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Effects of *Aloe vera* extract as a natural plant growth regulator 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 aesthetic and commercial value; however, its natural populations continue to decline due to habitat destruction and excessive exploitation. In vitro propagation offers an effective strategy for the large-scale production and conservation of this species. Natural plant growth regulators (PGRs), such as *Aloe vera* extract, have attracted increasing attention as environmentally friendly and cost-effective alternatives to synthetic PGRs because they contain naturally occurring auxins, cytokinins, vitamins, amino acids, and minerals that promote plant growth. This study aimed to evaluate the effects of different concentrations of *Aloe vera* extract on the in vitro regeneration and growth of *D. spectabile* plantlets. A Completely Randomized Design (CRD) with five treatments (0, 15, 30, 45, and 60 g L^{-1} of *Aloe vera* extract) was applied using Vacin and Went (VW) medium, with five replications per treatment. Plantlet survival, height, root length, number of leaves, shoots, roots, and fresh weight were evaluated 15 days after inoculation. Quantitative data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) test at the 5% significance level. All treatments maintained a 100% plantlet survival rate throughout the experimental period. The addition of *Aloe vera* extract significantly affected all growth parameters, with the 45 g L^{-1} treatment producing the greatest plantlet height (3.26 ± 0.26 cm), root length (1.58 ± 0.09 cm), number of leaves (4.00 ± 0.45), number of shoots (4.80 ± 0.58), number of roots (3.60 ± 0.51), and fresh weight (0.132 ± 0.009 g). These findings demonstrate that *Aloe vera* extract can serve as an effective natural plant growth regulator for the in vitro propagation of *D. spectabile*, with 45 g L^{-1} identified as the optimum concentration for promoting plantlet regeneration and vegetative growth.

Keywords: *Aloe vera* extract; *Dendrobium spectabile*; In vitro regeneration; Natural plant growth regulator; Orchid propagation

1. Introduction

Orchids are among the most valuable ornamental horticultural crops because of their remarkable diversity, unique floral characteristics, and high commercial value. Indonesia is recognized as one of the world's centers of orchid diversity, harboring approximately 6,000 species out of an estimated 26,000 orchid species worldwide [1]. Among these, *Dendrobium spectabile* (Blume) Miq. is one of the most attractive ornamental orchids due to its distinctive curly petals with yellow and maroon patterns, making it highly desirable in the ornamental plant market. However, increasing market demand, combined with habitat destruction and overexploitation, has contributed to the continuous decline of its natural populations. Furthermore, the species exhibits relatively slow natural propagation, making large-scale production through conventional methods inefficient [2].

In vitro culture has become an effective approach for the rapid propagation and conservation of orchids because it enables the production of large numbers of uniform and disease-free plantlets under controlled environmental

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conditions. The success of in vitro regeneration depends on the ability of plantlets to develop healthy vegetative organs, including shoots, leaves, and roots, which are widely recognized as important indicators of plantlet quality and subsequent acclimatization success [3]. Among the factors influencing plantlet regeneration, the composition of the culture medium and the application of plant growth regulators (PGRs) play a fundamental role in regulating cell division, differentiation, and organ development in Vacin and Went (VW) medium [4].

Although synthetic plant growth regulators, such as α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP), have been extensively used to promote in vitro plant growth, their relatively high cost and continuous dependence on synthetic chemicals have encouraged the exploration of natural alternatives. Natural plant growth regulators are considered more economical, environmentally friendly, and readily available for routine tissue culture applications. One promising natural source is *Aloe vera*, which contains naturally occurring auxins, cytokinins, vitamins, amino acids, carbohydrates, and minerals that collectively support cell division, cell elongation, tissue differentiation, and overall plant growth [5].

Previous research demonstrated that *Aloe vera* extract at concentrations of 25–75 g L^{-1} enhanced plant height, shoot formation, and root length in orchid plantlets derived from a cross between *Dendrobium* Morning Sun × *Dendrobium* Samarai [6]. These findings indicate that *Aloe vera* extract has considerable potential as a natural plant growth regulator for orchid micropropagation. However, information regarding its application for the in vitro regeneration of *D. spectabile* remains unavailable. Since different orchid species may exhibit distinct physiological responses to natural plant growth regulators, the optimum concentration of *Aloe vera* extract for promoting the regeneration and vegetative growth of *D. spectabile* plantlets has not yet been established.

Therefore, this study was conducted to evaluate the effects of different concentrations of *Aloe vera* extract on the in vitro regeneration of *D. spectabile* plantlets and to identify the optimum concentration for enhancing their vegetative growth. The findings of this study are expected to provide scientific evidence supporting the utilization of *Aloe vera* extract as a natural plant growth regulator for the efficient propagation and conservation of this economically important orchid species.

2. Materials and Methods

2.1. Equipment and Materials

The equipment used in this study included culture bottles, an autoclave, a Laminar airflow (LAF) cabinet, an analytical balance, Erlenmeyer flasks, a pH meter, glass stirring rods, beakers, funnels, measuring cylinders, a blender, scalpels, forceps, a magnetic stirrer, a hot plate, and a ruler.

The plant material consisted of *Dendrobium spectabile* plantlets. The culture medium was prepared using Vacin and Went (VW) basal medium supplemented with *Aloe vera* extract, sucrose, agar, activated charcoal, and distilled water. Additional materials included 70% ethanol, 10% sodium hypochlorite solution (commercial bleach), 1 N KOH, 1 N HCl, aluminum foil, heat-resistant plastic, rubber bands, sterile tissue paper, and labeling materials.

2.2. Experimental Design

This study employed a Completely Randomized Design (CRD) with a single experimental factor, namely the concentration of *Aloe vera* extract. Five treatment levels were evaluated: 0, 15, 30, 45, and 60 g L^{-1}. Each treatment consisted of five independent replications, resulting in a total of 25 experimental units. Each experimental unit comprised one culture bottle containing a single *D. spectabile* plantlet.

2.3. Procedure

2.3.1. Sterilization of Equipment and Work Area

All glassware and culture equipment were thoroughly washed with detergent and tap water, air-dried, wrapped in paper, and sterilized in an autoclave at 121°C for 30 min. Instruments used during inoculation, including forceps and scalpels, were sterilized by autoclaving, immersed in 70% ethanol, and flamed over a bunsen burner immediately before use to maintain aseptic conditions.

The work area was sterilized inside a laminar airflow (LAF) cabinet. The ultraviolet (UV) lamp was switched on for 30 min prior to use and then turned off before the blower and fluorescent light were activated. The working surface of the

LAF cabinet was sprayed with 70% ethanol and wiped with sterile tissue paper to ensure aseptic conditions before inoculation.

2.3.2. Preparation of Aloe vera Extract

Healthy and mature *Aloe vera* leaves were washed thoroughly under running water to remove adhering debris and latex, surface-sterilized with 70% ethanol, and dried using sterile tissue paper. The outer rind was carefully removed, and the inner gel was collected using a sterile spoon. The gel was cut into small pieces, weighed according to the required treatment concentrations, and homogenized using a blender to obtain a uniform extract. The prepared extract was immediately incorporated into the culture medium at concentrations of 0, 15, 30, 45, and 60 g L^{-1} [6].

2.3.3. Preparation of Treatment Media

VW basal medium, sucrose, activated charcoal, and *Aloe vera* extract were prepared according to the designated treatment concentrations. All medium components were dissolved in distilled water and mixed thoroughly until homogeneous. The pH of the medium was adjusted to 5.7 using either 1 N HCl or 1 N KOH. Agar was then added as the gelling agent, and the medium was heated while continuously stirred until completely dissolved.

The prepared medium was dispensed into culture bottles (20 mL per bottle), covered with heat-resistant plastic, and secured with rubber bands. The culture media were sterilized in an autoclave at 121°C and 1 atm for 15 min. Following sterilization, the media were incubated at room temperature for 3–4 days to confirm the absence of microbial contamination before inoculation. The composition of each treatment medium is presented in Table 1 [7].

Table 1 Composition of the culture media supplemented with different concentrations of *Aloe vera* extract.

***Aloe vera* extract concentration (g L^{-1})**	***Aloe vera* extract (g)**	**VW medium (mL)**
0	0	200
15	3	197
30	6	194
45	9	191
60	12	188

Note: The final volume of each culture medium was adjusted to **200 mL**. The volume of VW medium was reduced according to the amount of *Aloe vera* extract added.

2.4. Sterilization and Inoculation of Plantlets

D. spectabile plantlets were carefully removed from the stock cultures, and residual culture medium adhering to the roots was removed by rinsing with sterile distilled water for approximately 5 min. The plantlets were subsequently immersed in a 10% sodium hypochlorite solution for 2–3 min and rinsed three times with sterile distilled water. All inoculation procedures were performed aseptically inside a laminar airflow cabinet. One plantlet was transferred into each culture bottle, and each treatment consisted of five independent replications.

2.5. Observations Parameters

The responses of *D. spectabile* plantlets to different concentrations of *Aloe vera* extract were evaluated for 15 days after inoculation. Observations were conducted every three days for plantlet survival and visual appearance, whereas growth parameters were measured on the 15th day after inoculation.

2.5.1. Plantlet Survival and Visual Appearance

Plantlet survival was determined by comparing the number of surviving plantlets with the total number of plantlets cultured. Plantlets were considered viable when they remained green, exhibited normal growth, and showed no signs of browning, necrosis, or microbial contamination. The survival percentage was calculated using the following formula [8]:

$$\text{Percentage of Live Plantlets (\%)} = \frac{Number\ of\ Live\ Plantlets}{Initial\ Number\ of\ Plantlets} \times 100\%$$

In addition to survival, the visual appearance of the plantlets, particularly leaf color, was recorded to evaluate their general growth performance.

2.5.2. Plantlet Height (cm)

Plantlet height was measured from the base of the stem to the tip of the longest leaf using a ruler on the 15th day after inoculation.

2.5.3. Root Length (cm)

Root length was measured from the base of the longest root to its tip using a ruler on the 15th day after inoculation.

2.5.4. Number of Leaves

The number of fully expanded leaves produced by each plantlet was counted on the 15th day after inoculation.

2.5.5. Number of Shoots

The number of shoots is calculated by counting the number of shoots that have emerged on the 15th day after inoculation.

2.5.6. Number of Roots

The number of newly developed roots on each plantlet was counted on the 15th day after inoculation.

2.5.7. Fresh Weight (g)

Fresh weight was determined by weighing each plantlet individually using an analytical balance at the end of the observation period. Because this measurement was destructive, the plantlets were not returned to the culture medium after weighing.

2.6. Data Analysis

The data obtained from this study consisted of qualitative and quantitative observations. Qualitative data, including plantlet visual appearance, were presented descriptively and supported by photographic documentation. Quantitative data were analyzed using one-way analysis of variance (ANOVA) to determine the effect of different concentrations of *Aloe vera* extract on the growth of *D. spectabile* plantlets. Prior to ANOVA, the homogeneity of variance was evaluated using Levene's test. When significant differences among treatments were detected ($P < 0.05$), mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test at the 5% significance level.

3. Results

3.1. Percentage of Live Plantlets and Plantlet Visualization

The survival and visual appearance of *D. spectabile* plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract were evaluated every three days for 15 days after inoculation. Plantlets exhibiting green or yellowish-green tissues without browning or contamination were classified as surviving, whereas those showing brown to black tissues were classified as dead [8]. The survival percentage of the plantlets under each treatment is presented in Table 2.

Table 2 Survival percentage and visual appearance of *D. spectabile* plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract

***Aloe vera* Extract Concentration (g L^{-1})**	**Plantlet survival (%)**				
	3	**6**	**9**	**12**	**15**
0	100 H	100 H	100 H	100 H	100 H
15	100 H	100 H	100 H	100 H	100 H

30	100 H	100 H	100 H	100 H	100 HK
45	100 H	100 H	100 H	100 H	100 H
60	100 H	100 H	100 H	100 H	100 HK

Note: H = Green; HK = Yellowish-green.

As shown in Table 2, all *Aloe vera* extract treatments maintained a 100% survival rate of *D. spectabile* plantlets throughout the 15-day observation period. Although all plantlets survived, slight differences in visual appearance were observed among treatments. Plantlets cultured on media containing 0, 15, and 45 g L^{-1} *Aloe vera* extract remained green, whereas some plantlets grown on 30 and 60 g L^{-1} exhibited a yellowish-green coloration. Representative plantlets from each treatment are presented in Figure 1.

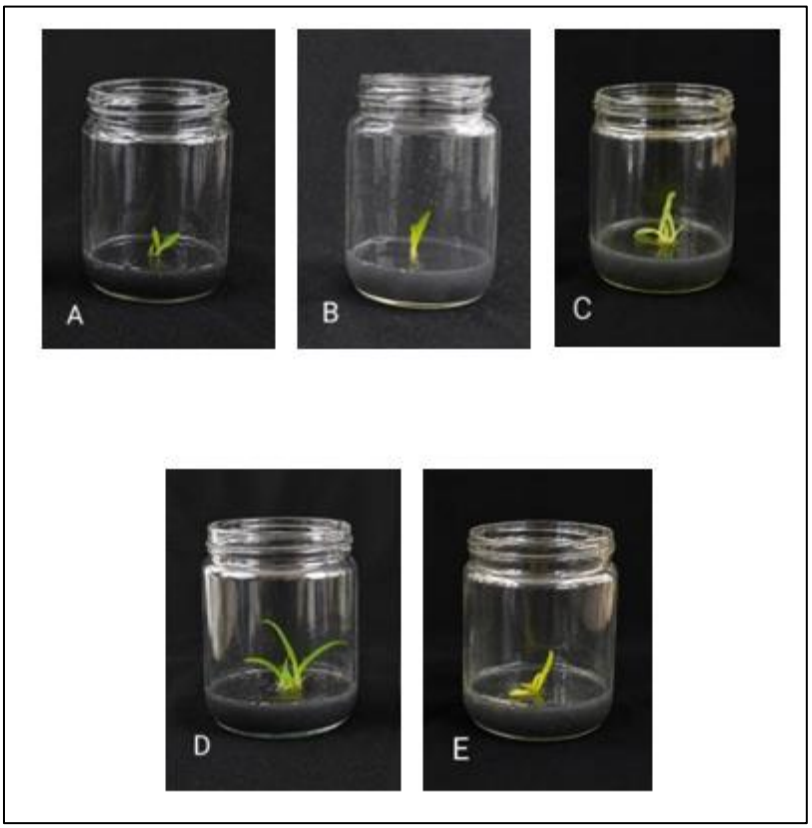

Figure 1 Representative *D. spectabile* plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract after 15 days of culture: (A) 0 g L^{-1}, (B) 15 g L^{-1}, (C) 30 g L^{-1}, (D) 45 g L^{-1}, and (E) 60 g L^{-1}

Some plantlets in the 30 g L^{-1}, and 60 g L^{-1}, treatments exhibited a yellowish-green coloration, whereas those in the 0 g L^{-1}, 15 g L^{-1}, and 45 g L^{-1} treatments remained green.

3.2. Plantlet Height

The effect of *Aloe vera* extract on the height of *D. spectabile* plantlets was evaluated on the 15th day after inoculation. The mean plantlet height obtained under each treatment is presented in Table 3.

Table 3 Mean plantlet height of *D. spectabile* cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Plantlet Height(cm)**
0	2.16 ± 0.07^{a}
15	2.14 ± 0.16^{a}

30	2.68 ± 0.21^{ab}
45	3.26 ± 0.26^{b}
60	2.56 ± 0.16^{ab}

Notes: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract influenced the height of *D. spectabile* plantlets after 15 days of culture (Table 3). The greatest plantlet height was obtained in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (3.26 ± 0.26 cm), whereas the lowest height was recorded in the 15 g L^{-1} treatment (2.14 ± 0.16 cm), which was comparable to the control treatment (2.16 ± 0.07 cm). Plantlets cultured on media containing 30 and 60 g L^{-1} exhibited intermediate heights and did not differ significantly from either the control or the 45 g L^{-1} treatment, as indicated by Tukey's HSD test.

3.3. Root Length

The effect of *Aloe vera* extract on the root length of *D. spectabile* plantlets was evaluated 15 days after inoculation. The mean root length of plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract is presented in Table 4.

Table 4 Mean root length of *D. spectabile* plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Root Length(cm)**
0	1.08 ± 0.12^{a}
15	0.88 ± 0.15^{a}
30	1.26 ± 0.07^{ab}
45	1.58 ± 0.09^{b}
60	1.11 ± 0.04^{a}

Note: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract significantly affected the root length of *D. spectabile* plantlets (P = 0.001) (Table 4). The greatest root length was obtained in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (1.58 ± 0.09 cm), whereas the shortest roots were recorded in the 15 g L^{-1} treatment (0.88 ± 0.15 cm). According to Tukey's HSD test, the 45 g L^{-1} treatment produced significantly longer roots than the 0, 15, and 60 g L^{-1} treatments, whereas the 30 g L^{-1} treatment did not differ significantly from the other treatments.

3.4. Number of Leaves

The effect of *Aloe vera* extract on the number of leaves of *D. spectabile* plantlets was evaluated 15 days after inoculation. The mean number of leaves per plantlet cultured on VW medium supplemented with different concentrations of *Aloe vera* extract is presented in **Table 5.**

Table 5 Mean number of leaves per plantlet of *D. spectabile* cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Number of leaves (plantlet^{-1})**
0	1.60 ± 0.24^{a}
15	1.80 ± 0.37^{a}
30	3.20 ± 0.58^{ab}
45	4.00 ± 0.45^{b}
60	2.20 ± 0.37^{a}

Note: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract significantly affected the number of leaves of *D. spectabile* plantlets (P = 0.003) (Table 5). The highest mean number of leaves was recorded in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (4.00 ± 0.45 leaves per plantlet), whereas the lowest value was observed in the control treatment (0 g L^{-1}) (1.60 ± 0.24 leaves per plantlet). According to Tukey's HSD test, the 45 g L^{-1} treatment produced a significantly greater number of leaves than the 0, 15, and 60 g L^{-1} treatments, whereas the 30 g L^{-1} treatment did not differ significantly from the other treatments.

3.5. Number of Shoots

The effect of *Aloe vera* extract on the number of shoots of *D. spectabile* plantlets was evaluated 15 days after inoculation. The mean number of shoots per plantlet cultured on VW medium supplemented with different concentrations of *Aloe vera* extract is presented in Table 6.

Table 6 Mean number of shoots per plantlet of *D. spectabile* cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Number of shoots (plantlet^{-1})**
0	1.80 ± 0.37^{a}
15	2.00 ± 0.45^{a}
30	2.40 ± 0.68^{a}
45	4.80 ± 0.58^{b}
60	2.00 ± 0.45^{a}

Note: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract significantly affected the number of shoots of *D. spectabile* plantlets (P = 0.003) (Table 6). The highest mean number of shoots was obtained in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (4.80 ± 0.58 shoots per plantlet), whereas the lowest value was recorded in the control treatment (0 g L^{-1}) (1.80 ± 0.37 shoots per plantlet). According to Tukey's HSD test, the 45 g L^{-1} treatment produced a significantly greater number of shoots than all other treatments, whereas the 0, 15, 30, and 60 g L^{-1} treatments did not differ significantly from one another.

3.6. Number of Roots

The effect of *Aloe vera* extract on the number of roots of *D. spectabile* plantlets was evaluated 15 days after inoculation. The mean number of roots per plantlet cultured on VW medium supplemented with different concentrations of *Aloe vera* extract is presented in **Table 7.**

Table 7 Mean number of roots per plantlet of *D. spectabile* cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Number of roots (plantlet^{-1})**
0	1.80 ± 0.37^{a}
15	2.00 ± 0.45^{ab}
30	2.20 ± 0.37^{ab}
45	3.60 ± 0.51^{b}
60	2.00 ± 0.32 ^{ab}

Note: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract significantly affected the number of roots of *D. spectabile* plantlets (P = 0.036) (Table 7). The highest mean number of roots was obtained in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (3.60 ± 0.51 roots per plantlet), whereas the lowest value was recorded in the control treatment (0 g L^{-1}) (1.80 ± 0.37 roots per plantlet). According to Tukey's HSD test, the 45 g L^{-1} treatment produced a significantly greater number of roots than

the control treatment, whereas no significant differences were observed between the 45 g L^{-1} treatment and the 15, 30, and 60 g L^{-1} treatments.

3.7. Fresh Weight of Plantlets

The effect of *Aloe vera* extract on the fresh weight of *D. spectabile* plantlets was evaluated 15 days after inoculation. The mean fresh weight of plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract is presented in Table 8.

Table 8 Mean fresh weight of *D. spectabile* plantlets cultured on VW medium supplemented with different concentrations of *Aloe vera* extract 15 days after inoculation

***Aloe vera* extract concentration (g L^{-1})**	**Fresh Weight (g)**
0	0.079 ± 0.006[a]
15	0.089 ± 0.006[a]
30	0.088 ± 0.011[a]
45	0.132 ± 0.009[b]
60	0.095 ± 0.010[a]

Note: Values are presented as mean ± SE. Means followed by the same superscript letter are not significantly different according to Tukey's HSD test at $P < 0.05$.

Application of *Aloe vera* extract significantly affected the fresh weight of *D. spectabile* plantlets (P = 0.003) (Table 8). The highest mean fresh weight was obtained in the medium supplemented with 45 g L^{-1} *Aloe vera* extract (0.132 ± 0.009 g), whereas the lowest value was recorded in the control treatment (0 g L^{-1}) (0.079 ± 0.006 g). According to Tukey's HSD test, the 45 g L^{-1} treatment produced a significantly greater fresh weight than all other treatments.

4. Discussion

The application of *Aloe vera* extract significantly influenced the in vitro growth of *D. spectabile* plantlets. Overall, plantlets cultured on VW medium supplemented with 45 g L^{-1} *Aloe vera* extract consistently exhibited superior performance across all measured growth parameters, including plantlet height, root length, leaf number, shoot number, root number, and fresh weight. These findings indicate that 45 g L^{-1} provided an optimal balance of naturally occurring bioactive compounds required for plantlet establishment and development.

Visual observations revealed that plantlets cultured on media supplemented with 30 and 60 g L^{-1} *Aloe vera* extract exhibited a yellowish-green coloration, whereas those grown in the 0, 15, and 45 g L^{-1} treatments remained green. This slight discoloration may indicate a reduction in chlorophyll content caused by changes in nutrient availability within the culture medium, resulting in mild chlorosis [9]. Nevertheless, no symptoms of tissue damage, such as wilting, browning, or necrosis, were observed, and all plantlets remained viable throughout the experimental period. The 100% survival rate demonstrates that all tested concentrations were within the physiological tolerance range of *D. spectabile* and did not induce phytotoxic effects. This favorable response is likely associated with the presence of auxins, cytokinins, vitamins, amino acids, and minerals in *Aloe vera*, which collectively support cell division, differentiation, metabolic activity, and stress adaptation under in vitro conditions [10].

The superior plantlet height obtained at 45 g L^{-1} suggests that this concentration supplied sufficient quantities of naturally occurring growth-promoting compounds to stimulate cell division and elongation. Similar responses have been reported previously, indicating that *Aloe vera* is most effective within an optimum concentration range rather than at either lower or higher concentrations [6]. The reduced growth observed at 15 g L^{-1} may have resulted from insufficient levels of natural growth regulators to effectively stimulate these physiological processes [11]. *Aloe vera* contains indole-3-acetic acid (IAA), gibberellins, vitamins, minerals, and carbohydrates that enhance plant metabolism and growth [10]. Auxins and gibberellins stimulate cell elongation, while gibberellin-induced α-amylase activity increases soluble sugar availability, facilitating water uptake and cell expansion. These physiological mechanisms likely contributed to the greater plantlet height observed at 45 g L^{-1} [12].

A similar response was observed for root growth, with the longest roots recorded at 45 g L^{-1}. This finding is consistent with previous studies demonstrating that *Aloe vera* application enhances root elongation and root development, with

effectiveness comparable to synthetic plant growth regulators [13]. Similar improvements have also been reported in *Hibiscus sabdariffa*, where *Aloe vera* extract enhanced nutrient uptake and photosynthetic activity [14]. Auxins promote proton extrusion into the cell wall, increasing cell-wall plasticity and facilitating cell elongation, while simultaneously stimulating meristematic activity in the root apex [15]. Gibberellins further enhance tissue responsiveness to auxins and stimulate α-amylase activity, increasing the availability of soluble sugars that promote water uptake and cell turgor, thereby accelerating root elongation [16,17]. Improved root development is likely to enhance nutrient and water absorption, which subsequently supports shoot growth and overall plantlet development. In addition, the strong rooting response observed in *D. spectabile* may be associated with its epiphytic growth habit, making this species highly responsive to externally supplied growth-promoting compounds [18].

Leaf formation was also enhanced by *Aloe vera* supplementation, with the highest number of leaves obtained at 45 g L^{-1}. This finding indicates that this concentration provided a favorable hormonal balance for leaf initiation and expansion. Although previous research reported that significant differences in leaf number became evident only after longer culture periods [6], the present study demonstrates that *D. spectabile* responded within only 15 days after inoculation. Such variation is likely attributable to differences in explant physiological condition, culture medium composition, and species-specific responsiveness to naturally occurring growth regulators. Leaf development is regulated through coordinated interactions between auxins and cytokinins, in which auxins promote cell enlargement while cytokinins stimulate cell division and shoot organogenesis [19]. Consequently, enhanced shoot formation contributes directly to increased leaf production. However, excessive auxin concentrations may suppress chlorophyll synthesis and reduce photosynthetic efficiency, thereby limiting subsequent leaf development [20].

Shoot proliferation also responded positively to *Aloe vera* supplementation, with the greatest number of shoots observed at 45 g L^{-1}. This response supports previous findings showing that *Aloe vera* effectively promotes shoot multiplication within an optimum concentration range [6]. The enhanced shoot formation is primarily associated with the cytokinin content of *Aloe vera*, which stimulates cell division and shoot initiation, whereas auxins regulate apical dominance and root formation [21]. A balanced interaction between these endogenous hormones is essential for successful organogenesis. Cytokinins have long been recognized as major regulators of shoot proliferation through their ability to stimulate cell division and the formation of new meristematic tissues [22]. Besides hormonal regulation, environmental factors such as light intensity, temperature, and humidity also influence shoot development by affecting photosynthetic efficiency and assimilate production [23].

The greater number of roots observed at 45 g L^{-1} further confirms the effectiveness of *Aloe vera* as a natural growth regulator. Similar responses have previously been reported in orchid plantlets cultured on media supplemented with *Aloe vera* extract [6]. Root initiation is primarily regulated by auxins, whereas vitamin B_1 contributes to respiratory metabolism and energy production required for active cell division during root formation [24]. However, excessive auxin concentrations may stimulate ethylene biosynthesis, reducing cell elongation in the root elongation zone and ultimately limiting root development [25]. These findings emphasize the importance of maintaining an optimal concentration of *Aloe vera* extract to maximize rooting efficiency.

Fresh weight accumulation reflected the integrated response of all vegetative growth parameters. The significantly greater fresh weight observed at 45 g L^{-1} is likely the result of enhanced shoot proliferation, leaf formation, and root development, which collectively improved nutrient absorption, photosynthetic capacity, and overall metabolic activity. Similar findings have been reported in honey guava cuttings treated with *Aloe vera* extract, although the optimum concentration differed among species because of variations in physiological characteristics and sensitivity to growth regulators [26]. The synergistic interaction between auxins and cytokinins, together with vitamins and soluble carbohydrates naturally present in *Aloe vera*, likely promoted both cell division and cell enlargement, resulting in greater biomass accumulation [27].

Overall, the present study demonstrates that *Aloe vera* extract can function as an effective natural plant growth regulator for the *In Vitro* propagation of *D. spectabile*. Among the concentrations evaluated, 45 g L^{-1} consistently produced the best performance across all measured growth parameters, indicating that this concentration provides an optimal balance of bioactive compounds required for early plantlet establishment. These findings highlight the potential of *Aloe vera* extract as a cost-effective, environmentally friendly, and sustainable alternative to synthetic plant growth regulators for orchid micropropagation.

5. Conclusion

The present study demonstrated that *Aloe vera* extract significantly enhanced the in vitro growth of *D. spectabile* plantlets. Among the concentrations evaluated, 45 g L^{-1} consistently produced the best growth performance, resulting

in greater plantlet height, root development, leaf production, shoot proliferation, root formation, and fresh weight while maintaining 100% plantlet survival. These findings indicate that 45 g L^{-1} *Aloe vera* extract provides an optimal balance of naturally occurring bioactive compounds required for plantlet establishment and development. Therefore, *Aloe vera* extract has considerable potential as a cost-effective, environmentally friendly, and sustainable natural alternative to synthetic plant growth regulators for the in vitro propagation of *D. spectabile*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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