JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 8 No. 2 May 2026
DOI :https://doi.org/10.36378/juatika.v8i2.5057
eissn 2656-1727
pissn 2684-785X
pages : 672 – 677

REVIEW ARTICLE Open Access

Composition of Naphthalene Acetate and Kinetin in Orchid Organogenesis (*Dendrobium biggibum*)

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Abstract

Dendrobium orchids are horticultural commodities with high market interest, so they are widely cultivated. The study aims to determine the composition of the best concentration of NAA and Kinetin growth regulators in MS planting medium for the organogenesis of Dendrobium orchid plantlets. This study was conducted with 2 (two) factors and repeated 4 times, which was compiled in a Completely Randomized Design (CRD). The first factor is the NAA concentration treatment (2, 3, and 4 ppm), while the second factor is the concentration of Kinetin (1, 2, and 3 ppm). The results showed that the combination of NAA concentration treatment of 4 ppm and Kinetin 1 ppm in MS planting medium produced the best root wet weight and root length and was significantly different from other treatment combinations. Therefore, the composition of NAA concentration of 4 ppm and Kinetin 1 ppm can be recommended as the best combination in increasing plantlet root growth.

Keywords: Concentration, MS Medium, Plantlets, Roots, Shoots

1. Introduction

Dendrobium orchids are a horticultural commodity with high economic value, widely cultivated by entrepreneurs as a source of income (Heriansyah & Sagiarti, 2014). Public interest in *Dendrobium* orchids has increased over time. Potted orchid production in Indonesia in 2023 (3,785,454) increased in 2024 (4,429,467). (Central Statistics Agency, n.d.). This is supported by the *Dendrobium orchid*, which is widely developed because it is in high demand in the market with a lower selling price than other types of orchids (Febryanti et al., 2017). Orchid propagation has obstacles because the seeds do not contain food reserves *(endosperm*), so only about 5% can germinate, and few can grow to maturity. This condition is an obstacle in the mass production of seeds and flowers to meet market needs (Pyati, 2019). This is because seed propagation cannot be carried out because they do not have endosperm, and the slow growth of orchid plants compared to other ornamental plants is caused by a low metabolic rate, which is a form of adaptation so that plants can grow in environments with low nutrient, water, and sunlight content (Sucandra et al., 2015). Slow growth in orchids is also influenced by genetic and environmental factors, such as light intensity, temperature, humidity, water, fertilizer, growing media, and pest and disease attacks.

Vegetative propagation through tissue culture can be applied because this method relies on the principle of totipotency, the ability of cells to regenerate into whole plants. Tissue culture techniques can produce large numbers of seedlings that are identical to the parent (true to type) in a relatively short time (Dasuha, 2022).

Previous research has revealed the potential of the combination of NAA and Kinetin in orchid plantlet organogenesis. Likewise, results vary depending on the response of various orchid varieties, but literature on orchid organogenesis discussing Dendrobium biggibum varieties is still minimal. Ulya's (2018) research showed that the addition of 3 ppm NAA concentration resulted in the best root length and higher plant growth, while the combination of 3 ppm NAA + 2 ppm Kinetin provided the highest number of Dendrobium sp. roots. Siburian's (2023) research showed that the addition of the best Kinetin concentration of 1 ppm resulted in the best plant height, and NAA concentrations of more than 1.5 ppm will result in the opportunity for more and longer roots in Wendrobium Woon Leng orchids at 12 WST. *Heriansyah's*

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research (2019) showed that the treatment of 50 g liter-1 sucrose and 1 ppm Kinetin affected the age of shoot emergence, number of shoots, shoot height, percentage of explants forming roots, number of roots, root length, and root-shoot ratio (T/R ratio). In contrast to previous research, this study provides an update by evaluating the combination of NAA concentrations of 2 ppm to 4 ppm and Kinetin 1 ppm to 3 ppm, factorially to see the interaction between NAA and kinetin growth regulators in *Dendrobium biggibum orchids* . In the tissue culture process, the addition of growth regulators (PGRs) plays an important role as non-nutrient organic compounds that can stimulate, inhibit, or change the direction of plant growth and development (Emilda, 2020). This study aimed to obtain the best combination of NAA and Kinetin concentration treatments for the callus growth of *Dendrobium biggibum orchid seedlings* using tissue culture.

2. Material and Methods

The research was conducted from June to November 2024 at the Biotechnology Laboratory, Faculty of Agriculture, National Development University "Veteran" East Java, Jl. Raya Rungkut Madya, Surabaya with location coordinates at 7°20'03"S 112°47'30"E which is located at an altitude of ± 5 meters above sea level, with the lowest to highest temperature of 25- 31 ℃ and air humidity between 50-80%.

Incubation maintenance is carried out by placing the plantlets on an incubation rack at room temperature of 24–27 ℃ with air humidity of around 70% and LED light intensity of 1000 lux, which is on for 24 hours. The equipment used includes: autoclave, sterile cabinet, Bunsen lamp, LAF (*Laminar Air Flow*), sprayer bottle, spatula, culture glass, stove, pan, beaker glass, measuring cup, *magic stirrer* , pipette, *scalpel*, petri dish, tweezers, oven, analytical balance, ruler and camera. The materials used include: *Dendrobium biggibum orchid plantlets, murashege and shoog (MS)* media, distilled water, 70% alcohol and iodine.

This study was a factorial experiment with 2 factors arranged in a Completely Randomized Design (CRD) with 4 replications. The first factor was the NAA concentration treatment, namely: 2, 3 and 4 ppm, and the second factor was the Kinetin concentration treatment, namely: 1, 2 and 3 ppm. The parameters observed were: percentage of live plantlets, number of shoots, number of leaves, number of roots, root length, shoot length, root fresh weight and shoot fresh weight.

Analysis of observation data using ANOVA *(Analysis of variance)*, if real or very real differences are found, further testing is carried out using the Honestly Significant Difference (HSD) test with a level of 5% using IBM SPSS Statistics 25. The following is a flow diagram of the research implementation:

Figure 1. Flowchart of research implementation

3. Results and Discussion

The combined effect of NAA and Kinetin concentrations on the percentage of live plantlets, number of leaves, and fresh shoot weight showed no significant interaction. Similarly, neither NAA nor Kinetin as a single factor significantly affected the percentage of live plantlets, number of leaves, or fresh shoot weight of 12-week-old orchid seedlings (Table 1).

Treatment of plant growth regulator concentrations of NAA and Kinetin added individually to the *Dendrobium orchid culture medium* did not increase the percentage of live plantlets, considering that the lowest concentration of NAA and Kinetin had already produced a high percentage of live plantlets (95%), so that further increases in concentration had no significant effect on the percentage of live plantlets.

Treatment of NAA and Kinetin concentrations individually on the number of leaves showed a tendency to increase the number of leaves with increasing NAA concentration from 2 ppm to 4 ppm. Likewise, the effect of Kinetin concentration also showed the same tendency, with an increase in the number of leaves of Dendrobium orchid seedlings from 1 ppm to 3 ppm (Table 1). This result is in accordance with the research of Rochmah & Rahayu (2021) that the number of leaves per plantlet indicates the stability of vegetative growth due to the balance of NAA and Kinetin hormones. The number of leaves parameter plays an important role in the photosynthesis process that supports the overall growth of the plant, as explained by Lestari (2011), that the response of plants to cytokinin and auxin hormones at various concentrations is highly dependent on the endogenous hormone content they have. Panwar et al. (2012) stated that the addition of NAA did not provide a significant increase in plant growth in individuals that already had sufficient endogenous hormone content.

Nurhanifah et al. (2021) added that the administration of exogenous auxin hormones that are not balanced with endogenous hormones can inhibit the process of division and elongation of plant cells.

The wet weight of orchid shoots did not show significant differences due to the influence of NAA and Kinetin concentration treatments, but increasing NAA concentrations up to 3 ppm and Kinetin up to 4 ppm individually resulted in a tendency to increase the wet weight of shoots. Kinetin is a synthetic cytokinin that plays an important role in tissue culture media to stimulate cell division, callus formation, and plantlet development. Tungga *et al*. (2023) reported that the combination of Kinetin 1 mg/l + 10% Coconut Water produced the heaviest wet weight of 0.15 g. The addition of appropriate cytokinin (Kinetin) PGR can stimulate leaf and shoot growth.

The effect of the combined treatment of NAA and Kinetin concentrations on the number of shoots and shoot length showed no significant interaction, likewise, the single factor of NAA concentration had no significant effect on the number and length of orchid seedling shoots. However, the single factor of Kinetin concentration had a significant effect on the number and length of dendrobium orchid seedling shoots (Table 2).

Table 1. Average Percentage of Live Plantlets, Number of Leaves and Fresh Weight of Orchid Seedling Shoots Aged 12 Weeks After Planting in NAA and Kinetin Concentration Treatments

Treatment	**Percentage of Live Plantlets (%)**	**Amount Leaf (Sheet)**	**Fresh Weight of Shoots (mg)**
NAA concentration			
2 ppm	95.00 ± 1.44	3.32 ± 0.22	151.08 ± 14.30
3 ppm	95.00 ± 2.50	3.30 ± 0.30	164.46 ± 4.02
4 ppm	95.00 ± 1.44	3.48 ± 0.29	183.92 ± 2.54
BNJ 5%	tn.	tn.	tn.
Kinetin Concentration			
1 ppm	93.33 ± 1.44	3.13 ± 0.22	156.17 ± 13.97
2 ppm	95.83 ± 63.75	3.44 ± 6.33	163.71 ± 11.84
3 ppm	95.83 ± 63.75	3.53 ± 6.32	179.58 ± 4.64
BNJ 5%	tn.	tn.	tn.

Description: tn = not significantly different.

Table 2. Average Number and Length of Orchid Seedling Shoots Aged 12 MST in NAA and Kinetin Concentration Treatments.

Treatment	**Number of Shoots**	**Shoot Length (mm)**
NAA concentration		
2 ppm	2.83 ± 0.44	21.42 ± 2.42
3 ppm	3.08 ± 0.22	20.00 ± 0.38
4 ppm	3.67 ± 0.22	18.17 ± 0.22
BNJ 5%	tn.	tn.
Kinetin Concentration		
1 ppm	2.67 ± 0.36 a	18.58 ± 0.44 a
2 ppm	3.25 ± 0.25 ab	19.25 ± 0.58 b
3 ppm	3.67 ± 0.17 b	21.75 ± 2.32 c
BNJ 5%	0.68	2.46

Description: Numbers accompanied by the same letter in the same treatment and column show no significant difference at BNJ 5%; tn = no significant difference.

NAA concentration alone showed a tendency towards increasing the number of shoots with increasing NAA concentration from 2 ppm to 4 ppm. However, the effect of Kinetin on the number of shoots showed that the Kinetin concentration of 3 ppm was significantly different from the treatment of kinetin concentration of 1 ppm, with a 37.45% increase in the number of shoots of dendrobium orchid seedlings (Table 2).

Treatment of NAA concentration alone on shoot length showed that there was a tendency to decrease shoot length with increasing NAA concentration from 2 ppm to 4 ppm. However, on the contrary, the effect of Kinetin concentration showed an increase with increasing Kinetin concentration from 1 ppm to 3 ppm, where the number of shoots of dendrobium orchid seedlings increased (Table 2). This is in accordance with, Sakina et al., (2019) showed that the number of shoots of orchid seedlings aged 16 MST was the highest at around 3 shoots and increased shoot formation through proper hormone concentration regulation, furthermore Mayura et al., (2017) that Cytokinin is a plant hormone that plays a role in various growth processes, such as apical dominance, shoot meristem activity, leaf aging, germination, and stress response.

Table 2. shows that there is a tendency for a decrease in the concentration of NAA 2 ppm to produce the longest shoot length. The treatment of Kinetin concentration 3 ppm produces the best shoot length and is significantly different from the treatment of Kinetin concentration 1 ppm. There is an increase in shoot length by the influence of Kinetin concentration 3 ppm compared to other concentration treatments. Anur et al. (2024) found that the length of the orchid shoots *of dendrobium sp*. with a height of 10.5 cm at 12 WAP indicates a synergistic relationship between the growth regulators auxin and cytokinin, because both compounds play a role in supporting plant growth and development. Cytokinin as a hormone that stimulates cell division and plays an important role in growth, leaf aging, root formation, and stress responses in plants (Barciszewski et al., 2007).

Pirmansyah (2022) found that the higher the auxin concentration given to orchid plantlets, the lower the plant height growth rate. Meanwhile, the lower the cytokinin concentration used, the higher the orchid plantlets' growth rate. This is in line with Tungga et al. (2023), who explained that plant growth regulators (PGRs) provide optimal effects at the right concentration, while at too low or too high concentrations, explant growth responses will be less than optimal. The concentration of NAA had a significant effect on the number of roots of orchid seedlings, while the concentration of Kinetin had no significant effect on the number of roots of dendrobium orchid seedlings (Table 3).

Treatment NAA concentration in single to amount root shown that there is improvement amount root with increasing NAA concentration from 2 ppm to with 4 ppm. However, different from influence Kinetin concentration shows the same decrease with increasing Kinetin concentration from 1 ppm to with 3 ppm occurring decline amount root seeds orchid dendrobium (Table 3). This is in accordance with the study by Siburian (2023), who reported that administration of NAA 1.5 ppm + Kinetin 1 ppm can influence the number of root plantlets of orchid Dendrobium sp. 12 MST, with 7.41 roots. This confirms that optimizing NAA concentration plays a crucial role in stimulating healthy root growth, which is crucial for successful orchid tissue culture.

The effect of the combination of NAA and Kinetin concentration treatments on root length showed a significant interaction (Table 4).

Table 3. Average Number of Roots of Orchid Seedlings Aged 12 MST in NAA and Kinetin Concentration Treatments

Treatment	Number of Roots
NAA concentration	
2 ppm	10.92 ± 0.46 a
3 ppm	12.25 ± 0.14 b
4 ppm	13.58 ± 0.22 c
BNJ 5%	1.54
Kinetin Concentration	
1 ppm	12.67 ± 0.73
2 ppm	12.33 ± 0.65
3 ppm	11.75 ± 0.95
BNJ 5%	Mr.

Description : Accompanied numbers letter The same on equal treatment show No different real on BNJ 5% test, tn = no different real

Table 4. Average Root Length of Orchid Seedlings Aged 12 Weeks After Planting with Combination of NAA and Kinetin Concentration Treatments

Treatment	Root Length (mm)		
	Kinetin Concentration (ppm)		
NAA concentration (ppm)	1 ppm	2 ppm	3 ppm
2 ppm	14.75 ± 0.85 cd	14.25 ± 1.84 c	7.25 ± 1.18 a
3 ppm	14.25 ± 0.85 c	13.25 ± 0.85 b	13.00 ± 1.29 b
4 ppm	**16.75 ± 0.85 e**	15.25 ± 1.65 d	15.00 ± 1.58 d
BNJ 5%		0.64	

Description: Numbers accompanied by the same letters show no significant difference in the 5% BNJ test.

The combination treatment of 4 ppm NAA and 1 ppm Kinetin concentrations produced the best root length and was significantly different from other treatment combinations (Table 4). Debitama et al., (2022) that, auxin play a role in spur elongation cell so that can increase growth shoot height. Combination NAA treatment 4 ppm and Kinetin 1 ppm, which shows result 16.75 mm compared on study Fathurrahman, (2013) which shows addition of 5 ppm NAA to orchid *Denrobium sp.* results long root orchid namely 3.66 cm at 6 MST, potentially increase long root through improvement proper concentration of NAA , thing This in accordance with Ranjan et al., (2018) that, the composition of the auxin ZPT is relatively tall influence growth on plantlet roots.

The effect of the combination of NAA and Kinetin concentration treatments on the wet weight of roots showed a significant interaction, and the single factor of NAA and Kinetin concentration had a significant effect on the number and wet weight of dendrobium orchid seedling roots (Table 5).

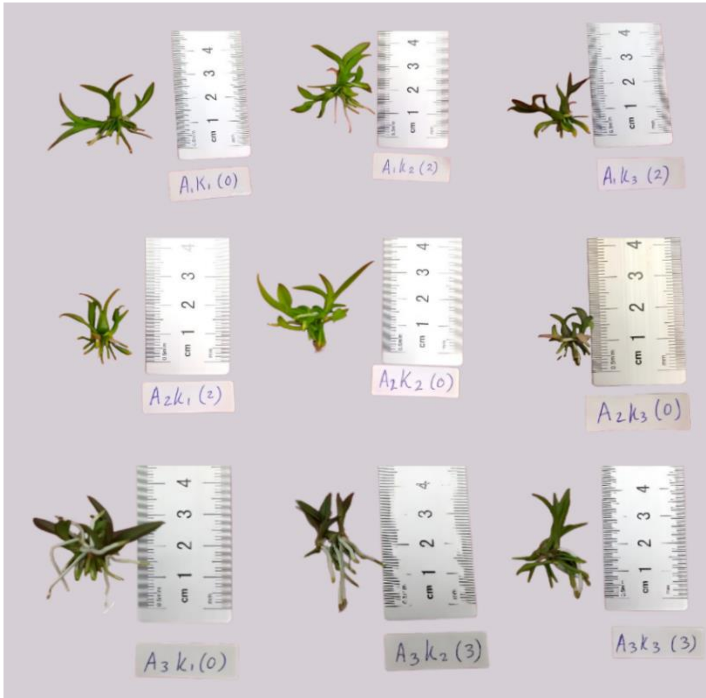

Figure 2. Results of the Root Length of *Dendrobium biggibum Orchid Plantlets*. Note: A_1 = NAA 2 ppm; A_2 = NAA 3 ppm; A_3 = NAA 4 ppm; K_1 = Kinetin 1 ppm; K_2 = Kinetin 2 ppm; K_3 = Kinetin 3 ppm .

Table 5. Average Wet Weight of Orchid Seedling Roots Aged 12 Weeks After Planting in Combination of NAA and Kinetin Concentration Treatments

Treatment	Wet Root Weight (mg)		
	Kinetin Concentration (ppm)		
concentration (ppm)	1 ppm	2 ppm	3 ppm
2 ppm	94.25 ± 17.66 cd	143.00 ± 38.23 e	43.50 ± 14.51 a
3 ppm	63.25 ± 16.94 b	100.75 ± 17.85 d	87.75 ± 18.51 c
4 ppm	**227.25 ± 18.90 f**	103.25 ± 14.61 d	109.25 ± 25.06 d
BNJ 5%		10.71	

Note: Numbers accompanied by the same letters indicate no significantly different in the 5% BNJ Test

Table 5 shows the combination of NAA and Kinetin concentrations on root wet weight, showing that root wet weight tends to increase with increasing NAA concentration from 2 ppm to 4 ppm. Thus, the effect of Kinetin concentration also shows the same tendency with increasing Kinetin concentration from 1 ppm to 3 ppm, there is an increase in the root wet weight of dendrobium orchid seedlings. So the best combination of NAA and Kinetin concentrations on root wet weight is shown at a concentration of 4 ppm NAA and 1 ppm Kinetin (Table 5). This is in line with the statement of Nurana et al. (2017) that the combination of NAA and 2-iP concentrations produces a total wet weight of Wong Ling hybrid Dendrobium orchids at 16 WAP weighing 0.47 g. Higher auxin concentrations than cytokinin concentrations will stimulate root growth, and vice versa. Genetic factors regulate root formation, while the number of leaves supports root growth through photosynthesis (Anur et al., 2024).

This is consistent with research by Arianbach et al. (2023), which reported a total wet weight of 790 mg of Phalaenopsis orchid seedlings at a NAA concentration of 10 ppm. This result indicates the potential for increasing root wet weight through appropriate increases in NAA concentration. This occurs because the interaction between exogenous and endogenous growth regulators determines the direction of a culture's development (Ariyanti et al., 2021) states that a high auxin composition focuses growth on the roots. Astutik et al., (2021).

4. Conclusion

The combination of 4 ppm NAA and 1 ppm Kinetin in MS growing media provided the best effect on root length and root fresh weight. 4 ppm NAA produced the best number of roots, while 3 ppm Kinetin produced the best number and length of shoots. Therefore, the combination of 4 ppm NAA and 1 ppm kinetin can be recommended as the best combination for increasing plantlet root growth.

Acknowledgement

Thank you to the Biotechnology Laboratory of the National Development University "Veteran" East Java for facilitating the infrastructure so that this research can be carried out.

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