In vitro propagation of "Kepok Grecek" banana (*Musa paradisiaca* L.) from East Kalimantan using sucker explants

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Abstract. The "Kepok Grecek" banana is a locally distinguished cultivar from East Kalimantan and is widely cultivated in Kutai Timur District, East Kalimantan Province, Indonesia. Its strong commercial demand and established presence in international markets position it as a promising commodity for large-scale cultivation. Achieving such expansion requires a dependable supply of high-quality, genetically uniform planting materials. This initial study on the in vitro propagation of Kepok Grecek examined the effects of various concentrations of the plant growth regulators 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) on shoot initiation and subsequent multiplication. A Completely Randomized Design (CRD) was used, consisting of 16 treatment combinations of BAP (0, 1, 2, and 3 mg L^{-1}) and IAA (0, 0.125, 0.150, and 0.175 mg L^{-1}), each replicated four times, with Murashige and Skoog (MS) medium as the basal medium. For the multiplication phase, BAP concentrations were increased tenfold. Observed parameters included explant survival, browning, contamination, morphological responses, and the number of nodules or shoots produced. Data were analyzed using ANOVA followed by Least Significant Difference (LSD) testing at the 5% level. Findings indicated that the interaction between BAP and IAA significantly affected early morphological development. The combination of 2 mg L^{-1} BAP with 0.175 mg L^{-1} IAA produced the most favorable initiation response, with all explants showing swelling and initial division within 7–10 days. During multiplication, treatment with 20 mg L^{-1} BAP and 0.175 mg L^{-1} IAA generated the highest nodule count (14.5 per explant). Overall, the results demonstrate that coordinated application of BAP and IAA markedly enhances the efficiency of micropropagation for the Kepok Grecek banana derived from sucker explants.

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1 Introduction

Indonesia is widely recognized as a major center of origin and diversity for bananas. Globally, the country ranks seventh in total banana production and contributes nearly half of Asia's overall banana supply. National banana cultivation is dominated by five key local cultivars: Pisang Susu, Pisang Tanduk, Pisang Raja, Pisang Ambon (a Cavendish subgroup), and Pisang Kepok [1]. One notable member of this group is the Kepok Grecek banana, a unique regional cultivar native to East Kalimantan, especially the Kutai Timur area. Genetically, this banana carries an ABB genome composition [2] and is classified as a cooking-type variety that must be processed before consumption. Its high tolerance to drought makes it particularly suitable for cultivation in dryland environments typical of East Kalimantan. In addition to its agronomic resilience, Kepok Grecek has already entered export markets such as Malaysia, Singapore, and Pakistan, generating an export value of approximately IDR 5.57 billion during the January–March 2023 period [3]. These combined economic and agronomic advantages underscore its strong potential for expanded commercial cultivation to serve both domestic and international market demands.

Traditional banana propagation depends on the use of suckers or corms from mature plants. Although common, this approach is slow, often results in plantlets with considerable variability, and carries a significant risk of spreading pathogens. To support the long-term production and preservation of Pisang Kepok Grecek in East Kalimantan, propagation methods capable of generating uniform, healthy, and high-performing plantlets are required—such as micropropagation through tissue culture.

In vitro culture is an important biotechnological method that facilitates plant propagation in sterile, highly controlled environments using cells, tissues, or organs as explants. This approach provides several benefits, such as the rapid generation of uniform, disease-free plantlets with high multiplication efficiency in a comparatively short timeframe [4]. As a result, in vitro propagation is regarded as a more dependable and effective alternative to traditional vegetative techniques. While extensive research has been conducted on banana micropropagation in general, studies specifically addressing optimal in vitro propagation protocols for the Kepok Grecek banana—particularly regarding the influence of plant growth regulators—remain unavailable.

In banana tissue-culture systems, suckers or corm pieces are commonly selected as explants because their meristematic regions show high cell division activity, allowing shoots to form quickly [5]. Using sucker-derived materials offers several benefits, including low cost, simple preparation, and their ability to regenerate into complete plantlets in a relatively short time. Nevertheless, because suckers collected from the field often carry microbial contaminants, rigorous surface-sterilization procedures are essential at the initiation stage to ensure successful culture establishment.

The success of in vitro plant propagation is strongly influenced by the formulation of the culture medium. Modifying Murashige and Skoog (MS) medium with targeted plant growth regulators (PGRs) can enhance tissue differentiation and improve developmental responses [6]. Among the different classes of PGRs, auxins and cytokinins play particularly critical roles, as their relative balance governs essential processes including organogenesis, cell division, and structural development. Auxins such as indole-3-acetic acid (IAA) are known to promote cell elongation, stimulate root formation, maintain apical dominance, and support the induction of reproductive structures [7]. Conversely, cytokinins—exemplified by 6-benzylaminopurine (BAP)—activate mitotic activity, facilitate shoot development, and encourage lateral bud growth due to their chemical stability and resistance to degradation [8]. This study investigated how various combinations of BAP and IAA affect the in vitro regeneration of Pisang Kepok Grecek derived from sucker explants, with the goal of

determining the most effective concentrations for promoting shoot initiation and subsequent multiplication.

2 Material and Method

2.1 Explants preparation

Banana suckers approximately 30 cm tall were trimmed by removing the outer leaf sheaths, reducing their size to about 15 cm to serve as explants. The trimmed suckers were first cleaned by soaking them for 5 minutes in a 0.005% (v/v) Tween-20 solution (five drops per 100 mL of distilled water), followed by a thorough rinse with sterile distilled water. They were then treated with a fungicide by submerging them for 30 minutes in Dithane M-45 (containing 80% mancozeb), rinsed once more, and subsequently placed in a bactericidal solution—Agrept 20WP (20% streptomycin sulfate)—for an additional 30 minutes before receiving the final rinse.

Additional sterilization steps were performed inside a laminar airflow cabinet to ensure aseptic conditions. The outer layers of the explants were carefully trimmed away, and the tissues were cut into pieces of approximately 1 cm^3. These pieces were then surface-sterilized by soaking in 70% ethanol for about 2 minutes, followed by a quick rinse with sterile distilled water. This was followed by immersion in a 30% (v/v) commercial bleach solution containing 5.2% NaOCl for 30 minutes. After the treatment, the explants were rinsed three times with sterile distilled water to remove any residual disinfectant before being placed onto the initiation medium.

2.2 Medium preparation

The components of the Murashige and Skoog (MS) medium, along with the required plant growth regulators (PGRs), were dissolved in distilled water, and the pH of the mixture was adjusted to 5.8 prior to sterilization. The prepared medium was dispensed into culture bottles at approximately 31–32 mL per bottle, covered with plastic film, fastened with rubber bands, wrapped, and labeled according to the respective treatments. Sterilization was performed in an autoclave at 121 °C for 30 minutes under 15–18 psi of pressure. All glassware and equipment were sterilized under the same autoclaving conditions (121 °C, 2 atm, 30 minutes). Petri dishes, forceps, scalpels, and scissors were wrapped in paper before autoclaving and subsequently stored in a drying oven to maintain aseptic conditions during culture handling.

2.3 Experimental design and data analysis

The research employed a factorial setup within a Completely Randomized Design (CRD) and was carried out in two sequential experiments: the initiation stage and the multiplication stage. During the initiation stage, two plant growth regulators (PGRs) were tested in a factorial arrangement. Factor C consisted of four BAP concentrations (0, 1, 2, and 3 mg L^{-1}), while Factor A included three levels of IAA (0.125, 0.150, and 0.175 mg L^{-1}). All media used in both stages were based on Murashige and Skoog medium enriched with 30 g L^{-1} sucrose and solidified with 7 g L^{-1} agar. The multiplication stage followed the same factorial design, but the BAP concentrations were elevated tenfold (0, 10, 20, and 30 mg L^{-1}). Across both experimental phases, the 16 resulting treatment combinations (4 × 4) were each replicated four times.

2.4 Shoot initiation

The sterilized explants were placed onto Murashige and Skoog (MS) medium supplemented with specific concentrations of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) to promote the initial phases of in vitro shoot formation. Shoot initiation generally took place within 4–6 weeks after culture establishment (weeks after shoot initiation, WSI), marked by the elongation of leaf sheaths and the appearance of axillary shoots. Observations were carried out to evaluate the percentage of surviving explants, the degree of tissue browning, the occurrence of contamination, as well as changes in explant color and morphological characteristics.

2.5 Shoot Multiplication

After the initiation stage, the developing shoots were transferred to a multiplication medium designed to promote the formation of additional shoots. This medium had the same basic formulation as the initiation medium, with the exception that the concentration of 6-benzylaminopurine (BAP) was increased by a factor of ten. Within roughly 4–6 weeks of culture, the number of shoots typically expanded from about two initial shoots to approximately four to eight shoots per explant. During this phase, observations included the percentages of living, browned, and contaminated explants, along with the total number of nodules (shoots) produced per explant.

2.6 Plant incubation

All explants on both the initiation and multiplication media were maintained in a controlled growth chamber under continuous fluorescent illumination (600–1000 lux), with environmental conditions set at 25 °C and a relative humidity of 78–80%.

2.7 Data analysis

All collected data were subjected to analysis of variance (ANOVA), and differences among treatment means were assessed using the Least Significant Difference (LSD) test at the 5% significance level. To meet the assumption of normality, percentage-based variables were transformed using the $\sqrt{(x + 0.5)}$ method prior to statistical analysis.

3 Results and Discussion

3.1 Results

3.1.1 Impact of Plant Growth Regulator Treatments on Shoot Induction and Subsequent Multiplication

The analysis of variance (ANOVA) revealed that varying concentrations of Benzyl Adenine Purine (BAP) and Indole-3-acetic acid (IAA) produced significant effects only on the percentage of morphological responses during the initiation phase and on nodule number during the multiplication phase (Table 1). These results indicate that the applied plant growth regulators primarily influenced morphogenetic processes, while exerting minimal impact on explant survival, contamination levels, or tissue browning. BAP alone significantly affected both the proportion of explants exhibiting morphological changes and the number of nodules formed, whereas IAA did not show a comparable effect. Additionally, no significant BAP–IAA interactions were observed for any of the in vitro regeneration variables assessed. Collectively, the findings suggest that the early phase of banana in vitro regeneration is

strongly driven by shoot development, underscoring the critical role of cytokinins during this stage.

Table 1. Analysis of variance (ANOVA) at initiation and multiplication stages.

	Initiation stages (*p-value*)				**Multiplication stages (*p-value*)**			
Variance sources	**Survive explants**	**Contaminated explants**	**Browning explants**	**Explant morphology**	**Survive Explant**	**Contaminated explants**	**Browning explants**	**Number of nodules**
Treatment	0.239 ns	0.051 ns	0.051 ns	0.003 **	0.270 ns	0.139 ns	0.011 ns	0.002 **
BAP	0.105 ns	0.350 ns	0.309 ns	0.003 **	0.076 ns	0.936 ns	0.083 ns	0.000 **
IAA	0.923 ns	0.587 ns	0.309 ns	0.092 ns	0.761 ns	0.443 ns	0.725 ns	0.319 ns
BAP*IAA	0.215 ns	0.075 ns	0.766 ns	0.186 ns	0.354 ns	0.051 ns	0.053 ns	0.242 ns

Note: **: very significant with p-value ≤ 0.01, ns: not significant

3.1.2 Shoot initiation

Preliminary assessments were carried out to evaluate explant survival, rates of contamination, and the extent of browning during the initiation phase (Tables 2, 3, and 4). The ANOVA results showed that neither BAP nor IAA, nor the interaction between them, had a significant influence on any of these parameters (Table 1). Overall survival across treatments reached 84.38%, indicating that the in vitro regeneration approach operated at roughly 84% efficiency (Table 2). Browning occurred in only 4.69% of explants, while contamination was observed in 10.94% (Table 3). Considering that the explants were derived from field-grown material, these outcomes demonstrate that the regeneration protocol and sterilization steps were well optimized and effective.

Table 2. Influence of PGR Treatments on Explant Survival (%)

IAA concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	**1 mg L^{-1}**	**2 mg L^{-1}**	**3 mg L^{-1}**	
0 mg L^{-1}	50	100	100	100	87.50
0.125 mg L^{-1}	100	100	75	50	81.25
0.150 mg L^{-1}	75	100	75	75	81.25
0.175 mg L^{-1}	50	100	100	100	87.50
Mean value	68.75	100	87.50	81.25	**84.38**

Note: Data were transformed using the √(x + 0.5) transformation prior to ANOVA

Table 3. Influence of PGR Treatments on Explant Contamination (%)

IAA Concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	1 mg L^{-1}	2 mg L^{-1}	3 mg L^{-1}	
0 mg L^{-1}	50	0	0	0	12.50
0.125 mg L^{-1}	0	0	25	50	18.75
0.150 mg L^{-1}	0	0	25	0	6.25
0.175 mg L^{-1}	25	0	0	0	6.25
Mean value	12.50	0	12.50	12.50	**10.94**

Note: Data were transformed using the √(x + 0.5) transformation prior to ANOVA

Table 4. Effect of plant growth regulator (PGR) on browning explants (%)

IAA Concentration	BAP concentration				Mean value
	0 mg L^{-1}	1 mg L^{-1}	2 mg L^{-1}	3 mg L^{-1}	
0 mg L^{-1}	0	0	0	0	0
0.125 mg L^{-1}	0	0	0	0	0
0.150 mg L^{-1}	25	0	0	25	12.50
0.175 mg L^{-1}	25	0	0	0	6.25
Mean value	12.50	0	0	6.25	**4.69**

Note: For analysis of variance, data were transformed using the √(x + 0.5) transformation

The analysis of variance showed that BAP concentrations had a significant effect on explant morphological responses (Tables 1 and 5). The treatment containing 1 mg L^{-1} BAP without IAA produced the highest proportion of peeling and swollen explants, achieving a 100% response rate, with shoot emergence occurring at an average of 10.25 days after inoculation (DAI) (Table 6; Fig. 1). Similarly, the combination of 2 mg L^{-1} BAP and 0.175 mg L^{-1} IAA also resulted in a complete response, with the earliest shoot initiation recorded at 8.25 DAI (Table 6; Fig. 1). Overall, BAP application exerted a strong influence on early morphogenic changes, with 1 mg L^{-1} BAP producing the highest frequency of visible morphological development during the initiation stage (Table 5).

The analysis of variance showed that the combination of BAP and IAA had a significant effect on explant morphology (Tables 1 and 5). The highest proportion of peeling and swollen explants was obtained with the treatment containing 1 mg L^{-1} BAP + 0 mg L^{-1} IAA, which resulted in a 100% response and an average of 10.25 days after inoculation (DAI) for the first shoot emergence (Table 6; Fig. 1). Likewise, the treatment with 2 mg L^{-1} BAP + 0.175 mg L^{-1} IAA also produced a 100% response, with the earliest shoot emergence recorded at 8.25 DAI (Table 6; Fig. 1). Overall, BAP application significantly affected morphological changes during the initiation stage, with the 1 mg L^{-1} concentration yielding the highest proportion of explants exhibiting visible morphological responses (Table 5).

During the initiation stage, the explants gradually shifted in color from yellow to green (Fig. 1). Yellow explants showed no visible morphological changes up to 6 weeks after inoculation (WAI) but remained viable and turgid. In contrast, light-green and dark-green explants began to exhibit morphological changes—such as splitting and swelling—at 2–3 WAI (Fig. 1).

Table 5. Effect of plant growth regulator (PGR) on morphological changes

IAA Concentration	BAP concentration				Mean value
	0 mg L^{-1}	1 mg L^{-1}	2 mg L^{-1}	3 mg L^{-1}	
0 mg L^{-1}	0	100	25	25	37.50
0.125 mg L^{-1}	0	75	25	0	25.00
0.150 mg L^{-1}	0	50	50	75	43.75
0.175 mg L^{-1}	25	75	100	50	62.50
Mean value	6.25 a	75.00 d	50.00 c	37.50 b	**42.19**

Note: Prior to analysis of variance (ANOVA), data were transformed using √(x + 0.5). Values followed by the same letter within the same row do not differ significantly according to the LSD test at the 5% significance level.

Table 6. Color and morphological changes of explants at the initiation stage.

Treatment	Morphological change		
	Color	**Description**	**Time for shoot emergence (DAI)**
C_0A_0	Yellow	No visible change	0.00
C_1A_0	Dark green	Divided and swelling	10.25
C_2A_0	Light green	Sloughing off	14.00
C_3A_0	Light green	Sloughing off	12.00

C_0A_1	Yellow	Death explant	0.00
C_1A_1	Dark green	Divided and swelling	9.67
C_2A_1	Yellow	No change	0.00
C_3A_1	Yellow	No change	0.00
C_0A_2	Yellow	No change	0.00
C_1A_2	Light green	Swelling	19.00
C_2A_2	Light green	Swelling	14.00
C_3A_2	Dark green	Divided and swelling	10.33
C_0A_3	Light green	Sloughing off	21.00
C_1A_3	Dark green	Divided and swelling	11.75
C_2A_3	Dark green	Divided and swelling	8.25
C_3A_3	Dark green	Divided and swelling	21.50

Note: DAI: Days after inoculation

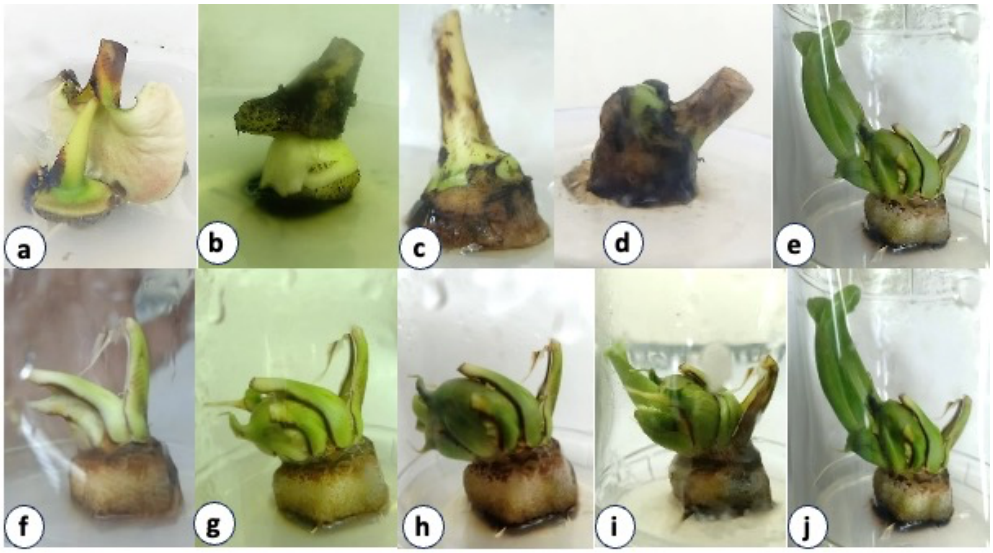

Fig. 1. Morphological changes of explants at 6 WAI; a. and b. peeling, c and d. swelling, and e. splitting and swelling at 6 WAI. Morphological changes of explants under treatment A15 (2 mg L^{-1} BAP + 0.175 mg L^{-1} IAA) at f. 2 WAI, b. 3 WAI, c. 4 WAI, d. 5 WAI, and e. 6 WAI. WAI: weeks after inoculation.

3.1.3 Shoot multiplication

Explants that survived the initiation stage were transferred to multiplication media to induce shoot proliferation. The survival, contamination, and browning rates of explants were subsequently evaluated (Table 7, 8, and 9). Analysis of variance showed that the application of BAP and IAA concentration, as well as their interaction, did not influence the variables observed (Table 1). The survival rate across all treatments was 50%, while 14.07% of the explants exhibited contaminated and 26.56% were browning (Table 7, 8, and 9). These findings indicate that the efficiency of the in vitro regeneration system during the multiplication stage declined compared with that observed during the initiation stage.

Table 7. Effect of plant growth regulators (PGRs) on explant survival

IAA Concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	
0 mg L^{-1}	25	100	25	50	50.00
0.125 mg L^{-1}	0	75	50	25	37.50

0.150 mg L^{-1}	50	75	50	50	56.25
0.175 mg L^{-1}	50	50	100	25	56.25
Mean value	31.25	62.50	56.25	37.50	**50.00**

Note: For analysis of variance (ANOVA), the data were transformed using the √(x + 0.5) transformation.

Table 8. Effect of plant growth regulator (PGR) on contaminated explants

IAA Concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	
0 mg L^{-1}	25	0	0	0	6.25
0.125 mg L^{-1}	0	25	25	0	12.50
0.150 mg L^{-1}	0	25	25	0	12.50
0.175 mg L^{-1}	25	0	0	75	25.00
Mean value	12.50	12.50	12.50	18.75	**14.07**

Note: For analysis of variance (ANOVA), data were transformed using the √(x + 0.5) transformation.

Table 9. Effect of plant growth regulator (PGR) on browning explants

IAA Concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	
0 mg L^{-1}	0	0	75	50	31.25
0.125 mg L^{-1}	100	0	0	25	31.25
0.150 mg L^{-1}	50	0	0	50	25.00
0.175 mg L^{-1}	25	50	0	0	18.75
Mean value	43.75	12.50	18.75	31.25	**26.56**

Note: For analysis of variance (ANOVA), data were transformed using the (x + 0.5) transformation.

Table 10. Effect of plant growth regulators (PGRs) on the number of nodules

IAA Concentration	**BAP concentration**				Mean value
	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	
0 mg L^{-1}	0	13.00	2.25	4.50	4.94
0.125 mg L^{-1}	0	9.25	2.75	0	3.00
0.150 mg L^{-1}	0	12.25	5.25	2.00	4.87
0.175 mg L^{-1}	1.00	9.00	14.50	3.25	6.94
Mean value	0.25 a	10.87 d	6.19 c	2.44 b	**4.94**

Note: For analysis of variance (ANOVA), data were transformed using the √(x + 0.5) formula. Values followed by the same letter within the same row are not significantly different according to the LSD test at the 5% significance level.

The ANOVA results showed that there was no significant interaction between BAP and IAA concentrations in influencing nodule production (Table 10). However, the combined application of these regulators resulted in the highest nodule formation in the treatment containing 20 mg L^{-1} BAP and 0.175 mg L^{-1} IAA, which produced an average of 14.50 nodules per explant. This outcome indicates that shoot proliferation is promoted when a high cytokinin concentration is paired with a relatively low level of auxin. Nodule development at six weeks after inoculation (WAI) is presented in Figure 2.

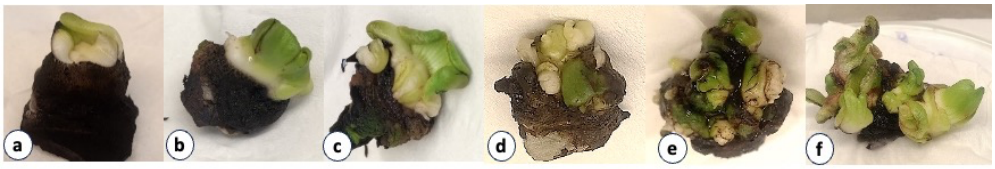

Fig. 2. Nodule development under treatment B15 (2 mg L^{-1} BAP + 0.175 mg L^{-1} IAA) at: a) 2 WAI, b) 3 WAI, c) 4 WAI, d) 6 WAI, e) 8 WAI, and f) 10 WAI.

3.2 Discussion

The ANOVA results indicated that varying concentrations of BAP and IAA significantly influenced explant morphogenesis during the initiation phase and nodule production during the multiplication stage, whereas these plant growth regulators had no effect on explant survival, browning, or contamination. Similar findings have been reported previously, where different combinations of cytokinin and auxin markedly shaped morphogenetic responses and proliferation capacity in banana tissue cultures [9]. Their work demonstrated that specific BAP–IAA formulations promoted shoot multiplication and stimulated the formation of actively dividing nodule-like meristems in several Malaysian cultivars. Consistent with this, [10] observed that supplementing culture media with BAP alongside IAA increased shoot number, leaf development, root growth, and total biomass, underscoring the pivotal influence of these PGRs on early developmental and multiplication processes in bananas.

Analysis of variance (ANOVA) revealed that the application of plant growth regulators (BAP and IAA) at different concentrations significantly affected the percentage of morphological changes during the initiation stage and the number of nodules during the multiplication stage. However, these regulators showed no influence on explant survival, contamination, or browning. Previous studies support these findings, showing that varying concentrations of BAP and IAA strongly regulate in vitro morphogenesis and proliferation in bananas [9]. Their work demonstrated that specific combinations of BAP and IAA significantly enhanced shoot multiplication and promoted the formation of highly proliferative nodule-like meristems in several Malaysian banana cultivars. Similarly, BAP in combination with IAA has been reported to increase shoot production, leaf formation, root initiation, and overall biomass of banana explants under micropropagation, confirming the essential role of these growth regulators in directing early morphogenetic and multiplication responses [10].

The initiation stage resulted in a high explant survival rate (84.38%), characterized by fresh, yellowish to green tissues that were free from browning and microbial contamination. The use of juvenile planting material derived from banana suckers likely contributed to this outcome, as young tissues possess a greater regenerative capacity and higher totipotency than mature tissues [11].

Contamination levels were relatively low (10.94%) and were mostly detected at 1 week after inoculation (WAI), indicating external sources such as inadequate sterilization of culture vessels, instruments, or the working environment. The most common fungal contaminants identified were *Aspergillus* sp., *Monilia* sp., and *Penicillium* sp., while *Pseudomonas solanacearum* was recognized as a bacterial contaminant frequently associated with banana tissues. These microorganisms proliferate rapidly in nutrient-rich culture media when aseptic techniques are not strictly maintained.

Browning occurred at a low frequency (4.69%), which may be attributed to a limited accumulation of phenolic compounds. Excessive oxidation of phenolics can hinder nutrient absorption and inhibit regeneration, whereas low browning intensity generally favors explant development. The lack of a significant effect of BAP and IAA on browning suggests that endogenous physiological mechanisms, rather than exogenous hormonal application, were primarily responsible for phenolic oxidation. Notably, elevated cytokinin concentrations may suppress auxin activity and induce necrosis, while IAA can promote ethylene synthesis, which in turn inhibits cell proliferation [12]. Periodic subculturing to fresh media remains an effective strategy to minimize browning by removing oxidized tissues.

Visual observations showed that explants changed color from white to yellow at 1 week after inoculation (WAI) and turned green by 2–3 WAI, indicating a metabolic activation and chlorophyll development stimulated by light exposure. Chlorophyll biosynthesis proceeds via the photoreduction of protochlorophyllide to chlorophyllide *a*, followed by phytol esterification catalysed by chlorophyl synthase [13]. In addition to promoting cell elongation,

auxin also contributes to chlorophyll formation. Greening was accompanied by morphological changes such as splitting, peeling, and swelling. The outermost sheath detached as nodules developed from the central part of the explant due to nutrient and water absorption from the culture medium. Approximately 40.63% of explants exhibited these changes with an average occurrence time of 14.97 days after initiation (DAI), indicating responsiveness to the combined BAP and IAA treatments.

The ANOVA results showed that the interaction between BAP and IAA did not significantly influence explant morphological changes. However, two treatment combinations—1 mg L^{-1} BAP without IAA and 2 mg L^{-1} BAP with 0.175 mg L^{-1} IAA—produced the highest proportion of peeling and swollen explants, each achieving a 100% response rate. Auxin–cytokinin interactions are known to regulate processes such as cell division and tissue formation, rather than determining the precise timing of specific developmental events. Together, these hormones modulate the balance between cell proliferation and differentiation by influencing gene expression in meristematic tissues and developing organs, thereby guiding organogenesis (e.g., shoot versus root formation) and overall tissue patterning. Numerous studies have demonstrated that altering the auxin-to-cytokinin ratio can shift cellular developmental pathways, with higher auxin concentrations promoting root formation and elevated cytokinin levels favoring shoot development. This underscores their central role in developmental patterning rather than temporal regulation [14].

The application of BAP had a significant effect on morphological changes during the initiation stage, with the concentration of 1 mg L^{-1} producing the highest proportion of explants exhibiting visible morphogenic responses. Cytokinin (BAP) promotes cell expansion by enhancing protein and nucleic acid synthesis, altering cellular metabolism, and facilitating water uptake [15]. The use of relatively low BAP concentrations (1–3 mg L^{-1}) during initiation enabled the explants to acclimate before exposure to a tenfold increase in cytokinin levels during the multiplication phase.

The survival rate of explants during the multiplication stage reached 50.00%, indicating that half of the explants from the initiation stage successfully adapted to the multiplication medium. Surviving explants were characterized by fresh, yellowish to green tissues free from browning and microbial contamination. The combination of BAP and IAA did not significantly affect explant survival, and a tenfold increase in BAP concentration relative to the initiation stage had no influence on viability. Nevertheless, the hormonal combination promoted further morphogenesis, as reflected by the formation of multiple shoots or nodules (scalps). Contamination levels were relatively low (14.07%) and mostly occurred at 1 week after inoculation, likely caused by inadequate sterilization or poor sealing of culture vessels. Browning was observed in 26.56% of explants, likely due to increased phenolic accumulation compared with the initiation stage. Longitudinal splitting and horizontal placement of explants, used to enhance nodule induction, may have intensified browning through wounding stress and oxygen exposure, which accelerate phenolic oxidation. Excessive phenolic accumulation inhibits nutrient uptake and regeneration, while low browning intensity favors explant development. Browning may also arise from hormonal imbalance, as IAA can enhance ethylene synthesis leading to tissue browning, while excessive BAP may suppress auxin activity and cause necrosis [12]. Subculturing to fresh media and trimming browned tissues remain effective strategies to minimize phenolic oxidation.

The application of BAP and IAA significantly affected the number of nodules produced. Nodule formation serves as a key indicator of in vitro developmental progress, particularly during the multiplication stage. In this study, nodulation was further stimulated by vertically splitting the explants and positioning them horizontally on the culture medium, which increased the surface area in contact with the medium, enhanced nutrient absorption, and ultimately promoted shoot proliferation. A tenfold increase in cytokinin (BAP) concentration

effectively boosted multiplication and nodule development by reducing apical dominance and encouraging the emergence of lateral buds. As a cytokinin, BAP promotes active cell division and morphogenesis, aligning with its common use in banana micropropagation at concentrations ranging from 0.1 to 20 mg L^{-1} [10]. The multiplication rate was influenced by both cytokinin concentration and genotype; banana cultivars containing one or two B genomes typically produced more shoots per subculture cycle than A-genome cultivars, reflecting genotype-specific responsiveness to BAP. The highest number of nodules (14.50) was observed in the treatment containing 20 mg L^{-1} BAP combined with 0.175 mg L^{-1} IAA, confirming this combination as the most effective for inducing morphogenesis. These findings are consistent with previous reports indicating that high cytokinin and low auxin levels favor optimal shoot proliferation [5].

Overall, the findings indicate that successful multiplication requires higher exogenous cytokinin levels than in the initiation stage, enabling effective interaction with endogenous auxin to regulate morphogenesis under in vitro conditions.

4 Conclusion

The application of BAP and IAA significantly influenced explant morphological responses during the initiation phase and nodule formation during the multiplication phase of in vitro propagation in Kepok Grecek banana. The combination of 2 mg L^{-1} BAP with 0.175 mg L^{-1} IAA produced the most effective initiation response, yielding 100% peeling and swelling of explants. During the multiplication stage, the treatment containing 20 mg L^{-1} BAP and 0.175 mg L^{-1} IAA resulted in the highest proliferation, producing an average of 14.50 nodules per explant. Further research is needed to refine the subsequent stages of regeneration, particularly shoot elongation and root induction, to ensure successful acclimatization of regenerated plantlets.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement
Yustika: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. Rudarmono: Data curation, Writing- Original draft preparation. Muhammad Saleh: Validation, Writing- Reviewing and Editing. Nurhasanah: Validation, Writing- Reviewing and Editing. Widi Sunaryo: Validation, Writing- Reviewing and Editing.

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