

Effect of Cavendish Banana Filtrates and BAP (6-Benzylaminopurine) in Vacin and Went (VW) Media on the Growth of *Cattleya* sp. Orchid Planlet In Vitro

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Abstract

The production of *Cattleya* sp. orchids has declined due to environmental changes and limitations in cultivation techniques, necessitating propagation methods using *in vitro* tissue culture. This study aims to determine the effect and optimal concentration of Cavendish banana filtrate and BAP on VW medium for *in vitro* growth of *Cattleya* orchid planlets. The research used a completely randomized design with five treatments and five replications; negative control (K), P1 (10g/L Cavendish banana filtrate + 2 ppm BAP), P2 (15g/L Cavendish banana filtrate + 1.5 ppm BAP), P3 (20g/L Cavendish banana filtrate + 1 ppm BAP), and P4 (25g/L Cavendish banana filtrate + 0.5 ppm BAP). Growth was observed from parameters including the number of leaves, leaf color, and plantlet height, which were analyzed using one-way ANOVA and Duncan's multiple range test. Results show that P1 (10g/L Cavendish banana filtrate + 2 ppm BAP) and P2 (15g/L Cavendish banana filtrate + 1.5 ppm BAP) had optimal effect on number and color of leaves, while P3 (20g/L Cavendish banana filtrate + 1 ppm BAP) and P4 (25g/L Cavendish banana filtrate + 0.5 ppm BAP) resulted on best height of *Cattleya* orchid planlets.

leaves; tissue culture; plantlet height; leaf color; plant species

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INTRODUCTION

Indonesia is a tropical country with very high biodiversity, including a wealth of ornamental plant species such as orchids. These plants not only have visual appeal through the beautiful shapes and colors of their flowers but also have high commercial value. Data from the Central Statistics Agency shows that national orchid production has experienced a significant decline, from 11.35 million pots in 2021 to only 2.52 million in 2023. Factors such as climate change and habitat destruction are believed to be the main causes of this decline (Setiawati *et al.*, 2016).

One of the most well-known and highly valued types of orchids is *Cattleya* sp., often dubbed as the "Queen of Orchids." This type has large, striking, and fragrant flowers that make it popular in the international market. However *Cattleya* sp. is also included in CITES Appendix I, which means it is classified as an endangered species and requires strict protection from international trade (CITES, 2022). This makes *ex situ* conservation efforts increasingly important, for example tissue culture (Arti and Mukarlina, 2017). Tissue culture is a method of plant propagation by growing parts of the plant (explants) *in vitro* in sterile artificial media. This technique allows for the mass production of seedlings without relying on a large number of parent plants. Another advantage is relatively short propagation time and uniform results. In tissue culture, the subculture process is necessary to extend the growth of explants that have developed into a new, more suitable medium (Niazian *et al.*, 2017).

One of the commonly used culture media in orchid tissue culture is the VW medium, which contains essential macro and micro nutrients to support plant growth (Wiraatmaja, 2017). This medium is often enriched with organic compounds and plant growth regulators (PGRs) such as cytokinins and auxins. 6-Benzyl Amino Purine (BAP) is a type of synthetic cytokinin that is effective in stimulating bud formation, as demonstrated in the study by Yulia *et al.* (2020), which showed an increase in the number of buds on *Cymbidium* orchids. Meanwhile, auxin plays a role in the process of cell elongation and enlargement. One of the natural sources of auxin is bananas. Arditti (1992) mentioned that bananas contain natural growth hormones such as auxin, and Mahadi (2016) and Yulia *et al.* (2020) showed that the application of auxin can increase the height and number of orchid shoots. Nurfadilah *et al.* (2018) also showed that 2.5% Ambon banana extract significantly increased the number of shoots and leaves

on Black Orchids. Cavendish bananas are rich in nutrients such as potassium, magnesium, and vitamin B6 (USDA, 2020) and have great potential to be used as a source of organic plant growth regulators. Putri *et al.* (2024) demonstrated that the combination of banana and potato extracts can increase the number of leaves, roots, and height of *Dendrobium* orchid plantlets. In addition to being effective, the use of banana filtrate is also more environmentally friendly and economical compared to synthetic plant growth regulators.

Based on various previous studies, this article aims to examine the effect of the combination of Cavendish banana filtrate and BAP in *Vacin and Went* (VW) medium on the in vitro growth of *Cattleya* sp. orchid plantlets.

MATERIALS AND METHODS

This research was conducted at the Tissue Culture Laboratory, Food Security and Agriculture Office of Surabaya City, from November to December 2024 to analyze the effect of adding Cavendish banana filtrate and BAP (6-Benzyl Amino Purine) to *Vacin and Went* (VW) medium on the in vitro growth of *Cattleya* Sp. orchid plantlets. The type of this research was experimental research that used a Completely Randomized Design (CRD) with one factor, which was the combination of various concentrations of plant growth regulators (PGR) added to the *Vacin and Went* medium, including Cavendish banana filtrate and BAP (6-Benzylaminopurine). This consisted of 5 treatments: P1 (10 g/L Cavendish banana + 2 ppm BAP), P2 (15 g/L Cavendish banana + 1.5 ppm BAP), P3 (20 g/L Cavendish banana + 1 ppm BAP), P4 (25 g/L Cavendish banana + 0.5 ppm BAP), and Control (0 g/L Cavendish banana + 0 ppm BAP). In this study, 5 replications were obtained for each treatment, resulting in 25 experimental units.

The initial stage of this research began with preparing the necessary tools and materials. Several procedures were carried out, the first being the sterilization of equipment such as culture bottles, tweezers, and petri dishes using an autoclave set at 121°C in solid mode for 20 minutes. Next 1000 mg of BAP was weighed using an Ohaus balance and then placed into a beaker containing 100 ml of distilled water. The mixture was homogenized using a magnetic stirrer, then 1 N NaOH was added gradually until the solution became clear. After that, distilled water was added until the total volume reached 1000 ml. Cavendish banana filtrate was prepared by weighing the fruit according to the treatment concentration using an Ohaus balance, then placing them in a mortar and pestle to be ground (Harahap and Sihotang, 2025).

The preparation of 1 L VW medium requires macro and micro nutrient stocks including ammonium sulfate (stock A) 10 ml, potassium nitrate (stock B) 10 ml, calcium phosphate (stock C) 10 ml, magnesium sulfate heptahydrate, potassium dihydrogen phosphate (stock D) 10 ml, ferrous sulfate (stock E) 10 ml, manganese sulfate heptahydrate (stock F) 10 ml, as well as vitamin stocks including myo-inositol, pyridoxine HCL, thiamin HCL, nicotinic acid glycine 10 ml, which were added to 200 ml of distilled water and homogenized using a magnetic stirrer. Then, 20 g of sugar and 7 g of agar were added, followed by distilled water until the volume reaches 1000 ml, and boiled.

The media treatment was prepared by adding 10 ml of each stock solution A, B, C, D, E, and F into a 200 ml glass beaker containing aquades, and the mixture was stirred evenly for 10 minutes using a magnetic stirrer. A 10 mL vitamin solution was added and homogenized again for 10 minutes. Then, a combination of Cavendish banana filtrate and BAP concentrations according to the predetermined treatment was added, and homogenized again for 10 minutes. Next, 7 grams of agar were added, and the solution volume was adjusted with aquades to reach 1000 mL, then stirred evenly for 5 minutes. The pH of the medium was measured and adjusted to a range of 5–6; if the pH was too basic, HCl was added, and if it was too acidic, NaOH was added. The prepared medium was then placed into sterilized bottles according to the volume and combination of Cavendish banana filtrate and BAP treatment. The medium was then sterilized using an autoclave at 121°C for 15 minutes.

The leaf color parameter was analyzed qualitatively by matching the leaf color of each planlet bud in each bottle using the Munsell Color Charts for Plant Tissue reference book, while the number of leaves and planlet height parameters were analyzed quantitatively. The obtained quantitative data were subjected to the Normality Test to determine data distribution, the Homogeneity Test to determine variance equality, and the Analysis of Variance (ANOVA) Test at a significance level of 0.05 (α) to determine the presence of a significant effect between treatments. Duncan's Multiple Range Test was used for post-hoc test at significance level of 5%.

RESULTS

Supplementation of Cavendish banana filtrate and BAP to VW media had a significant effect on the growth of the number of leaves of *Cattleya* Sp. orchids, in which P1, P2, P3, and P4 were significantly different compared to control (K) (Table 1). Treatments P1 and P2 were not significantly different from each other, but were significantly different from treatments K, P3, and P4. The number of leaves was the highest at 28.60 in treatment P2, which had a concentration of 15 g of banana filtrate and 1.5 ppm BAP. However this is not significantly different from P1 (Table 1)

Cavendish banana filtrate and BAP added to the VW medium also had a significant effect on the height of *Cattleya* orchid planlets. The control treatment (K) did not show a significant difference compared to treatments P1, P2, P3, and P4. The height of *Cattleya* sp. orchid planlets was the highest at 7.54 cm in P4, which involved 25 g banana filtrate and 0.5 ppm BAP. However this is not significantly different from P3 (Table 1).

Table 1. Treatment effect on the number of leaves and height of *Cattleya* orchid plantlets.

Type of Filtrates	Parameter*	
	Number of Leaves	Planlet Height
K	11.40 ± 3.84 ^a	4.64 ± 0.75 ^a
P1	27.00 ± 6.59 ^c	5.88 ± 0.43 ^b
P2	28.60 ± 7.23 ^c	5.92 ± 0.46 ^b
P3	18.20 ± 3.19 ^b	6.94 ± 0.40 ^c
P4	19.20 ± 3.03 ^b	7.54 ± 0.74 ^c

Note: *) The results of the Duncan test (5%) are indicated by the notation (a, b, c). The same notation in the same column indicates no significant difference, while different notations in the same column indicate a significant difference. K = VW media, P1 = 10 g/L filtrate Cavendish banana + 2 ppm BAP, P2 = 15 g/L Cavendish banana filtrate + 1.5 ppm BAP, P3= 20 g/L Cavendish banana filtrate + 1 ppm BAP, P4 = 25 g/L filtrate of Cavendish banana + 0.5 ppm BAP.

Based on the Figure 1, it shows that the color of *Cattleya* sp. orchid leaves was at a hue value of 5.0 Green Yellow (GY). The control treatment (K) has a value and chroma of 6/6, which is a bright green color with lower chlorophyll content. Treatment P1 has a value and chroma of 5/6, with a lower value, greener leaves, and higher chlorophyll content than treatment K. Treatment P2 has higher value and chroma scores of 5/8, with greater leaf brightness and higher chlorophyll content, indicating better leaf color compared to other treatments. Treatment P3 has a value and chroma of 6/6, with a higher value and lower chroma than treatments P1 and P2, and slightly lower chlorophyll content and leaf brightness. Treatment P4 has a value and chroma of 7/6, with chlorophyll content lower than the other treatments and leaf brightness equal to treatment K.

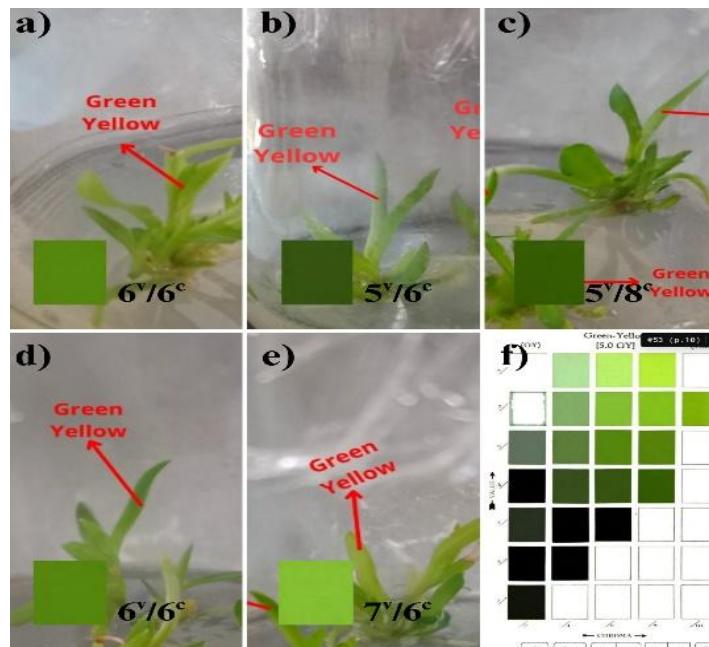


Figure 1. Results of the physiological visual observation of leaf color. a)= control (K), b)= P1, c)= P2, d)= P3, e)= P4, f)= Munsell color charts, v= value, c= chroma. A low value indicates a high chlorophyll content, while a high chroma value indicates a more optimal green intensity.

DISCUSSION

The growth of *Cattleya* sp. plantlets in vitro culture is greatly influenced by the growth medium and the addition of plant growth regulators (PGRs). VW medium contains basic nutrients, but it is not quite optimal for supporting the maximum growth of plantlets (Zulkarnaen, 2009). Therefore, modifying the media by adding natural substances such as Cavendish banana filtrate and synthetic plant growth regulators like BAP becomes an effective strategy to enhance growth quality. This study shows that the combination of Cavendish banana filtrate and BAP significantly increases the number of plantlet leaves. Treatment P2 (15 g/L banana filtrate and 1.5 ppm BAP) resulted in the highest number of leaves at 28.60 leaves. This combination provides a hormonal balance that supports leaf cells optimal division and elongation. These results indicate that banana filtrate enriches the medium with organic nutrients and natural auxins, while BAP promotes cytokinin activity thereby accelerating leaf growth (Sitompul and Guritno, 1995). Andany and Ratnasari (2023) showed that the use of BAP in vitro was able to increase the number of shoots and leaves in yellow kepok banana plantlets. These findings provide a basis that BAP has the potential to exert a similar influence on other plant species, including *Cattleya* sp. orchids, although in this study, a combination of Cavendish banana filtrate was used as an organic growth regulator source and VW medium as an alternative to MS medium.

Auxin plays an important role in determining the location of leaf primordia formation through the distribution of its concentration gradient in the apical meristem tissue. This distribution creates a pattern of polar transport that allows for the formation of leaf primordia at specific positions in the meristem dome. High concentrations of auxin at specific points will serve as the initial trigger to start the organogenesis of new leaves, thus auxin functions as the main positional signal in leaf initiation (Xiong and Jiao, 2019). After the location of the leaf primordia is formed, the role of cytokinins becomes dominant. Cytokinins stimulate rapid cell division in that area by activating cell cycle regulatory genes, such as cyclin D and cyclin-dependent kinases (CDKs). This activation accelerates the G1 phase to S phase in the cell cycle, supporting optimal cell proliferation and leaf organ development (Liang *et al.*, 2023). The combination of auxin and cytokinin, which work synergistically but at different stages, ensures the formation of organized and efficient leaves. Auxin determines where leaves will form, while cytokinin determines how leaf growth proceeds. Higher number of leaves indicates better growth, which is directly related to photosynthetic capacity. The more leaves formed, the larger the surface area to capture light and increase the rate of photosynthesis. Higher rate of photosynthesis result in greater carbon assimilation to supports tissue growth and chlorophyll production. This is related to the leaf color observed in this study, where leaves with a higher number tend to have a higher brightness of green color, indicating a higher chlorophyll content (Ramdani *et al.*, 2024).

Leaf color is also an important indicator when assessing planlet growth. Treatment P2 produced the greenest leaf color with a value and chroma of 5/8 based on the Munsell Color Chart Tissue. The dark green color indicates a high chlorophyll content, which plays key role in photosynthesis. Optimal photosynthesis generates more energy to support vegetative growth (Dyarta and Parawita, 2023; Widyawati *et al.*, 2023). In the filtrate of Cavendish bananas, natural auxins such as indole-3-acetic acid (IAA) support chlorophyll metabolism by enhancing enzyme activity involved in its biosynthesis, such as chlorophyll synthase and chlorophyllase, and delay leaf senescence (Luo *et al.*, 2023). Meanwhile, BAP supports cell division and inhibits chlorophyll degradation, also helping extend leaf lifespan by suppressing genes associated to chlorophyll degradation, such as Senescence-Associated Genes (SAG) (Mutui *et al.*, 2013). Balancing these hormones is important to maintain leaf color quality of the leaves. Improper hormone level can cause metabolic stress and hinder plant growth (Romeida, 2013; Prisco *et al.*, 2016). This can be seen in P2 which had the most positive effect on leaf color because it approaches the ideal hormonal balance.

The most optimal growth height of the planlet was shown in treatment P4 (25 g/L banana filtrate and 0.5 ppm BAP) with an average height of 7.54 cm. Cavendish banana filtrate contains natural auxin bioactive compounds that play an important role in cell elongation by increasing enzyme activity that supports cell wall elasticity. Auxin, particularly indole-3-acetic acid (IAA), plays a key role in stimulating cell elongation in the stem tissue of planlets through the acid growth hypothesis mechanism. Auxin activates proton pumps (H^+) on the plasma membrane, which lowers the pH of the cell wall, allowing expansin enzymes to loosen the cell wall and enabling the cells to elongate (Jacobs and Gilbert, 1983). This process results in stem elongation and increased height of the planlet. Meanwhile, cytokinins indirectly support height growth. Cytokinins promote cell division in the meristematic region, directly contributing to the vertical growth of the plant, thereby increasing the number of cells. The newly produced cells will undergo elongation under the influence of auxin,

accelerating the vertical growth of the planlet (Taiz and Zeiger, 2002). The taller planlets show a positive response to well-formulated media conditions. On the other hand, treatment P1 with a higher concentration of BAP resulted in planlets with the lowest height (5.88 cm), presumably due to hormonal imbalance that caused the formation of lateral buds to be more dominant than the elongation of the main stem (Hapsoro and Yusnita, 2018). These results highlight the importance of the proper auxin-cytokinin ratio in optimally stimulating the vertical growth of planlets.

According to Harahap and Yusnita (2018), excessively high concentrations of cytokinins can trigger uncontrolled cell division, causing energy allocation to be more focused on the formation of lateral shoots rather than the elongation of the main stem. In the control treatment that only used *Vacin and Went* medium without the addition of Cavendish banana filtrate and BAP, the average height of the planlets was the lowest compared to the other treatments. This is due to the absence of exogenous hormones that play a role in stimulating cell division and cell elongation. Although the VW medium had contains sufficient basic nutrients to support growth, it was not enough to promote optimal plant growth without the aid of growth regulators. Additionally, without the addition of auxins and cytokinins, plant growth relies solely on the endogenous hormones produced by the plantlets themselves. The concentration of endogenous hormones is often insufficient to support optimal growth, especially under in vitro culture conditions. Planlets with the control treatment showed slower growth and lower plantlet height compared to other treatments that received additional exogenous hormones.

Overall, the application of treatment P2 (15 g/L Cavendish banana filtrate + 1.5 ppm BAP) and P1 (10 g/L Cavendish banana filtrate + 2 ppm BAP) on the VW medium affected the leaf number parameter by providing an optimal increase in leaf number. Treatment P2 (15 g/L Cavendish Banana filtrate + 1.5 ppm BAP) also affects the leaf color of *Cattleya* Sp. orchids by producing a greener color with low value and high chroma. The application of treatment P3 (20 g/L Cavendish Banana Filtrate + 1 ppm BAP) and P4 (25 g/L Cavendish Banana Filtrate + 0.5 ppm BAP) affected the height of the planlets, yielding optimal results. This treatment can provide balanced hormonal support, resulting in the tallest planlets compared to other treatments.

CONCLUSION

In vitro growth of *Cattleya* sp. orchid plantlets was affected by the addition of Cavendish banana filtrate and BAP (6-Benzylaminopurine) to VW medium. The best results for the number of leaves were obtained with treatments P1 (10 g/L Cavendish banana filtrate + 2 ppm BAP) and P2 (15 g/L Cavendish banana filtrate + 1.5 ppm BAP), which produced an average of 27.00 and 28.60 leaves, respectively. The most favorable leaf colour of green-yellow 5v/8c, was obtained with treatment P2 (15 g/L Cavendish banana filtrate + 1.5 ppm BAP). The highest plantlet height at 7.54 cm, was observed with treatment P4 (25 g/L Cavendish banana filtrate + 0.5 ppm BAP).

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CONFLICT OF INTEREST

There is no conflict of interest.

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