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Analysis of moon orchid response to osmotic stress through measurement of chlorophyll content and tolerance index

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Abstract

Orchidaceae is a group of flowering plants with a very high level of diversity, comprising more than 28,000 species spread across the globe. One of the most well-known species is the moon orchid (*Phalaenopsis amabilis*). In cultivation practices, drought is a limiting factor that can inhibit plant growth. One strategy considered effective in overcoming this condition is the use of varieties that are tolerant to drought stress. This study aims to: (1) identify the concentration of PEG 6000 that still supports the growth of P. amabilis in vivo under drought stress, and (2) characterize the response of plants treated with PEG 6000, which includes the stress tolerance index and the content of chlorophyll a, chlorophyll b, and total chlorophyll. Data were analyzed using ANOVA and followed by the BNJ test at a 5% level. The results showed that a PEG 6000 concentration of 40% was still tolerable by P. amabilis. Increasing PEG 6000 concentration tended to decrease chlorophyll a, chlorophyll b, and total chlorophyll content, while the tolerance index at 40% treatment showed that the plants were still able to survive drought stress.

Keywords: Moon orchid; Drought; PEG 6000; Stress Tolerance Index; Chlorophyll

1. Introduction

The moon orchid (*Phalaenopsis amabilis* [L.] Blume) is one of the most popular monopodial epiphytic orchids in the ornamental plant industry due to its long-lasting beautiful flowers, distinctive aroma, and wide variety of colors. Additionally, the moon orchid has been designated as Indonesia's national flower. The genus Phalaenopsis encompasses around 60 species and 140 varieties, which are widely distributed in various countries such as Malaysia, the Philippines, Indonesia, Papua, and Australia. Topographically, moon orchids can grow in lowland areas up to an altitude of 600 meters above sea level. As epiphytic plants, moon orchids grow by attaching themselves to hosts and obtaining moisture and nutrients from their surroundings (1,2). Based on data from the Central Statistics Agency (3), national orchid production was recorded at 11.68 million units in 2020, decreasing to 11.35 million units in 2021, and then experiencing a significant decline to 6.79 million units in 2022. According to Latifah *et al.* (4), the main factors causing low orchid production in Indonesia are the limited availability of quality seedlings, inefficient cultivation systems, and suboptimal post-harvest handling. Drought stress is one of the limiting factors in orchid cultivation outside their natural habitat. This condition affects almost all phases of plant growth, from germination, shoot and root elongation, to the flowering process (5). According to Sujinah and Ali (6), drought stress occurs when the water content in the soil is at a minimum level that is insufficient to support optimal plant growth and production. The negative effects of drought stress on plants include changes in morphological, physiological, and biochemical aspects. One approach that is considered effective and efficient in overcoming drought stress in plants is through the use of varieties that are resistant to drought conditions (7). To test plant tolerance to these stresses, modifications can be made to the environment to minimize the impact of

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drought, improvements can be made to plant genotypes to make them more adaptive to drought, or stress simulations can be applied using Polyethylene Glycol (PEG) compounds. Garri *et al*. (8) reported that the application of PEG 6000 on green mustard plants had a negative effect on leaf length and width, dry weight, and chlorophyll a, chlorophyll b, and total chlorophyll content. Several studies also show that the use of PEG can help orchids adapt to stressed environments. Feriza *et al*. (9) reported that the administration of PEG 6000 in certain concentrations in vitro on *Dendrobium* Sp. orchids had an effect on chlorophyll content and stomatal index. The effectiveness of PEG 6000 in simulating drought conditions has also been proven in previous studies on 55 tangerines (10), bean planlets (*Phaseolus vulgaris* L.) (11), and long bean planlets (*Vigna unguiculata* (L.) Walp) (12). Based on these results, further research on moon orchids needs to be conducted in a relevant environment, namely in vivo in a greenhouse.

2. Material and methods

2.1. Tools and Materials

The tools and materials used in this study were moon orchids (*Phalaenopsis amabilis*), kadaka media, distilled water, 70% alcohol, polyethylene glycol (PEG), 96% alcohol, plastic cups, spray bottles, Ohaus analytical scales, 250 mL culture bottles, 100 mL measuring cups, 1 L beakers, aluminum foil, mortars and pestles, test tubes, test tube racks, filter paper, tissues, label paper, funnels, Shimadz UV spectrophotometers, and cameras. This study used a completely randomized design (CRD) with one factor, namely the addition of PEG 6000, which consisted of five concentration levels: 0%, 10%, 20%, 30%, and 40%. Each treatment was repeated five times.

2.2. Preparing the Planting Medium and Planting Moon Orchids (*Phalaenopsis amabilis*)

The growing medium used was kadaka, which was then placed into 25 plastic cups weighing 1/4 kg each and arranged in each plot. The *P. amabilis* seedlings were then planted in the 25 plastic cups provided.

2.3. Preparation of PEG 6000 Solution

PEG 6000 was prepared by dissolving PEG 6000 in distilled water at concentrations of 0%, 10%, 20%, 30%, and 40%. At a concentration of 10%, 10 grams of PEG was dissolved in 100 ml of distilled water. at a concentration of 20%, 20 grams of PEG is dissolved in 100 ml of distilled water, at a concentration of 30%, 30 grams of PEG is dissolved in 100 ml of distilled water, and at a concentration of 40%, 40 grams of PEG is dissolved in 100 ml of distilled water.

2.4. Stress Tolerance Index (STI)

The value of the stress tolerance index can be calculated using the following formula (13).

$$\text{STI} = \frac{\text{Ypi x Ysi}}{(\text{Yp})2} \text{x } 100\%$$

Explanation :
STI : Stress tolerance index
Ypi : Wet weight of plants under normal conditions
Ysi : Wet weight of plants under stress conditions
Yp : Average wet weight of all plants under optimal conditions

The criteria for determining the level of plant tolerance to drought stress are as follows.
- If the STI value is 0.5, the plant is sensitive to drought stress.
- If 1.0 ≤ STI > 0.75, the plant is moderately tolerant to drought stress.
- If STI > 1.0, the plant is tolerant to drought stress.

2.5. Chlorophyll Content

The material used for chlorophyll analysis was moon orchid leaves that had been selected with PEG 6000 using the Miazek method (14) with a spectrophotometer. A total of 0.1 grams of uniform moon orchid leaves were ground with a mortar and 10 ml of 96% ethanol was added. The solution was filtered with Whatman No. 1 paper and placed in a flask, which was then tightly closed. One milliliter of the sample solution and standard solution (96% ethanol) were taken and placed in a cuvette. After that, the absorbance was read with a UV spectrophotometer at wavelengths of 664 nm and 648 nm with three repetitions for each sample. The chlorophyll content was calculated using the Wintersmans and De Mots formula:

Chla = 13.36 A664 – 5.19 A648
Chlb = 27.43 A648 – 8.12 A664
Chltotal = 22.24 A648 + 5.24 A664

Explanation :
Chla = Chlorophyll a
Chlb = Chlorophyll b
Chltotal = Total Chlorophyll
A664 = Absorbance at a wavelength of 664 nm
A648 = Absorbance at a wavelength of 648 nm

3. Results

3.1. Stress Tolerance Index (STI)

The stress tolerance index (STI) is used to determine which concentration of PEG 6000 is tolerable for moon orchids under drought stress. Plants that meet the tolerance criteria indicate that they are able to survive even under stressful conditions. The results of the drought tolerance index of moon orchids with the addition of PEG 6000 at different concentrations are presented in **Table 1** and **Figure 1**.

Table 1 Stress Tolerance Index of Moon Orchids

PEG 6000 concentration (%)	Stress tolerance index (%) (ẏ± SE)	Criteria
10	0.503 ± 0.063[a]	Sensitive to stress
20	0.680 ± 0.001[b]	Sensitive to stress
30	0.788 ± 0.031[b]	Moderately Tolerant
40	1.015 ± 0.032[c]	Tolerant

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

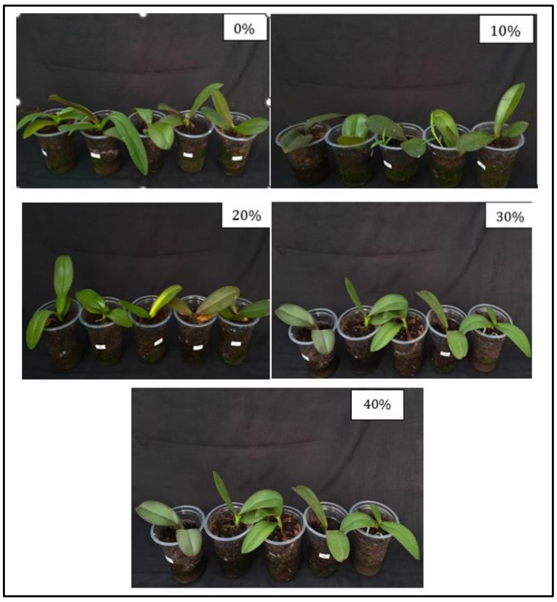

Figure 1 *Phalaenopsis Amabilis* [L.] Blume treated with PEG 6000 at several concentrations, namely 0%, 10%, 20%, 30%, and 40%

3.2. Chlorophyll Content

The chlorophyll content of *P. amabilis* selected with PEG 6000 at various concentrations is presented in **Table 2.**

Tabel 2 Average chlorophyll content of *P. amabilis* plants at several concentrations of PEG 6000

PEG 6000 concentration (%)	Average Chlorophyll a (mg/g) (ẏ ± SE)	Average Chlorophyll b (mg/g) (ẏ ± SE)	Average Total Chlorophyll (mg/g) (ẏ ± SE)
0	6.065 ± 0.064[a]	7.145 ± 0.119[a]	13.211 ± 0.173[a]
10	5.411 ± 0.058[b]	6.145 ± 0.117[b]	11.689 ± 0.156[b]
20	4.740 ± 0.063c	5.368 ± 0.245[c]	10.088 ± 0.308[c]
30	3.406 ± 0.053[d]	2.897 ± 0.101[d]	6.303± 0.155[d]
40	2.451 ± 0.026[e]	2.268 ± 0.043[e]	4.715± 0.065[e]

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

4. Discussion

Based on **Table 1**, the stress tolerance index (STI) in moon orchids shows that PEG 6000 treatment at a concentration of 10% produced an STI value of 0.503%, placing the plants in the sensitive category. At a concentration of 20%, the STI value of 0.680% was also still in the sensitive category. Furthermore, at a concentration of 30%, the ITC value increased to 0.788% and was classified as a plant with a moderate level of tolerance to drought stress. Meanwhile, at a concentration of 40%, an ITC value of 1.015% was obtained, indicating that the plant was in the category of tolerant to drought stress.

The results of this study indicate that moon orchids respond differently to drought stress levels, which are influenced by the concentration of PEG 6000, thereby limiting the volume of water that can be absorbed by the plants. At a concentration of 40%, the water absorbed by the plant was much lower than in the 10%, 20%, and 30% treatments, so *P. amabilis* had to optimize the use of available water. The higher the ITC value produced, the higher the level of tolerance of the moon orchid to drought stress conditions.

Research conducted by Soni et al. (15) states that plants respond to drought stress by accumulating soluble substances to prevent excessive water loss and protect cells from damage. The accumulation of proline is associated with a plant's tolerance to drought; tolerant plants accumulate more proline than plants that are sensitive or susceptible to drought stress (16). This is in line with the research by Mudhor *et al.* (17), which found that drought stress tolerance has a positive correlation with proline accumulation.

Plants experiencing water deficit exhibit root growth that becomes longer, thereby maximizing nutrient absorption to meet the plant's nutritional needs (18). In addition, the plant's tolerance response to drought stress can also be seen from the higher stomatal density, whereas sensitive plants have lower stomatal density (19).

Drought occurs when the water supply to plant roots is limited, while salinity occurs when the soil has a high salt concentration, inhibiting water absorption by plants. Nitrogen deficiency can increase plant susceptibility to salinity stress, while salinity stress can inhibit nitrogen absorption by plants. These conditions result in hyperosmotic pressure and physiological drought in plants, which interfere with their ability to absorb water optimally. In line with this, in selecting hybrid rice genotypes that are tolerant to drought stress in the seedling phase with a 25% concentration of PEG 6000 treatment (20).

Based on **Table 2**, the results of the analysis at a 5% significance level show that the addition of PEG 6000 to the kadaka medium at various concentrations had a significant effect on the chlorophyll a, chlorophyll b, and total chlorophyll content of *P. amabilis.* Based on the BNJ test at a 5% significance level, the chlorophyll content in *P. amabilis* decreased as the concentration of PEG 6000 administered increased. The chlorophyll content in orchids with a concentration of 0% (control) had the highest value compared to other concentrations (10%, 20%, 30%, and 40%). Thus, the difference in total chlorophyll content between the control and all other treatments was significant.

PEG 6000 added to the medium can bind water because it is polar and causes a decrease in water potential. Limited water availability in the orchid growth medium can cause drought stress. Drought stress is a limiting factor in growth because it can cause stomata to close. Stomata close to reduce water evaporation by plants. Water deficiency can result in a decrease in chlorophyll. This is caused by the inhibition of chlorophyll formation, a decrease in the rubisco enzyme, and the inhibition of the absorption of nutrients such as nitrogen and magnesium, which play an important role in chlorophyll synthesis. Water deficiency due to the addition of PEG to the medium will inhibit chlorophyll synthesis in the leaves, thereby also decreasing the rate of photosynthesis (21).

The addition of PEG 6000 to the growing medium at a concentration of 40% significantly reduced the content of chlorophyll a, chlorophyll b, and total chlorophyll in moon orchids. This is because chlorophyll biosynthesis is closely related to photosynthesis, which is sensitive to water deficiency, and chlorophyll content is one of the indicators of plant tolerance to drought stress (22). The results of this study are in line with the research by Nurcahyani *et al*. (7) on moon orchid (*Phalaenopsis amabilis*) shoots, which showed that the higher the addition of PEG concentration, the lower the chlorophyll content in plants. In addition, Putri *et al*. (24) conducted research on chlorophyll as an indicator of the level of drought tolerance in rice seedlings. From this study, a decrease in chlorophyll content was found. Dalal and Tripathy (25) stated that drought stress during the germination process can cause a decrease in chlorophyll content. Chlorophyll enables plants to obtain energy from light and plays an important role in the process of photosynthesis (26).

Media with low water content can inhibit the rate of photosynthesis, resulting in a decrease in chlorophyll synthesis. Induction of PEG 6000 at increasingly higher concentrations causes water stress in the medium, significantly reducing chlorophyll content (27). These results are supported by research conducted by Sarasmi *et al.* (28), who found that the decrease in total chlorophyll content in the leaves of Situ Bagendit variety upland rice was caused by a decrease in water content due to high concentrations of PEG 6000. This is because water is an important reagent for the continuity of photosynthesis and hydrolysis reactions in plants.

5. Conclusion

Based on the results of the study, the concentration of PEG 6000 that is still tolerable for *P. amabilis* for optimal growth is 40%. An increase in the concentration of PEG 6000 causes a decrease in the content of chlorophyll a, chlorophyll b, and total chlorophyll in plants. The stress tolerance index shows that PEG 6000 treatments at concentrations of 10% and 20% are sensitive to drought stress, 30% is moderate, while 40% is categorized as tolerant to drought stress.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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