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Response of regulation of resistance genes, reactive oxygen species, and antioxidant enzymes to salicylic acid treatments in drought tolerant rice

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Abstract. Drought is one of the most important issues in crop production which may disrupt physiological processes and biochemical metabolism in rice plants, including the emergence of plant resistance gene expression such as OsAB13 and OsLEA, the formation of ROS (Reactive Oxygen Species), namely hydrogen peroxide (H_2O_2), as well as the emergence of gene expression related to antioxidant enzyme activity such as OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic. Besides the emergence of plant resistance gene expression, ROS, and changes in gene expression related to antioxidant enzymes, rice plants also produce salicylic acid which acts as an endogenous signal to activate plant resistance gene responses and can encourage plant resistance responses such as antioxidant enzyme activity. The treatments in this study included: control, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM. The results showed that the interaction between treatments and rice plant varieties significantly affected plant height, root length, total chlorophyll, and H_2O_2 content. The expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes increased in the 15% PEG 6000 treatment compared to the control. In the 15% PEG 6000 + SA 1 mM treatment, there was an increase in gene expression of APX Cytosolic, Cu/Zn-SOD, and OsCATA compared to the 15% PEG 6000 treatment. The OsLEA is expressed in Siakraya and Sertani 1 as an indicator of resistance to drought stress, and the OsAB13 is expressed in Indragiri, Sertani 1, and Siakraya varieties as an indicator of resistance to drought stress.

Key words: drought stress, antioxidant enzymes, resistance genes, reactive oxygen species, and rice plants.

INTRODUCTION

Drought is one of the most critical constraints in crop production, including rice, which may reduce the chlorophyll content (Niu et al., 2022) as well as the absorption of

water and nutrients by plant roots. That conditions will disrupt the physiological and biochemical processes of the chemical metabolism of rice plants. Physiological and biochemical processes that occur due to drought stress conditions is a part of plant survival mechanism (Ubaidillah et al., 2019).

Rice plants under drought stress produce ROS as a result of the accumulation of oxygen (O_2) in the cells (Wang et al., 2005; (Ubaidillah et al., 2013). ROS consists of free radical groups that can trigger cell damage. The freest radicals formed in plant tissues are superoxide, which will later be converted into H_2O_2 and converted into hydroxyl radicals (OH-) which cause lipid peroxidation in cell membranes (Nahar et al., 2016). Excessive ROS production causes plants to make efforts to maintain their survival by producing enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) (Farssi et al., 2022), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and peroxiredoxin (PrxR) (Miller et al., 2010).

Rice plants also produce salicylic acid, a compound that acts as an endogenous signal to activate the response of genes related to plant resistance, including drought stress (Roumani et al., 2019), and is able to induce plant resistance responses such as the activation of antioxidant enzymes (Liu et al., 2015). Based on previous studies, salicylic acid plays a role in plant resistance.

Salicylic acid (SA) is a phenolic endogenous growth regulator and also a signaling molecule that participates in the regulation of physiological processes in plants such as growth, photosynthesis and other metabolic processes. Salicylic acid can modulate the antioxidant defense system thereby reducing oxidative stress. Salicylic acid regulates plant-water relations, photosynthetic rate, nitrogen metabolism, and proline metabolism when under abiotic stress conditions (Jayakannan et al., 2015).

This research was conducted to carried out the response of antioxidant genes and other genes that correlated with the resistance factor of Indonesian local tolerant rice. It should be noted that the IR64 variety was a moderately resistant control used as a comparison in this study. This research was expected to be able to provide information about physiological and biochemical changes in several varieties of rice plants that experience drought.

91 rice germplasm have been characterized and evaluated for resistance to drought stress based on the Standard Evaluation System for Rice IRRI (2013), and 8 candidates for rice varieties that are resistant to drought stress have been obtained, of which 3 rice were used in this study including Siak Raya, Sertani 1 and Indragiri. This study aims to evaluate the tolerance level of rice varieties to drought stress and to determine the growth response and expression of antioxidant genes in drought tolerant rice under drought stress conditions and what is the role of SA in increasing the resistance response.

MATERIALS AND METHODS

Plant material and Treatment

The three varieties used in this study are Siakraya, Indragiri, Sertani 1 and IR64 used as moderate resistance control. Rice seeds were soaked with fungicide for 1 week before ready to be planted in the pot tray for 14 days. Healthy rice plants were selected, with characteristic of leaves are green and free from disease, the number of leaves is 4 to 5, and the stems are upright. Plant treatment was carried out by placing each experimental unit portray into a tub containing the treatment solution and keeping it from

changing in concentration. The treatment solutions were in the form of control, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM. Plants that have been treated are adjusted with water every day up to the initial volume limit when the treatment is applied. Plants were maintained by controlling their environmental conditions from pests and ensuring the water and nutrients are maintained and the room is controlled. Each plant then being observed for its height, root length, total chlorophyll content, H_2O_2 content, as well as the resistance gene expression.

Morphological characterization

The plant height dan root length was determined after 8 days treatment. Plant height was measured from the root at ground level to the tip of the tallest shoot. Root length was measured from the base of the root to the tip of the root.

Chlorophyll and hydrogen peroxide content analysis

The total leaf chlorophyll content was calculated using leaf samples that were taken 8 days after treatment with the spectrophotometric method using 80% acetone and measuring the absorbance of chlorophyll using a spectrophotometer at a wavelength of 645 nm and 663 nm (Ahmad et al., 2019). The formula for calculating chlorophyll is as follows:

$$\text{Total chlorophyll (mg g}^{-1} = \frac{20.2\ (\text{A}\ 645)\ +\ 8.02\ (\text{A663})\ \times\ \text{V}}{1{,}000} \times \text{W} \qquad (1)$$

The content of hydrogen peroxide was measured according to the method (Christou et al., 2014). A total of 0.1 g of leaf sample was homogenized into 1 mL of 0.1% Trichloroacetoacid (TCA), then centrifuged at 10,000× g for 15 m. A total of 0.5 mL of the supernatant was taken and adjusted to 0.5 mL of 10 M phosphate buffer with pH 7.0 and 1 mL of 1 M potassium iodide. The solution was then incubated at room temperature for 30 m, then the absorbance was measured at a wavelength of 390 nm. The calibration standard curve was used as a standard in determining the content of H_2O_2.

Gene Expression analysis

The callus samples were taken on the 14th and 28th days of medium regeneration and frozen immediately in liquid nitrogen. Then the isolated total RNA with some modifications to the manufacturer's Gene All Ribospin Plant RNA Mini Kit (GeneAll Biotech, Korea). The 260 nm/280 nm measurement at a level between 1.8 and 2.2 for cDNA synthesis and RT-PCR was obtained using nanodrop (TECAN® Infinite M200 Multi-Detection Microplate Reader Part).

After verifying RNA quality, The RNA sample is converted into cDNA by reverse transcription using ReverTra Ace® RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan) treats 0.5 μg of total RNA. This process eliminates and replaces genomic DNA with a single-stranded cDNA. Total RNA was incubated at 37 °C for the DNase reaction for 5 m and reverse transcription reaction. Finally, gene-specific primers for the expression of OsAB13, OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD and APX Cytosolic genes (Table 1) have been used in real-time PCR applications.

Table 1. Primer Sequences for Gene Expression Analysis

Gene	Primer	Source
OsACTIN	Forward: 5' TCCATCTTGGCATCTCTCAG 3' Reverse: 5' GTACCCGCATCAGGCATCTG 3'	Kim et al. (2007)
OsAPX1	Forward: 5' CCAAGGGTTCTGACCACCTA 3' Reverse: 5' CAAGGTCCCTCAAAACCAGA 3'	Kim et al. (2007)
OsCATA	Forward: 5' CGGATAGACAGGAGAGGTTCA 3' Reverse: 5' AATCTTCACCCCCAACGACT 3'	Kim et al (2007)
Mn-SOD	Forward: 5' GGAAACAACTGCTAACCAGGAC 3' Reverse: 5' GCAATGTACACAAGGTCCAGAA 3'	Kim et al. (2007)
Cu/Zn-SOD	Forward: 5' CAATGCTGAAGGTGTAGCTGAG 3' Reverse: 5' GCGAAATCCATGTGATACAAGA 3'	Kim et al. (2007)
APX Cytosolic	Forward: 5' AGTACATTGCCCGTGGTACTCT 3' Reverse: 5' CGCATTTCATACCAACACATCT 3'	Kim et al. (2007)
OsAB13	Forward: 5' CCC AAC AAC AAA AGC AGG AT 3' Reverse: 5' CCT TTG TAT TGG ACG AGA CG 3'	Zhou et al. (2020)
OsLEA	Forward: 5' CCC AAG CTT AAA ATG GCG TCG AGG CAG GAC A 3' Reverse: 5' TGC TCT AGA TCA TGG CAA GAC TGC TGA TGT ATG g 3'	Zhou et al. (2020)

PCR analysis is performed in a total volume of 10 µL containing 5 µL of 2 × GoTaq® Green Master Mix, 1 µL cDNA templates, 2 µL Nuclease-free water and 1 µL Forward (F) and Reverse (R) primer to detect the presence of a specific nucleic acid sequence using the GoTaq® Green Master Mix kit (Promega). The PCR amplification profile consisted of an initial denaturation of 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. An electrophoresed 2% agarose gel in 1 X TAE buffer stained with GreenStar™ was used for PCR analysis. In addition, the UV transilluminator was used to visualize the banding patterns.

Data analysis

(ANOVA). If there is a significant difference between the treatments, a further test is carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Data obtained from gel electrophoresis visualization were analyzed using qualitative descriptive analysis with visual presentation.

RESULTS AND DISCUSSION

The effect of drought stress and salicylic acid treatment of morphological character

The rice plants be grown under salicylic acid treatments implemented to see its response under drought stress. Salicylic acid plays a role in the response of plant resistance to drought stress as evidenced by the result of plant high, root length, total chlorophyll, hydrogen peroxide content, as well as the expression of responsive genes towards treatment.

Generally, drought stress caused a decrease (Fig. 1) in plant height for all varieties compared to those without stress treatment.

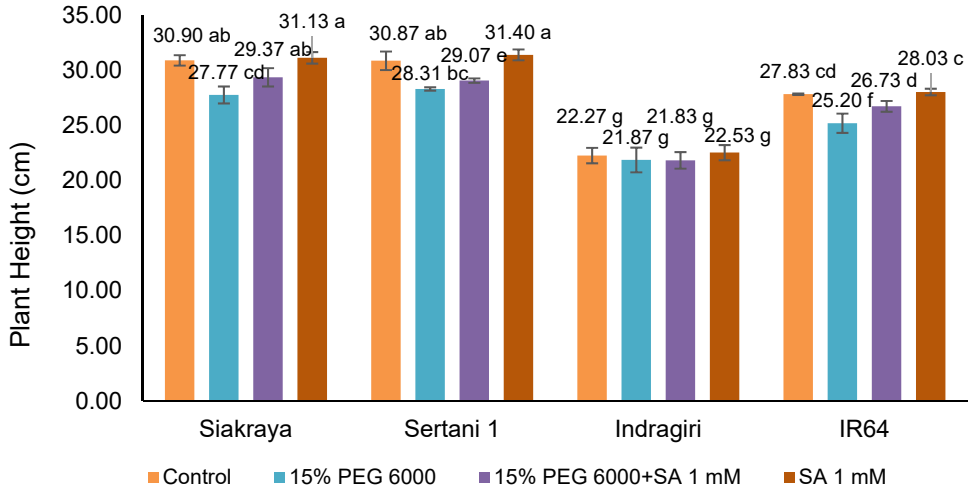

Figure 1. Response of rice plant height under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

The decrease in plant height in the 15% PEG 6000 treatment significantly occurred in the Siakraya variety by 3.13% compared to the control. The decrease in IR64 had a moderate level of resistance to drought stress decreased by 2.63% compared to the control. The decrease in Sertani 1 was 2.56%, compared to the control while the decrease was not significant for the Indragiri by 0.4%. Drought stress causes rice plants to experience a reduced cell size, inhibiting plant vegetative growth and decreasing plant height (Li et al., 2022).

The 15% PEG 6000 + SA 1 mM treatment generally increased plant height compared to the 15% PEG 6000 treatment, namely Siakraya, IR64, and Sertani 1 by 1.6%, 1.53%, and 0.76% respectively, while Indragiri experienced a not significant decrease by 0.04%. Rice plants treated with 15% PEG 6000 + SA 1 mM generally had plant heights that tended to be more stable than those treated with 15% PEG 6000, this was due to the response of the application of salicylic acid which was able to maintain membrane stability during drought stress (Ubaidillah et al., 2016). Salicylic acid also plays a role in regulating plant water relations and the rate of photosynthesis under abiotic stress conditions (Khalvandi et al., 2021).

The response of rice plants to drought stress also can be observed through its root length. Rice plants treated with drought stress generally experienced an increase in root elongation for all varieties compared to those without stress (Fig. 2). Root elongation with 15% PEG 6000 treatment significantly occurred at Siakraya by 3.73%, Sertani 1 by 2.77%, and Indragiri by 2.47% compared to the control treatment. The IR64 variety was not significant with a change occurring by 0.77% compared to the control because the IR64 variety had a moderate level of resistance to drought stress.

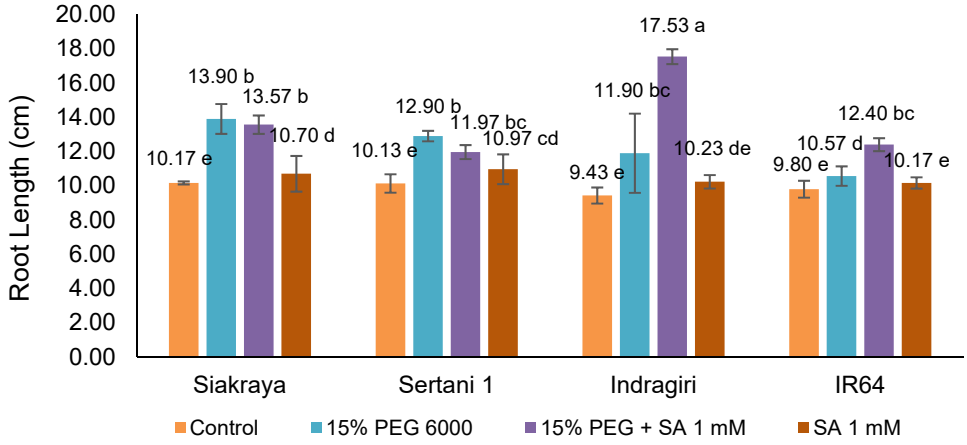

Figure 2. Response of Root Length to Treatment under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

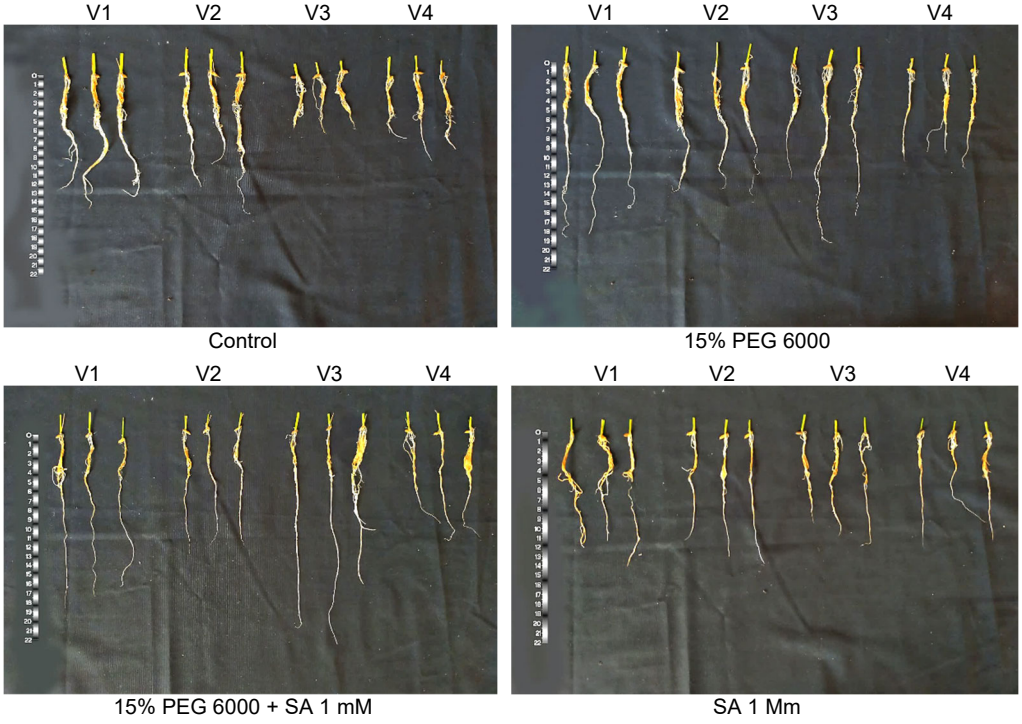

Figure 3. The morphology of root under several treatment condition V1; Siak raya, V2; Sertani, V3; Indragiri and V4; IR64.

Increasing root elongation occurs with the aim of expanding the root absorption area for water and nutrients (Noelle et al., 2018). In the rice variety Siakraya and Sertani 1 (Fig. 3), the 15% PEG 6000 + SA 1 mM treatment reduced root elongation compared to the 15% PEG 6000 treatment by 5.63% and 1.83%. The varieties of Indragiri and IR64

in the 15% PEG 6000 + SA 1 mM treatment increased root elongation by 0.33% and 0.93% compared to the 15% PEG 6000 treatment. Several varieties in the 15% PEG 6000 + SA 1 mM treatment generally experienced an increase in root elongation compared to those treated with 15% PEG 6000 due to a response from the application of salicylic acid which is an endogenous growth regulator. Salicylic acid is phenolic and also a signaling molecule that participates in regulating physiological processes in plants such as root growth, increased root volume, and the rate of photosynthesis (Jayakannan et al., 2015).

The effect of drought stress dan salicylic acid treatment on chlorophyl content and H_20_2

Due to the function of salicylic acid as a regulator of some physiological processes in plants including the rate of photosynthesis, the result of total chlorophyll in rice plant under the drought stress also varied. Rice plants treated with drought stress generally experienced a decrease in total chlorophyll content compared to those without stress treatment (Fig. 4), however, there were certain varieties treated with drought stress but experienced an increase in total chlorophyll content.

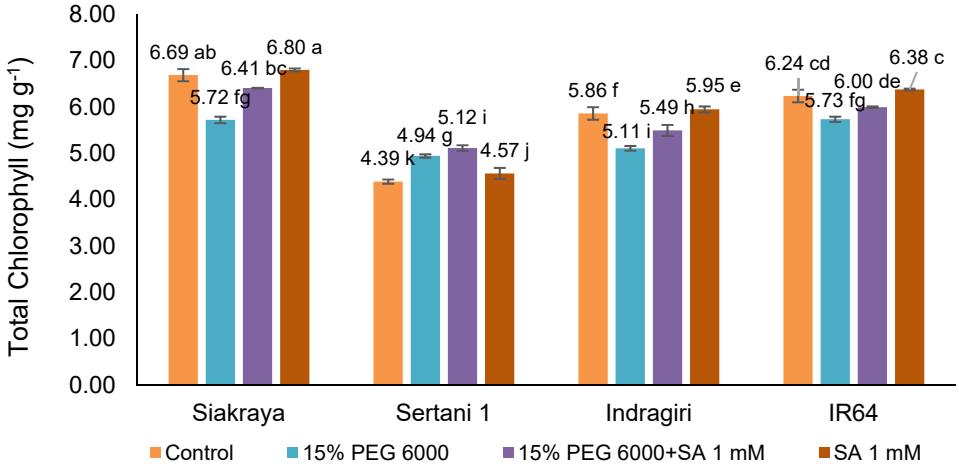

Figure 4. Response of Total Chlorophyll Content under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

The significant decrease of total chlorophyll content in the 15% PEG 6000 treatment occurred in Siakraya by 0.97%, Indragiri by 0.75%, and IR64 by 0.51% compared to the control treatment. The decrease in total chlorophyll content occurs because drought stress may have an impact in the form of accelerating the rate of leaf aging, abscission of old leaves, and inhibited opening of stomata, thereby reducing leaf dilation and inhibiting the rate of photosynthesis. The increase in total chlorophyll content in the 15% PEG 6000 treatment, namely Sertani 1, was 1.39% compared to the control. The increase in total chlorophyll content is due to the stimulus for chlorophyll synthesis in young leaves caused by the activation of enzymes in light-dependent biosynthesis and changes in chlorophyll levels in certain varieties against drought stress (Shin et al., 2021).

Treatment of 15% PEG 6000 + SA 1 mM generally increased the total chlorophyll content compared to the 15% PEG 6000 treatment, namely the Siakraya variety by 0.69%, Indragiri by 0.38%, IR64 by 0.27%, but the increase was not significant in Sertani 1 of 0.18%. The 1 mM SA treatment indicated the same response in general as the control treatment. The increase in total chlorophyll content in the 15% PEG 6000 + SA 1 mM treatment was due to salicylic acid acting to block the flow of electron transfer in photosystem II (PS II). Salicylic acid competes with quinone B (QB) for binding sites in the photosystem II (PSII) reaction center, thereby reducing the rate of electron flow from H_2O to NADP+ via electron carriers in the PS II reaction. Salicylic acid induces the production of singlet and triplet chlorophyll in photosystem II, therefore salicylic acid can increase the content of chlorophyll a, b, and the rate of photosynthesis (Radwan et al., 2019).

Drought stress generally increase the content of H_2O_2 in all varieties of rice plants (Fig. 5). The significant increase in the content of H_2O_2 in the 15% PEG 6000 treatment occurred in Siakraya by 13.21%, Sertani 1 by 7.12%, Indragiri by 5.28% and IR64 by 3.93% compared to the control. The increase in H_2O_2 content occurs because H_2O_2 is not immediately decomposed in the process of photosynthesis resulting in the accumulation of toxic H_2O_2 in plants (Hasanuzzaman et al., 2019).

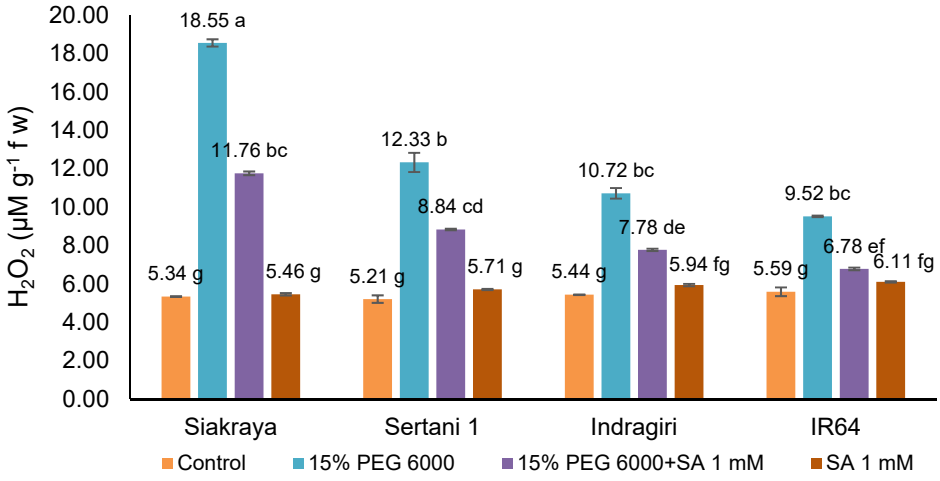

Figure 5. Response of Hydrogen Peroxide Content (H_2O_2) under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

The 15% PEG 6000 + SA 1 mM treatment generally decreased the content of H_2O_2 compared to the 15% PEG 6000 treatment, the decrease occurred in Siakraya by 6.79%, Sertani 1 by 3.49%, Indragiri by 2.94%, and IR64 by 2.74%. The decrease in the content of H_2O_2 was due to the addition of salicylic acid in the drought stress treatment which helped maintain the stability of the membranes in the plant. Salicylic acid also increases the rate of photosynthesis, increases the synthesis of secondary metabolism, and regulates the activity of antioxidant enzymes so that it is possible to reduce excess ROS production (Hasanuzzaman et al., 2017).

Hydrogen peroxide is one of the ROS product molecules that can cause oxidative damage to proteins, DNA, and lipid peroxidation. One of the main cells responsible for ROS production is the chloroplast (Sarker & Oba, 2020). During photosynthesis, energy from sunlight is fixed and transferred to two light-harvesting complexes (photosystem II and photosystem I) in the chloroplast membrane. In addition, the electron transport components of the thylakoid chloroplasts on the PSI side such as the Fe-S center and thioredoxin can be reduced automatically resulting in a reduction to form superoxide (O_2-) and H_2O_2. The rubisco enzyme, which carboxylatesribulose-1.5-bisphosphate (RuBP) during carbon assimilation, also uses oxygen to oxygenateribulose-1.5-bisphosphate. This reaction produces glycolate which is then transported from the chloroplast to the peroxisomes where they are oxidized by glycolate oxidase to produce H_2O_2. The mitochondrial electron transport chain is also responsible for ROS production under normal conditions, although to a lesser extent than chloroplasts and peroxisomes in the photosynthetic process (Nahar et al., 2016).

The effect of drought stress dan salicylic acid treatment on antioxidant gene and abiotic stress related gene

Gene expression of OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic was used as a research parameter to see the response of antioxidant enzyme activity to drought stress in all varieties when compared to OsACTIN, considering that OsACTIN was used as a housekeeping gene which was used as an internal control for gene expression analysis that had no effect on stress. The increase in gene expression in the 15% PEG 6000 treatment occurred in the expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes compared to the control treatment (Fig. 6), but the *APX Cytosolic* gene expression has a lower expression level in the control treatment.

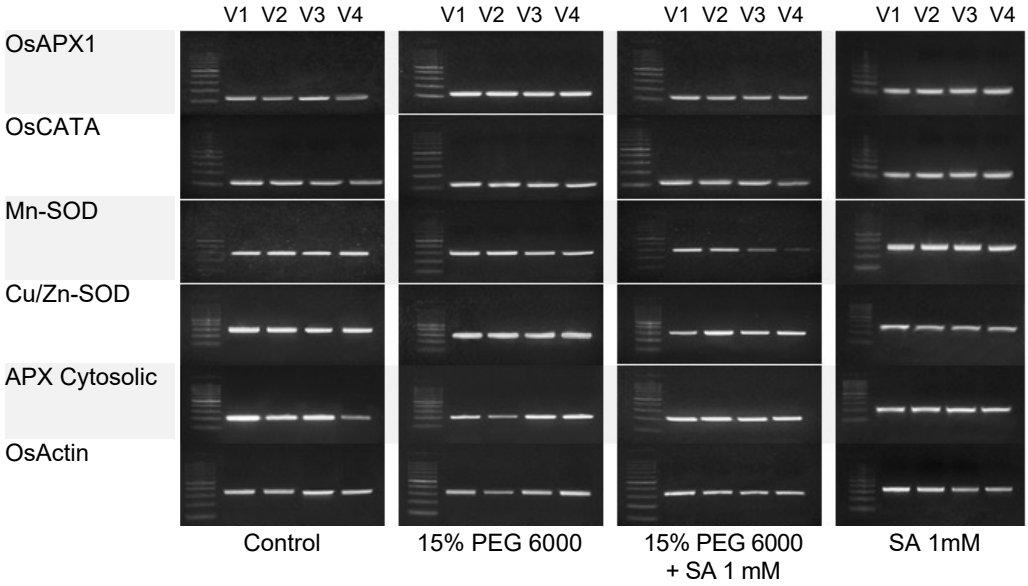

Figure 6. Expression of OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic genes were performed from total RNA samples for PCR analysis were isolated from leaf under several condition at 8 DAT. V1: Siakraya, V2 : Sertani 1, V3: Indragiri, and V4: IR64.

The varieties that had an average volume increase in band thickness were higher in the 15% PEG 6000 compared to the control treatment, namely the Siakraya and Sertani 1 varieties. In the treatment of 15% PEG 6000 + SA 1 mM, high gene expression values were obtained in APX Cytosolic, Cu/Zn-SOD, and OsCATA, but OsAPX1 and Mn-SOD had lower gene expression levels. The Sertani1 had an average volume increase in band thickness higher than other varieties in the 15% PEG 6000 + SA 1 mM treatment. In the 1 mM SA treatment, the expression of OsCATA, Cu/Zn-SOD, and Cytosolic APX genes also had average expression levels that were almost the same as the 15% PEG 6000 + SA 1 mM treatment, but the average expression level increased in OsAPX1 and Mn-SOD genes compared with 15% PEG 6000 + SA 1 mM treatment.

Increased expression of the OsAPX1 and Mn-SOD genes occurred in the 1 mM SA compared to the 15% PEG 6000 + SA 1 mM treatment. This may be because the application of salicylic acid to plants without stress can upregulate several ROS-related genes by activating ROS metabolic pathways, but when salicylic acid is treated on plants with drought stress, salicylic acid responds lower. The addition of salicylic acid to plants can directly increase the activity of antioxidant enzymes in an effort to increase protection against oxidative stress and increase the salicylic acid content in cells (Mostofa et al., 2020).

The sizes of the DNA fragments of the OsCATA and OsAPX1 genes in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM, were between 100–200 bp. The sizes of the DNA fragments of the Mn-SOD, Cu/Zn-SOD, and APX Cytosolic genes in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM, were between 250–300 bp, 250–350 bp, and 150–250 bp respectively. The sizes of the DNA fragment of the OsACTIN gene in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, or SA 1 mM, were between 300–400 bp.

Rice plants adapting to drought stress require a number of genes to be expressed in plant cells, including genes involved in signaling pathways (Nahar et al., 2016). These genes include OsCATA, OsAPX, Mn-SOD, Cu/Zn-SOD, and Cytosolic APX. In addition, during abiotic stress conditions, salicylic acid becomes a signal for plants to activate the expression of defense genes from stress. Salicylic acid also interacts with other signaling molecules, such as ABA, to coordinate the maintenance of membrane stability in plants under stress conditions. Salicylic acid also increases the rate of photosynthesis, increases the synthesis of secondary metabolism, and regulates the activity of antioxidant enzymes, impacting the activity of ROS which are toxic, so it is possible to reduce excess ROS production due to stress conditions (Hasanuzzaman et al., 2017).

Drought stress causes a reduction in CO_2 intake in plants resulting in an accumulation of O_2 which causes the formation of ROS. ROS may cause severe damage to metabolic processes in photosystem I and photosystem II. Efforts to reduce ROS production in rice plants are by activating signal transduction such as salicylic acid to help produce and activate enzymatic antioxidant systems, such as CAT, APX, and SOD. SOD forms the first line of defense against drought-induced ROS under drought conditions, in which SOD dismutase superoxide radicals into H_2O_2, then CAT reacts with H_2O_2 to catalyze the formation of H_2O and O_2 and APX decomposes H_2O_2 into H_2O by involving GR, MDHAR, DHAR in the AsA/GSH cycle. (Das & Roychoudhurry, 2014; Sarker & Oba, 2018a, 2018b).

Expression of OsAB13 and OsLEA genes is generally used as a research parameter to see whether there is the resistance of rice plants in all varieties to drought stress compared to control treatments.

Based on Fig. 7, the expression of the OsACTIN gene is used as a housekeeping gene which is used as an internal control for the analysis of gene expression that has no effect on stress. The figure shows that several plant varieties have resistance to drought stress on the expression of the OsAB13 gene. The rice variety with high expression of the OsAB13 gene in the 15% PEG 6000 compared to the control treatment was Indragiri, while Siakraya and Sertani 1 had lower gene expression levels than the control treatment, and OsAB13 in 1R64 was not expressed in the 15% PEG 6000 treatment. The expression level of OsAB13 which appeared in the 15% PEG 6000 treatment showed that Indragiri, Sertani 1, and Siakraya varieties had a correlation of resistance to drought stress in the OsAB13 gene. The OsAB13 (abscisic acid insensitive 3) gene has an important role in the development of plant growth against drought stress because its expression increases when plants suffered drought stress (Vashisth et al., 2021).

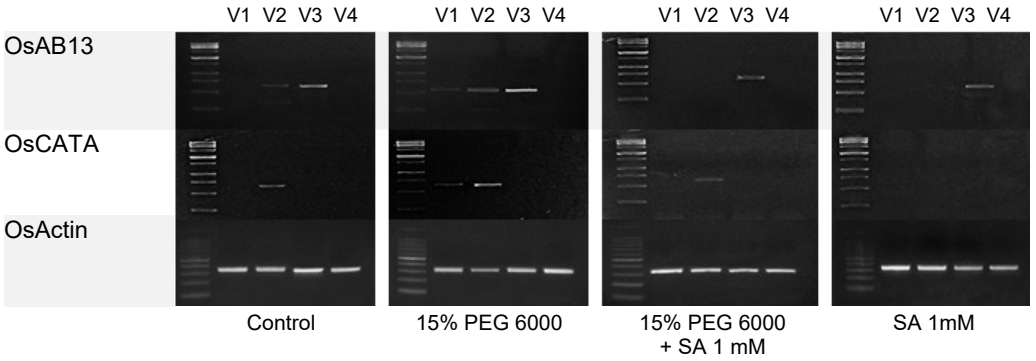

Figure 7. Expression of of OsAB13 and OsLEA genes were performed from total RNA samples for PCR analysis were isolated from leaf under several condition at 8 DAT. V1: Siakraya, V2 : Sertani 1, V3: Indragiri, and V4: IR64.

In the 15% PEG 6000 treatment, only the Sertani 1 variety had a high expression level of OsLEA gene compared to the control treatment, while the gene was not expressed in Siakraya, Indragiri, and IR64 varieties in the 15% PEG 6000 treatment. The expression levels of the OsLEA gene in the 15% PEG treatment 6000 indicate that Sertani 1 has a correlation of resistance to drought stress in the OsLEA gene. The OsLEA (late embryonic proteins abundant) gene has an important role in drought stress. LEA enzymes are important in increasing plant tolerance to drought in correlation with abscisic acid and other hormones as a form of signaling to drought stress (Wang et al., 2007).

CONCLUSIONS

The response to the treatment and variety of rice plants significantly affected plant height, root length, total chlorophyll, and H_2O_2 content. Increased expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes occurred in the 15% PEG 6000 treatment compared to the control treatment. The 15% PEG 6000 + SA 1 mM treatment had high gene expression values in APX Cytosolic, Cu/Zn-SOD, and OsCATA, but had lower gene expression levels of OsAPX1 and Mn-SOD. In the 1 mM SA treatment, the gene expression of OsCATA, Cu/Zn-SOD, and APX Cytosolic had an expression level that was almost the same as that of the 15% PEG 6000 + SA 1 mM treatment, but the expression

level of OsAPX1 and Mn-SOD increased. The rice variety Sertani 1 expressed the OsLEA gene as an indicator of resistance to drought stress, while the OsAB13 gene was expressed in rice varieties Indragiri, Sertani 1, and Siakraya as an indicator of resistance to drought stress. Based on this study, it is necessary to carry out further research by adding other research variables to support current data such as the content of antioxidant enzymes during drought stress or any study related to salicylic acid treatment.

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