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Induction Of In Plantain (*Musa acuminata* L.) Using The Growth Regulators 2,4-Dichlorophenoxyacetic & BAP (Benzyl Amino Purin)

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Abstract

Plat propagation through is a method of prodycing new plants by growing unspecified clusters (callus) from small pieces of tissue (explants) such as Gapi banana flowers. This study seeks to determine the appropriate doses of 2,4-D and BAP for callus formation. The study was conducted from September to October 2025 at the Tissue Culture Laboratory of Gorontalo State University, using male Gapi banana flowers explants. The experiment used a randomized block design (RBD) involving two factors: 2,4-D (2ppm) and BAP (5, 10, 15 ppm). The percentage of live explants (%) was observed weekly for 30 days. Final observation included browning, The results showed that the addition of 2,4-D and BAP to the medium greatly affected the percentage of live explants. Without these substances, the explants turned brown. Fungal contamination was dominant at D0 (control) and D2 (2 ppm 2,4-D + 10 ppm BAP). The callus was white/cream to greenish in color, with a firm texture. The percentage of callus-forming explants (%) increased with the addition of BAP (5, 10 and 15ppm), significantly affecting callus formation.

Keyword: Gapi Banana, Induction, Callus.

INTRODUCTION

Gapi bananas, or pisang doa as the Gorontalo people call them, have received support from IFAD (International Fund for Agricultural Development) and IsDB (Islamic Development Bank) for their development. Gapi bananas have the advantage of being priced between Rp 10,000 and Rp 25,000 per bunch, while the price per bunch ranges from IDR 80,000 to IDR

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100,000. In terms of nutritional content, gapi bananas contain 1.60g of protein, 0.10g of fat, 131 kcal of energy, and 31.10g of carbohydrates. Gapi bananas have a fairly long shelf life of around 14 days (Project, 2023).

Despite their high potential value, the development of gapi bananas is still hampered by the lack of attention to the rapid propagation of local varieties that are free from pests and diseases. Previous research has only focused on kepok bananas, with the combination of 2,4-D and BAP being effective in the callus induction stage from banana heart explants (Ardhani et al., 2024), but the response in local gapi banana varieties has not been studied. Another study on gapi bananas using carrot extract has not shown the desired results in the in vitro growth of gapi banana planlets (Galib et al., 2024).

The use of tissue culture techniques by utilising callus induction is very useful in developing the growth of Gapi banana explants. Gapi banana flowers were selected as explants that are free from fungal or bacterial contamination from the soil (Roostika et al., 2017). The ovaries of these banana flowers were then taken for planting in a growth medium that had been combined with the growth regulators 2,4-D and BAP.

The use of these PGs can support the success rate in tissue culture. The function of plant growth regulators is to stimulate and manipulate plant growth and development, directing tissue development and plant propagation in a sterile (controlled) environment. Although the medium used is basic MS medium, the addition of ZPT to this medium is essential to provide the elements that can be absorbed by the explants in the formation and development of callus (Ardhani et al., 2024) (Philips, G & Garda, 2019).

Auxin 2,4-D functions as a synthesis that is often used in cell division (cell dedifferentiation) in inducing callus. This hormone can stimulate cell division and maintain the pluripotent status of tissue to form callus mass (Biswas et al., 2025). Meanwhile, the cytokinin BAP functions to stimulate cell division and cell differentiation towards shoot organogenesis when the doses of cytokinin and auxin are appropriate. In other words, BAP functions to increase cell division activity and can influence callus morphology (colour and texture) (Putri et al., 2024). The optimal combination of 2,4-D and BAP can determine the colour and texture that appear during the induction process (Cheng et al., 2025).

Ardhani et al., (2024) The addition of auxin 2,4-D and cytokinin BAP to Kepok banana flowers showed that this combination could form compact callus on growth media containing 2,4-D 2ppm and BAP 5ppm, with compact callus texture of white-yellowish colour, appearing on average 13 days after induction and an explant callus rate of 79.17%. Meanwhile, the study by Dwiyani et al., (2018), The addition of auxin 2,4-D and cytokinin BAP to Kepok banana flowers

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showed that this combination could form compact callus on growth media containing 2,4-D 2ppm and BAP 5ppm, with compact callus texture of white- yellowish colour, appearing on average 13 days after induction and an explant callus rate of 79.17%. Meanwhile, the study by (Latunra et al., 2017).

This study aims to find the appropriate dose to induce callus formation by combining ZPT 2,4-D and BAP. The use of banana flower explants serves as explants to minimise the use of Gapi banana suckers, which are difficult to obtain. Banana flowers that grow far above the ground minimise contamination by microorganisms (fungi and bacteria) from the soil.

METHOD

Research Location

This research was conducted from August to December 2025. It took place at the Tissue Culture Laboratory, Faculty of Agriculture, Gorontalo State University on Jl. Prof. Dr. Ing. BJ. Habibie, Moutong Village, Tilongkabila Subdistrict, Bone Bolango Regency. The laboratory coordinates are 0033'26'N 123008'06"E, with an optimal temperature of 25°C. Lighting conditions are 16 hours of light and 8 hours of darkness, with a medium pH ranging from 5.8 to 6.2.

Materials and Equipment

Research equipment consisting of Laminar Air Flow (LAF), autoclave, Petri dishes, culture bottles, stirrers, pots, hot plates, rubber, sterile plastic, planting tools (dental tweezers, small anatomical tweezers, large anatomical tweezers, and scalpels), analytical scales, pH meters, micro pipettes, Erlenmeyer flasks, measuring cups, cameras, labels, and writing tools (notebooks and pens). Meanwhile, the materials used include Gapi banana male flowers, MS powder, agar-agar, ZPT 2,4-Dichlorophenoxyacetic & BAP (Benzyl Amino Purin), sugar, 70% and 95% alcohol, 1N NaOH, 1N HCl, disinfectants (NaClO) 10 and 20%, and sterile distilled water.

Research Design

The experiment used a Randomised Block Design (RBD) with a combination of 2,4-Dichlorophenoxyacetic acid at 2ppm and BAP (5ppm, 10ppm, and 15ppm). Treatment combinations were D0 (control), D1 (2ppm 2,4-D + 5ppm BAP), D2 (2ppm 2,4-D + 10ppm BAP), and D3 (2ppm 2,4-D + 15ppm BAP). The treatment was repeated three times with four treatment levels, each treatment consisting of one culture bottle, resulting in a total of 12 treatment bottles. The explants were observed for four weeks (30 days after planting).

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Research Procedure

The medium was prepared by weighing ingredients such as MS (4.4 g), agar-agar (5 g), and sugar (30 g). Then, the weighed sugar and MS were placed in a measuring cup and 800 mL of distilled water was added. The mixture was stirred until homogeneous, after which the solution was divided into 4 parts (for treatments D0, D1, D2, and D3) of 200 mL each. Then, in treatments D1, D2, and D3, ZPT BAP + 2,4-D was added according to the treatment. After mixing with ZPT, the solution was measured using a pH meter until the desired pH (5.8-6.2) was reached. After that, the solution was made up to 250 mL and then heated (add agar) to boiling point using a hot plate. The solution was poured into 25-30 mL bottles, then sealed tightly with plastic. The media was sterilised using an autoclave for 20 minutes at a temperature of 1210C and a pressure of 15 Psi.

Planting was carried out in a LAF that had been sterilised using 95% alcohol. The explants are cleaned using soap and rinsed, then placed in the LAF. The clean explants are soaked in 20% disinfectant (20 minutes), rinsed 3 times, then soaked in 10% disinfectant (15 minutes) and rinsed 3 times until clean. After that, the explants were separated from the bract layer (outer layer/skin covering the banana flower). Once separated, the banana flower explants were soaked in 10% disinfectant (5 minutes) and rinsed three times, then soaked in 70% alcohol (3 minutes) and rinsed three times. The explants are cut using a scalpel to separate the male flowers (ovaries) from the other parts. Plant the explants in bottles according to treatments D0, D1, D2, and D3. Plant 3 explants (ovaries) in each bottle.

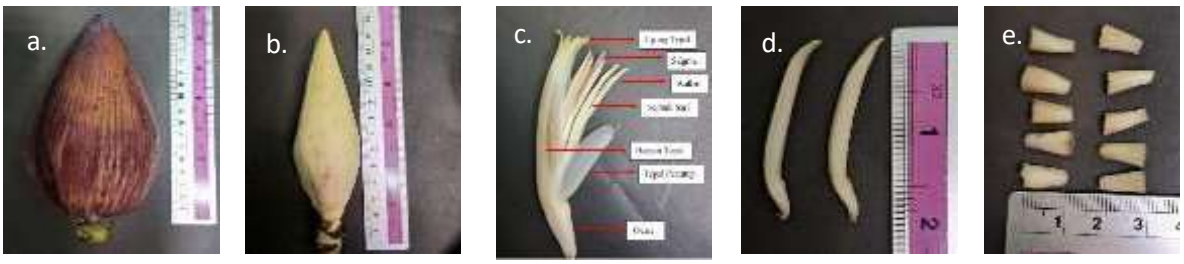


Figure 1. Gapi Banana Flowers (*Musa acuminata* L.)

Data Analysis

The development and growth of explants were observed by collecting data such as the percentage of live explants (%), browning, contamination, callus colour and texture, and explants with callus (%). The F test was used to analyse the data at a level of 5%. If the Pr(>F) data was less than 0.05 (5%), further analysis was performed using the BNT (Smallest Significant Difference) at a level of 5%. The data was analysed using the STAR (*Statistical Tool for Agricultural Research*)

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application.

Khairan et al., (2021) Percentage Of Live Explants (%)

$$\% \text{ Number of living explants} = \frac{\text{Number of living explants}}{\text{Total explants}} X\ 100\%$$

Percentage Of Callus Explants

$$\% \text{ Number of callus explants} = \frac{\text{Number of callus explants}}{\text{Total explants}} X\ 100\%$$

Karunia, et al., (2022) Percentage Of Browning

$$\% \text{ Number browning} = \frac{\text{Number browning}}{\text{Total explants}} X\ 100\%$$

Percentage Of Contamination

$$\% \text{ Number of contaminated explants} = \frac{\text{Number of contaminated explants}}{\text{Total explants}} X\ 100\%$$

RESULTS AND DISCUSSION

The results of the study show that the addition of ZPT to the culture medium can cause the explants to develop and survive. The percentage of surviving explants (%) can be seen in Table 1:

Table 1. Percentage Of Live Explants (%)

Treatment	Observation Time			
	1 MSI	2 MSI	3 MSI	4 MSI
D0	0,00c	0,00c	0,00b	0,00b
D1 (2ppm 2,4-D + 5ppm BAP)	0,78b	0,78b	0,78b	0,78b
D2 (2ppm 2,4-D + 10ppm BAP)	1,00a	1,00a	0,89a	0,67a
D3(2ppm 2,4-D + 15ppm BAP)	1,00a	1,00	1,00a	1,00a
BNT (5%)	0,19	0,19	0,24	0,57
CV	13,71	13,71	18,42	47,17

Note 1 MSI = Week after incubation

The addition of 2,4-D and BAP to the medium resulted in average live explant percentages

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of D1 (0.78%), D2 (0.67%), and D3 (1.00%). These values indicate that the addition of hormones such as 2,4-D and BAP resulted in a higher percentage of live explants compared to the treatment without PHT (D0). The combination of 2,4-D (2ppm) and BAP (15ppm) is the ideal combination for explant growth and development. The higher the BAP (15ppm) added to the growth medium, the higher the percentage of live explants. The cytokinin (BAP) added to this medium functions to trigger enzyme activity, while auxin (2,4- D) functions to maintain cell division activity. Ardhani et al., (2024) explained that the application of BAP (15ppm) and 2,4-D (2ppm) can increase callus formation and explant survival rate in Kepok bananas. This was followed by D1 (2ppm 2,4-D + 5ppm BAP), a combination that was quite low compared to the D2 combination (2ppm 2,4-D + 10ppm BAP), but the D1 combination had a 0.78% higher explant survival rate than D2 (0.67%). This is because the lower combination can produce moderate growth and development of explants (callus). Adero et al., (2023), Meanwhile, growth in explant D2 experienced oxidative stress due to less than sterile culture conditions, causing the explant to release exudate that could trigger microorganism contamination (Zhao et al., 2025).

Browning can be seen in Table 2. The browning that occurred in the D0 medium (without ZPT) illustrates that culture media without ZPT cannot be used as a growth medium for explants. The percentage of explants growing on D0 medium is 0%, indicating that culture media containing MS (Murashige Skoog) cannot provide adequate nutrients for explant growth. The browning that occurs in the D0 medium is caused by phenolic compounds that are released when the tissue is damaged. Rahmadi et al., (2021) explain that phenolic compounds can be released from damaged explant tissue, causing browning or discolouration of the explants. Handayani et al., (2024) also added that the addition of auxins such as 2,4-D in the culture medium can help reduce the level of browning in the medium because 2,4-D has enzymes that can function as detoxification (antidote).

Table 2. Browning and Microorganism Contamination

Treatment	Browning	Contamination
D0	100%	33%
D1 (2ppm 2,4-D + 5ppm BAP)	11%	0%
D2 (2ppm 2,4-D + 10ppm BAP)	33%	33%
D3(2ppm 2,4-D + 15ppm BAP)	0%	0%

Contamination in the explants can be seen in Table 2. The level of contamination caused by fungi occurred in media D0 and D2, indicating that the level of cleanliness of the culture

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environment greatly influences the occurrence of microorganism contamination. The microorganisms found in media D0 and D2 are fungi that have white, cotton-like hyphae. Contamination in D0 can be influenced by the absence of physiological activity (dead explants), which can release toxic compounds, thereby creating an environment conducive to fungal growth. Othmani et al., (2024) found that dead explants can release toxic compounds (exudates) that can create an environment for the growth of fungi such as Aspergillus, Penicillium, and even Fusarium. This contamination is divided into two types: internal and external. Internal contamination is caused by explants that have been infected by microorganisms, while external contamination is related to the cleanliness of the culture environment and the cleanliness during the planting process in the LAF (Sulichantini, E et al., 2024).

Table 3. Percentage of Callus Explants (%), Colour and Texture of Callus

Treatment	Percentage of callus formation (%)	Callus colour	Callus texture
D0	0,0	Chocolate	-
D1 (2ppm 2,4-D + 5ppm BAP)	0,67	White	Compact
D2 (2ppm 2,4-D + 10ppm BAP)	0,56	White	Compact
D3(2ppm 2,4-D + 15ppm BAP)	1,00	White and Green	Compact
CV	65,37		

Note: - (browning/dead explant)

The percentage of callus formation can be seen in Table 3. The percentage of callus formation on D3 medium (1.00) indicates that the medium with 15 ppm BAP + 2.4-D 2 ppm was able to produce callus at the highest rate compared to other treatments. This is because the addition of 2,4-D and BAP to the medium provides good synergy, resulting in the explants forming callus with a percentage of callus-forming explants of 100% or 1.00. In Dwiyani et al., (2018) the addition of 2.4-D 2ppm to the medium resulted in a callus formation percentage of 67%, with a total of 6 out of 9 planted explants forming callus, and the fastest callus growth time with an average of 4.83 days. Ardhani et al., (2024) found that the addition of 2.4-D 2 ppm and BAP 15 ppm to the medium could form callus with a callus formation percentage of 70.83%. In medium D1, 0.67% of explants formed callus, and in medium D2, 0.56% formed callus. This indicates that

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the addition of BAP and 2,4-D plant growth regulators to the culture medium proves that plant growth regulators greatly influence the percentage of explants that form callus. Fitri, M & Armaini, (2019) and Rasud, (2020) stated that the addition of BAP and 2,4-D to the culture medium can stimulate cell division and development and can enhance the process of cytokinesis, especially during RNA synthesis.

The colour of the callus formed in this study ranged from white/cream to green. The colour of the callus indicates that the explants in vitro culture have cells that are still actively dividing. White/cream-coloured callus indicates that the callus has high embryogenic potential due to the interaction between the ZPTs administered. The green colour of the callus indicates that it has chlorophyll that can develop into shoots. Isda & Salsabilla, (2022) stated that the green colour can form shoots and the white or cream colour indicates that the callus can form embryogenic callus. Meanwhile, Zhao et al., (2025) revealed that the green colour of callus formed in tissue culture can be influenced by the light index. The green colour of callus also indicates that it contains chlorophyll pigment and has high regeneration potential compared to white/cream-coloured callus.

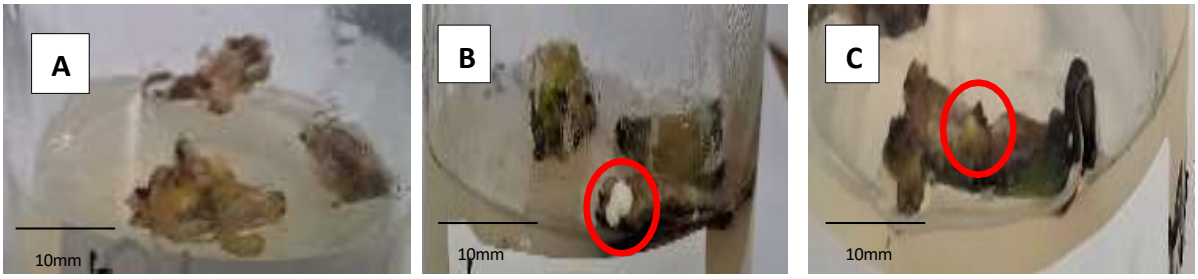


Figure 2. A D1 (2ppm 2,4-D + BAP 5ppm), D2 (2ppm 2,4-D + 10ppm BAP), and D3 (2ppm 2,4-D + 15ppm BAP)

Meanwhile, the texture of the callus obtained in treatments D1, D2 and D3 was compact. The formation of compact callus indicates that the use of media and ZPT had an effect on the explants. The most common types of callus formed were compact and crumbly. The compact texture in the explants is caused by the concentration of ZPT (cytokinin), which can stimulate rapid cell division (Isda & Salsabilla, 2022). Meanwhile, Mahadi et al., (2016) found that compact callus formation is caused by the lignification process, resulting in a harder texture, and that the addition of ZPT also significantly influences phytohormones in the nutrient transport process.

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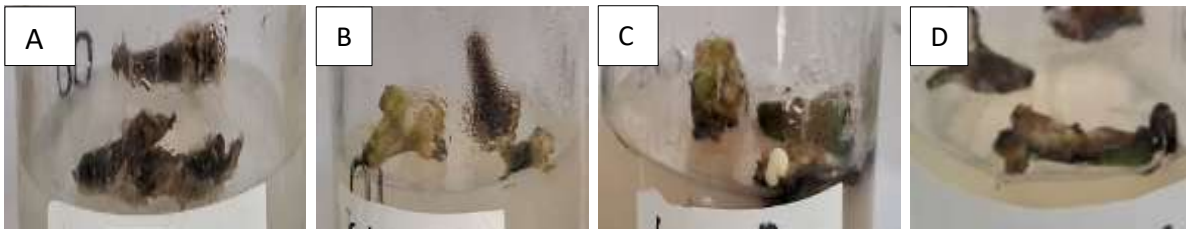


Figure 3. A (control) did not form callus, B (2 ppm 2,4-D + 5 ppm BAP) formed compact callus with a light green colour, C (2 ppm 2,4-D + 10 ppm BAP) formed compact callus with a white colour, D (2 ppm 2,4-D + 15 ppm BAP) formed callus with a green colour.

CONCLUSION

The addition of ZPT to the culture medium can increase the percentage of live explants and the percentage of explants with callus formation. In the D0 medium, the explants were found to be dead and no callus was formed. D1 (2 ppm 2,4-D + 5 ppm BAP) resulted in a live explant percentage of 0.78% (78%), D2 (2 ppm 2,4-D + 10 ppm BAP) had a percentage of living explants of 0.67% (67%), and D3 (2 ppm 2,4-D + 15 ppm BAP) had a percentage of living explants of 1.00% (100%). Browning in the D0 medium was caused by phenolic compounds released due to tissue damage. Contamination in treatments D0 (33%) and D2 (33%) indicated that tissue undergoing stress due to treatment could release exudates that could cause microbial growth in the culture medium. The fungi observed in the D0 and D2 media were predominantly white in colour with cotton-like hyphae. The colour and texture of the callus formed in treatments D1, D2, and D3 were predominantly white/cream to green with a compact, dense texture.

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