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Effects of Different Concentrations of 6-Benzylaminopurine (BAP) on the In Vitro Growth of *Dendrobium spectabile* (Blume) Miq. Plantlets

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Abstract

Dendrobium spectabile (Blume) Miq. is an ornamental orchid with high economic value and considerable potential for commercial cultivation. However, conventional propagation is relatively slow, making *in vitro* culture an effective alternative for producing large numbers of uniform plantlets. This study aimed to evaluate the effects of different concentrations of 6-benzylaminopurine (BAP) on the growth of *D. spectabile* plantlets and to determine the optimal BAP concentration for in vitro plantlet development. The experiment was arranged in Completely Randomized Design (CRD) consisting of five BAP concentrations (0, 0.5, 1.0, 1.5, and 2.0 mg/L) using Vacin and Went (VW) medium. The observed parameters included plantlet survival percentage, visual appearance, plantlet height, number of leaves, number of shoots, root length, number of roots, and fresh weight. Data were analyzed using analysis of variance (ANOVA), followed by Tukey's test at the 5% significance level. The results showed that BAP concentrations significantly affected the growth of *D. spectabile* plantlets. A concentration of 1.5 mg/L BAP was the most effective for promoting plantlet height, number of leaves, number of shoots, and fresh weight, whereas 0.5 mg/L BAP produced the greatest root length and number of roots. These findings indicate that 1.5 mg/L BAP is the optimal concentration for enhancing shoot growth, while 0.5 mg/L BAP is more suitable for root development of *D. spectabile* plantlets cultured on VW medium.

Keywords: *Dendrobium spectabile*; In vitro culture; 6-Benzylaminopurine (BAP); Plantlet; Vacin and Went (VW) medium.

1. Introduction

Orchids are horticultural commodities with high economic value and are widely recognized as ornamental plants because of their diverse floral forms, attractive colors, and high market demand (1). Indonesia is one of the world's major centers of orchid diversity, harboring approximately 6,000 orchid species distributed across its tropical regions (2). One species with considerable ornamental and economic importance is *Dendrobium spectabile* (Blume) Miq., which is highly valued for its distinctive and attractive floral morphology. However, the increasing commercial demand for this species may contribute to overexploitation of its natural populations. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), *D. spectabile* is listed in Appendix II, indicating that international trade must be regulated to prevent threats to its survival in the wild (3). Therefore, effective conservation and propagation strategies through both *in situ* and *ex situ* approaches are essential.

Conventional propagation of orchids remains limited by their slow growth rate and low seed germination success, primarily because orchid seeds lack endosperm (4). Consequently, in vitro culture has become an effective alternative for overcoming these limitations, as it enables the large-scale production of uniform plantlets under controlled

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environmental conditions (5). The success of in vitro propagation is strongly influenced by the composition of the culture medium and the application of plant growth regulators. Among the cytokinins commonly used in plant tissue culture, 6-benzylaminopurine (BAP) plays an important role in promoting cell division, shoot multiplication, and plantlet development. However, the effectiveness of BAP depends on its concentration, as insufficient concentrations may fail to stimulate growth, whereas excessive concentrations may inhibit plantlet development (6).

Previous studies have reported that 1.5 ppm BAP resulted in the best growth response in *D. spectabile* plantlets cultured on Murashige and Skoog (MS) medium (6). However, information regarding the optimization of BAP concentrations for the in vitro growth of *D. spectabile* plantlets cultured on Vacin and Went (VW) medium remains limited. Since the nutrient composition of VW medium differs from that of MS medium, plantlet responses to BAP may also vary. Therefore, further investigation is needed to determine the optimum BAP concentration for promoting the growth of *D. spectabile* plantlets cultured on VW medium. Accordingly, this study was conducted to evaluate the effects of different BAP concentrations on the in vitro growth of *D. spectabile* plantlets and to identify the optimal BAP concentration for plantlet development on VW medium.

2. Material and methods

2.1. Research Materials and Experimental Design

This study was conducted at the Tissue Culture Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung. The main plant material used in this study consisted of *Dendrobium spectabile* (Blume) Miq. plantlets cultured on Vacin and Went (VW) medium. The experiment was arranged in a completely randomized design (CRD) with one factor, namely the concentration of 6-Benzylaminopurine (BAP), consisting of five levels: 0, 0.5, 1.0, 1.5, and 2.0 mg/L. Each treatment was repeated five times, resulting in a total of 25 experimental units.

2.2. Preparation of culture medium

VW medium was prepared by adding sugar at 30 g/L, activated charcoal at 2 g/L, and agar at 7 g/L. The pH of the medium was adjusted to 5.7 using 1 N HCl or 1 N KOH, and the medium was then sterilized in an autoclave at 121°C for 15 minutes. A BAP stock solution with a concentration of 100 mg/L was sterilized using a 0.22 µm *syringe filter* and added aseptically to the sterile medium according to the treatment concentrations. The medium was then poured into sterile culture bottles at 20 mL per bottle and incubated for 3–4 days to ensure that no contamination occurred.

2.3. Plantlet inoculation and observation

Sterilized *Dendrobium spectabile* (Blume) Miq. plantlets were inoculated onto the treatment medium, with one plantlet placed in each culture bottle. The plantlets were incubated at 24–26°C under a light intensity of approximately 1000 lux. The observed parameters included plantlet survival percentage, plantlet visual appearance, plantlet height, number of leaves, number of shoots, root length, number of roots, and plantlet fresh weight.

The percentage of surviving plantlets was observed every four days for 16 days after planting and was calculated using the formula of Nurcahyani et al. (7): Plantlet survival percentage : $\frac{Number\ of\ surviving\ plantlets}{Total\ number\ of\ plantlets} \times 100\%$

Plantlets were considered alive when they were green and fresh, exhibited good turgor, and showed no signs of decay or contamination. The visual appearance of the plantlets was assessed based on color, freshness, necrosis, and contamination. Plantlet height was measured from the surface of the medium to the growing point using a ruler (8). The number of leaves was counted based on fully opened leaves, while the number of shoots was counted based on new shoots formed during the observation period. Root length was measured from the base to the tip of the longest root using millimeter paper (9), and the number of roots was determined by counting the newly formed roots on each plantlet. Observations of plantlet height, number of leaves, number of shoots, root length, number of roots, and fresh weight were conducted on the 16th day after planting. Plantlet fresh weight was measured using an analytical balance to determine plantlet biomass accumulation.

2.4. Data analysis

The observed data were analyzed using analysis of variance (ANOVA). When a significant effect was detected, *Tukey's* test was performed at the 5% significance level.

3. Results and Discussion

3.1. Plantlet Survival Percentage and Visual Appearance

The survival percentage and visual appearance of *Dendrobium spectabile* (Blume) Miq. plantlets were observed every four days for 16 days on Vacin and Went (VW) medium supplemented with BAP at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 mg/L. The results are presented in **Table 1.**

Table 1 Survival percentage of *Dendrobium spectabile* (Blume) Miq. plantlets

BAP Concentration (mg/L)	Plantlet survival percentage by day (%)			
	4	8	12	16
0	100	100	100	100
0.5	100	100	100	100
1.0	100	100	100	100
1.5	100	100	100	100
2.0	100	100	100	100

Note: The value 100 indicates that all plantlets survived until the 16th day after planting.

Based on **Table 1**, all plantlets in all treatments showed a survival percentage of 100% until the end of the observation period. This condition indicates that VW medium was able to support plantlet survival during the culture period. VW medium contains macro- and micronutrients as well as other nutritional components, such as vitamins, amino acids, organic compounds, carbohydrates, plant growth regulators, and water as a solvent, which meet the nutritional requirements of the plantlets and optimize explant growth (10).

The visual appearance of *Dendrobium spectabile* (Blume) Miq. plantlets during the 16-day observation period are presented in Table **2.**

Table 2 Visual appearance of *Dendrobium spectabile* (Blume) Miq. plantlets after the addition of 6-Benzylaminopurine (BAP) at different concentrations

BAP Concentration (mg/L)	Survival Percentage and Visual Appearance of Plantlets by Day (%)			
	4	8	12	16
0	100G	100G	80G 20YG	80G 20YG
0.5	100G	100G	100G	100G
1.0	100G	100G	100G	100G
1.5	100G	100G	100G	100G
2.0	100G	100G	80G 20YG	60G 40YG

Note: G = green; YG= yellowish-green; Y = yellow; B = brown.

Based on **Table 2**, all *Dendrobium spectabile* (Blume) Miq. plantlets showed 100% survival with green coloration (G) in all BAP treatments until the 8th day. This condition indicates that the plantlets were able to adapt well to Vacin and Went (VW) medium and the culture environment. Visual changes began to appear on the 12th day, particularly in the 0 mg/L and 2.0 mg/L BAP treatments, in which 80% of the plantlets remained green and 20% turned yellowish-green (YG).

On the 16th day, the 0.5, 1.0, and 1.5 mg/L BAP treatments resulted in 100% of the plantlets remaining green, while the 2.0 mg/L BAP treatment showed a decrease, with 60% green plantlets and 40% greenish-yellow plantlets. The highest

BAP concentration was presumed to reduce the visual quality of the plantlets compared with the other treatments. These results indicate that BAP concentrations of 0.5–1.5 mg/L represent the best concentration range for maintaining the viability and visual quality of *Dendrobium spectabile* (Blume) Miq. plantlets during the observation period.

The visual appearance of *Dendrobium spectabile* (Blume) Miq. plantlets are shown in **Figure 1.**

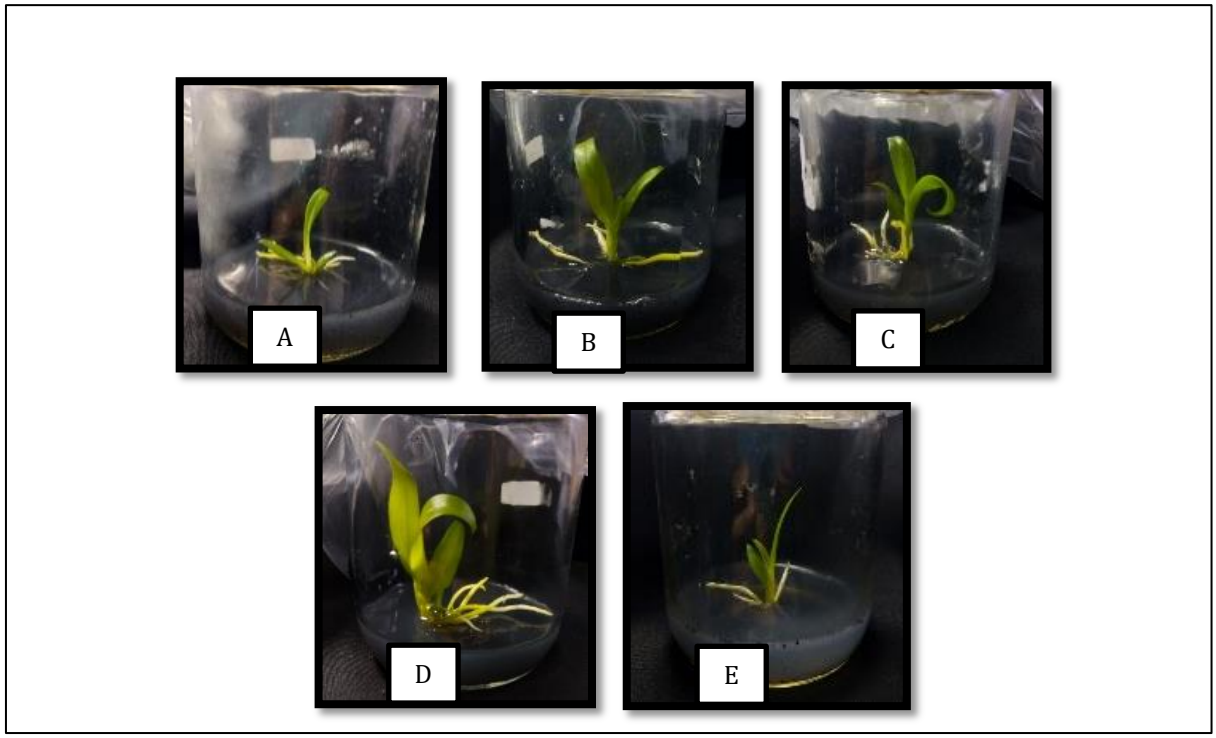

Figure 1. *Dendrobium spectabile* (Blume) Miq. plantlets on VW medium supplemented with different BAP concentrations (A = 0 mg/L, B = 0.5 mg/L, C = 1.0 mg/L, D = 1.5 mg/L, and E = 2.0 mg/L) on the 16th day after planting.

Figure 1. shows the visual appearance of *Dendrobium spectabile* (Blume) Miq. plantlets after 16 days of culture on VW medium supplemented with different concentrations of BAP. Plantlets treated with 0.5, 1.0, and 1.5 mg/L BAP remained green, whereas 20% of the plantlets cultured without BAP exhibited a yellowish-green coloration. The highest proportion of discoloration was observed in plantlets treated with 2.0 mg/L BAP, with 40% of the plantlets exhibiting a yellowish-green coloration. These results indicate that BAP concentrations ranging from 0.5 to 1.5 mg/L were more effective in maintaining the green coloration and visual quality of *D. spectabile* plantlets.

3.2. Plantlet Height

Data on the average plantlet height on the 16th day after planting are presented in **Table 3.**

Table 3 Average plantlet height of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Plantlet Height $\bar{Y}$±SE
0 mg/L	4.54 ± 0.641^{ab}
0.5 mg/L	4.74 ± 0.522^{ab}
1.0 mg/L	5.44 ± 0.472^{b}
1.5 mg/L	5.98 ± 0.471^{b}
2.0 mg/L	2.98 ± 0.208^{a}

Note: $\bar{Y}$ = mean plantlet height; SE = standard error. Values followed by different letters indicate significant differences among treatments.

The results showed that the height of *Dendrobium spectabile* (Blume) Miq. plantlets on the 16th day after planting varied among the BAP concentration treatments. The 1.5 mg/L BAP treatment produced the greatest mean plantlet height (5.98 ± 0.471 cm), while the 2.0 mg/L BAP treatment produced the lowest average height, namely 2.98 ± 0.208 cm. The ANOVA results showed that BAP significantly affected plantlet height ($p < 0.05$). Tukey's test at the 5% significance level showed that the 1.5 mg/L treatment was not significantly different from the 1.0 mg/L and 0.5 mg/L treatments, but was significantly different from the 2.0 mg/L treatment (Table 3).

These findings are in line with Arafah et al. (11), who reported that 1.5 ppm BAP produced the greatest plantlet height. BAP, as a cytokinin, plays a role in stimulating cell division, thereby supporting vegetative plantlet growth (12). However, increasing the BAP concentration to 2.0 mg/L reduced plantlet height. According to Wuriesyliane and Sawaluddin (13), the application of plant growth regulators at concentrations exceeding plant requirements may inhibit plant growth. These results indicate that 1.5 mg/L BAP is the optimal concentration for promoting the growth in height of *D. spectabile* plantlets in vitro.

3.3. Number of leaves

Data on the average number of leaves on the 16th day after planting are presented in **Table 4.**

Table 4 Average number of leaves of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Number of Leaves $\bar{Y}$±SE
0 mg/L	2.96 ± 0.264[a]
0.5 mg/L	3.60 ± 0.510[ab]
1.0 mg/L	4.00 ± 0.548[ab]
1.5 mg/L	5.00 ± 0.316[b]
2.0 mg/L	2.46 ± 0.227[a]

Note: $\bar{Y}$ = mean number of leaves; SE = standard error. Values followed by different letters indicate significant differences among treatments.

Based on the ANOVA results, 6-Benzylaminopurine (BAP) significantly affected the number of leaves of *Dendrobium spectabile* (Blume) Miq. plantlets ($p < 0.05$). The 1.5 mg/L BAP treatment produced the highest number of leaves, namely 5.00 ± 0.316 leaves, while the 2.0 mg/L BAP treatment produced the lowest number, namely 2.46 ± 0.227 leaves. Tukey's test showed that the 1.5 mg/L treatment was significantly different from the control and the 2.0 mg/L treatment, but was not significantly different from the 0.5 mg/L and 1.0 mg/L treatments (Table 4).

These results are in line with Purita et al. (14), who reported that 1.5 ppm BAP produced the highest number of leaves. This condition indicates that the addition of exogenous cytokinin at 1.5 mg/L could interact effectively with endogenous plant hormones to support leaf formation and development. At 2.0 mg/L BAP, the number of leaves decreased. According to Ario and Setiawan (6), the use of plant growth regulators above the optimum concentration may inhibit plant growth due to hormonal imbalance in explant tissues.

3.4. Number of Shoots

Data on the average number of shoots on the 16th day after planting are presented in **Table 5.**

Based on **Table 5,** the average number of shoots of *Dendrobium spectabile* (Blume) Miq. plantlets differed among BAP concentration treatments. The 1.5 mg/L BAP treatment produced the highest number of shoots, namely 3.80 shoots, while the 2.0 mg/L BAP treatment produced the lowest number, namely 1.80 shoots. The ANOVA results showed that BAP significantly affected the number of shoots ($p < 0.05$). Tukey's test showed that the 1.5 mg/L treatment was significantly different from the 2.0 mg/L treatment, but was not significantly different from the 0, 0.5, and 1.0 mg/L treatments (Table 5).

These findings are in line with Adhikari and Pant (15), who reported that the application of 1.5 mg/L BAP was optimal for inducing shoot formation. BAP is a cytokinin that plays a role in stimulating shoot formation and proliferation, as well as breaking apical dominance, thereby increasing lateral shoot growth (16). Increasing the BAP concentration to

2.0 mg/L tended to reduce the number of shoots formed. This condition was presumably caused by hormonal imbalance in plant tissues, which inhibited cell differentiation and meristematic activity, resulting in suboptimal shoot formation.

Table 5 Average number of shoots of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Number of Shoots $\bar{Y}$±SE
0 mg/L	2.20 ± 0.374^{ab}
0.5 mg/L	3.00 ± 0.447^{ab}
1.0 mg/L	3.20 ± 0.374^{ab}
1.5 mg/L	3.80 ± 0.490^{b}
2.0 mg/L	1.80 ± 0.374^{a}

Note: $\bar{Y}$ = mean number of shoots; SE = standard error. Values followed by different letters indicate significant differences among treatments.

3.5. Root Length

Data on the average root length on the 16th day after planting are presented in **Table 6.**

Table 6 Average root length of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Root Length $\bar{Y}$±SE
0 mg/L	3.48 ± 0.585^{ab}
0.5 mg/L	4.62 ± 0.240^{b}
1.0 mg/L	4.06 ± 0.556^{ab}
1.5 mg/L	3.32 ± 0.567^{ab}
2.0 mg/L	2.22 ± 0.225^{a}

Note: $\bar{Y}$ = mean root length; SE = standard error. Values followed by different letters indicate significant differences among treatments.

Based on **Table 6**, the average root length of *Dendrobium spectabile* (Blume) Miq. plantlets differed among BAP concentration treatments. The 0.5 mg/L BAP treatment produced the longest roots, namely 4.62 cm, while the 2.0 mg/L BAP treatment produced the shortest roots, namely 2.22 cm. The ANOVA results showed that BAP significantly affected root length ($p < 0.05$). Tukey's test showed that the 0.5 mg/L treatment was significantly different from the 2.0 mg/L treatment, while the 0, 1.0, and 1.5 mg/L treatments were not significantly different from either treatment (Table 6).

The results showed that the application of 0.5 mg/L BAP produced the greatest root length. This condition was presumably due to the low cytokinin concentration, which could still maintain the balance of endogenous hormones, particularly auxin, which plays a role in root cell division and elongation. Karimah et al. (17) stated that root elongation is influenced by endogenous hormone activity in the root region, which promotes cell division and elongation. Increasing the BAP concentration to 2.0 mg/L tended to reduce root length, presumably due to increased cytokinin dominance, which inhibited auxin-mediated root growth.

3.6. Number of Roots

Data on the average number of roots on the 16th day after planting are presented in **Table 7.**

Based on **Table 7,** the average number of roots of *Dendrobium spectabile* (Blume) Miq. plantlets differed among BAP concentration treatments. The 0.5 mg/L BAP treatment produced the highest number of roots, namely 5.80 roots, while the 2.0 mg/L BAP treatment produced the lowest number, namely 3.00 roots. The ANOVA results showed that BAP significantly affected the number of roots ($p < 0.05$). Tukey's test showed that the 0.5 mg/L treatment was significantly different from the 2.0 mg/L treatment, while the 0, 1.0, and 1.5 mg/L treatments were not significantly different from either the 0.5 mg/L or 2.0 mg/L treatments.

These findings are in line with Arafah et al. (11), who reported that 0.5 ppm BAP produced the highest number of roots in orchid plantlets. This condition was presumably due to the low cytokinin concentration, which was still able to maintain the balance of endogenous hormones, particularly auxin, which plays an important role in root formation. Noli et al. (18) stated that auxin plays a role in stimulating adventitious root formation, root elongation, and cell division. Increasing the BAP concentration to 2.0 mg/L tended to reduce the number of roots formed due to hormonal imbalance in plantlet tissues, resulting in less optimal root formation. According to Arnilan and Mardaleni (19), excessive use of plant growth regulators may inhibit plant growth and organ formation.

Table 7 Average number of roots of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Number of Roots $\bar{Y}$±SE
0 mg/L	3.60 ± 0.510^{ab}
0.5 mg/L	5.80 ± 0.583^{b}
1.0 mg/L	4.40 ± 0.748^{ab}
1.5 mg/L	4.00 ± 0.548^{ab}
2.0 mg/L	3.00 ± 0.316^{a}

Note: $\bar{Y}$ = mean number of roots; SE = standard error. Values followed by different letters indicate significant differences among treatments.

3.7. Fresh Weight

Data on the average fresh weight of plantlets on the 16th day after planting are presented in **Table 8.**

Table 8 Average fresh weight of *Dendrobium spectabile* (Blume) Miq. at different BAP concentrations on the 16th day after planting

Treatment	Average Fresh Weight $\bar{Y}$±SE
0 mg/L	0.340 ± 0.045^{a}
0.5 mg/L	0.408 ± 0.043^{ab}
1.0 mg/L	0.551 ± 0.085^{ab}
1.5 mg/L	0.733 ± 0.161^{b}
2.0 mg/L	0.251 ± 0.037^{a}

Note: $\bar{Y}$ = mean fresh weight; SE = standard error. Values followed by different letters indicate significant differences among treatments.

Based on **Table 8,** the average fresh weight of *Dendrobium spectabile* (Blume) Miq. plantlets differed among BAP concentration treatments. The 1.5 mg/L BAP treatment produced the highest fresh weight, namely 0.733 g, while the 2.0 mg/L BAP treatment produced the lowest fresh weight, namely 0.251 g. The ANOVA results showed that BAP significantly affected fresh weight ($p < 0.05$). Tukey's test showed that the 1.5 mg/L treatment was significantly different from the 0 mg/L and 2.0 mg/L treatments, but was not significantly different from the 0.5 mg/L and 1.0 mg/L treatments.

The maximum fresh weight obtained at 1.5 mg/L BAP indicates that this concentration provided favorable culture conditions that supported the physiological requirements of the plantlets. The efficient absorption of macro- and micronutrients could stimulate cell division and cell enlargement. According to Zanirah et al. (20), the increase in tissue mass in orchid explants is closely related to cell expansion and elongation. A balanced medium composition allows plantlets to optimize water and nutrient absorption to support the differentiation of new organs, such as shoots and leaves, which cumulatively increases total biomass (21). The significant decrease in fresh weight at 2.0 mg/L BAP indicates a negative effect of an excessive cytokinin concentration. High concentrations of plant growth regulators may trigger hormonal imbalance, disrupt cellular metabolism, reduce nutrient absorption efficiency, and induce physiological stress, thereby inhibiting explant growth and reducing overall biomass accumulation.

4. Conclusion

Based on the results of this study, it can be concluded that the application of different BAP concentrations produced distinct growth responses in *Dendrobium spectabile* (Blume) Miq. plantlets cultured *in vitro* on VW medium. These responses were reflected in the plantlet survival percentage and visual condition, plantlet height, number of leaves, number of shoots, root length, number of roots, and fresh weight. A BAP concentration of 1.5 mg/L was the optimal concentration for promoting plantlet height, number of leaves, number of shoots, and fresh weight, whereas 0.5 mg/L BAP produced the best results for root length and number of roots.

Compliance with ethical standards

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Disclosure of Conflict of Interest

The authors declare that there is no conflict of interest regarding this study.

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