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***In vitro* regeneration protocol for commercially important Banana cultivar through meristem culture**

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Abstract

Micropropagation through meristem culture is defined as the aseptic excision and *in vitro* culture of the shoot apical meristem to produce genetically uniform, disease-free planting material. This technique holds particular promise for banana (*Musa* spp.), where conventional vegetative propagation through suckers is slow and carries the risk of transmitting systemic pathogens. The present research standardised an *in vitro* regeneration protocol for banana cv. Grand Naine (AAA genome group) at the tissue culture laboratory of Jomo Kenyatta University of Agriculture and Technology, Juja, Kiambu County, during March-October 2023. Shoot-tip explants (3-5 mm) were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of 6-benzylaminopurine (BAP, 0-6 mg L⁻¹) and indole-3-acetic acid (IAA, 0-1 mg L⁻¹). The highest multiplication ratio (5.7 shoots per explant) was obtained on MS + 4.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ IAA after the fourth subculture cycle. Rooting was best on half-strength MS with 1.0 mg L⁻¹ IBA (92.3% rooting, 4.8 roots per shoot). Hardened plantlets achieved 88.6% survival after 30 days in the greenhouse. The protocol can produce over 3,000 uniform plantlets from a single meristem within 8-10 months.

Keywords: *In vitro* regeneration, banana, meristem culture, tissue culture, micropropagation, BAP, multiplication ratio, Grand Naine, hardening, shoot-tip culture

Introduction

Meristem culture—the excision and *in vitro* growth of a tiny dome of undifferentiated cells at the shoot apex—was originally developed to eliminate viruses from clonally propagated crops and has since become the backbone of commercial banana micropropagation worldwide ^[1]. Kenya is a major producer of banana in East Africa, with an annual output exceeding 1.5 million tonnes, yet the demand for high-quality, disease-free planting material far outstrips supply, particularly for the Cavendish subgroup cultivar Grand Naine that dominates export markets ^[2].

Conventional propagation through sword suckers yields only 5-15 plants per mother plant per year and risks transmitting Fusarium wilt (TR4), banana bunchy top virus, and bacterial soft rot ^[3]. *In vitro* multiplication can amplify this rate several hundred-fold while ensuring pathogen-free stock, but success depends on the precise combination of growth regulators, subculture frequency, and acclimatisation conditions, which vary across genotypes and laboratory environments ^[4]. BAP is the most commonly used cytokinin for banana shoot multiplication, yet optimum concentrations reported in the literature range from 2 to 8 mg L⁻¹ depending on cultivar and explant age ^[5].

This research aimed to standardise a reliable, reproducible meristem culture protocol for cv. Grand Naine, optimise BAP and IAA concentrations for maximum shoot multiplication, evaluate rooting media, and assess plantlet survival during hardening under the sub-humid conditions of central Kenya.

Material and Methods

Material

Healthy, virus-indexed mother plants of cv. Grand Naine were obtained from a certified tissue culture nursery in Nairobi. Sword suckers (15-20 cm height) were collected, trimmed to a 3 cm corm block, and surface-sterilised by sequential treatment with 70% ethanol (1 min), 0.1% mercuric chloride (8 min), and three rinses in sterile distilled water.

Shoot-tip explants (3-5 mm, including 1-2 leaf primordia) were excised under a laminar air flow cabinet and placed on initiation medium. The work was carried out from March to October 2023 ^[6].

Methods

Initiation medium was MS basal salts with 30 g L⁻¹ sucrose, 8 g L⁻¹ agar, and 3.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ IAA (pH 5.8). After 4 weeks, initiated cultures were transferred to multiplication media containing BAP at 0, 2, 3, 4, 5, and 6 mg L⁻¹ combined with 0.5 mg L⁻¹ IAA. Five subculture cycles of 4 weeks each were completed. Multiplication ratio

(shoots per explant), shoot length, and leaf number were recorded at each cycle. For rooting, individual shoots (3-4 cm) were transferred to half-strength MS with IBA at 0.5, 1.0, or 1.5 mg L⁻¹. Rooted plantlets were washed, planted in plastic cups with a 1:1:1 mixture of soil, sand, and vermicompost, and kept in a greenhouse (28 ± 2 °C, 80% RH) for 30 days. Survival, plant height, and leaf number were recorded. Each treatment had 20 replicates in a completely randomised design. Data were analysed by ANOVA and DMRT ($p < 0.05$) in R v 4.3.1.

Results

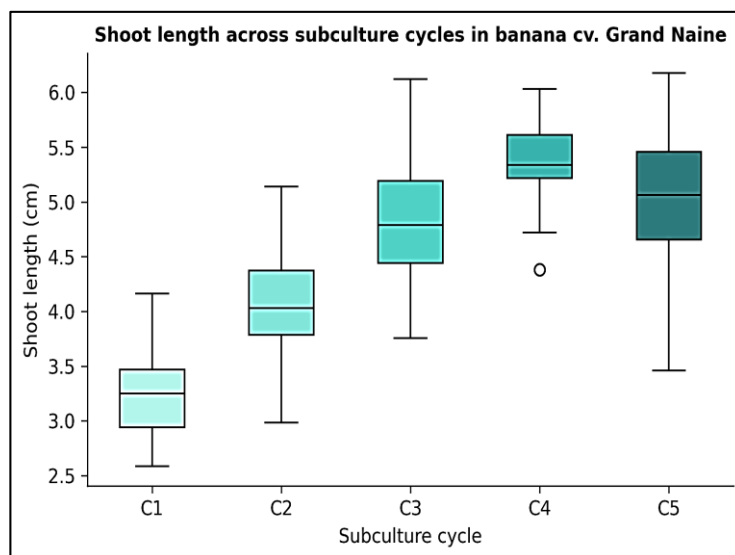


Fig 1: Shoot length distribution across five subculture cycles on MS + 4 mg L⁻¹ BAP

Explant initiation on MS + 3.0 mg L⁻¹ BAP produced visible swelling within 10-12 days, with the first adventitious shoots emerging by day 18-21. By the end of the initiation phase (4 weeks), 87.5% of explants had produced at least one shoot.

Table 1: Effect of BAP concentration on shoot multiplication over five subculture cycles

BAP (mg/L)	C1	C2	C3	C4	C5	Mean
0.0 (control)	1.0	1.0	1.1	1.0	1.0	1.0
2.0	1.8	2.4	2.7	2.8	2.6	2.5
3.0	2.6	3.4	4.1	4.3	4.0	3.7
4.0	3.2	4.1	5.2	5.7	5.3	4.7
5.0	3.0	3.8	4.7	5.1	4.8	4.3
6.0	2.4	3.1	3.8	3.9	3.6	3.4
CD (p=0.05)	0.4	0.5	0.6	0.5	0.5	—

C1-C5 = subculture cycles 1 through 5. Values are mean shoots per explant (n = 20).

Table 2: Rooting response on half-strength MS with different IBA concentrations

IBA (mg/L)	Rooting (%)	Root no./shoot	Root length (cm)	Days to root
0.5	68.4	2.7	2.1	18
1.0	92.3	4.8	3.6	14
1.5	84.1	5.3	2.8	12
CD (p=0.05)	8.7	0.9	0.4	2.1

Table 3: Hardening performance of tissue-cultured banana plantlets after 30 days

Parameter	Mean ± SD	Range
Survival (%)	88.6 ± 3.2	84-94
Plant height (cm)	12.4 ± 1.8	9.2-16.1
Leaf number	4.6 ± 0.7	3-6
Pseudostem girth (cm)	2.1 ± 0.3	1.6-2.8

SD = standard deviation; n = 50 plantlets.

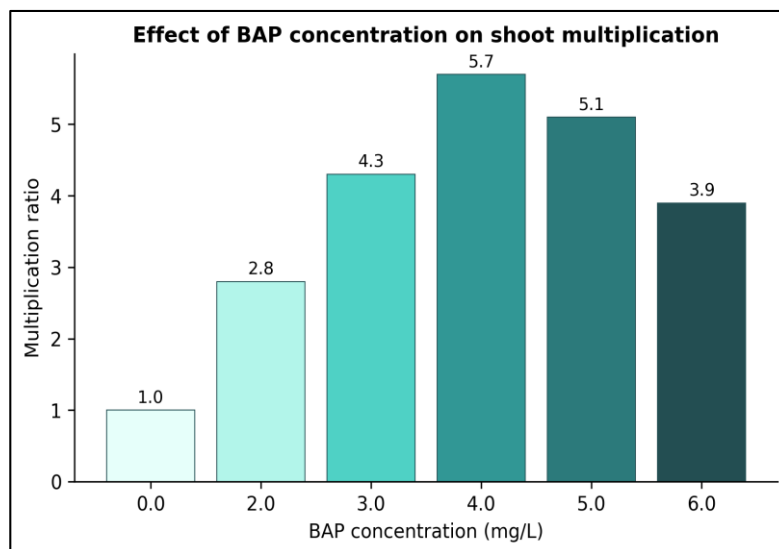


Fig 2: Effect of BAP concentration on mean shoot multiplication ratio

Comprehensive Interpretation

BAP at 4.0 mg L⁻¹ combined with 0.5 mg L⁻¹ IAA produced the highest multiplication ratio (5.7 at cycle 4), and multiplication peaked at the fourth subculture before declining slightly in the fifth cycle. IBA at 1.0 mg L⁻¹ on half-strength MS gave the best balance of rooting percentage (92.3%) and root quality. Hardened plantlets showed 88.6% survival with vigorous growth after 30 days in greenhouse conditions.

Discussion

The results of this research can be summarised as follows: 4.0 mg L⁻¹ BAP was the optimum cytokinin concentration; multiplication improved progressively through four subculture cycles before plateauing; and 1.0 mg L⁻¹ IBA was best for rooting. The literature broadly agrees with these findings. Ali *et al.* [4] reported peak multiplication at 4-5 mg L⁻¹ BAP for Grand Naine in Bangladesh, and Vuylsteke and De Langhe [7] placed the optimum at 4.5 mg L⁻¹ for AAA cultivars.

Where the present data diverge is in the decline at 6 mg L⁻¹ BAP, which other authors have attributed to vitrification (hyperhydricity) and the formation of stunted, glassy shoots that fail to elongate [8]. The possible explanation is that supra-optimal cytokinin levels disrupt the auxin-cytokinin ratio needed for normal shoot organogenesis, diverting meristematic activity towards callus formation rather than axillary bud proliferation [5].

The high hardening survival (88.6%) is encouraging for commercial adoption under semi-arid conditions. The soil-sand-vermicompost mix likely provided adequate aeration and moisture retention, and the controlled humidity (80%) during the first two weeks prevented desiccation—a common cause of hardening failure in tropical tissue culture [9].

Conclusion

The semi-arid plains of western Kiambu County demand large volumes of disease-free banana planting material that conventional sucker propagation cannot supply. This research established that MS medium supplemented with 4.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ IAA supports a multiplication ratio of 5.7 shoots per explant by the fourth subculture, while half-strength MS with 1.0 mg L⁻¹ IBA achieves 92.3%

rooting. Hardened plantlets survived at 88.6% in greenhouse conditions. On a practical level, this protocol can generate over 3,000 uniform plantlets from a single meristem within 8-10 months, making it suitable for scaling up through institutional or commercial tissue culture laboratories. Looking ahead, testing this protocol under field conditions over a full crop cycle and evaluating its cost-effectiveness relative to sucker-based planting would strengthen the case for large-scale adoption.

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