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Characterization of Drought Stress Responses in *Phalaenopsis amabilis* Based on Survival Percentage and Stomatal Density

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Abstract

The moon orchid [*Phalaenopsis amabilis* (L.) Blume] is a highly valued ornamental plant designated as one of Indonesia's national flowers due to its exceptional aesthetic appeal. Despite its economic importance, its growth is strongly influenced by environmental conditions, particularly drought stress. Drought can inhibit various physiological processes, such as reduced turgor pressure, stomatal closure, and disruptions in photosynthesis due to limited water availability. These conditions may decrease plant quality and reduce the success rate of seedling production, which is a crucial aspect for the commercial development of orchid commodities. This study aimed to determine the percentage of *Phalaenopsis amabilis* plants that survived under different concentrations of PEG 6000 used to induce drought stress and to characterize the changes in stomatal density on the leaves as an adaptive physiological–morphological response. The experiment was arranged in a Completely Randomized Design (CRD) with a single factor of PEG 6000 concentration: 0%, 10%, 20%, 30%, and 40%, each with five replications. Data were analyzed using ANOVA at a 5% significance level followed by the Honestly Significant Difference (HSD) test. The results showed that increasing PEG 6000 concentrations reduced plant survival percentages, yet led to an increase in stomatal density as an adaptive response. The 40% concentration exhibited the highest tolerance response, providing insights into the adaptive mechanisms of *P. amabilis* under drought stress.

Keywords: Drought stress; Survival Percentage; Stomatal Density; *Phalaenopsis amabilis;* PEG 6000.

1. Introduction

Indonesia is home to approximately 4,000 orchid species distributed across its islands, many of which possess high ornamental and economic value due to their long-lasting floral freshness [1]. *Phalaenopsis amabilis* (L.) Blume, commonly known as the moth orchid, is one of Indonesia's national flowers and a member of the Orchidaceae family with distinctive aesthetic traits [2–4]. However, *P. amabilis* is listed in Appendix II of CITES, indicating its vulnerable conservation status and the need for sustainable cultivation efforts [5].

Despite its potential, *P. amabilis* production in Indonesia remains low compared to countries such as Thailand, Taiwan, and Australia, mainly due to limited propagation technology and environmental constraints [6]. Among these, drought stress is a major abiotic factor affecting orchid growth and productivity by reducing leaf area, disrupting stomatal function, and inhibiting photosynthesis [7–9]. Drought-induced physiological stress is widely recognized as one of the most detrimental environmental stresses to agricultural productivity worldwide [10–12].

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An effective approach to improving drought tolerance is the selection of resistant genotypes through controlled stress simulation using polyethylene glycol (PEG) 6000, a non-toxic osmotic agent that accurately mimics water deficit conditions [13–17]. PEG-induced stress enables early screening of tolerant plants under reproducible conditions, making it a reliable tool in physiological and biochemical studies [18].

Therefore, physiological response of *P. amabilis* under PEG-induced drought stress is vital to understanding its tolerance mechanisms and supporting future orchid breeding and conservation programs.

2. Material and methods

The tools and materials used in this research were moon orchids (*Phalaenopsis amabilis*), kadaka media, 70% alcohol, distilled water, polyethylene Glycol (PEG) 6000, plastic cups, spray bottles, object glass, cover glass, microscope, optilab, tweezers, pipettes, measuring glass, stirring rod and camera.

The study used a completely randomized deign (CRD) with one factor, namely the addition of Polyethylene Glycol (PEG) 6000, which consisted of five concentration levels : 0% (A1), 10% (A2), 20% (A3), 30% (A4), and 40% (A5). Each of treatment was repeated five times.

2.1. Polyethylene Glycol 6000 Treatment

The preparation of PEG 6000 solutions was carried out by dissolving PEG 6000 in 100 mL of distilled water according to the desired concentration levels of 0%, 10%, 20%, 30%, and 40%. A 10% PEG 6000 solution was prepared by dissolving 10 g of PEG 6000 in 100 mL of distilled water, a 20% solution by dissolving 20 g of PEG 6000, a 30% solution by dissolving 30 g of PEG 6000, and a 40% solution by dissolving 40 g of PEG 6000 in 100 mL of distilled water. The 0% PEG 6000 solution (control) consisted of 100% distilled water without PEG 6000. All solutions were stirred until completely homogeneous and stored in labeled plastic containers prior to use.

PEG 6000 treatment was applied to *Phalaenopsis amabilis* plants aged three months. Each plant was watered once with 20 mL of PEG 6000 solution according to the treatment concentration indicated on the label of each planting cup. For the control treatment (0% PEG 6000), watering was carried out using distilled water only. Observations were conducted 30 days after treatment to evaluate plant responses. The data obtained were used to determine drought tolerance levels with stress response mechanisms in *P. amabilis*.

2.2. The Percentage of Surviving and Visualization Plants

2.2.1. The percentage of surviving plants

The survival percentage of *Phalaenopsis amabilis* was calculated by comparing the number of surviving plants to the total number of plants per treatment using the formula 1 [19]:

$$\textit{The percentage of surviving plants: } \frac{\textit{Number of Surviving Plants}}{\textit{Total plants}} \times 100\ \%$$

2.2.2. The visualization of plants

Plant visualization was observed at the end of the treatment based on color appearance (green, yellow, or brown) as indicators of plant vitality and stress response [20]

2.3. Stomatal Density Analysis

Leaf samples of *P. amabilis* were fixed in 70% ethanol. The abaxial surface of the leaf was sectioned thinly using a scalpel and placed on a glass slide, stained with safranin, and covered with a cover glass. Observations were carried out using a microscope at 40× magnification connected to an optilab camera. Stomatal density was calculated using the Image Raster 3 software [21] with the formula:

$$\textit{Stomatal Density: } \frac{\textit{Number of Stomata}}{\textit{Observation Field Area (mm)}}$$

3. Results and discussion

3.1. Percentage of Surviving Plants

The survival percentage of *Phalaenopsis amabilis* plants under various PEG 6000 concentrations observed over four weeks can be seen in Table 1.

Table 1 Percentage of Surviving Plants from PEG 6000 Selection

PEG 6000 Concentration	**Percentage of Surviving Plants in Week (%)**			
(%)	**I**	**II**	**III**	**IV**
0	100	100	100	100
10	100	100	100	100
20	100	100	100	100
30	100	100	100	100
40	100	100	100	100

Based on the data in **Table 1**, the results show that from the first to the fourth week of observation, the survival rate of *Phalaenopsis amabilis* plants under PEG 6000 concentrations of 0%, 10%, 20%, 30%, and 40% remained at 100%, indicating that no plant mortality occurred. The success of drought stress selection is influenced by the high regeneration capacity of the plants and the effectiveness of the selective agent used. PEG 6000-induced drought stress functions by reducing the water potential of the growth medium without penetrating plant tissues, thereby creating osmotic stress conditions. In response, plants under go osmoadaptation by accumulating compatible solutes such as proline, soluble sugars, and polyamines, along with the activation of antioxidant enzymes to stabilize membranes and mitigate damage caused by Reactive Oxygen Species (ROS) [22]. This response is considered a form of stress memory, where plants temporarily slow their growth but maintain cellular viability. When PEG 6000 treatment is discontinued, the plants recover fully due to these protective mechanisms that preserve tissue integrity during the stress period. Therefore, the sustained survival of *P. amabilis* under PEG 6000 treatment indicates not the absence of stress but a controlled physiological adaptation that enhances drought tolerance without inducing lethality [23].

3.2. The Visualization Percentage of *P. amabilis*

During the treatment period, visual assessment included the observation of plant color categorized as green, greenish-brown, or brown [20]. The visualization variation under different PEG 6000 concentrations is presented in **Table 2.**

Table 2 Visualization percentage under PEG 6000 selection

Concentration of PEG 6000 (%)	**Visualization Percentage of P. amabilis at week (%)**			
	I	**II**	**III**	**IV**
0	G : 100	G : 100	G : 100	G : 100
10	G : 100	G : 100	G : 100	G : 100
20	G : 100	G : 100	G : 100	G : 60 GB : 40
30	G : 100	G : 100	G : 80 GB : 20	G : 60 GB : 40
40	G : 100	G : 100	GB : 60 GB : 40	G : 40 GB : 60

Note: G = Green, GB = Greenish-brown

Based on **Table 2**, it can be seen that during the first and second weeks of observation, all *P. amabilis* plants (100%) exhibited green coloration across all PEG 6000 treatments. The effects of PEG 6000 application began to appear in the third week, where plants treated with 30% PEG 6000 showed 80% green and 20% greenish-brown coloration. At a concentration of 40%, 60% of the plants remained green, while 40% appeared greenish-brown. In the fourth week, the effect of drought stress became more apparent. At 30% PEG 6000, 60% of the plants remained green and 40% turned greenish-brown, whereas at 40% PEG 6000, only 20% of the plants were green and 80% appeared greenish-brown. The treatment response of *P. amabilis* plants after four weeks of exposure to various PEG 6000 concentrations is presented in **Figure 1**.

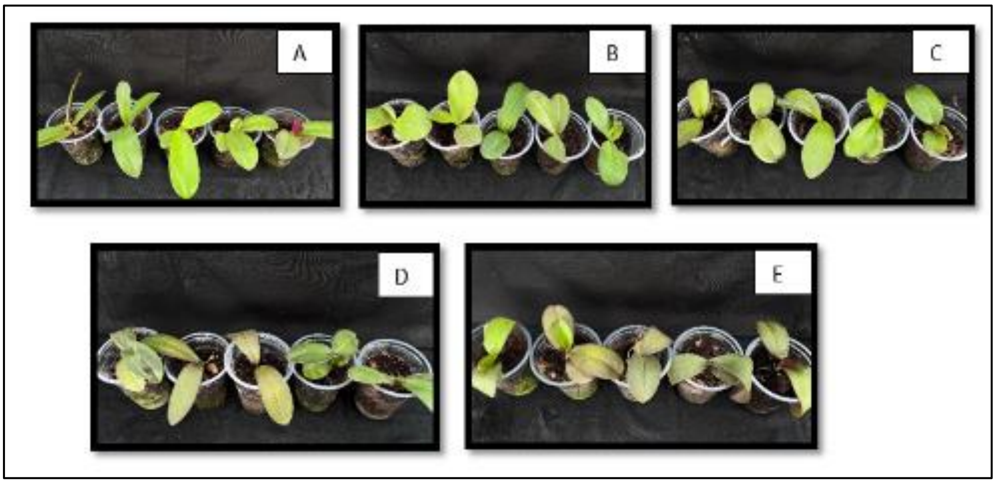

Figure 1. *P. amabilis* with PEG 6000 treatment at the 4th week. (A) = 0%, (B) = 10%,(C) = 20%, (D) = 30% and (E) = 40%.

The results of this study indicated that the application of PEG 6000 as a drought stress-inducing agent affected *Phalaenopsis amabilis*, leading to wilting symptoms and changes in leaf coloration. This finding is consistent with previous studies on *P. amabilis* [24], Cavendish banana [25], *Dendrobium* orchids [26] and cassava [27], all of which reported drought stress conditions caused by reduced water potential in the media treated with PEG 6000.

Leaf discoloration to brown indicates that the plant could not tolerate high PEG 6000 concentrations. This phenomenon is associated with the oxidation of phenolic compounds by the enzyme polyphenol oxidase in damaged tissues. Phenolic compounds containing aromatic benzene rings are oxidized into quinone compounds, which are characterized by the appearance of brown pigmentation in plant tissues [28].

3.3. Stomatal Density

The stomatal density of *Phalaenopsis amabilis* under different PEG 6000 concentrations is presented in **Table 3.**

Table 3 Stomatal Density

PEG 6000 concentration (%)	Stomatal Density (mm^2) ẏ ± SE
0	185 ± 74.1 ^{a}
10	300 ± 39.5 ^{b}
20	400 ± 61.2 ^{bc}
30	435 ± 60.2 ^{c}
40	580 ± 48.0 ^{d}

Note: ẏ = Average, SE = Standard Error; Values followed by the same letter are not significantly different at the 5% level BNJ (0. 05) = 0.05

Based on **Table 3**. the highest stomatal density of *Phalaenopsis amabilis* was observed in plants treated with 40% PEG 6000, reaching 580 mm^2, followed by 30% PEG 6000 at 435 mm^2, while the lowest density was recorded in the control (0% PEG 6000) at 185 mm^2. These results indicate that the application of PEG 6000 had a significant effect on stomatal density in *P. amabilis*. This variation may be influenced by the young developmental stage of the plants, which were approximately 30 days after planting (DAP) and still in the early growth phase. At this stage, plant organs including leaves are still developing, and stomatal structures are not yet fully mature. This observation aligns with the findings [29], reported that stomatal development progresses in parallel with leaf growth and organ maturation. Consequently, younger tissues tend to exhibit lower stomatal density compared to mature leaves.

The results showed that stomatal density increased in line with the higher concentrations of PEG 6000 applied. This condition indicates that the application of PEG 6000 as a drought-inducing agent can influence the plant's physiological responses, particularly in stomatal development. Moreover, the increase in stomatal density may also be affected by environmental factors such as light intensity. Higher light intensity tends to promote the formation of a greater number of stomata as an adaptive mechanism to optimize photosynthesis and gas exchange [30]. The microscopic observation of the abaxial leaf surface of *P. amabilis* under different PEG 6000 concentrations is presented in **Figure 2**.

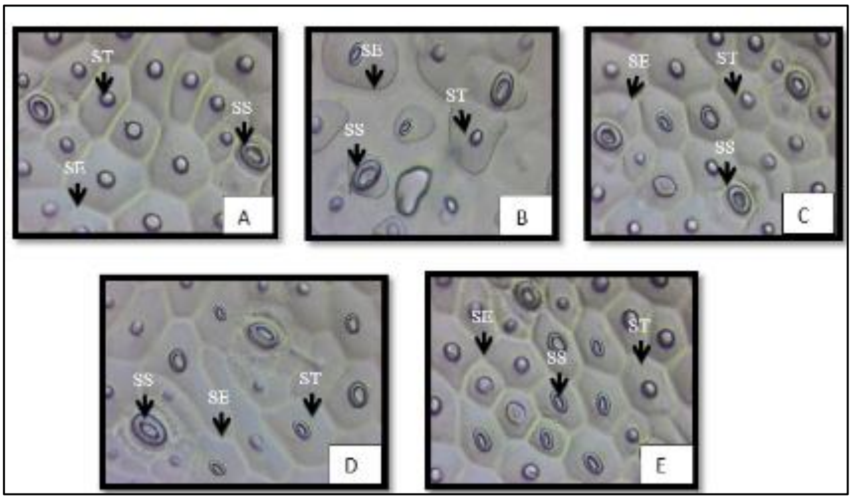

Figure 2 Abaxial leaf surface of *P. amabilis* at 40X magnification under PEG 6000 treatment (A) = 0% , (B) = 10%, (C) = 20%, (D) = 30% and (E) = 40%

Based on **Figure 2**, it can be observed that the distance between stomata in plants treated with 40% PEG 6000 is closer, resulting in higher stomatal density compared to plants treated with 0% PEG 6000. This indicates that increasing PEG 6000 concentration tends to enhance stomatal density on the leaf surface. This finding aligns with previous reports stating that the number of stomata is directly proportional to stomatal density, meaning that a greater number of stomata per unit area corresponds to higher stomatal density [31].

Furthermore, it has been reported that stomatal size plays a significant role in determining overall stomatal density within a given microscopic field. When stomatal size is relatively small, the distance between stomata becomes shorter, leading to a greater number of stomata observed per field of view and, consequently, a higher stomatal density. Conversely, larger stomata result in wider spacing and fewer visible stomata within the same area, which ultimately decreases the measured stomatal density [32].

4. Conclusion

The results of the study showed that the tolerant concentration of PEG 6000 for the growth of *Phalaenopsis amabilis* under drought stress was 40%, as indicated by an increase in stomatal density. The expression characteristics of *P. amabilis* under drought stress induced by PEG 6000 also demonstrated that stomatal density increased with higher PEG 6000 concentrations. In addition, the percentage of surviving plants decreased as the PEG 6000 concentration increased.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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