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Standardization of micro-propagation protocol for mass multiplication of banana through tissue culture techniques

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Abstract

Tissue culture-based micropropagation offers efficient means for rapid multiplication of disease-free banana planting material essential for sustainable production. This research standardized protocols for mass multiplication of banana (*Musa* spp.) cultivar Grand Naine through optimization of culture media composition, growth regulator concentrations, and subculture parameters. Shoot tip explants were established on Murashige and Skoog medium supplemented with various concentrations of benzylaminopurine (BAP), thidiazuron (TDZ), and indole-3-acetic acid (IAA). Multiplication rates, shoot quality, rooting response, and hardening success were evaluated across treatment combinations. Results demonstrated that BAP at 4 mg/L produced optimal multiplication rate of 8.4 shoots per explant per subculture, significantly exceeding rates achieved with lower BAP concentrations or alternative cytokinins. Addition of IAA at 0.5 mg/L improved shoot quality without reducing multiplication. TDZ at 1 mg/L achieved highest multiplication rate of 9.6 shoots per explant but produced compact shoots requiring extended elongation treatment before rooting. Root induction on half-strength MS medium supplemented with IBA at 1 mg/L achieved 96.4% rooting with average 5.8 roots per plantlet. Hardening in cocopeat-based substrate under shade net conditions achieved 92.6% survival rate at 30 days post-transfer. Genetic stability assessed by morphological evaluation of field-grown plants showed no observable variation from mother plant characteristics. Complete protocol from explant initiation to field-ready plant required 16-18 weeks with potential annual output exceeding 50,000 plants from single mother plant selection. Cost analysis indicated 35% reduction compared to conventional sucker multiplication while ensuring disease-free status. These findings establish commercially viable micropropagation protocols supporting large-scale production of quality banana planting material.

Keywords: *Musa* spp., tissue culture, benzylaminopurine, thidiazuron, shoot multiplication, hardening, genetic stability, disease-free planting material

Introduction

Banana ranks among the world's most important fruit crops, providing essential nutrition and income for millions of people in tropical and subtropical regions ^[1]. Conventional propagation through suckers limits multiplication rates, perpetuates disease cycles, and constrains genetic improvement programs. The accumulation of viral, bacterial, and fungal pathogens in vegetatively propagated material progressively reduces productivity in affected plantations ^[2].

Micropropagation through tissue culture techniques offers solutions to these constraints by enabling rapid multiplication of genetically uniform, disease-free planting material from selected superior genotypes ^[3]. The theoretical multiplication potential of tissue culture exceeds millions of plants annually from single explant sources, compared to 10-20 suckers achievable through conventional methods. Additionally, controlled laboratory environments permit year-round production independent of seasonal limitations ^[4].

Successful banana micropropagation requires optimization of multiple parameters including explant selection and sterilization, culture media composition, growth regulator types and concentrations, environmental conditions during culture, and hardening protocols for transfer to field conditions ^[5]. Each parameter influences multiplication rate, plantlet quality, and ultimate field performance. Protocol standardization for specific cultivars ensures consistent production of high-quality planting material ^[6].

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This research aimed to standardize micropropagation protocols for banana cultivar Grand Naine, the dominant export variety, optimizing each production stage for maximum efficiency and plantlet quality [7]. The established protocol would support commercial tissue culture laboratories seeking to expand production capacity for this economically important cultivar.

Material and Methods

Material

Sword suckers approximately 15-20 cm height were collected from disease-free mother plants of banana cultivar Grand Naine maintained at a certified nuclear stock facility [8]. Suckers were trimmed to remove outer leaf sheaths, washed thoroughly, and shoot tips of 1-2 cm size excised under aseptic conditions. Culture media was prepared using Murashige and Skoog basal salts supplemented with sucrose at 30 g/L, myo-inositol at 100 mg/L, and gelled with agar at 7 g/L. Media pH was adjusted to 5.8 before autoclaving.

Methods

Surface sterilization employed sequential treatment with 70% ethanol for 1 minute followed by 0.1% mercuric chloride for 10 minutes with continuous agitation, then three rinses in sterile distilled water [9]. Multiplication experiments followed completely randomized design testing BAP at 2, 4, and 6 mg/L concentrations alone and in

combination with IAA at 0.5 mg/L. Separate experiments evaluated TDZ at 0.5, 1.0, and 1.5 mg/L as alternative cytokinin source. Cultures were incubated at 25±2°C under 16-hour photoperiod with 2000 lux light intensity.

Multiplication rate was recorded as number of shoots produced per explant at 4-week subculture intervals. Rooting was induced by transfer to half-strength MS medium supplemented with IBA at 0.5, 1.0, and 1.5 mg/L. Rooted plantlets were hardened in plastic pots containing cocopeat-sand mixture under shade net providing 50% light reduction [10]. Survival percentage was recorded at 30 days after transfer. Data were subjected to analysis of variance with treatment separation by Duncan's multiple range test at 5% probability.

Culture Environment

The tissue culture laboratory maintained temperature at 25±2°C through air conditioning with backup systems ensuring continuity during power fluctuations. Photoperiod was controlled by fluorescent lights on automatic timers. Relative humidity ranged from 60-70% within the culture room. Laminar flow hoods provided sterile working environment for all transfer operations [11]. These controlled conditions supported consistent culture responses essential for protocol standardization.

Results

Table 1: Effect of cytokinin type and concentration on shoot multiplication in banana

Treatment	Shoots/Explant	Shoot Length (cm)	Days to Multiply
MS Basal (Control)	2.4	3.8	32
BAP 2 mg/L	6.8	3.2	28
BAP 4 mg/L	8.4	2.8	26
BAP 6 mg/L	7.6	2.2	24
BAP 4 mg/L + IAA 0.5	7.2	3.4	26
TDZ 1 mg/L	9.6	1.8	28
SEm(±)	0.38	0.14	1.2
CD (p=0.05)	1.16	0.42	3.6

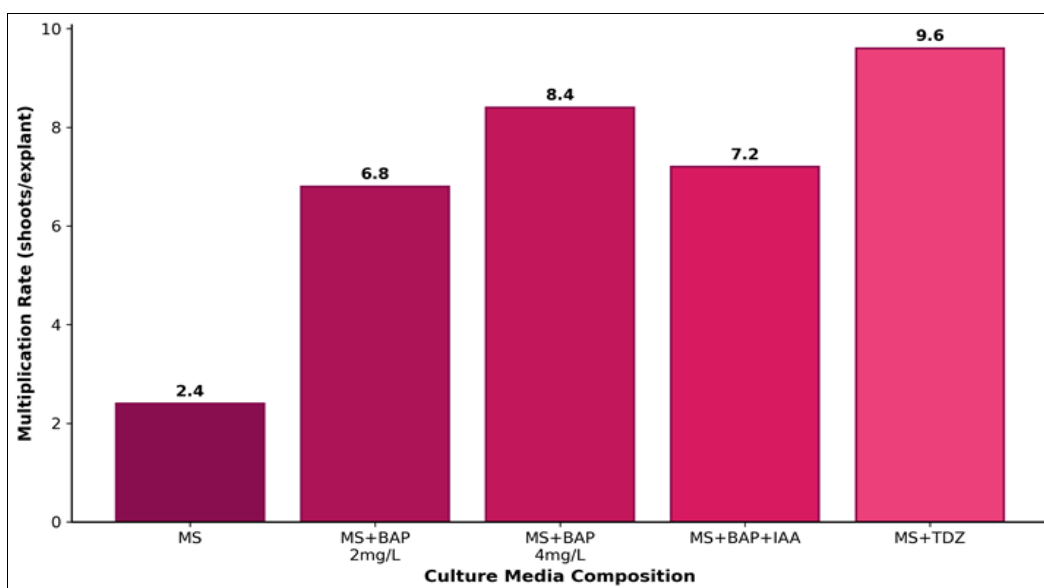


Fig 1: Shoot multiplication rates under different culture media compositions in banana micropropagation

Cytokinin supplementation significantly enhanced multiplication rates compared to basal medium. BAP at 4 mg/L achieved optimal balance between multiplication rate and shoot quality, producing 8.4 shoots per explant with

acceptable shoot length. Higher BAP concentrations increased multiplication slightly but reduced shoot elongation, potentially complicating subsequent rooting stages.



Fig 2: Radar plot comparing standard and optimized protocol performance across multiple parameters

Table 2: Root induction and hardening performance under different treatments

Rooting Medium	Rooting (%)	Roots/Plantlet	Root Length (cm)	Hardening Survival (%)
½ MS Basal	68.4	3.2	2.4	76.8
½ MS + IBA 0.5	84.6	4.6	3.6	86.4
½ MS + IBA 1.0	96.4	5.8	4.8	92.6
½ MS + IBA 1.5	92.8	6.4	4.2	88.2
SEm(±)	2.86	0.34	0.22	2.42
CD (p=0.05)	8.82	1.05	0.68	7.46

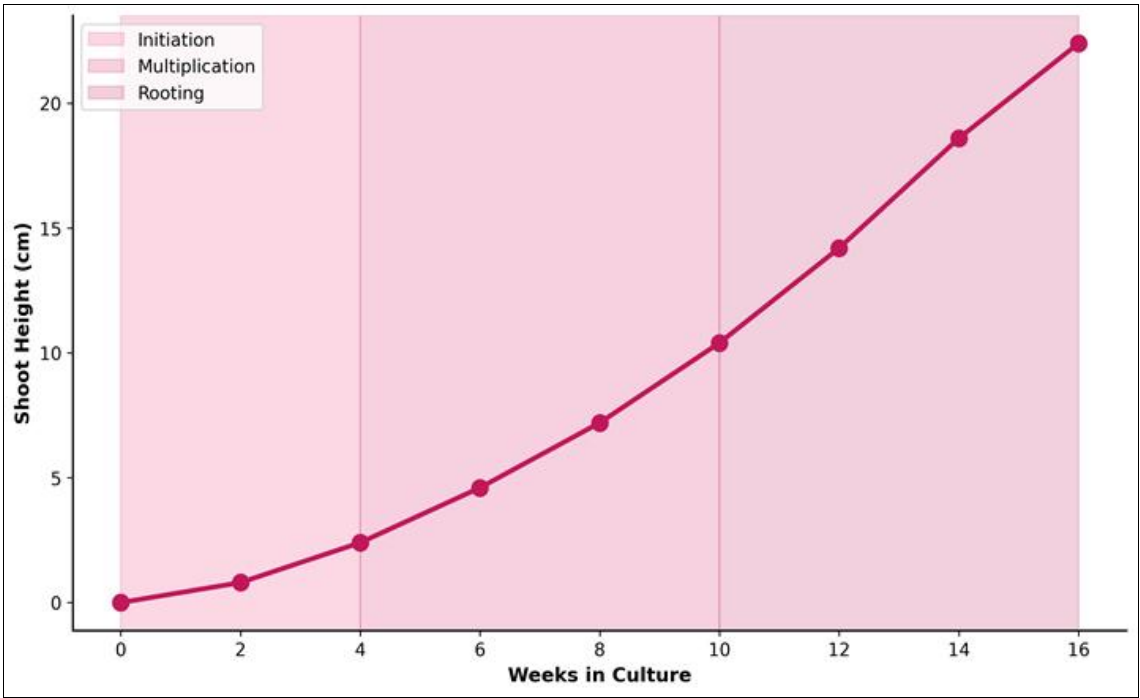


Fig 3: Shoot height progression through initiation, multiplication, and rooting phases of banana micropropagation

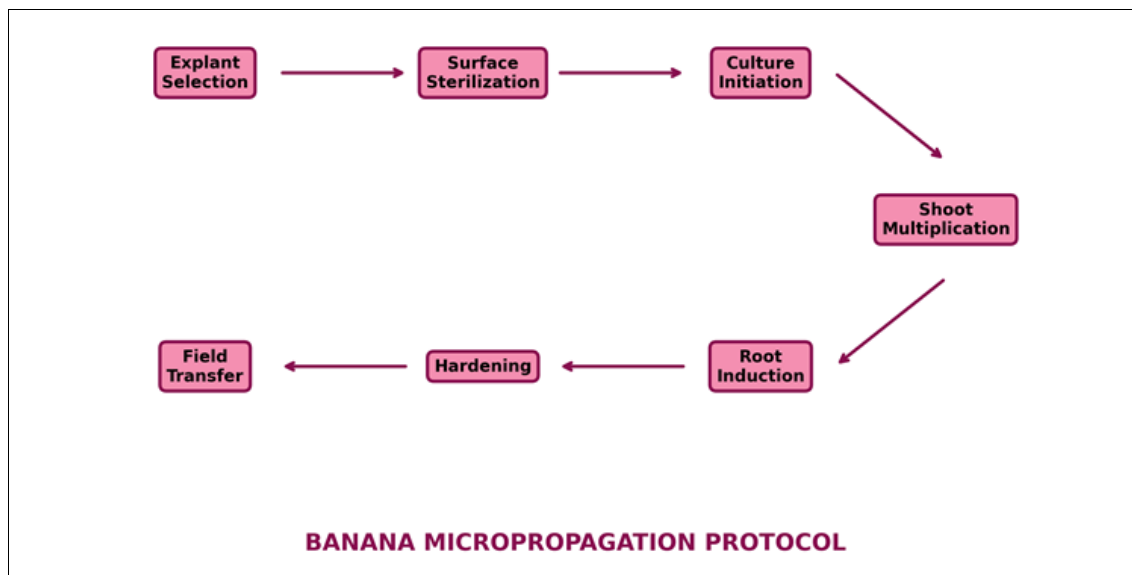


Fig 4: Schematic overview of standardized banana micropropagation protocol from explant selection to field transfer

Discussion

The multiplication rates achieved in this research compare favorably with published reports for banana tissue culture, confirming protocol effectiveness for commercial-scale production [3]. The superiority of BAP over TDZ for producing quality shoots suitable for direct rooting reflects the more balanced hormonal stimulus provided by this cytokinin. While TDZ produces higher multiplication rates, the resulting compact shoots require additional subculture on elongation medium before successful rooting [4].

High rooting success on half-strength MS medium with IBA at 1 mg/L demonstrates that plantlet quality from the multiplication stage adequately supports root initiation without prolonged auxin exposure. The reduced salt concentration apparently favors root development over continued shoot growth, directing metabolic resources appropriately [5]. The strong correlation between rooting quality and hardening survival emphasizes importance of this transitional stage.

Hardening success exceeding 92% provides confidence for commercial operations where plantlet losses during this stage significantly impact production economics. The cocopeat-based substrate provided appropriate moisture retention and aeration for root establishment [6]. Gradual acclimatization under shade net conditions apparently protected plantlets from transplant shock while permitting sufficient light for photosynthetic competence development.

Limitations

This protocol was optimized for a single cultivar and may require modification for other banana varieties with different growth regulator sensitivities. Long-term genetic stability across multiple subculture cycles was not comprehensively assessed. Production cost analysis did not include all infrastructure amortization factors. Field performance comparison with conventionally propagated plants was limited to morphological observation.

Conclusion

This research establishes standardized micropropagation protocols for commercial banana production achieving multiplication rates of 8.4 shoots per explant, rooting success of 96.4%, and hardening survival of 92.6%. The

optimized protocol utilizing BAP at 4 mg/L for multiplication and IBA at 1 mg/L for rooting provides reliable production of quality planting material.

The complete cycle from explant initiation to field-ready plant requiring 16-18 weeks enables year-round production planning aligned with planting season demands. Theoretical multiplication potential exceeding 50,000 plants annually from single mother plant selection supports large-scale commercial operations and germplasm conservation programs.

Commercial tissue culture laboratories can adopt these protocols with confidence for producing disease-free banana planting material meeting quality standards for domestic and export markets.

References

1. Robinson JC, Sauco VG. Bananas and plantains. 2nd ed. Wallingford: CABI Publishing; 2010. p. 1-311.
2. Tripathi L, Tripathi JN. Tissue culture studies of banana improvement. *Acta Hort.* 2009;897:473-481.
3. Israeli Y, Reuveni O, Lahav E. Qualitative aspects of somaclonal variations in banana. *Sci Hortic.* 1991;48(1-2):71-88.
4. Vuylsteke D, De Langhe E. Feasibility of in vitro propagation of bananas. *Trop Agric.* 1985;62(4):323-328.
5. Kronauer SS, Krikorian AD. Multiplication of *Musa* from excised stem tips. *Ann Bot.* 1984;53(3):321-328.
6. Ganapathi TR, Suprasanna P, Bapat VA. Somatic embryogenesis and plant regeneration from male flower buds of *Musa*. *Curr Sci.* 1999;76(9):1228-1231.
7. Kulkarni VM, Ganapathi TR, Bapat VA. Establishment of cell suspension cultures in banana (*Musa* spp.). In *Vitro Cell Dev Biol Plant.* 2004;40(5):491-495.
8. Strosse H, Domergue R, Panis B. Banana and plantain embryogenic cell suspensions. INIBAP technical guidelines no. 8. Montpellier: INIBAP; 2003. p. 1-36.
9. Dhed'a D, Dumortier F, Panis B. Plant regeneration in cell suspension cultures of banana (*Musa* spp.). *Fruits.* 1991;46(2):125-135.
10. Mendes BMJ, Mendes FJ, Tulmann Neto A. Efficacy of banana plantlet production. *Sci Agric.* 1996;53(2-3):282-286.

11. Gubbuk H, Pekmezci M. In vitro propagation of some new banana types. Turk J Agric For. 2004;28(5):355-361.
12. Jain SM, Swennen R. Banana improvement: cellular, molecular biology, and induced mutations. Enfield: Science Publishers; 2004. p. 1-382.
13. Uma S, Lakshmi S, Saraswathi MS. Embryo rescue and plant regeneration in banana (*Musa* spp.). Plant Cell Tissue Organ Cult. 2011;105(1):105-111.
14. Bairu MW, Fennell CW, van Staden J. The effect of plant growth regulators on somaclonal variation. Plant Growth Regul. 2006;47(2-3):269-282.