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Induction Of Callus Formation In Gapi Banana (*Musa Acuminata* L.) Ovaries At Various Concentrations Of Iaa And Bap In Vitro

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Abstract

Gapi banana (Musa acuminata L.) is a valuable tropical fruit limited by a shortage of quality planting material. In vitro callus induction from floral ovary explants was examined in this work to determine the effects of coupled IAA and BAP. Sterilized explants were cultured on MS media across five IAA+BAP combinations: A0 (0/0 ppm), A1 (2/8 ppm), A2 (5/8 ppm), A3 (8/8 ppm), and A4 (11/8 ppm). Using a completely randomized design, data on explant viability, callus formation, and browning were evaluated via ANOVA and Chi-square tests. While treatment combinations did not significantly alter explant survival, they significantly affected callus formation and browning. Treatments featuring 8–11 ppm IAA alongside 8 ppm BAP consistently maximized callus induction through 7 weeks post-inoculation, highlighting this hormone combination as an effective strategy for Gapi banana micropropagation.

Keyword: Plant tissue culture, PGR Browning, MS Medium

INTRODUCTION

Banana plants are an important agricultural commodity with significant influence both domestically and internationally. In addition to taste, banana fruits possess high nutritional and economic value, which serve as key advantages of this crop. In Indonesia, bananas rank first among fruits with the highest production volume (Dwivanny et al., 2021) and rank third in Gorontalo Province (Khasanah et al., 2024). The high market demand for bananas is naturally accompanied by an increased demand for high-quality plantlets. However, the demand for banana plantlets cannot be fulfilled relying solely on conventional propagation, particularly in

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Gorontalo, where conventional propagation methods are still predominantly used alongside a shortage of seedling suppliers.

The high demand for banana plantlets can be effectively met by utilizing in vitro culture as an alternative propagation method. Mitotic cell division is the sole means of cell, tissue, and organ proliferation in in vitro culture. This process generates cells, tissues, and whole individuals that possess identical genetic material to the mother plant (Cardoso et al., 2019). The in vitro culture method offers distinct advantages in producing uniform, disease-free seedlings in large quantities within a relatively short period. In the in vitro propagation of bananas, shoot tip and floral bud explants are commonly employed (Elma et al., 2017; Wahidah & Hasrul, 2017).

The selection of explant types significantly influences regeneration success. The floral ovary of the banana represents a potential explant type because it contains somatic cells that are highly responsive to hormonal triggers. Furthermore, using floral buds yields a higher quantity of explants per inflorescence with a minimal risk of contamination (Darvari et al., 2010; Dwiyani & Yuswati, 2018). In in vitro propagation, plant growth regulators (PGRs) are frequently applied to stimulate explants to develop roots, shoots, or calli. The application of PGRs produces varying outcomes depending on the specific type and concentration supplied. Applying IAA alone without combination with other PGRs is not recommended for achieving optimal outcomes (Wahidah & Hasrul, 2017). Conversely, a study by Dwiyani & Yuswati (2018) demonstrated that applying IAA in combination with NAA is recommended as it successfully induces plantlet formation. In a previous study by Rohman et al. (2024), applying 5 ppm IAA + 8 ppm BAP on shoot tip explants enhanced plant height at 3–7 weeks after planting (WAP) and increased shoot number at 7–12 WAP. Therefore, it is hypothesized that specific concentration combinations of IAA and BAP will elicit an optimal callus growth response in Gapi banana ovary explants. This study was conducted to evaluate the response and identify suitable concentration combinations of IAA and BAP for inducing callus formation from Gapi banana floral ovaries in vitro. Additionally, this study aims to serve as a baseline reference regarding the in vitro response of Gapi banana ovary explants to IAA and BAP combinations.

METHOD

Study Site and Period

From 2025 to 2026, the study took place at the Tissue Culture Laboratory of the Faculty of Agriculture at Universitas Negeri Gorontalo in Moutong Village, Tilongkabila District, Bone Bolango Regency, Gorontalo Province, Indonesia. The professor in charge of the lab was Jl. Prof. Dr. Ing. B.J. Habibie. Methods: Five different combinations of IAA and BAP were tested in this

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research using a CRD. With three explants per culture bottle and three replications of each treatment, a total of 45 explants were used in 15 experimental units. Here are the therapies that were used:

- A0: 0 ppm IAA + 0 ppm BAP (Control)
- A1: 2 ppm IAA + 8 ppm BAP
- A2: 5 ppm IAA + 8 ppm BAP
- A3: 8 ppm IAA + 8 ppm BAP
- A4: 11 ppm IAA + 8 ppm BAP

Exploitation and Sterilization Procedure

Banana male buds (banana heart) were washed thoroughly using liquid detergent under running tap water. Subsequently, Surface sterilization of intact male buds involved consecutive 15-minute immersions in 20% and 10% disinfectant solutions, each followed by three rinses in sterile distilled water. Subsequently, initiation took place under a laminar airflow cabinet by stripping the outer bracts to reveal the inner floral buds. The isolated buds were further disinfected in a 10% solution for 5 minutes, rinsed thrice, and subjected to a final 3-minute treatment with 70% ethanol, concluding with three sterile water rinses (Djarot & Gati, 2024). Inoculation and Culture Conditions: The explants were dissected to isolate ovaries measuring 0.8 to 1.0 cm in length. The separated ovaries were then inoculated onto Murashige and Skoog (MS) media corresponding to their respective treatment groups at a depth of less than 1 cm. After planting, the mouths of the culture bottles were sealed with plastic sheets, rubber bands, and cling wrap. Each culture bottle was labeled with the treatment code and planting date, afterwards, the cultures were moved to an incubation chamber that followed a photoperiod of 16 hours of light and 8 hours of darkness. Subculturing was conducted twice at two-week intervals.

Observation Parameters and Statistical Analysis

Among the criteria that were recorded were the percentages of live explants, explants that formed calluses, browning, and contamination. The ANOVA was used for the statistical analysis. When we found treatment effects that were statistically significant, researcher used DMRT to compare means at the 5% level of significance again. Conversely, data showing non-significant differences or uniform values assesses through Chi-Square Analysis.

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RESULTS AND DISCUSSION

Percentage of Viable Explants

To find significant differences, the percentages of transplant viability were analysed using ANOVA, followed by DMRT at $p < 0.05$. Figure 1 shows a chart of the observed percentages of viable explants.

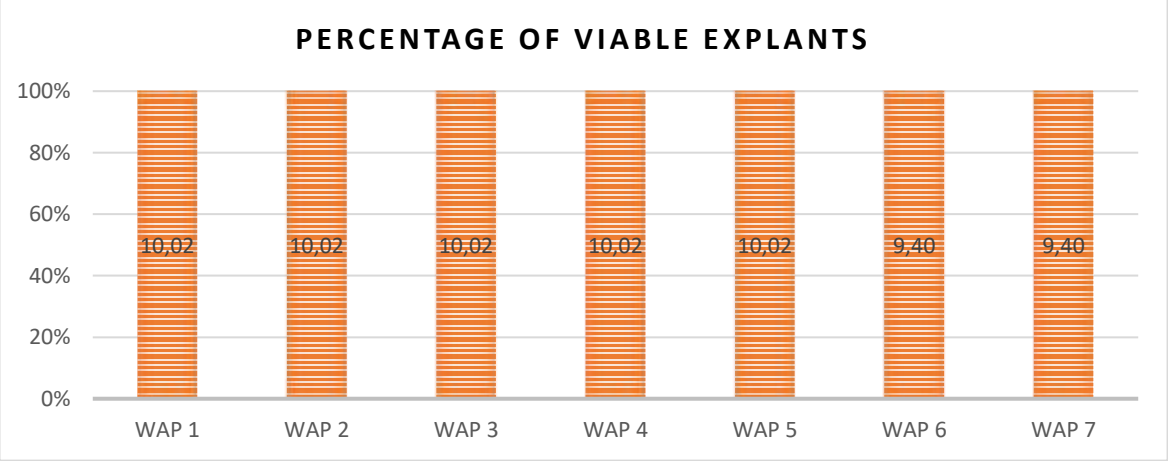


Figure 1. Percentage of Viable Explants, Weeks 1–7
Note: This data has been transformed using the square root of ((x + 0.5))

Table 1. Result Chi-Square of persentase of viable explant

Week	χ^2Value	Df	p-value	Significance
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	4.191	4	1	ns (p>0,05)
7	4.191	4	1	ns (p>0,05)

Note: Fisher's Exact Test was used for data analysis because several cells had expected values less than 5. WAP = weeks after planting, df = degrees of freedom, ns = not significant, s = significant, (-) dead/non-viable/constant data

The data regarding the percentage of viable explants were examined using Chi-Square tests and ANOVA. The proportion of viable explants was not significantly affected by the IAA + BAP treatments, according to analysis of variance; therefore, only the mean values are

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presented. From weeks 1 to 5 after planting (WAP), the percentage of viable explants remained consistent at 10.02% (square-root transformed data, √(x + 0.5)). Viable explants were characterized by greening and tissue swelling, whereas non-viable explants turned brown or pale brown. The survival of explants across all treatments during 1–5 WAP indicates that the basal MS medium alone provided sufficient nutrients to maintain explant viability, rendering the PGR additions statistically non-significant during this initial stage (Sari et al., 2013).

Statistical evaluation using Chi-Square analysis could not be processed for 1–5 WAP because all explants remained viable (100% survival rate, resulting in constant data). Constant data lack frequency variation, which violates the primary prerequisite of Chi-Square testing (Zhang, 2024). At 6–7 WAP, a slight decrease in explant viability to 9.40% was observed. This reduction was due to explant mortality in control treatment A0. Explant death in treatment A0 likely occurred due to the absence of exogenous PGRs in the growth medium, which failed to stimulate cell division, ultimately triggering browning, tissue necrosis, and cell death (Cardoso et al., 2019).

Percentage of Explants Forming Callus

Callus formation, marked by an increase in cell proliferation, serves as a key indicator of successful explant growth in in vitro culture. Which PGRs are added to the medium and at what concentrations have a significant impact on callus induction and development. To evaluate treatment effects, data on the percentage of callus-forming explants were analyzed using ANOVA and DMRT at α = 5% (Table 2).

Table 2. ANOVA results for the percentage of explants forming callus across 1–7 WAP

Treatment	WAP 1	WAP 2	WAP 3	WAP 4	WAP 5	WAP 6	WAP 7
A0	0,71	0,71^{a}	0,71^{a}	0,71	0,71^{a}	0,71^{a}	0,71^{a}
A1	3,69	7,19^{c}	7,19^{c}	4,69	6,80^{bc}	3,69^{bc}	3,69^{ab}
A2	3,69	6,19^{c}	6,19^{c}	0,71	7,80^{c}	2,20^{ab}	4,69^{abc}
A3	6,19	6,19^{c}	6,19^{c}	5,18	5,18^{b}	6,19^{cd}	8,41^{c}
A4	3,69	3,69^{b}	3,69^{b}	2,20	5,18^{b}	8,20^{d}	8,20^{bc}
KK	60%	37%	37%	76%	32%	43%	46%

Note: According to DMRT at α = 5%, there is a significant difference between values within the same column followed by different superscript letters. Information changed by √(x + 0.5).

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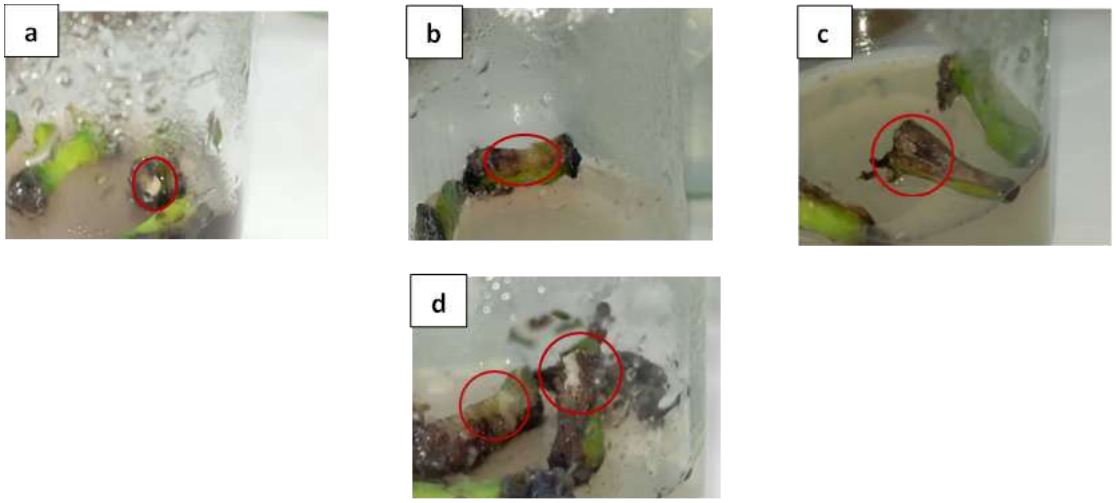


Figure 2: Callus on explants: (a) callus in treatment A1, (b) callus in treatment A2, (c) callus in treatment A3, (d) callus in treatment A4

At 1 WAP, treatments showed non-significant effects (ns) with a Coefficient of Variation (CV) of 60%, indicating that explants were still in an adaptation phase to the new environmental conditions and had not yet exhibited morphogenetic responses to IAA and BAP (Isah, 2015). By weeks 2 and 3, PGR treatments exerted a statistically significant influence; treatment A1 produced the highest value (7.19), followed by A2 and A3 (6.19), which were not significantly different from each other but significantly superior to A4 (3.69) and control A0 (0.71). This demonstrates that explants had adapted and initiated active cell division triggered by exogenous PGRs boosting endogenous hormone levels (Lestari, 2011). At 4 WAP, data again showed non-significant results with a high CV of 76%. High coefficients of variation in tissue culture studies are typically caused by data variance resulting from contamination, tissue browning, and explant death, which introduce zero values into the dataset (George et al., 2008).

At 5 WAP, significant responses re emerged, with A1 achieving the highest value (6.80). At 6 WAP, treatment A4 displayed the highest value (8.20), followed by A3 (6.19), whereas A2 declined to 2.20, indicating that higher IAA concentrations require a longer induction period to achieve maximum performance. By the end of the observation period (7 WAP), treatments A3 (8.41) and A4 (8.20) emerged as the best treatments, proving that higher IAA concentrations (8–11 ppm) combined with 8 ppm BAP are most optimal for supporting sustained, long-term callus formation.

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Percentage of Browning

At the 5% level of significance, the percentage data from Browning were examined using an ANOVA followed by DMRT. Table 3 displays the browning percentages for the explants, while Figure 3 illustrates the browning observed across treatments.

Table 3. ANOVA results for the percentage of explant browning across 1–7 WAP

Treatment	WAP 1	WAP 2	WAP 3	WAP 4	WAP 5	WAP 6	WAP 7
A0	10,02^{b}	10,02^{b}	10,02^{b}	10,02^{b}	10,02^{b}	10,02^{b}	10,02^{b}
A1	2,20^{a}	2,20^{a}	2,20^{a}	2,20^{a}	2,20^{a}	3,81^{a}	3,81^{a}
A2	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}	2,20^{a}	2,20^{a}	2,20^{a}
A3	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}
A4	0,71^{a}	0,71^{a}	0,71^{a}	0,71^{a}	2,20^{a}	2,20^{a}	2,20^{a}
KK	40%	40%	40%	40%	58%	77%	77%

Note: Values followed by different letters within the same column differ significantly at α = 5% (DMRT). Data transformed via √(x + 0.5).

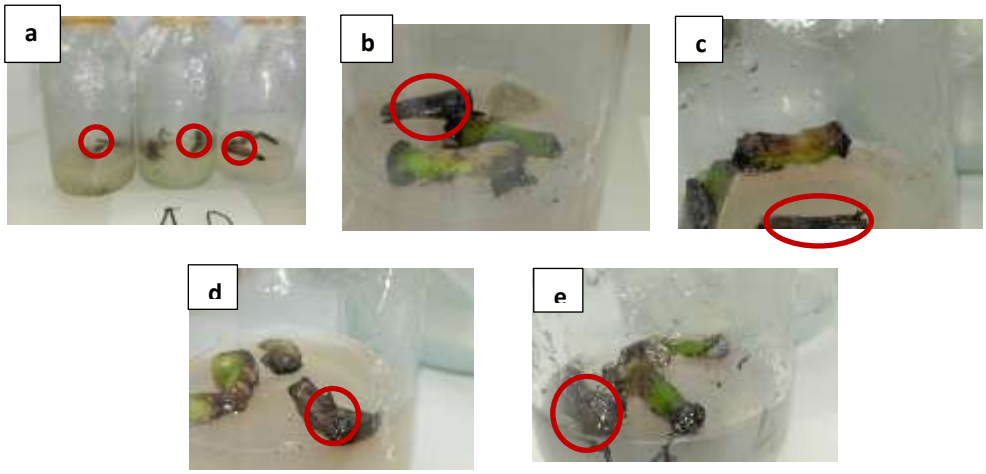


Figure 3. (a) Browning in treatment A0, (b) Browning in treatment A1, (c) Browning in treatment A2, (d) Browning in treatment A3, (e) Browning in treatment A4

According to the findings of the variance analysis for WAPs 1–7, treatment A0 consistently showed the highest browning percentage, namely 10.02, with a “b” notation indicating a significant difference compared to treatments A1, A2, A3, and A4, which had an “a” notation. Although there were differences in numerical values among A1, A2, A3, and A4,

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statistically, there were no significant differences among them. The increase in KK values (58–77%) at 5–7 WAP indicates greater variation in response among replicates during the later growth stages; however, overall, the control treatment without plant growth regulators remained the most susceptible to oxidation.

Browning in tissue culture stems from the elevated production and subsequent enzyme-mediated oxidation of phenolic compounds (Febriani, 2022; Hutami, 2008). The reduced browning observed in the IAA + BAP treatment relative to the control likely reflects the ability of auxin and cytokinin to lower oxidative stress by maintaining cellular redox balance (Faisal et al., 2018). Claims made by Thiry et al. (2026) and Liu et al. (2024) are in agreement with this, who demonstrated that exogenous plant growth regulators (PGRs) can inhibit phenolic oxidation, as well as Sadat's (2018) assertion that combined PGRs optimize cellular metabolism to limit browning in kapok banana cultures.

CONCLUSION

The in vitro application of IAA+BAP plant growth regulators to Gapi banana ovary explants produced a significant response in terms of the percentage of explants forming callus and the browning rate; however, it had no significant effect on the percentage of viable explants, indicating that the application of plant growth regulators did not affect explant viability. Treatments with 8 to 11 ppm IAA and 8 ppm BAP were found to be optimal for inducing callus formation in Gapi banana ovary explants. Regarding the browning percentage, no specific treatment had a significant effect; however, all treatments with plant growth regulators yielded better results than those without.

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