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Optimization of *in vitro* multiplication of *Dendrobium* sp. through application of Gibberellin (GA_3)

Mita Ardelia, Endang Nurcahyani *, Yulianty and Mahfut

Applied Biology Study Program, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia.

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Abstract

Dendrobium sp. is a widely popular ornamental orchid valued for its aesthetic appeal and diverse varieties. *In vitro* multiplication is a method of propagating new plants by stimulating shoot growth, either directly or via callus induction. Gibberellic acid (GA_3), a type of plant growth regulator (PGR), is commonly used to enhance plant growth and development. This study aimed to evaluate the effect and optimal concentration of GA_3 on the *in vitro* growth of *Dendrobium* sp. plantlets. The experiment was arranged in a Completely Randomized Design (CRD) with five concentrations of GA_3: 0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L. Each treatment was replicated five times, with one plantlet per replicate. Data were analyzed using one-way ANOVA in SPSS, followed by Tukey's post hoc test at a 5% significance level. The results indicated that GA_3 application significantly affected plantlet survival rate, height, number of leaves, and number of roots. However, no significant effect was observed on total carbohydrate content. Among the tested concentrations, 2 mg/L GA_3 was identified as the most effective in promoting the growth and development of *Dendrobium* sp. plantlets *in vitro*.

Keywords: *Dendrobium* sp.; Gibberellic Acid; *In vitro* Culture; Orchid Propagation; Plant Growth Regulator

1. Introduction

Dendrobium sp. is one of the most popular orchid genera due to its adaptability to lowland environments, minimal maintenance requirements, ease of flowering, diversity of floral forms, and the rapid blooming characteristics of certain hybrids [1]. Most *Dendrobium* species exhibit racemose-type inflorescences, in which the flowers bloom sequentially from the base to the apex along a single main axis [2, 3]. Furthermore, large compound flowers are particularly favored by consumers, as they enhance the ornamental appeal of the plant [3, 4].

Plant tissue culture is an *in vitro* propagation technique conducted aseptically on artificial media. This method involves culturing explants derived from various parts of the parent plant, such as shoots, roots, callus, seeds, embryos, pollen, seedlings, or even single cells [5]. The culture medium is typically supplemented with plant growth regulators (PGRs), including auxins, cytokinins, gibberellins, ethylene, abscisic acid, and other related hormones [6]. Multiplication refers to the phase of *in vitro* propagation aimed at generating new plantlets by stimulating shoot proliferation prior to acclimatization. This process is performed by transferring the developing plantlets to fresh culture vessels containing media enriched with PGRs that promote shoot formation and development [7].

One of the plant growth regulators (PGRs) commonly used to stimulate plant growth and development is gibberellic acid (GA_3) [8]. Gibberellin, specifically GA_3, plays a vital role in promoting growth by significantly enhancing cell

* Corresponding author: Endang Nurcahyani

elongation in the apical meristem. In addition to stimulating cell elongation, gibberellins also activate cell division and differentiation, break dormancy, and accelerate flowering [9].

Research on the *in vitro* multiplication of *Dendrobium* sp. plantlets supplemented with gibberellic acid (GA_3) remains limited. Therefore, this study aims to investigate the effect and optimal concentration of GA_3 on the *in vitro* growth of *Dendrobium* sp. plantlets.

2. Materials and Methods

2.1. Materials and Equipment

The materials used in this study included *Dendrobium* sp. plantlets, Vacin and Went (VW) ready-to-use solution, 1 N potassium hydroxide (KOH), 1 N hydrochloric acid (HCl), 70% ethanol, agar, sucrose, activated charcoal, gibberellic acid (GA_3) at various concentrations, glucose solution, sulfuric acid (H_2SO_4), standard glucose solution, and phenol.

The equipment used consisted of an autoclave, culture bottles, heat-resistant plastic, rubber stoppers, stirring rods, beakers, Bunsen burners, Petri dishes, measuring cylinders, Erlenmeyer flasks, hot plate, oven, laminar air flow (LAF) cabinet, magnetic stirrer, pH meter, forceps, scalpels, analytical balance, Whatman No. 1 filter paper, millimeter ruler, and UV-Vi's spectrophotometer.

2.2. Experimental Design

The experiment was arranged in a completely randomized design (CRD) with a single factor: GA_3 concentration. Five levels of GA_3 were tested: 0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L. Each treatment was replicated five times. Observations were conducted over a period of four weeks after planting to evaluate the most effective GA_3 concentration for *in vitro* cultivation of *Dendrobium* sp.

The data collected comprised both qualitative and quantitative variables. Qualitative data were presented descriptively and supported by photographic documentation. Quantitative data for each parameter were analyzed using the Statistical Package for the Social Sciences (SPSS) with analysis of variance (ANOVA). When significant differences were found, Tukey's post hoc test was applied at a 5% significance level.

2.3. Preparation and Dilution of GA_3

Gibberellic acid (GA_3) stock solutions were prepared by dissolving GA_3 powder in 1 liter of distilled water for each concentration level: 0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L. The 0 mg/L solution, used as the control, consisted of distilled water without GA_3. The 1 mg/L solution was prepared by dissolving 1 mg of GA_3 powder in 1 liter of distilled water; similarly, 2 mg, 3 mg, and 4 mg of GA_3 were dissolved in 1 liter of distilled water to obtain the 2 mg/L, 3 mg/L, and 4 mg/L solutions, respectively.

Once fully dissolved and homogeneous, the solutions were filtered using Whatman No. 1 filter paper into sterilized culture bottles. Subsequently, 1 mL of each GA_3 solution was added to the Vacin and Went (VW) medium prepared for *in vitro* culture.

2.4. Sterilization of *Dendrobium* sp.

Dendrobium sp. plantlets were individually transferred into an Erlenmeyer flask using sterile forceps and initially rinsed with sterile distilled water for 5 minutes. They were then surface-sterilized using 10 mL of Bayclin solution (a commercial bleach containing 5.25% sodium hypochlorite, NaOCl) for 3 minutes. After sterilization, the plantlets were rinsed twice with 100 mL of sterile distilled water to remove any residual disinfectant.

2.5. Multiplication of *Dendrobium* sp. Plantlets

The washed plantlets were placed in Petri dishes lined with millimeter grid paper to measure their initial height. Each culture bottle received a single explant, which was sealed near a flame from a Bunsen burner and covered with plastic wrap to maintain sterility. Planting was carried out in a laminar air flow (LAF) cabinet at room temperature (24°C–26°C). The culture bottles containing the explants were then incubated on shelves in a growth room under optimal lighting conditions (1,000 lux, equivalent to a 40-watt fluorescent lamp) and maintained at a constant temperature of 24°C–26°C [10].

2.6. Percentage of Live Plantlets and Plantlet Visualization

Observations were carried out four weeks after planting to determine the effective concentration of GA_3 for the *in vitro* multiplication of *Dendrobium* sp. The following parameters were assessed [11]:

$$\text{Percentage of live plantlets} = \frac{\text{Number of surviving plantlets}}{\text{Total number of plantlets}} \text{ x } 100\%$$

2.7. Plantlet Height

The height of the plantlets was measured from the base of the stem to the tip of the longest leaf. Measurements were taken at both the beginning and the end of the observation period [12].

2.8. Number of Leaves

The number of leaves was determined by manually counting all leaves present on each plantlet, including both newly emerged and fully developed leaves [12].

2.9. Number of Roots

The number of roots was determined by observing the root development of each plantlet. All roots emerging from the base of the plantlet were counted manually, including those that were newly formed or still short in length [12].

2.10. Total carbohydrate analysis

Total carbohydrate content was analyzed using the phenol-sulfuric acid method. Leaves from *Dendrobium* sp. plantlets were collected and weighed to 0.1 grams. The samples were ground using a mortar and pestle, then mixed with 10 mL of distilled water. The mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was transferred into a test tube. Subsequently, 1 mL of the filtrate was mixed with 1 mL of concentrated sulfuric acid (H_2SO_4) and 2 mL of phenol solution. The reaction mixture was transferred to a cuvette and its absorbance was measured at 490 nm using a UV-Vis spectrophotometer. Total soluble carbohydrate content was quantified using a standard glucose calibration curve prepared from known glucose concentrations and measured at the same wavelength [13].

3. Results and discussion

3.1. Percentage of Live Plantlets and Plantlet Visualization

The survival of *Dendrobium* sp. plantlets was monitored weekly from the first to the fourth week after planting. Plantlets exhibiting green to yellowish coloration were classified as alive, whereas those showing brown discoloration were considered dead [14].

Table 1 Survival Percentage of *Dendrobium* sp. Plantlets at Various GA_3 Concentrations

Treatment	**Percentage of Live Plantlets at Week (%)**			
GA3 concentrations	I	II	III	IV
0 mg/L	100	100	100	100
1 mg/L	100	100	100	100
2 mg/L	100	100	100	100
3 mg/L	100	100	100	100
4 mg/L	100	100	100	80

Note 100 indicates 100% percentage of live *Dendrobium* sp. planlets from the first to fourth week.

Based on Table 1. it was observed that from the first to the third week, all treatments—including the control (0 mg/L) and GA_3 concentrations ranging from 1 to 4 mg/L—exhibited a 100% survival rate of plantlets. This suggests that during the early stages of the culture period, *Dendrobium* sp. plantlets were able to tolerate the applied GA_3 concentrations effectively.

Differences in survival began to emerge in the fourth week, particularly at the 4 mg/L GA_3 concentration, where the survival rate declined to 80%. In contrast, all other treatments maintained a 100% survival rate. The decline at 4 mg/L is likely due to the toxic effects of excessive GA_3, which may disrupt metabolic processes or induce physiological stress in the plantlets [15]. This trend is illustrated in **Figure 1.**

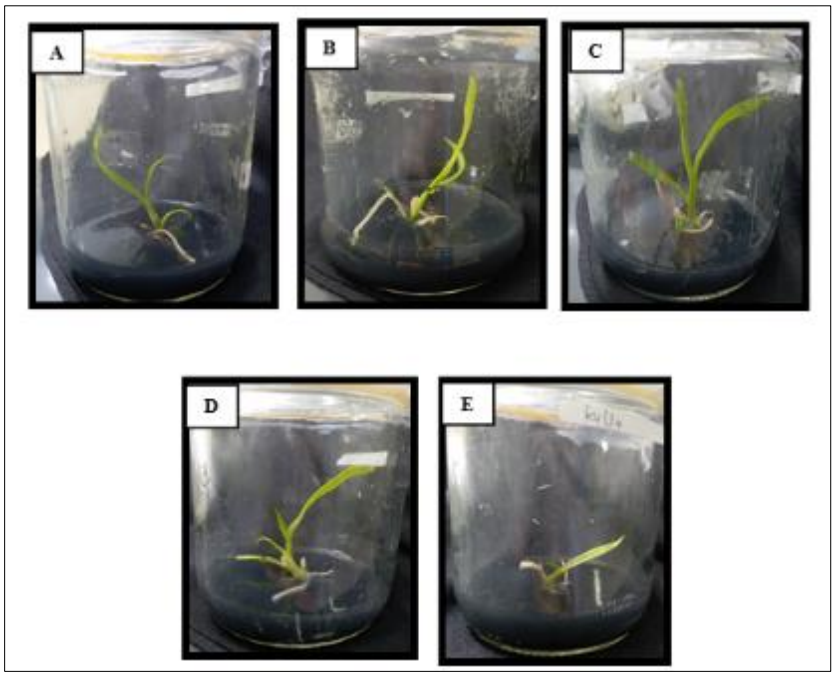

Figure 1 Visualization of *Dendrobium* sp. plantlets in the fourth week on Vacin and Went (VW) medium with varying concentrations of GA_3. A = 0 mg/L (control), B = 1 mg/L, C = 2 mg/L, D = 3 mg/L, E = 4 mg/L

3.2. Plantlet Height

Plantlet height was measured in the fourth week after the application of various GA_3 concentrations to the Vacin and Went (VW) medium. The effect of different GA_3 concentrations on plantlet height is presented in Table 2.

Table 2 Average Height of *Dendrobium* sp. Plantlets in the Fourth Week After Application of Various GA_3 Concentrations

Treatment	**Average height of planlets y± SE**
	Fourth week
0 mg/L	4.56 ± 0.31^{ab}
1 mg/L	5.58 ± 0.35^{b}
2 mg/L	5.68 ± 0.31^{b}
3 mg/L	4.86 ± 0.48^{b}
4 mg/L	3.28 ± 0.32^{a}

Notes y= Average height of planlets**;** SE= *Standard Error;* Numbers followed by different letters indicate significant differences between treatments.

Based on Table 2. the application of various concentrations of GA_3 to the VW medium had a significant effect on the average height of *Dendrobium* sp. plantlets after four weeks of treatment. The concentration of 2 mg/L GA_3 resulted in the highest average plantlet height, reaching 5.68 cm, indicating it was the most effective in promoting growth.

This increase in height is likely attributed to the application of GA_3 at low to moderate concentrations, which can optimally stimulate growth in *Dendrobium* sp. plantlets. This finding is consistent with previous research, which reported that low concentrations of GA_3 promote both cell division and cell elongation [16]. In general, phytohormones at low concentrations tend to stimulate plant growth, while excessively high concentrations may inhibit plant height due to potential physiological stress or hormonal imbalance [15].

3.3. Number of Leaves

The number of leaves is a critical parameter influencing various aspects of plant physiology and growth. Leaf number was recorded in the fourth week following the application of GA_3 to *Dendrobium* sp. plantlets. The results are presented in **Table 3.**

Table 3 Average number of *Dendrobium* sp. leaves in the fourth week after GA3 addition

Treatment	Average number of leaves y± SE
	Fourth week
0 mg/L	3.8 ± 0.38[a]
1 mg/L	5.0 ± 0.45[ab]
2 mg/L	7.4 ± 0.83[b]
3 mg/L	5.6 ± 0.51[ab]
4 mg/L	3.8 ± 0.38[a]

Notes y = Average number of leaves**;** SE= *Standard Error***;** Numbers followed by different letters indicate significant differences between treatments.

Based on Table 3. the application of various concentrations of GA_3 had a significant effect on the average number of leaves produced by *Dendrobium* sp. plantlets. The 2 mg/L concentration was found to be the most effective in promoting leaf formation. The increase in leaf number following GA_3 application aligns with the findings of [16], which reported that GA_3 at optimal concentrations enhances vegetative growth by stimulating leaf production.

Gibberellic acid plays a vital role in promoting cell division and elongation, particularly in meristematic tissues, thereby accelerating the initiation of leaf primordia that develop into mature leaves. Additionally, GA_3 can stimulate shoot meristem activity, supporting the development of new vegetative organs, including leaves [16].

However, the use of GA_3 at excessively high concentrations may not only be ineffective in increasing leaf number but may also inhibit overall plantlet growth. This inhibition is attributed to phytotoxic effects, where hormonal imbalance leads to physiological stress, such as oxidative damage, disruption of photosynthetic processes, and suppression of cell division in meristematic regions. As a result, the formation of new leaves slows down, and the quality of the leaves declines, often appearing abnormally elongated, thin, or pale. Prolonged exposure to such conditions can significantly hinder plantlet development, including leaf production [16].

3.4. Number of Roots

The number of roots was recorded in the fourth week following the application of various GA_3 concentrations to *Dendrobium* sp. plantlets. The results are presented in Table 4.

Table 4 Average Number of Roots in *Dendrobium* sp. Plantlets Treated with Various GA_3 Concentrations

Treatment	Average number of roots y± SE
	Fourth week
0 mg/L	4.40±0,51[ab]
1 mg/L	5.00±0,31[ab]
2 mg/L	6.00±054[b]
3 mg/L	4.60±0,67[ab]
4 mg/L	3.40±0,51[a]

Notes y = Average number of roots; SE = *Standard Error*; Numbers followed by different letters indicate significant differences between treatments.

Based on Figure 4. the application of various GA_3 concentrations had a significant effect on the average number of roots produced by *Dendrobium* sp. plantlets. The 2 mg/L GA_3 treatment resulted in the highest root number, indicating it was the most effective concentration for promoting root development.

This increase can be attributed to the role of GA_3 in enhancing meristematic activity in root tissues and accelerating cell division. GA_3 is also known to stimulate the activity of enzymes and proteins associated with root initiation. However, excessive concentrations of GA_3 may induce phytotoxic effects, such as reduced physiological responses or hormonal imbalances particularly with hormones like auxins that are also involved in root formation ultimately inhibiting root development [17].

3.5. Total Carbohydrate Analysis

Total carbohydrate content serves as an indicator of the effectiveness of GA_3 treatment on plant metabolic activity. The total soluble carbohydrate content of *Dendrobium* sp. plantlets following the application of GA_3 to the Vacin and Went (VW) medium is presented in Figure 2.

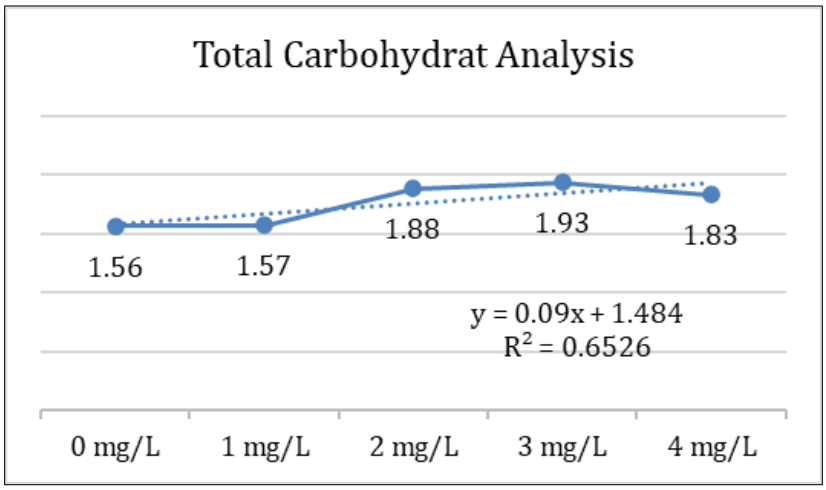

Figure 2 Average Total Carbohydrate Content in *Dendrobium* sp. Plantlets Treated with Various GA_3 Concentrations

Based on Figure 2. the application of various GA_3 concentrations had no significant effect on the average total carbohydrate content of *Dendrobium* sp. plantlets. GA_3 is primarily effective in stimulating cell division in meristematic tissues and promoting stem elongation. However, it does not significantly influence leaf development after the budding stage, which may explain its limited impact on carbohydrate accumulation.

Although GA_3 can enhance stem diameter through cell enlargement, this morphological change does not directly correlate with an increase in total carbohydrate content. Since leaves are the main organs responsible for photosynthesis, the limited number of leaves restricts the plant's ability to produce and accumulate carbohydrates efficiently [9].

4. Conclusion

Based on the results and discussion of this study, it can be concluded that GA_3 has a significant effect on the survival rate, root number, leaf number, and height of *Dendrobium* sp. plantlets cultured *in vitro*. The most optimal GA_3 concentration for promoting the growth of *Dendrobium* sp. plantlets was 2 mg/L.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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