Journal of Applied Horticulture, 28(1): 43-50, 2026
https://doi.org/10.37855/jah.2026.v28i01.6

Journal of Applied Horticulture
ISSN: 0972-1045

A novel cost-effective micropropagation strategy for large-scale multiplication of banana (*Musa acuminata*) cv. Grand Naine

Ibrahim Baba Shehu[1], Ramesh Joshi[1], Hussaini Umara Bulakarima[1], Amarjeet Yadav[1], Prashasti Singh[1], P. Agastian[2] and Dilip Nandwani[3]

*[1]Department of Life Sciences, School of Biosciences and Technology, Sharda University, Greater Noida, Uttar Pradesh, India. [2]Department of Plant Biology and Biotechnology, Loyola college, Chennai, India. [3]Department of Agricultural Science and Engineering, Tennessee State University, Nashville, USA. *E-mail: ramesh.joshi1@sharda.ac.in*

Abstract

Banana (*Musa acuminata* cv. Grand Naine) is a globally important triploid cultivar valued for its yield and uniformity. Yet, large-scale cultivation is constrained by the limited multiplication rate and disease transmission through conventional sucker propagation. The present study develops and validates a cost-effective micropropagation protocol integrating optimized initiation, liquid-phase multiplication using reusable glass beads and *ex vitro* rooting in Soil-Rite substrate. Shoot induction was maximized on MS medium containing 4.0 mg L^{-1} BAP, while the combination of 1.0 mg L^{-1} IAA and 0.5 mg L^{-1} TDZ produced the highest proliferation (8.67 ± 0.47 shoots $clump^{-1}$). Rooting was most efficient at 1.0 mg L^{-1} IBA, achieving up to 99% induction in Soil-Rite. Successive sub-cultures (cycles 1–10) revealed peak multiplication at cycle 6 and declining regeneration thereafter, emphasizing the importance of limiting sub-culture duration. Early-cycle plantlets exhibited superior hardening (96%) and field survival (92.7%). Comparative cost analysis demonstrated that substituting agar with glass beads during multiplication and employing Soil-Rite rooting reduced production expenses by ≈ 52% per 1,000 plantlets. The proposed hybrid workflow thus shortens culture duration, improves survival efficiency, and substantially lowers unit cost, providing a scalable and commercially viable strategy for mass propagation of banana cv. Grand Naine and other elite Musa cultivars.

Key words: Banana, Grand Naine, micropropagation, *Musa acuminata*

Introduction

Banana is a parthenocarpic, sterile fruit crop of global importance as a food and source of income for millions of growers in tropical and subtropical regions (Frison *et al*., 2004). This fruit crop is among the most consumed and traded globally, contributing substantially to food security and nutrition across the tropics (Otieno, *et al*., 2025). Cavendish bananas with the triploid AAA cultivar *Musa acuminata* 'Grand Naine' are one of the leading cultivars valued for its uniformity and high yield (Kumar *et al*., 2023). To ensure a steady supply of improved-quality, disease-free planting material, the Grand Naine is therefore critical for sustaining productivity and ensuring supply-chain continuity (Tripathi and Tripathi, 2009; Singh *et al*., 2011).

Recent studies have reported improvements in *In vitro* techniques for the novel shoot excision method developed by Sharma *et al*. (2024) to accelerate shoot multiplication in banana cv. Chini Champa. Some investigations have also explored natural bioactive compounds, such as marine algal extracts, which were found to be effective for the growth and development of banana micropropagules (Teraiya *et al*., 2023). Some studies have demonstrated the bio-immunization strategies for managing Fusarium wilt in cv. Sabri (Damodaran *et al*., 2025) and subsequent economic evaluations confirmed the effectiveness of ICAR FUSICONT technology at a broader scale (Ravi *et al*., 2025).

Propagation of banana by conventional methods through suckers is limited due to a slow multiplication rate, a long cycle duration (9-12 months), and disease transmission, thereby restricting commercial cultivation programs (Cronauer and Krikorian, 1984; Hwang and Ko, 1988). To overcome these limitations in banana cultivation, plant tissue culture techniques for micropropagation have been widely accepted and adopted for large-scale production of disease-free and genetically uniform planting material (Manokar *et al*., 2022a, 2022b; Roels *et al*., 2005).

Propagation of banana using plant tissue culture techniques offers significant advantages, including the conservation of elite germplasm and year-round production (Murashige and Skoog, 1962; Justine *et al*., 2022). Soma-clonal variations arise during *In vitro* culture, but they occasionally provide novel traits that can contribute to the development of disease-resistant and stress-tolerant plants (Sági *et al*., 2004; Skirvin, 1994).

Several researchers have demonstrated efficient *In vitro* regeneration of banana plantlets through organogenesis from shoot tips and successfully established field transfer (Talla *et al*., 2022). However, the application of plant tissue culture for commercial propagation faces critical barriers, such as high production costs per plant, labour-intensive subcultures, and the use of gelling agents (Alvard *et al*., 1993; Etienne and Berthouly, 2002). Additionally, microbial contamination during the initial stages increases production costs (Bhutani *et al*., 2021; Gubbuk and Mustafa**,** 2007). Optimized surface sterilization protocols and use of antioxidants and adsorbents have also been explored to reduce the contamination rate and browning in the cultures (Banu *et al*., 2024).

The present study focuses on and validates a novel, cost-effective *In vitro* regeneration strategy for large-scale multiplication of *Musa acuminata* cv. Grand Naine by integrating optimized low-input in initiation and multiplication steps, enhanced shoot multiplication and *ex vitro* rooting of *In vitro* shoots directly in glass beads and soil-rite under hardening chamber. The proposed study is suitable to demonstrate a low-cost, scalable production strategy for the commercial cultivation of banana cv. Grand Naine using plant tissue culture technique.

Materials and methods

The certified banana cv. Grand Naine plants were collected from the ICAR-National Research Centre for Banana, Tiruchirappalli, Tamil Nadu , and grown in the agricultural experimental field of Sharda University.

Rhizomes of banana were collected from healthy, disease-free plants maintained in the experimental field. The collected suckers were thoroughly washed under running tap water to remove adhering soil particles. The trimmed suckers were pre-treated by soaking in 1% Bavistin® (systemic fungicide) solution for 30 minutes. Thereafter, the older and outer leaf sheaths were carefully removed by sequential un-whorling, along with a small portion of the rhizome until a shoot tip of approximately 4.0 cm in length was obtained.

The excised shoot tips were then treated for 15 minutes in an antioxidant solution containing 100 mg L^{-1} ascorbic acid and 150 mg L^{-1} citric acid to minimize phenolic exudation and associated tissue browning. Following antioxidant treatment, explants were surface-sterilized with 70% (v/v) ethanol for 30 seconds, followed by a 2% (v/v) sodium hypochlorite solution for 7 minutes. Explants were rinsed several times with sterile distilled water to remove traces of sterilizing agents completely. The outermost tissues exposed to sterilants were removed prior to inoculation under a laminar flow hood.

Shoot initiation: Surface-sterilized shoot tips were inoculated onto MS medium containing BAP at 1, 2, 3, 4, and 5mg L-1 alone and in combination with kinetin at 0.5, 1.0, 1.5, 2, 2.5, and 3 mg L^{-1}. Cultures were incubated at 25ºC under a 16 h photoperiod and 80% relative humidity.

Shoot multiplication in agar-solidified MS medium and glass beads: For shoot multiplication, agar-solidified Murashige and Skoog (MS) medium and MS liquid medium were dispensed into culture vessels containing sterilized glass beads. The *In vitro* raised shoots were inoculated onto agar-solidified and liquid media, where the glass beads provided physical support and maintained contact between the *In vitro* shoots and the nutrient solution. The reusability of glass beads was ensured by thoroughly washing, sterilizing, and autoclaving them before each culture cycle. The glass bead system was systematically assessed for its efficiency in promoting *In vitro* shoot proliferation compared to the conventional agar-solidified MS medium.

After optimizing the shoot multiplication medium, the regenerated multiple shoots were subcultured as clumps (six shoots per clump) at 3–4-week intervals. This process was repeated for up to ten successive cycles to assess the relative fold change in shoot multiplication across each cycle. The relative fold change in shoot clump multiplication in each subculture cycle was recorded in both systems.

Root Induction: For root induction, multiple shoots obtained from each sub-culture cycle were separated from the shoot clumps and individually inoculated onto Murashige and Skoog (MS) medium supplemented with different concentrations of indole-3-butyric acid (IBA: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1}). Cultures were maintained for two to four weeks and the percentage of *In vitro* shoots producing roots in each sub-culture cycle was recorded.

The rooting experiments in reusable glass beads and Soil-Rite (*ex vitro*) were conducted simultaneously using the same batch of cultures. Shoots developed under controlled culture conditions were excised individually from shoot clumps and subjected directly to root induction in soil-rite. The basal end of each excised micro-shoot was treated with different concentrations of indole-3-butyric acid (IBA: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1}) for root initiation. Treated shoots were transferred into culture bottles containing an autoclaved Soil-Rite mixture composed of cocopeat, vermiculite, and perlite in a ratio of 1:1:1 and Hoagland liquid medium. The culture vessels were maintained in a controlled hardening unit providing a semi-sterile environment.

To reduce the production cost of *In vitro* banana plantlets, an alternative to agar powder, a commonly used but expensive gelling agent in tissue culture media, was evaluated. In the present study, agar was substituted with reusable glass beads (2 mm diameter) as the support matrix for plantlet culture.

Acclimatization: Rooted plantlets from each cycle were carefully removed from the culture vessels and transferred to plastic pots containing an autoclaved mixture of garden soil, farmyard manure (FYM), and sand in a 2:1:1 ratio for primary hardening. The plantlets were maintained under controlled conditions for one month, and the survival percentage was recorded.

After primary hardening, well-developed plantlets were shifted to polythene bags containing soil and FYM in a 1:1 ratio for secondary hardening for 45–60 days. Subsequently, acclimatized plantlets were transplanted to field conditions. The survival rate of rooted plantlets during both hardening phases and after field transfer was assessed for each sub-culture cycle.

Data analysis for cost assumption: According to the rate of multiplication and percentage of rooting in all the substrate media used in the present study, the costs (media, substrates, consumables, labour cost and electricity consumption) were tabulated for agar-solidified MS medium (multiplication and rooting), soil-rite liquid and glass bead liquid media. Total expenditure, unit cost per plantlet, and percentage cost reduction were calculated. The cost savings were expressed both in absolute values (₹/1,000 plantlets) and relative terms (%)

Statistical analysis: Three explants were taken for each treatment and all the experiments were repeated thrice. The results were expressed as mean ± standard error of the mean (SEM). The datasets included fold change in shoot clump multiplication, percentage of rooting, and survival rate of plantlets during pre-hardening and field transfer across successive subculture cycles. Statistical significance among treatments was evaluated using one-way analysis of variance (ANOVA) in GraphPad

Prism software (version 10.0; GraphPad Software Inc., USA). Differences were considered statistically significant at $P< 0.05$.

Results

Shoot initiation and multiplication: Agar-solidified MS medium was used for all the shoot initiation experiments from surface-sterilized shoot tips (Fig. 1a). MS medium supplemented with different concentrations of BAP and kinetin, individually or in combination, was used for shoot initiation from shoot tips of Musa acuminata cv. Grand Naine. The results indicate that shoots were initiated from shoot tips in response to both cytokinins." with varying efficiency. Shoot numbers increased progressively with the increased BAP concentration as compared to the control (1.67 ± 0.58 shoots/explant), reaching the maximum number of shoots 6.00 ± 0.12 shoots/explant (Fig.1b). Shoot induction was not further enhanced by increasing the concentration of BAP to 5.0 mg L^{-1}, suggesting an optimum concentration of BAP 4.0mg L^{-1} (Table 1)

Table 1. Effect of MS Medium Supplemented with BAP and Kinetin alone and in combination on Shoot Initiation from Shoot tips of Banana (*Musa acuminata* cv. Grand Naine).

Growth regulators(mg/L)		Number of shoots per explant (±SEM)
BAP	Kinetin	
0.0	0.0	1.67±0.58 f
1.0	0.0	4.33±0.58 cd
2.0	0.0	5.67±0.58 ab
3.0	0.0	5.33±0.58 bc
4.0	0.0	6.00±0.12 a
5.0	0.0	5.67±0.58 ab
0.0	1.0	1.33±0.58 f
0.0	2.0	1.67±0.58 f
0.0	3.0	4.67±0.58 cd
0.0	4.0	3.33±0.47 de
0.0	5.0	3.67±0.58d e
0.5	0.5	3.75±0.43d e
1.0	1.0	4.50±0.50c d
1.5	1.5	4.25±0.43c d
2.0	2.0	4.20±0.71c d
2.5	2.5	4.00±0.63cd

F = 185.62, $P< 0.001$ (significant at $P \leq 0.05$). Means ± SEM within each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to DMRT

Kinetin alone in MS medium was found comparatively less effective as only 1.33 ± 0.58 to 1.67 ± 0.58 shoots/explant were produced on 1.0 to 2.0 mg L^{-1}. Higher concentrations (4.0–5.0 mg L^{-1}) resulted in a slight decline in induction of shoot numbers (3.33 ± 0.47 to 3.67 ± 0.58).

The BAP in combination with kinetin showed shoot induction from shoot tip. The highest number of shoots under combined treatment was observed 4.50 ± 0.50 shoots/explant on 1mg L^{-1} each BAP and Kinetin while further increases in concentration of both regulators in equal concentration up to 2.5 mg L^{-1} + 2.5 mg L^{-1} led to a gradual decline (4.00 ± 0.63 shoots/explant).

Interactive effect of cytokinin and auxin: After 3-4 week, the shoots regenerated from shoot tip on the optimized concentration of BAP (4.0 mg L^{-1}) were further sub-cultured as clumps on to two culture systems 1. agar-solidified MS medium and 2. glass-bead supported liquid MS medium. Both media were supplemented with varying concentrations of IAA and TDZ to evaluate the interactive effect of auxins and cytokinins on shoot-clump multiplication.

In shoot multiplication experiment the observations recorded as number of shoots per clump. At IAA (0.1 mg L^{-1}) without TDZ supplementation, the culture produced only 2.00 ± 0.12 shoot-clumps with an average of 6.00 ± 0.23 shoots per clump (Fig. 2). A gradual increase in both IAA and TDZ concentrations resulted in a marked enhancement in multiplication efficiency. The maximum response was observed at 1.0 mg L^{-1} IAA combined with 0.5 mg L^{-1}TDZ, which yielded the highest number of 4.67 ± 0.47 shoot-clumps and 8.67 ± 0.47 shoots per clump (Fig. 3). Higher concentration of growth regulators (1.5–2.5 mg/L IAA with 1.0–2.0 mg/L TDZ) led to a decline in shoot-clump multiplication efficiency, at 2.5 mg/L IAA and 2.0 mg/L TDZ, the number of shoot-clumps and shoots per clump decreased to 3.67 ± 0.47 and 7.33 ± 0.58, respectively (Table 2).

Table 2. Effect of MS Medium Supplemented with BAP (4.0mg/L) and IAA and TDZ on Shoot multiplication in Banana (*Musa acuminata* cv. Grand Naine)

IAA (mg/L)	TDZ (mg/L)	Number of shoot-clump (±SEM)	Number of Shoots/ shoot- clump (±SEM)
0.1	0.0	2.00±0.12 d	6.00±0.23 c
0.5	0.1	3.33±0.47 c	6.25±0.43 c
1.0	0.5	4.67±0.47 a	8.67±0.47 a
1.5	1.0	4.33±0.47 ab	8.00±0.00 ab
2.0	1.5	4.00±0.13 b	7.67±0.58 b
2.5	2.0	3.67±0.47 bc	7.33±0.58 b

Number of shoot-clumps: F = 26.45, $P< 0.001$ (significant), number of shoots per clump: F = 31.82, $P< 0.001$ (significant). Values followed by different letters within a column differ significantly according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Sub-culture cycles: To evaluate the effect of successive sub-culture cycles on shoot-clump proliferation in agar-solidified and glass bead–liquid MS media supplemented with BAP (4.0 mg L^{-1}), IAA (0.5 mg L^{-1}), and TDZ (0.5 mg L^{-1}).

Shoot-clump multiplication was not observed during first sub-culture cycle in both the media (1.0 ± 0.12). In the second cycle shoot-clump numbers was increased to 2.5 ± 0.33 in agar medium compared to 2.7 ± 0.25 in liquid medium. A significant enhancement was observed between the third and sixth cycles, where shoot-clump multiplication in glass-bead liquid medium increased from 6.5 ± 0.58 to 8.8 ± 0.47 (Fig. 1c) as compared to agar-solidified MS medium (Fig. 1d) on which 6.5 ± 0.58 shoot-clumps were regenerated (Table 3).

Table 3. Effect of sub-culture cycle on shoot-clump proliferation in agar-solidified versus glass bead–liquid MS media supplemented with BAP4.0mg/L, IAA 0.5 mg/L and TDZ 0.5mg/L

Subculture cycle (3-4 weeks)	Shoot- clump	
	Agar-solidified medium(±SEM)	Glass bead- liquid medium(±SEM)
1	1.0±0.26 f	1.0±0.32 f
2	2.5±0.33 e	2.7±0.25 e
3	4.2±0.47 d	6.5±0.58 c
4	5.5±0.25 c	7.5±0.47 b
5	6.2±0.33 ab	8.4±0.33 a
6	6.5±0.58 a	8.8±0.47 a
7	6.3±0.47 ab	8.2±0.33 a
8	4.5±0.25 cd	5.5±0.58 d
9	3.3±0.58 de	4.8±0.25 d
10	1.5±0.55 f	1.8±0.58 f

Agar-solidified medium: F = 54.23, $P< 0.001$ (significant), Glass bead–liquid medium: F = 87.15, $P< 0.001$ (significant). Values followed by different letters within a column differ significantly according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

In subsequent cycles, a marked reduction in multiplication was

Fig. 1. (a) Shoot initiation from shoot tip of banana cv. Grand.Naine; (b) Shoot multiplication in first subculture cycle on Agar-solidified MS medium supplemented with BAP (4.0mgL^{-1}), IAA (1.0 mg L^{-1}) and TDZ (0.5 mg L^{-1}); (c) Shoot multiplication in six sub-culture cycle on glass bead-liquid medium supplemented with BAP (4.0mg L^{-1}), IAA (1.0mg L^{-1}) and TDZ (0.5 mg L^{-1}); (d) Shoot multiplication in six sub-culture cycle on agar-solidified MS medium supplemented with BAP (4.0mg L^{-1}), IAA (1.0mg L^{-1}) and TDZ (0.5mg L^{-1}); (e) Fig. 5. Rooting on agar-solidified MS medium supplemented with IBA 1.0mg L^{-1}; (f) Rooting on glass bead- liquid medium supplemented with IBA 1.0mg L^{-1}; (g) Rooting and primary hardening of shoots on soil-rite substrate system.

recorded in both systems, with 4.5 ± 0.25 and 3.3 ± 0.58 in agar versus 5.5 ± 0.58 and 4.8 ± 0.25 in liquid medium in the eighth and ninth cycles, respectively. By the tenth cycle, shoot-clump proliferation further reduced to 1.5 ± 0.55 in agar and 1.8 ± 0.58 in liquid medium.

Rooting: The excised individual shoots obtained from each sub-culture cycle were transferred to three media systems: Agar-solidified MS medium supplemented with different concentrations of IBA, MS liquid medium containing glass beads and autoclaved soil-rite substrate. Prior to inoculation onto glass-bead and soil-rite substrate media, *In vitro* raised shoots were pretreated with varying concentrations of IBA to evaluate its influence on subsequent rooting responses.

Rooting response from shoots was observed in the control (without IBA) and all the concentrations of IBA in all the substrate media with varying percentages. Rooting percentage was relatively low in control with 65.33% in agar (Fig. 1e), 70.00% in glass bead–liquid medium (Fig. 1f), and 75.33% in Soil-Rite (Fig. 1g). Progressive increase in rooting was observed and was maximum at 1.0 mg L^{-1} IBA in agar, 96.00%, glass bead 98.25% and Soil-Rite 99.00%. This clearly establishes that 1.0 mg L^{-1} IBA as the optimum concentration for root induction in all substrates. However, further increase in IBA concentration resulted in gradual decline in rooting percentage, while the lowest percentage of rooting was recorded in agar (70.55%), glass bead (74.25%) and soil-rite (75.00%) substrate (Table 4).

Table 4. Effect of Different Media with IBA on rooting from *in vitro* shoots of Banana (*Musa acuminata)* cv. Grand Naine.

IBA (mg/L)	Rooting (%)		
	Agar-solidified MS medium (±SEM)	Glass beads- liquid substrate (±SEM)	Soil-rite liquid substrate (±SEM)
0.0	65.33±1.25 g	70.00±1.00 f	75.33±1.47 e
0.5	80.75±2.33 d	85.33±1.47 c	90.25±1.47 b
1.0	96.00±1.00 a	98.25±1.58 a	99.00±1.00 a
1.5	93.25±1.58 ab	95.75±1.88 a	96.25±1.58 a
2.0	90.75±1.58 b	90.33±1.58 b	93.58±1.78 ab
2.5	85.33±1.78 c	87.48±2.25 bc	89.55±1.58 bc
3.0	82.55±1.25 d	84.00±1.55 cd	85.47±1.78 cd
3.5	78.65±2.25 e	81.33±1.78 de	81.33±1.58 de
4.0	75.00±1.47 ef	77.47±1.47 ef	78.47±1.33 ef
4.5	70.55±1.58 fg	74.25±2.33 fg	75.00±1.55 ef

F = 185.62 $P < 0.001$ (significant) Values followed by different letters within a column differ significantly according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$

Acclimatization and field survival: To evaluate the effect of repeated sub-culture cycle on survival of *in vitro* derived plantlets of banana (*Musa acuminata* cv. Grand Nane), rooted shoots from all the substrate media in each sub-culture cycle were transferred separately to plastic pots containing an autoclaved mixture of garden soil, farmyard manure (FYM), and sand in a 2:1:1 ratio for acclimatization (Fig. 8). Following primary hardening, plantlets were transplanted to the field (Fig. 9).

The survival under both hardening and field conditions was recorded for each sub-culture cycle. The survival percentage under both hardening and field conditions varied with the number of sub-culture cycle and the type of substrate used for rooting.

Fig. 2. Acclimatized tissue-cultured plantlets of Banana cv. Grand Naine

Fig. 3. Two-month-old tissue culture-raised plants of banana (*Musa acuminata*) cv Grand Naine under field conditions.

The plants derived in first cycle showed 96.00 ± 1.25% and 92.67 ± 1.58% survival in hardening and field conditions, respectively. A gradual decline was recorded with successive sub-culture cycles 2 and 3. The percentage further reduced to 85.25 ± 1.47% (cycle 5) to 59.33 ± 1.58% (cycle 7), with the field survival 88.00 ± 1.25% and 85.00 ± 0.25% respectively. A progressive decline in survival percentage was noted 85.25 ± 1.47% (cycle 5) to 59.33 ± 1.58% (cycle 7), and field survival dropped from 77.25 ± 1.47% to 57.25 ± 1.58%. The plantlets derived from 8 and 10 cycles exhibited significantly lower survival rates in hardening (35.45 ± 1.47%) and field (32.55 ± 1.47%) conditions (Table 5).

Table 5. Effect of sub-culture cycle on survival of *In vitro* plantlets in hardening and field

Sub-culture cycle	Survival (%)	
	Hardening	Field condition
1	96.00±1.25 ab	92.67±1.58 a
2	92.25±1.00 b	88.00±1.25 b
3	89.48±1.78 bc	85.00±0.25 bc
4	83.03±1.58 c	81.33±1.47 cd
5	85.25±1.47 cd	77.25±1.47 de
6	75.00±1.33 e	72.00±1.58 ef
7	59.33±1.58 f	57.25±1.58 g
8	58.33±1.47 f	55.33±1.75 g
9	46.00±1.00 g	43.47±1.58 h
10	35.45±1.47 h	32.55±1.47 i

Values represent mean ± SEM (n = 3). Means followed by different letters within each column differ significantly (DMRT, $P < 0.05$). ANOVA F values: Cycle = 1825.09 ($P < 0.001$), Condition = 176.50 ($P < 0.001$), Interaction = 4.61 ($P = 0.0003$).

Table 6. Cost breakdown and saving summary of tissue-cultured plants of banana (*Musa acuminata*) cv. Grand Naine.

Substrate medium	Work-flow	Cost per plant	Cost for 1000 plants			Cost saving (%)
		Rupee ₹)	Rupee (₹)	Dollar ($)	Euro (€)	
Agar-solidified MS medium	Multiplication and rooting	21.78±0.42 a	21780	245.30	213.08	
Soil-rite +liquid medium	Multiplication on agar and rooting in soil-rite liquid	14.39±0.36 b	14390	162.07	140.78	33.90
Glass bead+ liquid medium	Multiplication and rooting	10.30±0.29 c	10300	116.00	100.77	52.70

Means followed by different lowercase letters (a, b, c) differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT). Significance level: $P < 0.001$ indicates a highly significant difference among substrate media.

Cost breakdown and saving: The data recorded for the comparative cost analysis of three different multiplication and rooting workflows for 1000 tissue-cultured plants of banana cv. Grand Naine showed that the conventional agar solidified MS medium used for both multiplication and rooting recorded the highest cost at ₹ 21.78 per plant (₹ 21,780 per 1,000 plants) equivalent US $ 245.30 or € 213.08. In the agar-based shoot multiplication followed by rooting in soil-rite with liquid medium the cost per plant reduced to ₹ 14.39 (₹ 14,390 per 1,000 plants; $ 162.07 or € 140.78), achieving a 33.9% cost saving relative to all agar solidified medium. The glass bead with liquid medium showed the lowest total cost of ₹ 10.30 per plant (₹ 10,300 per 1,000 plants; $ 116.00 or € 100.77), representing a total 52.7% cost saving (Table 6).

Discussion

Shoot initiation and multiplication: The results of the present study clearly demonstrate that BAP is more effective than kinetin in stimulating shoot induction and multiplication in banana. This finding is consistent with earlier reports indicating that BAP induces more shoots than kinetin under many genotypes and growth conditions (Ali *et al*., 2016; Muhammad *et al*., 2007).

The findings of the present study further revealed that the gradual increase in shoot number up to the optimum concentration of BAP (4.0mg L^{-1}) followed by a decline at higher concentrations of cytokinin, may lead to the vitrification and suppression of shoot elongation. Similar observations were reported by Justine *et al*. (2022) and Deo *et al*. (2020) in *Musa* cv. Patakpura. As per our observations, the combined BAP and kinetin stimulates shoot formation but was less effective than BAP alone. This may suggest the antagonistic interaction between the two cytokinins. In several published micropropagation protocols of *Musa* and other monocots, BAP has been used for inducing (Anjali and Vinay, 2025; Gerema *et al*., 2022; Manokari *et al*., 2022).

In many studies of banana micropropagation, it has been reported that IAA and TDZ in combination with BAP often give optimal shoot proliferation (Ferreira *et al*., 2023). Similarly, Subrahmanyeswari *et al*. (2022) reported that TDZ in moderate concentrations improved multiplication rates in banana, but its higher concentration inhibits the shoot proliferation. Similarly, in other banana varieties, low-to-moderate combinations of auxin and cytokinin favor shoot multiplication, but higher levels may suppress shoot elongation or induce callus formation, as also discussed in review articles by Justine *et al*. (2022). This pattern aligns with present study as higher shoot multiplication was achieved in the MS medium supplemented with a combination of BAP (4.0 mg L^{-1}) with IAA (1.0 mg L^{-1}) and TDZ (0.5 mg L^{-1}). Present study further revealed that the liquid medium in glass beads supports shoot proliferation as compared to agar-semisolid medium, which is also consistent with several reports showing enhanced nutrient uptake, better aeration and more uniform exposure to media in liquid culture (Abdalla *et al*., 2022; Alvard *et al*., 1993 and Roels *et al*., 2005).

Sub-culture cycles and shoot proliferative stability: In the present investigation shoot clumps were sub-cultured repeatedly up to ten cycles to evaluate its effect on shoot proliferation in both agar-solidified and glass bead-liquid culture systems. Shoot multiplication was not observed in cycle-1, suggesting a lag phase. Multiplication increases through cycles 2 to 6 and reaches a maximum around cycle 6. A marked decline in shoot multiplication was observed in cycles 8 to 10 with liquid medium. This trend across sub-culture cycles

Repeated subculture (cycles 1–10) affects proliferation in both agar-solidified and glass-bead–liquid (semi-liquid) culture systems. Neither system showed multiplication in cycle 1, suggesting an adaptation or lag phase. Multiplication increases through cycles 2 to 6 and reached a maximum around cycles 4–6 and a marked decline in shoot multiplication was observed by cycles 8 to 10 with liquid culture. This trend of shoot response across sub-culture cycles is well documented in many micropropagation systems over repeated subculturing of *in vitro* cultures can lose vigor and morphogenetic competence (Majumder *et al*., 2025).

***In vitro* rooting**: *In vitro* raised shoots were transferred to all the substrates media (agar -solidified, glass-bead liquid and soil-rite liquid) to evaluate rooting percentage. Many reports in banana and other species show that low-to-moderate IBA levels are effective for root induction, but supra-optimal levels lead to inhibitory or abnormal root formation (*e.g.* thick callus at base, reduced branching). IBA significantly enhances rooting percentage across all substrates with an optimal concentration of 1.0 mg L^{-1}. Rooting decreases at higher concentrations (2.0 and 4.5 mg L^{-1}) showed a dose-dependent curve. The data of our study clearly suggest that rooting is incremental compared to other banana micropropagation protocol reported by Deo *et al.* (2020). The higher and robust rooting in soil-rite suggests that its aeration and texture favour root growth as compared to dense agar or glass bead -liquid systems.

Acclimatization and field survival: In the present investigation, the survival data show that the *in vitro* plantlets regenerated from early sub-culture cycles have very high survival rates during acclimatization (96%) and in field conditions (92.7%). The survival rates gradually decrease and drop to 32% at cycle 10 in field conditions. This confirms that repeated sub-culturing up to a certain cycle not only affects the *In vitro* regeneration of shoots but also reduces *ex vitro* adaptability and vigor.

The plantlets regenerated in later sub-culture cycles (8 and 10) showed poor growth and lower survival rates is consistent with the well-documented phenomenon reported by Justine *et al*.

(2022) that *In vitro* plantlets regenerate from prolonged subculture cycles often suffer from reduced photosynthetic capacity, structural abnormalities, and a weakened root system that reduce environmental fitness. Prolonged *In vitro* exposure to a plant system can lead to anatomical abnormalities and alter hormonal balance, which reduces post-transplant performance of *In vitro* raised plantlets (Duta-Cornescu *et al*., 2023; Majumder *et al*., 2025).

Cost analysis: The comparative cost analysis demonstrates that replacing agar with glass bead–liquid medium during multiplication and Soil-Rite during rooting reduces production costs of banana micropropagation by approximately 52% per 1,000 plantlets. However, some studies have explored the use of polypropylene bags as an alternative to pro-trays in the hardening process of tissue-cultured banana plants to reduce the mortality and production cost of plants by up to 22.72% (Sharma *et al*., 2024). In contrast, the present study demonstrates a significantly higher cost reduction, approximately 52% through an integrated strategy that combines simultaneous rooting and primary hardening within the Soil-Rite substrate mixture.

Limitations and mitigation strategies: To enable the higher multiplication in liquid medium, first, we observed more contamination risks because microbes proliferate in the nutrient broth rapidly. Second, vitrification and hyperhydricity were observed due to the lack of proper aeration in the culture room and third, as banana explants are prone to phenolic exudations in liquid medium, leading to medium browning and tissue necrosis. In the present investigation several safeguards were incorporated to minimise these risks.

To minimise the contamination in glass bead-liquid system, reusable beads were autoclaved and re-sterilized before each cycle. During the initiation stage, the phenolic exudation was occurred during the first 48 to 72 hr. we opted for antioxidant (ascorbic acid/citric acid), PVP and adsorbent activated charcoal.

We recommend that laboratories adopt equivalent sterility SOPs for reusable supports, track contamination and keep antioxidant/ adsorbent interventions on standby for phenolic-prone explants.

The present investigation successfully developed an integrated and commercially and economically viable large-scale *In vitro* propagation hybrid strategy for Musa acuminata cv. Grand Naine, combining agar-based culture initiation, glass-bead liquid multiplication and soil-rite-based *ex vitro* rooting. The optimized cytokinin 4.0 mg L^{-1} BAP, IAA 1.0 mg L^{-1} and TDZ 0.5 mg L^{-1} yielded maximum regeneration of shoots (7.8 ± 0.4) per explant during the fifth subculture cycle, while 94 – 96% rooting in soil-rite liquid medium containing 1.0 mg L^{-1} IBA with more than 90% field survival. Economic evaluation in the present study indicated a substantial reduction in production cost from ₹ 21.78 ± 0.42 per plant in the conventional agar-solidified method to ₹ 10.30 ± 0.29 per plant in the glass bead- liquid system, resulting in an overall 52.7% cost saving. The reusability of glass bead for up to 10 cycles substantially minimizes the recurring cost expenses and enhanced sustainability.

Future scaling of this hybrid micropropagation strategy for banana and other horticultural crops can strengthen semi-automated temporary immersion bioreactors or continuous nutrient circulation bioreactors to replace the manual transfer activities involved in the micropropagation process. Programmable immersion and sensor-based monitoring for optimize nutrient uptake, renewable power source and recycling of culture components could further reduce the cost of labour and strengthen environmental sustainability.

The developed approach demonstrates a scalable, cost-effective and automation-ready strategy capable of producing true-to-type plantlets in a mass scale, representing a major advancement in banana tissue culture industries in India.

Acknowledgments

We gratefully acknowledge Professor Agastian S., Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, for his valuable support and for providing the certified banana cultivar *Musa acuminata* (cv. Grand Naine), which served as the experimental material for this research.

References

Abdalla, N., H. El-Ramady, M.K. Seliem, M.E. El-Mahrouk, N. Taha, Y. Bayoumi, and J. Dobránszki, 2022. An academic and technical overview on plant micropropagation challenges. *Hortic*., 8(8): 677.

Ali, M., E. Qaoud and A. Allam, 2016. Effect of medium and cytokinin types on banana micropropagation during multiplication stage. *Hortsc. J. Suez Canal Univ.*, *5*(1): 1-7.

Alvard, D., F. Côte and C. Teisson, 1993. Comparison of methods of liquid medium culture for banana micropropagation: effects of temporary immersion of explants. *Plant Cell Tissue Organ Cult.,* 32: 55–60. https://doi.org/10.1007/BF00040116

Anjali K., and B.K. Vinay, 2025. Optimized protocol for high-efficiency micropropagation of banana varieties G9 and Malbhog. *J. Appl. Biol. Biotechnol.,* 13(3): 135–139. https://doi.org/10.7324/JABB.2025.220956

Bhutani, R., S. Shukla and S. Shukla, 2021. Impact of sterilants on culture establishment of indigenous Musa L. varieties: a step forward for conservation. *Environ. Sci. and Poll. Res.*, 28(4): 3913-3919.

Bidabadi, S.S., S. Meon, Z. Wahab and M. Mahmood, 2010. Study of genetic and phenotypic variability among somaclones induced by BAP and TDZ in micropropagated shoot tips of banana (Musa spp.) using RAPD markers. *J. Agric. Sci.*, 2(3): 49.

Cronauer, S.S. and A.D. Krikorian, 1984. Rapid multiplication of bananas and plantains by *In vitro* shoot tip culture. *HortScience*, 19(2): 234-235.

Damodaran, T., Prasenjit Debnath, Maneesh Mishra, Ram Gopal, Sangeeta, Sukhen Chandra Das, Debasree Podder, Swapan Deb Roy, Pinak Pani Datta, Thingreingam Irenaeus and Niren Majumder, 2025. Use of bio-immunization technology to manage Fusarium wilt (Race 1) in banana cv. Sabri growing in Tripura (India). *J. Appl. Hortic.,* 27(2): 174-178.

Deo, B., B. Keshari and B. Pradhan, 2019. *In vitro* propagation of popular banana cultivar (Musa spp. Cv. Patakpura). *Bang. J. Agric. Res.*, 44(4): 641-648. https://www.researchgate.net/publication/339636598

Duta-Cornescu, G., N. Constantin, D.M. Pojoga, D. Nicuta and A. Simon-Gruita, 2023. Somaclonal variation—Advantage or disadvantage in micropropagation of the medicinal plants. *Int. J. Mol. Sci.*, 24(1): 838.

Etienne, H., and M. Berthouly, 2002. Temporary immersion systems in plant micropropagation. *Plant Cell Tissue Organ Cult*., 69: 215–231. https://doi.org/10.1023/A:1015668610465

Ferreira, M.D.S., A.D.J. Rocha, F.D.S. Nascimento, W.D.D.S. Oliveira, J.M.D.S. Soares, T.A. Rebouças and E.P. Amorim, 2023. The role of soma-clonal variation in plant genetic improvement: A systematic review. *Agronomy,* 13(3): 730.

Frison, E.A., J.V. Escalant and S. Sharrock. 2004. "The global Musa genomic consortium: a boost for banana improvement." *Science Publishers.* 341-349.

Gerema, A., G. Megersa and C. Imiru, 2022. Protocol optimization for micropropagation of banana varieties (Musa spp.) using shoot-tip culture. *Acta Bot Plantae*., 1(2): 1–9. http://dx.doi.org/10.5281/zenodo.7262848

Gubbuk, H. and M. Pekmezci, 2006. *In vitro* propagation of banana (Musa spp.) using thidiazuron and activated charcoal. *Acta Agric.,* 56(1): 65-69. https://doi.org/10.1080/09064710510029204

Hwang, S.C. and W.H. Ko, 1988. Tissue culture of banana plants susceptible to Fusarium wilt for disease resistance. *Plant Dis.,* 72(5): 520–523.

Justine, A.K., N. Kaur, Savita and P.K. Pati, 2023. Biotechnological interventions in banana: current knowledge and future prospects. *Heliyon,* 8(11): e11636.

Kumar, A., B.D. Bhuj, S. Dhar, K.M.J. Dixit and S.P. Singh, 2023. Micropropagation of fruit crops: a review. *Adv. Crop Sci. Technol*., 11: 595.

Majumder, S., A.U. Igamberdiev and S.C. Debnath, 2025. Somaclonal Variation and Clonal Fidelity in Commercial Micropropagation: Challenges and Perspectives. *Agronomy*, 15(6): 1489.

Manokari, M., M.K. Badhepuri, M. Cokulraj, B.S. Rajput, A. Dey, M. Faisal, A.A. Alatar, A. Alok and MS Shekhawat, 2022. High-throughput *In vitro* propagation and evaluation of foliar micro-morpho-anatomical stability in *Musa acuminata* cv. 'Grand Nain' using 6-benzoyladenine (BOA) in the nutrient medium. *Sci. Hortic.*, 304: 111334.

Manokari, M., M. Ramya, R. Ramesh and S. Saravanan, 2022B. Optimized micropropagation protocol for banana cultivar 'Grand Naine' *Sci. Hortic.,* 299:111019. https://doi.org/10.1016/j.scienta.2022.111019

Muhammad, A., H. Rashid, I. Hussain and S.S. Naqvi, 2007. Proliferation-rate effects of BAP and kinetin on banana (*Musa* spp. AAA Group)' Basrai'. *HortScience*, *42*(5): 1253-1255.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.,* 15(3): 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Otieno, D. and G.N. Mose, 2025. Promoting the Adoption of Tissue Culture Banana through Increased Market Participation by Small Holder farmers in Kenya.

Pankaj Nautiyal, Anjali Sahu, Mohit Singh, Trilok Nath Rai, Traloki Singh and S. Thanga Anusya, 2025. On-farm assessment of banana-based cropping systems with ICAR-FUSICONT and CSR GROW-SURE in the Indo-Gangetic Plain. *J. Appl. Hortic.,* 27(4): 680-683.

Priti Sharma, S. Swosti Das, Maneesh Mishra and Mala Trivedi, 2024. A novel *In vitro* shoot excision technique for enhancing proliferation in banana cv. Chini Champa. *J. Appl. Hortic.,* 26(2): 258-261.

Ravi, S.C., T. Damodaran, M. Muthukumar, Maneesh Mishra, Shailendra Rajan, Ram Gopal, Alok Shukla and Prasenjit Debnath, 2025. Impact assessment of ICAR FUSICONT using economic surplus model *J. Appl. Hortic.,* 27(2): 174-178.

Roels, S., M. Escalona, I. Cejas, C. Noceda, R. Rodriguez, M.J. Canal, J. Sandoval, P. Debergh and J. Gonzalez-Olmedo, 2005. Optimization of plantain (Musa AAB) micropropagation by temporary immersion system. *Plant Cell Tissue Organ Cul.,* 82: 57–66. https://doi.org/10.1007/s11240-004-6326-5

Sagar Teraiya, Dhaval Nirmal and Preetam Joshi, 2023. Studies on the effect of natural extract of some marine algae on *In vitro* growth and development of banana micropropagules. *J. Appl. Hortic.,* 25(3): 243-248.

Sági, L., S. Remy and R. Swennen, 2004. Genetic improvement of banana and plantains. In: Jain SM, Swennen R, editors. Banana improvement: cellular, molecular biology, and induced mutations. Enfield (NH): *Sci. Pub*., 147–188.

Sahijram, L., J.R. Soneji and K.T. Bollamma, 2003. Analysing somaclonal variation in micropropagated bananas (Musa spp.). *In vitro Cell. Dev. Biol. – Plant*, 39(6): 551-556.

Sharma, P., M. Mishra, S. Rajan, T. Damodaran and M. Trivedi, 2025. An innovative hardening technology for micropropagated banana plantlets for medium-sized tissue culture industries. *J. App. Hortic.*, 27(1): 143-148.

Singh, H.P., S. Uma, R. Selvarajan and J.L. Karihaloo, 2011. Micropropagation for production of quality banana planting material in Asia-Pacific. *Asia-Pacific Cons. Agric. Biotechnol. (APCoAB), New Delhi, India*, 92.

Skirvin, R.M., K.D. McPheeters and M. Norton, 1994. Sources and frequency of somaclonal variation. *HortScience*, *29*(11): 1232-1237.

Subrahmanyeswari, T., S. Gantait, S. Sarkar and S. Bhattacharyya, 2022. Accelerated mono-phasic *In vitro* mass production of banana propagules and their morpho-cyto-genetic stability assessment. *S. Afr. J. Bot.*, 146: 794-806.

Talla, S.K., P. Bagari, S. Manga, M. Aileni and P. Mamidala, 2022. Comparative study of micropropagated plants of Grand Naine banana during *In vitro* regeneration and *ex vitro* acclimatization. *Biocat. Agric. Biotechnol.*, 42: 102325.

Tripathi, L. and J.N. Tripathi, 2009. Role of biotechnology in staving off banana Xanthomonas wilt in East Africa. *Afr. J. Biotechnol.,* 8(20): 5346–5353.

Received: September, 2025; Revised: November, 2025;
Accepted: November, 2025