival. The treatment SS-10 also effectively controlled contamination (38.00%) aseptic culture and (53.13%) survival. The least aseptic culture establishment (3.33%) was observed in SS-3, whereas SS-1 and SS-2 recorded no aseptic culture. Overall, the aseptic culture establishment ranged between 0.00-45.00%, with SS-12 proving to be the most efficient sterilization protocol for *P. nigra* explants.

Table 2: Effect of surface sterilizing agents on aseptic culture establishment of *Phyllostachys nigra* nodal explant

Tr. No.	Tr. Code	Treatment Details	Time (min)	Per cent aseptic culture	Per cent survivability
1	SS-1	DDW (Washing) + 70% alcohol (Control)	3 times wash + 1	00.00 (00.00)	00.00 (00.00)
2	SS-2	0.2% Carbendazim +1.250mg/l Streptomycin + 5% Sodium hypochlorite + 70% alcohol	60+15+2+30sec	00.00 (00.00)	75.26 (44.98)
3	SS-3	0.2% Carbendazim +1.250mg/l Streptomycin + 5% Sodium hypochlorite + 70% alcohol	60+15+5+1	03.33 (10.39)	63.50 (45.84)
4	SS-4	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 70% alcohol	60+15+5+1	7.33 (15.65)	62.06 (46.77)
5	SS-5	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 70% alcohol	60+15+10+1	12.66 (20.80)	60.50 (47.56)
6	SS-6	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 0.1% $HgCl_2$ + 70% alcohol	60+15+20+2+1	17.33 (24.56)	59.03 (48.48)
7	SS-7	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 0.1% $HgCl_2$ + 70% alcohol	60+15+10+5+1	23.33 (28.86)	57.50 (49.29)
8	SS-8	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 0.1% $HgCl_2$ + 70% alcohol	60+15+10+10+1	27.33 (31.50)	56.10 (50.18)
9	SS-9	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 0.15 $HgCl_2$ + 70% alcohol	60+15+10+5+1	33.66 (36.70)	54.50 (51.04)
10	SS-10	0.2% Carbendazim +1.250mg/l Streptomycin + 10% Sodium hypochlorite + 0.15% $HgCl_2$ + 70% alcohol	60+15+10+10+1	38.00 (38.03)	53.13 (51.96)
11	SS-11	0.2% Carbendazim + 0.2% Streptomycin + 5% Sodium hypochlorite + 0.2% H_2O_2 + 0.15% $HgCl_2$ + 70% alcohol	60+60+10+3+9+1	44.66 (41.92)	51.51 (52.81)
12	SS-12	0.2% Carbendazim + 0.2% Streptomycin + 5% Sodium hypochlorite + 0.2% H_2O_2 + 0.15% $HgCl_2$ + 70% alcohol	60+60+12+5+6+1	45.00 (42.11)	50.00 (60.15)
SE (m)				0.91	0.57
CD (at 1%)				2.70	1.70

Note: SS - Surface Sterilization (Figures in parentheses indicate arcsine values)

Effect of Plant Growth Regulators on Shoot Initiation

For shoot initiation, nodal explants were cultured on MS medium supplemented with ascorbic acid (20 mg/L), PVP (50 mg/L), + BAP 4.5 mg/L) with (83.53%) initiation. The lowest initiation (47.93%) was recorded in SIM-1 (MS control) (Table 3, Plate 2, Fig 1). Thus, 4.0 mg/L BAP was found to be optimal for rapid and efficient shoot initiation in *Phyllostachys nigra*.

Table 3: Effect of plant growth regulators on shoot initiation response

Tr. No.	Tr. code	Composition of media	% Shoot initiation response
1	SIM-1	MS Media (control)	47.93 (43.79)
2	SIM-2	MS + BAP 2.0 mg/	55.26 (48.00)
3	SIM-3	MS + BAP 2.5 mg/1	65.13 (53.78)
4	SIM-4	MS + BAP 3.0 mg/1	73.66 (59.10)
5	SIM-5	MS + BAP 3.5 mg/1	81.63 (64.60)
6	SIM-6	MS + BAP 4.0 mg/1	86.50 (68.43)
7	SIM-7	MS + BAP 4.5 mg/1	83.53(66.03)
8	SIM-8	MS + BAP 5 mg/1	80.80(63.98)
9	SIM-9	MS + BAP 5.5 mg/1	77.76 (61.84)
10	SIM-10	MS + BAP 6.0 mg/l	74.8 (59.84)
SE (m)			0.43
CD (at 1%)			1.29

Note: SIM - Shoot initiation medium (Figures in parentheses indicate arcsine values)

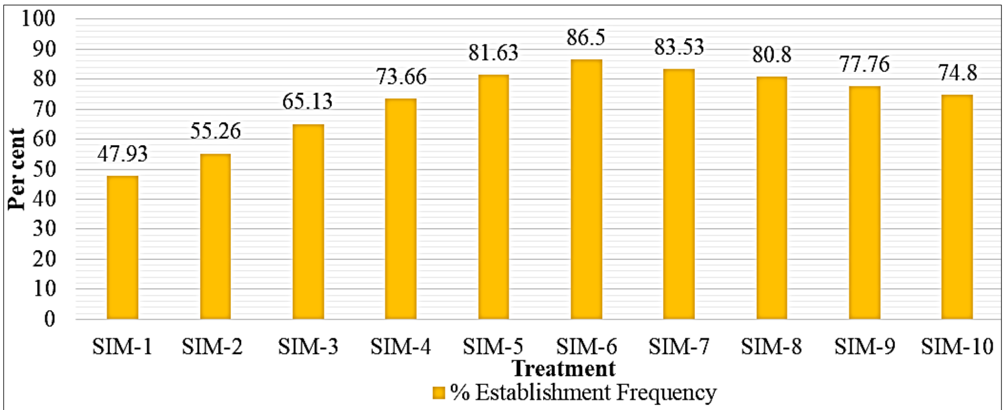

Fig 1: Effect of plant growth regulators on shoot initiation response

Effect of Plant Growth Regulators on Number of Shoots per Explant

BAP concentrations between 2.0-6.0 mg/L significantly affected multiple shoot induction. The maximum number of 9.00 shoots per explant with 3.06 cm average shoot length was obtained in SIM-7 (BAP 4.5 mg/L), followed by SIM-6 (BAP 4.0 mg/L) with 8.40 shoots and 4.53 cm length. Higher BAP levels (≥ 5.0 mg/L) reduced shoot length, though shoot numbers remained high. The longest shoots (6.83 cm) were observed in SIM-2 (BAP 2.0 mg/L). Increasing BAP concentration enhanced shoot proliferation but reduced elongation, indicating an inverse relationship between shoot number and length (Table 4, Plate 3).

Table 4: Effect of plant growth regulators on the number of shoots induction per explant.

Tr. No.	Tr. code	BAP (mg/l)	Average No. of shoots/explants	Average Shoot Length (cm)
1	SIM-GR-1	MS Control	1.86	6.66
2	SIM-GR -2	MS + 2.0 mg/l BAP	2.23	6.83
3	SIM-GR -3	MS + 2.5 mg/l BAP	3.06	6.53
4	SIM-GR -4	MS + 3.0 mg/l BAP	3.86	6.10
5	SIM-GR -5	MS + 3.5 mg/l BAP	6.10	5.16
6	SIM-GR -6	MS + 4.0 mg/l BAP	8.40	4.53
7	SIM-GR -7	MS + 4.5 mg/l BAP	9.00	3.06
8	SIM-GR -8	MS+ 5.0 mg/l BAP	7.73	3.03
9	SIM-GR -9	MS + 5.5 mg/l BAP	6.83	2.76
10	SIM-GR -10	MS + 6.0mg/l BAP	6.26	2.53
SE (m)			0.41	0.54
CD (at 1%)			1.23	1.61

Note: SIM-GR-Shoot induction medium-Growth regulators

Effect of Plant Growth Regulators on Shoot Multiplication

Shoots established after 45 days were transferred to multiplication media supplemented with adenine sulfate (10 mg/L), ascorbic acid (20 mg/L), PVP (50 mg/L), Plantomycin (50 mg/L), sodium hypochlorite (300 µL), carbendazim (200 mg/L) and varying concentrations of BAP (0.5-3.0 mg/L) and kinetin (0.5-3.0 mg/L). Among all treatments, SM-5 (MS + 2.0 mg/L BAP) exhibited the highest multiplication rate (5.60), with an average of 38.66 shoots/explant and a mean shoot length of 3.23 cm, followed by SM-6 (MS + 2.5 mg/L BAP) with a multiplication rate of 5.23 and 34.66 shoots/explant (2.86 cm length). The highest response to kinetin was observed in SM-13 (MS + 3.0 mg/L Kin), showing a multiplication rate of 4.90. The lowest multiplication (1.76) and highest shoot length (7.93 cm) were recorded in the control (SM-1). BAP proved more effective than Kinetin for inducing shoot proliferation. Hence, BAP at 2.0 mg/L was most effective for shoot multiplication (Table 5, Fig 2).

Table 5: Effect of plant growth regulators on shoot multiplication

Tr. No.	Tr. code	MS media supplemented with Cytokinin (mg/l)		Multiplication rate	Average No. of Shoots	The average length of the shoot (cm)
		BAP	Kin			
1	SM-1	0.0	0.0	1.76	11.33	7.93
2	SM-2	0.5	0.0	2.30	26.66	7.36
3	SM-3	1.0	0.0	3.16	34.33	6.16
4	SM-4	1.5	0.0	4.26	36.66	5.20
5	SM-5	2.0	0.0	5.60	38.66	3.23
6	SM-6	2.5	0.0	5.23	34.66	2.86
7	SM-7	3.0	0.0	4.06	26.66	2.70
8	SM-8	0.0	0.5	1.20	21.33	7.06
9	SM-9	0.0	1.0	2.23	28.66	5.83
10	SM-10	0.0	1.5	3.20	31.33	5.06
11	SM-11	0.0	2.0	3.80	34.33	2.86
12	SM-12	0.0	2.5	4.33	28.66	1.70
13	SM-13	0.0	3.0	4.90	23.66	1.23
SE (m)				0.58	0.60	0.08
CD (at 1%)				1.58	1.77	0.24

Note: SM - Shoot Multiplication

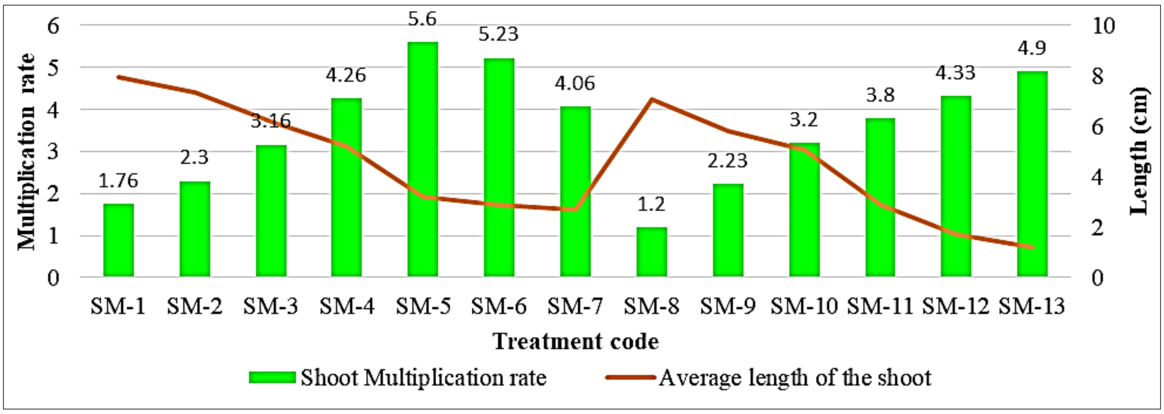

Fig 2: Effect of plant growth regulators on shoot multiplication

Effect of Number of Shoots per Clump on Shoot Multiplication

Ten clump sizes were evaluated to determine their effect on shoot multiplication. The clump with five shoots per explant (SC-SM-5) exhibited the highest multiplication rate (5.8) and produced healthy, vigorous green shoots. Clumps with six (SC-SM-6) and seven (SC-SM-7) shoots also performed well (5.3 and 5.0, respectively), whereas the single-shoot clump had the lowest multiplication (1.2) and showed early browning. Thus, five shoots per clump were identified as optimal for maintaining vigor and enhancing multiplication efficiency (Table 6, Plate 4).

Table 6: Effect of number of shoots per clump on shoot multiplication in MS medium

Tr. No.	Tr. Code	No. of shoots/ clump	Multiplication rate: (Avg. No. of shoots produced)	Nature of shoots
1	SC-SM-1	1	1.2	Main shoot turned brown after 4 days
2	SC-SM-2	2	1.6	Main shoot turned brown after 5 days
3	SC-SM-3	3	3.9	Healthy shoots with less multiplication
4	SC-SM-4	4	4.6	Healthy shoots with better multiplication
5	SC-SM-5	5	5.8	Healthy green shoots with highest multiplication rate
6	SC-SM-6	6	5.3	Healthy shoots with good multiplication
7	SC-SM-7	7	5.0	Healthy shoots with better multiplication
8	SC-SM-8	8	4.6	Healthy shoots but old shoots turned to brown
9	SC-SM-9	9	4.3	Main shoots turned brown and dead
10	SC-SM-10	10	2.6	Main shoots turned brown and dead
SE(m)			0.18	
CD (at 1%)			0.54	

Note: SC-SM -Size of clump in shoot multiplication

Effect of Plant Growth Regulators on Root Initiation

For root induction, elongated shoots were transferred to MS medium containing IBA (0.5-2.0 mg/L) and NAA (0.5-1.5 mg/L) singly or in combination. The treatment RIM-13 (MS + IBA 1.0 mg/L + NAA 1.0 mg/L) yielded the highest rooting (87.50%) and 9.66 roots per propagule within 12 days, followed by RIM-14 (84.50%, 9.00 roots). Root initiation was delayed and less effective at low hormone levels and absent in the control (RIM-1). Hence, the combined use of IBA and NAA at 1.0 mg/L each was optimal for rooting of *P. nigra* (Table 7, Plate 5, Fig 3).

Table 7: Effect of plant growth regulators on root initiation frequency

Tr. No.	Tr. Code	Treatments	Auxin		Avg. No of roots/ propagule	% Rooting response	Day of root induction
			IBA	NAA			
1	RIM-1	Full MS (control)	0	0	0	0 (0.00)	0
2	RIM-2	MS + IBA	0.5 mg/1	0.00	1.66	14 (21.93)	25
3	RIM-3	MS + IBA	1.0 mg/1	0.00	4.33	37.5 (37.74)	22
4	RIM-4	MS + IBA	1.5 mg/l	0.00	5.66	50.5 (45.26)	19
5	RIM-5	MS + IBA	2.0 mg/l	0.00	6.33	67.00 (54.92)	15
6	RIM-6	MS + NAA	0.00	0.5 mg/l	1.33	10.01 (18.44)	28
7	RIM-7	MS + NAA	0.00	1.0 mg/l	3.33	30.16 (33.29)	25
8	RIM-8	MS + NAA	0.00	1.5 mg/l	5.33	41.00 (39.79)	20
9	RIM-9	MS + NAA	0.00	2.0 mg/l	5.66	60.00 (50.75)	18
10	RIM-10	MS + IBA + NAA	0.5 mg/1	0.5 mg/l	4.66	55.53 (48.16)	14
11	RIM-11	MS + IBA + NAA	1.0 mg/l	0.5 mg/1	7.66	64.60 (53.47)	12
12	RIM-12	MS + IBA + NAA	0.5 mg/l	1.0 mg/l	8.66	69.63 (56.53)	13
13	RIM-13	MS + IBA + NAA	1.0 mg/l	1.0 mg/l	9.66	87.50 (69.32)	12
14	RIM-14	MS + IBA + NAA	1.0 mg/l	1.5 mg/l	9.00	84.50 (66.79)	12
SE (m)					0.37	1.75	
CD (at 1%)					1.10	2.61	

Note: RIM - Root induction medium. (Figures in parentheses indicate arcsine values)

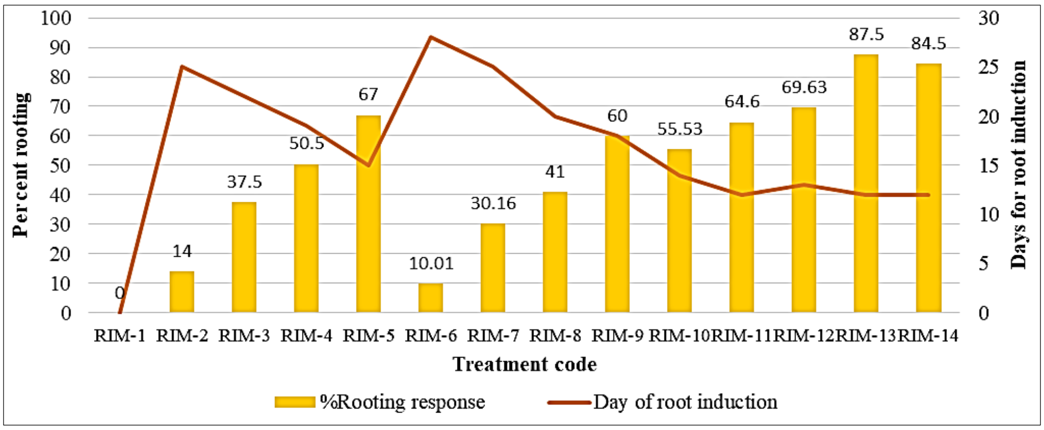

Fig 3: Effect of plant growth regulators on rooting response

Hardening of Regenerated Plantlets

Rooted plantlets were acclimatized in a greenhouse using nine potting mixtures of red soil, cocopeat, and vermicompost in different ratios. The maximum survival (83.70%) was obtained in H-9 (1:1:1 ratio of red soil:cocopeat:vermicompost), followed by H-2 (cocopeat 100%) with 79.33% survival. Lower survival was recorded in soil-only mixtures. The balanced combination of soil, cocopeat and vermicompost improved aeration and moisture retention, leading to higher survival of hardened *P. nigra* bamboo plantlets (Table 8, Plate 6).

Table 8: Per cent survival after 45 days of hardening in bamboo plantlets

Tr. No.	Potting code	Potting Mixture	Per cent of survival
1	H-1	Red Soil	14.83 (22.64)
2	H-2	Cocopeat	79.33 (62.93)
3	H-3	Red soil: Cocopeat (1:1)	68.33 (55.73)
4	H-4	Red Soil: Cocopeat (3:2)	65.1 (53.76)
5	H-5	Red Soil: Cocopeat (3:1)	61.00 (51.33)
6	H-6	Red soil: Vermicompost (3:1)	46.20 (42.80)
7	H-7	Red soil: Cocopeat: Vermicompost (2:5:3)	70.66 (57.18)
8	H-8	Red soil: Cocopeat: Vermicompost (2:1:1)	5 6.00 (48.42)
9	H-9	Red soil: Cocopeat: Vermicompost (1:1:1)	83.73 (66.18)
SE(m)			0.20
CD (at 1%)			0.61

Note: H - Hardening (Figures in parentheses indicate arcsine values)

Discussion

The present investigation established a reliable *in vitro* propagation protocol for *Phyllostachys nigra* using nodal explants. The choice of explant type significantly influenced culture establishment and regeneration efficiency. In this study, nodal segments exhibited a higher survival and sprouting rate than apical explants, which turned brown and failed to survive. Similar observations were reported in other bamboo species where nodal segments served as an ideal explant source for direct organogenesis due to the presence of pre-formed axillary buds and less susceptibility to oxidative browning (Arya and Sharma, 1999; Shroti *et al*., 2012) [2, 23]. The high frequency of shoot initiation in nodal explants of *P. nigra* indicates their superior morphogenic potential and responsiveness to cytokinin enriched media.

Explant size was found to be another critical factor affecting regeneration. In the present study, medium-sized explants (3.0 cm × 2.5 mm) produced the highest sprouting percentage and shoot number compared to smaller or larger explants. This could be attributed to the optimal balance between nutrient absorption and surface area exposure, ensuring effective uptake of plant growth regulators from the medium. Larger explants may accumulate more phenolic compounds leading to browning, while smaller ones may lack sufficient meristematic tissue to initiate multiple shoots. These findings are similar with previous reports on *Dendrocalamus strictus* and *Bambusa vulgaris*, where intermediate sized nodal segments produced better regeneration (Rout and Das, 1994; Mudoi *et al*., 2013) [20, 14].

Surface sterilization is a crucial prerequisite for successful *in vitro* culture establishment, especially in field-grown bamboo, which often leads to endogenous and exogenous contaminants. In this study, a combined sterilization treatment using Carbendazim, Streptomycin, NaOCl, H_2O_2 and $HgCl_2$ was most effective in minimizing contamination and enhancing survival. The synergistic use of fungicides, antibiotics and surface disinfectants has previously been demonstrated to increase asepsis in bamboo cultures (Borah *et al*., 2008; Ray *et al*., 2011) [7, 19]. The optimized sterilization protocol (SS-12) provided the highest aseptic culture percentage, confirming the importance of sequential and mild sterilization steps to balance disinfection efficacy and tissue viability.

Shoot initiation and multiplication in bamboo are primarily regulated by cytokinins, particularly 6-benzylaminopurine (BAP). In the current investigation, moderate BAP concentrations (4.0-4.5 mg/L) significantly enhanced shoot initiation frequency, while higher concentrations reduced shoot elongation. This trend confirm the findings of (Shroti *et al*., 2012; Sawant *et al*., 2016) [23, 22], who reported that intermediate cytokinin levels promote efficient bud proliferation, whereas excessive concentrations lead to physiological stress or vitrification. The decrease in shoot length with increasing BAP concentration observed here suggests an inverse relationship between cytokinin concentration and shoot elongation. Such outcomes are commonly associated with hormonal imbalance affecting apical dominance.

In the multiplication phase, BAP proved more effective than kinetin, with the medium containing 2.0 mg/L BAP yielding the highest multiplication rate and shoot number. Similar results were documented in *Bambusa balcooa* and *Dendrocalamus asper*, where BAP-induced cytokinin activity enhanced axillary shoot proliferation and the production of uniform shoots (Gunasena *et al*., 2024; Rahman *et al*., 2024) [9, 16]. The superior performance of BAP compared to kinetin may be due to its stronger affinity for cytokinin receptors in bamboo tissues, promoting faster cell division and bud differentiation. Additionally, shoot clump size played an essential role in multiplication; a clump of five shoots per explant resulted in optimal regeneration, indicating that the presence of neighboring shoots facilitates mutual hormonal stimulation and nutrient sharing within the culture vessel.

Root induction in *Phyllostachys nigra* was effectively achieved using a combination of auxins IBA and NAA where equal concentrations (1.0 mg/L each) produced the maximum rooting percentage and number of roots per shoot. The combined effect of IBA and NAA in root initiation has been reported in several bamboo species, where its use enhances rhizogenesis and root quality (Nath and Baruah, 2012; Rahman *et al*., 2024) [15, 16]. IBA promotes root primordia formation, while NAA assists in root elongation, explaining the superior performance of the combined treatment in this study. The control treatment without auxins showed no rooting response, confirming that endogenous auxin levels were insufficient for root induction in *P. nigra*.

The acclimatization and hardening of *in vitro* derived plantlets is often a critical phase determining the success of micropropagation. In the present study, the highest survival rate (83.70%) was recorded in a potting mixture containing equal proportions of red soil, cocopeat and vermicompost (1:1:1). The combination provided adequate aeration, drainage and nutrient availability, supporting better establishment under greenhouse conditions. Similar reports in *Bambusa bambos* and *Dendrocalamus giganteus* have emphasized the importance of balanced organic substrates for minimizing transplant shock and improving survival (Singh *et al*., 2010; Rahman *et al*., 2024) [24, 16]. Cocopeat, in particular, maintained favorable moisture levels and was observed to be the most effective component for survival, consistent with previous findings.

Overall, the results of the present study demonstrate that nodal explants of *Phyllostachys nigra* can be successfully utilized for rapid *in vitro* propagation through optimized cytokinin and auxin treatments. The established protocol comprising effective surface sterilization (SS-12), ideal explant size (3.0 cm × 2.5 mm), BAP concentration (4.0-4.5 mg/L) for shoot induction and combined IBA + NAA (1.0 mg/L each) for rooting ensures a high rate of regeneration and plantlet survival. This standardized approach can significantly contribute to the large-scale multiplication and conservation of *P. nigra*, which holds immense ornamental and commercial potential. The findings align with prior studies on other bamboo species, confirming that species-specific optimization of growth regulators and culture conditions is crucial for successful micropropagation. The developed system can further serve as a foundation for genetic improvement, conservation and sustainable plantation programs of *Phyllostachys nigra*.

Plate 1: (A) Healthy mother plant of *Phyllostachys nigra*, (B) Various explants sizes, (C) Standardized size of explant with 3 cm in length and 2.5 mm in diameter.

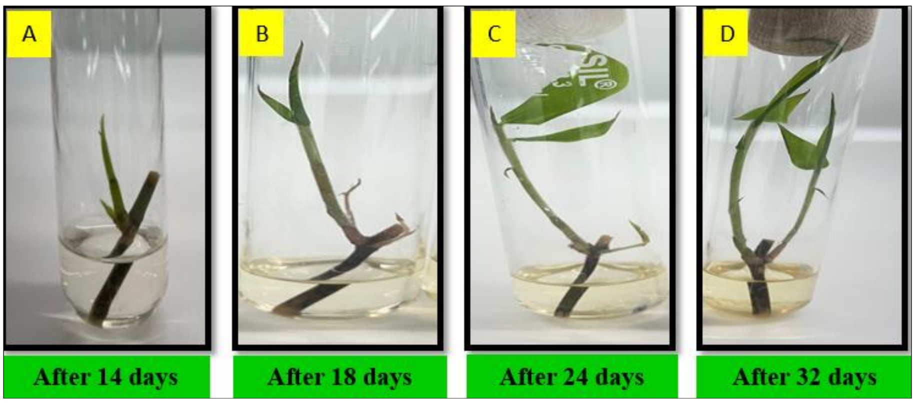

Plate 2: Effect of cytokinin (MS + 4.0 mg/l BAP) on shoot initiation, (A-D) Growth recorded of *Phyllostachys nigra* after certain intervals.

Plate 3: Effect of cytokinin (MS + 4.5 mg/l BAP) on shoot induction.

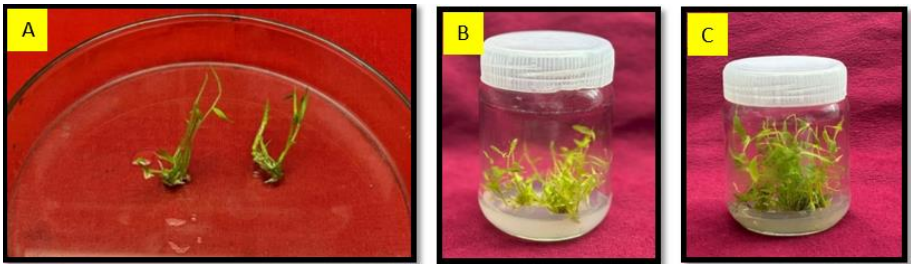

Plate 4: (A) Clump size of 5 shoots/explant, (B-C) Effect of cytokinin (MS + 5.0 mg/l BAP) shoot multiplication

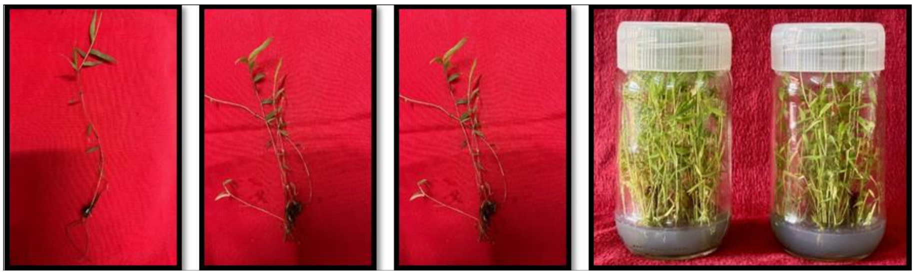

Plate 5: Effect of plant growth regulators (MS + 1.0 mg/l IBA + 1.5 mg/l NAA) on root induction

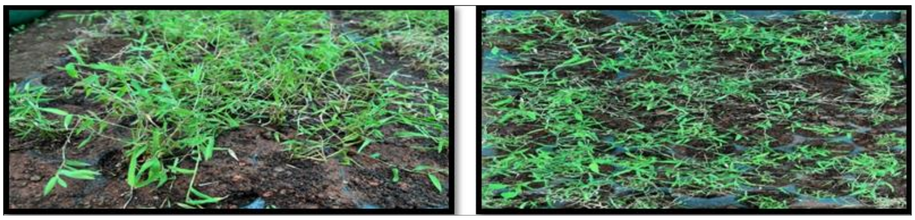

Plate 6: Hardening of *P.nigra* bamboo plantlets

Conclusion

The present study successfully established an efficient and reproducible *in vitro* organogenesis and micropropagation protocol for black bamboo (*Phyllostachys nigra*). The sequential sterilization treatment comprising (0.2% Carbendazim for 60 min, 0.2% Streptomycin for 60 min, followed by 5% Sodium hypochlorite for 12 min, 0.2% H_2O_2 for 5 min, 0.15% $HgCl_2$ for 6 min and 70% alcohol for 1 min) was found to be most effective for obtaining aseptic culture with the highest survival rate. Among the explants tested, nodal segments of 3.0 cm length and 2.5 mm diameter exhibited the maximum sprouting and the highest average number of shoots within the shortest culture duration.

For shoot initiation, Murashige and Skoog (MS) medium supplemented with 4.0 mg/L BAP proved to be the most suitable, resulting in maximum bud break, while MS + 4.5 mg/L BAP induced the highest average number of shoots per explant and longest shoot length. During shoot multiplication, MS medium containing 2.0 mg/L BAP resulted in the highest multiplication rate and number of shoots per explant. Subsequent subculturing with five shoots per culture enhanced shoot clump formation and produced healthy green shoots with vigorous growth. Repeated subculturing for 4-5 cycles further increased the number of shoot clumps suitable for rooting. The best rooting response was achieved on MS medium fortified with 1.0 mg/L IBA and 1.0 mg/L NAA, which resulted in the maximum number of roots and highest rooting percentage. During acclimatization, the potting mixture consisting of soil, vermicompost and cocopeat in equal ratio (1:1:1) gave the best survivability (83.70%) of hardened plantlets under greenhouse conditions.

Hence, the developed *in vitro* regeneration protocol offers a reliable method for rapid clonal propagation and large-scale production of *Phyllostachys nigra*. This technique can serve as an effective means for conservation, commercial multiplication and sustainable cultivation of this economically valuable bamboo species.

References

1. Ansari I, Gupta S, Soni S, Baig A. *In vitro* regeneration of *Bambusa balcooa* through nodal segments. Int J Curr Microbiol Appl Sci. 2017;6:4901-4905.
2. Arya S, Sharma S, Kaur R, Dev Arya I. Micropropagation of *Dendrocalamus asper* by shoot proliferation using seeds. Plant Cell Rep. 1999;18(10):879-882.
3. Bae RN, Kim KW, Kim TC, Lee SK. Anatomical observations of anthocyanin rich cells in apple skins. HortScience. 2006;41(3):733-736.
4. Banik RL. Bamboo silviculture. In: Bamboo: The Plant and its Uses. Cham: Springer; 2015. p.113-174.
5. Banik RL. Review of conventional propagation research on bamboos and future strategies. INBAR Rep No. 5. New Delhi: INBAR; 1994. p.115-142.
6. Bisht P, Pant M, Kant A. *In vitro* propagation of *Gigantochloa atroviolaceae* through nodal explants. J Am Sci. 2010;6(10):1019-1026.
7. Borah A, Borah P, Talukdar B. Optimization of surface sterilization protocol for bamboo micropropagation. Indian J Biotechnol. 2008;7:394-398.
8. Devi WS, Bengyella L, Sharma GJ. *In vitro* seed germination and micropropagation of *Dendrocalamus giganteus*. Biotechnol. 2012;11(2):74-80.
9. Gunasena MDKM, Chandrasena PH, Senarath WTPSK. *In vitro* mass propagation of *Dendrocalamus asper* through direct organogenesis. Adv Bamboo Sci. 2024;8:100090.
10. Hassan AA, Debergh P. Embryogenesis and plantlet development in *Phyllostachys viridis*. Plant Cell Tissue Organ Cult. 1987;10:73-77.
11. Huang SQ, Yang CF, Lu B, Takahashi Y. Honeybee-assisted wind pollination in *Phyllostachys nidularia*. Bot J Linn Soc. 2002;138(1):1-7.
12. Kumar A. Mass production of field planting stock of *Dendrocalamus strictus* through macroproliferation. Indian For. 1991;117(12):146-152.
13. Li B, Hu WP, Sun N. Past and future of *Phyllostachys nigra* in Putuo Mountains. Appl Mech Mater. 2011;44:3677-3680.
14. Mudoi KD, Saikia SP, Goswami A, Gogoi A, Bora D, Borthakur M. Micropropagation of important bamboos: A review. Afr J Biotechnol. 2013;12(20):2770-2785.
15. Nath S, Baruah A. Effect of auxins on root induction of bamboo microshoots. Indian J Plant Physiol. 2012;17(2):172-178.
16. Rahman M, Parvin W, Sultana N, Bashar KK, Islam J. Micropropagation and development of high yielding *Bambusa balcooa* BB1. J Earth Environ Sci Res. 2024;6(12):2-7.
17. Rajput BS, Jani MD, Gujjar MR, Shekhawat MS. Large-scale *in vitro* propagation of *Dendrocalamus strictus* using nodal segments. World Sci News. 2019;(130):238-249.
18. Raju RI, Roy SK. Mass propagation of *Bambusa bambos* through *in vitro* culture. Jahangirnagar Univ J Biol Sci. 2016;5(2):15-26.
19. Ray PK, Tripathi AK, Singh S. Surface sterilization and micropropagation of *Bambusa* species. J Environ Biol. 2011;32:379-382.
20. Rout GR, Das P. Micropropagation and regeneration of bamboo through nodal culture. Plant Cell Tissue Organ Cult. 1994;39:105-111.
21. Saini H, Arya ID, Arya S, Sharma R. *In vitro* micropropagation of *Drepanostachyum falcatum*. Am J Plant Sci. 2016;7(9):1317-1327.
22. Sawant SS, Gokhale NB, Sawardekar SV, Bhave SG. *In vitro* propagation technique for bamboo of Western Ghats. J Tree Sci. 2016;35(1):53-58.
23. Shroti RK, Upadhyay R, Niratkar C, Singh M. Micropropagation of *Dendrocalamus asper* through internodal segment. BEPLS. 2012;1(4):58-60.
24. Singh R, Sharma S, Bhattacharya S. Acclimatization and field performance of *in vitro* raised bamboo plantlets. Indian For. 2010;136(5):693-699.
25. Tewari DN. A monograph on bamboo. Dehra Dun: International Book Distributors; 1992. 498 p.
26. Varmah JC, Bahadur KN. Country report and status of research on bamboos in India. 1980.
27. Waikhom SD, Louis B. Micropropagation of *Bambusa tulda* and *Melocanna baccifera* through nodal culture. Sci World J. 2014;2014:345794.