RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

COCONUT WATER AS A COST-EFFECTIVE ALTERNATIVE TO SYNTHETIC CYTOKININS IN BANANA MICROPROPAGATION AND OPTIMIZATION OF AUXIN-CYTOKININ RATIOS

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ABSTRACT

Optimization of growth regulator combinations is critical for developing cost-effective banana micropropagation protocols. This study compared the effects of coconut water combined with indole-3-acetic acid and indole-3-butyric acid on in vitro banana multiplication and rooting. Analysis of variance revealed distinct response patterns between auxin types: IAA treatments showed significant main effects of coconut water on shoot emergence time, shoot height, shoot number, and root number, whereas IBA treatments primarily exhibited significant interaction effects on temporal parameters (shoot, root, and leaf emergence) and shoot multiplication. The fastest organogenesis occurred with 75 mL/L coconut water + 0.5 ppm IBA, while maximum shoot multiplication was achieved with 225 mL/L coconut water without IBA supplementation. IBA treatments produced substantially longer roots than IAA, with maximum root proliferation at 75 mL/L coconut water + 0.5 ppm IBA. A critical hormonal imbalance was identified at 150 mL/L coconut water + 0.5 ppm IBA, which delayed shoot emergence to 16.00 days and leaf emergence to 29.00 days. These findings demonstrate that optimal coconut water concentration depends on auxin type and developmental objective, with high concentrations favoring shoot multiplication when synthetic auxin is absent, while moderate concentrations combined with IBA optimize rooting and accelerate organogenesis. The superior performance of IBA over IAA for root development, coupled with the cost-effectiveness of coconut water as a natural cytokinin source, provides preliminary evidence for optimizing banana micropropagation protocols, although acclimatization trials and field performance evaluation are needed before commercial-scale adoption.

Keywords: Musa spp., Tissue culture, Plant growth regulators, Auxin-cytokinin interaction, Coconut water, IAA, IBA, Micropropagation optimization.

Article History (ABR-26-047) || Received: 17-Feb-2026 || Revised: 26-Apr-2026 || Accepted: 29-Apr-2026 || Published Online: 06-May-2026
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1. INTRODUCTION

Banana (*Musa* spp.) represents one of the world's most significant fruit crops, with annual global production exceeding 130 million tonnes and serving as both a primary food source and cash crop for millions of smallholder farmers across tropical and subtropical regions (Emmanuel et al., 2025; Maseko et al., 2024; Tripathi et al., 2024). In Indonesia, banana cultivation contributes substantially to agricultural income and food security, accounting for approximately 35% of total national fruit production (Kurniati et al., 2022; Nugraha et al., 2023; Sutanto et al., 2024). Among the diverse banana cultivars grown in Indonesia, Mas Kirana (*Musa acuminate* L.) and Rajabulu (*Musa paradisiaca* L.) are particularly valued for their superior organoleptic qualities, commanding premium market prices and exhibiting strong domestic and export demand. However, expanding commercial production of these premium cultivars faces significant constraints related to the availability of high-quality, disease-free planting material.

Conventional banana propagation through vegetative suckers presents multiple limitations that restrict production scalability and sustainability. The low multiplication rate of 2–3 suckers per mother plant annually cannot meet the rapidly growing demand for planting material (Mekonen et al., 2021). Furthermore, sucker-based propagation carries substantial risks of transmitting systemic diseases, including banana bunchy top virus (BBTV),

Citation: Samanhudi, Hartati S, Rahayu M, Setyawati A, Arniputri RB, Iswahyudi, Widiana I, Wulandari IR and Bashir H, 2026. Coconut water as a cost-effective alternative to synthetic cytokinins in banana micropropagation and optimization of auxin-cytokinin ratios. Agrobiological Records 24: 60-73. https://doi.org/10.47278/journal.abr/2026.025

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

banana streak virus (BSV), and fungal pathogens such as *Fusarium* wilt, which can devastate entire plantations (Dato et al., 2021; Guy et al., 2020; Mrope & Jeeva, 2024). These challenges have intensified interest in micropropagation as a viable alternative for the commercial-scale production of uniform, pathogen-free banana plantlets.

Plant tissue culture micropropagation offers transformative advantages over conventional propagation methods, including year-round production capacity, rapid multiplication rates (potentially 5–10 fold per four-week subculture cycle), production of disease-free plantlets, genetic uniformity, and minimal space requirements for mass propagation (Abdalla et al., 2022; Chaudhari, 2023; Hasnain et al., 2022; Kumar et al., 2024). Recent advances in understanding tissue culture plant regeneration mechanisms have further improved protocol efficiency, with optimized cytokinin–auxin combinations achieving remarkable multiplication rates while maintaining genetic stability (Talitha et al., 2022; Samanhudi et al., 2021b; Marković et al., 2023; Setyawati et al., 2023; Chen et al., 2024; Reshi et al., 2025; Wang et al., 2025). Despite these technical achievements and proven efficacy, widespread adoption of micropropagation technology remains limited, particularly among small-scale commercial producers and public sector propagation facilities in developing countries. The primary barrier to adoption is the high operational cost of synthetic plant growth regulators (PGRs), which can account for 30–50% of total medium preparation costs.

Synthetic cytokinins, particularly 6-benzylaminopurine (BAP) and kinetin, are essential components of shoot multiplication media but carry substantial cost burdens. With high market prices these compounds significantly increase production costs per plantlet, making commercial micropropagation economically prohibitive for many applications. In developing countries, where profit margins for agricultural enterprises are narrow and access to high-quality reagents may be limited, the cost of synthetic PGRs presents a critical bottleneck to technology adoption. This economic constraint has stimulated research into natural, locally available alternatives that could maintain regeneration efficiency while substantially reducing production costs (Purwanto et al., 2023).

Coconut water (CW), the liquid endosperm of *Cocos nucifera* L., has emerged as a promising and cost-effective natural supplement in plant tissue culture applications. This abundant agricultural by-product, widely available in tropical coconut-producing regions at minimal cost, contains a complex mixture of growth-promoting substances. Phytochemical analyses have detected naturally occurring cytokinins (zeatin, kinetin) and auxins (IAA) in coconut water, although reported concentrations vary widely depending on coconut variety, maturity stage, geographic origin, and analytical methodology. Some Indonesian coconut varieties have been reported to contain relatively high concentrations of zeatin and kinetin, whereas other investigations have reported substantially lower concentrations in the µg/L range (Yong et al., 2009). Regardless of absolute phytohormone concentrations, the functional cytokinin-like activity of coconut water in tissue culture systems is well documented (Akhiriana et al., 2019). Coconut water also contains vitamins (thiamin, pyridoxine, nicotinic acid), amino acids, minerals (potassium, calcium, magnesium), and organic acid (Amritha et al., 2022; Mu et al., 2024; Shayanthavi et al., 2025; Bello et al., 2025). This rich composition provides both hormonal and nutritional support for plant tissue growth and development in vitro.

The application of coconut water in tissue culture has been documented across numerous plant species, with varying degrees of success. In banana micropropagation specifically, several studies have reported positive effects of coconut water supplementation on shoot proliferation, plantlet vigor, and rooting parameters (Al-Khayri et al., 1992; Andini et al., 2020; Setyawati et al., 2024; Bello et al., 2025; Pontillas et al., 2025; Santosa et al., 2025). However, the optimal concentration ranges and the critical interaction effects between natural cytokinins present in coconut water and exogenously supplied synthetic auxins remain incompletely characterized. This knowledge gap limits the development of standardized, reproducible protocols that could be confidently adopted by commercial propagators.

The morphogenic response of plant tissues in vitro is fundamentally governed by the ratio and absolute concentrations of cytokinins and auxins, following the classical principle that high cytokinin-to-auxin ratios favor shoot organogenesis while high auxin-to-cytokinin ratios promote root formation. Recent molecular and physiological studies have elucidated the complex signaling networks through which these hormones regulate cell division, differentiation, and organ formation, revealing cultivar-specific sensitivities and dose-dependent response curves (Yuniastuti et al., 2018; Mariotti et al., 2021; Samanhudi et al., 2022; Sun et al., 2023). When using coconut water as a natural cytokinin source, determining the appropriate exogenous auxin concentration becomes crucial for achieving optimal hormonal balance and maximizing regeneration efficiency. Both indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), the two most commonly employed auxins in tissue culture protocols, exhibit different chemical stability profiles, metabolic pathways, and biological activities (Epstein & Ludwig-Müller, 1993; Nissen & Sutter, 1990; Samanhudi et al., 2021a). Understanding how these auxins interact with the natural cytokinin pool provided by coconut water is essential for protocol optimization.

Previous investigations of coconut water in banana tissue culture have typically examined limited concentration ranges, focused on single cultivars, or assessed a restricted set of morphological parameters. Comprehensive optimization studies that evaluate multiple coconut water concentrations across different auxin types and concentrations, while simultaneously assessing temporal dynamics (emergence timing), quantitative responses

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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(shoot and root numbers), and quality parameters (shoot height, root length) are notably lacking in the published literature. Furthermore, rigorous economic analyses comparing coconut water-based protocols with standard synthetic cytokinin protocols are rarely presented, despite being critical for practical adoption decisions. These knowledge gaps hinder the translation of research findings into commercially viable, reproducible protocols.

This study was designed to address these critical knowledge gaps through a systematic evaluation of coconut water as a cost-effective alternative to synthetic cytokinins in banana micropropagation. We employed a comprehensive experimental approach utilizing Murashige and Skoog (MS) basal medium supplemented with foliar fertilizer as a semi-organic nutrient source, combined with three concentrations of coconut water (75, 150, and 225 mL/L) and two auxin types (IAA and IBA) across practical concentration ranges.

The specific objectives were to: (1) determine the optimal concentration of coconut water for maximizing shoot multiplication efficiency while maintaining plantlet quality; (2) evaluate the interaction effects between coconut water and different auxin types and concentrations on morphogenic responses throughout the culture cycle; (3) compare the temporal dynamics of shoot emergence, root formation, and leaf development under different hormonal regimes; (4) conduct a detailed economic analysis comparing coconut water-based protocols with conventional synthetic cytokinin protocols; and (5) provide evidence-based, practical recommendations for cost-effective commercial banana micropropagation that can be readily adopted by propagation facilities in resource-limited settings. Through this comprehensive investigation, we aim to contribute both fundamental knowledge regarding auxin–cytokinin interactions in banana morphogenesis and practical solutions to overcome economic barriers limiting micropropagation technology adoption in developing countries.

2. MATERIALS AND METHODS

2.1. Plant Material and Maintenance

In vitro banana shoot cultures were obtained from the Tissue Culture Laboratory at Salaman Horticultural Seed Farm, Central Java, Indonesia, and maintained through regular subculture cycles at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia. Two banana cultivars were used in this study: Mas Kirana (*Musa acuminata* L., genome group AA), a premium cultivar originating from Lumajang, East Java, was used for the coconut water × IBA experimental series; and Rajabulu (Musa paradisiaca L., genome group AAB), sourced from Kebun Benih Hortikultura Salaman, Central Java, was used for the coconut water × IAA experimental series. Both cultivars were maintained as separate, genetically uniform clonal lines throughout the experimental period. Stock cultures were subcultured every 4 weeks on standard Murashige and Skoog (MS) medium supplemented with 30 g/L sucrose and 8 g/L agar to maintain actively growing shoot clumps. All cultures were incubated under controlled environmental conditions: continuous photoperiod (24 h) provided by cool-white fluorescent lamps with light intensity of 40 µmol m^{-2} s^{-1}, temperature of 22 ± 2 °C, and relative humidity of 60–70%.

2.2. Experimental Design

The experiment was designed to comprehensively evaluate the interaction between coconut water and synthetic auxins across practical concentration ranges. The study comprised two separate experimental series, each arranged as a factorial experiment in a completely randomized design (CRD). Series 1 (coconut water × IAA) used Rajabulu cultivar, and Series 2 (coconut water × IBA) used Mas Kirana cultivar. Both series shared identical coconut water concentration treatments but employed different auxin types and concentration ranges, reflecting the distinct biological potencies of IAA and IBA. Each series was analyzed independently by two-way ANOVA. The two factors in each series were: coconut water concentration and auxin supplementation.

Factor A (Coconut water): Three concentrations (75, 150, and 225 mL/L) were evaluated to represent low, medium, and high cytokinin supplementation levels.

Factor B (Auxin supplementation): To assess auxin–cytokinin interactions across a broad physiological range, two auxin types at different concentration series were evaluated: indole-3-acetic acid (IAA) at 0, 0.25, 0.50, and 0.75 ppm, and indole-3-butyric acid (IBA) at 0, 0.5, 1.0, and 1.5 ppm. This design allowed evaluation of hormonal interactions from minimal auxin supplementation through moderate concentrations, encompassing the typical range employed in commercial banana micropropagation protocols.

The complete design yielded 12 treatment combinations per series (3 coconut water concentrations × 4 auxin concentrations), totaling 24 treatment combinations across both series. In the IBA series (Mas Kirana), each treatment was replicated ten times; from the surviving replicates, the three best-performing samples per treatment were selected for statistical analysis. In the IAA series (Rajabulu), each treatment was replicated three times with five culture vessels per replication. The experimental design enabled comprehensive assessment of main effects and interaction effects between the natural cytokinin source (coconut water) and exogenous auxin supplementation on multiple morphogenic parameters.

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2.3. Preparation of Culture Medium

The basal culture medium consisted of MS salts with macro- and micronutrient compositions as originally formulated. Young coconut water (*Cocos nucifera* L.) was obtained from freshly harvested coconuts at 6-8 months maturity, representing the developmental stage with optimal growth-promoting hormone content. The coconuts were surface-sterilized with 70% ethanol, and the water was aseptically extracted using sterile syringes, immediately filtered through pre-sterilized 0.22 µm membrane filters to remove particulate matter while preserving heat-sensitive bioactive compounds, and stored at 4°C until use (within 24 h of extraction). Coconut water was incorporated into the medium at specified concentrations (75, 150, or 225 mL/L) prior to pH adjustment.

Auxin stock solutions were prepared by dissolving analytical-grade IAA (Sigma-Aldrich, St. Louis, MO, USA) or IBA (Sigma-Aldrich) in small volumes of 1 N NaOH, then diluting with sterile distilled water to the appropriate concentrations and filter-sterilizing through 0.22 µm membranes. Auxin solutions were stored in amber bottles at 4 °C and used within one week of preparation to minimize oxidative degradation.

For medium preparation, MS salts, Gandasil D foliar fertilizer (Kalatham, Indonesia; composition: N 20%, P_2O_5 15%, K_2O 15%, $MgSO_4$ 1%, plus micronutrients Mn, B, Cu, Co, Zn) at 1 g/L (half the manufacturer's recommended rate of 2 g/L), and sucrose (30 g/L) were dissolved in approximately 700 mL distilled water with continuous stirring. The MS medium was combined with Gandasil D in a 2:1 ratio to provide supplementary micronutrients at reduced cost, following the semi-organic medium approach previously validated for banana micropropagation at this laboratory (Mardhikasari et al., 2020). Coconut water was then added at the designated concentration, and the volume was adjusted to 950 mL. The pH was measured using a digital pH meter and adjusted to 5.8 ± 0.1 using 1 N NaOH or 1 N HCl. After pH adjustment, agar (Oxoid bacteriological agar, 8 g/L) was added, and the medium was heated with continuous stirring until the agar completely dissolved and the medium reached boiling point. The medium was then dispensed in 40 mL aliquots into 350 mL transparent polypropylene culture vessels fitted with vented polypropylene closures. The media were autoclaved at 121 °C and 1.05 kg/cm² for 20 min, then allowed to cool and solidify under laminar airflow conditions. After cooling to approximately 50 °C, filter-sterilized auxin solutions were added aseptically to achieve the desired final concentrations. Control treatments (0 ppm auxin) received equivalent volumes of sterile distilled water. Prepared media were stored at room temperature (22 ± 2 °C) in the dark for 24–48 h prior to use to allow detection of any contamination.

2.4. Explant Preparation and Inoculation

Actively growing shoot clumps from 4-week-old stock cultures were used as source material. Under aseptic conditions in a laminar airflow cabinet, individual shoots were carefully separated from the clumps using sterile forceps and scalpels. Each explant was trimmed to remove older, yellowing, or necrotic tissues, excess roots, and outer leaf sheaths, resulting in clean shoot tips weighing 1.0–1.5 g fresh weight and containing 1–2 visible shoot primordia. This standardization of explant size and developmental stage was critical for ensuring comparable initial physiological status across treatments and minimizing variation in morphogenic response.

The prepared explants were immediately placed onto the solidified culture medium, with the basal cut surface in firm contact with the medium surface to facilitate nutrient and hormone uptake. One explant was cultured per vessel to allow individual monitoring and prevent competition effects. All culture vessels were clearly labelled with treatment codes, replication numbers, and inoculation dates using permanent markers. The inoculated cultures were arranged in a completely randomized pattern on culture room shelves to eliminate any positional effects related to light intensity or temperature gradients.

2.5. Culture Conditions and Maintenance

All cultures were incubated in a dedicated culture room maintained at 22 ± 2 °C with continuous illumination (24 h photoperiod) provided by cool-white fluorescent tubes positioned 40 cm above the culture shelves, delivering approximately 40 µmol m^{-2} s^{-1} photosynthetically active radiation at shelf level. The continuous photoperiod and lower temperature regime were adopted following the established protocol at the Plant Physiology and Biotechnology Laboratory, UNS, which has consistently supported satisfactory banana micropropagation outcomes (Mardhikasari et al., 2020; Prabowo et al., 2018). Light intensity was verified periodically using a quantum sensor. Relative humidity in the culture room was maintained at 60–70% through natural air conditioning. Culture vessels were inspected daily during the first two weeks for signs of contamination or physiological stress, then every 2–3 days thereafter. Contamination rates were approximately 15.8% (19 of 120 vessels) in the IBA series (Mas Kirana) and approximately 2.8% (1 of 36 vessels) in the IAA series (Rajabulu). Contaminated cultures were removed and, where possible, replaced from reserve replicates only within the first week post-inoculation under pre-established replacement rules. Subculture or data collection occurred at 8 weeks (56 days) after inoculation, representing two standard multiplication cycles and allowing sufficient time for complete expression of morphogenic responses.

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2.6. Data Collection and Measurements

A comprehensive set of temporal, quantitative, and morphometric parameters were assessed to characterize the morphogenic responses. The time (days) from inoculation to first observable shoot emergence (shoot ≥5 mm height with visible green coloration), first root emergence (root ≥5 mm length emerging from explant base), and first leaf expansion (leaf blade fully separated from pseudostem with visible lamina) were recorded through daily observations during the first four weeks of culture. At weekly intervals from week 2 through week 8, the number of shoots (individual shoots ≥1 cm height originating from the explant), number of roots (individual roots ≥1 cm length), and number of fully expanded leaves (leaves with completely opened laminae) were counted for each explant under a stereomicroscope when necessary to distinguish small structures. At harvest (8 weeks post-inoculation), shoot height was measured from the explant base to the apex of the tallest shoot using digital calipers (accurate to 0.1 mm), and root length was measured from the explant base to the tip of the longest root after carefully removing the plantlet from the medium and gently washing away adhering agar under running distilled water.

2.7. Economic Analysis

To assess the practical viability of coconut water-based protocols, a detailed cost analysis was conducted comparing optimized experimental treatments with a standard commercial protocol using synthetic cytokinins. The analysis included direct costs for: (1) MS basal salts (PhytoTechnology Laboratories, USA, catalog price); (2) Gandasil D foliar fertilizer (local market price, Indonesia); (3) coconut water (local market price based on fresh coconut purchase); (4) synthetic auxins IAA and IBA (Sigma-Aldrich catalog prices); (5) synthetic cytokinins for comparison (6-benzylaminopurine, Sigma-Aldrich); (6) sucrose (analytical grade); (7) agar (bacteriological grade); and (8) estimated energy costs for autoclaving and lighting based on standard laboratory consumption rates. Labor costs were excluded from primary analysis as they are relatively constant across protocols but were considered in sensitivity analysis. All costs were calculated per liter of medium and subsequently per 1000 plantlets produced, based on observed multiplication rates and assuming 85% survival through acclimatization (industry standard). Prices were obtained from 2024–2025 quotations and converted to USD using exchange rates current at the time of analysis.

2.8. Statistical Analysis

Data normality and homogeneity of variance were verified using Shapiro–Wilk and Levene's tests, respectively, prior to analysis of variance (ANOVA). For parameters meeting parametric assumptions, two-way ANOVA was performed to evaluate main effects of coconut water concentration, auxin treatment, and their interaction. Given the exploratory nature of auxin treatment evaluation (different types and concentrations), auxin treatments were analyzed as discrete categories rather than continuous variables in the primary ANOVA model. For significant effects ($P<0.05$), means were separated using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Where significant interactions were detected, simple main effects were examined to interpret the nature of the interaction. All statistical analyses were conducted using SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA), and graphs were prepared using Python.

3. RESULTS

3.1. Overall Analysis of Variance

Analysis of variance revealed that coconut water concentration and auxin type (IAA vs IBA) differentially affected banana micropropagation parameters (Table 1). Coconut water significantly affected shoot emergence time ($P<0.01$), shoot height ($P<0.05$), shoot number ($P<0.01$) and root number ($P<0.05$). IAA concentration showed significant effects only on shoot number ($P<0.05$). The interaction between coconut water and IAA was significant for shoot number ($P<0.01$).

Table 1: Summary of ANOVA results for coconut water × auxin effects on banana micropropagation

Parameter	IAA Treatment			IBA Treatment		
	CW	IAA	CW×IAA	CW	IBA	CW×IBA
Shoot emergence time	**	ns	Ns	ns	ns	*
Shoot height	*	ns	Ns	ns	ns	ns
Shoot number	**	*	**	ns	ns	*
Root emergence time	ns	ns	Ns	ns	ns	*
Root number	*	ns	Ns	ns	ns	ns
Leaf emergence time	ns	ns	Ns	ns	ns	*
Leaf number	ns	ns	Ns	ns	ns	ns
Root length	ns	ns	Ns	ns	ns	ns

Note: CW = coconut water; ** = P<0.01; * = P<0.05; ns = not significant.

Citation: Samanhudi, Hartati S, Rahayu M, Setyawati A, Arniputri RB, Iswahyudi, Widiana I, Wulandari IR and Bashir H, 2026. Coconut water as a cost-effective alternative to synthetic cytokinins in banana micropropagation and optimization of auxin-cytokinin ratios. Agrobiological Records 24: 60-73. https://doi.org/10.47278/journal.abr/2026.025

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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3.2. Shoot Emergence Time

Shoot emergence occurred 3.00–16.00 days after inoculation (DAI) across all treatments (Fig. 1). Under IAA treatments, coconut water significantly affected emergence time ($P<0.01$), while under IBA treatments only the interaction was significant ($P<0.05$). The fastest emergence (3.00 days) was achieved with both 75 mL/L + 0.25 ppm IAA and 75 mL/L + 0.5 ppm IBA. The slowest (16.00 days) occurred with 150 mL/L + 0.5 ppm IBA, representing a 5.3-fold delay.

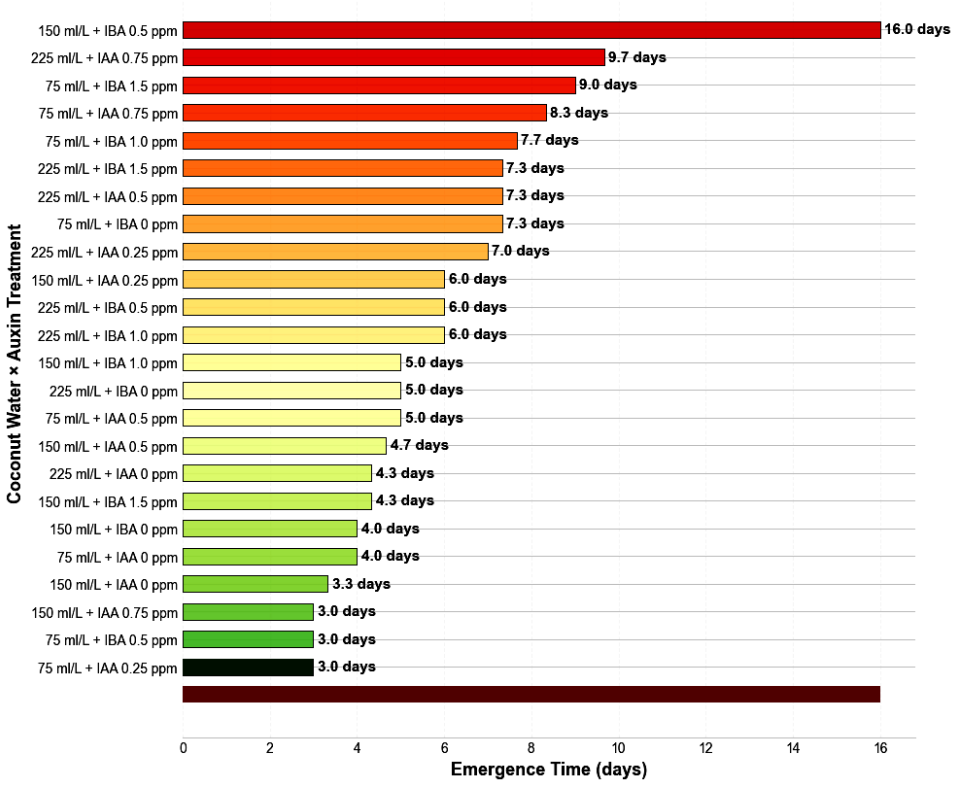

Fig. 1: Shoot emergence time (days after inoculation (DAI)) under coconut water × auxin treatments.

3.3. Root Emergence Time

Root emergence ranged from 6.00 to 16.67 days across treatments (Fig. 2). IAA treatments showed no significant effects ($P>0.05$), while IBA treatments exhibited significant interaction effects ($P<0.05$). The fastest root emergence (6.00 days) occurred at 150 mL/L + 1.5 ppm IBA, while the most delayed (16.67 days) was at 225 mL/L + 0.25 ppm IAA.

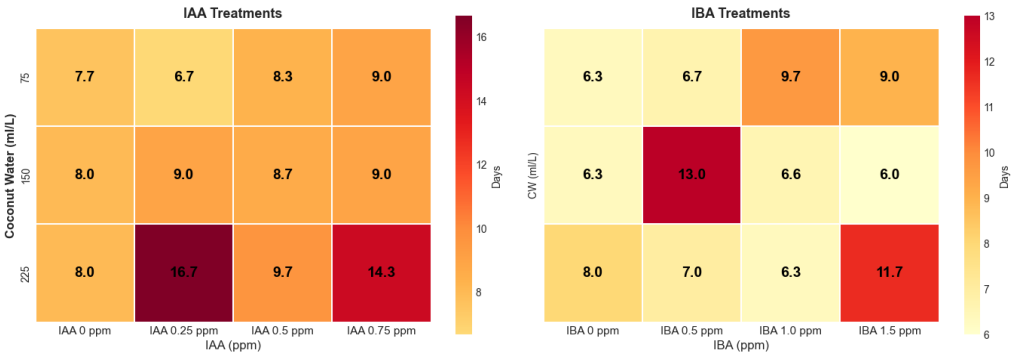

Fig. 2: Heatmaps of root emergence time (days after inoculation (DAI)) under coconut water × auxin interactions.

3.4. Leaf Emergence Time

Leaf emergence time ranged from 14.00 to 32.33 days after inoculation across both experimental series (Table 2). Under IAA treatments (Rajabulu), leaf emergence ranged from 18.00 to 32.33 days, with a mean of 23.47 ± 7.64 days; neither coconut water concentration, IAA concentration, nor their interaction significantly affected leaf emergence time ($P>0.05$). The slowest leaf emergence under IAA (32.33 days) occurred at 225 mL/L + 0.75 ppm

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IAA, while the fastest (18.00 days) was at 150 mL/L + 0.50 ppm IAA. Under IBA treatments (Mas Kirana), leaf emergence ranged from 14.00 to 29.00 days. The coconut water × IBA interaction significantly affected leaf emergence ($P<0.05$). The fastest emergence (14.00 days) occurred at 75 mL/L + 0.5 ppm IBA, while the most delayed (29.00 days) was at 150 mL/L + 0.5 ppm IBA. Across both series, the 150 mL/L coconut water + 0.5 ppm IBA treatment consistently delayed leaf emergence, paralleling the inhibitory pattern observed for shoot and root emergence at this combination.

Table 2: Leaf emergence time (days after inoculation (DAI)) under coconut water × auxin treatments

Coconut Water (mL/L)	IAA Concentration (ppm)				IBA Concentration (ppm)			
	0	0.25	0.50	0.75	0	0.5	1.0	1.5
75	19.33	18.67	22.67	22.00	16.67	14.00	19.00	23.00
150	27.33	22.00	18.00	23.33	14.33	29.00	17.33	14.67
225	27.67	27.33	21.00	32.33	19.67	20.00	16.33	20.00

3.5. Shoot Number

Shoot number varied from 1.67 to 13.33 shoots per explant across all treatments (Table 3). Under IAA treatments, coconut water ($P<0.01$), IAA ($P<0.05$), and their interaction ($P<0.01$) all significantly affected shoot multiplication. Under IBA treatments, only the interaction was significant ($P<0.05$). The highest shoot production (13.33) was achieved with 225 mL/L + 0 ppm IBA, while the lowest (1.67) occurred at 225 mL/L with 0 or 0.75 ppm IAA.

Table 3: Shoot number per explant under coconut water × auxin treatments

Coconut Water (mL/L)	IAA Concentration (ppm)				IBA Concentration (ppm)			
	0	0.25	0.50	0.75	0	0.5	1.0	1.5
75	4.33	3.33	2.33	4.00	6.67	5.67	11.33	4.67
150	4.00	2.00	3.00	3.00	8.67	11.00	8.33	7.67
225	1.67	2.67	2.00	1.67	13.33	6.67	9.33	8.00

3.6. Root Number and Length

Root number ranged from 11.33 to 40.00 roots per explant (Fig. 3a). Under IAA treatments, coconut water significantly affected root production ($P<0.05$), producing 11.33–18.67 roots. Under IBA treatments, no significant effects were detected, with values ranging from 12.00 to 40.00 roots and high variability. Root length showed no significant response to coconut water, auxin concentration, or their interaction in either treatment series ($P>0.05$). Under IAA treatments, root length ranged from 5.73 to 7.43 cm (mean: 6.36 ± 0.56 cm). Under IBA treatments, roots were substantially longer, ranging from 13.57 to 32.50 cm (Fig. 3b).

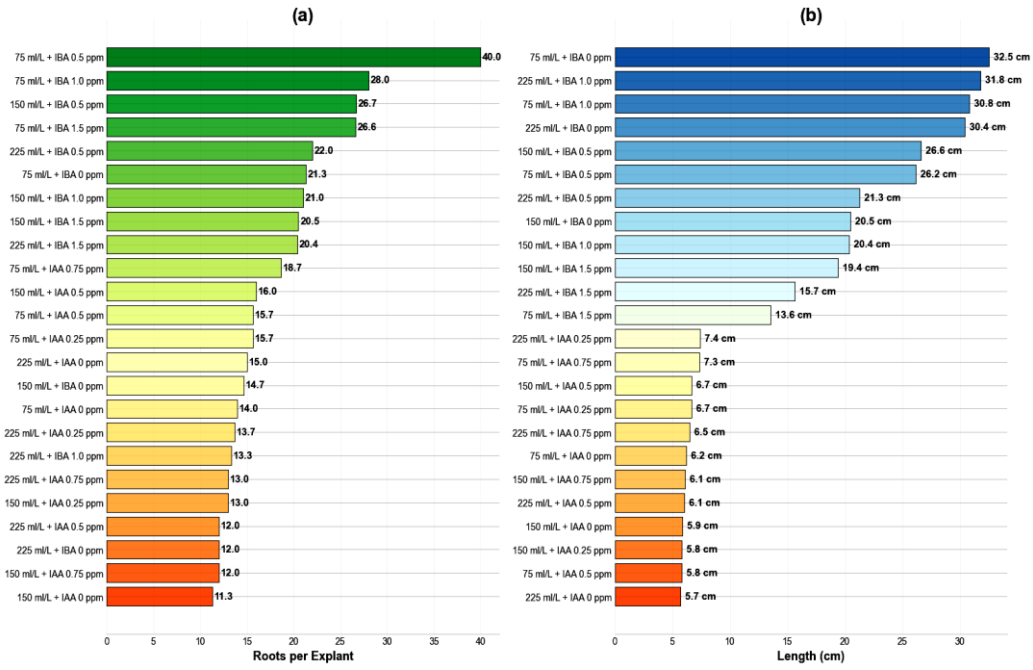

Fig. 3: Root morphology under coconut water × auxin treatments (a) Root number and (b) Root length (cm).

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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IBA treatments numerically produced roots approximately 3.8 times longer than IAA treatments on average, although a direct statistical comparison between auxin types was not performed as the two series involved different cultivars and were analyzed independently. The longest roots (32.50 cm) occurred at 75 mL/L + 0 ppm IBA, while the shortest IAA-treated roots (5.73 cm) were at 225 mL/L + 0 ppm IAA. Notably, maximum root number (40.00 roots) was achieved at 75 mL/L CW + 0.5 ppm IBA, whereas maximum root length (32.50 cm) occurred without IBA supplementation (75 mL/L CW + 0 ppm IBA), indicating a trade-off between root proliferation and elongation under IBA supplementation.

3.7. Leaf Number

Leaf number was not significantly affected by coconut water, auxin concentration, or their interaction in either treatment series (*P>0.05*). Under IAA treatments, leaf number ranged from 6.67 to 10.33 (Fig. 4a). Under IBA treatments, the range was 9.00–22.67 leaves. The highest leaf production (22.67) was at 75 mL/L + 1.0 ppm IBA, while the lowest (6.67) occurred at 75 mL/L + 0.50 ppm IAA and 150 mL/L + 0.25 ppm IAA.

3.8. Shoot Height

Shoot height varied from 12.23 to 21.97 cm across treatments. Under IAA treatments, coconut water significantly affected shoot height (P<0.05), ranging from 17.67 to 21.53 cm. Under IBA treatments, no significant effects were detected, with heights ranging from 12.23 to 21.97 cm. The tallest shoots (21.97 cm) were produced at 150 mL/L + 0 ppm IBA, while the shortest (12.23 cm) occurred at 225 mL/L + 1.5 ppm IBA (Fig. 4b).

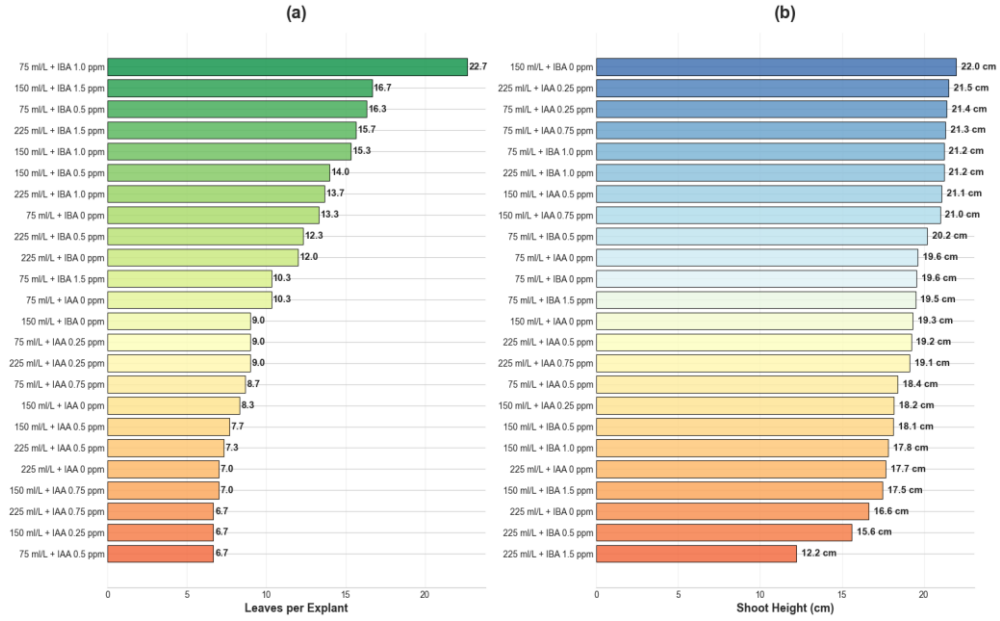

Fig. 4: Aerial morphology of explants under coconut water × auxin treatments. (a) Leaf number per explant (b) Shoot height (cm).

3.9. Economic Analysis

A comparative cost analysis of culture media is presented in Table 4. The optimized coconut water-based multiplication medium (MS + ½ Gandasil D + 225 mL/L CW, without synthetic auxin) cost approximately USD 2.85 per liter, compared with USD 8.40 per liter for a standard MS + 2.0 ppm BAP protocol, representing a 66% reduction in medium cost. When IBA was included for the rooting phase (75 mL/L CW + 0.5 ppm IBA), the medium cost was approximately USD 3.20 per liter. Based on the observed multiplication rate of 13.33 shoots per explant at 225 mL/L CW + 0 ppm IBA, and assuming 85% survival through acclimatization (an industry benchmark, not experimentally verified in this study), the estimated cost per 1,000 plantlets using the coconut water-based protocol was USD 252, compared with USD 743 for the standard BAP protocol. These estimates include costs for MS basal salts, Gandasil D (at local market price of approximately USD 1.50 per kg), coconut water (approximately USD 0.20 per liter from fresh coconuts at local markets in Central Java), synthetic auxins (IAA and IBA at Sigma-Aldrich catalog prices), sucrose, agar, and estimated energy costs for autoclaving and lighting. Labor costs were excluded as they are relatively constant across protocols. The substantial cost advantage of coconut water as a natural cytokinin source is primarily attributable to the elimination of expensive synthetic cytokinins (BAP at approximately USD 45–65 per gram) from the multiplication medium.

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Table 4: Comparative cost analysis of coconut water-based and standard BAP-based banana micropropagation media

Cost Component	CW Multiplication	CW Rooting	Standard BAP
MS basal salts (USD/L)	1.20	1.20	1.20
Gandasil D (USD/L)	0.10	0.10	—
Coconut water (USD/L)	0.05	0.02	—
BAP / Synthetic cytokinin (USD/L)	—	—	4.50
IBA (USD/L)	—	0.38	—
Sucrose + Agar (USD/L)	1.20	1.20	1.20
Energy costs (USD/L)	0.30	0.30	0.30
Total medium cost (USD/L)	2.85	3.20	8.40
Est. cost per 1,000 plantlets (USD)	252	283	743

CW Multiplication = MS + ½ Gandasil D + 225 mL/L coconut water + 0 ppm auxin; CW Rooting = MS + ½ Gandasil D + 75 mL/L coconut water + 0.5 ppm IBA; Standard BAP = MS + 2.0 ppm BAP. Plantlet cost assumes 85% acclimatization survival.

4. DISCUSSION

4.1. Differential Responses to Auxin Type

The contrasting response patterns between IAA and IBA treatments represent a key finding of this study. Under IAA supplementation, coconut water exerted significant main effects on multiple parameters (shoot emergence time, shoot height, shoot number, and root number), whereas IBA treatments primarily showed significant interaction effects with coconut water (Lakho et al., 2023; Irmayanti et al., 2025). This differential behavior reflects fundamental differences in auxin metabolism and stability between these two compounds. IAA, being a natural auxin, is rapidly metabolized by IAA-oxidase enzymes in plant tissues via oxidative decarboxylation, resulting in a shorter biological half-life and more transient effects (Gao et al., 2024; Pasternak & Steinmacher, 2024). In contrast, IBA is more resistant to enzymatic degradation and can be slowly converted to IAA *in vivo*, providing a sustained auxin supply (Gao et al., 2024; Musazade et al., 2025). The significant interaction effects observed with IBA treatments suggest that the optimal IBA concentration is highly dependent on the cytokinin level provided by coconut water, whereas IAA effects appear more concentration-dependent and less influenced by cytokinin availability. The dramatic inhibition observed at 150 mL/L coconut water + 0.5 ppm IBA across all temporal parameters represents a specific hormonal imbalance that warrants detailed consideration. This combination may have created a cytokinin-to-auxin ratio that induced callus formation or arrested morphogenic differentiation, as evidenced by the delayed organogenesis across all measured developmental stages (Muthi'ah et al., 2023; Pasternak and Steinmacher, 2024; Irmayanti et al., 2025).

4.2. Coconut Water as a Cytokinin Source

Coconut water serves as a natural source of cytokinins, particularly zeatin and zeatin riboside, along with other growth-promoting factors including amino acids, vitamins, and mineral nutrients (Aguila, 2024; Yong et al., 2009). Recent evidence shows that coconut water's effect in vitro depends on concentration and interacts with other growth regulators, so the "optimal" level varies by targeted growth parameter and hormonal context (Kovács et al., 2024; Mu et al., 2024). For shoot multiplication under IAA treatments, lower coconut water concentrations (75 mL/L) without exogenous auxin produced the highest shoot number, consistent with the principle that high cytokinin-to-auxin ratios favour shoot proliferation. However, under IBA treatments, the highest multiplication was achieved with 225 mL/L coconut water without IBA, suggesting that when a more stable auxin is absent, higher cytokinin levels can be tolerated and even beneficial for shoot induction. The concentration-dependent effects of coconut water on shoot height were particularly evident in IAA treatments, where increasing coconut water from 75 to 225 mL/L generally increased shoot elongation. This aligns with previous reports that coconut water at 150 mL/L promoted maximum shoot length in banana micropropagation (Kovács et al., 2024; Mu et al., 2024). The cytokinin-mediated cell division and expansion in apical meristems likely accounts for this elongation response. Interestingly, excessive coconut water combined with synthetic auxins tended to reduce shoot multiplication efficiency in IAA treatments. This inhibition may result from supraoptimal cytokinin levels that disrupt normal auxin-cytokinin homeostasis, as reported by studies that coconut water concentrations above 100 mL/L decreased morphological variables in banana culture (Do et al., 2018; Rachmi et al., 2020; Tamimi and Othman, 2023).

4.3. Temporal Dynamics of Organogenesis

Plant organogenesis timing reflects hormone-regulated competence of cells to switch from meristematic to organ-specific growth, with auxin/cytokinin interactions being key drivers of temporal developmental progression. Leaf emergence patterns further reinforced the differential responses between auxin types. Under IAA treatments, leaf emergence was uniformly non-significant across all factor combinations, with a relatively narrow range (18.00–32.33 days) and high mean (23.47 days), suggesting that IAA-treated explants exhibited a more gradual and less

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treatment-responsive leaf developmental programme. In contrast, IBA treatments produced a significant coconut water × IBA interaction for leaf emergence, with the 75 mL/L + 0.5 ppm IBA combination yielding the fastest leaf emergence (14.00 days) — nearly 9 days earlier than the IAA series mean. The marked delay at 150 mL/L + 0.5 ppm IBA (29.00 days) once again highlights the critical hormonal imbalance at this specific combination, consistent with the inhibitory effects observed across shoot and root emergence parameters.

This sequence reflects the programmed developmental progression from meristematic activation to organ differentiation and maturation (Pasternak and Steinmacher, 2024; Zhai et al., 2024). Auxin and cytokinin signaling orchestrate temporal organogenesis, with shoot organ induction typically preceding root initiation and later leaf expansion, reflecting meristem activation and differentiation cascades. This combination likely maintains an appropriate auxin-cytokinin ratio that promotes cell division in meristematic zones while preventing callus formation. A balanced auxin–cytokinin interplay accelerates organogenic responses; moderate cytokinin levels complement stable auxin (like IBA) in enhancing rapid shoot and root initiation relative to unbalanced treatments (Pasternak and Steinmacher, 2024; Zhai et al., 2024). For commercial micropropagation, accelerating these early stages can substantially reduce production costs and increase throughput, making the identification of optimal hormone combinations economically important.

4.4. Auxin Effects on Root Proliferation and Elongation

Exogenous IBA often promotes more pronounced rooting than IAA across plant species, with root number and morphology showing greater variability depending on IBA concentration, reflecting differences in uptake and stability between auxins. Studies indicate that IBA can have a broader range of effects on root proliferation than IAA, likely due to its greater stability and less rapid catabolism in culture media, and its interactions with other growth regulators can strongly influence outcomes (Ercan Oğuztürk et al., 2025; Muleo et al., 2025). Auxin type influences root length, with IBA frequently producing longer and more abundant roots than IAA in multiple species, an outcome attributed to IBA's stability and slow conversion to active forms that sustain elongation. Research has shown that root elongation responses to IBA are concentration dependent, with low-to-moderate IBA levels increasing root length while higher levels may inhibit elongation or cause adverse physiological responses (Ercan Oğuztürk et al., 2025; Muleo et al., 2025). IBA's effectiveness as a rooting agent is attributed to its stability, slower uptake kinetics, and partial conversion to active IAA in tissues, which maintains auxin signaling over a longer period compared with IAA (Muleo et al., 2025). Auxin, including IBA and its derivatives, influences root elongation and cell expansion by modulating cell wall loosening mechanisms and transport processes that can affect water uptake and elongation zones.

4.5. Biological Variation and Methodological Considerations

Several parameters (leaf emergence time under IAA, leaf number, root length under IAA, shoot height under IBA) showed no significant treatment effects despite numerical ranges of 55–152% between minimum and maximum values. The high coefficients of variation for these parameters (24.1% for leaf number, 42.3% for root length under IBA) indicate substantial within-treatment heterogeneity. This variation may arise from several sources: (1) individual explant differences in endogenous hormone levels and physiological state at the time of culture initiation, (2) microenvironmental variation within culture vessels affecting light exposure and nutrient access, (3) differential uptake of exogenous hormones depending on explant size and vigor, and (4) positional effects of explants relative to the medium surface. Similar high variability in morphometric parameters has been reported in other banana micropropagation studies and represents an inherent challenge in achieving complete uniformity in tissue culture systems. The lack of significant effects on leaf number (7.86 ± 1.14 leaves for IAA, 14.28 ± 3.38 for IBA treatments) likely reflects that leaf production is a secondary consequence of shoot proliferation rather than a direct hormogenic response. Once shoots are formed, their capacity to produce leaves may be more dependent on overall vigor and nutrient availability than on specific growth regulator concentrations.

4.6. Optimal Protocol Recommendations

Based on the integrated analysis of temporal and quantitative responses, distinct optimal protocols emerge for different micropropagation objectives. For maximum shoot multiplication 225 mL/L coconut water without IBA supplementation produced 13.33 shoots per explant, representing a 3.1-fold advantage over the best IAA treatment. This protocol is recommended for the multiplication phase when the goal is to maximize shoot proliferation. The high cytokinin level in coconut water at this concentration, without competing auxin effects, creates an optimal hormonal environment for shoot induction. For rapid organogenesis 75 mL/L coconut water + 0.5 ppm IBA accelerated shoot emergence to 3.00 days and root emergence to 6.67 days. This protocol would be advantageous when minimizing the time from subculture to rooted plantlet is critical, such as in high-throughput commercial operations. The moderate cytokinin level combined with stable auxin appears to provide balanced

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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signaling for coordinated shoot and root development. For balanced growth with IAA 75 mL/L coconut water without IAA or with 0.75 ppm IAA produced 4.00–4.33 shoots with moderate rooting (14.00–18.67 roots) and acceptable shoot height (19.63–21.33 cm). This protocol offers a cost-effective alternative using the less expensive IAA auxin while achieving satisfactory multiplication rates. For enhanced rooting 75 mL/L coconut water + 0.5 ppm IBA generated up to 40.00 roots per explant with an average root length of 26.17 cm. This protocol would be valuable during the rooting stage prior to acclimatization, ensuring robust root systems that enhance transplant survival. The combination of moderate cytokinin and optimal IBA concentration creates conditions favoring both root number and elongation.

4.7. Economic and Practical Implications

The successful use of coconut water as a partial or complete substitute for synthetic cytokinins has significant economic implications for micropropagation protocols. Coconut water is inexpensive and readily available in banana-growing regions, whereas synthetic cytokinins such as BAP and kinetin represent a substantial cost component in tissue culture media. The finding that 225 mL/L coconut water alone (without synthetic auxin) produced the highest shoot multiplication (13.33 shoots) suggests that a simplified, lower-cost medium could be effective for the multiplication phase. However, the addition of IBA at 0.5–1.0 ppm during rooting and acclimatization stages may justify the additional cost given the dramatic improvement in root development (3.8-fold longer roots and up to 40 roots per explant) (Samanhudi et al., 2024). The differential responses between IAA and IBA also have cost implications. IAA is generally less expensive than IBA, but the superior rooting performance of IBA may offset its higher cost through improved transplant success rates and reduced acclimatization losses. The cost analysis presented in Table 4 demonstrates that the coconut water-based multiplication medium (USD 2.85/L) achieves a 66% cost reduction compared with the standard BAP protocol (USD 8.40/L), with an estimated saving of approximately USD 491 per 1,000 plantlets. It should be noted that the 85% acclimatization survival rate used in these estimates is an assumed industry benchmark and was not experimentally verified in this study. Future investigations should include formal cost-benefit analyses to determine the most economically optimal protocols for commercial-scale production.

5. CONCLUSION

This comparative study demonstrates that coconut water concentration and auxin type (IAA and IBA) exert differential and interactive effects on banana micropropagation efficiency. The key findings and their practical implications-based protocol recommendations for commercial application: 1) Multiplication phase: 225 mL/L coconut water + 0 ppm IBA for maximum shoot proliferation (13.33 shoots per explant), 2)Rooting phase: 75 mL/L coconut water + 0.5–1.0 ppm IBA for enhanced root development (up to 40 roots, 26.17 cm average length), 3) Cost-effective alternative: 75 mL/L coconut water + 0–0.75 ppm IAA for balanced growth with acceptable multiplication rates (4.00–4.33 shoots). Thus, this study provides preliminary guidance for optimizing banana micropropagation protocols through strategic manipulation of coconut water concentration and auxin type. However, these findings are based on in vitro performance and further validation through acclimatization trials, greenhouse transfer experiments, and batch-to-batch reproducibility assessments is necessary before commercial-scale adoption can be confidently recommended.

Declarations

Funding: This research was funded by the Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology, under the Priority Research Downstreaming Program (Program Hilirisasi Riset Prioritas), specifically the Integrated Technology Transfer-Based Research Downstreaming Scheme (Skema Hilirisasi Riset Berbasis Transfer Teknologi Terintegrasi), Fiscal Year 2025, under Contract No. 3260/UN27.22/PT.01.03/2025.

Acknowledgement: The authors gratefully acknowledge the financial support provided by the Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology, under the Priority Research Downstreaming Program (Program Hilirisasi Riset Prioritas), specifically the Integrated Technology Transfer-Based Research Downstreaming Scheme (Skema Hilirisasi Riset Berbasis Transfer Teknologi Terintegrasi), Fiscal Year 2025, under Contract No. 3260/UN27.22/PT.01.03/2025.

Conflicts of Interest: All authors declare that they do not have any potential conflict of interest.

Data Availability: All the data is available in the article.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Ethics Statement: Not applicable.

Author's Contributions: Samanhudi: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing; Sri Hartati: Supervision, Resources, Writing – review & editing; Muji Rahayu: Supervision, Methodology, Resources; Andriyana Setyawati: Supervision, Resources; Retna Bandriyati Arniputri: Supervision, Resources; Iswahyudi: Supervision, Resources; Iga Widiana: Resources, Investigation; Itsnani Ratna Wulandari: Investigation, Methodology, Data curation, Writing – original draft; Hassan Bashir: Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Citation: Samanhudi, Hartati S, Rahayu M, Setyawati A, Arniputri RB, Iswahyudi, Widiana I, Wulandari IR and Bashir H, 2026. Coconut water as a cost-effective alternative to synthetic cytokinins in banana micropropagation and optimization of auxin-cytokinin ratios. Agrobiological Records 24: 60-73. https://doi.org/10.47278/journal.abr/2026.025

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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