

EFFECT OF 2,4-D AND BAP ON IN VITRO INDUCTION OF ORCHID AXILLARY SHOOTS *Dendrobium sp.*

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Article Info	ABSTRACT
Article history: Received 13-12-2024 Revised 13-01-2025 Accepted 17-04-2025 Keyword: 2,4-Dichlorophenoxyacetic Axillary Buds Benzyl Amino Purine <i>Dendrobium sp.</i> Node Position Tissue Culture	Limited efficiency in conventional propagation and inconsistent responses to growth regulator combinations remain major constraints in large-scale orchid production, highlighting the need for optimized in vitro culture strategies. The market demand for orchids is increasing every year; however, this trend is not accompanied by a corresponding increase in orchid production in Indonesia. This study aimed to determine the effect of different combinations of growth regulator concentrations (2,4-D + BAP) and node positions on the induction of axillary shoots of <i>Dendrobium sp.</i> orchids under in vitro conditions, in order to identify the most effective culture medium composition to enhance orchid production in Indonesia. The experiment employed a two-factor design consisting of combinations of plant growth regulator (PGR) concentrations (2,4-D + BAP) and node positions (top, middle, and bottom). Axillary bud growth was evaluated based on bud emergence time, bud growth, leaf development, and root growth. These findings showed that the combination of 1 ppm 2,4-D + 2 ppm BAP in NP + AK medium was the most effective treatment, resulting in the fastest bud emergence and the highest values for bud number, bud diameter, fresh weight, number of leaves, leaf length and width, number of roots, and root length. In addition, the combination of 1.5 ppm 2,4-D + 1 ppm BAP produced the largest bud diameter, while the middle node position yielded the best overall response. No interaction was observed between PGR combinations and node positions with respect to bud height.
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1. INTRODUCTION

Orchids are among the most economically important ornamental plants worldwide, with consistently high market demand due to their aesthetic value and potential applications in medicinal and economic sectors. This growing interest has led to an increasing demand for orchids each year, making orchid cultivation a popular activity in horticultural agriculture. Data from the Central Statistics Agency (2018) indicate that orchid production in Indonesia showed a positive trend, increasing from 3.43 million stalks in 2000 to 24.70 million stalks in 2018, reflecting an average annual increase of approximately 1–2 million stalks.

Despite this trend, orchid production has not been able to keep pace with market demand. In 2020, national orchid demand reached approximately 15 million stalks, while total production accounted for only 11.68 million stalks, representing a 37% decline compared to 2019 production levels (Central Statistics Agency, 2020). This imbalance has resulted in unmet market demand and is further exacerbated by the limited availability of high-quality orchid seedlings. Prolonged propagation periods and various technical constraints in conventional cultivation methods hinder

large-scale production, highlighting the urgent need for efficient and reliable propagation techniques, such as optimized in vitro culture, to support sustainable orchid production.

Efforts to increase orchid production primarily depend on the availability of high-quality seedlings in sufficient quantities. Conventional propagation methods, including vegetative division and seed-based hybridization, are often constrained by slow multiplication rates, low uniformity, and limited scalability, making them inadequate to meet increasing market demand. Although hybridization techniques are widely used to generate new orchid varieties with superior physiological and biological traits, the subsequent propagation of selected elite genotypes remains a major bottleneck due to the lengthy propagation cycle and low propagation efficiency under conventional cultivation. To overcome these limitations, in vitro propagation offers a reliable alternative, as it enables the rapid and mass production of uniform plantlets within a relatively short period. In vitro culture provides controlled environmental conditions and allows the manipulation of plant growth through the addition of plant growth regulators (PGRs). The use of auxin and cytokinin combinations, particularly 2,4-D and BAP, plays a crucial role in regulating cell division, differentiation, and axillary shoot induction, making this approach suitable for optimizing orchid propagation efficiency.

According to Pierik (1997), auxin is a hormonal substance that can increase cell prolongation and division as well as the formation of adventitious roots. Auxin also has an influence on the formation of adventitious shoots and axillary shoots. The type of auxin used in this study is 2,4-D. The compound 2,4-D dichlorophenoxy acetic acid (2,4-D) is a potent auxin that is widely used in embryogenetic induction. According to Wattimena (2001), cytokinin is a plant hormone substance that has the function of accelerating cell division. Cytokinins will stimulate the growth of lateral shoots, prevent aging and increase the amount of chlorophyll. The type of cytokinin used in this study is 6-Benzyl Amino Purine (BAP). BAP is a cytokinin group of ZPT which is a synthetic ZPT that has stable properties, namely not easily broken down by heating in the sterilization process, and is relatively cheap BAP hormone has a function to increase cell division, shoot proliferation, and shoot morphogenesis (Zulkarnain, 2009).

Therefore, research is required to evaluate the effect of different combinations of 2,4-D and BAP on the induction of axillary shoots of *Dendrobium sp.* under in vitro conditions, in order to determine the optimal growth regulator concentrations for shoot induction in hybrid orchids derived from crosses between *Dendrobium* Black Apple and *Dendrobium* Vian. In addition to growth regulator composition, the position of the node used as an explant (top, middle, or bottom) is expected to influence axillary shoot induction due to differences in physiological maturity, endogenous hormone distribution, and meristematic activity; however, this factor has not been adequately addressed in previous studies.

2. RESEARCH METHOD

This study is an experimental study employing a two-factor factorial Randomized Complete Design (RCD) focusing on orchid propagation and cultivation in response to increasing market demand for orchids. The research examines the effect of combinations of 2,4-D and BAP on the induction of axillary shoots of *Dendrobium sp.* under in vitro conditions, with the aim of determining the optimal growth regulator concentrations for enhancing axillary bud induction.

This study is an experiment using a 2-factor factorial RCD, namely: Variation in the combination of ZPT 2,4-D + BAP concentration and orchid node position *Dendrobium sp.* The combination variation of ZPT 2,4-D + BAP concentration consisted of 5 concentration combination treatments. The variation in the position of the node consists of 3 positions. Each treatment was repeated 5 times using a jar bottle. So there are a total of 25 jars. In each jar bottle, 3 orchid node explants *Dendrobium sp.* of the 3 node positions on the plant source of the explant. Thus, there are 75 explants of *Dendrobium sp.* orchid.

Table 1. Treatment Plan

Code of Conduct	Node Position	2,4-D (ppm)	BAP (ppm)
A	Upper	0	4
	Middle	0	4
	Lower	0	4
B	Upper	0,5	3
	Middle	0,5	3
	Lower	0,5	3
C	Upper	1	2
	Middle	1	2
	Lower	1	2
D	Upper	1,5	1
	Middle	1,5	1
	Lower	1,5	1
E	Upper	2	0
	Middle	2	0
	Lower	2	0

Culture work procedure in vitro conducted using a table Laminar Air Flow (LAF) in a sterile room, using a sterile device that has been autoclaved at a temperature of 121°C for 30 minutes at a pressure of 1 atm, and is carried out aseptically and sterilely. In addition, the explants used have been prepared in advance by having character uniformity, namely having 3 nodes to be cut into 3 parts.

This study used the treatment medium of New Phalaenopsis (NP) + coconut water (AK) coupled with a variation of the combination of ZPT 2,4-D + BAP concentrations. To make 100 ml of medium, an NP medium of 2.22 grams (22.2 g.L-1) is required. Sterile aqueducts of 70 ml are put into the Erlenmeyer and then NP medium is added. The medium is heated using a hotplate magnetic stirrer. 15 ml of coconut water is added and then homogenized (150 ml.L-1). Growth regulators 2,4-D and BAP were added to the NP+AK medium according to the combination of ZPT concentrations in the study design. After that, 0.325 grams of norite is added and homogenized. then an acidity test was carried out using pH paper. The pH of the medium should be between 5 and 6.

Sterile aqueducts were then added up to 100 ml and then 0.7 grams (7 g.L-1) agarose was added. The medium is homogenized and heated to a boil. It was then sterilized using an autoclave at a temperature of 121°C with a pressure of 1 atm for 15 minutes. The sterilized medium is then poured into jars and petri dishes with a volume of 20 ml/bottle and petri dishes. The medium is stored in the storage room for 1 week before use to detect contamination. Work procedure for planting orchid explants *Dendrobium sp.* carried out aseptically and sterilely on a Laminar Air Flow table. The explants are removed from the culture bottle and placed in a petri dish and then separated from the other explants, also the medium that is still attached to the explant. The explant is cut at the position of the upper (a), middle (b), and lower (c) nodes, each of which is 1 node. Nodes of the same explant are planted on the medium in a jar bottle.

After inoculation, the culture bottles were placed on cleaned incubation racks and arranged according to a previously established completely randomized design. The cultures were incubated at a controlled room temperature is set to 18°C. under a photoperiod of 16 h light and 8 h dark, with a light intensity of approximately 1,000–2,000 lux provided by cool white fluorescent lamps. Observations were conducted once a week on the same day for a period of 90 days. Parameters observed included the time of bud emergence, number of shoots, number of leaves, and number of roots. On the 90th day, final growth measurements were recorded, including the number of shoots, shoot height, number of leaves, leaf length, leaf width, stem diameter, number of roots, root length, and root diameter.

The data analysis was performed using non-parametric statistical methods because the data did not meet the normality assumption based on preliminary normality testing. Differences among treatments were analyzed using the Kruskal–Wallis H test, with significance determined at $p < 0.05$. All statistical analyses were conducted using SPSS software. Descriptive statistics were also applied to summarize and present detailed information for each observed parameter.

3. RESULTS AND ANALYSIS

3.1. Result

The results showed that the addition of a combination of 1 ppm 2,4-D + 2 ppm BAP in the NP + AK medium was the best combination of ZPT concentrations because it was able to induce the fastest bud emergence time, number of buds, bud diameter, wet weight, number of leaves, leaf length, leaf width, number of roots, and root length was the best. The combination of 1.5 ppm 2,4-D + 1 ppm BAP yields the best bud diameter. The best node position is the middle node position.



0 ppm 2,4-D + 4 ppm BAP
lower node



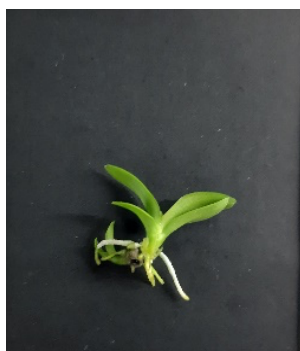
0 ppm 2,4-D + 4 ppm BAP
middle node



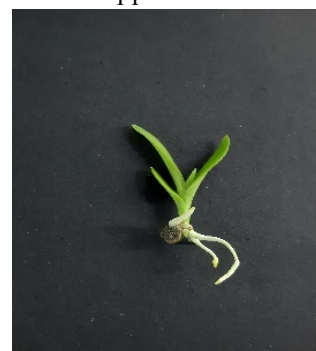
0 ppm 2,4-D + 4 ppm BAP
upper node



0.5 ppm 2,4-D + 3 ppm BAP
lower node



0.5 ppm 2,4-D + 3 ppm BAP
middle node



0.5 ppm 2,4-D + 3 ppm BAP
upper node



1 ppm 2,4-D + 2 ppm BAP
lower node



1 ppm 2,4-D + 2 ppm BAP
middle node



1 ppm 2,4-D + 2 ppm BAP
upper node

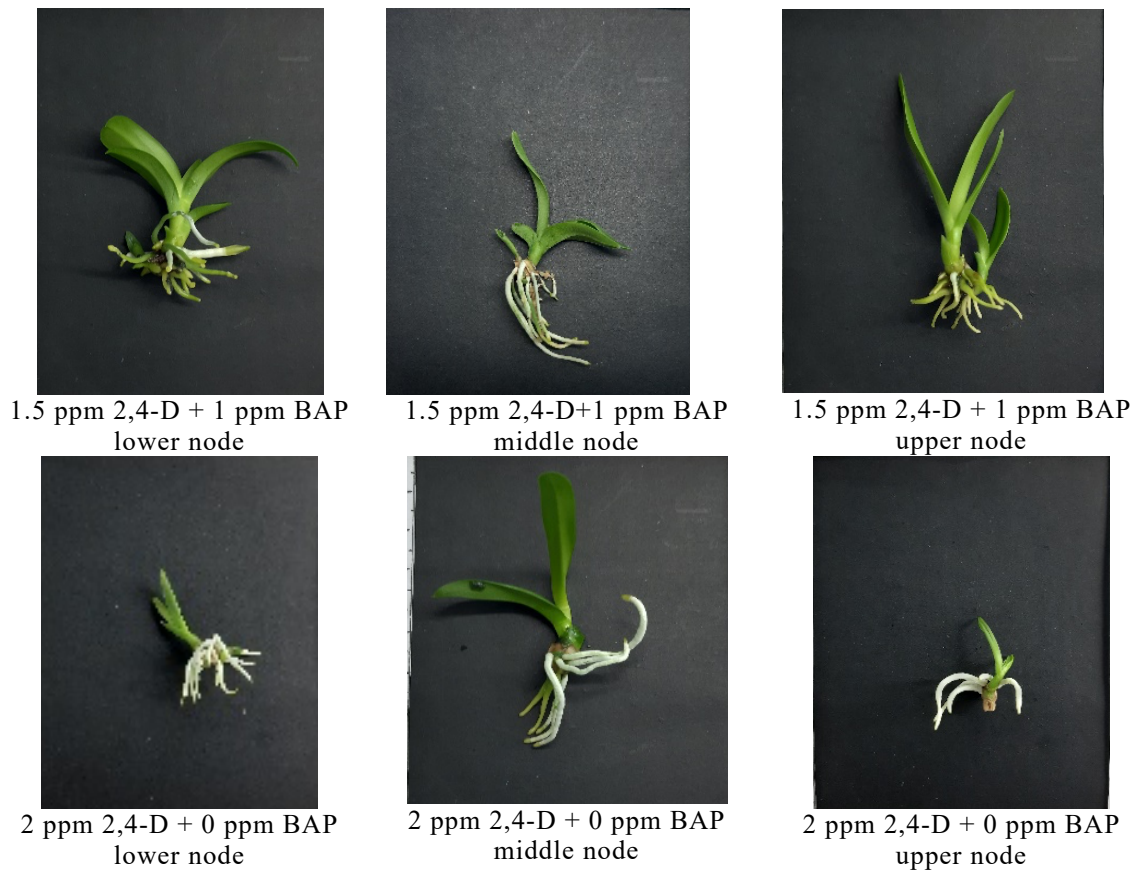


Figure 1. Harvest of orchid axillary bud growth *Dendrobium sp.* with a measuring scale of 4 cm using the scale bar.

Table 1. Time data on the emergence of orchid buds *Dendrobium sp.*

Table 1. Time data on the emergence of <i>Bombus terrestris</i> sp.						
Node Position	Average Length of Bud Emergence Time (days)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	5,6	5,6	15,4	9,8	7	1,24
Middle	11,2	19,6	19,6	-	7	1,64
Upper	5,6	-	23,8	4,2	5,6	1,12
Average	7.4	8.4	19.6s	4,6	6.5	

Table 2. Descriptive Test and Test Results *Kruskall-Wallis H* Bud Emergence Time

Source of Deviation	Value
Average	9,333
Mode (without 0)	21
Variansi	173,928
Standard Deviation	13,188174
Asymp.Sig Combination Concentrate	0,018
Asymp.Sig Node Position	0,595

Table 3. Data on the number of orchid buds *Dendrobium sp.*

Table 3. Data on the number of shoots buds <i>Denaria sp.</i>						
Node Position	Average Number of Shoots (shoots)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,60	0,20	0,80	0,80	0,40	0,56
Middle	1,00	1,40	1,40	0	0,20	0,80
Upper	0,40	0	1,00	0,40	0,20	0,40
Average	0,67	0,63	1,06s	0,40	0,26	

Table 4. Descriptive Test and Test Results *Kruskall-Wallis H* Number of Buds

Source of Deviation	Value
Average	0,58667
Mode (without 0)	1
Variansi	0,840
Standard Deviation	0,916712
Asymp.Sig Combination Concentrate	0,040
Asymp.Sig Node Position	0,367

Table 5. High data of orchid buds *Dendrobium sp.*

Table 3. High data of blend buds <i>Delonix regia</i> sp.						
Node Position	Average Bud Height (cm)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,05	0,09	0,23	0,20	0,11	0,13
Middle	0,18	0,05	0,36	0	0,22	0,34
Upper	0,09	0	0,31	0,16	0,11	0,13
Average	0,11	0,34	0,30	0,12	0,15	

Table 6. Descriptive Test Results and *Uji Kruskall-Wallis H* Bud Height

Source of Deviation	Value
Average	0,207
Mode (without 0)	0,4 – 0,6
Variansi	0,141
Standard Deviation	0,375608
Asymp.Sig Combination Concentrate	0,069
Asymp.Sig Node Position	0,295

Table 7. Orchid bud diameter data *Dendrobium sp.*

Node Position	Average Bud Diameter (cm)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,04	0,06	0,23	0,13	0,07	0,11
Middle	0,16	0,23	0,31	0	0,08	0,15
Upper	0,04	0	0,27	0,07	0,05	0,09
Average	0,08	0,09	0,27	0,07	0,07	

Table 8. Descriptive Test and Test Results *Kruskall-Wallis H* Diameter Tunas

Source of Deviation	Value
Average	0,12113
Mode (without 0)	0,3
Variansi	0,033
Standard Deviation	0,180314
Asymp.Sig Combination Concentrate	0,006
Asymp.Sig Node Position	0,392

Table 9. Wet weight data of orchid buds *Dendrobium sp.*

Node Position	Average Wet Weight of Buds (grams)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,07	0,07	0,44	0,43	0,14	0,23
Middle	0,25	0,22	0,46	0,12	0,34	0,28
Upper	0,06	0,03	0,26	0,40	0,24	0,20
Average	0,13	0,11	0,39s	0,32	0,24	

Table 10. Descriptive Test and Test Results *Kruskall-Wallis H* Heavy Wet Buds

Source of Deviation	Value
Average	0,24049
Mode (without 0)	0,014 – 0,082
Variansi	0,080
Standard Deviation	0,282000
Asymp.Sig Combination Concentrate	0,027
Asymp.Sig Node Position	0,637

Table 11. Data on the number of leaves of orchid buds *Dendrobium sp.*

Table 11: Data on the number of leaves of <i>Ornithoglossum densifolium</i> sp.						
Node Position	Average Number of Leaves (leaves)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	1,80	1	3,80	2,20	0,80	1,92
Middle	3,40	4,80	4,80	0	0,40	2,68
Upper	1	0	2,80	1,20	0,60	1,12
Average	2.06	1.93	3.80s	1.13	0.60	

Table 12. Descriptive Test and Test Results *Kruskall-Wallis H* Number of Leaves

Source of Deviation	Value
Average	1,90667
Mode (without 0)	5-6
Variansi	9,761
Standard Deviation	3,124331
Asymp.Sig Combination Concentrate	0,002
Asymp.Sig Node Position	0,366

Table 13. Leaf length data of orchid buds *Dendrobium sp.*

Node Position	Average Leaf Length (cm)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,29	0,23	1,35	1,11	0,32	0,66
Middle	0,72	1,64	1,80	0	0,89	1,60
Upper	0,34	0	1,05	0,76	0,36	0,50
Average	0,45	0,62	1,43s	0,62	0,50	

Table 14. Descriptive Test and Test Results *Kruskall-Wallis H* Leaf Length

Source of Deviation	Value
Average	0,73327
Mode (without 0)	1-2
Variansi	1,265
Standard Deviation	1,124599
Asymp.Sig Combination Concentrate	0,025
Asymp.Sig Nodusnns Position	0,274

Table 15. Orchid bud leaf width data *Dendrobium sp.*

Node Position	Average Leaf Width (cm)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	0,07	0,09	0,36	0,32	0,08	0,18
Middle	0,26	0,46	0,51	0	0,17	0,28
Upper	0,11	0	0,42	0,17	0,08	0,15
Average	0,14	0,18	0,43s	0,16	0,11	

Table 16. Descriptive Test and Test Results *Kruskall-Wallis H* Leaf Width

Source of Deviation	Value
Average	0,21053
Mode (without 0)	0,5 – 0,6
Variansi	0,092
Standard Deviation	0,302801
Asymp.Sig Combination Concentrate	0,018

Table 17. Data on the number of root buds of orchids *Dendrobium sp.*

Asymp.Sig Node Location						0,322
Table 17. Data on the number of root buds of orchids <i>Dendrobium</i>						
Node Position	Average Number of Roots (roots)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	1,60	1,80	5,40	4,40	2	3,08
Middle	2,80	5	6	0	2	3,16
Upper	0,60	0	1,80	3,60	1	1,40
Average	1,67	2,26	4,40	2,73	1,67	

Table 18. Descriptive Test and Test Results *Kruskall-Wallis H* Number of Roots

Source of Deviation	Value
Average	2,54667
Mode (without 0)	8
Variansi	18,440
Standard Deviation	4,294224
Asymp.Sig Combination Concentration	0,043
Asymp.Sig Node Location	0,186

Table 19. Root length data of orchid buds *Dendrobium sp.*

Node Position	Average Root Length (cm)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	2,20	1,44	15,70	7,16	2,54	5,81
Middle	3,91	6,05	11,78	0	3,84	5,12
Upper	0,62	0	4,88	3,01	1,71	2,04
Average	2,24	2,50	10,79s	3,39	2,70	

Table 20. Descriptive Test and Test Results *Kruskall-Wallis H* Root Length

Source of Deviation	Value
Average	4,32695
Mode (without 0)	10-15
Variansi	62,514
Standard Deviation	7,906570
Asymp. Sig Combination Concentrate	0,025
Asymp.Sig Node Position	0,298

Table 21. Orchid bud root diameter data *Dendrobium sp.*

Node Position	Average Root Diameter (root)					Average
	Combination of 2,4-D + BAP (ppm)					
	0 – 4	0,5 – 3	1 – 2	1,5 – 1	2 – 0	
Lower	1,60	1,80	5,40	4,40	2	0,10
Middle	2,80	5	6	0	2	0,07
Upper	0,60	0	1,80	3,60	1	0,03
Average	1,67	2,26	4,40	2,73	1,67	

Table 22. Descriptive Test and Test Results *Kruskall-Wallis H* Diameter Akar

Source of Deviation	Value
Average	0,07107
Mode (without 0)	1,6 – 1,8
Variansi	0,025
Standard Deviation	0,158733
Asymp.Sig Combination Concentrate	0,361
Asymp.Sig Node Position	0,303

3.1. Discussion

a. Effect of Variation in 2,4-D + BAP Concentration and Node Position on the Emergence of *Dendrobium sp.* Orchid Buds.

The time of sprouting for all the treatments tried occurred almost simultaneously, between the 21st day and the 28th day after planting with a total number of non-growing explants reaching 49 explants. The addition of a combination of 1 ppm 2,4-D + 2 ppm BAP is able to induce orchid shoots the fastest and maximum, namely 21 days after planting, so as to cause an acceleration of bud formation while being able to maximize bud growth as evidenced by the number of shoots that grow at this concentration, which is 9 shoots. Meanwhile, in other concentration combinations, although the time of emergence of shoots is faster, the number of shoots that grow is only 4.5 shoots. Not as much as in the variation of the concentration combination of 1 ppm 2,4-D + 2 ppm BAP. According to Mahadi (2016), shoots are formed due to cell lengthening, cell division, morphogenesis and growth regulation are important processes that are followed by the formation of shoots. This shows that the formation of buds requires auxin ZPT (including 2,4-D) and cytokinin (including BAP).

Based on the position of the nodus, the fastest bud emergence time tends to be in the position of the upper node, which is 5.6 days after planting. However, this is not in accordance with the results of Dwiyani (2016) research which shows that in *Vanda tricolor* Lind orchids, the explant slices at the base of the stem provide the best results for the time of bud emergence compared to the base of the leaves, the tips of the leaves and the tips of the stems.

b. Effect of Variation in 2,4-D + BAP Concentration and Node Position on the Growth of *Dendrobium sp.* Orchid Shoots.

Effect of variation in 2,4-D + BAP concentration and node position on the growth of *Dendrobium sp.* orchid shoots. It has several parameters including the number of buds, bud height, bud diameter, and wet weight. The results of the non-parametric *Kruskal-Wallis H* test showed that there was a difference between several bud growth parameters of *Dendrobium sp.* orchids to the addition of variations in concentration of 2,4-D + BAP, namely the number of shoots, the diameter of shoots and the wet weight. While one other parameter, namely the height of the shoot, does not show a difference in the variation in the concentration of 2,4-D + BAP and the position of the node.

Number of Buds

Based on the data obtained, the two concentration combinations that were able to produce the highest number of shoots were the combination of lower auxins and higher cytokinins, namely the addition of a combination of 1 ppm 2,4-D + 2 ppm BAP to the average total number of buds growing as much as 1.06 buds and 0.5 ppm 2,4-D + 3 ppm BAP with an average total number of buds of 0.53 buds. This is in accordance with the opinion of Wattimena (2002) who states that low auxin and higher cytokinin administration tend to promote the formation of adventitious shoots and the proliferation of axillary shoots.

Based on the position of the nodus, the number of axillary shoots of the *Dendrobium sp.* The highest tends to be in the position of the middle node, which is 0.80 buds. This is inconsistent with the theory that the number of shoots is strongly influenced by the position of the explant. The older the age of the explant tissue, the more shoots are produced. The number of shoots is also affected by the initial phase of growth. Shoots will undergo a callus formation phase first while *internodes* and axillary shoots directly form shoots (Saputri, 2019).

Bud Height

The height of the orchid buds that grew in this study was quite varied between each variation of the concentration of 2,4-D + BAP. However, based on this study, the addition of variations in the concentration of 2,4-D + BAP did not have a significant effect on the height growth of shoots. On the other hand, the position of the nodes and the interaction between hormones and nodes have a real and significant effect. The addition of a combination of 0.5 ppm 2,4-D + 3 ppm BAP concentrations showed results that tended to be higher than other combinations with an average height of 0.34 cm of sprouts. Meanwhile, the addition of a combination of 0 ppm 2,4-D + 4 ppm BAP concentrations showed the worst results with the average height of buds growing only 0.11 cm.

Based on the position of the nodus, the best bud growth tends to be in the central node position with a high growth of 0.34 cm. The high growth rate of shoots in the middle node is because the middle node is more responsive to growth signals than the upper and lower nodes. In the upper node, the growth height of the shoots is less than optimal due to the young age of the nodes, so the absorption of nutrients is used to mature the cells in the nodes first before being used to grow shoots.

Bud diameter

The diameter of the buds that grew in this study was quite diverse between each variation of the concentration of 2,4-D + BAP. However, based on this study, the addition of a concentration variation of 1 ppm 2,4-D + 2 ppm BAP had a real effect on the growth of bud diameter with the best result of 0.27 cm. Meanwhile, four other concentration combinations were seen to produce a fairly poor orchid bud diameter with concentrations of 1.5 ppm, 2,4-D + 1 ppm BAP, and 2 ppm 2,4-D + 0 ppm BAP, being the concentration with the worst bud diameter because it only produced a similar average growth of 0.07 cm. This is in accordance with the opinion of Darmanti (2008), an increase in cytokinin levels can spur the improvement of the vascular bundle between the lateral shoots and the plant stem. When the cambium fusiform initials form the xylem and secondary phloem, the diameter of the plant stem will increase. The formation of this secondary vascular network increases the diameter of the plant.

Based on the position of the node, the best diameter growth is found in the position of the central node with a height growth of 0.15 cm. This shows that the central node explant has the appropriate endogenous hormone content so that it can increase the growth of the axillary diameter of the orchid *Dendrobium sp.* Chambell and Reece (2012) stated that the position of the explant, the composition of the medium and the environment affect the swelling of the explant stem. Endogenous auxin and cytokinin hormones can affect cell division.

Wet Weight

The wet weight of orchids grown in this study was quite varied between each variation of 2,4-D + BAP concentration. However, based on this study, the addition of a concentration variation of 1 ppm 2,4-D + 2 ppm BAP gave the best results with an average wet weight of 0.39 grams. Meanwhile, the two concentration combinations were seen to produce a fairly poor orchid wet weight with concentrations of 0.5 ppm, 2,4-D + 4 ppm, BAP, and 0 ppm, 2,4-D + 3 ppm, BAP, being the concentration with the worst wet weight results, because it only produced a fairly similar average wet weight, namely 0.11 grams and 0.13 grams. Based on the data produced, it can be seen that the higher the addition of 2,4-D, the better the wet weight of the orchid produced. Reached its optimum point at a combination of 1 ppm 2,4-D + 2 ppm BAP and began to decline again when the dose of 2,4-D was increased. This is in accordance with the opinion in Mastuti's research. (2018) which states that the administration of 2,4-D as an exogenous auxin source can initiate the formation of calluses and cells in plants. Giving Auxin at the right concentration can give the best effect, but too high an auxin concentration will not have an effect on the plant's weight.

Based on the position of the node, the best wet weight tends to be in the position of the middle node with an average wet weight of 0.28 grams. Then followed by the lower node with an average wet weight of 0.23 grams and finally the upper node with an average wet weight of 0.20 cm.

c. Effect of Variation in Concentration of 2,4-D + BAP and Node Position on the Growth of Orchid Buds *Dendrobium sp.*

Effect of variation in 2,4-D + BAP concentration and node position on the growth of orchid bud leaves *Dendrobium sp.* It has several parameters including the number of leaves, leaf length, and leaf width. The results of the non-parametric *Kruskal-Wallis H* test showed that there was a difference between all the growth parameters of *Dendrobium sp* orchid shoots to the addition of 2,4-D + BAP concentration variations, namely the number of leaves, the length of leaves and the width of leaves.

Number of Leaves

Based on the results of the descriptive test, the addition of a concentration variation of 1 ppm 2,4-D + 2 ppm BAP gave the best results with an average number of leaves of 3.80 leaves. After that, the number of leaves in the concentration combination tends to decrease as the concentration of 2,4-D increases. This is in accordance with the opinion of Wattimena (2001) who states that 2,4-D exogenous will affect endogenous auxin levels, giving ZPT at a concentration of 0.5 ppm to 1 ppm will stimulate growth, because it changes the level of endogenous hormones (Wattimena, 2001).

Based on the position of the nodes, the greatest number of leaves tends to be found in the position of the central node with an average number of leaves of 2.68 leaves. Then followed by the lower node with an average number of leaves of 1.92 leaves and finally the upper node with an average number of leaves of 1.12 leaves.

Leaf Length

The effect of applying ZPT concentration on the growth of the leaf length of the orchid *Dendrobium sp.* increased starting from the beginning of the addition of concentration variations of 0 ppm 2,4-D + 4 ppm BAP until reaching its optimal point at a combination of 1 ppm 2,4-D + 2 ppm BAP with a leaf length of 1.43 cm and began to decrease in concentration variations afterwards. At a concentration of 1 ppm 2,4-D + 2 ppm BAP there is a balance between endogenous hormones and exogenous hormones added to the medium as well as a balance between auxins and cytokinins added. This makes the average leaf length result in the combination of concentrations the best compared to other concentration combinations. The results of Alitalia (2008) research stated that the administration of BAP at concentrations of 0.5 ppm and 1 ppm resulted in a maximum leaf length of 19.8 mm compared to the use of 2 ppm BAP which only produced a leaf length of 9.1 mm in *Nepenthes mirabilis shoots*.

Based on the position of the nodus, the middle is the position of the nodes that tends to grow the best in leaf length with an average leaf length of 1.02 cm. This shows that the central node has a hormonal balance so that it can spur leaf growth optimally. This is in accordance with the opinion of Taiz and Zeiger (2002) who stated that the length of the leaves produced by each explant can vary depending on its position. The farther the leaf is from the shoot, the longer the leaf will be, because the leaves that are close to the shoots are young leaves that will continue to grow to a certain leaf length while the leaves that are far from the shoots are mature leaves so that their growth is maximized and cannot grow any longer.

Leaf Width

The addition of 2,4-D + BAP to the whole treatment has a different effect on leaf width. The effect of applying ZPT concentration on the leaf width growth of *Dendrobium sp. orchids*. increased starting from the beginning of the addition of the concentration variation of 0 ppm 2,4-D + 4 ppm BAP until reaching its optimal point at the concentration combination of 1 ppm 2,4-D + 2 ppm BAP with a leaf length of 0.43 cm and began to decrease in concentration variation afterwards. At a concentration of 1 ppm 2,4-D + 2 ppm BAP there is a balance between auxins and cytokinins added. This makes the average leaf length result in the combination of concentrations the best compared to other concentration combinations.

Based on the position of the nodus, the best leaf width tends to be in the central node position with an average leaf width of 0.28 cm. Then followed by the upper node with an average leaf width of 0.18 cm and finally the lower node with an average leaf width of 0.15 cm.

d. Effect of Variation in 2,4-D + BAP Concentration and Node Position on the Root Growth of *Dendrobium sp.* Orchid Shoots.

Effect of variation in 2,4-D + BAP concentration and node position on root growth of *Dendrobium sp. orchid shoots*. It has several parameters including the number of roots, root length, and root diameter. The results of the non-parametric *Kruskal-Wallis H* test showed that there was a difference between several parameters of the root growth of *Dendrobium sp orchid shoots* to the addition of variations in concentration of 2,4-D + BAP, namely the number and length of the roots.

Meanwhile, the root diameter showed no difference in the variation in the concentration of 2,4-D + BAP and nodus.

Number of Roots

Based on the results of this study, the addition of a concentration variation of 1 ppm 2,4-D + 2 ppm BAP gave the best results with an average number of roots of 4.40 roots. Meanwhile, the combination of concentrations that produced the least average number of orchid bud roots was a concentration of 2 ppm 2,4-D + 0 ppm BAP and 0 ppm 2,4-D + 4 ppm BAP by only producing the average number of roots of the same orchid shoots, which was 1.67 roots. This is also in accordance with research on Mokara orchid explants, the highest number of roots was found in a combination of treatment of 1 ppm BAP + 1 ppm NAA + 0.1 ppm 2,4-D with an average number of roots of 6.25 roots (Widiastoety, 2014).

Based on the position of the nodes, the average number of roots tends to be in the middle node with an average of 3.16 roots, followed by the lower node with an average number of 3.08 roots and the lower node with an average number of 1.40 roots. According to Mansur (2019), auxins given to cuttings of young plant tissue can stimulate root release, but in old tissues it only stimulates cell division.

Root Length

The root length of orchid shoots grown in this study was quite varied between each variation of 2,4-D + BAP concentration. Based on the results of this study, the addition of a concentration variation of 1 ppm 2,4-D + 2 ppm BAP gave the best results with an average root length of 10.39 cm. Meanwhile, the combination of concentrations that produced the shortest average root length was 0 ppm 2,4-D + 4 ppm BAP with only an average root length of 2.24 cm of orchid shoots. The addition of 2,4-D exerts a diverse influence on root length. Treatment of 1 ppm 2,4-D tends to give the best results for root length which is 11.92 cm. This is in accordance with the results of a study (Yusnita et al., 2014) that 1 ppm of 2,4-D added to the medium can increase the growth of orchid plantlets *Cattleya* sp. shown by the highest results in the variables of plantlet height of 5.67 cm, root length of 2.07 cm, and wet weight of 0.36 g.

Based on the position of the nodus, the root length of the orchid shoots is best suited to be in the position of the lower node with an average root length of 5.81 cm. This is in accordance with the opinion of Djumali (2012) who states that auxin hormone plays a role in cell lengthening, auxin is found at the growth point of plants, namely the end of the root and the end of the stem (Djumali, 2012).

Root Diameter

The root diameter of the orchid buds that grew in this study was quite diverse between each variation of the concentration of 2,4-D + BAP. Based on the results of this study, the root diameter of orchid shoots is best compared to a combination of 1.5 ppm 2,4-D + 1 ppm BAP with an average diameter of 0.10 cm, then followed by a combination of 1 ppm 2,4-D + 2 ppm BAP with an average diameter of 0.09 cm of orchid bud roots. This proves that a balance between auxins and cytokinins is needed to maximize the growth of the root diameter because when one of the auxins or cytokinins is not added which ends up making one of them dominant, the root diameter result cannot be maximized.

Based on the position of the nodes, the root diameter of the orchid shoots is best found in the position of the lower node with an average root diameter of 0.10 cm. Then followed by the middle node with an average root diameter of 0.07 cm and finally the upper node with an average root diameter of 0.03 cm. young tissues has a greater auxin content than young tissues were auxins trigger root growth. Auxin hormone plays a role in cell lengthening, auxin is found at the plant growth point, namely the root end and stem end (Djumali, 2012).

4. CONCLUSION

The results of this study demonstrate that the combination of 1 ppm 2,4-D and 2 ppm BAP in the NP + AK medium is the most effective treatment for inducing axillary shoot growth in *Dendrobium* sp. under in vitro conditions, as it produced the fastest bud emergence, the highest number of shoots, greater shoot diameter, higher fresh weight, increased leaf number and size, as well as superior root number and length. In addition, the middle node position was identified as the most suitable explant source for axillary shoot induction. Future studies are recommended to evaluate the long-term growth stability and acclimatization success of plantlets produced using this treatment, as well as to explore alternative growth regulator combinations and concentrations to further optimize large-scale orchid propagation.

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