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The Effect of Growmore and BAP (Benzyl Amino Purine) Concentrations on The Growth of *Dendrobium bigiante agrihorti* Orchid In-Vitro

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ABSTRACT

The increase in production of Dendrobium bigiante agrihorti orchids by in vitro culture is to produce numerous and uniform seedlings. This study aimed to determine the effect of Growmore and BAP concentrations on the growth of orchid plantlets. The experiment consisted of two factors namely Growmore concentration (0; 3; 6; 9 ppm), and BAP concentrations (0; 1; 3; 5 ppm). The factors combination on ½ MS media was repeated 3 times and obtained 48 experimental units. Observation parameters included the percentage of live plantlets, number of roots, number of leaves, plant height (cm), and wet weight of plantlets (g). Results showed that a Growmore concentration of 3 ppm had a significant effect on the number of roots and plant height at 4.36 units and 4.17 cm. BAP concentration of 3 ppm also had a significant effect on the number of roots, plant height, and wet weight of respectively 4.72 units, 3.22 cm, and 0.63 g. A combination of Growmore 3 ppm + BAP 0 ppm produced the largest percentage of live planets at 100% and plant height at 7.37 cm, Growmore 9 ppm + BAP 0 ppm produced the largest number of roots at 6.33 units and Growmore 6 ppm + BAP 3 ppm produced the largest number of leaves at 8.78 strands.

1. INTRODUCTION

Indonesia is one of the countries with significant potential in increasing the floriculture industry, especially orchid ornamental plant commodities which have great opportunities for horticultural production. One type of ornamental plant that has an attractive flower shape and color and has a long shelf life is the Dendrobium orchid ornamental plant. The Dendrobium orchid, which belongs to the *Orchidaceae* family, is a favorite orchid genus for orchid lovers in general. Especially the *Dendrobium* var. *bigiante agrihorti*, which is an orchid resulting from crosses at Balai Penelitian Tanaman Hias (the Ornamental Plant Research Center), originating from *Dendrobium bigibum* and *Dendrobium antennatum*.

Based on data from the Central Statistics Agency (BPS, 2020), orchid production reached 11.68 million stalks. This number decreased by 37.22% compared to 2019 which

reached 18.61 million stalks. Orchid production from 2016 to 2020 experienced instability. In 2016, orchid production was 19.98 million stalks, up 0.35% in 2017 and again increased by 23.3%, in 2018 which reached 24.72 million stalks. After that, in 2019 it fell by 24.71% and decreased again by 37.22% in 2020. The increased demand for ornamental plants has driven up the demand for *Dendrobium* orchid seeds. However, until now the high demand for plant materials has not been fulfilled, so other alternatives are needed to provide plant materials in sufficient quantities. According to Latifah *et al*. (2017) the low production of orchids in Indonesia is caused by the lack of availability of quality seeds, inefficient cultivation and poor post-harvest handling. Thus, to overcome the problem of orchid production in Indonesia, it is possible to increase production through efficient plant propagation in vitro to produce seeds or flowers in large and uniform quantities.

The successes of in vitro orchid culture is influenced, among others, by the culture medium used to produce plantlets. The culture media commonly used for orchids is VW (Vacin and Went) media, because it basically is effective for supporting the conservation of orchid species (Kartikaningrum *et al*., 2017). However, according to Jualang *et al*. (2014), MS (Murashig-Skoog) media is the best medium, because MS media is a medium with the most complete nutritional composition compared to VW and Knudson C, and therefore is often used for culturing various types of plants. In addition to preparing media, in orchid plant culture a combination of ZPT and nutrients is also carried out to support plantlet growth by mixing ingredients that contain nutrients and humic substances. One of the ZPT that is often used in mixed in-vitro culture media is BAP (benzyl amino purine). BAP is a PGR (plant growth regulator) group of cytokinins that are active in cell division. Likewise with foliar fertilizers which play an important role in providing macro and micro nutrients needed for the growth and development of orchid plants. Therefore, this study will utilize Growmore 32-10-10 and ZPT BAP nutrients in culture media.

Based on the research results of Syamsiah *et al*. (2020), the addition of BAP with a concentration of 2.00 mg/L was the best treatment with the fastest shoot emergence time of 16.80 DAP (day after planting) and the fastest leaf emergence time of 25.20 DAP and the best number of leaves with an average of 14.4 leaves at the end of the observation, which was a week to 12. Hartati *et al*. (2016), in their research also revealed that the addition of ZPT BAP 3 ppm had a significant effect on the increase in root length and increase in plantlet height, so that increasing the concentration level of BAP could increase the vegetative growth response of orchid explants. Meanwhile, according to the results of Priatna (2019) the most proliferative *D. dian agrihorti* orchid proliferation rate resulted from the addition of 4 ppm Growmore, namely with the indicator of adding the most wet weight of culture. Based on this description, it is necessary to conduct research on the effect of Growmore and BAP nutrient concentrations on the growth of *Dendrobium* var. *bigiante agrihorti* in-vitro. The purpose of this study was to determine the effect and optimum dose of Growmore and BAP concentrations on the vegetative growth of orchid plantlets.

2. MATERIALS AND METHOD

2.1. Time, Place, and Plant Material

The experiment was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional (UPN) "Veteran" Jawa Timur, Surabaya, from September to December 2022. The main material consisted of ±1 year old *Dendrobium*

bigiante orchid plantlets obtained from the Balai Penelitian Tanaman Hias, Cianjur, West Java.

The *Dendrobium bigiante* orchid plantlets were previously stored in the incubation room of the Biotechnology Laboratory, UPN "Veteran", for explant culture (planting) activities into each treatment combination. Storage in the incubation room must also always be sterile or routinely sprayed with 70% alcohol. Cutting and planting of orchid plantlets was carried out aseptically in a previously sterilized laminar air flow (LAF) by spraying with 70% alcohol and irradiated with UV light for ±1 hour. Explants were cut (in the form of pseudobulbs size of ±1 cm) using tweezers and a scalpel (Figure 1.). The planting process also used tweezers carried out on a bunsen by opening the plastic at the mouth of the culture bottle and inserting one explant in each bottle of treatment medium.

Figure 1. The size of the pseudobulb plantlet of the *Dendrobium bigiante* orchid

The basic medium used was Murashige-Skoog (MS) medium with a size of ½ MS consisting of macro, micro, iron, myo-inositol and vitamin stock solutions. The preparation of ½ MS media is almost the same as MS0 (full strength-MS macronutrients) media, the only different in each stock solution is concentration (half the concentration in mg/L), namely, 50 ml of macro stock solution, 5 ml of macro stock solution, 5 ml of iron stock solution, 5 ml of vitamin stock solution, 5 ml of myo-inositol, 15-grams of sugar, and 7-grams of agar as solids. The acidity of the media was adjusted at pH 5.6-5.8 by adding 1N KOH or HCl. Then, the media was transferred into culture bottles that had been sterilized each ±30 ml and closed the culture bottles using plastic and glued with heat-resistant rubber, after that, put the culture bottles into the autoclave with a temperature of 121 °C and a pressure of 1.5 atm for 30 minutes.

2.2. Experimental Design and Observation

The factorial experiment was arranged based on a Completely Randomized Design (CRD) which consisted of 2 factors, namely the concentration of Growmore nutrients (G) and the concentration of BAP (B). Factor 1 Growmore concentration consisted of 4 levels, namely (0; 3; 6; 9 ppm). Factor 2 BAP concentration also consists of 4 levels, namely (0; 1; 3; 5 ppm). The combination of the 2 factors on the ½ MS media resulted in 16 treatments which were repeated 3 times, so that 48 experimental units were obtained. Labeling was performed after planting. After that, store the culture bottles on the culture rack in the incubation room and irradiate them with a 40-watt TL lamp with a temperature of 20-24 °C.

Observation of the growth of *Dendrobium bigiante* orchids in vitro was carried out quantitatively. Quantitative observation was carried out by collecting measurable data which aims to show the percentage of live plantlets, number of roots, number of leaves, plant height (cm), and plantlet wet weight (g) against Growmore and BAP administration. Quantitative observation variables were carried out in the last week of the study (8 DAP). In addition, the percentage live was calculated for each treatment by using the Equation (1):

$$\%\ \text{live plantlets} = \frac{(\Sigma\ \text{live plantlets})}{(\Sigma\ \text{all plantlets})} \times 100\% \quad (1)$$

2.3. Data analysis

The research data were analyzed by analysis of variance (ANOVA) according to the terms and conditions of the factorial Completely Randomized Design (CRD). Data analysis uses variance which aims to determine whether or not there is a difference in one factor, and the interaction of the two factors. If from the results of the CRD analysis of variance it is known that there is a significant difference, then a further test is carried out according to the Honest Significant Difference (HSD) test which is used to reconfirm which experimental units are significantly different.

3. RESULTS AND DISCUSSIONS

3.1. Percentage Live of Planlets

The results showed that there was a significant interaction between the concentration of Growmore and BAP on the percentage of live plantlets of Dendrobium bigiante orchids. Meanwhile, single treatment of Growmore and BAP concentrations did not have a significant effect on the percentage of live plantlets. The average results of Growmore and BAP interactions on the percentage of living orchid plantlets are presented in Table 1.

Table 1. The interaction effect of Growmore and BAP treatments on the percentage live (%) of *Dendebium* plantlets at 8 WAP

Growmore (ppm)	BAP			
	0	1	3	5
0	77.78 ± 19.25 abc	100.00 ± 0.00 c	100.00 ± 0.00 c	100.00 ± 0.00 c
3	100.00 ± 0.00 c	100.00 ± 0.00 c	77.78 ± 19.25 abc	100.00 ± 0.00 c
6	55.56 ± 19.25 a	100.00 ± 0.00 c	100.00 ± 0.00 c	66.67 ± 33.33 ab
9	100.00 ± 0.00 c	88.89 ± 19,25 bc	100.00 ± 0.00 c	77.78 ± 19.25 abc
HSD 5%	23.13			

Note: Numbers followed by the same letter in the same column were not significantly different in the 5% HSD test.

Table 1 shows that the combination treatment of Growmore and BAP had a positive and significant effect on the growth of orchid plantlets in the height range of 88.89% - 100%. The addition of various concentrations of Growmore and ZPT fertilizers in orchid culture media is proven to accelerate the growth of propagules. According to Hasanah *et al*. (2014) fertilizer concentration has a very significant effect on the height of orchid plantlets. Thus, it allows a real interaction to occur on the vegetative growth of orchid plantlets. This is also due to the fact that orchids are still in the form of plantlets that require elements of N, P, and K that are large enough to grow into adults. Pebriana *et al*. (2019) in his research also stated that cytokinin ZPT is a compound that functions to

increase cell division in plant cell tissues and regulate plant growth and development. Therefore, the addition of BAP which is also a cytokinin ZPT plays a very important role in stimulating the vegetative growth of orchids.

3.2. Number of Roots

The results showed that there was a significant interaction between the concentration of Growmore and BAP on the number of roots of the Dendrobium bigiante orchid. Whereas in the single treatment the concentration of Growmore and BAP also had a significant effect on the number of roots. The average results of the interaction and single treatment of Growmore and BAP on the number of orchid roots are presented in Tables 2 and 3.

Table 2 shows the highest number of roots with an average of 6.33 root units. Growmore concentration which is quite high with or without BAP interaction has a major effect on root growth. According to Pitriyanto *et al*. (2014), the addition without Benzyladenine + Growmore (32 : 10 : 10) had a significant effect on the number of *Dendrobium* orchid roots with the highest yield of 6.9 root units. The high concentration of Growmore on the number of roots in the results of the study was also reinforced by the research of Costa (2012), that fertilizers with high nitrogen play an important role in metabolic processes where the results of the metabolism are used for the growth of seedling organs such as roots. Table 2 also shows the lowest yield of 0.89 units with a deviation value that is also not much different, so that this combination of treatments has an unfavorable effect on the growth of Dendrobium orchid plantlets. According to research by Lestari *et al*. (2013) BAP is classified as a synthetic cytokinin whose use is influenced by other nutrients, so the addition of BAP can also affect growth hormone in orchids.

Table 2. The interaction effect of Growmore and BAP treatments on the number of roots of *Dendebium* plantlets at 8 WAP

Growmore (ppm)	BAP			
	0	**1**	**3**	**5**
0	3.67 ± 0.58 cdef	4.89 ± 1.07 defg	6.11 ± 0.19 g	2.11 ± 0.84 abc
3	4.78 ± 0.84 defg	5.89 ± 1.64 fg	3.56 ± 2.91 bcde	3.22 ± 0.84 bcd
6	2.11 ± 1.17 abc	2.56 ± 0.77 abc	5.44 ± 1.90 efg	0.89 ± 0.77 a
9	6.33 ± 1.15 g	5.56 ± 1.90 efg	3.78 ± 0.19 cdef	1.44 ± 0.77 ab
HSD 5%	2.19			

Note: Numbers followed by the same letter were not significantly different in the 5% HSD test.

Table 3. Effect of single factors *Growmore* and BAP on the number of roots of *Dendebium* plantlets

Growmore (ppm)	**0**	**3**	**6**	**9**
Number of Roots	4.19 ± 1.67 b	4.36 ± 1.87 b	2.75 ± 2.04 a	4.28 ± 2.21 b
BAP (ppm)	**0**	**1**	**3**	**5**
Number of Roots	4.22 ± 1.81 b	4.72 ± 1.82 b	4.72 ± 1.87 b	1.92 ± 1.14 a

Note: Numbers followed by the same letter in the same row were not significantly different in the 5% HSD test.

A single treatment with a higher Growmore concentration resulted in the number of orchid roots at concentrations of 3 ppm and 9 ppm, namely 4.36 and 4.28 root units (Table 3). In general, the growth of orchids in the multiplication process is relatively

long. According to Suradinata *et al*. (2012), the growth and development of orchid seedlings can be stimulated by a fertilization process that contains micro and macro nutrients so that the addition of Growmore can spur root growth even though it is still in the multiplication process or before acclimatization.

Table 3 also shows that the higher the concentration of BAP, the lower the average number of roots in the orchid plantlets. The optimum results for the number of roots are found at BAP concentrations of 0 to 3 ppm. So, according to the research by Erisa *et al*. (2022), the range of 1 to 2 ppm BAP is able to produce a number of roots compared to a concentration of 0 ppm BAP. This is presumably because the concentration of 1 to 2 ppm of BAP given has been able to stimulate the growth of orchid roots. Nurhanifah *et al*. (2021) in his research also stated that MS media treatment without BAP and coconut water had a very significant effect, in which the control media produced quite a large number of roots with an average number of roots of 3.5. Basically the BAP hormone functions to trigger shoot growth and cell division so that it is active in cell differentiation, and will inhibit root formation. Therefore it is in accordance with the results of the study that increasing the BAP hormone can actually inhibit the growth of orchid plantlet roots.

3.3. Number of Leaves

The results showed that there was a significant interaction between the concentration of Growmore and BAP on the number of leaves of the Dendrobium bigiante orchid. The effect of single treatment (concentration of Growmore and BAP) did not significant on the number of leaves. The average results of Growmore and BAP interactions on the number of orchid leaves are presented in Table 4.

Table 4. The interaction effect of Growmore and BAP treatments on the number of leaves of *Dendebium* plantlets at 8 WAP

Growmore (ppm)	BAP			
	0	1	3	5
0	4.11 ± 2.01 ab	7.33 ± 0.67 de	6.56 ± 0.77 bcde	6.33 ± 1.33 bcde
3	6.44 ± 0.69 bcde	6.33 ± 1.15 bcde	4.78 ± 1.58 abcd	7.33 ± 0.88 de
6	4.44 ± 2.50 abc	6.78 ± 1.17 cde	8.78 ± 1.07 e	3.67 ± 1.45 a
9	6.22 ± 1.07 abcde	5.44 ± 2.91 abcde	6.67 ± 0.88 bcde	5.11 ± 1.71 abcde
HSD 5%	2.56			

Note: Numbers followed by the same letter were not significantly different in the 5% HSD test.

Table 4 shows the results of the highest number of leaves with an average number of leaves of 8.78, compared to other treatments with an average number of 6-7 leaves. Leaf formation on orchid plantlets increased with increasing concentrations of Growmore and BAP. According to Ayuningtyas *et al*. (2020), the formation of leaves for orchid seedlings increased with the higher concentration of treatments used in the study, so that fertilizer treatment at these concentrations also resulted in an increase in the number of new leaves formed during the study period. The N element contained in Growmore is also very influential in the formation of leaf organs. This was supported by the research of Apsari *et al*. (2020), that during the vegetative period, plants need large amounts of nitrogen.

Table 4 also shows that BAP 0 ppm in its use can increase the growth and development of plant organs, especially leaves. This is in accordance with the research by Erisa *et al*. (2022), that giving a concentration of 0 ppm BAP produced more leaves

than the other 4 concentrations. This is presumably because plants are able to produce enough natural endogenous hormones (endogenous cytokinins) to stimulate the development of leaf numbers. The effect of BAP on orchid multiplication was also found in the study by Fithriyandini *et al*. (2015) who stated that the addition of BAP to Murishage and Skoog media on the moon orchid (Phalaenopsis amabilis) gave a good number of large leaves.

3.4. Plant height

There was a significant interaction between the concentration of Growmore and BAP on the height of the *Dendrobium bigiante* orchid. The single factor of Growmore and BAP concentration also had a significant effect on plant height. The average results of the interaction and single treatment of Growmore and BAP on orchid plant height are presented in Tables 5 and 6.

Table 5. The interaction effect of Growmore and BAP treatments on the plant height (cm) of *Dendebium* plantlets at 8 WAP

Growmore (ppm)	BAP			
	0	**1**	**3**	**5**
0	4.08 ± 0.44 f	3.23 ± 0.50 cdef	3.44 ± 0.38 def	2.08 ± 0.75 abc
3	7.37 ± 0.78 g	3.81 ± 0.87 ef	2.52 ± 1.04 abcd	2.97 ± 0.06 cdef
6	1.69 ± 0.60 ab	2.98 ± 0.04 cdef	3.50 ± 0.50 def	1.54 ± 0.51 a
9	3.59 ± 0.52 def	2.87 ± 1.50 bcdef	3.41 ± 0.52 def	2.66 ± 1.16 abcde
HSD 5%	1.25			

Note: Numbers followed by the same letter were not significantly different in the 5% HSD test.

Table 6. Effect of single factors *Growmore* and BAP on the plant height (cm) of *Dendebium* plantlets

Growmore (ppm)	**0**	**3**	**6**	**9**
Number of Roots	3.21 ± 0.88 bc	4.17 ± 2.10 d	2.43 ± 0.96 a	3.13 ± 0.96 ab
BAP (ppm)	**0**	**1**	**3**	**5**
Number of Roots	4.18 ± 2.20 d	3.22 ± 0.86 b	3.22 ± 0.70 bc	2.31 ± 0.85 a

Note: Numbers followed by the same letter in the same row were not significantly different in the 5% HSD test.

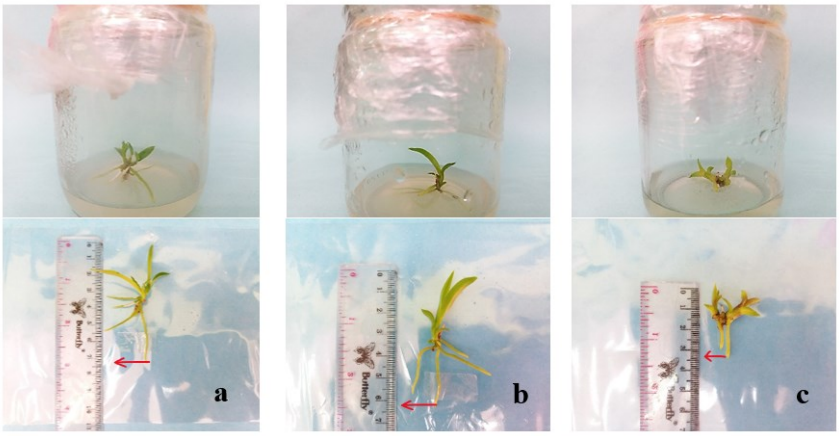

Figure 2. The height of *Dendrobium bigiante* orchid plantlet at 8 WAP with different treatments: a) Growmore 3 ppm + BAP 0 ppm; b) no Growmore + no BAP; c) Growmore 6 ppm + BAP 5 ppm

Table 5 shows the addition or without the addition of Growmore concentration affected the speed of growth of orchid plantlets, but the highest average yield was affected by the addition of 3 ppm Growmore. Thus, nitrogen is a macro nutrient that is needed by Dendrobium orchid plantlets. According to Zasari *et al*. (2015), if there is a shortage of nitrogen, it will inhibit the vegetative growth of plants, especially in orchid plantlets. However, the results of the study also showed that giving very high concentrations of Growmore and BAP actually caused a decrease in the height of the orchid plantlets, as evidenced by the lowest yield of 1.54 cm (Figure 2c.), so that in this case the absorption of excess nutrients can reduce the growth of the height of the orchid plantlets.

A single treatment of 3 ppm Growmore gave more optimum results for plantlet height (Table 6.). According to Ayuningtyas *et al*. (2020), the concentration of foliar fertilizer treatment affected the growth rate of *Dendrobium* var. *dian agrihorti*, and foliar fertilizer concentration of 2.25 mlL-1 was the best concentration which produced the highest average for seedling height. So that is in accordance with the results of the study which showed the combination of 3 ppm Growmore could provide the highest average of 4.17 cm.

Table 6 also shows that a single treatment with high ZPT BAP concentrations caused a decrease in plant height. However, there is also an optimum concentration dose, namely at BAP 3 ppm, so that it is in accordance with the research of Hartati *et al*. (2016) that the addition of ZPT BAP 3 ppm had a significant effect on the increase in root length and increase in plantlet height. Samanhudi *et al*. (2021) in his research also stated that the administration of cytokinins into the culture media could increase the concentration of endogenous ZPT in plant cells so that it became a triggering factor for the response to growth and development of plant tissues, but this was not in accordance with the research that had been conducted, due to the addition of higher concentrations of BAP inhibits plantlet height.

3.5. Wet Weight of Plantlets

The results showed that there was no interaction in the combination of Growmore and BAP concentration treatments on the wet weight of Dendrobium bigiante orchids. Whereas in the single treatment the concentration of Growmore also had no significant effect, however in the single treatment BAP gave a significant effect on the wet weight of the orchids. The average results of the single factor of Growmore and BAP on the wet weight of orchid plantlets are presented in Table 7.

Table 7. Effect of single factors *Growmore* and BAP on the plant weight (g) of *Dendebium* plantlets

Growmore (ppm)	**0**	**3**	**6**	**9**
Number of Roots	0.38	0.34	0.50	0.50
BAP (ppm)	**0**	**1**	**3**	**5**
Number of Roots	4.18 ± 2.20 d	3.22 ± 0.86 b	3.22 ± 0.70 bc	2.31 ± 0.85 a

Note: Numbers followed by the same letter in the same row were not significantly different in the 5% HSD test.

Figure 3 shows the concentration of BAP can trigger the growth of plant organs such as roots and leaves in orchid plantlets, thereby affecting the high average wet weight with a yield of 0.63 g. According to Hidayat (2020), the development of the wet weight of orchid plantlets is influenced by the enlargement and elongation of the cells

contained in the explants. According to Agriani (2010) in his research also stated that naturally some explants are capable of producing sufficient amounts of endogenous auxin.

Figure 3. Wet weight of *Dendrobium bigiante* orchid plantlets at 8 WAP with different treatments: a) BAP 0 ppm; b) BAP 1 ppm; c) BAP 3 ppm; d) BAP 5 ppm

The presence of endogenous auxin itself can stimulate organogenesis. The small average wet weight is also affected by the low number of growing organs (shoots and leaves) or physiological processes in the orchid plantlets (Figure 3a.). According to Yuswanti *et al*. (2014) in the presence or occurrence of physiological processes in these plants, the cell mass content will increase, which can be identified by an increase in plantlet dry weight. BAP (6-Benzylaminopurine) is a cytokinin hormone that is very active in triggering shoot formation and also works very effectively in cell division and shoot multiplication in certain plants (Akbar *et al*., 2017). So according to table 7, that the addition of 3 ppm BAP can trigger the formation of shoots followed by an increase in fresh weight (Figure 3c.).

4. CONCLUSIONS

Growmore concentration of 3 ppm had a very significant effect on the number of roots and plantlet height of Dendrobium bigiante orchids. Further test results proved that the largest number of roots and plant height obtained with the effect of a 3 ppm Growmore concentration were 4.36 units and 4.17 cm. Meanwhile, the BAP concentration of 3 ppm also had a very significant effect on the number of roots, plant height and wet weight of orchid plantlets. Further test results proved that the largest number of roots, plant height and fresh weight obtained with the influence of 3 ppm BAP concentrations were 4.72 units, 3.22 cm and 0.63 g. There is an interaction between Growmore and BAP which affects the percentage of living plantlets, the number of roots, the number of leaves, and the height of the orchid plantlets. The combination of 3 ppm Growmore + 0 ppm BAP produced the highest percentage of live plantlets and plant height, namely 100% and 7.37 cm. The combination of Growmore 9 ppm + BAP 0 ppm produced the largest number of roots, namely 6.33 units. Meanwhile, the combination of Growmore 6 ppm + BAP 3 ppm produced the largest number of leaves, namely 8.78 leaves. For all interactions, the combination of 3 ppm Growmore + 0 ppm BAP is the optimum dose for the growth of Dendrobium bigiante orchid plantlets. It is recommended that further research trials be carried out to reduce the dosage concentration of using nutrients and synthetic ZPT with optimal concentrations (0; 1; and 3 ppm) as well as the alternative use of organic matter as a growth medium for Dendrobium bigiante orchid plantlets needed to obtain optimal results.

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