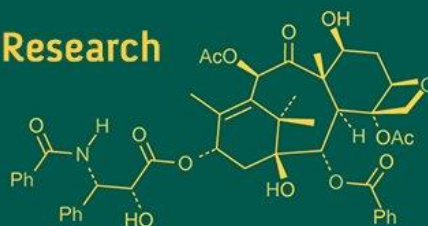
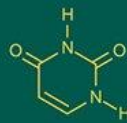
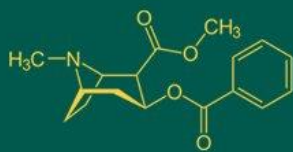


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In vitro organogenesis techniques in golden bamboo (*Phyllostachys aurea*)

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Abstract

The present investigation aimed to develop a standardized *in vitro* organogenesis protocol for the production of disease and virus free plantlets of Golden Bamboo (*Phyllostachys aurea*). The study was conducted in a Completely Randomized Design at the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkani Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) during the academic years 2023-2025. Among the various surface sterilization treatments of axillary nodal explants. The sequential treatment comprising Tween-20 (5.0%), Alcohol (70%), Carbendazim (0.2%), Streptomycin (0.2%), Ethanol (60%), NaOCl (3.0%), H₂O₂ (0.2%) and HgCl₂ (0.1%) recorded the highest aseptic culture establishment (43.66%) and survival rate (87.33%). The nodal explant with 3 cm in length and 3 mm in diameter exhibited the highest bud sprouting response achieved 85.66% with 87.33% survival rate. Murashige and Skoog (MS) medium supplemented with 5.0 mg/l BAP and additives facilitated 90.66% shoot initiation with shoot induction, producing an average of 6.66 shoots per explant with a mean shoot length of 3.0 cm. The highest shoot multiplication rate (4.66) was obtained on MS medium fortified with 0.5 mg/l BAP with 6.0 cm shoot length. The rooting was most effective (88.66%) on MS medium containing 1.0 mg/l IBA and 1.5 mg/l NAA, resulting in an average of 9.66 roots per explant within 12 days. The plantlets acclimatized in a 1:1:1 potting mixture of red soil: cocopeat: vermicompost achieved a plantlet survival rate of 80.33%.

Keywords: Golden Bamboo, *Phyllostachys aurea*, Organogenesis and axillary nodal explant

Introduction

Golden Bamboo (*Phyllostachys aurea*), also known as “Golden Chinese Bamboo” or “Yellow Bamboo,” is distinguished by its bright golden-yellow culms that deepen in color with age and sunlight. It is a highly adaptable and multipurpose forest species valued for its extensive use “from cradle to coffin.” The culms have a distinctive tortoiseshell-like pattern near the base and the plant thrives in diverse soil types and climatic conditions, making it ideal for erosion control. Golden Bamboo is widely used for living fences, furniture, ceiling construction and as an attractive indoor ornamental plant.

India produces about 9.5 million tonnes of bamboo annually, with around 4.9 million tonnes utilized in the paper industry to yield approximately 600,000 tonnes of pulp each year (Varmah and Pant, 1981) [16]. Environmentally, bamboo plays a vital role in biodiversity conservation, soil and water protection, carbon sequestration and maintaining atmospheric balance. It also reduces light intensity, serving as a natural shield against harmful ultraviolet rays (Venkatachalam *et al.*, 2015) [17].

Conventional propagation methods such as seeds, offsets and culm cuttings face challenges. Bamboo's long flowering cycle (up to 120 years), poor seed viability, monocarpic nature and high seed predation hinder seed-based propagation. Vegetative methods are limited by bulky planting material, low survival rates and seasonal dependency. Due to these constraints, traditional techniques are inadequate to meet large-scale reforestation and commercial demands (Singh *et al.*, 2013) [15]. In contrast, tissue culture provides a reliable and efficient approach for mass production of disease-free, genetically uniform Golden Bamboo plantlets.

Materials and Methods

Source of Explants

In vitro organogenesis techniques were conducted on Golden Bamboo (*Phyllostachys aurea*) during 2024-25 at Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri (MS) 415712 and India. The Explants were collected from healthy mother plant of bamboo (Plate 1) from the Biodiversity Park, College of Forestry, Dapoli.

Explant Preparation and Sterilization

Axillary nodal explants (3 cm long) were collected from mature mother plants using sterile secateurs and excised with a scalpel. After removing leaf sheaths, explants were rinsed under running tap water and kept in distilled water for 45 min. Surface sterilization included wiping with 70% ethanol, rinsing twice with distilled water and treat with 5% Tween-20. Explants were then immersed in 0.2% Carbendazim + 0.2% Streptomycin solution (200 ml) for 2 hr, followed by three rinses with sterile distilled water in a laminar air flow (LAF) cabinet. Sequential sterilization inside LAF included 60% ethanol (45 sec), 3% NaOCl (10 min, no rinse), 0.2% H₂O₂ (8 min, two rinses) and 0.1% HgCl₂ (6 min) followed by 4-5 rinses with sterile distilled water to remove the HgCl₂ traces. Sterilized explants were then transferred to sterile bottles and kept in LAF, ready for inoculation.

Culture Media Preparation

The sterilized nodal explants were inoculated on MS medium supplemented with 40 gm/l sucrose as a carbon source and 1 gm/l gelrite + 3 gm/l agar as solidifying agents. Various plant growth regulators such as: cytokines (BAP & Kin) and auxins (IBA & NAA) were used to prepare MS medium for culture establishment, shoot initiation and induction, shoot multiplication and root induction from the base of excised new shoots. The pH 5.8 was adjusted by

using 0.1N NaOH or 0.1N HCl before adding gelrite and the medium was sterilized in autoclave at 121°C for 20 minutes.

Statistical Analysis

The study was conducted under controlled laboratory conditions with standardized aseptic media, growth parameters and temperature and light regimes. A Completely Randomized Design (CRD) was employed following the methodology of Panse and Sukhatme (1954)^[10]. All experimental data were statistically analyzed using OPSTAT, an online analytical software platform. Three replications were considered for each treatment and repeated thrice.

Results and Discussion

Selection of Explant types

In this study, healthy and disease-free explants were selected for organogenesis using two explant types- axillary nodal and apical explant. MS medium supplemented with additives and BAP (5.0 mg/l) was used for establishment. After two weeks, axillary nodal explants showed a high response of survival and sprouting within 12 days, whereas apical explants turned brown or black and died within a week. Therefore, axillary nodal explants were found suitable for *in vitro* organogenesis, which aligns with observations reported by Sawant *et al.* (2016)^[12], Jha *et al.* (2013)^[4], Bisht *et al.* (2010)^[1] and Mehta *et al.* (2010)^[7].

Standardization of explant size

Axillary nodal explants of different sizes were cultured on MS medium supplemented with antioxidants, antibiotics and 5.0 mg/l BAP. Explants measuring 3.0 cm × 3.0 mm (ES-11) showed the highest response with 85.66% sprouting within 12 days and an average of 6.66 sprouts per explant. Medium-sized nodal segments were thus found most suitable for culture establishment, corroborating earlier findings by Sanjaya *et al.* (2005), Kapruwan *et al.* (2014)^[5], Malini and Ananda Kumar (2013)^[6], Shrotri *et al.* (2012)^[13], Singh *et al.* (2012)^[14] and Sawant *et al.* (2016)^[12].

Table 1: Effect of explants size on bud sprouting

Tr. No.	Treatment code	Explant size		Sprouting (%)	Days required for sprouting	Number of sprouts per explant
		Length (cm)	Diameter (mm)			
T ₁	ES-1	2.5	3.0	58.66 (49.97)	25	0.66
T ₂	ES-2	2.5	3.5	61.66 (51.72)	22	1.33
T ₃	ES-3	4.0	4.0	64.00 (53.11)	20	1.66
T ₄	ES-4	3.5	3.0	66.66 (54.71)	20	2.66
T ₅	ES-5	3.5	3.5	68.33 (55.73)	18	3.66
T ₆	ES-6	3.0	3.5	70.33 (56.98)	20	3.00
T ₇	ES-7	3.0	4.5	74.66 (59.76)	16	4.66
T ₈	ES-8	3.5	4.5	78.66 (62.47)	19	4.00
T ₉	ES-9	3.5	3.5	80.33 (63.66)	14	5.33
T ₁₀	ES-10	3.0	4.0	83.33 (65.88)	13	5.66
T ₁₁	ES-11	3.0	3.0	85.66 (67.72)	12	6.66
SE (m)				0.70	-	-
CD 1%				2.80	-	-

(Figures in parenthesis are arcsine values.)

Surface sterilization of explants

Different surface sterilization treatments were evaluated for axillary nodal explants of Golden Bamboo to minimize fungal and bacterial contamination (Table 2). Among twelve treatments tested, the combination of 0.2% Carbendazim (60 min) + 0.2% Streptomycin (60 min) + 3.0% NaOCl (10 min) + 0.2% H₂O₂ (8 min) + 0.1% HgCl₂ (6 min) + 60%

ethanol (45 sec) (SS-12) gave the best results, achieving 43.66% aseptic culture establishment and 87.33% survival rate. These findings align with earlier reports by Sawant *et al.* (2016)^[12], Wei *et al.* (2015)^[18] and Goyal *et al.* (2015)^[2], confirming the effectiveness of sequential sterilization treatments for contamination control.

Table 2: Effect of surface sterilizing agents on aseptic culture establishment of *Phyllostachys aurea* nodal explants

Tr. No.	Tr. Code	Treatment details	Concentration (%)	Time	Per cent aseptic culture	Per cent survival
T ₁	SS-1	Control (DDW Washing)	-	30 min	0.00 (0.00)	0.00 (0.00)
T ₂	SS-2	Ethanol	70%	30sec	4.66 (12.45)	56.00 (48.42)
		NaOCl	5%	2min		
T ₃	SS-3	Ethanol	70%	60sec	6.66 (14.94)	65.33 (53.90)
		NaOCl	5%	5min		
T ₄	SS-4	Ethanol	70%	60sec	8.66 (17.10)	67.66 (55.33)
		NaOCl	10%	5min		
T ₅	SS-5	Ethanol	70%	60sec	10.66 (19.05)	68.66 (55.94)
		NaOCl	10%	10min		
T ₆	SS-6	Ethanol	70%	1min	13.33 (21.39)	69.66 (56.56)
		NaOCl	10%	20 min		
		HgCl ₂	0.1%	2 min		
T ₇	SS-7	Ethanol	70%	60sec	17.33 (24.59)	70.66 (57.18)
		NaOCl	10%	10min		
		HgCl ₂	0.1%	5min		
T ₈	SS-8	Ethanol	70%	60sec	21.66 (27.72)	71.33 (57.62)
		NaOCl	10%	10min		
		HgCl ₂	0.1%	10min		
T ₉	SS-9	Ethanol	70%	60sec	27.66 (31.72)	75.66 (60.42)
		NaOCl	10%	10min		
		HgCl ₂	0.15%	5min		
T ₁₀	SS-10	Ethanol	70%	60sec	33.66 (35.45)	80.00 (63.42)
		NaOCl	10%	10min		
		HgCl ₂	0.15%	10min		
T ₁₁	SS-11	Ethanol	70%	60 sec	39.66 (39.01)	84.33 (66.66)
		NaOCl	10%	15 min		
		HgCl ₂	0.15%	10 min		
		H ₂ O ₂	0.2%	10 min		
T ₁₂	SS-12	Ethanol	60%	45 sec	43.66 (41.34)	87.33 (69.13)
		NaOCl	3%	10 min		
		HgCl ₂	0.1%	6 min		
		H ₂ O ₂	0.2%	8 min		
SE(m)					0.61	1.00
CD 1%					2.43	3.95

(Figures in parenthesis are arcsine values.)

Effect of plant growth regulators on shoot initiation

Ten treatments comprising MS medium with different concentrations of BAP and additives [Ascorbic acid (20 mg/l), PVP (50 mg/l), Plantomycin (50 mg/l), Sodium hypochlorite (300 µl/l) and Carbendazim (200 mg/l)] were tested for shoot initiation (Table 3, Plate 3). Shoot emergence and sprouting were influenced by explant

texture, seasonal factors and culture conditions. Among the treatments, SIM-8 (MS + 5.0 mg/l BAP) showed the best response, achieving 90.66% shoot initiation within 12 days under controlled conditions. These findings are consistent with those of Goyal *et al.* (2015)^[2], Sawant *et al.* (2016)^[12] and Muthukumaran *et al.* (2015)^[9], who also reported BAP as an effective cytokinin for *in vitro* shoot initiation.

Table 3: Effect of plant growth regulators on shoot initiation

Tr. No.	Tr. code.	Treatments	Per cent shoot initiation
T ₁	SIM-1	MS (control)	48.66 (44.21)
T ₂	SIM-2	MS+ 2.0 mg/l BAP	51.33 (45.74)
T ₃	SIM-3	MS+ 2.5 mg/l BAP	69.33 (56.35)
T ₄	SIM-4	MS+ 3.0 mg/l BAP	75.33 (60.20)
T ₅	SIM-5	MS+3.5mg/l BAP	79.00 (62.61)
T ₆	SIM-6	MS+4 mg/l BAP	82.66 (65.35)
T ₇	SIM-7	MS+4.5 mg/l BAP	86.00 (68.03)
T ₈	SIM-8	MS+5 mg/l BAP	90.66 (72.21)
T ₉	SIM-9	MS+ 5.5 mg/l BAP	82.33 (65.14)
T ₁₀	SIM-10	MS+6.0 mg/l BAP	76.00 (60.65)
SE(m)			0.91
CD 1%			3.69

(Figures in parenthesis are arcsine values.)

Effect of plant growth regulators on shoot proliferation

MS medium supplemented with varying concentrations of BAP and additives [Ascorbic acid (20 mg/l), PVP (50 mg/l), Plantomycin (50 mg/l), Sodium hypochlorite (300 µl/l) and

Carbendazim (200 mg/l)] was tested for shoot proliferation (Table 4, Plate 4). Among ten treatments, SIM-8 (MS + 5.0 mg/l BAP) showed the best response, producing an average of 6.66 shoots per explant with a mean shoot length of 3 cm.

These results confirm that BAP is an effective cytokinin for shoot multiplication, aligning with the findings of Goyal *et al.* (2015)^[2] reported that MS medium enriched with plant growth regulators, especially BAP (6-Benzylaminopurine),

was highly effective in inducing shoot regeneration. Similarly, Sawant *et al.* (2016)^[12] and Muthukumaran *et al.* (2015)^[9] used BAP as effective plant growth regulator for *in vitro* shoot proliferation with their different concentration.

Table 4: Effect of plant growth regulators on shoots induction

Tr. No.	Tr. Code	BAP (mg/l)	Average number of shoots / explants	Average shoot length (cm) (After 30 days of sprouting)
T ₁	SIM-1	0.0	0.66	7.33
T ₂	SIM-2	2.0	1.33	6.33
T ₃	SIM-3	2.5	1.66	6.00
T ₄	SIM-4	3.0	2.66	5.66
T ₅	SIM-5	3.5	3.66	5.00
T ₆	SIM-6	4.0	3.00	4.33
T ₇	SIM-7	4.5	4.66	4.00
T ₈	SIM-8	5.0	6.66	3.00
T ₉	SIM-9	5.5	5.66	3.66
T ₁₀	SIM-10	6.0	5.33	2.33
SE(m)			0.44	0.48
CD 1%			1.79	1.94

(Figures in parenthesis are arcsine values.)

Effect of plant growth regulators on shoot multiplication

After 45 to 60 days of inoculation, regenerated shoots were transferred to MS medium supplemented with Adenine sulfate (10 mg/l), PVP (50 mg/l), Plantomycin (50 mg/l), Sodium hypochlorite (300 µl/l), Carbendazim (200 mg/l) and varying concentrations of BAP and Kinetin (Table 5, Plate 5). Among thirteen treatments, MS + 0.5 mg/l BAP (SM-2) produced the best results, with 4.66% shoot multiplication, an average of 38.66 shoots per explant and a mean shoot length of 6.00 cm after 60 days. Once the shoots

regenerated, shoot clusters were separated and individually subculture on fresh shoot multiplication medium every 1 to 2 weeks for 2 to 3 months. During each subculture, dead leaves, damaged tissues, old shoots and sheaths were removed to provide space for the underlying buds to grow which promoted healthy growth, while lower PGR concentrations enhanced shoot proliferation. These results agree with Sawant *et al.* (2016)^[12], who also reported optimal shoot multiplication at lower BAP concentrations.

Table 5: Effect of plant growth regulators on shoot multiplication

Tr. No.	Tr. code	Plant growth regulators (mg/l)		Shoot Multiplication rate	Average Number of Shoots (60 days after shoot initiation)	Average length of the shoot(cm) (60 days after shoot initiation)
		BAP	Kin			
T ₁	SM-1	MS (control)	MS (control)	2.33 (8.74)	11.33	7.00
T ₂	SM-2	0.5 mg/l	0.00	4.66 (11.99)	38.66	6.00
T ₃	SM-3	1.0 mg/l	0.00	4.00 (11.53)	35.33	5.33
T ₄	SM-4	1.5 mg/l	0.00	3.66 (11.01)	34.66	4.66
T ₅	SM-5	2.0 mg/l	0.00	3.33 (10.49)	32.66	3.66
T ₆	SM-6	2.5 mg/l	0.00	2.66 (10.49)	28.66	3.33
T ₇	SM-7	3.0 mg/l	0.00	2.33 (8.74)	28.00	2.66
T ₈	SM-8	0.00	0.5 mg/l	1.00 (5.73)	13.33	5.00
T ₉	SM-9	0.00	1.0 mg/l	1.66 (7.33)	18.00	4.66
T ₁₀	SM-10	0.00	1.5 mg/l	2.66 (9.35)	24.33	3.33
T ₁₁	SM-11	0.00	2.0 mg/l	2.33 (8.74)	10.66	3.00
T ₁₂	SM-12	0.00	2.5 mg/l	1.66 (7.33)	9.66	2.66
T ₁₃	SM-13	0.00	3.0 mg/l	1.33 (6.53)	9.33	2.00
SE(m)				0.34	0.63	1.30
CD 1%				1.40	2.49	0.30

(Figures in parenthesis are arcsine values.)

Effect of plant growth regulators on root induction

After the shoot multiplication, the shoot clumps were subculture for root induction. The highest results were shown by the treatment combination (Table 6, Plate 6). The medium RIM-14 (MS + IBA 1.0 mg/l + NAA 1.5 mg/l) recorded 88.66% of rooting frequency with maximum number of roots (9.66) induced per explant after 12 days,

followed by medium RIM-13 containing (MS + IBA 1.0 mg/l + NAA 1.0 mg/l) showed 83.66% of rooting frequency and 9.00 roots per explant after 13 days and treatment RIM-12 (MS + IBA 0.50mg/l + NAA 1.00 mg/l) recorded 76.66% of rooting frequency with maximum number of roots (8.66) initiated per explant after 14 days. This result was demonstrated by Mudoi *et al.* (2009)^[8].

Table 6: Effect of plant growth regulators on root induction

Tr. No.	Tr. code	MS medium supplemented with Auxin		Avg. No of roots/ propagules	Rooting frequency (%)	Day of root induction
		IBA	NAA			
T ₁	RIM-1	MS (control)	MS (control)	0.00	0.00 (0.00)	-
T ₂	RIM-2	0.5 mg/l	0.00	1.33	12.33 (20.54)	22
T ₃	RIM-3	1.0 mg/l	0.00	3.33	28.33 (32.13)	20
T ₄	RIM-4	1.5 mg/l	0.00	4.33	39.33 (38.82)	18
T ₅	RIM-5	2.0 mg/l	0.00	5.33	53.66 (47.08)	17
T ₆	RIM-6	0.00	0.5 mg/l	2.66	16.66 (24.08)	19
T ₇	RIM-7	0.00	1.0 mg/l	4.66	32.33 (34.63)	21
T ₈	RIM-8	0.00	1.5 mg/l	5.66	47.33 (43.45)	20
T ₉	RIM-9	0.00	2.0 mg/l	6.00	60.66 (50.94)	26
T ₁₀	RIM-10	0.5 mg/l	0.5 mg/l	6.66	56.66 (48.81)	16
T ₁₁	RIM-11	1.0 mg/l	0.5 mg/l	7.33	65.33 (53.90)	13
T ₁₂	RIM-12	0.5 mg/l	1.0 mg/l	8.66	76.66 (61.09)	14
T ₁₃	RIM-13	1.0 mg/l	1.0 mg/l	9.00	83.66 (66.15)	13
T ₁₄	RIM-14	1.0 mg/l	1.5 mg/l	9.66	88.66 (70.31)	12
SE(m)				0.45	0.79	
CD 1%				1.77	3.09	

(Figures in parenthesis are arcsine values.)

Hardening of bamboo plantlets

The *in vitro* regenerated plantlets were removed from the medium and treated with 0.1% Bavistin (1 gm/l for 30 min) to prevent fungal infections and transplanted into different potting mixtures composed of red soil, cocopeat and vermicompost in varying proportions. Among the fourteen

combinations the mixture with a 1:1:1 ratio of soil, cocopeat and vermicompost resulted in the highest plantlet survival rate (80.33%) under controlled conditions (Table 7, Plate 7). Whereas, the results obtained by Gunasena *et al.* (2024) ^[3] were reported emphasizing gradual acclimatization and proper substrate selection.

Table 7: Per cent survival after 45 days of hardening

Tr. No.	Tr. Code	Potting Mixture	Survival (%)
T ₁	PM-1	Red Soil	40.00 (39.21)
T ₂	PM-2	Cocopeat	54.66 (47.65)
T ₃	PM-3	Red Soil: Cocopeat (1:1)	59.33 (50.36)
T ₄	PM-4	Red Soil: Cocopeat (3:2)	62.00 (51.14)
T ₅	PM-5	Red Soil: Cocopeat (3:1)	64.00 (53.11)
T ₆	PM-6	Red Soil: Vermicompost (3:1)	68.33 (55.73)
T ₇	PM-7	Red Soil: Cocopeat: Vermicompost (2:5:3)	70.33 (56.98)
T ₈	PM-8	Red Soil: Cocopeat: Vermicompost (2:1:1)	75.00 (59.97)
T ₉	PM-9	Red Soil: Cocopeat: Vermicompost (1:1:1)	80.33 (63.66)
SE(m)			0.91
CD 1%			3.72

(Figures in parenthesis are arcsine values.)





Plate 1: Healthy mother plant of *Phyllostachys aurea*, **Plate 2:** Standardized size of explant with 3 cm in length and 3 mm in diameter, **Plate 3:** Effect of cytokinin (MS + 5.0 mg/l BAP) on shoot initiation, **Plate 4:** Effect of cytokinin (MS + 5.0 mg/l BAP) on shoot induction, **Plate 5:** Effect of cytokinin (MS + 5.0 mg/l BAP) shoot multiplication, **Plate 6:** Effect of plant growth regulators (MS + 1.0 mg/l IBA + 1.5 mg/l NAA) on root induction, **Plate 7:** Hardening of bamboo plantlets.

Conclusion

In this study, axillary nodal explants proved most suitable for culture establishment. Sequential surface sterilization using 0.2% Carbendazim + 0.2% Streptomycin, 60% ethanol, 3.0% NaOCl, 0.2% H₂O₂ and 0.1% HgCl₂ effectively eliminated contamination. Explants measuring 3.0 cm × 3.0 mm showed the best sprouting, while MS medium with 5.0 mg/l BAP and additives promoted maximum bud break, shoot initiation and shoot growth and medium with 0.5 mg/l BAP gives the best shoot multiplication result. Optimal rooting was achieved on MS medium containing 1.0 mg/l IBA, 1.5 mg/l NAA and 40 g/l sucrose. During hardening, a soil:cocopeat:vermicompost (1:1:1) mixture gave the highest survival rate, indicating the protocol's effectiveness for large-scale clonal propagation of *Phyllostachys aurea*.

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