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In vitro growth of *Dendrobium* orchid on different concentrations of sucrose and myo-inositol

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ABSTRACT

Introduction: The limitation of endosperm in seeds requires the propagation of *Dendrobium* orchid plants to be carried out using in vitro culture techniques. The composition of in vitro culture media needs to be considered because it greatly determines the success of plant propagation. The addition of sucrose and myo-inositol to the growth media can support the growth of explants planted in vitro. This study aimed to determine the best concentration of myo-inositol and sucrose added to the multiplication medium to increase the growth of *Dendrobium* orchid explants. **Methods:** This study used a Completely Randomized Design. The experimental treatments were the concentration of myo-inositol and sucrose added to the VW media consisting of four levels, namely myo-inositol 50 mg/l + sucrose 20 mg/l, myo-inositol 50 mg/l + sucrose 40 mg/l, myo-inositol 100 mg/l + sucrose 20 mg/l and myo-inositol 100 mg/l + sucrose 40 mg/l. Observation variables include plant height, shoot emergence time, number of shoots and shoot growth rate. Data were analyzed using analysis of variance (ANOVA) at 5% α level and Duncan's Multiple Range Test (DMRT) at 5% level. **Results:** The addition of 40 g/l sucrose + 50 mg/l myo-inositol to the in vitro culture medium was the best treatment in producing optimal explant growth. **Conclusion:** The addition of 40 g/l sucrose can reduce the addition of myo-inositol to VW media by up to 50% to propagate *Dendrobium* orchids in vitro.

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INTRODUCTION

Dendrobium orchids have high commercial potential as potted flowers and cut flowers. (Janakiram & Baskaran, 2018). *Dendrobium* orchids have beautiful flower clusters in a variety of colors, sizes and shapes that have a relatively long blooming period of several weeks to several months (Pammai *et al.*, 2022). *Dendrobium* orchids can generally be propagated generatively and vegetatively. Generatively, orchid seeds do not have endosperm, so they cannot grow naturally without mutualistic symbiosis with mycorrhizal fungi. The existence of these fungi is very limited, making it difficult for orchid plants to be propagated naturally, making it difficult to meet the need for many seeds in a relatively short time (Puri *et al.*, 2022). Therefore, one alternative to overcome this problem is to propagate orchid plants using an in vitro culture method known as tissue culture.

In vitro culture is a plant propagation technique under controlled laboratory conditions, which allows for rapid and efficient plant propagation. The growth medium plays an important role in supporting plant growth and development. Two components that are often used in in vitro culture media are myo-inositol and sucrose. These two components have different but equally important roles in supporting successful plant propagation.

Myo-inositol plays a role in the synthesis of cell wall components such as pectin and hemicellulose. Strong and healthy cell walls are essential for plant growth and development. Myo-inositol is a precursor for inositol phosphate and phosphatidylinositol, which function as signaling molecules in various signal transduction pathways (Sharma *et al.*, 2020). This pathway regulates many aspects of plant physiology, including responses to hormones and environmental stress. Myo-inositol can act as an osmoprotectant, helping plants maintain osmotic pressure and protecting cells from damage (Al-Mushhin *et al.*, 2021). The use of myo-inositol in in vitro culture media can increase the efficiency of plant propagation. The addition of myo-inositol in the right concentration can accelerate the growth of shoots and roots, improve plant quality, and increase propagation results. The addition of 100 ppm of myo-inositol to the in vitro media for propagation of *Dendrobium* orchids significantly increased shoot growth, number and length of roots. (Widiastoety *et al.*, 2012).

Sucrose provides the energy needed for various metabolic processes in plant cells. This energy is used for the synthesis of proteins, nucleic acids, and other cellular components that are essential for growth and development (Pires *et al.*, 2023). Sucrose helps regulate osmotic pressure in cells. Sucrose can act as an osmoprotectant that protects cells from damage due to drought or salinity (da Silva *et al.*, 2020). The addition of appropriate sucrose in the media can enhance explant growth, shoot regeneration, and root formation. The addition of 40 g/l of sucrose to the in vitro media significantly increased the shoot height, shoot number, root length, and root number of *Dendrobium* orchids (Karimah *et al.*, 2021).

Previous studies have demonstrated synergistic effects when myo-inositol is combined with other compounds. For instance, the combination of myo-inositol with corn steep liquor enhanced cold tolerance and early seedling growth in cucumber and tomato, while also improving salt stress resistance in cabbage (Zhang *et al.*, 2023). In addition, in tomato protoplast cultures, media supplemented with both myo-inositol and sucrose significantly increased plating efficiency and cell division compared to media containing only one of these components (Bellini *et al.*, 1990). Moreover, sucrose and myo-inositol act as essential substrates for raffinose family oligosaccharides biosynthesis in legumes, highlighting their complementary roles in plant metabolism (Akiyama *et al.*, 2004). These findings suggest that myo-inositol may exhibit synergistic or additive effects when combined with carbon sources such as sucrose. However, the combined effect of myo-inositol and sucrose on the in vitro propagation of *Dendrobium* orchids has not yet been explored, and this knowledge gap is what the present study aims to address.

The effect of various concentrations of myo-inositol and sucrose given singly on the in vitro propagation of *Dendrobium* orchids has been widely studied. However, the effect of the combination of the use of both materials on the regeneration of *Dendrobium* orchid explants has not been widely studied. Investigating the combined effect of myo-inositol and sucrose is essential, not only to optimize culture media formulation but also to reveal whether their interaction results in synergistic or antagonistic effects on explant growth. A synergistic interaction could accelerate shoot multiplication and improve plantlet quality beyond the effect of each compound alone, while an antagonistic interaction would caution against their excessive use in combination. Moreover, identifying the optimal balance between myo-inositol and sucrose could lead to cost-efficient culture media by reducing the concentration of one component without compromising growth performance. Therefore, a study was conducted on the effect of adding myo-inositol and sucrose on the in vitro multiplication of *Dendrobium* orchids. This study aimed to determine the best concentration of myo-inositol and sucrose added to the multiplication medium to increase the growth of *Dendrobium* orchid explants.

METHODS

Research materials and tools

The research was conducted from October to November 2023 at the Tissue Culture Laboratory of Jember State Polytechnic. The tools used include laminar air flow cabinet (LAFC), dissecting set, bunsen lamp, hand sprayer, petri dish and culture bottle. The materials used include Vacin and Went (VW) media, *Dendrobium* orchid explants, myo-inositol and sucrose. VW medium was selected because it is widely used and proven effective for orchid culture, particularly for the multiplication of *Dendrobium* species. Compared to Murashige and Skoog (MS) medium, VW contains lower total nitrogen and a different ammonium-to-nitrate ratio, which has been reported to promote better protocorm and shoot development in orchids (Khasim *et al.*, 2020).

The explants used in this study were shoots from contamination-free *Dendrobium* orchid plantlets. Shoots are considered an ideal explant source for in vitro multiplication because they exhibit high regenerative potential, genetic stability, and lower susceptibility to contamination compared to other explant types (Hossain *et al.*, 2013).

Experimental design and treatment

The experimental design used in this study was a Completely Randomized Design. The experimental treatments were the concentration of myo-inositol and sucrose added to VW media consisting of four levels, namely myo-inositol 50 mg/l + sucrose 20 mg/l, myo-inositol 50 mg/l + sucrose 40 mg/l, myo-inositol 100 mg/l + sucrose 20 mg/l and myo-inositol 100 mg/l + sucrose 40 mg/l. The last treatment based on earlier studies proposed by Karimah *et al.* (2021) and Widiastoety *et al.* (2012). Each treatment was repeated five times so that there were 20 experimental units. Each experimental unit consisted of two bottles containing five explants planted in vitro.

Preparation of culture media

VW media was modified with myo-inositol and sucrose concentrations according to the treatment. Myo-inositol 50 and 100 mg, each dissolved in 1 liter of VW media. Sucrose was weighed as much as 20 and 40 mg, each dissolved in 1 liter of VW media. The pH of the medium was adjusted to 5.6–5.8 using NaOH or HCl, following the general recommendations for orchid tissue culture media (Corbellini, 2020). Distilled water was added to reach a final volume of 1 L, followed by the addition of 8 g/L agar. The medium was heated to boiling, dispensed into culture bottles at 25 mL per bottle, and sterilized in an autoclave at 121 °C for 30 minutes.

Explant planting and incubation

Explants were planted in culture bottles containing VW media that had been added with myo-inositol and sucrose at concentrations according to the treatment. To avoid contamination, planting was carried out in LAFC that had been sterilized using alcohol and ultraviolet light for one hour. Each bottle contained five *Dendrobium* orchid explants that were planted in vitro. The bottles containing the explants were then stored in an incubation room with a temperature of 27^{o} C and a humidity of 53%. These conditions are commonly used for orchid tissue culture and have been reported to support optimal explant survival and regeneration (Hossain *et al.*, 2013)

Observation variables

Observation variables were plant height, shoot emergence time, number of shoots and shoot growth rate. Plant height and number of shoots are calculated based on the addition of growth at the observation time and the initial condition of the explant. Shoot emergence time is calculated based on the emergence of new shoots on at least one planted explant. Shoot growth rate is calculated based on the number of shoots added per day.

Data analysis

Data were analyzed using analysis of variance (ANOVA) with F test at α level of 5%. Data showing significant effects were further tested using Duncan's Multiple Range Test (DMRT) at 5%. Data analysis was performed using SAS 9.4 application.

RESULTS AND DISCUSSION

General effect of treatment on explant growth

The application of myo-inositol and sucrose at several concentrations tested significantly affected the time of emergence of *Dendrobium* orchid explant shoots. This treatment had a very significant effect on the height of shoots at the age of 1 to 3 weeks after planting (WAT) and the rate of shoot growth. However, the administration of myo-inositol with a concentration ranging from 50 - 100 mg / l combined with sucrose with a concentration ranging from 20 - 40 mg/l had no significant effect on the number of *Dendrobium* orchid explant shoots (Tabel 1).

Table 1. Analysis of variance of the effect of myo-inositol and sucrose concentrations on explant growth of *Dendrobium* orchid

No.	Observations Variables	Pr > F	Treatment Effect
1.	Shoot emergence time	0.03	Significant
2.	The number of shoots	0.19	Not significant
3.	Plant height 1 WAT	<0.01	Very significant
4.	Plant height 2 WAT	<0.01	Very significant
5.	Plant height 3 WAT	<0.01	Very significant
6.	Shoot growth rate	<0.01	Very significant

Time of shoot emergence and number of shoots

Dendrobium orchid explants grown in vitro on media supplemented with 50 mg/l Myo-inositol + 40 g/l Sucrose can produce new shoots significantly faster than media supplemented with 50 mg/l Myo-inositol + 20 g/l Sucrose and 100 mg/l Myo-inositol + 20 g/l Sucrose. The emergence of *Dendrobium* orchid shoots on media with 40 g/l sucrose did not show any significant difference even though the concentration of myo-inositol was increased to 100 mg/l. The administration of myo-inositol and sucrose at the tested concentrations did not show any significant difference in the number of shoots from *Dendrobium* orchid explants grown in vitro (Table 2). Orchid plants, which often have a long breeding period and require special conditions for propagation, show significant changes in the time of emergence of new shoots based on the concentration of sucrose in the culture medium. The addition of sucrose at the right level provides sufficient energy for metabolic processes that support cell division and the synthesis of plant hormones, which are important in shoot formation (Sari *et al.*, 2018). High sucrose levels can increase cytokinin synthesis, thereby accelerating shoot emergence. Conversely, sucrose deficiency can reduce cytokinin synthesis and inhibit shoot formation (Chaidir *et al.*, 2019).

Functionally, sucrose mainly serves as a carbon and energy source, influencing growth vigor and elongation, while myo-inositol supports cell division, membrane and cell-wall biosynthesis, and osmotic regulation. However, both compounds are not primary morphogens for shoot induction, which may explain why shoot number was not significantly affected (Hu *et al.*, 2020). Although myo-inositol and sucrose are key components of in vitro media, neither directly triggers axillary bud release in the same way as cytokinin. In orchids, shoot multiplication is primarily regulated by exogenous cytokinin such as BAP or kinetin; without them, the number of newly formed shoots often remains limited even when basal medium and carbon supply are sufficient. Previous studies have shown that cytokinin markedly enhance shoot formation in *Dendrobium*, whereas cytokinin-free media tend to favor elongation of existing shoots rather than the initiation of new buds (Arli & Noli, 2024).

Table 2. Shoot emergence time and the number of shoots at several concentrations of myo-inositol and sucrose

Treatment	Shoot emergence time (weeks after planted)	The number of shoots
50 mg/l myo-inositol + 20 g/l sucrose	1.80±0.13 a	0.44±0.05
50 mg/l myo-inositol + 40 g/l sucrose	0.40±0.05 b	0.20±0.02
100 mg/l myo-inositol + 20 g/l sucrose	2.40±0.08 a	0.22±0.02
100 mg/l myo-inositol + 40 g/l sucrose	1.50±0.11 ab	0.68±0.04

note: numbers followed by the same letter within column indicate no significant difference based on the 5% DMRT test.

Growth rate and shoot height

In vitro cultivation of *Dendrobium* orchids in the treatment of 100 mg/l Myo-inositol + 40 g/l Sucrose showed a significantly higher shoot growth rate compared to the treatments of 50 mg/l Myo-inositol + 20 g/l Sucrose and 100 mg/l Myo-inositol + 20 g/l Sucrose. However, in vitro culture medium that had been added with 40 g/l sucrose did not show a significantly different shoot growth rate with the addition of 50-100 mg/l myo-inositol (Figure 1). Sucrose provides the energy needed for plant metabolism. During the active growth phase, plants require sufficient energy supply to support cell division and growth. In in vitro culture media, plants cannot produce carbon from photosynthesis effectively due to insufficient light and limited leaf structure. Therefore, sucrose in the culture media replaces the function of photosynthesis by providing direct energy in the form of glucose and fructose. Optimal sucrose levels ensure that orchid plants have enough energy to support root, stem, and shoot growth, which directly contributes to faster growth rates (Ayub *et al.*, 2019). The right level of sucrose in the culture medium can increase cytokinin synthesis, which contributes to increased growth rate. Conversely, sucrose deficiency can reduce the synthesis of these hormones, inhibiting plant growth and slowing down the growth rate (Harahap *et al.*, 2021).

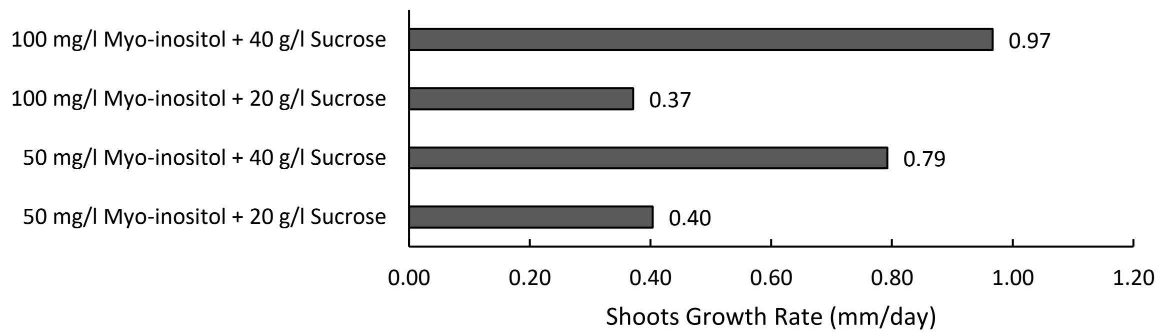

Figure 1. Shoots growth rate at various concentrations of myoinositol and sucrose
(note: numbers followed by the same letter indicate no significant difference based on the DMRT test 5%)

Sucrose also plays a role in cell wall synthesis. Strong, healthy cell walls are essential for plant growth and development, as they provide structural support and protect cells from damage. Sucrose supports the synthesis of polysaccharides such as cellulose and hemicellulose that make up cell walls. In in vitro cultures, orchids grown in media with optimal sucrose levels produce more robust cell walls, supporting faster and stronger shoot growth (Karimah *et al.*, 2021). The enhancement of shoot growth observed in media containing both sucrose and myo-inositol may be attributed to their cooperative roles in cell wall formation. Sucrose supplies the carbon skeletons for cellulose and hemicellulose, while myo-inositol acts as a precursor for pectin biosynthesis and regulates inositol phosphates involved in wall assembly (Hu *et al.*, 2020; Sharma *et al.*, 2020). When present together, these compounds ensure balanced synthesis of the main structural carbohydrates, producing stronger and more flexible cell walls that support shoot elongation. This synergy explains why higher sucrose levels improved plantlet growth even with reduced myo-inositol concentration, highlighting their complementary contribution to the efficient in vitro propagation of *Dendrobium* orchids (Karimah *et al.*, 2021).

Orchid explants grown in vitro on media supplemented with 100 mg/l myo-inositol + 40 g/l sucrose showed significantly higher shoot growth compared to treatments of 50 mg/l myo-inositol + 20 g/l sucrose and 100 mg/l myo-inositol + 20 g/l sucrose at 1-3 WAP. However, the addition of 50-100 mg/l myo-inositol did not show a significant increase in shoot height in explants grown on media supplemented with 40 g/l or 20 g/l sucrose at 1 and 3 WAP (Figure 2). Sucrose provides the energy needed for cell growth. Under in vitro conditions, plants are heterotrophic. The addition of sucrose to the culture medium helps plants to produce the energy needed for cell division and shoot growth. (Gago *et al.*, 2022). In addition, the right concentration of sucrose in the culture medium can help maintain water balance within the cells, which is essential for optimal growth. Adequate water availability supports plant physiological processes, including nutrient transport (Bayhan & Yücesan, 2024).

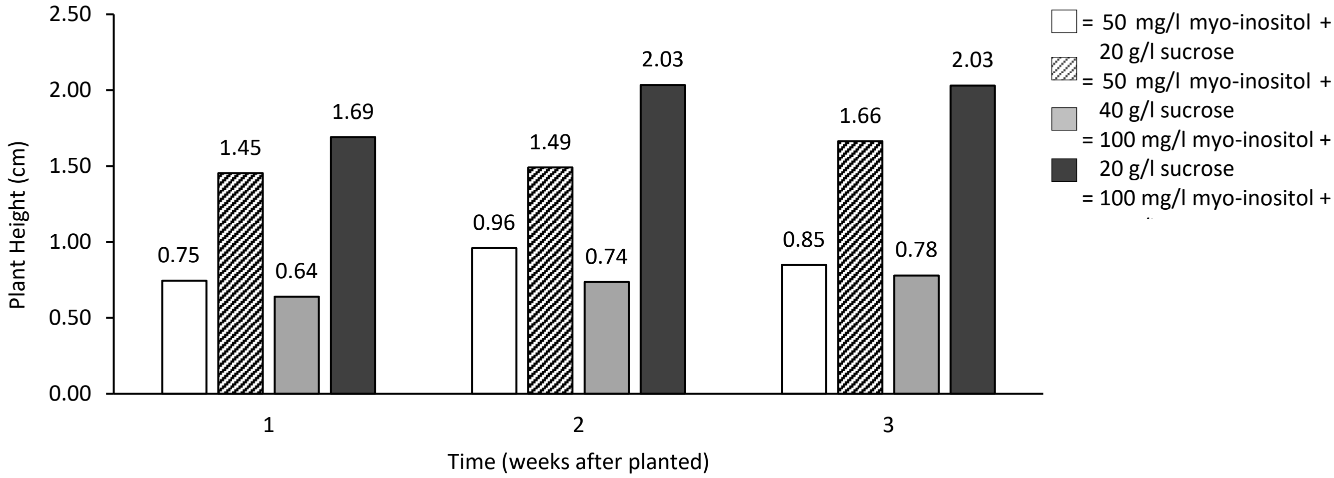

Figure 2. Plant height at various concentrations of myoinositol and sucrose
(note: numbers followed by the same letter indicate no significant difference based on the DMRT test 5%)

The addition of 100 mg/l myo-inositol to media containing 40 g/l sucrose significantly increased the height of orchid explant shoots compared to the addition of 50 mg/l myo-inositol to media containing 40 g/l sucrose (Figure 2). Myo-inositol can improve the structure of cell walls, making it easier for cells to absorb nutrients from the culture medium (Sharma *et al.*, 2020). A research conducted by Widiastoety *et al.* (2012) showed that the addition of 50-100 ppm myo-inositol can increase shoot height by 92-111% due to the role of myo-inositol in regulating the availability of auxin during plantlet growth. Myo-inositol promotes the balance of osmotic pressure by providing sorbitol influx into cells so that it can maintain water and nutrient absorption (Hu *et al.*, 2020).

Santika *et al.* (2023) showed that the addition of 150 mg/l of myo-inositol significantly increased the height of *Dendrobium* orchid shoots grown in vitro compared to no myo-inositol in vitro media. Al-Mushhin *et al.* (2021) proposed that inositol plays a role in the phosphatidylinositol signaling pathway, storage and distribution of auxin and cytokinin. Inositol conjugated with IAA functions as a place to store or transport auxin and can regulate the availability of IAA during plantlet growth, inositol is also often used in culture media to increase growth and morphogenesis. Furthermore, Hu *et al.* (2020) stated that myo-inositol in apple plants stimulates the synthesis of 62.5% more pectin maintaining the bonds between cell walls.

Relationship between growth variables

Plant growth is a complex process influenced by various biological and environmental factors. One important aspect of plant growth is the shoot, which is the center of vegetative growth. Shoot height, shoot emergence time, and shoot growth rate are important parameters that can provide information about the quality of plant growth (Lisnawati *et al.*, 2022; Wulannanda *et al.*, 2023). Sucrose influence the synthesis and regulation of growth hormones in plants, such as auxin and cytokinin, which are very important in regulating plant growth (Chaidir *et al.*, 2019; Hajibehzad *et al.*, 2023). Meanwhile, myo-inositol, apart from playing a role in the synthesis of pectin between cell walls, also plays a role in helping the absorption of nutrients, such as Fe and Zn (Amaral & Brown, 2022). The height of *Dendrobium* orchid shoots grown in vitro showed a fairly strong relationship with shoot emergence time and a very strong relationship with shoot growth rate. (Figure 3). The relationship between shoot height and shoot emergence time can be understood through the basic principles of plant growth. Early shoot emergence time allows plants to utilize light and nutrients earlier than other plants. Plants with earlier shoot emergence time tend to achieve more optimal shoot height (Fajar *et al.*, 2018; Nuraini *et al.*, 2022). Tomasoa (2023) stated that high shoot growth rates tend to produce taller shoots in a shorter time.

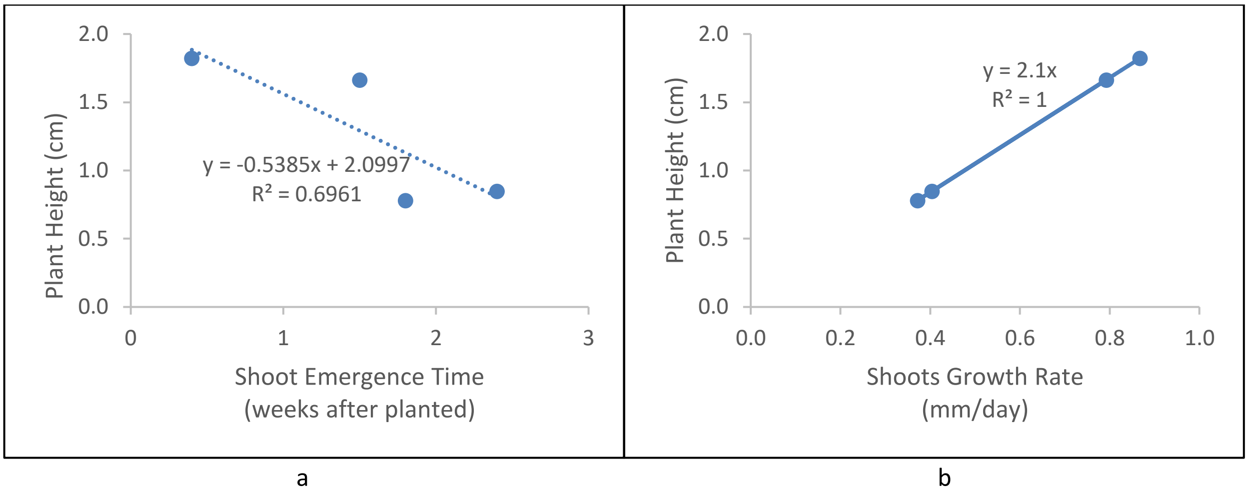

Figure 3. The relationship between several growth variables and the plant height, a.) shoot emergence time, b.) shoot growth rate

The positive correlations observed among shoot emergence time, shoot height, and shoot growth rate in this study can be directly linked to the sucrose treatments applied. Media supplemented with 40 g/L sucrose consistently produced earlier shoot emergence, faster growth rates, and taller shoots compared to 20 g/L sucrose, regardless of the myo-inositol concentration. This suggests that sucrose not only supplies energy for cell division but also indirectly influences developmental timing, allowing shoots to establish and elongate earlier. The correlation analysis therefore reinforces the role of sucrose as the primary driver of growth performance in this experiment. In contrast, variations in myo-inositol concentration mainly fine-tuned growth responses but did not alter the fundamental relationships between the variables. These findings emphasize that sucrose concentration is a critical determinant of coordinated growth responses in *Dendrobium* orchid explants, linking the general growth principles to the specific outcomes of this study.

Research implications and efficiency of myo-inositol use

The results of this study informed that the addition of sucrose as much as 40 g/l can reduce the addition of myo-inositol in VW media by up to 50% to multiply *Dendrobium* orchids in vitro, according to the shoot emergence time (Table 1), shoot growth rate (Figure 1) and plant height (Figure 2). VW formulations supply nitrogen primarily via potassium nitrate and ammonium sulfate then present an NH_4^+ : NO_3^- balance and overall ionic strength. These compositional can alter tissue osmotic conditions, nitrogen availability and pH drift during culture, all of which influence early establishment and growth of orchid explants. Physiologically, the relative forms and amounts of available nitrogen (NH_4^+ and NO_3^-) affect carbon–nitrogen interactions, root/shoot allocation; balanced NH_4^+:NO_3^- ratios often promote biomass accumulation (Hao *et al.*, 2023). Given that many orchids are epiphytic and adapted to relatively low-nutrient environments, a medium with an ionic composition more similar to VW may better match their physiological requirements and therefore favor earlier shoot emergence and vigorous elongation under our sucrose/myo-inositol treatments.

In practice, adjustments to the sucrose content in the culture medium can be made based on the specific needs of the orchid plants being propagated. Too high sucrose levels can cause sugar accumulation in plant tissues, which can affect plant metabolism and osmotic balance, and vice versa (Stein & Granot, 2019). Besides sucrose, other factors such as hormone types, media composition, and environmental conditions such as temperature and lighting also need to be considered to achieve optimal results. Researchers and tissue culture practitioners need to conduct trials and adjustments to find the most effective in vitro culture conditions for propagating *Dendrobium* orchids.

CONCLUSION

The addition of 40 g/l sucrose + 50 mg/l myo-inositol to the in vitro culture medium was the best treatment that produces *Dendrobium* orchid explants with the most optimal growth, according to the shoots emergence time, shoot growth rate and plant height, but was not significantly different from the addition of 40 g/l sucrose + 100 mg/l myo-inositol. The addition of 40 g/l sucrose could reduce the addition of myo-inositol to VW media by up to 50% to propagate *Dendrobium* orchids in vitro.

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