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Morphological, physiological, and anatomical responses of *Cattleya labiata* Lindl. plantlets to mannitol-induced drought stress under in vitro conditions

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Abstract

Cattleya labiata Lindl. is a high-value ornamental orchid whose growth and development are highly dependent on adequate water availability. Drought stress disrupts cellular water balance, reduces photosynthetic activity, and restricts plant growth. Under in vitro conditions, drought stress can be simulated by incorporating mannitol into the culture medium as an osmotic agent. This study evaluated the effects of different mannitol concentrations on the morphological, physiological, and anatomical responses of *C. labiata* plantlets cultured in vitro. The experiment was arranged in a Completely Randomized Design (CRD) with five mannitol concentrations (0, 50, 100, 150, and 200 mM), each with five replications. The evaluated parameters included plantlet survival, morphological characteristics (root length, plantlet height, and leaf number), chlorophyll content, and stomatal density. Quantitative data were analyzed using analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test at the 5% significance level. Mannitol concentrations ranging from 50 to 200 mM did not significantly affect plantlet survival, morphological growth, or chlorophyll content. In contrast, stomatal density decreased significantly with increasing mannitol concentration, indicating that anatomical responses were more sensitive to osmotic stress than morphological and physiological traits during the three-week culture period. These findings demonstrate that mannitol effectively induced drought stress responses in *C. labiata* plantlets under in vitro conditions, although the applied concentrations were insufficient to produce significant morphological and physiological alterations within the experimental period.

Keywords: *Cattleya labiata* Lindl.; Drought Stress; In Vitro Culture; Mannitol; Stomatal Density.

1. Introduction

Indonesia is recognized as one of the world's tropical biodiversity hotspots, harboring approximately 10,000 orchid species, many of which possess high ornamental and commercial value [1]. Among the members of the Orchidaceae family, *Cattleya labiata* Lindl. is widely appreciated for its large flowers, attractive coloration, distinctive floral morphology, and pleasant fragrance, earning it an important position in the ornamental plant industry [2]. The increasing market demand for *C. labiata* has encouraged efforts to improve its propagation and cultivation. However, successful cultivation depends not only on genetic characteristics but also on appropriate environmental conditions. Water availability is considered one of the most critical factors influencing orchid growth, development, and flowering [3].

Water deficit is one of the major abiotic stresses limiting orchid cultivation because it disrupts various physiological and morphological processes. Drought stress reduces cell turgor pressure, inhibits photosynthesis, restricts leaf expansion, and suppresses flower development, ultimately decreasing plant productivity and survival [4]. Under prolonged water deficiency, plants experience osmotic imbalance that interferes with nutrient uptake and metabolic activities. Consequently, evaluating plant responses to drought stress is essential for identifying adaptive characteristics

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associated with stress tolerance. Such information is particularly valuable for improving orchid cultivation under increasingly variable environmental conditions.

The in vitro culture technique provides a controlled experimental system for evaluating plant responses to abiotic stress while minimizing environmental variation. This method enables researchers to manipulate culture conditions precisely and observe morphological, physiological, and anatomical responses under simulated stress environments [5]. In addition to supporting rapid propagation of genetically uniform plantlets, in vitro culture offers an effective platform for screening drought tolerance at an early developmental stage [6]. Therefore, this approach has become widely applied in studies investigating stress adaptation in economically important ornamental plants, including orchids.

One of the most commonly used osmotic agents for simulating drought stress under in vitro conditions is mannitol. Mannitol reduces the osmotic potential of the culture medium, thereby limiting water availability to plant tissues and inducing osmotic stress that resembles drought conditions [7]. As water uptake decreases, plantlets initiate various adaptive mechanisms to maintain cellular homeostasis and physiological stability. Previous studies demonstrated that mannitol concentrations between 200 and 300 mM effectively induced drought stress in *Camellia sinensis* by decreasing tissue water content, increasing membrane permeability, and stimulating osmolyte accumulation [8]. These findings indicate that mannitol is a reliable osmoticum for drought simulation in plant tissue culture.

Plant adaptation to drought stress can be evaluated through several growth-related indicators, including morphological, physiological, and anatomical characteristics. Morphological responses are commonly reflected by changes in root length, plantlet height, and leaf development, whereas physiological adaptation can be assessed through chlorophyll content because chlorophyll is closely associated with photosynthetic efficiency [9]. Anatomical responses, particularly stomatal density, provide additional information regarding plant water-use regulation and gas exchange under stress conditions [10]. These parameters collectively provide a comprehensive understanding of plant tolerance mechanisms during drought stress.

Several studies have reported the effectiveness of osmotic agents such as polyethylene glycol (PEG) and mannitol in inducing drought stress in different orchid species and other horticultural crops [11][12]. Nevertheless, information regarding the drought response of *Cattleya labiata* Lindl. under mannitol-induced osmotic stress remains limited. In particular, the optimal mannitol concentration capable of inducing drought stress while maintaining plantlet viability has not yet been clearly established. This lack of information restricts the development of efficient screening methods for drought-tolerant *C. labiata* plantlets cultivated in vitro.

Therefore, this study was conducted to evaluate the growth responses of *Cattleya labiata* Lindl. plantlets cultured under different concentrations of mannitol-induced drought stress. Morphological traits, chlorophyll content, and stomatal density were analyzed to characterize plant adaptation under osmotic stress conditions. Furthermore, the study aimed to identify the level of drought tolerance exhibited by *C. labiata* plantlets and determine the concentration of mannitol that effectively simulates drought stress without causing plantlet mortality. The findings are expected to provide valuable information for improving in vitro drought screening and supporting the development of adaptive orchid cultivation strategies under water-limited environments.

2. Materials and Methods

2.1. Materials and Experimental Design

The study was conducted from February 2026 to March 2026 at the Botany Laboratory (In Vitro Culture Room), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia. The experiment was arranged in a Completely Randomized Design (CRD) with five mannitol concentration treatments and five replications. The treatments consisted of supplementing Vacin and Went (VW) culture medium with mannitol at concentrations of 0 mM (control), 50 mM, 100 mM, 150 mM, and 200 mM. Each treatment consisted of five experimental units, resulting in a total of 25 experimental units.

Culture equipment was sterilized in an autoclave at 121°C for 20 min. The workbench and Laminar Air Flow (LAF) cabinet were disinfected with 70% ethanol to maintain aseptic conditions throughout the culture process. Vacin and Went (VW) basal medium was prepared by dissolving 1.63 g of VW medium in distilled water, followed by the addition of 30 g/L sucrose and 3 g activated charcoal. The pH of the medium was adjusted to 5.6–5.8 using HCl or KOH solution, after which 7 g agar was added. The medium was heated until completely homogenized and then divided into five mannitol concentration treatments (0, 50, 100, 150, and 200 mM), with 200 mL prepared for each treatment. Finally, the media were sterilized in an autoclave at 121°C for 15 min.

2.2. Preparation of Culture Media and Planting of Plantlets

The work area and all culture equipment were sterilized using 70% ethanol to maintain aseptic conditions during the culture process. The Vacin and Went (VW) basal medium was prepared by dissolving 1.63 g of VW powder in 800 mL of distilled water until homogeneous. Subsequently, 30 g L^{-1} sucrose and 3 g activated charcoal were added, and the solution was mixed until all components were completely dissolved.

The medium pH was measured using a pH meter and adjusted to 5.6–5.8 using NaOH or HCl solutions. Distilled water was then added to achieve a final volume of 1 L. Agar (7 g) was added, and the medium was heated until completely homogenized. The medium was then divided into five mannitol concentration treatments: 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM, with 200 mL prepared for each treatment.

Approximately 20 mL of each treatment medium was dispensed into culture bottles and sterilized using an autoclave at 121°C for 15 min. The sterilized media were incubated for seven days to ensure the absence of contamination before being used for *Cattleya labiata* plantlet cultivation. The planting of *Cattleya labiata* Lindl. plantlets were carried out aseptically inside a Laminar Air Flow Cabinet (LAFC), which had been sterilized using UV irradiation and 70% ethanol. The plantlets were sterilized by washing with sterile distilled water, soaking in 0.5% NaOCl solution, and rinsing three times with sterile distilled water. Subsequently, one plantlet was placed in each culture bottle containing the treatment medium. The bottles were tightly sealed, labeled according to the treatment, and incubated in a culture room under a photoperiod of 16 h light and 8 h dark for growth observation. Observation Parameters.

2.3. Observation Parameters

2.3.1. Plantlet Survival Percentage

Plantlet survival percentage was determined at the end of the observation period. Survival percentage was calculated according to [12] using the following equation:

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

2.3.2. Plantlet Morphology

Morphological observations included root length, stem height, and number of leaves. Observations were conducted periodically and supported by visual documentation of changes in plantlet morphology.

2.3.3. Chlorophyll Content

Chlorophyll content analysis was performed using an extraction method with 96% ethanol. A 0.1 g leaf sample was extracted, and the absorbance was measured using a UV spectrophotometer at wavelengths of 648 nm and 664 nm. Chlorophyll content was calculated based on the equation described by Miazek (2002).

2.3.4. Stomata Density

Observations were conducted using the epidermal imprint method with transparent nail polish. The prepared samples were observed using a light microscope at 400× magnification, and the number of stomata was counted based on the microscope field of view area. Stomatal density values were calculated using the following formula:

$$\text{Stomatal Index} = \frac{\text{Number of stomata}}{\text{Area of microscope field of view } (\text{mm}^2)}$$

2.4. Data Analysis

The research data consisted of qualitative and quantitative data. Qualitative data were analyzed descriptively through documentation of morphological, physiological, and anatomical changes in the plantlets. Quantitative data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. If significant differences were found among treatments, the analysis was continued with Tukey's Honestly Significant Difference (HSD) test at a 5% confidence level.

3. Results and Discussion

3.1. Plantlet Survival Percentage

Observations on the growth of *Cattleya labiata* Lindl. orchid plantlets treated with mannitol for three weeks showed plantlet survival and differences in morphological visualization among treatments. Mannitol was applied at five different concentrations, namely 0 mM (control), 50 mM, 100 mM, 150 mM, and 200 mM. Each treatment consisted of five replications with one plantlet in each culture bottle. The percentage of surviving plantlets is presented in Table 1.

Table 1 Percentage of surviving *Cattleya labiata* Lindl. plantlets after mannitol treatment at different concentrations.

Mannitol concentration (mM)	**Percentage of surviving plantlets at week (%)**		
	I	II	III
0	100	100	100
50	100	100	100
100	100	100	100
150	100	100	100
200	100	100	100

Based on Table 1, all plantlets remained alive until the third week in all treatments. The results showed that mannitol application up to 200 mM for three weeks did not cause plantlet mortality. This condition was presumably because the stress duration was relatively short, allowing the plantlets to maintain water balance and metabolic activity. This result is consistent with Putri et al. (2022), who reported that *Dendrobium* orchid plantlets maintained a survival percentage of 100% even under high PEG concentrations used to simulate drought stress [18]. Surviving orchid plantlets were characterized by green to yellowish-green coloration, indicating that chlorophyll was still present and physiological activities were still occurring. According to Sandy et al. (2022), living explants are generally green to yellowish-green, whereas brown or black coloration indicates tissue damage or death [19].

The visual condition of *C. labiata* plantlets subjected to mannitol-induced drought stress is presented in Table 2. Visual observations were carried out for three weeks based on changes in plantlet color, namely green (H) and yellowish-green (HK), as indicators of plantlet responses to the treatments.

Table 2 Visualization of *Cattleya labiata* Lindl. plantlets after mannitol treatment at different concentrations

Mannitol Concentration (mM)	**Plantlet Visualization (%) by Week**					
	I		II		III	
	H	HK	H	HK	H	HK
0	100	0	100	0	100	0
50	100	0	100	0	100	0
100	100	0	100	0	80	20
150	100	0	80	20	40	60
200	100	0	15	85	0	100

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

Based on Table 2, color changes began to appear in the second week, indicating that the response of plantlets to drought stress did not occur immediately. During the first week, water reserves and metabolic capacity were presumably still sufficient to maintain chlorophyll synthesis. As the duration of stress increased, this capacity gradually decreased, and chlorosis symptoms began to appear [20].

The results showed that increasing mannitol concentration had a greater effect on the visual condition of the plantlets. Higher mannitol concentrations reduced the water potential of the culture medium, thereby decreasing water uptake by the plantlets. This condition interfered with physiological processes, including chlorophyll formation, due to impaired uptake of nitrogen (N) and magnesium (Mg), which are important for chlorophyll biosynthesis [21]. Consequently, chlorophyll content decreased, causing the plantlets to change from green to yellowish-green (chlorosis). Green coloration indicates that chlorophyll content is still maintained, whereas yellowish-green coloration indicates the beginning of chlorophyll degradation due to osmotic stress [22]. Therefore, the higher the mannitol concentration applied, the greater the osmotic stress experienced by the plantlets, making the yellowish-green discoloration more pronounced. Mannitol at an optimal concentration may support the growth requirements of planlets, but excessive concentrations can inhibit growth and reduce physiological condition [23]. The condition of *C. labiata* plantlets after three weeks is shown in Figure **1.**

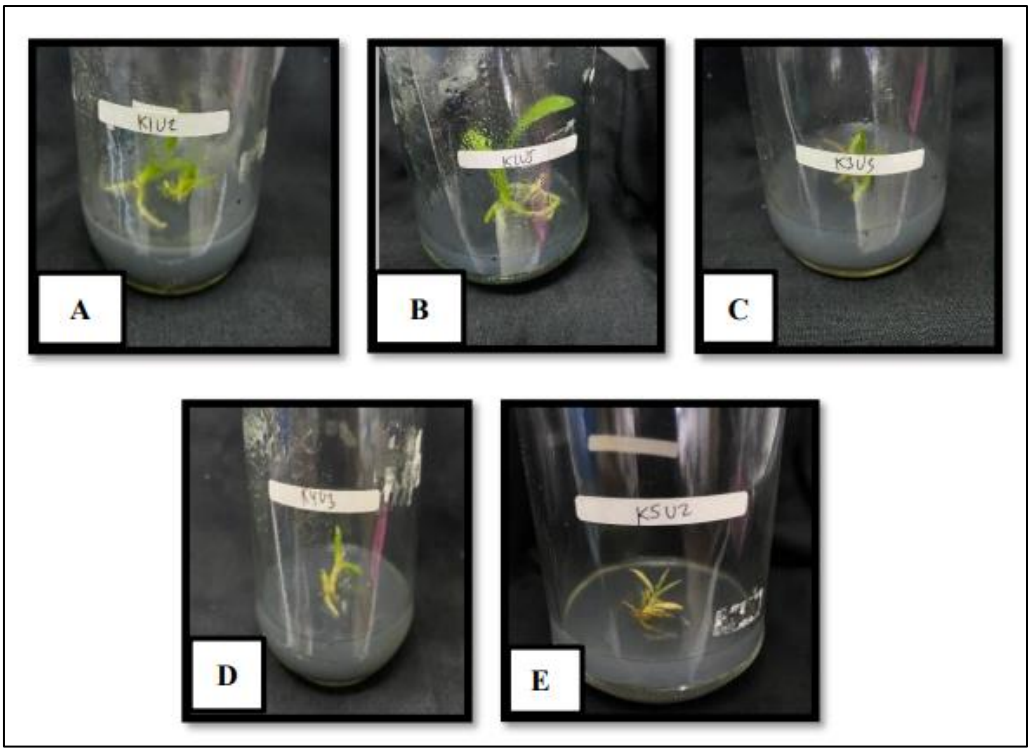

Figure 1 Visualization of *Cattleya labiata* Lindl. plantlets cultured on VW medium supplemented with different mannitol concentrations at week 3**.** (A) 0 mM, (B) 50 mM, (C) 100 mM, (D) 150 mM, and (E) 200 mM

Based on Figure 1, increasing mannitol concentration increased the effect of drought stress on plantlet condition. At low concentrations, plantlets were still able to maintain green coloration and relatively normal growth, whereas higher concentrations caused discoloration and growth reduction due to increased osmotic stress. This result is consistent with Apherta et al. (2025), who reported that increasing PEG 6000 concentrations caused visual changes in *Phalaenopsis amabilis* plantlets under drought stress. In addition to concentration effects, plantlet responses became more apparent with increasing observation time [24]. Initially, plantlets maintained green coloration, but after several weeks those exposed to higher concentrations gradually became yellowish-green. This indicates that the response of plantlets to drought stress developed progressively during the in vitro culture period. Similar findings were reported by Mustaqim et al. (2023), who stated that growth and physiological responses of plantlets to drought stress become increasingly evident with prolonged exposure to mannitol during in vitro culture [25].

3.2. Plantlet Morphology

The observation data for growth parameters of *Cattleya labiata* Lindl. plantlets, including number of leaves, plantlet height, and root length at different mannitol concentrations, are presented in Table 3.

The results showed that different mannitol concentrations produced different growth responses in terms of root length, plantlet height, and number of leaves. However, ANOVA analysis indicated that these differences were not statistically significant. The observed variation therefore could not yet be considered a definite effect of the treatments.

Table 3 Mean growth of *Cattleya labiata* Lindl. plantlets at different mannitol concentrations.

Concentration (mM)	Root length (cm)	Plantlet height (cm)	Number of leaves
0	1.78	0.86	4.8
50	2.94	0.60	4.6
100	1.94	0.58	5.4
150	1.70	0.56	3.8
200	1.58	0.54	6.0

Based on Table 3, the increase in root length at 50 mM indicates that mild drought stress was still able to stimulate root elongation as an adaptive response to improve water acquisition. However, when mannitol concentration increased to 100–200 mM, osmotic stress became more severe, and cell division and elongation in the roots began to be inhibited. Similar results were reported by Manalu et al. (2024), who found that increasing PEG 6000 concentration reduced root length in black orchid plantlets because water uptake became limited under drought stress. The higher mannitol concentration reduced the water potential of the medium, thereby decreasing the ability of root cells to absorb water. Restricted water uptake can interfere with cell division and elongation in root meristem tissues, resulting in inhibited root growth [22].

In addition to affecting root length, mannitol application also tended to reduce the height of *Cattleya labiata* Lindl. plantlets. The decrease in plantlet height became apparent at 50 mM and continued up to 200 mM. This indicates that stem growth was relatively sensitive to the reduction in medium water potential caused by mannitol addition. Similar findings were reported by Manalu et al. (2024), who observed that increasing PEG concentration reduced the height of black orchid plantlets because of restricted water uptake under drought stress [22]. Reduced cell turgor pressure causes stem cells to lose the driving force required for elongation, so cell division, enlargement, and elongation do not occur optimally, leading to reduced plantlet height as mannitol concentration increases [28].

Mannitol application also produced different responses in the number of leaves of *C. labiata* plantlets. The data in Table 3, show that leaf number did not follow a consistent pattern across treatments but fluctuated during the observation period. This indicates that mannitol up to 200 mM did not produce a uniform response in leaf formation. Leaf development is a morphological process that generally requires a longer time than physiological responses to drought stress. Increasing mannitol concentration lowered the water potential of the medium, restricted water uptake, and induced osmotic stress, causing the plantlets to prioritize maintenance of metabolic processes rather than formation of new leaves. According to Nio and Banyo (2011), water deficiency inhibits physiological processes, thereby limiting the energy available for the development of new organs. This is consistent with Putri et al. (2022), who reported that physiological responses to osmotic stress in orchid plantlets cultured in vitro appear earlier than morphological changes.

3.3. Chlorophyll Content

Chlorophyll analysis was performed to evaluate the photosynthetic capacity of *Cattleya labiata* Lindl. plantlets in relation to their growth. The chlorophyll content of plantlets is presented in Table 4.

Table 4 Chlorophyll content of *Cattleya labiata* Lindl. plantlets at week 3**.**

Mannitol concentration (mM)	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Total chlorophyll (mg/L)
0	4.807	3.899	3.792
50	5.277	4.294	3.551
100	4.376	3.555	3.456
150	4.435	3.616	3.514
200	3.826	3.088	3.005

ANOVA results at the 5% significance level indicated that different mannitol concentrations did not significantly affect chlorophyll a, chlorophyll b, or total chlorophyll content in *C. labiata* plantlets. Nevertheless, chlorophyll content tended to decrease as mannitol concentration increased. This suggests that during the three-week observation period the plantlets were still able to maintain chlorophyll content, so the decrease was not large enough to produce statistically significant differences. This ability was presumably related to physiological adaptation mechanisms that maintained photosynthetic activity during the early stage of drought stress.

The statistical analysis showed that mannitol application at different concentrations did not significantly affect chlorophyll content. The differences among treatment means were relatively small, resulting in non-significant statistical differences. However, chlorophyll content generally tended to decline with increasing mannitol concentration. Similar results were reported by Apherta et al. (2025), who found that PEG 6000 treatment caused a decrease in chlorophyll a, chlorophyll b, and total chlorophyll in *Phalaenopsis amabilis* as concentration increased.

The reduction in chlorophyll content at higher mannitol concentrations was associated with the lower water potential of the medium, which restricted water uptake by the plantlets. This osmotic stress can inhibit chlorophyll biosynthesis, causing chlorophyll content to decrease as mannitol concentration increases. In addition, the chlorophyll response may depend on the ability of the plantlets to maintain the stability of photosynthetic pigments under stress conditions, so chlorophyll reduction had not yet occurred markedly.

Increasing mannitol concentration lowered the water potential of the medium, thereby reducing water uptake and inducing osmotic stress. This condition interferes with the uptake of nutrients, particularly nitrogen (N) and magnesium (Mg), which are essential for chlorophyll biosynthesis. Nitrogen is a major component of the chlorophyll molecule, whereas magnesium forms the central atom of the chlorophyll ring. Consequently, chlorophyll formation is inhibited and chlorophyll content tends to decrease [9]. Osmotic stress also promotes the formation of reactive oxygen species (ROS), and when ROS production exceeds the capacity of the antioxidant defense system, oxidative stress occurs, leading to damage of cell membranes and chloroplasts where photosynthesis takes place [24].

3.4. Stomatal Density

Stomatal density of *Cattleya labiata* Lindl. plantlets differed among mannitol treatments. ANOVA showed that mannitol application had a significant effect on stomatal density, as indicated by an F-value of 30.98 and a P-value of 0.000 ($p < 0.05$). This indicates that significant differences occurred in stomatal observations. Therefore, mannitol application significantly affected the anatomical response of the plantlets, particularly stomatal formation. The stronger response in stomatal density compared with chlorophyll content suggests that the anatomical response of *C. labiata* plantlets to mannitol-induced stress was more pronounced than the physiological response. The stomatal density values are presented in Table 5.

Table 5 Stomatal density of *Cattleya labiata* Lindl. plantlets at week 3 after mannitol treatment.

Mannitol concentration (mM)	Stomatal density $\mu = (\bar{Y} \pm SE)$
0	35.32 ± 2.27 [a]
50	23.37 ± 2.62 [b]
100	16.61 ± 1.44 [bc]
150	13.80 ± 1.04 [c]
200	10.416 ± 0.72 [c]

Note: $\mu = (\bar{Y} \pm SE)$ $\bar{Y}$ = Mean SE = Standard Error

Based on Table 5, Tukey's test at the 5% level showed that mannitol treatments differed significantly. Mannitol application at different concentrations significantly affected stomatal density in *C. labiata* plantlets. The highest stomatal density was observed in the control treatment (0 mM), reaching 35.32 ± 2.27, whereas the lowest value was observed at 200 mM, reaching 10.416 ± 0.72. In general, stomatal density tended to decrease as mannitol concentration increased. The control treatment differed significantly from all mannitol treatments. The 50 mM treatment differed significantly from the control as well as from the 150 mM and 200 mM treatments, whereas the 100 mM treatment did not differ significantly from 50 mM, 150 mM, or 200 mM. These results indicate that increasing mannitol concentration from 50 to 200 mM gradually affected stomatal density in *C. labiata*.

The effect of mannitol concentration on stomatal density is shown in Figure 2.

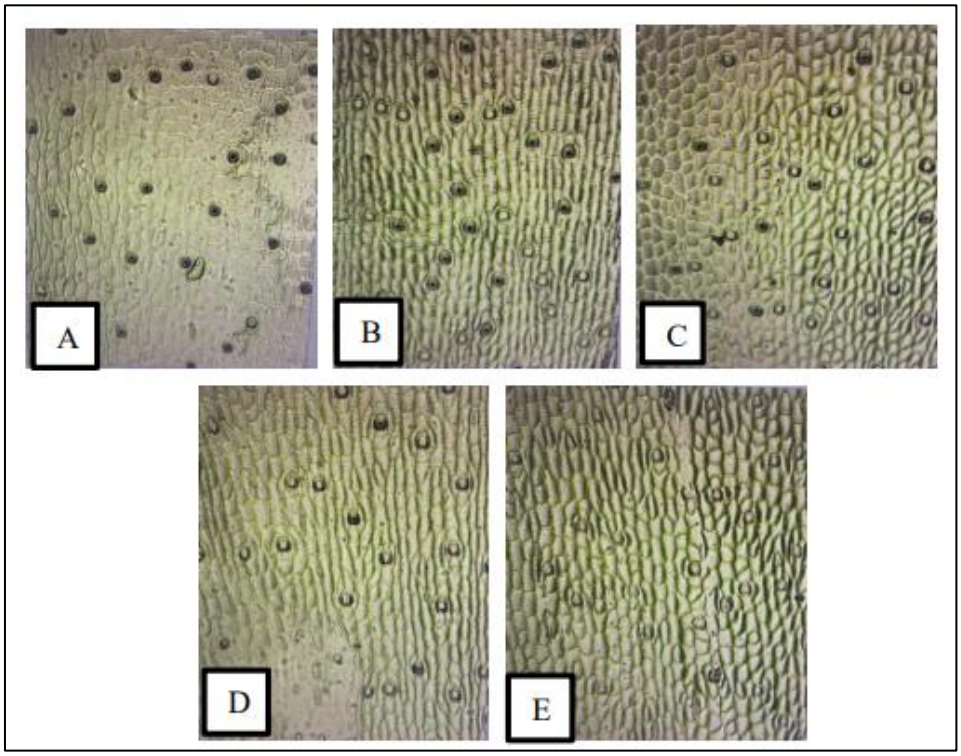

Figure 2 Abaxial leaf surface of *Cattleya labiata* Lindl. plantlets observed at 100× magnification: A = 0 mM, B = 50 mM, C = 100 mM, D = 150 mM, E = 200 mM.

Based on Figure 2 mannitol application at different concentrations affected stomatal density in *C. labiata* plantlets. Higher mannitol concentrations resulted in fewer stomata on the abaxial leaf surface. In the control treatment, stomata were denser and more abundant than in treatments with higher mannitol concentrations. At 150 mM and 200 mM, stomatal density was lower and the spacing between stomata was wider. This indicates that drought stress simulated by mannitol affected stomatal density in orchid plantlets.

Stomatal density in *C. labiata* plantlets tended to decrease at higher mannitol concentrations. This is consistent with Nurcahyani et al. (2026), who explained that reduced stomatal density is an adaptive response of plants to drought stress induced by PEG 6000. Under such conditions, plants reduce stomatal number to limit water loss through transpiration and improve water-use efficiency. The stress caused by mannitol addition results from reduced medium water potential, which restricts water uptake and lowers cell turgor pressure. This condition interferes with cell division and elongation involved in growth and leaf tissue formation, including stomatal development [23].

The reduction in stomatal density is presumed to be an adaptive mechanism of *C. labiata* plantlets to mannitol-induced drought stress. Increasing mannitol concentration lowers the water potential of the medium and induces osmotic stress. This condition promotes the accumulation of abscisic acid (ABA), which inhibits stomatal formation and induces stomatal closure to reduce water loss through transpiration. As a result, stomatal density decreases, helping maintain water balance within plant tissues during drought stress [26].

This mechanism may have contributed to the ability of *C. labiata* plantlets to survive throughout the observation period despite exposure to drought stress. According to Hasanuzzaman et al. (2023), regulation of stomatal density is an important adaptive mechanism for plants under osmotic stress. Plants with lower stomatal density generally exhibit better water-use efficiency and greater survival ability under drought conditions. However, reduced stomatal density may also limit CO_2 entry into the leaves, thereby decreasing photosynthesis and plant growth if the stress persists for a longer period [24].

Stomata are physiological parameters that reflect plant responses to environmental conditions through changes in stomatal number, density, and opening activity on the leaf surface. Such changes indicate physiological adjustment of

plants to environmental stress and therefore stomatal characteristics can be used as indicators of stress level and plant adaptation [28].

4. Conclusion

This study demonstrated that mannitol-induced drought stress significantly affected the anatomical response of *Cattleya labiata* Lindl. plantlets by reducing stomatal density as mannitol concentration increased, whereas morphological parameters (root length, plantlet height, and leaf number) and physiological parameters (chlorophyll a, chlorophyll b, and total chlorophyll content) did not show significant differences during the three-week observation period. Although all plantlets remained viable under mannitol concentrations ranging from 50 to 200 mM, the observed responses indicated that these concentrations had not yet induced a level of drought tolerance that could be clearly characterized under in vitro conditions. Therefore, further studies are recommended using lower mannitol concentration intervals (e.g., 0–50 mM with 10- or 25-mM increments), longer observation periods, and additional physiological and biochemical indicators, such as proline accumulation, reducing sugar content, and antioxidant enzyme activity, to more accurately determine the drought tolerance threshold and adaptive mechanisms of *Cattleya labiata* Lindl. plantlets under osmotic stress.

Compliance with Ethical Standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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