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Evaluation of Drought Tolerance in In Vitro potato (*Solanum tuberosum* L.) cv. Dayang Sumbi Plantlets Using Polyethylene Glycol (PEG) 6000

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Abstract

Potato (*Solanum tuberosum* L.) is an important food crop whose productivity tends to decline under drought stress conditions. Water deficit limits water uptake, reduces cell turgor pressure, and disrupts photosynthesis, resulting in inhibited plant growth. Development of drought-tolerant potato cultivars can be achieved through in vitro culture using polyethylene glycol (PEG) 6000 as a selective agent that lowers the water potential of the culture medium without causing direct damage to plant cells. This study aimed to evaluate the effect of PEG 6000 on the growth of in vitro potato plantlets of the Dayang Sumbi cultivar and to determine the concentration that could still be tolerated under drought stress conditions. The experiment was conducted at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lampung, using a Completely Randomized Design (CRD) with five PEG 6000 concentrations (0%, 5%, 10%, 15%, and 20%) and five replications. The observed parameters included plantlet survival percentage, plantlet height, number of leaves, and stomatal index. The data were analyzed using analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test at a 5% significance level. The results showed that all plantlets remained viable, with a 100% survival rate across all PEG 6000 concentrations during the two-week observation period. Significant differences were observed in plantlet height, leaf number, and stomatal index, all of which decreased progressively with increasing PEG 6000 concentration and reached their lowest values at 20% PEG 6000. A PEG 6000 concentration of 10% was found to support the growth tolerance of *Solanum tuberosum* L. cv. Dayang Sumbi plantlets under in vitro drought stress conditions.

Keywords: Potato; Drought Stress; In Vitro Culture; PEG 6000.

1. Introduction

Food crops are an important source of carbohydrates and proteins and serve as the primary source of energy for human consumption. Among these crops, potato (*Solanum tuberosum* L.) is widely cultivated and consumed due to its high nutritional value, including carbohydrates, proteins, amino acids, vitamins, and minerals [1]. According to [2], potato production in Indonesia has fluctuated over the years, with declines observed during certain periods and relatively low and unstable production in Lampung Province. These conditions indicate that improving potato productivity remains an important objective to support national food security.

Climate change has become one of the major challenges affecting agricultural production worldwide. Rising temperatures, irregular rainfall patterns, and prolonged drought periods have increased the occurrence of abiotic stresses that negatively affect crop growth and productivity, including that of potato plants. Drought stress limits plant growth, disrupts physiological processes, and inhibits tuber formation, ultimately reducing potato yield [3]. Potato is also recognized as a drought-sensitive crop and is therefore highly susceptible to yield losses under conditions of limited

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water availability [4]. Such impacts threaten not only agricultural production but also food security and farmers' livelihoods [5].

Development of drought-tolerant potato cultivars can be facilitated through in vitro culture techniques using polyethylene glycol (PEG) 6000 as an osmotic agent to simulate water-deficit conditions [6]. PEG 6000 is an inert and non-toxic compound that reduces the water potential of the culture medium, thereby restricting water uptake by plant tissues [7]. Previous studies have demonstrated that increasing PEG concentrations can reduce plantlet growth, chlorophyll content, and stomatal index as a consequence of disrupted physiological processes [7,9,10]. PEG 6000 has also been widely used as a selective agent for identifying and developing plant materials with improved adaptation to drought stress [11].

Plant responses to PEG-induced drought stress may vary among species and cultivars. Information regarding the response of *Solanum tuberosum* L. cv. Dayang Sumbi to PEG 6000 under in vitro conditions remains limited. This study was conducted to evaluate the effects of PEG 6000 on the growth of potato plantlets and to determine the level of drought stress tolerance of the Dayang Sumbi cultivar under in vitro conditions.

2. Materials and Methods

2.1. Materials and Experimental Design

The study was conducted from November 2025 to January 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 a single factor consisting of five concentrations of polyethylene glycol (PEG) 6000: 0%, 5%, 10%, 15%, and 20%. Each treatment was replicated five times, resulting in a total of 25 experimental units, with each culture bottle representing one experimental unit.

The materials used in this study included certified potato (*Solanum tuberosum* L.) cv. Dayang Sumbi plantlets obtained from Bogor, Murashige and Skoog (MS) basal medium, sucrose, agar, polyethylene glycol (PEG) 6000, 70% and 96% ethanol, distilled water, Whatman No. 1 filter paper, chloral hydrate, Bayclin solution, aluminum foil, and tissue paper. The equipment included a Laminar Air Flow Cabinet (LAFC), autoclave, analytical balance, microscope, camera, Petri dishes, culture bottles, graduated cylinders, Erlenmeyer flasks, beakers, funnels, glass stirring rods, culture racks, oven, microscope slides, cover slips, scalpels, forceps, labeling paper, and scissors.

2.2. Preparation of Culture Media and Planting of planlets

The PEG 6000 stock solution was prepared by dissolving 100 g of PEG 6000 in 100 mL of sterile distilled water until completely homogeneous. The solution was filtered through Whatman No. 1 filter paper and tightly covered with aluminum foil to maintain sterility.

The Murashige and Skoog (MS) basal medium was prepared by dissolving 4.43 g of MS powder in 1 L of distilled water, followed by the addition of 30 g L^{-1} sucrose. The solution was mixed thoroughly, and the pH was adjusted to 5.7 using a pH meter. Agar was then added at a concentration of 7 g L^{-1}, and the medium was heated until all components were completely dissolved. The prepared medium was divided into five treatments by adding PEG 6000 stock solution to obtain final concentrations of 0% (0 mL), 5% (10 mL), 10% (20 mL), 15% (30 mL), and 20% (40 mL).

Approximately 20 mL of each treatment medium was dispensed into individual culture bottles and sealed with heat-resistant plastic before sterilization in an autoclave at 121°C and 1 atm for 15 minutes. Sterilized media were incubated for seven days to confirm the absence of contamination. The explants consisted of potato (*Solanum tuberosum* L.) cv. Dayang Sumbi plantlets cultured on the respective treatment media. Planting was performed aseptically inside a Laminar Air Flow Cabinet (LAFC), with one plantlet placed in each culture bottle. Each bottle was tightly sealed, labeled according to its treatment, and incubated for growth observation throughout the experimental period.

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 Height

Plantlet height was measured at the end of the observation period, two weeks after PEG 6000 treatment. Measurements were taken using a ruler from the base of the root to the tip of the shoot.

2.3.3. Number of Leaves

The number of leaves was recorded at the end of the observation period by counting all fully expanded leaves produced by each plantlet in a culture bottle, following the method described by [13].

2.3.4. Indeks Stomata

The stomatal index was determined at the end of the culture period using leaf samples collected from two-week-old potato (*Solanum tuberosum* L.) plantlets. Leaf tissues from the abaxial (lower) surface were fixed in 70% ethanol for 5 minutes to remove the wax layer and terminate enzymatic activity. The samples were then immersed in a chloral hydrate solution prepared at a 5:2 ratio with sterile distilled water and heated for 5–10 minutes until the epidermal tissue became transparent. The cleared leaf samples were rinsed with sterile distilled water to remove any remaining solution and stained with 1% safranin for 1–3 minutes to enhance the visibility of epidermal cell structures. Excess stain was removed by rinsing the samples again with sterile distilled water. The stained specimens were mounted on microscope slides with the abaxial surface facing upward, supplemented with 10% glycerol, covered with a cover slip, and observed under a light microscope at 100× magnification (10 × 10). The stomatal index was calculated as the ratio of the number of stomata to the total number of stomata and epidermal cells within a single field of view and expressed as a percentage according to the method described in [14]:

$$\text{Stomatal Index} = \frac{\text{Number of stomata}}{\text{Number of stomata + Number of epidermal cells}} \times 100\%$$

2.4. Data Analysis

The collected data consisted of qualitative and quantitative observations. Qualitative data were presented descriptively and supported by photographic documentation. Quantitative data for each parameter were analyzed statistically using Analysis of Variance (ANOVA). Statistical significance was evaluated at the 5% probability level, and significant differences among treatments were further analyzed using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level.

3. Results and Discussion

3.1. Plantlet Survival Percentage

The survival percentage of potato (*Solanum tuberosum* L.) plantlets was recorded at one-week intervals over a two-week observation period under different concentrations of PEG 6000 (0%, 5%, 10%, 15%, and 20%). The survival percentages of Dayang Sumbi potato plantlets under each treatment are presented in **Table 2**.

Table 2 Survival Percentage of Potato (*Solanum tuberosum* L.) cv. Dayang Sumbi Plantlets Cultured under Different PEG 6000 Concentrations In Vitro.

PEG 6000 Concentration (%)	Plantlet Survival Percentage at Week -	
	I	II
0	100%	100%
5	100%	100%
10	100%	100%
15	100%	100%
20	100%	100%

Based on the observations presented in **Table 2**, the application of PEG 6000 at concentrations of 0%, 5%, 10%, 15%, and 20% to potato (*Solanum tuberosum* L.) cv. Dayang Sumbi plantlets for two weeks resulted in a 100% survival rate during both the first and second weeks of observation. These findings indicate that the plantlets were able to maintain their viability under drought stress conditions simulated by PEG 6000 within the tested concentration range.

The 100% survival rate observed across all treatments suggests that the plantlets were able to maintain their viability despite experiencing osmotic stress induced by PEG 6000. This response may be associated with the ability of the plantlets to undergo physiological adjustments under water-deficit conditions. The non-ionic nature of PEG 6000 allows it to reduce the water potential of the culture medium without causing direct toxic effects on plant tissues [15]. Plant survival under drought stress is also supported by the activation of physiological and biochemical adaptation mechanisms that enable plants to cope with water deficiency [16].

The present findings are consistent with those reported by [9], who demonstrated that potato plantlets remained viable at PEG 6000 concentrations of up to 20%, indicating a certain degree of adaptation to drought stress under in vitro conditions. Visual observations further showed that the plantlets retained green to greenish-brown coloration, indicating that they remained alive despite the occurrence of tissue discoloration in some treatments. Such discoloration may represent an early indicator of stress responses but does not necessarily indicate complete tissue death. Morphological changes, including leaf rolling and browning symptoms, were also observed following PEG 6000 treatment. Increased osmotic pressure in the culture medium simulates drought stress and may trigger browning through the oxidation of phenolic compounds into brown-colored quinones mediated by polyphenol oxidase activity in damaged tissues [17]. The appearance of potato plantlets cultured under different PEG 6000 concentrations is presented in **Figure 5.**

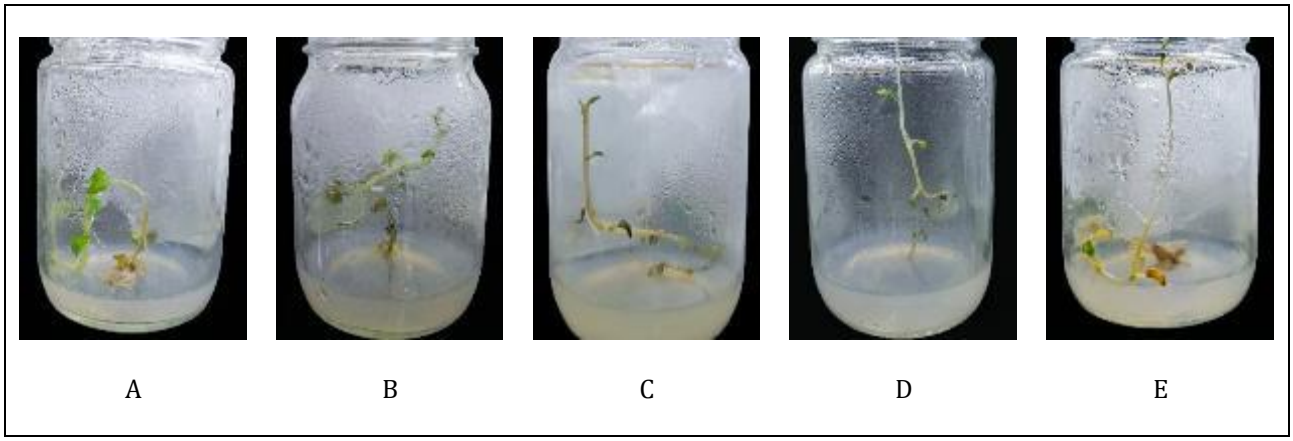

Figure 5 Potato (*Solanum tuberosum* L.) cv. Dayang Sumbi plantlets cultured under different PEG 6000 concentrations: (A) 0%, (B) 5%, (C) 10%, (D) 15%, and (E) 20%.

3.2. Plantlet Height

The mean height of potato plantlets grown under different PEG 6000 concentrations is presented in **Table 3.**

Table 3 Mean Height of Potato (*Solanum tuberosum* L.) Plantlets Cultured under Different PEG 6000 Concentrations In Vitro at Week 2.

PEG 6000 Concentration (%)	Plantlet Height (cm) $\bar{Y}$ ± SE
0	15.4 ± 0.27^{d}
5	11.8 ± 0.26^{c}
10	9.9 ± 0.41^{b}
15	8.0 ± 0.50^{a}
20	7.0 ± 0.22^{a}

Note: $\bar{Y}$: Mean plantlet height; SE: Standard Error

Table 3 shows that potato plantlets grown in the absence of PEG 6000 (0%) exhibited the greatest mean height, reaching 15.4 cm. Plantlet height gradually decreased with increasing PEG 6000 concentration, measuring 11.8 cm at 5%, 9.9 cm at 10%, 8.0 cm at 15%, and 7.0 cm at 20%. These results indicate that increasing PEG 6000 concentration significantly reduced the height of potato plantlets. Similar findings were reported by [8], who demonstrated that in vitro application of PEG 6000 inhibited plantlet growth, particularly at higher concentrations compared with the control treatment, indicating the occurrence of drought-induced stress that restricted plant development.

The reduction in plantlet height was likely associated with the ability of PEG 6000 to decrease the water potential of the culture medium, thereby limiting water availability to the plantlets. Restricted water availability reduces water uptake by plant tissues, resulting in cellular water deficiency and decreased turgor pressure. Reduced turgor pressure limits cell expansion and interferes with both cell division and cell elongation, which are essential processes for normal plant growth. Osmotic stress induced by PEG 6000 may also disrupt various physiological processes, leading to suboptimal growth and development. Increasing PEG 6000 concentrations therefore impose greater osmotic constraints on the plantlets, resulting in progressively reduced plant height [18,19].

3.3. Number of Leaves

The mean number of leaves of potato plantlets grown under different PEG 6000 concentrations is presented in **Table 4.**

Table 4 Mean Number of Leaves of Potato (*Solanum tuberosum* L.) Plantlets Cultured under Different PEG 6000 Concentrations In Vitro at Week 2.

PEG 6000 Concentration (%)	Number of Leaves $\bar{Y}$ ± SE
0	17.2 ± 0.58^{d}
5	14.6 ± 0.50^{c}
10	13.0 ± 0.70^{c}
15	9.2 ± 0.37^{b}
20	6.6 ± 0.74^{a}

Note: $\bar{Y}$: Mean plantlet height; SE: Standard Error

Table 4 shows that potato plantlets grown without PEG 6000 (0%) produced the highest mean number of leaves, reaching 17.2 leaves per plantlet. The number of leaves gradually decreased with increasing PEG 6000 concentration, reaching 14.6 leaves at 5%, 13.0 leaves at 10%, 9.2 leaves at 15%, and the lowest value of 6.6 leaves at 20%. These results indicate that increasing PEG 6000 concentration significantly reduced leaf production in potato plantlets. The present findings are consistent with those reported by [20], who demonstrated that increasing PEG 6000 concentrations reduced vegetative growth under in vitro conditions, including leaf formation, compared with the control treatment. The control treatment produced the highest number of leaves because the plantlets developed under normal conditions without osmotic stress. Plantlets exposed to PEG 6000 experienced reduced growth as a consequence of decreased water availability in the culture medium, which adversely affected their physiological activities. Similar observations were reported by [21], who stated that PEG 6000 negatively affects plantlet growth and development, resulting in a progressive decline in leaf production as the level of osmotic stress increases.

The reduction in leaf number is closely associated with physiological disturbances caused by drought stress. Leaf formation depends on active cell division and cell expansion, both of which require adequate water availability. Water deficit reduces cellular turgor pressure, thereby limiting cell enlargement and inhibiting the development of leaf tissues. [22] reported that PEG 6000, as a drought stress simulator, interferes with water uptake and reduces physiological activity in plants, resulting in a significant decline in vegetative growth, including leaf development. Reduced leaf production consequently slows the formation of new leaves compared with plants grown under non-stress conditions. A decline in leaf number may also represent an adaptive response to drought stress. Fewer leaves reduce the transpiration surface area, thereby limiting water loss and helping maintain water balance within plant tissues under unfavorable environmental conditions. Increasing PEG 6000 concentrations therefore impose greater osmotic stress on the plantlets, resulting in progressively stronger inhibition of vegetative growth, particularly leaf formation [20,21].

3.4. Stomatal Index

The mean stomatal index of potato plantlets grown under different PEG 6000 concentrations is presented in **Table 5.**

Table 5 Mean Stomatal Index of Potato (*Solanum tuberosum* L.) Plantlets Cultured under Different PEG 6000 Concentrations *In Vitro* at Week 2.

PEG 6000 Concentration (%)	Stomatal Index $\bar{Y}$ ± SE
0	28.9 ± 0.34^{d}
5	27.3 ± 0.17^{c}
10	25.4 ± 0.33^{b}
15	25.2 ± 0.26^{b}
20	20.2 ± 0.43^{a}

Note: $\bar{Y}$: Mean stomatal index; SE: Standard Error

Based on **Table 5**, potato plantlets grown without PEG 6000 (0%) exhibited the highest stomatal index, with a mean value of 28.9. The stomatal index gradually decreased with increasing PEG 6000 concentration, reaching 27.3 at 5%, 25.4 at 10%, 25.2 at 15%, and the lowest value of 20.2 at 20%. These results indicate that PEG 6000 significantly affected the stomatal index of potato plantlets. The present findings are consistent with those reported by [8], who demonstrated that increasing PEG 6000 concentrations tend to reduce the stomatal index under drought stress conditions. The observed reduction is associated with impaired leaf growth and development resulting from limited water availability. Variations in water availability also influence stomatal activity as part of the plant's adaptive response to environmental stress. Under water-deficit conditions, a lower stomatal index contributes to reducing water loss through transpiration, thereby enhancing plant survival under drought stress [23,24].

Drought stress induces a range of physiological adaptations that enable plants to maintain internal water balance, one of which involves the regulation of stomatal opening and closure. Stomata are microscopic pores located on the leaf surface that facilitate gas exchange and water vapor release through transpiration. Adequate water availability allows stomata to remain open, supporting photosynthesis and gaseous exchange. Limited water availability causes stomata to close in order to minimize excessive water loss. Stomatal movement is regulated by the turgor pressure of guard cells. Loss of water from guard cells reduces turgor pressure and results in stomatal closure, whereas water uptake restores turgor pressure and promotes stomatal opening. This mechanism represents an important adaptive strategy that reduces transpiration and helps maintain water balance within plant tissues during drought stress [24].

A reduction in stomatal index may also represent an adaptive mechanism that improves water use efficiency under drought conditions. A relatively lower number of stomata reduces the potential for water loss through transpiration, thereby increasing the ability of plants to maintain internal water balance during periods of water deficit. This adaptation occurs concurrently with a series of physiological responses activated under drought stress conditions [25,26].

4. Conclusion

The results of this study demonstrate that the application of polyethylene glycol (PEG) 6000 affected the growth of potato (*Solanum tuberosum* L.) cv. Dayang Sumbi plantlets under *in vitro* drought stress conditions. Increasing PEG 6000 concentrations resulted in progressive reductions in plantlet height, leaf number, and stomatal index. Plantlet survival remained at 100% throughout the observation period across all treatments. A PEG 6000 concentration of 10% was identified as the most suitable level for supporting the growth tolerance of *Solanum tuberosum* L. cv. Dayang Sumbi plantlets, as it maintained relatively favorable growth performance under simulated drought stress conditions.

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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