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Morphophysiological and Biochemical Characteristics of Local Rice MSP 17 to Different Durations of PEG-Induced Drought Stress

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Abstract: Drought stress is one of the main factors that hinder rice crop productivity. Drought stress limits the availability of water to plants, causing them to undergo morphological, physiological, and biochemical changes. Changes in the morphological characteristics of plants, such as plant height, number of roots, and root length. From a physiological perspective, water stress can reduce chlorophyll levels, increase carotenoid levels, increase electrolyte leakage, and decrease plants' relative water content. Meanwhile, from a biochemical perspective, drought stress can trigger changes in proline content, the formation of reactive oxygen species (ROS). These changes indicate that plant responses to drought are not only related to physical growth but also involve adjustments to physiological functions and metabolic processes to ensure the plants survival. The extent of the impact of drought stress on plants depends on the duration of exposure. The longer plants are subjected to water stress, the greater the likelihood of change in their morphology, physiology, and biochemistry, although these responses may vary depending on the plant's genetic characteristics and adaptive capabilities. Short-term stress can trigger an initial response to water limitations, while longer-lasting stress has the potential to cause greater disruption to water balance, photosynthesis, growth, and plant metabolism. Therefore, observations over various stress durations are necessary to understand the patterns of plant responses to drought over time. The method used in this study to investigate plant responses to drought stress involved the use of polyethylene glicol (PEG). In this study, PEG-induced drought stress was applied for durations of 2, 4, 8, 16 days to evaluate changes in the response of the local rice variety MSP 17 to the duration of stress exposure. It is hoped that these differences in duration will provide insight into the plant's initial response, the changes that occur during the stress period, and the possibility of more severe disturbances occurring during longer treatment periods. Simultaneous observation of morphological, physiological, and biochemical characteristics is necessary to gain a more comprehensive understanding of the response of the local rice MSP 17 to water stress.

Keywords: Duration of drought stress, PEG, Local rice MSP 17, Morphology, Physiology, Biochemical

INTRODUCTION

Rice (*Oryza sativa* L.) is a food commodity that plays a key role in both national and global food security. Rice, as the primary product of the rice plant, is a staple food consumed by half the world's population [1]. However, productivity is currently facing various challenges due to climate change, one of which is drought stress. Drought is one of the main factors limiting rice production because it can cause decline in yields and results in significant economic losses [2].

Drought stress disrupts various physiological processes in plants, causing changes in their normal metabolism [3]. Drought stress limits water availability to plants, affecting gene expression and causing morphological, physiological, biochemical, and other changes in rice, which inhibit growth, development, and yield [4]. Plant responses to drought conditions involve changes in the plants' morphological, anatomical, physiological, and biochemical characteristics as adaptive mechanisms to maintain water balance and improve water use efficiency [5]. In rice plants, morphological changes caused by drought stress are characterized by inhibited seed germination, a reduced number of tillers, earlier maturation, reduced plant height, and reduced biomass [6]. These physiological changes result in inhibited cell division and elongation, stomatal closure, reduced ability to maintain turgor, decreased photosynthesis rates, slower growth rates, and reduced yields [7].

The duration of drought stress is one of the factors that influence plant growth and physiological and biochemical responses. The longer plants are subjected to drought stress, the greater the potential for changes in various metabolic processes as a response to limited water availability. The plant's response to drought stress is the closure of stomata, which serves to reduce water loss through transpiration [8]. This closure of the stomata affects the diffusion of carbon dioxide (CO_2) into the cells, thereby reducing the rate of photosynthesis and the production of assimilates [9]. A decrease in CO_2 availability causes an imbalance between the absorption of light energy and its utilization in the carbon fixation process, resulting in excess energy in the photosystem, which triggers the formation of reactive oxygen species (ROS) and increased lipid peroxidation, as indicated by the accumulation of malondialdehyde (MDA) [10]. ROS which consists of superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen, hydroxyl radicals (·OH), and organic oxygen radicals (RO· and ROO·), accumulate when they exceed the capacity of the cell's defense system and cause oxidative damage [11]. This accumulation damages the integrity of the membrane and disrupts the stability of the photosystem complexes and the electoral transport chain, thereby limiting the production of ATP and NADPH which serve as energy sources and reducing agents for the Calvin cycle and ultimately reducing the plants photosynthetic efficiency.

Therefore, this study aims to examine the effects of the durations of drought stress (2, 4, 8, and 16 days) induced using PEG 6000 on the morphological, physiological, and biochemical responses of the local rice MSP 17.

MATERIAL AND METHODS

This study was conducted in May 2026 at the Center of Excellence for Science and Technology in Industrial Plant Biotechnology (PUI-PT BioTIn), University of Jember, Indonesia (8°11′12,2″ S, 113°38′08,6″ E).

Plant Materials

The rice seeds (*Oryza sativa* L.) used were the local cultivar MSP 17. The seeds were soaked for 48 hours in distilled water. The imbibed seeds were sown on rice husk paper that had been moistened with distilled water and placed in the dark at room temperature. Uniform 3-day old rice seedlings were selected and transferred to rockwool before excessive root elongation occurred.

Growth Conditions and Drought Stress Treatments

Rice seedlings 7 days after germination were planted in AB Mix hydroponic medium without added PEG 6000 as the control, while seedlings in the stress treatment were transferred to AB Mix hydroponic medium containing 10% PEG 6000. Control and treatment plants were maintained and harvested according to the treatment duration, namely 2, 4, 8,16 days. The leaves and roots of the plants were harvested according to the duration of each treatment and analyzed for morphological, physiological, and biochemical characteristics.

Morphological Parameter

Morphological observations were conducted on control plants and plants treated with 10% PEG 6000 at the respective treatment durations (2, 4, 8, and 16 days) following the drought stress treatment. Plant height is measured from the base of the stem to the tip of the longest leaf, the number of roots is counted based on all visible roots, while root length is measured from the base of the root to the tip.

Pigment Content Photosynthesis

The photosynthethic pigment was determined using the method described by Porra [12]. Chlorophyll content was determined using an 80% methanol extraction method. A total of 0,3 g of fresh rice leaves was weighed and homogenized using a mortar, then 1,5 ml of 80% methanol was added, with a solid to solvent ratio of 1:5 (w/v). The extract was then transferred to a microtube and centrifuged at 10.000 rpm and 4°C for 10 minutes. The supernatant obtained was used to measure absorbance using a spectrophotometer at a wavelength of 665 nm for chlorophyll a, 652 nm for chlorophyll b, and 470 nm for carotenoids. Next, it is calculated using the following equation:

$$\text{Chlorophyll a (mg/g)} = \frac{[(16{,}29\ x\ Abs665)-(8{,}54\ x\ Abs652)]}{1000} x\ volume/weight \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = \frac{[(30{,}66\ x\ Abs652)-(13{,}58\ x\ Abs665)]}{1000} x\ volume/weight \quad (2)$$

$$\text{Carotenoid (mg/g)} = \frac{[(1000\ x\ Abs470)-(22{,}12\ x\ Abs665)-(2{,}71\ x\ Abs652)]}{227} x\ volume/weight \quad (3)$$

$$\text{Total chlorophyll (mg/g)} = \frac{[(22{,}12\ x\ Abs665)+(2{,}71\ x\ Abs652)]}{1000} x\ volume/weight \quad (4)$$

Relative Water Content (RWC), Electrolyte Leakage (EL)

RWC in rice leaf and root samples was measured based on the differences in fresh weight (SW), saturated weight (SW), and dry weight (DW). A 0,3 g sample of fresh leaves and root was weighed, then soaked in distilled water for 24 hours at 4°C, drained of surface water, and weighed again as SW. The sample was then dried at 65°C for 6 hours until a constant weight was reached to determine DW. The RWC value is calculated using the formula: RWC (%) = [(FW − DW) / (SW − DW)] × 100% [13].

Electrolyte leakage is measured using the method employed by [8]. Samples of rice leaves and roots, each weighing o,3 g, were cut into pieces approximately 0,5 cm long and then soaked in 10 ml of deionized water for 24 hours in the dark. The initial conductivity

(EC_1) was measured, the sample was then autoclaved at 121°C for 15 minutes and measured again to determine the final conductivity (EC_2). The percentage of electrolyte loss is calculated using the formula (EC_1/EC_2) × 100%.

H_2O_2 and MDA Content

The H_2O_2 content in rice leaves and roots was analyzed using the following method [14]. A 0,3 g fresh sample was homogenized with TCA 0,1% (1:5, w/v), then centrifuged at 12.000 rpm at 4°C for 15 minutes. A total of 250 µL of the supernatant was mixed with 250 µL potassium phosphate buffer (pH 7) and 1 ml of 1 M KI, then incubated at 4°C for 15 minutes. Absorbance was measured using a spectrophoto meter at a wavelength of 390 nm.

The malondialdehyde (MDA) content in the leaf and root tissues plants was analyzed using method [15]. Fresh leaf and root samples, each weighing 0,3 g, were weighed ground and homogenized with 0,1% TCA in a 1:5 (w/v) ratio. The homogenate was then centrifuged at 1.000 xg for 15 minutes at 4°C. A total of 250 µL of the supernatant was mixed with 1 ml of o,5% TBA solution in 20% TCA, then incubated on dry block at 250 µL 95°C for 30 minutes. After cooling to room temperature, the absorbance of the solution was measured using a spectrophotometer at wavelengths of 532 and 600 nm.

Protein and Prolin Content

The soluble protein content in rice leaf and root tissues was analyzed using the Bradford method. A0,3 g sample of fresh material was homogenized with a potassium phosphate buffer (pH 7,8) in a 1:3 (w/v) ratio, it was then centrifuged at 14.000 rpm and 4°C for 5 minutes. The supernatant was incubated at 4°C for 12 hours. A total of 5µL of the supernatant was mixed with 45 µL of distilled water and 950 µL of Bradford reagent, then incubated until a blue color developed. Absorbance was measured at 595 nm using a spectrophotometer. The dissolved protein content was determined based on a standard curve.

The proline content in the leaf and root tissues of the rice plant was analyzed based on the concentration of soluble protein to determine the ratio of the mixture of samples, distilled water, and ethanol. The mixture was then combined with 800 µL of reaction mix, homogenized using a vortex mixer, and incubated at 95°C for 20 minutes using a dry block. After cooling to room temperature, the samples were centrifuged at 10.000 rpm for 25 minutes. Absorbance was measured using a spectrophotometer at 520 nm [16]. The proline content is determined based on absorbance and calculated using a proline standard curve.

Total Amino Acid Content

Free amino acid content in leaves and roots was determined according to the method described in [15]. Fresh tissue samples (0.3 g) were extracted with potassium phosphate buffer (pH 7.8) at a ratio of 1:3 (w/v), followed by centrifugation at 14,000 rpm for 5 min at 4°C. subsequently, 25 µL of the supernatant was mixed with 400 µL of potassium phosphate buffer (pH 8.0) and 200 µL of 0.1% 2,4,6-trinitrobenzenesulfonic acid (TNBS), followed by incubation at 50°C for 1 h. The reaction mixture was the supplemented with

400 µL of Na_2SO_3 and incubated at room temperature for 15 min. Absorbance was measured at 420 nm using a spectrophotometer.

Statistical Analysis

Data was analyzed using two-way analysis of variance (ANOVA). Comparisons between the control (0% PEG) and 10% PEG treatment at each treatment duration were performed using Šídák's multiple comparisons test. Statistical significance was set at $p < 0.05$, corresponding to a 95% confidence level.

RESULT AND DISCUSSION

The Effect of Drought Stress Duration on Plant Morphology

Drought stress simulated using 10% polyethylene glycol (PEG) reduced the morphological growth of rice plants compared with the control (0% PEG) (Figure 1). After 16 days of treatment, plant height under 10% PEG reached 25.97 cm, which was 17.74% lower than that of the control (31.57 cm). Root number was also lower under 10% PEG, averaging 7.33 compared with 10.33 in the control, representing a reduction of 29.03%. Root length reached 11.77 cm under 10% PEG, compared with 13.07 cm in control, corresponding to a reduction of 14.11%. PEG induced osmotic stress may reduce water uptake by roots, thereby limiting plant growth.

Drought stress can inhibit plant growth by limiting water uptake, thereby reducing cell turgor and expansion [17][18]. These effects may restrict organ elongation and consequently limit plant height. Root growth may also be constrained by reduced water availability to developing tissues under drought conditions [19][20]. Thus, reduced shoot and root growth represents a morphological response to limited water availability.

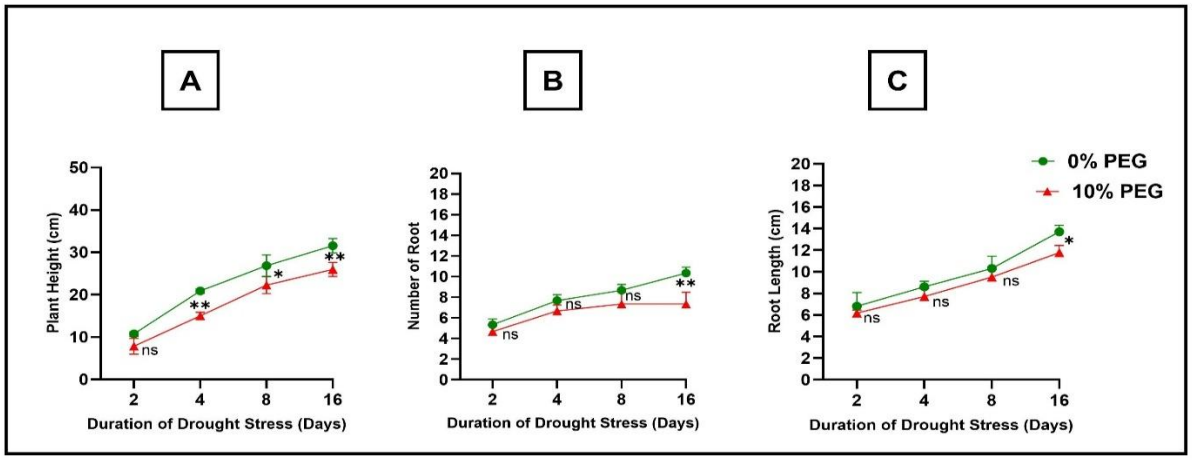


Fig. 1: Morphological traits of rice plants under different durations of drought stress. (A) Plant height, (B) Number of roots, and (C) Root length. Data are presented as means ± standard error (SE; n=3). Asterisks indicate significant differences between the control (0% PEG) and 10% PEG treatment at each stress duration (*p < 0.05; **p < 0.01), whereas ns indicates no significant difference.

The Effect of Drought Stress Duration on Plant Physiology

Table 1: Effect of Drought Stress Duration on Chlorophyll and Carotenoid Contents.

Treatment		Parameter			
		Klorofil a (mg/g FW)	Klorofil b (mg/g FW)	Karotenoid (mg/g FW)	Total Klorofil (mg/g FW)
0% PEG	2	3.86 ± 0.02	0.95 ± 0.01	2.81 ± 0.00	4.81 ± 0.01
	4	5.19 ± 0.02	1.18 ± 0.01	2.83 ± 0.01	6.36 ± 0.03
	8	7.48 ± 0.03	1.62 ± 0.02	2.45 ± 0.01	9.10 ± 0.02
	16	9.37 ± 0.01	1.83 ± 0.00	2.44 ± 0.00	11.20 ± 0.02
10% PEG	2	3.29 ± 0.01**	0.85 ± 0.00*	3.01 ± 0.00**	4.15 ± 0.00**
	4	2.97 ± 0.00**	1.10 ± 0.00*	3.66 ± 0.00**	4.06 ± 0.00**
	8	2.74 ± 0.00**	1.11 ± 0.01**	4.57 ± 0.00**	3.85 ± 0.00**
	16	1.21 ± 0.04**	1.67 ± 0.04**	6.91 ± 0.001**	2.88 ± 0.00**

Note: Values are expressed as means ± SE (n = 3). Significant differences between the control and 10% PEG treatments at each drought stress duration are denoted by * ($p < 0.05$) and ** ($p < 0.01$). The abbreviation ns indicates a nonsignificant difference.

The duration of drought stress significantly inhibited photosynthesis in rice plants [21]. According to Table 1, chlorophyll content decreased significantly under 10% PEG treatment compared with the control at all duration. After 16 days of treatment, the control plant contained 9.37 mg/g FW of chlorophyll a, whereas it decreased to 1.21 mg/g FW under 10% PEG treatment.

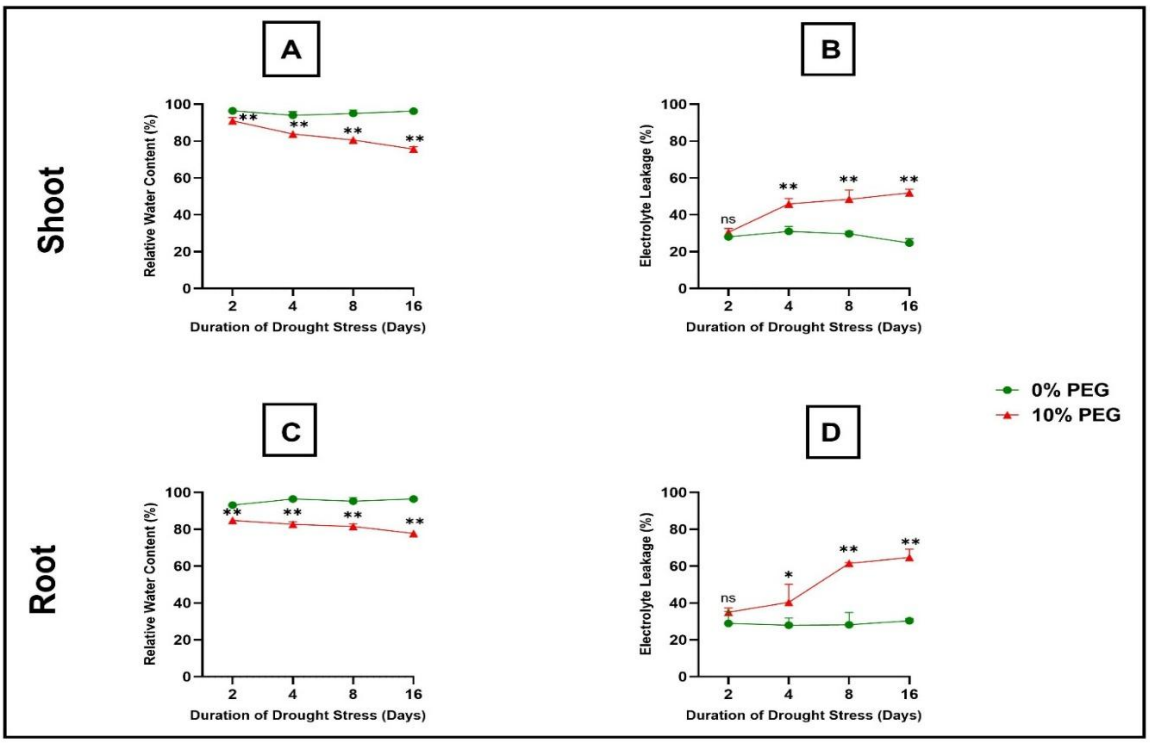


Fig 2: (A-B) RWC and EL in the shoot; (C-D) RWC and EL in the root. Values are expressed as means ± standard error (SE; n = 3). Statistical differences between the control and 10% PEG treatments at each stress duration are indicated by * ($p < 0.05$) and ** ($p < 0.01$), while ns denotes a nonsignificant difference.

Chlorophyll b and carotenoid contents increased under 10% PEG treatment as the duration of drought stress extended. Total chlorophyll content exhibited an increasing trend in control plants as the treatment period progressed, whereas a progressive decline was observed in plants subjected to 10% PEG treatment. Carotenoid content reached its highest level (6.91 mg/g FW) after 16 days of drought stress under 10% PEG treatment, representing an increase of 183.2% relative to the control (2.44 mg/g FW). Drought stress may induce chloroplast damage through the excessive accumulation of reactive oxygen species (ROS) [22].

Prolonged drought stress reduces the relative water content (RWC) of plant tissues due to limited water availability and transport [23]. The graph in Figure 3 shows a decline in RWC in both leaves and roots as the duration of drought stress increases. In the shoots, relative water content (RWC) under 10% PEG treatment was 91.03%, 83.87%, 80.65%, and 75.71% on days 2, 4, 8, and 16, respectively, compared with 96.43%, 94.00%, 95.05%, and 96.30% in the control. Similarly, root RWC under 10% PEG treatment was 84.85%, 82.67%, 81.57%, and 77.78%, whereas the corresponding values in the control were 93.10%, 96.43%, 95.32%, and 96.43%, respectively. Osmotic stress occurs when the osmotic balance between the intracellular and extracellular environments is disrupted [24]. Under PEG treatment, the reduction in water potential of the growth medium impairs root water uptake and limits water supply to the shoot. Drought stress also increases electrolyte leakage (EL) in plants [25]. After 16 days of treatment, EL in the shoots of plants exposed to 10% PEG reached 51.95%, compared with 24.68% in control. Similarly, root EL reached 64.74% under 10% PEG treatment, compared with 30.37% in control. Prolonged drought stress (16 days) exposes plants to sustained osmotic stress, thereby exacerbating cellular water imbalance and disrupting physiological processes. When plant tissues experience dehydration, as indicated by a substantial decline in RWC, cellular membrane integrity progressively deteriorates, increasing membrane permeability and promoting the leakage of intracellular ions [26].

The Effects of Drought Stress Duration on Plant Biochemical

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were measured to evaluate plant oxidative stress responses under osmotic stress. Under 10% PEG treatment, H_2O_2 and MDA contents in the shoots and roots differed significantly from those in the control (0% PEG) at all drought stress durations (Fig. 3). Shoot H_2O_2 content increased from 1.08 μM g^{-1} FW on day 2 to 3.08 μM g^{-1} FW on day 16, while root H_2O_2 content increased from 1.10 to 2.40 μM g^{-1} FW. In contrast, H_2O_2 content in the control remained relatively stable throughout the experimental period. The increase in H_2O_2 indicates the accumulation of reactive oxygen species (ROS), potentially resulting from an imbalance between ROS production and the plant's antioxidant defense capacity [27]. Under normal conditions, the balance between ROS production and antioxidant defense helps maintain cellular homeostasis [28]. However, osmotic stress can disrupt this balance by inducing stomatal closure, which restricts CO_2 uptake and limits photosynthesis, thereby potentially increasing ROS production [29]. Disruptions in the electron transport chain and cellular metabolism may also contribute to ROS accumulation [30]. Therefore, the increase in H_2O_2 under 10% PEG treatment may reflect disruption of cellular redox homeostasis.

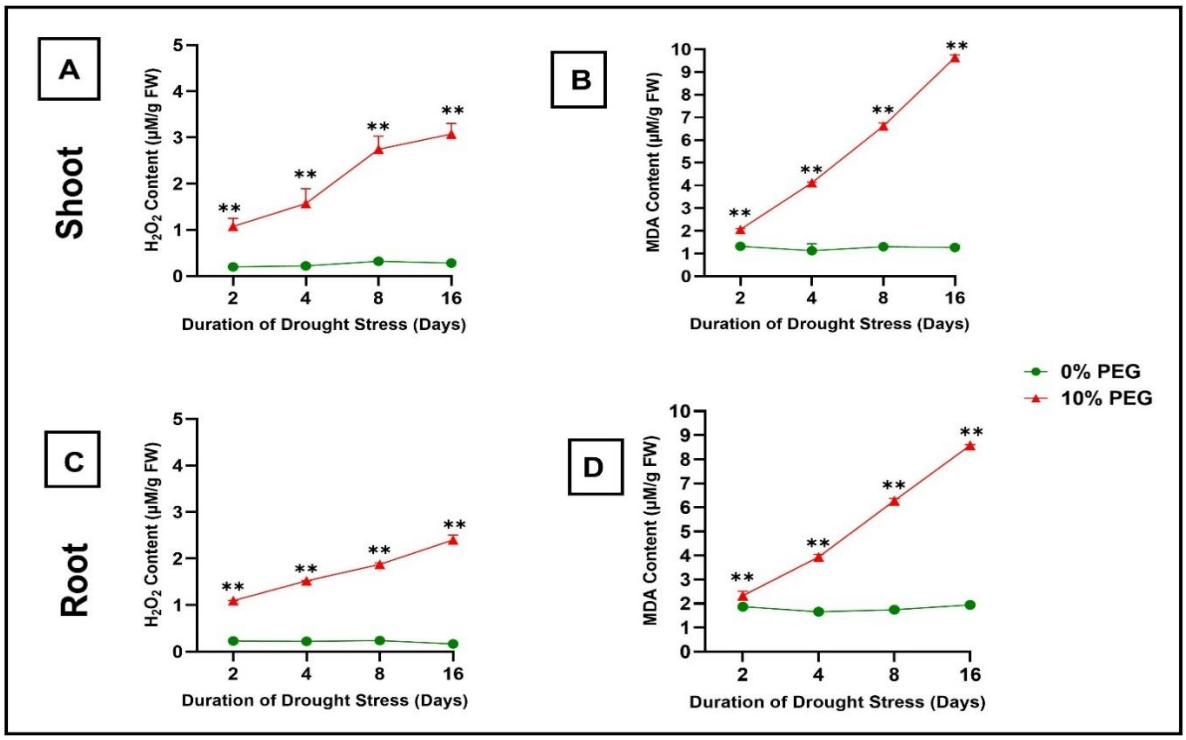


Fig. 3: (A-B) H_2O_2 dan MDA in the shoot plant, (C-D) H_2O_2 dan MDA in the root plant. Data are presented as means ± standard error (SE; n=3). Asterisks indicate significant differences between the control (0% PEG) and 10% PEG treatment at each stress duration (*p < 0.05; **p < 0.01), whereas ns indicates no significant difference.

A similar pattern was observed for MDA content. In the shoots, MDA content increased from 2.06 µM g^{-1} FW on day 2 to 9.63 µM g^{-1} FW on day 16, while in the roots, it increased from 2.32 to 8.58 µM g^{-1} FW. In contrast, MDA content in the control remained relatively stable throughout the experimental period. The higher MDA content under 10% PEG treatment indicates increased lipid peroxidation, which can occur when ROS accumulate excessively[31]. MDA is a widely used indicator of oxidative damage to cell membranes because it is a product of lipid peroxidation [32]. Therefore, increased MDA content in both shoots and roots suggests that osmotic stress may compromise cell membrane structure and function.

The increases in H_2O_2 and MDA contents under 10% PEG treatment indicate that oxidative stress intensified with increasing stress duration. This response was accompanied by changes in plant metabolism, as reflected by decreased soluble protein content and increased total amino acid and proline contents. These biochemical changes may reflect the plant's adaptive responses to osmotic stress.

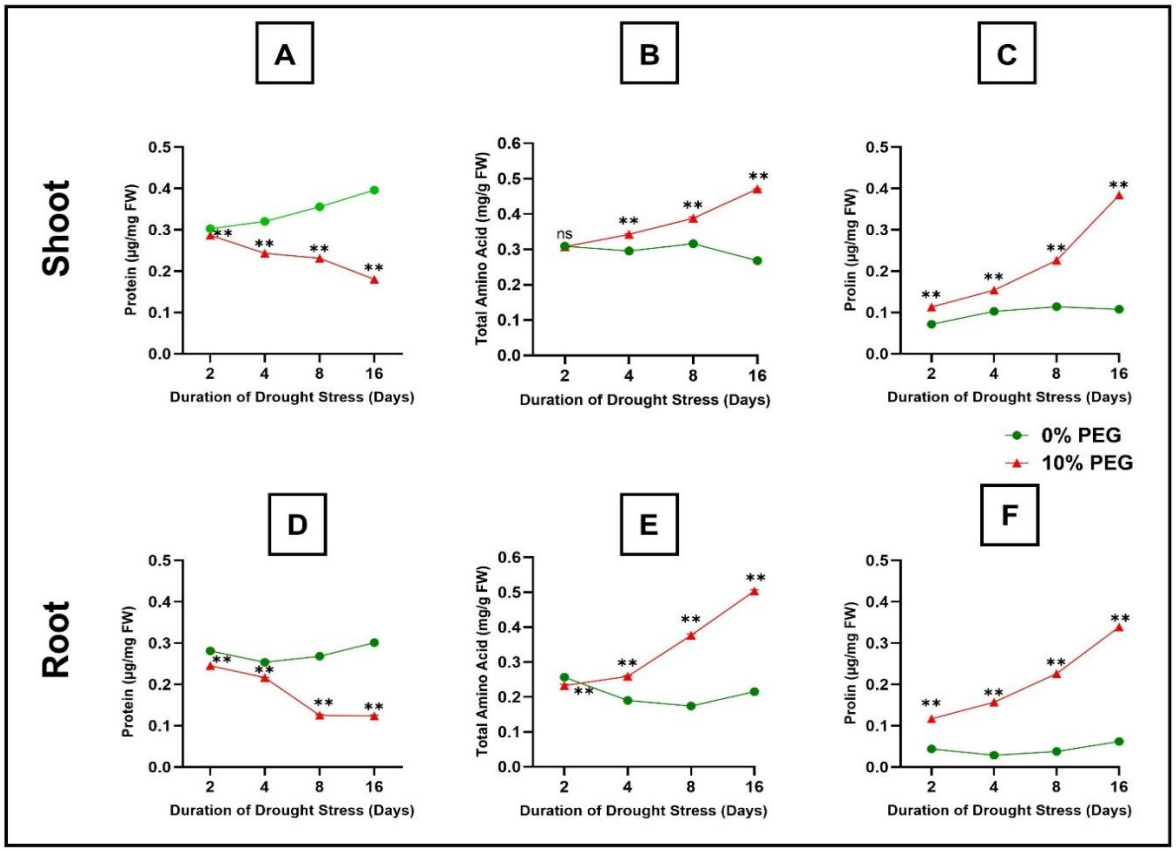


Fig. 4: (A-C) protein, total amino acid content and prolin in the shoot plant, (D-F) protein, total amino acid content and prolin in the root plant. Data disajikan sebagai rata-rata ± SE (n = 3). Data are presented as means ± standard error (SE; n=3). Asterisks indicate significant differences between the control (0% PEG) and 10% PEG treatment at each stress duration (*p < 0.05; **p < 0.01), whereas ns indicates no significant difference.

Soluble protein content decreased under 10% PEG treatment in both shoots and roots (Fig. 4A, D). In shoots, protein content declined from 0.29 µg g^{-1} FW on day 2 to 0.18 µg g^{-1} FW on day 16, whereas the control increased from 0.30 to 0.40 µg g^{-1} FW, with significant differences observed from day 4 to day 16. In roots, protein content decreased from 0.24 µg g^{-1} FW on day 2 to 0.12 µg g^{-1} FW on day 8 and remained relatively stable thereafter, while the control fluctuated and reached 0.30 µg g^{-1} FW on day 16. This decline may reflect impaired cellular metabolism and protein synthesis under limited water availability. Moreover, excessive ROS accumulation may induce protein oxidation, potentially disrupting enzymatic activity and other protein functions [33].

Total amino acid content increased under 10% PEG treatment in both shoots and roots (Fig. 4B, E), rising from 0.31 to 0.47 mg g^{-1} FW in shoots and from 0.23 to 0.50 mg g^{-1} FW in roots between days 2 and 16. In contrast, the control decreased to 0.27 mg g^{-1} FW in shoots by day 16, while root content declined from 0.25 mg g^{-1} FW on day 2 to 0.17 mg g^{-1} FW on day 8 before increasing to 0.21 mg g^{-1} FW on day 16. These changes may reflect metabolic adjustments to osmotic stress, as amino acids contribute to protein synthesis, metabolic regulation, and osmotic adjustment [34][35] . Proline is another amino acid involved in plant responses to osmotic stress. Under 10% PEG treatment, proline content

increased progressively in both shoots and roots (Fig. 4C, F). In shoots, it increased from 0.11 to 0.38 µg g^{-1} FW between days 2 and 16, whereas the control remained relatively stable (0.07–0.11 µg g^{-1} FW). Similarly, root proline content increased from 0.04 to 0.34 µg g^{-1} FW, while the control remained low with minor fluctuations. Significant differences between treatment and control were observed at all sampling times in both organs. This accumulation may contribute to osmotic adjustment, cellular protection, and defense against oxidative stress [36][37].

CONCLUSION

The duration of drought stress imposed using 10% PEG 6000 significantly influenced the morphological, physiological, and biochemical characteristics of the local rice cultivar MSP 17. Prolonged exposure to drought stress, particulary for 16 days, resulted in the most pronounced effect on several parameters, including growth inhibition, reduced chlorophyll content and relative water content (RWC), and increased carotenoid content and electrolyte leakage. At the biochemical level, prolonged drought stress led to increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents, accompanied by a reduction in soluble protein content, alterations in total amino acid content, and an increase in proline accumulation. These changes indicate physiological distruption and enhanced oxidative stress, while also reflecting the activation of biochemical responses associated with plant adaptation. Overall, the finding demonstrates that prolonged drought stress induces progressively greater alterations in the morphological, physiological, and biochemical traits of the local rice cultivar MSP 17.

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