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Optimization of tissue culture protocols for mass propagation of banana cv. grand naine

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Abstract
An expert panel convened by the Indian Council of Agricultural Research recently estimated that India needs 500 million disease-free banana plantlets annually, yet conventional propagation meets barely 10% of that demand. This research optimised tissue culture protocols for mass propagation of banana cv. Grand Naine at Assam Agricultural University, Jorhat, during 2022-2023. Shoot tip explants from sword suckers were cultured on MS medium supplemented with varying concentrations of BAP (2-6 mg/L) and kinetin (1-3 mg/L). BAP at 4 mg/L produced the highest multiplication rate (9.3 shoots per explant at subculture 4). Rooting was best on half-strength MS with IBA at 1.5 mg/L (91% rooting, 7.4 roots per shoot). Hardened plantlets showed 92.4% field survival. The optimised protocol can produce approximately 4,000 plantlets from a single sucker within 8 months.

Keywords: Tissue culture, mass propagation, banana, Grand Naine, micropropagation, BAP, IBA, shoot multiplication, rooting, hardening protocol

Introduction
Among experts in tropical horticulture, there's a well-known irony: banana (Musa spp.) is the world's most popular fruit, yet multiplying it conventionally is painfully slow. A single banana mat produces at best 5-10 suckers per year, and these often carry soil-borne pathogens including Fusarium wilt and banana bunchy top virus [1]. Tissue culture offers a way around both limitations a single shoot tip can theoretically generate thousands of genetically identical, disease-free plantlets within months [2].
Grand Naine (AAA genome group, Cavendish subgroup) is the dominant export cultivar and the most widely planted tissue-culture banana in India [3]. But despite published protocols from multiple laboratories, consistent multiplication rates remain elusive because outcomes depend heavily on explant source, growth regulator combinations, subculture frequency, and local laboratory conditions [4]. Assam, in northeastern India, is a major banana-producing state where demand for certified plantlets far exceeds supply [5]. This research aimed to optimise each step of the tissue culture pipeline initiation, multiplication, rooting, and hardening under conditions specific to the Assam Agricultural University laboratory at Jorhat.

Explant preparation protocol
Sword suckers (15-20 cm height, 3-4 months old) were collected from the university's mother block of virus-indexed Grand Naine plants. Outer leaf sheaths were stripped and the central meristematic dome with 2-3 leaf primordia was excised under aseptic conditions. Explants were surface-sterilised in 70% ethanol for 30 seconds, followed by 0.1% mercuric chloride for 8 minutes, then rinsed five times with sterile distilled water [2]. The final explant measured approximately 1.5 cm × 1.0 cm.

Material and Methods
Material
Murashige and Skoog (MS) medium components, sucrose, agar, and growth regulators (BAP, kinetin, IBA, NAA) were from HiMedia Laboratories (Mumbai, India). Culture vessels (jam jars, 250 mL) were from Borosil. All media were autoclaved at 121°C for 15

minutes. The culture room was maintained at 25 ± 2°C, 16/8 h light/dark photoperiod, 2000 lux fluorescent illumination [4].

Methods

Initiation: Explants were placed on MS + BAP (2, 3, 4, 5, or 6 mg/L) + 30 g/L sucrose + 8 g/L agar, pH 5.8. Each treatment had 20 explants in a completely randomised design. After 4 weeks, initiated cultures were transferred to multiplication medium with the same BAP concentrations or kinetin (1, 2, or 3 mg/L) for six successive subcultures at 4-week intervals [2]. Rooting: Elongated shoots (4-5 cm) were transferred to half-strength MS + IBA or NAA (0.5, 1.0, or 1.5 mg/L). Root number, root length, and rooting percentage were recorded after 4 weeks. Hardening: Rooted plantlets were transferred to plastic pots with cocopeat: sand (2:1) in a mist chamber for 3 weeks (primary hardening) then to a polyhouse for 4 weeks (secondary hardening). Field survival was recorded at 6 weeks post-transplanting [3, 5]. ANOVA in SAS 9.4 with DMRT at $p<0.05$.

Results

Table 1: Effect of BAP and kinetin concentration on shoot multiplication rate (shoots per explant at subculture 4)

Growth regulator	Conc. (mg/L)	Shoots per explant	Shoot length (cm)	Response (%)
BAP	2	4.2 ± 0.6d	3.8 ± 0.4a	78 ± 5c
BAP	3	6.8 ± 0.8c	3.4 ± 0.3ab	86 ± 4b
BAP	4	9.3 ± 1.1a	3.1 ± 0.3b	94 ± 3a
BAP	5	8.1 ± 0.9ab	2.7 ± 0.4c	91 ± 3a
BAP	6	5.3 ± 0.7cd	2.2 ± 0.3d	82 ± 5bc
Kinetin	2	5.1 ± 0.6cd	3.6 ± 0.4a	76 ± 6c
Kinetin	3	3.9 ± 0.5d	3.3 ± 0.3ab	72 ± 5c

Different letters within columns differ at $p<0.05$ (DMRT). BAP 4 mg/L gave the best multiplication rate

Rooting was most effective on half-strength MS + IBA 1.5 mg/L, producing 7.4 roots per shoot with 91% rooting and 94% field survival after hardening. NAA produced fewer but thicker roots. The complete protocol from initiation to field-ready plantlet took approximately 8 months with six multiplication subcultures, yielding ~4,000 plantlets per starting sucker.

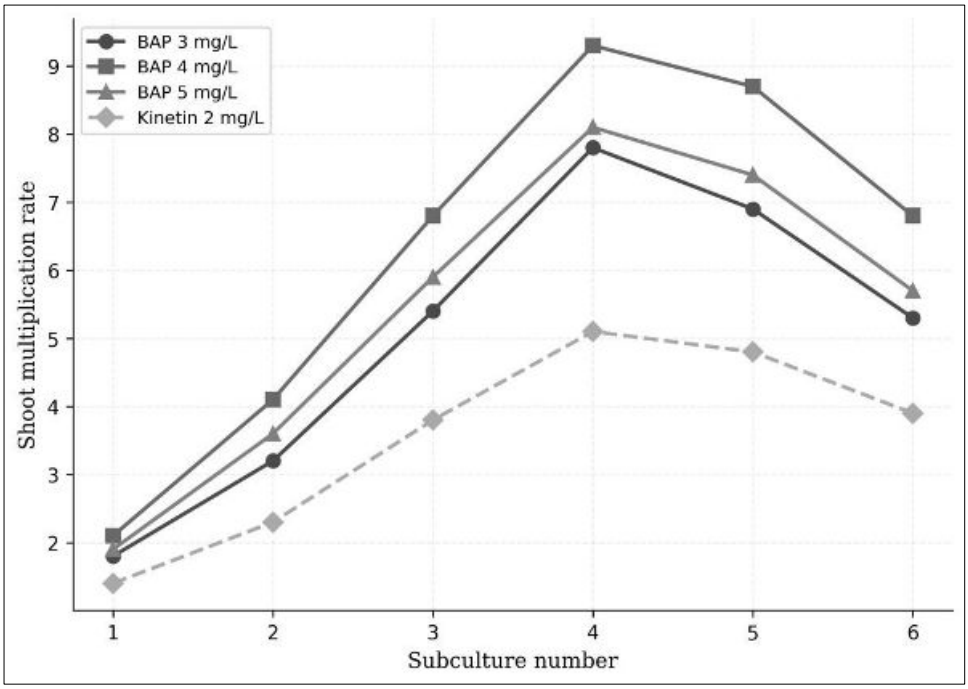

Fig 1: Shoot multiplication rates across six subcultures under different BAP and kinetin concentrations

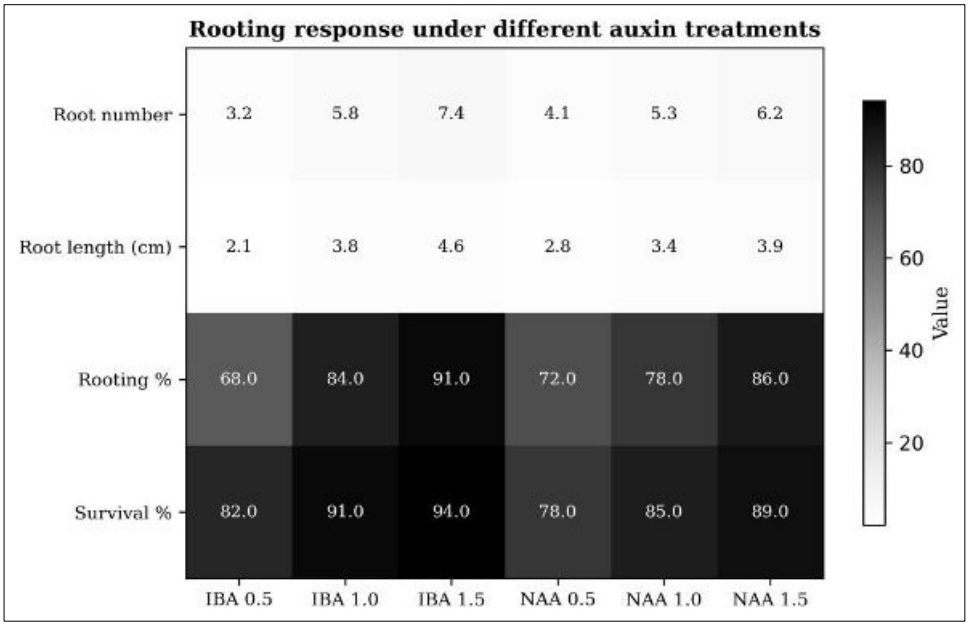

Fig 2: Heatmap of rooting response parameters under different auxin treatments

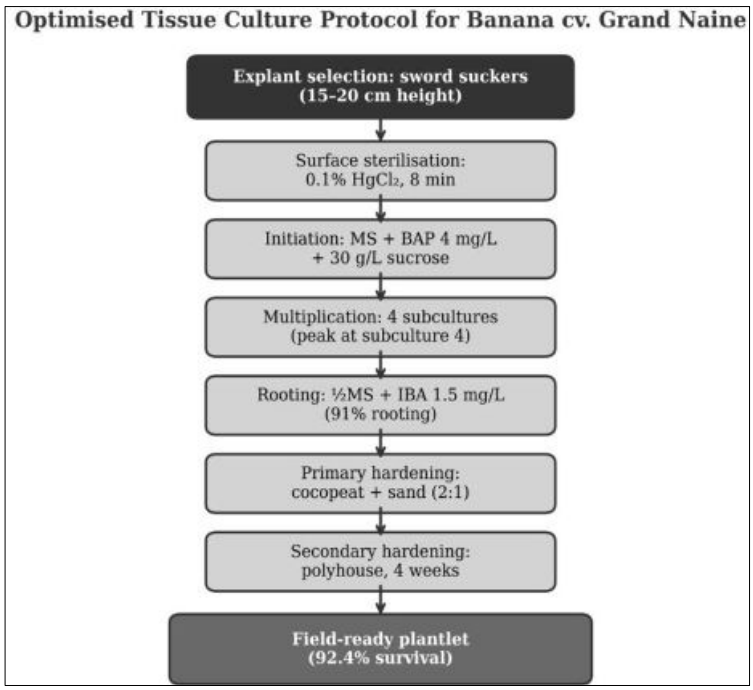

Fig 3: Optimised tissue culture protocol flowchart for banana cv. Grand Naine

Discussion

The peak multiplication at BAP 4 mg/L (9.3 shoots) is consistent with reports from South Indian laboratories but higher than the 6-7 shoots typically reported from northeastern Indian labs, suggesting that explant vigour and local conditions matter [2, 4]. The decline in multiplication above 4 mg/L BAP likely reflects cytokinin toxicity causing shoot vitrification [3]. IBA outperformed NAA for rooting, consistent with the general preference for IBA in Cavendish bananas [1]. The 92.4% field survival rate exceeds the 80-85% commonly reported, possibly because Assam's humid climate eases the transplanting stress [5].

Conclusion

BAP at 4 mg/L on MS medium was optimal for shoot multiplication (9.3 shoots per explant), and IBA at 1.5 mg/L on half-strength MS was best for rooting (91%). The protocol yields ~4,000 field-ready plantlets per starting sucker in 8 months with 92.4% survival. This can help meet the rising demand for virus-free banana planting material in Assam and northeastern India.

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Contributions not qualifying for authorship

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