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(Research Article)

Response of Vanilla (*Vanilla planifolia* Andrews) to Osmotic Stress Induced by PEG 6000 Treatment

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Abstract

Vanilla (*Vanilla planifolia* Andrews) is a member of the Orchidaceae family with high economic value, yet it is highly sensitive to drought, which significantly hampers its cultivation. To simulate drought stress under controlled conditions, high-molecular-weight osmotic agents such as Polyethylene Glycol (PEG) are widely used. This study aimed to determine the maximum tolerable concentration of PEG 6000 for vanilla plants and to analyze their physiological responses, including chlorophyll a, b, and total content; stomatal index; total soluble carbohydrate content; reducing sugar levels; and stress tolerance index. The experiment was designed using a Completely Randomized Design (CRD) with five PEG 6000 concentrations: 0% (A1), 10% (A2), 20% (A3), 30% (A4), and 40% (A5). Data were analyzed using one-way ANOVA at a 5% significance level, followed by Tukey's post hoc test. Results indicated that PEG 6000 concentrations up to 40% are still applicable for in vivo selection of vanilla plants. At this concentration, plants demonstrated drought tolerance as indicated by the stomatal index and stress tolerance index (STI). Increasing PEG 6000 concentrations reduced chlorophyll content (a, b, and total) while enhancing total soluble carbohydrate content in vanilla plants.

Keywords: *Vanilla planifolia*; Osmotic Stress; PEG 6000; Drought Tolerance; Physiological Response

1. Introduction

Vanilla (*Vanilla planifolia* Andrews) is one of the high-value plantation commodities with a strategic role in Indonesia's economy. In addition to being a leading export commodity, vanilla also serves as a primary source of income for farmers in various regions. However, vanilla cultivation faces significant challenges, particularly due to the plant's low tolerance to drought conditions. Vanilla requires a high-humidity environment to support optimal growth; thus, prolonged periods of drought can induce abiotic stress that negatively affects both vegetative growth and plant productivity.

One of the efficient, effective, and environmentally friendly approaches to mitigating drought stress is the use of cultivars that are tolerant to such conditions [1]. The development of superior varieties that are adaptive to drought stress is a crucial strategy in addressing the increasingly unpredictable impacts of climate change. To support early selection of drought-tolerant plants, artificial stress induction under in vitro conditions is commonly applied using high-molecular-weight osmotic agents such as Polyethylene Glycol (PEG) [2–6].

Polyethylene Glycol (PEG) is a chemical compound composed of ethylene oxide units and possesses osmotic activity. This compound can lower the osmotic potential of the medium by binding water molecules through hydrogen bonding,

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thereby creating simulated drought conditions for plants [7–9]. The application of PEG in in vitro culture media aims to reduce water availability, inducing osmotic stress conditions that mimic field drought. PEG 6000 has been widely used in drought tolerance screening studies across various plant species. Examples include rice with PEG concentrations of 5–25% [10]; Batu 55 mandarin orange with a combination of 1 mL/L atonik and 3% PEG [11]; Pontianak Siam orange with 3 mL/L atonik and 4% PEG [12]; Dendrobium orchids with 0–20% PEG [13]; Cattleya orchids with 0–20% PEG [14]; Phalaenopsis orchids with 3 mL/L atonik and 10% PEG [15]; Dendrobium with 2 mL/L atonik and 25% PEG [16]; Cattleya with 3 mL/L atonik and 20% PEG [17]; common beans with 0%, 15%, and 30% PEG [18]; yardlong beans with the same concentrations [19]; cassava with 0–40% PEG [20,21]; and Cavendish banana with 0–20% PEG [22].

Polyethylene Glycol (PEG) is a chemical compound composed of ethylene oxide units and exhibits active osmotic properties. It reduces the osmotic potential of the medium by binding water molecules through hydrogen bonding, thereby simulating drought conditions in plants [7–9]. The application of PEG in in vitro culture media aims to decrease water availability, thus inducing osmotic stress conditions that mimic drought in field environments. PEG 6000 has been widely employed in drought tolerance screening studies across various plant species. Examples include rice with PEG concentrations ranging from 5% to 25% [10]; Batu 55 mandarin orange with a combination of 1 mL/L atonik and 3% PEG [11]; Pontianak Siam orange with 3 mL/L atonik and 4% PEG [12]; Dendrobium orchids with 0–20% PEG [13]; Cattleya orchids with 0–20% PEG [14]; Phalaenopsis orchids with 3 mL/L atonik and 10% PEG [15]; Dendrobium with 2 mL/L atonik and 25% PEG [16]; Cattleya with 3 mL/L atonik and 20% PEG [17]; common beans with 0%, 15%, and 30% PEG [18]; yardlong beans with the same concentrations [19]; cassava with 0–40% PEG [20,21]; and Cavendish banana with 0–20% PEG [22].

Previous studies have explored the in vitro response of vanilla plants to osmotic stress induced by polyethylene glycol (PEG). A study by [23] reported that increasing PEG concentrations led to a reduction in growth, chlorophyll content, soluble protein levels, stomatal index, and the percentage of open stomata in vanilla. Conversely, the levels of dry matter, proline, and glycine betaine increased with higher PEG concentrations, indicating the significant role of these osmolytes in drought tolerance mechanisms. Furthermore, research by [24] compared the morphological and physico-chemical responses of *V. planifolia* and a *V. pompona* hybrid to PEG-induced water stress. The results showed that the hybrid exhibited smaller reductions in morphophysiological responses compared to *V. planifolia*, along with increased accumulation of metabolites such as carbohydrates, amino acids, purines, phenols, and organic acids, all of which contributed to enhanced drought stress tolerance.

Although studies using PEG have provided insights into the physiological responses of vanilla to drought, a deeper understanding of physiological parameters—such as chlorophyll a, b, and total content; stomatal index; total soluble carbohydrate content; reducing sugars; and stress tolerance index—can serve as important indicators in the selection of vanilla varieties more adaptive to drought conditions. This study aims to characterize vanilla plants treated with PEG 6000 based on these physiological parameters as determinants of drought tolerance. Through this approach, it is expected that superior mutant vanilla varieties tolerant to drought stress can be developed, thereby enhancing the competitiveness of Indonesia's vanilla commodity in the global market.

2. Material and methods

2.1. Materials and Equipment

The materials and equipment used in this study included: a microscope, spectrophotometer, test tubes, test tube rack, analytical balance, measuring glass, stirring rod, polybags, water bath, and 4-month-old vanilla plants. The chemicals used were 96% ethanol, distilled water, Polyethylene Glycol (PEG) 6000, organic fertilizer, tissue paper, filter paper, soil, sucrose, H_2SO_4, phenol, and glucose.

2.2. Experimental Design

This study was conducted at the Greenhouse and Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. A Completely Randomized Design (CRD) was employed with five levels of PEG 6000 concentration: 0% (A1), 10% (A2), 20% (A3), 30% (A4), and 40% (A5). Each treatment was replicated five times, with one vanilla plant (*Vanilla planifolia*) per replicate, resulting in a total of 25 experimental units.

Four-month-old vanilla seedlings were planted in polybags containing a mixture of soil and manure fertilizer at a 1:1 ratio, then placed separately in plots within a 3 m × 3 m shade house. PEG treatments were applied to simulate drought stress conditions. The observed parameters included chlorophyll a, b, and total content; stomatal index; total soluble carbohydrate content; reducing sugars; and stress tolerance index.

Chlorophyll content was analyzed using spectrophotometric methods, stomatal index was observed microscopically, while total soluble carbohydrate and reducing sugar contents were analyzed using the phenol-sulfuric acid and DNS (3,5-dinitrosalicylic acid) chemical methods, respectively. The stress tolerance index was calculated based on the ratio of growth in treated plants to control plants.

The observational data were analyzed using analysis of variance (ANOVA). If significant differences were found, a further test (Tukey's test) was conducted at a 5% significance level.

2.3. Preparation of Growing Medium

The plot was cleared of weeds and leveled to a size of 3 m × 3 m. A shade structure was constructed over the plot using paranet attached to a bamboo frame. A total of 25 plastic polybags were prepared and filled with a mixture of soil and organic fertilizer at a 1:1 ratio. After filling, the polybags were placed in the prepared plots.

2.4. Preparation of PEG Solution and Planting

PEG 6000 solutions were prepared at concentrations of 0%, 10%, 20%, 30%, and 40%, using distilled water as the solvent. The prepared solutions were filtered through a syringe filter to ensure purity. PEG solutions were applied by watering each polybag with 10 mL every 7 days for 4 weeks. Four-month-old vanilla plants were planted in polybags containing the soil and fertilizer mixture. Each treatment consisted of five replicates, with one vanilla plant per polybag in each replicate.

2.5. Stress Tolerance Index (STI)

2.5.1. The stress tolerance index was calculated using the formula developed by [25] as follows:

$$ITC = (Ypi \times Ysi)/Yp \times 100\%$$

where:

- STI : Stress tolerance index
- Ypi : Fresh weight of the plant under normal conditions (without stress)
- Ysi : Fresh weight of the plant under stress conditions (treated with PEG)
- Yp : Average fresh weight of all plants under optimum conditions

2.5.2. Criteria for Determining Plant Tolerance Levels to Drought Stress.

Based on the values of the Stress Tolerance Index (STI), plant tolerance levels to drought stress can be classified as follows:

- STI < 0,5 : Highly sensitive to drought stress
- 0,5 ≤ STI < 0,75 : Sensitive to drought stress
- 0,75 ≤ STI ≤ 1,0 : Moderately tolerant to drought stress
- STI > 1,0 : Tolerant to drought stress

2.6. Stomatal Index (SI)

2.6.1. Preparation of Leaf Impressions and Observation

The abaxial (lower) surface of the leaf was coated with transparent nail polish and allowed to dry. Once dry, the nail polish layer was carefully peeled off using adhesive tape to create a transparent impression of the leaf surface, which was then placed onto a glass slide. The preparation was observed under a microscope at 400× magnification.

The stomatal index was calculated using the following formula:

$$SI\ (\%) = \frac{\text{Number of Stomata}}{\text{Number of epidermal cells} + \text{Number of Stomata}} \times 100$$

2.7. Chlorophyll Content

Chlorophyll analysis was performed using the method described by [26] with a UV spectrophotometer. A volume of 1 mL of each sample solution and standard solution (80% acetone) was transferred into a cuvette. Absorbance was then measured at wavelengths of 646 nm and 663 nm using a UV spectrophotometer, with three replicates for each sample. Chlorophyll content was calculated using the following formulas:

- Total Chlorophyll = 17,30 × A646 + 7,18 × A663 mg/L
- Chlorophyll a = 12,21 × A663 – 2,81 × A646 mg/L
- Chlorophyll b = 20,13 × A646 – 5,03 × A663 mg/L

Note: A646 and A663 refer to the absorbance values at 646 nm dan 663 nm, respectively.

2.8. Soluble Carbohydrate Content

The analysis of total soluble carbohydrate content was carried out using the phenol-sulfuric acid method. A total of 0.1 grams of vanilla plant leaves was weighed, then ground using a mortar with the addition of 10 mL of distilled water. The resulting extract was filtered using Whatman No. 1 filter paper, and the filtrate was collected into a test tube. One milliliter of the filtrate was taken and mixed with 1 mL of H_2SO_4 solution and 2 mL of phenol solution. The mixture was transferred into a cuvette, and its absorbance was measured using a spectrophotometer at a wavelength of 490 nm.

To determine the carbohydrate concentration, glucose standard solutions with various concentrations were prepared and measured for absorbance at the same wavelength. The absorbance values of the standard solutions were used to construct a standard curve and determine the linear regression equation using the formula: **Y = ax + b**, where **Y** represents the absorbance value and **x** represents the glucose concentration.

2.9. Data Analysis

The research data were analyzed using a Completely Randomized Design (CRD). Quantitative data for each parameter were tested using Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). If the ANOVA results indicated significant differences among treatments, further analysis was conducted using Tukey's Honestly Significant Difference (HSD) test at the same significance level.

3. Results

3.1. Stress Tolerance Index (STI)

The Stress Tolerance Index (STI) is used to identify the level of plant tolerance to drought stress. In this study, the fresh weight of the plant was used as the basis for determining the STI value. The higher the STI value, the greater the plant's tolerance to drought conditions. The STI values of vanilla plants under various PEG 6000 concentrations are presented in Table 1.

Table 1 Stress Tolerance Index (STI) of Vanilla Plants at Various PEG 6000 Concentrations

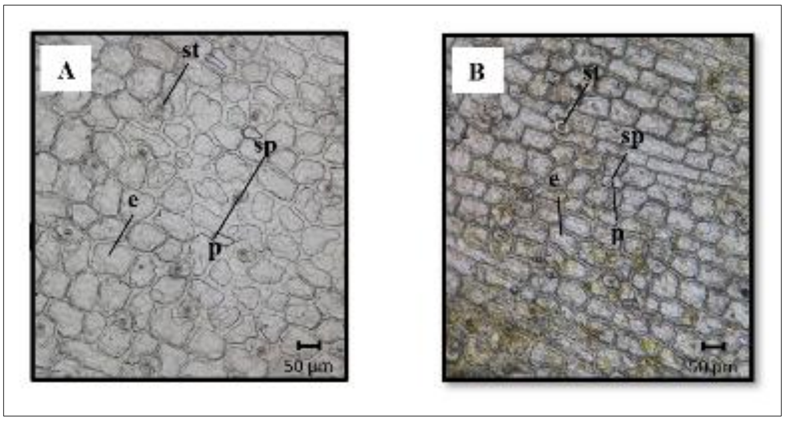
PEG 6000 Concentration (%)	Stress Tolerance Index (STI)
10	1,001 ± 0,016^{a}
20	1,001 ± 0,021^{a}
30	0,748 ± 0,062^{b}
40	0,254 ± 0,012^{c}

STI = Stress Tolerance Index, expressed as mean ± standard error (SE); Values followed by different letters (a, b, c) indicate significant differences at $\alpha = 0.05$ (Tukey test).

Based on Table 1, vanilla plants treated with 10% and 20% PEG 6000 showed STI values above 1.00, thus categorized as tolerant to drought stress. At a concentration of 30%, the STI value of 0.748 indicates a moderate tolerance category. Meanwhile, at 40%, the STI value of 0.254 indicates that the plants were highly sensitive to drought stress.

3.2. Stomatal Index (SI)

The observation results of the lower leaf surface of vanilla plants at 0% (control) and 40% PEG 6000 concentrations are shown in Figure 1.



Note: e = epidermis, st = stomata, p = pore, sp = guard cell.

Figure 1 Lower leaf surface of vanilla plant at 400× magnification, showing stomata (A) without PEG 6000 treatment and (B) after 40% PEG 6000 treatment

Based on Figure 1, it is evident that the stomatal index on vanilla leaves treated with PEG 6000 exhibits anatomical changes. In the control plants, the epidermal cells are larger with fewer stomata, whereas in plants treated with 40% PEG 6000, the epidermal cells are smaller. This difference indicates that the control plants are in normal conditions, while those exposed to 40% PEG experience drought stress as a morphological response to the stress. The stomatal index is calculated to measure the stomata's response to water deficit conditions by comparing the number of stomata and epidermal cells in a field of view, then converting it into a percentage. The results of stomatal index calculations in vanilla plants treated with PEG 6000 at concentrations of 0%, 10%, 20%, 30%, and 40% show varying stomatal index values.

The average stomatal index of vanilla plants treated with PEG 6000 is presented in Table 2.

Table 2 Stomatal Index of Vanilla Plants

PEG 6000 Concentration (%)	**Stomatal Index (%) $\bar{Y} \pm SE$**
0	10,580 ± 0,303[a]
10	14,018 ± 1,688[ab]
20	16,207 ± 1,589[ab]
30	20,463 ± 1,339[bc]
40	28,092 ± 3,154[c]

Note: Stomatal index is presented as mean ± standard error ($\bar{Y} \pm SE$); Values followed by different letters (a, b, c) indicate significant differences at $\alpha = 0.05$ (Tukey test).

The average stomatal index of vanilla plants treated with PEG 6000 is presented in Table 2. The stomatal index values increased with rising PEG concentrations, with the lowest value observed in the control (0%) at 10.58% and the highest at 40% concentration reaching 28.09%. The differences in stomatal index among treatments at several concentrations were statistically significant, as indicated by different letters following the mean values.

The increase in PEG 6000 concentration shows a linear relationship with the stomatal index values in vanilla plants. This relationship is illustrated in the regression curve between PEG 6000 concentration and stomatal index, as shown in Figure 2.

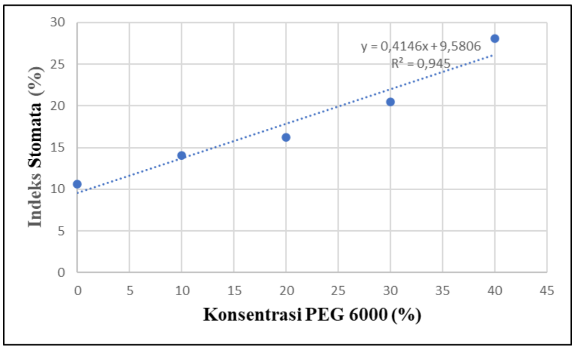


Figure 2 Regression curve showing the relationship between stomatal index of vanilla plants and PEG 6000 concentration

Based on Figure 2, the relationship between PEG 6000 concentration and the stomatal index of vanilla plants follows a positive linear regression pattern, with the regression equation *y = 0.4146x + 9.5806* and a coefficient of determination (R^2) of 0.945. This indicates that an increase in PEG 6000 concentration is strongly associated with an increase in stomatal index. The high R^2 value suggests that 94.5% of the variation in stomatal index can be explained by the variation in PEG 6000 concentration.

3.3. Chlorophyll Content

The analysis of chlorophyll content (a, b, and total) was conducted to assess the effect of various PEG 6000 concentrations on the photosynthesis of vanilla plants, considering that chlorophyll plays an important role in the plant's response to drought stress. Chlorophyll a content in vanilla plants treated with different concentrations of PEG 6000 is presented in Table 3.

Table 3 Chlorophyll a Content in Vanilla Plants at Various Concentrations of PEG 6000

PEG 6000 Concentration (%)	**Chlorophyll a Content (mg/g) Ȳ ± SE**
0	4,048 ± 0,077[a]
10	3,641 ± 0,212[ab]
20	3,049 ± 0,219 [ab]
30	3,007 ± 0,079[b]
40	1,882 ± 0,107[c]

Notes: Chlorophyll a content = Ȳ ± SE; Ȳ = Mean value of chlorophyll a content; SE = Standard error; Values followed by different letters (a, b, c) indicate significant differences at α = 0.05 (Tukey test).

Based on Table 3, the application of PEG 6000 at various concentrations had a significant effect on chlorophyll a content in vanilla plants (Tukey's test, $p < 0.05$). Chlorophyll a content tended to decrease as the concentration of PEG 6000 increased. The control (0%) showed the highest value. The decrease at 10% and 20% concentrations was not significantly different from the control, nor was there a significant difference among the 10%, 20%, and 30% concentrations. However, at 40% PEG 6000 concentration, chlorophyll a content significantly decreased compared to the control.

Chlorophyll b content in vanilla plants treated with various concentrations of PEG 6000 in soil media is presented in Table 4.

Table 4 Chlorophyll b Content of Vanilla Plants at Various PEG 6000 Concentrations

PEG 6000 Concentration (%)	Chlorophyll b Content (mg/g) Ȳ ± SE
0	5,352 ± 0,236[a]
10	5,215 ± 0,311[a]
20	4,788 ± 0,383 [a]
30	4,567 ± 0,335[a]
40	1,891 ± 0,595[b]

Note: Chlorophyll b content = Ȳ ± SE; Ȳ = Mean chlorophyll b content of vanilla plants; SE = Standard error; Values followed by different letters (a, b) indicate significant differences at α = 0.05 (Tukey test).

Based on Table 4, chlorophyll b content decreases as the concentration of PEG 6000 increases. The 0% concentration (control) shows the highest value. The decrease at concentrations of 10%, 20%, and 30% is not significantly different from the control; however, at 40% concentration, there is a significant reduction compared to the control.

The total chlorophyll content of vanilla plants treated with various concentrations of PEG 6000 in soil media is presented in Table 5.

Tabel 5 Total Chlorophyll Content of Vanilla Plants at Various PEG 6000 Concentrations

PEG 6000 Concentration (%)	Total Chlorophyll Content (mg/g) Ȳ ± SE
0	9,339 ± 0,204[a]
10	8,856 ± 0,162[ab]
20	8,198 ± 0,194[bc]
30	7,574 ± 0,337[c]
40	3,772 ± 0,165[d]

Note: Total chlorophyll content = Ȳ ± SE; Ȳ = Mean value of total chlorophyll content in vanilla plants; SE = Standard error; Values followed by different letters (a, b, c) indicate significant differences at α = 0.05 (Tukey test).

The analysis at the 5% significance level showed that the application of PEG 6000 at various concentrations had a significant effect on the total chlorophyll content of vanilla plants. Based on the Duncan's Multiple Range Test (DMRT), the total chlorophyll content decreased with increasing PEG 6000 concentration. The 0% concentration (control) had the highest value. The decreases at 10%, 20%, and 30% concentrations were not significantly different from the control, but at 40% concentration, there was a significant decrease compared to the control.

3.4. Carbohydrate Content

Total soluble carbohydrate content was used as a parameter to assess the drought tolerance of vanilla plants subjected to PEG 6000 treatments at various concentrations in soil media. The total soluble carbohydrate content increased with increasing concentrations of PEG 6000. The analysis results are presented in Table 6.

Table 6 Total Soluble Carbohydrate Content of Vanilla Plants at Various PEG 6000 Concentrations

PEG 6000 Concentration (%)	Total Soluble Carbohydrate Content (mg/g) Ȳ ± SE
0	40,775 ± 3,588[a]
10	42,975 ± 3,308[a]
20	65,025 ± 2,281[a]
30	69,675 ± 7,717[a]
40	142,325 ± 13,011[b]

Note: Total soluble carbohydrate content = Ȳ ± SE; Ȳ = Mean total soluble carbohydrate content of vanilla plants; SE = Standard error; Values followed by different letters (a, b, c) indicate significant differences at α = 0.05 (Tukey test).

Based on Table 6, the total soluble carbohydrate content of vanilla plants significantly increased at 40% PEG 6000 concentration compared to the control (0%). Increases at concentrations of 10%, 20%, and 30% were not significantly different from the control. The increase in PEG 6000 concentration in the soil medium showed a linear correlation with the increase in total soluble carbohydrate content in vanilla plants.

4. Discussion

Water is very important for plant metabolism [27]. Water deficiency causes physiological and biochemical changes in vanilla. To cope with drought stress, plants respond physiologically, morphologically, and biochemically, including a decrease in chlorophyll and an increase in soluble carbohydrates [28]. Measuring chlorophyll content is important to assess the impact of water deficit on growth and yield, as it is related to the rate of photosynthesis. Morphologically, leaves change color to brownish due to stress [29].

Vanilla is a plant sensitive to drought and can die if the drought persists for a long time without water availability. Studies show that treatment with low concentrations of PEG 6000 (0%, 10%, 20%) keeps the plants green, whereas high concentrations (30%, 40%) cause browning on parts of the plant. This color change indicates disrupted metabolism due to drought stress, which can lead to wilting and death in sensitive plants. However, even at high concentrations, some plants survive, indicating tolerance to stress. This finding aligns with [30], which reported that drought-sensitive rice plants exhibited changes in leaf and stem color as well as leaf rolling.

Leaves play an important role in the survival of plants through stomata, which function as the “mouth” of the leaf for the exchange of gases O_2, CO_2, and water vapor with the environment [31]. Stomata are crucial in photosynthesis, respiration, and transpiration, and their number per mm^2 varies among plant species [32]. Stomatal density is influenced by the number of stomata and environmental factors such as light intensity, water availability, temperature, and CO_2 concentration. For example, stomatal density increases with higher light intensity [33]. Stomata regulate the movement of water in and out of leaves to prevent excessive water loss due to high transpiration rates [34]. Observation of the stomatal index can be used to analyze the response of vanilla plants to drought stress and to determine plant tolerance.

Research results show that the stomatal index of vanilla plants increases with increasing concentrations of PEG 6000, indicating a response to drought stress. An increased stomatal index is related to the function of stomata as the site of gas exchange between the atmosphere and the intercellular spaces of the mesophyll [32]. However, according to [35], a high stomatal index in drought-stressed rice causes increased transpiration, making the plants more susceptible to wilting. One plant adaptation to drought stress is stomatal closure to reduce transpiration. According to [36], plants with a high stomatal index are less drought tolerant, whereas a low stomatal index is an adaptive strategy to prevent excessive water loss.

The tolerance criteria of plants are determined based on the Drought Tolerance Index (DTI), where a higher DTI value indicates greater tolerance to drought stress. Based on Table 1, vanilla plants treated with 10% and 20% PEG 6000 concentrations are classified as tolerant, 30% as moderately tolerant, while 40% is categorized as sensitive to drought stress. The increase in PEG 6000 concentration causes a decrease in the DTI of vanilla plants because PEG lowers the water potential of the growth medium, thereby inhibiting plant growth.

The addition of 40% PEG 6000 concentration in the soil medium significantly reduces chlorophyll a, chlorophyll b, and total chlorophyll content in vanilla plants. Chlorophyll a and total chlorophyll are often used as indicators of drought stress, as reported by [37], who found a decrease in total chlorophyll in drought-stressed wheat. The reduction in chlorophyll impacts the decline in plant production and food reserves. A decrease in chlorophyll content was also reported by [38], who observed similar effects in rice with increasing concentrations of PEG 6000. This is caused by the influence of water deficiency on plant growth, which inhibits chlorophyll synthesis and the uptake of essential nutrients such as nitrogen and magnesium [39]. Water deficit stress can inhibit photosynthetic activity and the distribution of assimilates to reproductive organs [40]. Soluble carbohydrates play a role in osmotic regulation and correlate positively with proline. [41] reported the accumulation of low molecular weight carbohydrates, such as glucose, fructose, inositol, and sucrose, in the leaves of plants experiencing water stress. These carbohydrate contents help maintain plant survival under drought stress [42].

5. Conclusion

Based on the research results, it can be concluded that the tolerant concentration of PEG 6000 for in vivo selection of vanilla plants is up to 40%. The stomatal index of vanilla plants at 40% PEG 6000 concentration indicates tolerance to drought stress. The drought tolerance index (DTI) of vanilla plants is categorized into tolerant, moderately tolerant, and sensitive according to the PEG 6000 concentration. As the concentration of PEG 6000 increases, the contents of chlorophyll a, chlorophyll b, and total chlorophyll decrease, while the total soluble carbohydrate content increases.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest.

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