Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 17, No. 1, 2025, pp 50 - 56
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: 10.25134/quagga.v17i1.360

Effect of Indole Butyric Acid (IBA) and Coconut Water on The Growth of Orchid Plant Shoots (*Cattleya* sp.) in Vitro

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APA Citation: Yasmint, A. A., & Harahap, F. (2025). Effect of Indole Butyric Acid (IBA) and Coconut Water on The Growth of Orchid Plant Shoots (Cattleya sp.) in Vitro. *Quagga: Journal of Education and Biology*, 17(1), 50-56. DOI: 10.25134/quagga.v17i1.360.

Received: 28-11-2024 Accepted: 11-12-2024 Published: 04-01-2025

Abstract: *This study aims to observe the effect of Indole Butyric Acid (IBA) concentration and coconut water on the growth of orchid plant shoots of Cattleya sp. In vitro using the tissue culture method. The research was conducted at the YAHDI Tissue Culture Laboratory from March to July 2024. There were 9 treatments with a combination of IBA concentrations (0, 1, 2 mg/l) and coconut water (0, 50, 100 ml/l), which were repeated 3 times each, resulting in a total of 27 experimental units. The variables observed included the time of emergence of shoots, the number of shoots, the number of leaves, and the height of the plantlet. The results showed that a coconut water concentration of 100 ml/l provided the best bud growth, while an IBA concentration of 1 mg/l and coconut water of 100 ml/l produced the highest number of leaves. However, variance analysis showed that the application of IBA and coconut water did not have a significant effect on the number of shoots, the number of leaves, and the height of orchid plantlets at the age of 6 MST. Observations showed that the interaction between IBA and coconut water was not optimal enough to stimulate bud growth, and the use of younger orchid leaves gave the best results. This study provides insight into the use of coconut water in tissue culture to improve the growth of Cattleya orchids, although its effectiveness is still limited to certain concentrations.*

Keywords: *Indole Butyric Acid, Coconut Water, Orchid Plant Sprouts*

1. INTRODUCTION

The Cattleya orchid is one of the most sought-after types of orchids by houseplant enthusiasts, mainly due to its large flower size and striking color, so it attracts a lot of attention. Although it has high popularity among orchid lovers, its existence is rarer when compared to other types of orchids, one of which is because of its relatively short blooming period. Cattleya orchids grow naturally in tropical America, including in countries such as Mexico and Brazil, and require warm temperatures as well as sufficient exposure to light to grow optimally. The bright red color of this orchid flower, coupled with the very beautiful and charming shape of the flower, makes it highly coveted by orchid collectors. One of the main characteristics of the Cattleya orchid is its large flower size and very attractive labellum (more prominent flower part), which further adds to the appeal of this plant (Darmono, 2003).

The name Cattleya was given in honor of William Cattley, an orchid farmer who was instrumental in the development of this orchid. He received a rare orchid from William Jackson Hooker, professor of botany at the University of Glasgow, who obtained it from plant collector William Swainson. John Lindley, a botanist, published a book *Collectania Botanica* containing illustrations of the orchid and named it Cattleya in honor of Cattley, who was born in 1787 in London and died in 1835 (Ning, 2013).

Although the Cattleya orchid has been quite widespread in various regions, it is still difficult to

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find in the wild due to its relatively short blooming process. This orchid is famous for its diversity of colors, as well as its distinctive aroma that is very striking, and can even be smelled from a considerable distance, making it very attractive and popular among ornamental plant lovers. To propagate Cattleya orchid plants, one of the methods used is through tissue culture techniques, where meristemic tissues such as shoot meristems or axillary shoots are used as starting material. This tissue culture technique has various purposes, including to efficiently propagate horticultural plants, produce disease-free seedlings, and preserve germplasm which is important for plant diversity. In addition, this technique can also be used to create superior varieties with desired characteristics and to produce secondary metabolite compounds that have high economic value, such as active compounds that can be utilized in various industries (Karjadi, 2016; Zullkarnain, 2014).

Indole Butyric Acid (IBA) is a compound that has high chemical stability and is effective in working for a long period of time. However, this compound has low mobility, so it only serves to stimulate root growth in plants. Research shows that the administration of IBA at a dose of 2 mg/l has been proven to be effective in increasing bud and root development in *Dendrobium* sp. (Karimah, 2021). In addition, some plant explants are naturally able to produce endogenous auxins that can support the growth process without the need for additional auxins from outside (Mahadi, 2016). To meet the need for large quantities of Cattleya orchids, tissue culture techniques are used as a faster and more efficient alternative compared to conventional methods. Conventional methods, although they have been used for a long time, require a long time and provide limited results. On the contrary, tissue culture allows plant propagation to be carried out in a shorter time with more and higher quality yields, so that it is the main choice for propagating Cattleya orchids more effectively and efficiently (Prayono, 2017). This technique allows the breeding process to be carried out under controlled conditions that support optimal growth for plants.

Tissue culture is a plant propagation technique that uses nutrient-rich media, such as banana or potato pulp, which aims to support and accelerate the plant growth process (Ning, 2013). One medium that has been shown to be effective in tissue culture is coconut water, which contains cytokinin compounds. This compound plays an important role in stimulating cell division and supporting the process of organogenesis, which is the formation of new plant organs. Research shows that coconut water can increase callus growth by up to 20%, which is one of the important stages in tissue culture. In terms of propagation of Cattleya orchids, the use of coconut water with a concentration of 100–150 ml/l has proven to be very effective in maximizing the growth of shoots and plantlets. The combination of the use of coconut water with the right growth regulators can increase success in plant propagation, create healthier shoots, and produce stronger seedlings. This technique provides advantages in creating superior varieties that are faster and more efficient.

2. RESEARCH METHODS

This research was conducted in March-July 2024 at the YAHDI Tissue Culture Laboratory, Medan Marelan. The population and research samples were in the form of *young Cattleya* sp. orchid plants obtained from the Alifah Agricultural Research Center (ALIFA_ARC), Medan Maimun, Medan City, North Sumatra.

This study aims to observe the growth of *Cattleya* sp. orchid shoots using the Factorial Complete Random Design (RAL) method with 9 treatments, namely a combination of Indole Butyric Acid (IBA) concentration and coconut water.

Table 1. Research Design

PITY	**AK Coconut Water (ml/l)**		
(mg/l)	**0**	**50**	**100**
0	I_0A_0	I_0A_{50}	I_0A_{100}
1	I_1A_0	I_1A_{50}	I_1A_{100}
2	I_2A_0	I_2A_{50}	I_2A_{100}

Information:
I1 : (IBA 0 mg/l), I2 : (IBA 1 mg/l), I3 : (IBA 2mg/l) AK0 : (Coconut Water 0 ml/l), AK50 : (Coconut Water 50 ml/l), AK100 : (Coconut Water 100 ml/l)

There were 9 treatment combinations (3x3), each repeated 3 times using a bottle as an experimental unit. Thus, the total treatment and repetition yielded 27 vials (9x3). The independent variables in this study were the concentration of ZPT IBA and Mulda Coconut

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Water, while the bound variables included the observed parameters, namely the time of bud appearance, number of shoots, number of leaves, and plantlet height.

The tools used in this study include culture bottles, beaker glasses, Bunsen lamps, tweezers, spatula, scalpels, petri cups, autoclaves, volume pipettes, hand sprayers, aluminum foil, Laminar Air Flow Cabinet (LAFC), stirring rods, measuring cups, pH meters, heaters, analytical scales, refrigerators, tissues, millimeter paper, labels, culture shelves, masks, gloves, laboratory coats, and stationery. The ingredients used include orchid explant (Cattleya sp.), MS medium (Murashige Skoog), agar, IBA (Indole Butyric Acid), young coconut water, 30% alcohol, 96% alcohol, sterile aquades, 0.1N HCl solution, 0.1N NaOH solution, detergent, chlorox solution (bayclin), and orchid leaves.

The data collection method in this study was carried out by observing the growth parameters of the buds of *the Cattleya* sp. orchid plant, including the time of bud formation, the number of shoots, the number of leaves, and the height of the plantlet. Rooms for tissue culture should always be sterile, with floor, wall, table, and appliance cleaning using 4% formalin and ventilation restrictions to avoid dust. Tools such as tweezers, knives, and culture bottles are sterilized by washing them using running water and soap, then putting them into an autoclave at 121°C for 1 hour. The sterile bottles are covered with plastic sprayed with 70% alcohol and stored in the refrigerator. The media and aquades are also sterilized by autoclave for 15 minutes to maintain the nutrient content, then stored in the culture room.

The medium used in this study was Murashige Skoog (MS) media with the addition of ZPT IBA (0 mg/l, 1 mg/l, 2 mg/l) and coconut water (0 ml/l, 50 ml/l, 100 ml/l) for the growth of *Cattleya* sp. orchid buds. The manufacturing process begins with preparing a stock solution of MS and ZPT IBA, as well as a special technique for dissolving FeSO4 and NaEDTA. MS media is prepared by mixing various ingredients, then adjusting the pH (4.8–5.8) by adding KOH or HCl. After that, it is added, and the media is cooked until boiling. The media is then poured into sterilized and autoclaved culture bottles at 121°C for 15 minutes. After that, the media is ready to use.

Sterilization of the culture media is carried out in an autoclave at 121°C for 15 minutes. After that, the bottles are covered with plastic and sprayed with 70% alcohol to maintain sterilization, then stored in the storage room. Leaf explant initiation is carried out in a sterilized Laminar Air Flow Cabinet (LAFC). Tools such as knives and tweezers were burned with Bunsen lamps. Orchid explants are cut and placed in culture bottles with suitable media, then placed on culture racks. Maintenance is carried out at a temperature of 18–20°C with lighting 16 hours a day. The room remains sterile to prevent contamination. Observations include the time of emergence of shoots, the number of shoots, the number of leaves, and the height of the plantlet, carried out every week to 6 weeks.

Visual data analysis uses a descriptive method with a factorial Complete Random Design and a 2-track ANAVA test. The significantly different results were further tested with the Duncan Multiple Range Test (DMRT) at a significance of 5%. If there is no obvious difference, the DMRT test is not carried out. The analysis was carried out with SPSS version 26.

3. RESULTS AND DISCUSSION

Based on observations in orchids aged 1-6 weeks after planting (MST), the fastest bud growth occurred in explants treated with I2A100 in the second week, which produced three buds. Meanwhile, other treatments only produce one to two buds in the same period. The I0A0 treatment showed the emergence of buds in the second week, while the I0A100 and I1A0 treatments only showed buds in the third week. The shoots appear after the explant is swollen, which is a response to the administration of Indole Butyric Acid (IBA). The results of this study show that the use of a concentration of 100 ml/l of Coconut Water produces the most optimal bud growth. This is in line with research conducted by Harahap (2011), which states that the concentration of coconut water of more than 15% can inhibit the growth of sprouts. Thus, the right concentration is essential to achieve maximum results in orchid propagation through tissue culture, given that too high a concentration can have a negative effect on plant growth.

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Table 2. Time of Emergence of Shoots

Treatment	Average
I0A0	2 MST
I0A50	2 MST
I0A100	3 MST
I1A0	2 MST
I1A50	2 MST
I1A100	2 MST
I1A0	2 MST
I2A50	2 MST
I2A100	2 MST

Furthermore, the results of the number of shoots where the observation of the impact of IBA and Coconut Water on the growth of Cattleya orchid buds at the age of 6 MST can be seen in Table 3.

Table 3. Number of Shoots

Treatment	Average
I0A0	1,33
I0A50	3,33
I0A100	1
I1A0	1
I1A50	1,33
I1A100	3,33
I2A0	2
I2A50	1,33
I2A100	1,33

Based on Table 3, the number of shoots that grew in the I0A50 and I1A100 treatments reached a total of 10 shots with an average of 3.33, while the I0A100 and I1A0 treatments only produced an average of 1 bud. In conclusion, Coconut Water affects the number of shoots that grow according to its maximum point concentration.

Table 4. ANAVA RAL Factorial Effect of IBA and Coconut Water on the Number of Orchid Shoots 6 MST

SK	DB	JK	KT	Fhit	F Table 5%	F Table 1%	Mr.
IBA	2	0,677	0.333	0.071	3,55	6,01	0.931
AK	2	104.667	0,778	0.167	3,55	6,01	0.848
I x AK	4	18,444	4,611	0.988	2,93	4,58	0.439
GALAT	18	84	4.667				
TOTAL	26	105					

The results of the variance analysis **in Table 4** showed that the application of ZPT IBA and Coconut Water did not have a significant effect on the number of shoots at the age of 6 MST ($\alpha \leq 0.05$). The test of the similarity hypothesis of two variances showed that F count IBA (0.071) and F count AK (0.167) were smaller than F table (3.55), and F count interaction (0.988) was smaller than F table (2.93). Since F counts ≤ F table, Ho is accepted, and Ha is rejected at a 95% confidence level. In conclusion, there was no significant effect of the administration of ZPT IBA and Coconut Water on the number of shoots at the age of 6 MST, so the DMRT test was not necessary.

Furthermore, the observation of the influence of IBA and coconut water on the growth of Cattleya orchid shoots at the age of 6 MST can be seen in Table 5.

Table 5. Number of Leaves

Treatment	Average
I0A0	1
I0A50	2,66
I0A100	1,33
I1A0	0,66
I1A50	1,66
I1A100	4,33
I2A0	1,33
I2A50	2
I2A100	3

Based on Table 5, the highest number of leaves at the age of 6 MST was found in the treatment of IBA 1 mg/l and Coconut Water 100 ml, with an average of 4.33 leaves, followed by I2A100 with an average of 3 leaves. I1A0 treatment produced the least number of leaves, which was an average of 0.66 leaves. In conclusion, a balance of IBA and Coconut Water concentrations is necessary for optimal results. Coconut water influenced leaf

p-ISSN 1907-3089, e-ISSN 2615-5869
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DOI: 10.25134/quagga.v17i1.360

count if the ZPT concentration was balanced, with the I1A100 treatment showing better results than I2A100 although the IBA concentration was higher in the treatment in Table 6.

Table 6. ANAVA RAL Factorial Effect of IBA and Coconut Water on the Number of Orchid Leaves 6 MST

SK	DB	JK	KT	Fhit	F Table 5%	F Table 1%	Mr.
IBA	2	1,556	0.778	0,2	3,55	6,01	0,821
AK	2	16	8.111	2,086	3,55	6,01	0,153
I x AK	4	14,222	3.556	0,914	2,93	4,58	0,477
GALAT	18	70	3.889				
TOTAL	26	102					

The results of the variance analysis in Table 6 showed that the application of ZPT IBA and Coconut Water did not have a significant effect on the number of leaves in orchids aged 6 MST ($\alpha < 0.05$). Based on the hypothesis test of the similarity of the two variances, the F value for IBA (0.2) and Coconut Water (2.086) was obtained which was smaller than the F value of the table (3.55), as well as the F calculation for the interaction (0.914) which was smaller than the F table (2.93). Since F is smaller than F table, the null hypothesis (Ho) is accepted, and the alternative hypothesis (Ha) is rejected. This shows that there is no significant effect on the growth of the number of leaves due to the application of ZPT IBA and Coconut Water. Therefore, further testing using DMRT is not necessary.

Furthermore, the observation of the influence of IBA and coconut water on the growth of Cattleya orchid shoots at the age of 6 MST can be seen in Table 7.

Table 7. High Plantlet

Treatment	Average
I0A0	0,83
I0A50	1,53
I0A100	0,86
I1A0	1,26
I1A50	1,03
I1A100	1,26
I2A0	1,4
I2A50	1,4
I2A100	1,2

Based on Table 7, the treatment with the best plantlet height at the age of 6 MST was obtained from I0A50 with an average of 1.53, followed by I2A0 and I2A50 with an average of 1.4. This shows that the concentration of 50 ml of coconut water provides the maximum effect compared to 100 ml without the highest IBA concentration. Meanwhile, the lowest concentration was recorded in control and 100 ml Coconut Water.

Table 8. ANAVA RAL Factorial Effect of IBA and Coconut Water on Orchid Plantlet Height 6 MST

SK	DB	JK	KT	Fhit	F Table 5%	F Table 1%	Mr.
IBA	2	0,636	0.318	0.254	3,55	6,01	0.779
AK	2	0.112	0.056	0.045	3,55	6,01	0.957
I x AK	4	0,968	0.242	0.193	2,93	4,58	0,939
GALAT	18	22,567	1.255				
TOTAL	26	24.303					

The results of the variance analysis in Table 8 showed that the administration of ZPT IBA and Coconut Water had no real effect ($\alpha < 0.05$) on the height of the plantlet at the age of 6 MST. Based on the calculations, the F calculation for IBA (0.254) and Coconut Water (0.045) is smaller than the F table (3.55 and 3.55), as well as for the interaction (0.193 vs 2.93). Since F counts < F

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DOI: 10.25134/quagga.v17i1.360

table, Ho is accepted, and Ha is rejected at a 95% confidence level. Thus, it can be concluded that there is no effect of ZPT IBA and Coconut Water on the growth of plantlet height at the age of 6 MST, so there is no need for further DMRT testing.

The results of observations on orchids aged 6 MST showed that the time of bud emergence was not significantly affected by the application of growth regulators (ZPT) and organic matter such as coconut water. This may be due to the less-than-optimal interaction between IBA and Coconut Water in stimulating bud growth. The use of the 3rd leaf as an explant in the treatment may not provide a maximum response because its meristematic tissue is less active compared to the first and second leaves, which tend to be more responsive in bud formation (Harahap, 2007). In addition, Harahap (2006) stated that to stimulate the formation of shoots, cytokinins are needed, but if the concentration of cytokinins is too high, this can inhibit the growth process. Shoots that begin to appear are usually preceded by a white bump that then turns green, indicating that the bud formation process has begun (Royani, 2015).

The treatment that gave the maximum results in sprouting was I2A100 on leaves 1, 2, and 3 in the 2nd week. High-dose treatments showed budding at 4 weeks; however, the process was longer due to longer swelling and explant rupture before buds appeared. In contrast, lower-dose treatments, such as I1A50 and I2A50 on leaf 2, showed faster results. This shows that the 50 ml/l dose of Coconut Water is not balanced with IBA 1 and 2 mg/l, while the interaction between 100 ml Coconut Water and IBA 1 and 2 mg/l is more effective in stimulating shoots (Harahap, 2006).

Based on observations of orchids that are 6 MST years old, the number of shoots produced does not show a significant difference. This is likely due to the interaction between ZPT and organic matter that does not work optimally in stimulating bud growth. The use of the 1st, 2nd, and 3rd leaves in the treatment also showed differences in response, where the 3rd leaf was less responsive to IBA and Coconut Water. This is due to the condition of the 3rd leaf meristematic tissue which is weaker compared to the 1st and 2nd leaves. I0A50 and I1A100 treatments showed that Coconut Water influenced the number of shoots, but only when combined with the right dose. A 50 ml/l Coconut Water concentration provides maximum results, while a concentration of 100 ml/l does not reach the optimal level in stimulating the desired number of buds. On the other hand, administration of IBA at a dose of 2 mg/l results in the maximum number of shoots. The combination of Coconut Water 100 ml/l and IBA 1 mg/l also showed optimal results for the number of shoots. In this process, cytokinins play an important role in cell division and are needed for the induction and multiplication of buds, as explained by Harahap (2008) and Dewi (2008).

Based on observations of orchids that are 6 MST old, the number of leaves formed does not show a significant difference. This is likely due to the interaction between ZPT and organic matter that is less than optimal in stimulating leaf growth. The use of the 1st, 2nd, and 3rd leaves in the treatment causes the 3rd leaf to be less responsive to IBA and Coconut Water, which can reduce the efficiency of the induction process. The I1A100 treatment produced the maximum number of leaves, which was 13 leaves, followed by the I2A100 treatment which produced 9 leaves, which shows the importance of the combination of IBA and Coconut Water doses in achieving optimal results. Meanwhile, the I1A0 treatment produced the least number of leaves, only 3 leaves, indicating that without the addition of Coconut Water, the results were not as good as with the combination. A dose of IBA of 2 mg/l was shown to give the best results in increasing the number of leaves, when compared to the less effective dose of 1 mg/l, as explained by Harahap (2008). Yunus (2007) also stated that cytokinins play a role in promoting an increase in leaf count, while Wethrell explains that kinetins can stimulate leaf formation. However, doses that are too high or too low can inhibit this process, demonstrating the importance of dose adjustment to achieve optimal results.

Based on observations of orchids aged 6 MST, the height of the formed plantlets did not show a significant difference, which may be due to the interaction between ZPT and organic matter that is not optimal in stimulating their growth. The use of the 1st, 2nd, and 3rd leaves in the treatment caused the 3rd leaf to be less responsive to the administration of IBA and Coconut Water, this may be due to differences in the activity of meristematic tissue in each leaf (Harahap, 2008).

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DOI: 10.25134/quagga.v17i1.360

The treatment with the combination of I0A50 resulted in a maximum plantlet height, which was 4.6 cm, followed by the I2A0 and I2A50 treatments which each produced a height of 4.2 cm. This shows that the use of Coconut Water with a concentration of 50 ml/l and IBA 2 mg/l has a significant effect on the increase in plantlet height. In contrast, the control treatment resulted in the lowest plantlet height, which indicates that without the use of ZPT, the growth is limited. IBA, which is a type of auxin commonly used in tissue culture, has been shown to stimulate root formation, but if used in excessive doses, it can cause inhibition of plant growth (Shofiana, 2013; Harahap, 2009). Young explants, which have an active cell network, are easier to grow and develop because their cells are more responsive to environmental stimuli, as explained by Harahap (2008).

4. CONCLUSION

Based on the results of the study, it can be concluded that the administration of IBA does not show a real effect on the growth of the number of shoots, the number of leaves, and the height of the plantlet but the administration of IBA 2 mg/l has the most dominant influence on the number of shoots and the number of leaves, while at the height of the plantlet, the administration of IBA 2 mg/l also shows better results. Likewise, coconut water does not have a real effect on the growth of the number of shoots, the number of leaves, and the height of the plantlet. Coconut water at a dose of 50 ml/l had the most dominant effect on the number of shoots and plant height, while a dose of 100 ml/l had the best effect on the number of leaves. The interaction between IBA and coconut water, especially in I2A50 treatment, did not show a significant effect on the growth of the number of shoots, the number of leaves, or the height of the plantlets. Overall, neither IBA nor coconut water, either separately or in combination, exerted no real effect on the observed parameters.

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