

Effect of Kinetin and 2,4-D Plant Growth Regulators on *In Vitro* Subculture Growth of *Dendrobium stockelbuschii*

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ABSTRACT

Dendrobium orchids are a popular genus due to their adaptability and wide variation in flower shapes and colors. Conventional propagation methods face challenges in producing large quantities of high-quality seedlings. This study aimed to produce superior-quality seedlings through tissue culture techniques using ½ Murashige and Skoog (MS) medium supplemented with kinetin and 2,4-D to enhance growth. The research was conducted at the Plant Physiology and Biotechnology Laboratory, Sebelas Maret University, from February to July 2024. The experimental design used was a completely randomized design (CRD) with two factors. The first factor was kinetin concentration (0, 0.5, 1, 1.5, 2 ppm), and the second factor was 2,4-D concentration (0, 0.5, 1, 1.5, 2 ppm). A total of 25 treatment combinations were tested, each replicated three times, resulting in 75 experimental units. Observed parameters included the number of roots, plant height, number of shoots, and root length. Data were analyzed using analysis of variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) for mean separation. The results showed that kinetin had a significant effect on the number of roots and plant height, while 2,4-D significantly affected root length. No significant interaction was observed between the two factors.

Keywords: Auxin; Cytokinin; Orchid; Ornamental crop; Tissue culture

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INTRODUCTION

Orchids are widely known as ornamental plants. They are among the most popular ornamental commodities due to their high aesthetic value, with a great diversity of shapes and colors. According to Anggraeni (2022), orchids have the largest number of species compared to other ornamental plants. These plants can survive in a wide range of temperatures, from subzero conditions to high desert heat. The long-lasting nature of orchid flowers is another reason they are favored as ornamental plants. There are already many naturally occurring orchid species, and the propagation process, often carried out through crossbreeding, has produced even more varieties. This has resulted in new orchid types with unique flower colors and forms, ensuring their continued popularity among consumers. The color range of *Dendrobium* orchids is particularly diverse. In general, hybrid orchids display colors such as light purple, white, golden yellow, or combinations of these hues (Widiastoety et al., 2010). The increasing market demand for orchids continues to encourage producers to maintain a steady supply. Orchid production in Indonesia between 2014 and 2018 showed fluctuations but still

indicated a strong market demand (Banu et al., 2023). Traditional cultivation techniques are often insufficient to meet this demand, highlighting the need for propagation methods capable of producing large quantities of plants in a shorter time. The tissue culture method offers an effective solution, as it can generate many seedlings using a limited number of parent plants and within a relatively short period (Basri, 2016). Thus, tissue culture is considered a suitable cultivation technique for orchid production.

The tissue culture technique is a cultivation method that produces new plants from isolated parts of existing plants, which are then grown in a specially formulated medium that stimulates growth. Plant tissue culture involves growing and developing plant parts such as cells, tissues, or organs, under aseptic conditions and *in vitro* (Marpaung et al., 2019). The plant parts that can be used in tissue culture include stems, leaves, roots, and shoots. Whole plants can be regenerated from living plant parts or sections of roots, stems, or leaves known as explants (Putri et al., 2021). Plant growth in tissue culture is strongly influenced by the composition of the

culture medium. Various types of media can be used in this technique, each with specific benefits, and the selection of a suitable medium depends on the desired growth parameters. Generally, culture media contain macro and micronutrients as well as plant growth regulators, particularly auxins and cytokinins. The source of plantlets and the culture medium are key factors determining the success of plant propagation through tissue culture. Numerous media formulations have been developed to optimize the growth and development of cultured plants (Supatmi et al., 2018). Auxins and cytokinins, as plant growth regulators, can be derived from synthetic or organic sources. Modifying the culture medium by adding these growth regulators is necessary to increase the success rate of tissue culture. Two types of plant hormones, auxins and cytokinins are commonly used in in vitro propagation (Rosita et al., 2015). One of the auxin types widely used is 2,4-D, while kinetin represents the cytokinin group.

The combination of auxin and cytokinin plant growth regulators added to the medium is one of the main factors determining the success of tissue culture. The balance and concentration of these hormones play a crucial role in regulating in vitro plant development (Damanik et al., 2017). Auxins are plant growth regulators involved in root and callus formation, as well as cell division and enlargement. They influence cell division, elongation, tissue expansion, and root formation. At high concentrations, auxins promote callus formation but inhibit shoot and root growth (Rahman et al., 2021). One of the most commonly used auxins in plant tissue culture is 2,4-dichlorophenoxyacetic acid (2,4-D). This type of auxin is often applied because of its stability, resistance to enzymatic degradation within plant cells, and tolerance to high temperatures during sterilization. Cytokinins play an essential role in promoting cell division and elongation, thereby accelerating plant growth and development. Cytokinins stimulate cytokinesis, encourage plant development, and delay senescence by regulating the degradation processes that lead to cell death (Ernita et al., 2023). One of the cytokinin types frequently used in tissue culture is kinetin, which effectively promotes cell division and morphogenesis. This study aims to determine the effect of kinetin and 2,4-D plant growth regulators on the subculture growth of *Dendrobium stockelbuschii*.

MATERIALS AND METHOD

Time and Place

This research was conducted from February 2024 to July 2024, located at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta.

Tools and Materials

The tools and materials used in this study included an autoclave, laminar air flow (LAF), Erlenmeyer flask, Bunsen burner, culture bottles, analytical balance, Petri dish, measuring cylinder, scalpel, tweezers, oven, sprayer, pH meter, tissue paper, magnetic stirrer, and filter paper, along with *Dendrobium stockelbuschii* orchid plantlets, agar, sugar, NaOH and HCl solutions, 70% alcohol, kinetin, 2,4-D, coconut water, banana, activated charcoal, Fe EDTA, vitamin solution, and micro and

macro nutrient solutions.

Research Design

This study used a Completely Randomized Design (CRD) with a factorial arrangement (two factors). The first factor was the concentration of kinetin (K), consisting of five levels: 0, 0.5, 1, 1.5, and 2 ppm. The second factor was the concentration of 2,4-D (D), also consisting of five levels: 0, 0.5, 1, 1.5, and 2 ppm. The experiment included 25 treatment combinations with three replications. Each replication consisted of one plantlet, resulting in a total of 75 plantlets used in the experiment. The combination treatments of kinetin and 2,4-D growth regulators are shown in Table 1.

Table 1. Combination of Kinetin and 2,4-D Growth Regulator Treatments

K/D	K1	K2	K3	K4	K5
D1	K1D1	K2D1	K3D1	K4D1	K5D1
D2	K1D2	K2D2	K3D2	K4D2	K5D2
D3	K1D3	K2D3	K3D3	K4D3	K5D3
D4	K1D4	K2D4	K3D4	K4D4	K5D4
D5	K1D5	K2D5	K3D5	K4D5	K5D5

Notes: K1: without Kinetin; K2: Kinetin 0.5 ppm; K3: Kinetin 1 ppm; K4: Kinetin 1.5 ppm; K5: Kinetin 2 ppm; D1: without 2,4-D; D2: 2,4-D 0.5 ppm; D3: 2,4-D 1 ppm; D4: 2,4-D 1.5 ppm; D5: 2,4-D 2 ppm.

Research Implementation

Tools Sterilization, The tools used, such as scalpels, tweezers, Petri dishes, and culture bottles, were washed with soap and running water, then dried and wrapped in newspaper before sterilization. Other materials, such as tissue and filter paper, were placed directly into culture bottles. The sterilization process included sterilizing the planting tools, culture bottles, and the *Laminar Air Flow* (LAF). Contaminated bottles were cleaned, and contaminated media were disposed of properly (Firmani et al., 2024).

Preparation of Stock Solution, Stock solutions were prepared to avoid repeated weighing during media preparation. Macronutrients, micronutrients, and vitamins were measured according to the media composition, diluted with distilled water, stirred evenly, labeled, and stored in a refrigerator (Khoriyah et al., 2023). Plant growth regulator extracts, such as banana juice and coconut water, were used as stock solutions. Banana juice was made by blending 12.5 grams of banana per treatment, while 25 mL of coconut water was used per treatment.

Subculture of Plantlets, The plantlet subculture was performed aseptically inside a *Laminar Air Flow* (LAF) cabinet. Equipment and materials were sterilized with alcohol, and the LAF was operated with the fan and UV lamp for 30 minutes. After switching to TL light, tools were sterilized in spirit, and plantlets were transferred from culture bottles to sterile Petri dishes using forceps. Explants were separated into individual plantlets by cutting the roots and leaving two leaves, then transferred to new culture bottles.

Research Variables

Observations were carried out by monitoring plantlet growth based on the parameters of number of leaves,

number of roots, number of shoots, plant height, root length, and fresh weight.

Data Analysis

The observed data were analyzed using analysis of variance (ANOVA) with an F-test at the 5% significance level. If the results showed a significant effect, further testing was conducted using Duncan's Multiple Range Test (DMRT) at the 5% level to determine significant differences between treatments. Standard deviation was calculated for quantitative data, while descriptive analysis was performed for qualitative data.

RESULT AND DISCUSSION

Number of Leaves

The number of leaves is an indicator of plant growth that reflects the overall growth process. According to Wardana et al. (2024), a higher number of leaves corresponds to increased photosynthetic activity, resulting in greater production of photosynthates essential for plant growth. A greater number of leaves in plantlets indicates better growth performance. Based on the analysis of variance (ANOVA) at the 5% F-test level, the application of kinetin and 2,4-D, as well as their interaction, had no significant effect on the number of leaves of *Dendrobium stocklebuschii* plantlets.

Table 2. The results of the DMRT 5% post-hoc test on the application of kinetin and 2,4-D to the number of leaves of *Dendrobium stocklebuschii* orchids at 60 days after culture (DAC).

Kinetin (ppm)	Number of Leaves
0	5.33 a $\pm$ 1.45
0.5	7.13 b $\pm$ 2.36
1	7.27 b $\pm$ 3.03
1.5	7.33 b $\pm$ 2.61
2	6.33 ab $\pm$ 1.35
2,4-D (ppm)	
0	7.47 a $\pm$ 2.92
0.5	6.87 a $\pm$ 2.50
1	6.53 a $\pm$ 2.53
1.5	6.07 a $\pm$ 1.49
2	6.47 a $\pm$ 2.00

Note: Numbers followed by the same letter within each treatment indicate no significant difference according to DMRT at the 5% level.

Based on the table 2, the application of kinetin to the plants resulted in a number of leaves ranging from 5.33 to 7.33. The application of 1.5 ppm kinetin produced the highest number of leaves, 7.33 leaves. This may be due to the higher cytokinin concentration, which accelerates cell division, leading to optimal leaf formation. This finding is consistent with Azizah (2021), who stated that leaf formation in tissue culture is influenced by cytokinin hormones, where sufficient nutrients promote cell elongation and stimulate plant growth. The treatment without kinetin resulted in the lowest number of leaves, 5.33 leaves. Treatment without kinetin (K0) resulted in the lowest number of leaves, which was caused by the low dose of kinetin that disrupted the process of cell division in plants, where kinetin acts as a key element in the cell division process in plant tissue culture.

Plants treated with 2,4-D produced between 6.07 and 7.47 leaves. Treatments without 2,4-D resulted in the highest number of leaves in a total of 7.47 leaves, while 1.5 ppm 2,4-D produced the lowest in a total of 6.07 leaves. Wahidah and Hasrul (2017) explained that control plants without auxin treatment showed better leaf development than those treated with 1.5 ppm concentration, likely because control plants possess sufficient endogenous auxin to support leaf formation. Low auxin concentrations tend to promote a higher number of leaves compared to higher concentrations. This aligns with Wulannanda et al. (2023), who noted that treatment without 2,4-D provides an optimal ratio with cytokinin concentration, making the physiological process of leaf formation more effective. Similarly, Nana and Salamah (2014) stated that auxin promotes growth up to an optimal concentration, but when exceeded, it disrupts plant metabolism and development, resulting in reduced growth.

Plants treated with 1.5 ppm kinetin and 1 ppm 2,4-D produced 14 leaves (Figure 1a), while those treated without kinetin and 1.5 ppm 2,4-D produced 4 leaves (Figure 1b). The application of cytokinin at a higher concentration than auxin can stimulate cell division, resulting in more optimal leaf formation. According to Nurana et al. (2017), a higher kinetin concentration compared to auxin promotes morphogenesis toward the formation of shoots and leaves. The application of kinetin at a sufficiently high concentration can stimulate cell division in leaf primordia, supporting an increase in the number of leaves. Tambun et al. (2023) stated that leaf formation requires cytokinin translocated through the roots, and providing the right concentration of cytokinin can enhance the optimal growth of explant leaves. This is also in accordance with Hairuddin et al. (2023), who explained that the addition of plant growth regulators, especially auxin and cytokinin at sufficiently high concentrations affects cell growth and development, roots, and the number of explant leaves. This is supported by Rineksane et al. (2020), who stated that increasing cytokinin concentration can enhance leaf formation and growth.

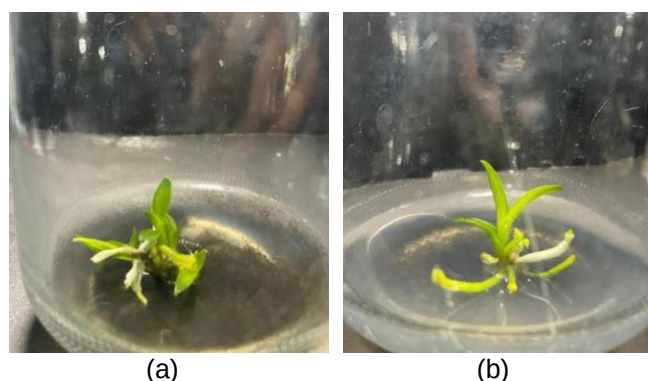


Figure 1. Results of leaf number in *Dendrobium stocklebuschii* orchids: (a) treatment with 1.5 ppm kinetin and 1 ppm 2,4-D, (b) treatment with 0 ppm kinetin and 1.5 ppm 2,4-D.

Number of Roots

The number of roots indicates the extent of the area reached by the plant in absorbing nutrients. The greater the number of roots, the more nutrients can be absorbed. According to Sofa et al. (2022), the number of roots that

develop affects the nutrient absorption process; therefore, the number of roots is an important factor in the success of tissue culture. Based on the results of the F-test at the 5% level, kinetin treatment had a significant effect on the number of roots, while 2,4-D treatment had no significant effect. The interaction between kinetin and 2,4-D also showed no significant effect on the number of roots.

Table 3. The results of the DMRT 5% post-hoc test on the application of kinetin and 2,4-D to the number of roots of *Dendrobium stocklebuschii* orchids at 60 days after culture (DAC)

Kinetin (ppm)	Number of Roots
0	6.93 ab $\pm$ 2.12
0.5	5.33 a $\pm$ 2.53
1	7.53 b $\pm$ 1.88
1.5	6.00 ab $\pm$ 1.98
2	5.33 a $\pm$ 2.23
2,4-D (ppm)	
0	5.47 a $\pm$ 1.85
0.5	6.93 a $\pm$ 2.49
1	7.00 a $\pm$ 2.40
1.5	5.87 a $\pm$ 1.98
2	5.87 a $\pm$ 2.50

Note: Numbers followed by the same letter within each treatment indicate no significant difference according to DMRT at the 5% level.

The results of the analysis in Table 3 show that plants treated with kinetin at various concentration levels produced an average number of roots ranging from 5.33 to 7.53. The application of 1 ppm kinetin resulted in an average of 7.53 roots, which was not significantly different from the treatments without kinetin and 1.5 ppm. This indicates that applying kinetin at a moderate concentration can increase the number of roots in *Dendrobium stocklebuschii* orchids. According to Riono (2019), the highest number of roots was observed at moderate kinetin concentrations, and the roots produced under these conditions exhibited good growth.

The results of the analysis in Table 3 also show that the application of 2,4-D produced a number of roots ranging from 5.47 to 7. Plants treated with 1 ppm 2,4-D produced an average of 7.47 roots, while those without 2,4-D produced an average of 5.47 roots. This demonstrates that applying auxin at an appropriate concentration results in better root formation. Auxin and gibberellin hormones play an important role in promoting plant growth, particularly in supporting root and shoot development. The application of 1 ppm exogenous auxin, combined with the endogenous auxin present in plantlets, resulted in a higher number of roots because plants require relatively high auxin concentrations for root initiation. According to Zulkarnain et al. (2018), the presence of auxin, either alone or in combination with kinetin at low concentrations, influences root proliferation.

Plants treated with 1 ppm kinetin and 0.5 ppm 2,4-D produced 10 roots (Figure 2a). The application of kinetin in the medium was able to stimulate cell division, which

enabled the plant to form roots. According to Sulichantini (2016), an adequate concentration of kinetin can activate the role of auxin in root formation and elongation. The addition of auxin and cytokinin at appropriate

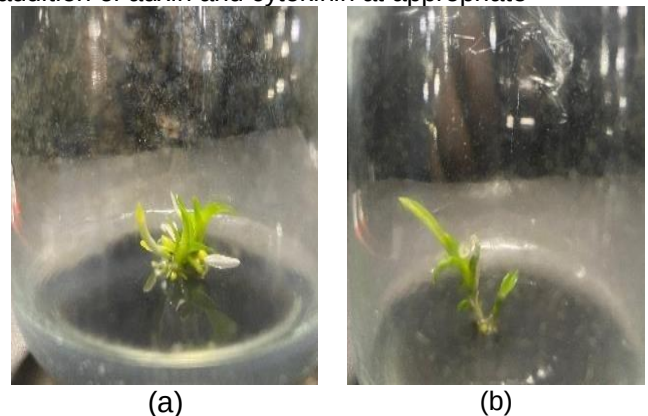


Figure 2. The number of roots of *Dendrobium stocklebuschii* orchids: (a) treatment with 1 ppm kinetin and 0.5 ppm 2,4-D; (b) treatment with 0.5 ppm kinetin and 2 ppm 2,4-D.

concentration of kinetin can activate the role of auxin in root formation and elongation. The addition of auxin and cytokinin at appropriate concentrations can enhance the performance of each hormone in the plant growth process. Mawaddah (2021) stated that the right combination of auxin and cytokinin concentrations can induce root formation in each explant. According to Tambun et al. (2023), cytokinin can stimulate cell division and influence differentiation pathways, so when applied at the proper concentration, it can promote root growth.

Plants treated with 0.5 ppm kinetin and 2 ppm 2,4-D produced 2 roots (Figure 2b). The addition of auxin at high concentrations can inhibit its own activity due to the accumulation of hormones in the medium. According to Rostiana and Seswita (2007), the decrease in root number with increasing auxin concentration may occur because a high dose of auxin can inhibit explant growth, both in shoots and roots. This is consistent with the statement of Rezaldi (2022) that a low number of roots results from excessively high auxin concentrations, which can stimulate the production of ethylene hormones. Ethylene is a hormone that inhibits the growth of roots, leaves, and flowers, depending on the species. This is supported by Avivi (2022), who stated that the use of excessively high auxin concentrations negatively affects root formation and may even cause plant death; however, if auxin is used at too low a concentration, it will not be effective in stimulating root formation.

Number of Shoots

Shoot formation is one of the indicators of success in plant tissue culture. According to Duri (2022), the ability of explants to form shoots is an indication that the plant propagation process has been successful. The shoots that appear on the plantlets represent a developmental response phase resulting from wounding during culture and the effects of treatments applied to regenerate into complete plants. Based on the results of the F-test at the 5% significance level, it was found that kinetin and 2,4-D treatments did not have a significant effect on the

number of shoots. The interaction between kinetin and 2,4-D also showed no significant effect on the number of shoots.

The application of kinetin to plants produced a number of shoots ranging from 0.93 to 1.6 shoots.

Table 4. Results of the 5% DMRT follow-up test on the application of kinetin and 2,4-D to the number of shoots of *Dendrobium stockelbuschii* orchids at 60 days after planting (DAP)

Kinetin (ppm)	Number of Shoots
0	0.93 a $\pm$ 0.97
0.5	1.40 a $\pm$ 1.35
1	1.33 a $\pm$ 1.11
1.5	1.60 a $\pm$ 1.30
2	1.27 a $\pm$ 0.60
2,4-D (ppm)	
0	1.27 a $\pm$ 1.10
0.5	1.73 a $\pm$ 1.39
1	1.20 a $\pm$ 1.21
1.5	1.07 a $\pm$ 0.46
2	1.27 a $\pm$ 1.10

Note: Numbers followed by the same letter within each treatment indicate no significant difference according to DMRT at the 5% level.

Plants treated with 1.5 ppm kinetin produced an average of 1.6 shoots, while those without kinetin treatment (0 ppm) produced an average of 0.93 shoots. The application of cytokinin at the appropriate concentration can induce the formation of proteins involved in shoot formation. The availability of these proteins in cells optimizes cell division, allowing shoots to develop in plantlets. This is presumably because higher kinetin concentrations cause cells to divide more rapidly. This is consistent with the statement of Elgedaway et al. (2020), who found that increased shoot and bud formation can be achieved by applying higher kinetin concentrations in MS medium. This finding is supported by Annisa (2022), who stated that the addition of an appropriate concentration of exogenous cytokinin can optimize the processes of differentiation and cell division in shoot formation.

The application of 2,4-D to plants produced a number of shoots ranging from 1.5 to 1.73. Plants treated with 0.5 ppm kinetin produced an average of 1.73 shoots, while plants treated with 1.5 ppm 2,4-D produced an average of 1.07 shoots. This is presumed to occur because only a small amount of auxin is required in the process of shoot formation. When plantlets already contain sufficient endogenous auxin, the addition of exogenous auxin may inhibit cytokinin activity, thereby limiting its ability to initiate new shoots. According to Pamungkas et al. (2009), exogenously applied auxin does not influence shoot formation, as shoot formation is more strongly affected by the presence of endogenous cytokinin.

Plants treated with 1.5 ppm kinetin and 1 ppm 2,4-D produced 5 shoots (Figure 3a). The growth of shoots in plantlets was influenced by the presence of kinetin at a higher concentration than 2,4-D. Kinetin belongs to the cytokinin group, which functions in the process of cell division within plant tissues and regulates plant growth and development. According to Dinika et al. (2021), the

application of cytokinin at a higher concentration than auxin tends to promote the formation of adventitious shoots and the proliferation of axillary shoots. This is also consistent with the statement of Kartikasari et al. (2013)

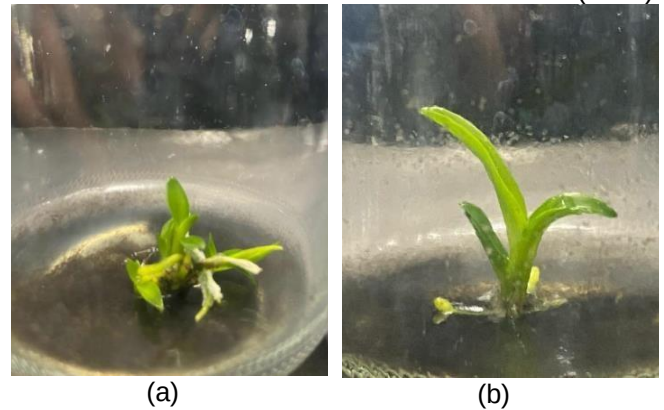


Figure 3. The number of shoots of *Dendrobium stockelbuschii* orchids: (a) treatment with 1.5 ppm kinetin and 1 ppm 2,4-D; (b) treatment without kinetin and without 2,4-D

that a lower concentration of 2,4-D compared to kinetin encourages optimal shoot induction. According to Wulansari et al. (2017), auxin and gibberellin hormones play an important role in supporting plant growth, particularly in root and shoot development. Meanwhile, plants treated without kinetin and without 2,4-D produced no shoots (Figure 3b). The absence of cytokinin hormones, in this case kinetin, in the culture medium caused the plants to be unable to initiate shoot formation optimally. According to Chika et al. (2021), cytokinin hormones play a more dominant role than auxin in promoting cell division that leads to shoot formation in *Dendrobium* sp. plantlets.

Plant Height

Plant height is a parameter that is often monitored to assess plant growth and to measure the effects of environmental factors or treatments applied to the plant, as it is the most easily observed parameter. According to Rani et al. (2023), plant height is a commonly observed parameter used as an indicator of growth and to evaluate the effects of applied treatments. Based on the results of the F-test at the 5% significance level, it was found that kinetin treatment had a significant effect on plant height, while 2,4-D treatment did not show a significant effect. The interaction between kinetin and 2,4-D also showed no significant effect on plant height.

The application of kinetin at various concentration levels produced plant heights ranging from 1.99 to 2.57 cm. Plants treated with 0.5 ppm kinetin had an average height of 2.57 cm, although this was not significantly different from the treatments with without, 1.5, and 2 ppm kinetin. According to Ningrum et al. (2024), kinetin is a type of cytokinin plant growth regulator that affects plant growth; therefore, an increase in kinetin concentration can influence plant height. This is supported by Hartati et al. (2016), who stated that increasing kinetin can lead to greater plant height because the cytokinin content in plantlets reaches an equilibrium point. Plant height is associated with the time of root emergence in explants, particularly in explants that are immediately subcultured, which tend to have greater height.

Plants treated with various concentrations of 2,4-D produced plant heights ranging from 2.24 to 2.53 cm.

Plants treated with 2 ppm 2,4-D reached an average height of 2.53 cm.

Table 5. Results of the 5% DMRT follow-up test on the application of kinetin and 2,4-D to the plant height of *Dendrobium stockelbuschii* orchids at 60 days after planting (DAP)

Kinetin (ppm)	Plant Height
0	2.51 b $\pm$ 0.65
0.5	2.57 b $\pm$ 0.50
1	1.99 a $\pm$ 0.51
1.5	2.53 b $\pm$ 0.46
2	2.44 b $\pm$ 0.58
2,4-D (ppm)	
0	2.24 a $\pm$ 0.38
0.5	2.45 a $\pm$ 0.70
1	2.52 a $\pm$ 0.57
1.5	2.28 a $\pm$ 0.60
2	2.53 a $\pm$ 0.58

Note: Numbers followed by the same letter within each treatment indicate no significant difference according to DMRT at the 5% level.

The growth rate of plantlets increased due to the availability of carbohydrate reserves required for plant growth and development. According to Mahadi et al. (2024), treatment with 0 ppm kinetin and 0 ppm IAA (K0I0), which served as the control and did not receive any plant growth regulators, resulted in the lowest average plantlet height of 1.1 cm. This finding is supported by Junairiah et al. (2019), who noted that both endogenous hormones synthesized by the plant and exogenous hormones applied to the plant have a significant influence on the process of cell elongation.

Plants treated with 0.5 ppm kinetin and 2 ppm 2,4-D produced plants with a height of 3.5 cm (Figure a). This was due to stem elongation resulting from cell division, elongation, and enlargement of new cells occurring in the apical meristem and stem nodes, which caused the plant to grow taller. According to Saepudin (2020), stem elongation occurs because of cell division, elongation, and enlargement processes in the apical meristem and stem nodes, leading to an increase in plant height. The addition of cytokinin to the culture medium influences tissue growth. According to Mardiyah et al. (2017), the increase in the length or height of an organ such as a shoot results from the activity of cell division, elongation, and enlargement in meristematic tissues. The application of an appropriate kinetin concentration can maximize cell division, leading to better plant growth, particularly in plant height. Kartiman et al. (2018) stated that the application of cytokinin at an optimal concentration, balanced with the plant's endogenous hormone levels, stimulates cell division that functions in organ formation. This is supported by Rustikawati et al. (2021), who explained that cytokinin plays a role in cell division, but when combined with auxin, it can induce cell elongation, one of the effects of which is expressed as an increase in shoot height.

Plants treated with 1 ppm kinetin and 0.5 ppm 2,4-D produced plants with a height of 1.4 cm (Figure b). The

relatively high auxin concentration without sufficient cytokinin balance caused less effective cell division, resulting in limited growth in plant height. According to Wahyuni and Susilowati (2017), the application of the auxin 2,4-D, which can influence cell elongation, is not effective when not supported by endogenous cytokinin, and therefore does not significantly affect shoot height growth in explants.

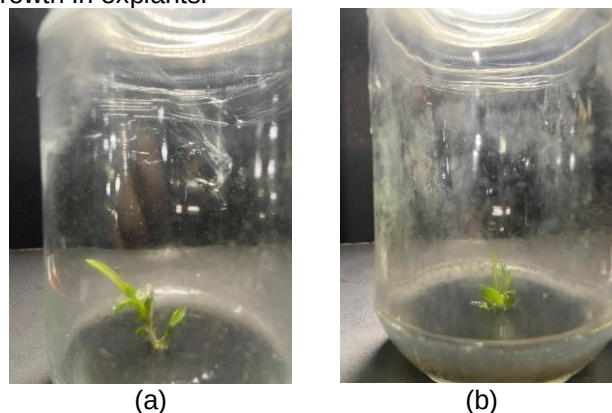


Figure 4. Plant height of *Dendrobium stockelbuschii* orchids: (a) treatment with 0.5 ppm kinetin and 2 ppm 2,4-D; (b) treatment with 1 ppm kinetin and 0.5 ppm 2,4-D

Root Length

Root length indicates the extent of a plant's ability to absorb nutrients; thus, the longer the roots, the wider the absorption range and the greater the amount of nutrients that can be taken up. In tissue culture growth, longer roots also indicate healthy plantlets capable of optimally absorbing nutrients from the medium. According to Darlis et al. (2021), root formation is one of the indicators of successful in vitro propagation, as roots are essential plant organs that function to absorb nutrients from the medium, which are then used to support growth. Based on the results of the F-test at the 5% significance level, it was found that 2,4-D treatment had a significant effect on root length, while kinetin treatment also had a significant effect on root length. The interaction between kinetin and 2,4-D, however, showed no significant effect on root length.

Table 6. Results of the 5% DMRT follow-up test on the application of kinetin and 2,4-D to the root length of *Dendrobium stockelbuschii* orchids at 60 days after planting (DAP)

Kinetin (ppm)	Root Length (cm)
0	1.10 a $\pm$ 0.76
0.5	0.93 a $\pm$ 0.60
1	1.33 a $\pm$ 0.66
1.5	1.03 a $\pm$ 0.48
2	0.87 a $\pm$ 0.57
2,4-D (ppm)	
0	1.17 ab $\pm$ 0.57
0.5	1.11 ab $\pm$ 0.59
1	1.39 b $\pm$ 0.69
1.5	0.87 a $\pm$ 0.65
2	0.74 a $\pm$ 0.50

Note: Numbers followed by the same letter within each

treatment indicate no significant difference according to DMRT at the 5% level.

The application of kinetin at various concentrations produced root lengths ranging from 0.87 to 1.33 cm. Tabel 6 shows that application of 1 ppm kinetin resulted in an average root length of 1.33 cm, while treatment with 2 ppm kinetin produced an average root length of 0.87 cm. This indicates that lower kinetin concentrations result in better root elongation compared to higher concentrations. According to Sualang et al. (2023), the addition of cytokinin-type plant growth regulators (PGRs) can inhibit or reduce root growth potential in explants.

The application of 2,4-D at various concentrations produced root lengths ranging from 0.74 to 1.39 cm. The application of 1 ppm 2,4-D resulted in a root length of 1.39 cm, which was not significantly different from treatments without 2,4-D and 0.5 ppm. Plants treated with 2 ppm 2,4-D produced an average root length of 0.74 cm. According to Arli and Noli (2023), high root growth in explants is caused by the proper interaction between endogenous and exogenous hormones. The application of an appropriate auxin concentration can stimulate better root development. Munthe et al. (2022) also stated that the application of auxin, either alone or in combination with cytokinin at certain concentrations, can enhance root elongation.

The application of 0 ppm kinetin and 1 ppm 2,4-D resulted in a root length of 2.5 cm (Figure 5a), while the treatment with 2 ppm kinetin and 2 ppm 2,4-D produced a root length of 0.1 cm (Figure 5b). The appropriate concentration of kinetin can stimulate cell division, which promotes organ elongation. However, a high concentration of kinetin can inhibit root elongation due to its antagonistic effect on auxin, thereby suppressing auxin's role in root elongation. According to Aljubori and Amery (2022), increasing the concentration of cytokinin in the medium reduces the role of auxin in promoting nuclear cell elongation, resulting in inhibited root growth. This is supported by Sosnowski et al. (2023), who stated that in root differentiation, cytokinin and auxin exhibit antagonistic effects, auxin promotes lateral root development, whereas cytokinin inhibits it.

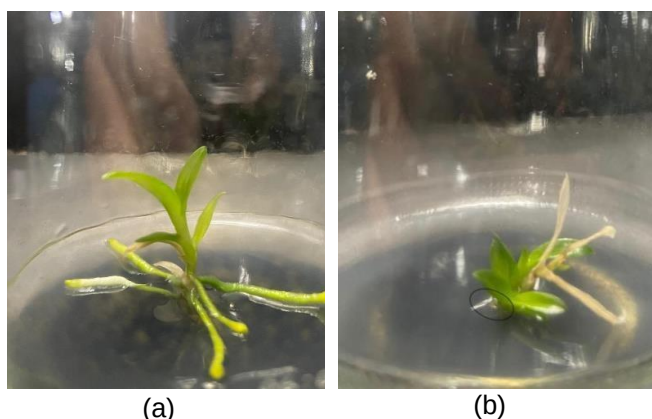


Figure 5. Root length of *Dendrobium stockelbuschii* orchid (a) treatment without kinetin and 1 ppm 2,4-D (b) treatment with 2 ppm kinetin and 2 ppm 2,4-D

Fresh Weight

Growth is characterized by an irreversible increase in mass; therefore, fresh weight measurement can be used as an indicator for evaluating growth parameters. The fresh weight of a plant reflects the accumulation of

photosynthetic products and water resulting from respiration. According to Abdillah et al. (2024), fresh weight is a growth parameter that represents the outcome of cell division and enlargement processes, and it is used to assess plant growth and productivity in tissue culture contexts. The interaction between kinetin and 2,4-D showed no significant effect on the fresh weight parameter. Similarly, the individual application of kinetin or 2,4-D had no significant effect on fresh weight.

Table 7. Results of the DMRT 5% follow-up test on the application of kinetin and 2,4-D on the fresh weight of *Dendrobium stockelbuschii* orchids at 60 days after planting (DAP)

Kinetin (ppm)	Fresh Weight
0	0.27 a ± 1.22
0.5	0.21 a ± 0.10
1	0.28 a ± 0.10
1.5	0.27 a ± 0.12
2	0.26 a ± 0.20
2,4-D (ppm)	
0	0.30 a ± 0.20
0.5	0.26 a ± 0.09
1	0.29 a ± 0.13
1.5	0.23 a ± 0.13
2	0.22 a ± 0.08

Note: Numbers followed by the same letter within each treatment indicate no significant difference according to DMRT at the 5% level.

Table 7 shows that plants treated with kinetin showed fresh weights ranging from 0.21 to 0.28 grams. Plants given 1 ppm kinetin produced a fresh weight of 0.28 grams, while those treated with 0.5 ppm kinetin had a fresh weight of 0.21 grams. In the development of fresh weight, an appropriate concentration of cytokinin can stimulate leaf and shoot growth, thereby increasing the overall fresh weight of the culture. According to Tungga et al. (2023), the addition of cytokinin hormones at appropriate concentrations can promote leaf and shoot growth, leading to higher fresh weight. This is consistent with the statement by Yoas et al. (2021) that fresh weight is strongly influenced by the rate of cell division induced by cytokinin application.

Application of various concentrations of 2,4-D on the plants resulted in fresh weights ranging from 0.22 to 0.30 grams. Plants treated without 2,4-D produced a fresh weight of 0.30 grams, while those treated with 2 ppm 2,4-D had a fresh weight of 0.22 grams. This is presumably because the endogenous auxin content in the plantlets was already sufficient for cell division, requiring only a very low concentration of exogenous auxin to support fresh weight accumulation. According to Khorayah et al. (2023), auxins can alter the activity of enzymes involved in the synthesis of cell wall components and reorganize them into a complete wall matrix, which affects cell weight.

Plants treated with 1 ppm kinetin and 1.5 ppm 2,4-D produced a fresh weight of 0.54 g (Figure 6a). According to Normasari et al. (2023), a higher auxin concentration compared to cytokinin results in greater callus fresh weight. The increase in fresh weight occurs because plant cells undergo division, leading to an increase in cell

mass. As the cell mass in plants increases, the plant's fresh weight also increases. Rizal et al. (2017) stated that culture fresh weight tends to increase with higher kinetin concentrations. This is in line with Zuraidassanaaz (2016), who explained that cytokinin-induced cell division and protein synthesis promote cell proliferation, thereby increasing weight as the volume of produced cells expands.

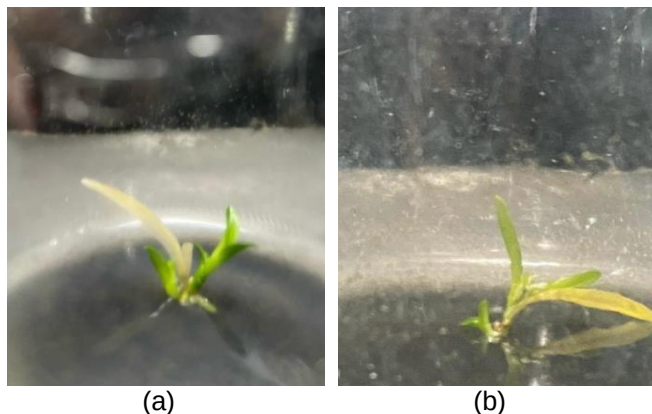


Figure 6. Fresh weight results of *Dendrobium stockelbuschii* orchids: (a) treatment with 1 ppm kinetin and 1.5 ppm 2,4-D, (b) treatment without kinetin and without 2,4-D.

Auxin addition also plays an important role in influencing fresh weight, in conjunction with cytokinin. A relatively high auxin concentration can stimulate plant growth through collaboration with cytokinin. Sulasiah et al. (2015) stated that the fresh weight produced depends largely on the rate of cell division and enlargement; media with higher auxin concentrations tend to show greater increases in shoot fresh weight. Similarly, Shofiyani and Damajanti (2017) reported that the rate of cell division is influenced by the specific combination of auxin and cytokinin concentrations, as well as plant species and other factors.

Meanwhile, plants treated with 0 ppm kinetin and 0 ppm 2,4-D produced a fresh weight of 0.08 g (Figure 6b). The absence of cytokinin and auxin addition resulted in suboptimal cell division, thereby limiting plant fresh weight. According to Puri et al. (2022), treatments without kinetin lead to the lowest plantlet fresh weight because the lack of kinetin disrupts stimulation processes in roots and shoots, reducing optimal biomass production and ultimately affecting plant weight.

CONCLUSION

The application of 0.5 ppm kinetin was able to increase plant height, while 1 ppm kinetin enhanced the number of roots in *Dendrobium stockelbuschii*. The application of 0.5 ppm 2,4-D was able to increase root length in *Dendrobium stockelbuschii*. The combination of kinetin and 2,4-D treatments did not significantly affect any growth parameters of *Dendrobium stockelbuschii*.

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