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Characterization of rice varieties under salinity level and the response of defense-related genes during the germination stage

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Abstract. *Hartatik S, Rozzita N, Wibowo S, Choirunnisa E, Sakanti SAS, Puspito AN, Ubaidillah M. 2024. Characterization of rice varieties under salinity level and the response of defense-related genes during the germination stage. Biodiversitas 25: 1536-1543.* Climate change causes sea level rise, leading to an increase in soil salinity. In the germination phase, rice plants are more sensitive to salinity stress. Under salinity stress, plants produce an increased content of Reactive Oxygen Species (ROS). Local rice (*Oryza sativa*) has complex gene properties to survive in salt-stress conditions. This research aims to study the potential of salt tolerance at the germination stage and provide a molecular analysis based on antioxidant genes in local rice. This study uses a factorial completely randomized design. The first factor is rice varieties (*IR64*, *Silaun*, *Ime Buci*, *Me Klan*, *Me Likan*, *Me Meak*, *Me Taek*, and *Umak Klete)*, while the second factor is the salt treatments (0, 100, 200, 300, 400, 500, and 600 mM NaCl). The results showed that *Ime Buci* became the most tolerant variety to salinity stress due to its ability to survive and germinate at the highest NaCl concentration (400 mM NaCl). In addition, the H_2O_2 content and Malondialdehyde (MDA) values of the *Ime Buci* variety were lower compared to the *IR64* variety. The expression of antioxidant genes (*Mn-SOD, Cu/Zn SOD, Cytosolic APX, OsAPX1, CAT, OsCATA*, and *GPOD*) was used as observational variables in this study. The results of antioxidant gene expression showed that the Ime Buci variety activated genes more than *IR64* variety based on real-time PCR. However, it still needs more investigation to analyze the potential of *Ime Buci* in salt conditions during the vegetative and generative stages to get more information about rice's mechanism in terms of tolerating stress conditions.

Keywords: Antioxidant genes, reactive oxygen species, rice, salt stress

INTRODUCTION

Rice is one of the most important staple foods for more than half of the world's population (Zhao et al. 2020). The demand for high-quality, high-yielding rice has increased rapidly in recent years as the population has grown. The Indonesian Ministry of Agriculture targets rice production of 55.20 million metric tons. This target is a challenge for the Ministry of Agriculture because rice production in 2021 has decreased compared to 2020. Rice production in 2021 is 54.42 million tons, down 233.91 thousand tons, or 0.43%, compared to 2020 rice production of 54.65 million tons (Statistics Indonesia 2022). The decline in rice production is caused by climate change. Climate change causes sea level rise, increasing soil salinity (Eswar et al. 2021). There is a high increase in evaporation, causing the flow of water from the soil surface containing seawater to the surface so that many agricultural lands in coastal areas experience an increase in salinity or salinization (Sukarman et al. 2018). Indonesian agricultural land has different levels of salinity, such as in arid and semi-arid areas where Indonesia's salinity is due to seawater intrusion for more than four months to a year, with soil nutrient levels varying between 8% and 15% (Sopandie 2014).

Salt stress in plants will cause plants to make an adaptation that causes changes in morphology, physiology, and biochemistry (Shahid et al. 2020). Salinity stress conditions in rice plants can have a negative impact so plants experience osmotic stress, oxidative stress, nutrient imbalance, and ion toxicity. The excessive content of Na^+ and Cl^- ions causes seed poisoning and inhibits the process of root formation (Isayenkov and Maathuis 2019). The imbalance in the absorption of water and nutrients by the roots results in a decrease in growth, especially during the germination phase (del Carmen Martínez-Ballesta et al. 2020). Rice plants are one of the plant groups with a medium level of tolerance to salinity stress. Salinity stress can raise osmotic pressure, which lowers water potential in plant cells and tissues, reduces water absorption by roots, and resulting in a slow pace of growth. Excessive salt in plant cells and tissues can hinder germination, photosynthesis, and the balance of plant nutrients (Quintao et al. 2023). These conditions can affect the growth and development of rice plants especially in the germination phase, which is characterized by a decrease in root length, plant height, and number of leaves, causing the plant to die.

One of the efforts to answer this challenge is the need to characterize rice as having superior genetic resistance. Local rice has special genetic characteristics depending on the distribution area (Wei et al. 2021). These genetic characteristics can be a very important asset in the development of plant breeding to produce tolerant rice

genotypes. The level of plant resistance to environmental stress will be regulated by the expression of several genes related to resistance traits (Imran et al. 2021; Herawati et al. 2024). One of the genes coding for resistance to environmental stress can be expressed through the expression of antioxidant genes. Antioxidant genes play an important role in reducing Reactive Oxygen Species (ROS) compounds formed due to environmental stress (Sachdev et al. 2021). Breeding salinity-tolerant rice varieties at the germination stage can help protect rice from permanent damage in the early stages of growth and can increase rice yields. This can produce new superior rice genotypes that are resistant to the effects of climate change and produce high production to meet future food needs. Salinity stress especially NaCl has a dramatic effect on rice and the ability of rice plant on salinity also different. Basically rice has a critical point on salt concentration between 50 to 300 mM NaCl in the germination stage (Chen et al. 2018; He et al. 2019). This study aimed to understand the potential of salt tolerance at the germination stage and provide a molecular analysis based on plant defence-related genes such as antioxidant enzymes in local rice. The local rice varieties used in this research were from Timor Leste, which has had extreme geographical conditions and led plants to enhance their defense mechanisms against several abiotic stresses, such as salt. This research also used a high level of salinity up to 600 mM NaCl to discover the highly resistant variety.

MATERIALS AND METHODS

Location and time

This research was conducted at the Agrotechnology Laboratory, Faculty of Agriculture, University of Jember, East Java, Indonesia from November 1 to February 17, 2023.

Plant materials and salt treatment

The local rice varieties used in this study were *IR64* (control), *Silaun, Ime Buci, Me Klan, Me Likan, Me Meak, Me Taek*, and *Umak Klete*. These rice seeds were obtained from Ministry of Agriculture and Fisheries (MAF) East Timor. The seeds are pre-oven at 55°C for 2 days. Then soak in a 1% NaCl solution for 20 minutes. Then it was washed with sterile water, placed on a tissue, and treated with a NaCl solution. The NaCl concentrations were 0 mM (control), 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, and 600 mM NaCl. A total of fifty seeds were used in each salinity treatment and placed in a petridish containing 25 mL of 100-600 mM NaCl solution or distilled water for control. Rice seeds were germinated at 28°C in the dark for 10 hours and in the light for 14 hours. Furthermore, the best rice variety that is tolerant to high salinity stress was selected and compared with *IR64* (control).

Relative germination potential and relative germination index

Observations were made to determine the potential and relative germination index. Calculations were made on the 9th day. The number of seeds that germinated was counted every day for statistical analysis. Salt stress tolerance was assessed using two parameters: Relative Germination Potential (RGP), defined as RGP = N1/N2 × 100%, where *N1* is the number of treated seeds that germinated on day 9 and *N2* is the number of control seeds that germinated on day-5; the days differed because the seeds in the control group ended up germinating on day-5.

Relative Germination Index (RGI), calculated as ∑(Gt/Dt), where Gt is the germination percentage on each day and Dt is the number of days it took the seeds to germinate (Duan et al. 2022). Germinating rice seeds are marked by the emergence of shoots. Based on the gradient level of salinity concentration, rice plants are classified into five groups: 80-100% is very tolerant of salinity. 60-80%: Tolerant to salinity (3). 40-60%: Moderately tolerant of salinity (4). 20-40%: Sensitive to salinity, and (5). 0-20%: Very sensitive to salinity (Duan et al. 2022).

Hydrogen Peroxide (H_2O_2) content and Malondialdehyde (MDA) value

Hydrogen peroxide (H_2O_2) analysis was carried out by homogenizing rice plant leaves (100 mg) in 1000 µL of 0.1% Trichloroacetic Acid (TCA) solution. The sample was then transferred into a 1.5-mL microtube and centrifuged for 15 minutes at 12,000 rpm. Then 0.5 µL of supernatant was added using a buffer containing 1 mL of 1 M potassium iodide (KI) and 0.5 µL of 10 mM phosphate buffer, pH 7. Incubate the solution for 30 minutes at room temperature and take measurements using a spectrophotometer at a wavelength of 390 nm (Christou et al. 2014).

MDA analysis was carried out by homogenizing rice plant leaves (100 mg) using 5,000 µL of 0.1% Trichloroacetic Acid (TCA) solution. The sample was then transferred into a 1.5-mL microtube to be centrifuged for 10 minutes at 12,000 rpm at 4°C. Add 4,000 µL TBA to 1000 µL supernatant and incubate for 30 minutes at 90°C. Measure the intensity results using a spectrophotometer with a wavelength of 532 nm and 600 nm on a spectrophotometer (UV-2550). The formula for calculating the MDA value can be as follows (Habib et al. 2020):

$$\text{MDA level (nmol)} = \Delta\,(\text{A 532 nm-A 600 nm})/1.56 \times 105$$

Gene expression analysis

The genes for antioxidant primers (*Mn-SOD, Cu/ZnSOD, Cytosolic APX, OsAPX1, CAT, OsCATA*, and *GPOD*) exhibited expression (Kim et al. 2018). *IR64* rice seeds and the best germination were collected nine days after the salinity stress treatment. The phases of gene expression analysis were RNA isolation, cDNA synthesis, and PCR. Total RNA was extracted from the callus using the *Ribospin*™ Plant Kit method (*GeneAll*). GoTaq® Green Master Mix kit (Promega) enhanced the target cDNA. 0.5 g of total RNA was treated using the ReverTra Ace® RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan). 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 minutes and reverse transcription reaction. In real-time PCR applications, an Eco™ Real-Time PCR System (Illumina, Cat. EC-900-1001, CA, United States) has been used with 2X Multi-Star OneStep qRT-

PCR Master Mix (BIOFACT, Cat. RQ351-50h, Seoul, Korea). Specific genes have been used with OsActin as housekeeping gene.

Data analysis

The data obtained was then analyzed using Analysis of Variance (ANOVA). If the results are significantly different, further analysis is carried out with Duncan's Multiple Range Test (DMRT) with $p<0.05$.

RESULTS AND DISCUSSION

Relative germination potential and relative germination index

Relative Germination Potential (RGP) is the number of treated seeds that germinated on day 9 divided by the number of control seeds that germinated on day 5. The Relative Germination Index (RGI) is the percentage of germination per day divided by the number of days needed for the seeds. RPG and RGI observations were carried out to determine the germination ability of each rice variety seed to adapt to salinity treatment. The addition of different salinity treatments and the use of different varieties gave different responses to the ability of rice plants to germinate.

Germination of rice plants under conditions of salinity stress showed growth inhibition (Figure 1). Comparing the average salinity concentrations yielded different effects on relative germination potential (Table 1) and relative germination index (Table 2). On the parameters of relative germination potential, it is known that the *IR64* variety has a value of 91.85% at 200 mM, but *IR64* wasn't able to survive at the higher concentration of salt. Meanwhile, the *Ime Buci* variety at 200 mM has a value of 94.52% and has become the most tolerant variety to salt since it can still germinate up to 400 mM of salt with a value of 13.00%. It was proven that the *Ime Buci* variety was able to tolerate high-salinity stress better than other varieties.

Hydrogen Peroxide (H_2O_2) content and Malondialdehyde (MDA) value

Hydrogen peroxide (H_2O_2) content and Malondialdehyde (MDA) values were carried out to determine the level of damage in rice plant tissue. The *IR64* rice variety is an Indonesian rice variety that has a moderate level of resistance to salinity stress, while the *Ime Buci* variety is a Timor Leste rice variety that has the highest level of resistance to salinity stress. The rice varieties *IR64* and *Ime Buci* were able to germinate and start to generate shoots and leaves at a salt concentration of 100 and 200 mM, respectively. The shoots and leaves will be analyzed for hydrogen peroxide (H_2O_2) content and Malondialdehyde (MDA) values to determine the level of damage in rice plant tissue.

Under salt stress, plants produce large amounts of ROS. Regarding the parameter of H_2O_2 content, the production of ROS content increased in rice varieties *IR64* and *Ime Buci* during salinity stress, but the application of salt treatments had no significant effect on the increase of ROS when compared to controls. In the *IR64* variety, the H_2O_2 concentration increased by 5.75 and 6.80 µmol/g at 100 and 200 mM NaCl concentrations, respectively. In Figure 2, the increasing content of H_2O_2 in the *Ime Buci* variety was 4.73 and 6.38 µmol at 100 and 200 mM NaCl, respectively.

The higher ROS production affects the increase in MDA accumulation in rice plant tissue. In terms of the MDA value parameter, although the application of NaCl had no significant effect compared to the control, the MDA value still increased in rice varieties *IR64* and *Ime Buci* (Figure 3). The values of MDA production in the *IR64* variety under 100 and 200 mM NaCl were 0.92 and 1.66 µmol/g, respectively. Meanwhile, in the *Ime Buci* variety, the MDA production increased by 0.36 and 0.47 µmol/g at 100 and 200 mM of NaCl, respectively (Figure 3).

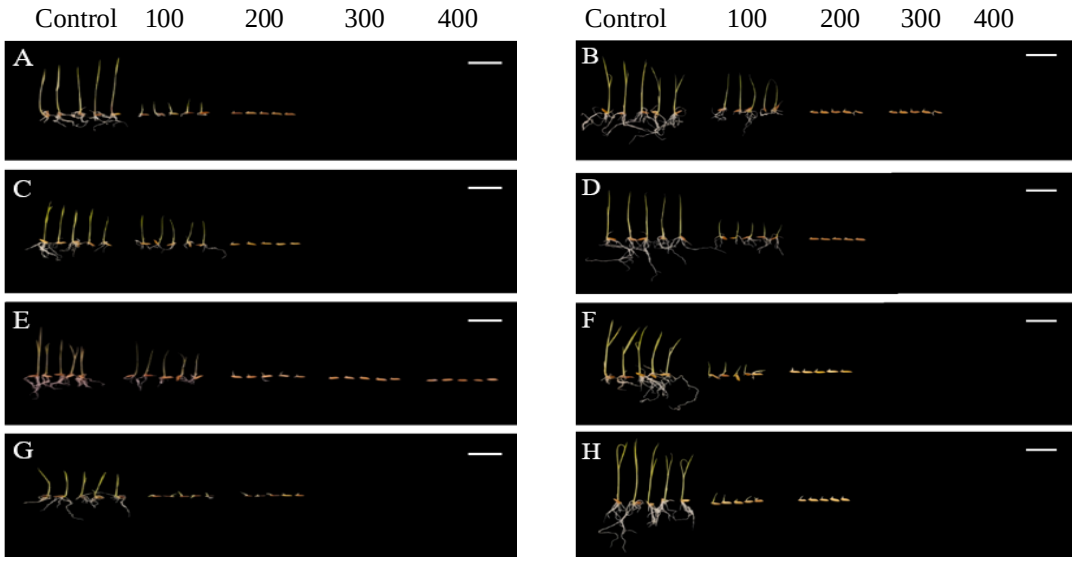

Figure 1. Effect of NaCl (mM) on rice plants after 9 days of treatment (scale bar: 2.5 cm). Pictures of rice varieties: A. *IR64*, B. *Silaun*, C. *Ime Buci*, D. *Me Klan*, E. *Me Likan*, F. *Me Meak*, G. *Me Taek*, H. *Umak Klete*

Table 1. Effect of NaCl treatment on Relative Germination Potential (RGP) of rice plants after 9 days of treatment. Different letters show significantly different results on the 5% DMRT test

Varieties	Relative germination potential			
	NaCl concentration	Mean±SD (%)	Range (%)	CV (%)
IR64	100 mM	97.04±1.28^{d}	95.56-97.78	1.32
	200 mM	91.85±1.28^{c}	91.11-93.33	1.40
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Silaun	100 mM	97.04±1.28^{d}	95.56-97.78	1.30
	200 mM	91.11±2.22^{c}	88.89-93.33	2.44
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Ime Buci	100 mM	95.90±3.52^{d}	91.84-97.96	3.67
	200 mM	94.52±1.22^{c}	93.75-95.92	1.29
	300 mM	34.92±1.71^{b}	33.33-36.73	4.90
	400 mM	13.00±3.03^{a}	10.42-16.33	23.28
	500 mM	-	-	-
	600 mM	-	-	-
Me Klan	100 mM	93.44±3.28^{d}	91.43-97.22	3.28
	200 mM	69.13±3.27^{c}	65.71-72.22	6.19
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Likan	100 mM	97.15±1.22^{d}	95.74-97.87	1.25
	200 mM	79.28±1.38^{c}	78.26-80.85	1.74
	300 mM	57.17±3.85^{b}	53.19-60.87	6.73
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Meak	100 mM	79.24±9.15^{d}	71.43-89.80	11.55
	200 mM	72.35±3.84^{c}	68.09-75.51	5.30
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Taek	100 mM	75.55±2.94^{d}	72.92-78.72	3.89
	200 mM	59.44±1.05^{c}	58.33-60.42	1.76
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Umak Klete	100 mM	96.51±2.39^{d}	93.75-97.92	2.48
	200 mM	76.94±1.87^{c}	75.00-78.72	2.43
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-

Table 2. Effect of NaCl treatment on Relative Germination Index (RGI) of rice plants after 9 days of treatment. Different letters show significantly different results on the 5% DMRT test

Varieties	Relative germination index			
	NaCl concentration	Mean±SD (%)	Range (%)	CV (%)
IR64	100 mM	96.42±0.43^{c}	95.93-96.67	0.44
	200 mM	51.30±0.16^{b}	51.11-51.39	0.31
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Silaun	100 mM	97.16±0.43^{c}	96.67-97.41	0.44
	200 mM	25.56±0.28^{b}	25.28-25.83	1.09
	300 mM	-	-	-
	400 mM	-	-	-
	500 Mm	-	-	-
	600 mM	-	-	-
Ime Buci	100 mM	93.70±1.70^{c}	91.85-95.19	1.81
	200 mM	85.93±0.74^{b}	85.19-86.67	0.86
	300 mM	11.85±0.13^{a}	11.78-12.00	1.08
	400 mM	0.78±0.19^{a}	0.62-0.99	24.12
	500 mM	-	-	-
	600 mM	-	-	-
Me Klan	100 mM	59.14±1.13^{c}	58.15-60.37	1.91
	200 mM	17.41±0.42^{b}	16.94-17.78	2.44
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Likan	100 mM	96.17±0.43^{c}	95.93-96.67	0.44
	200 mM	34.72±0.28^{b}	34.44-35.00	0.80
	300 mM	22.13±0.89^{a}	21.11-22.78	4.04
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Meak	100 mM	89.75±7.31^{c}	84.81-98.15	8.14
	200 mM	61.60±0.77^{b}	60.74-62.22	1.25
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Me Taek	100 mM	85.93±1.48^{c}	84.44-87.41	1.72
	200 mM	16.48±0.16^{b}	16.39-16.67	0.97
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-
Umak Klete	100 mM	70.00±0.37^{c}	69.63-70.37	0.53
	200 mM	27.96±0.16^{b}	27.78-28.06	0.57
	300 mM	-	-	-
	400 mM	-	-	-
	500 mM	-	-	-
	600 mM	-	-	-

Gene expression analysis of antioxidant enzyme

The expression of *OsActin*, *Mn-SOD*, *Cu/ZnSOD*, *Cytosolic APX*, *OsAPX1*, *CAT*, *OsCATA*, and *GPOD* genes was used as observational variables in this study to determine the response of antioxidant activity to differences in rice varieties and salinity stress concentrations (Figure 4). The visible gene expression will be compared with *OsActin*, where *OsActin* is used as a housekeeping gene, which is an internal control gene for the analysis of gene expression that does not respond to differences in the substance concentration of NaCl and rice plant varieties. In the 100 mM NaCl treatment on both *IR64* and *Ime Buci* varieties, the results of antioxidant gene expression showed that the *Ime Buci* variety activated those genes (*Mn-SOD*, *Cu/ZnSOD*, *CAT*, and *OsCATA*) more than *IR64* variety based on real-time PCR. This shows that the activity of antioxidant genes in rice varieties *Ime Buci* is greater compared to rice varieties *IR64*.

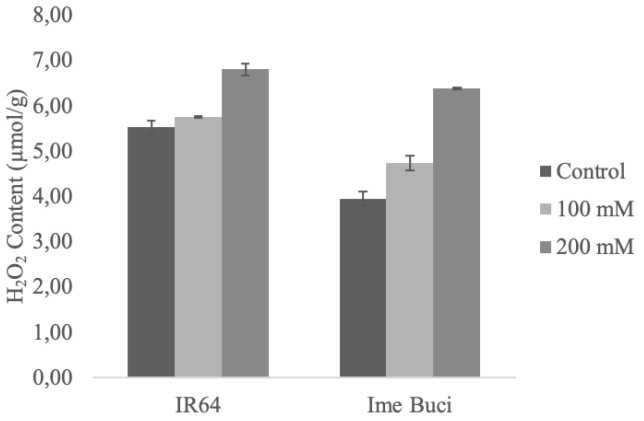

Figure 2. Effect of NaCl treatment on the hydrogen peroxidase (H_2O_2) content of rice plants after 9 days of treatment

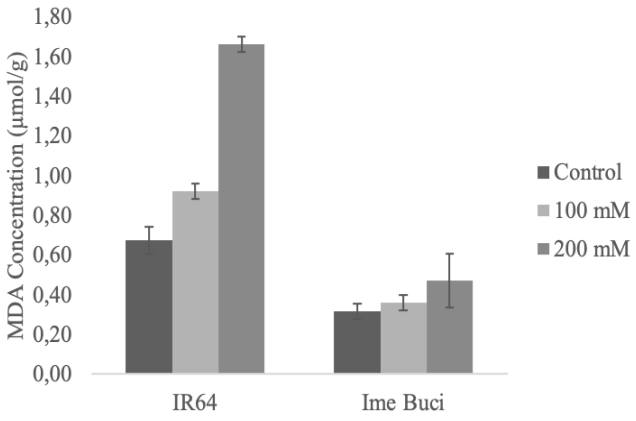

Figure 3. The effect of NaCl treatment on the MDA concentration of rice plants after 9 days of treatment

Discussion

Salinity stress causes osmotic and ionic effects in plants, which causes oxidative stress and depletion of plant cells (Liu et al. 2022). In addition to the toxic effects of certain ions, higher salinity can reduce the water potential in the media, thereby inhibiting the absorption of water by seeds and causing a decrease in germination (Hakim et al. 2010). The plants can defend themselves by maintaining osmotic balance and stabilizing proteins adapting to sustain cell expansion and growth (Nahar et al. 2016).

Salinity stress treatment can increase ROS production in rice plants. Reactive Oxygen Species (ROS) are free radicals found in plant tissues. ROS function as signaling molecules that can respond to various stimuli in plant cells (Niu and Liao 2016). ROS are produced in plant cells such as chloroplasts, mitochondria and peroxisomes (Miller et al. 2010). ROS compounds in plants are O_2, H_2O_2, O_2^- and OH· (Miller et al. 2008). Among the various types of ROS, only H_2O_2 has relatively high stability and a long half-life (Nurnaeimah et al. 2020). Therefore, H_2O_2 is often used as an important parameter in determining stress in plants. H_2O_2 plays an important role in various plant physiological processes including photosynthesis, senescence, stomata movement, cell growth, and development (Nasir et al. 2020).

The higher concentration of salinity affects the production of high ROS in rice plant tissue. Excessive accumulation of ROS causes phytotoxic reactions such as DNA mutations, protein degradation, and carbohydrate and lipid peroxides (Yang et al. 2018). Under normal conditions, plants can maintain low ROS levels due to a balance between antioxidant gene production and ROS production. In addition to increasing ROS production, salinity stress conditions in plants can also cause an increase in MDA values.

Malondialdehyde (MDA) is a substance produced by membrane lipids in response to ROS and can be used as an indicator to assess the level of damage to the plasma membrane and the plant's ability to withstand stress (Zhang et al. 2021). Plants with lower MDA under stress conditions are generally considered to be more tolerant (Ma et al. 2015). The level of MDA is not only related to stress resistance but also indicates a disturbance in the process of photosynthesis (Tulkova and Kabashnikova 2021). MDA accumulation caused by reduced water deficit in plants during salinity stress adversely affects photosynthesis. MDA can play a positive role in activating regulatory genes involved in plant defense and development, as well as protecting cells under conditions of oxidative stress (Morales and Munné-Bosch 2019). Therefore, the mechanism of antioxidant genes plays an important role in plant defense responses to future climate change, especially environmental stress.

Antioxidant genes have an important role in plants to remove ROS such as *SOD*, *APX*, *CAT*, and *GPOD*. In terms of the influence of salinity stress treatment on antioxidant gene expression, it was discovered that there is antioxidant gene activity in rice varieties *IR64* and *Ime Buci* during salinity stress (Figure 4). Superoxide Dismutase (SOD) is one of the most potent intracellular enzymatic antioxidants and catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide (Kurutas 2016). In the results of gene expression seen in the study showed the increased of enzyme activity in the salinity stress treatment, this shows that the administration of salinity stress treatment was able to show the activity of the *Mn-SOD* and *Cu/ZnSOD* antioxidant genes. The two antioxidants are SOD isoforms, which are differentiated, based on their production site, which is Mn-SOD which is located in the mitochondria and Cu/Zn-SOD which is located in the chloroplast, peroxisomes, and cytosol (Mittler 2002). SOD has two similar isoforms that specifically catalyze the dismutation of the superoxide anion to oxygen and water (Kurutas 2016). Increases in SOD activity often correlate with increases in plant tolerance to environmental stresses (Sharma et al. 2012).

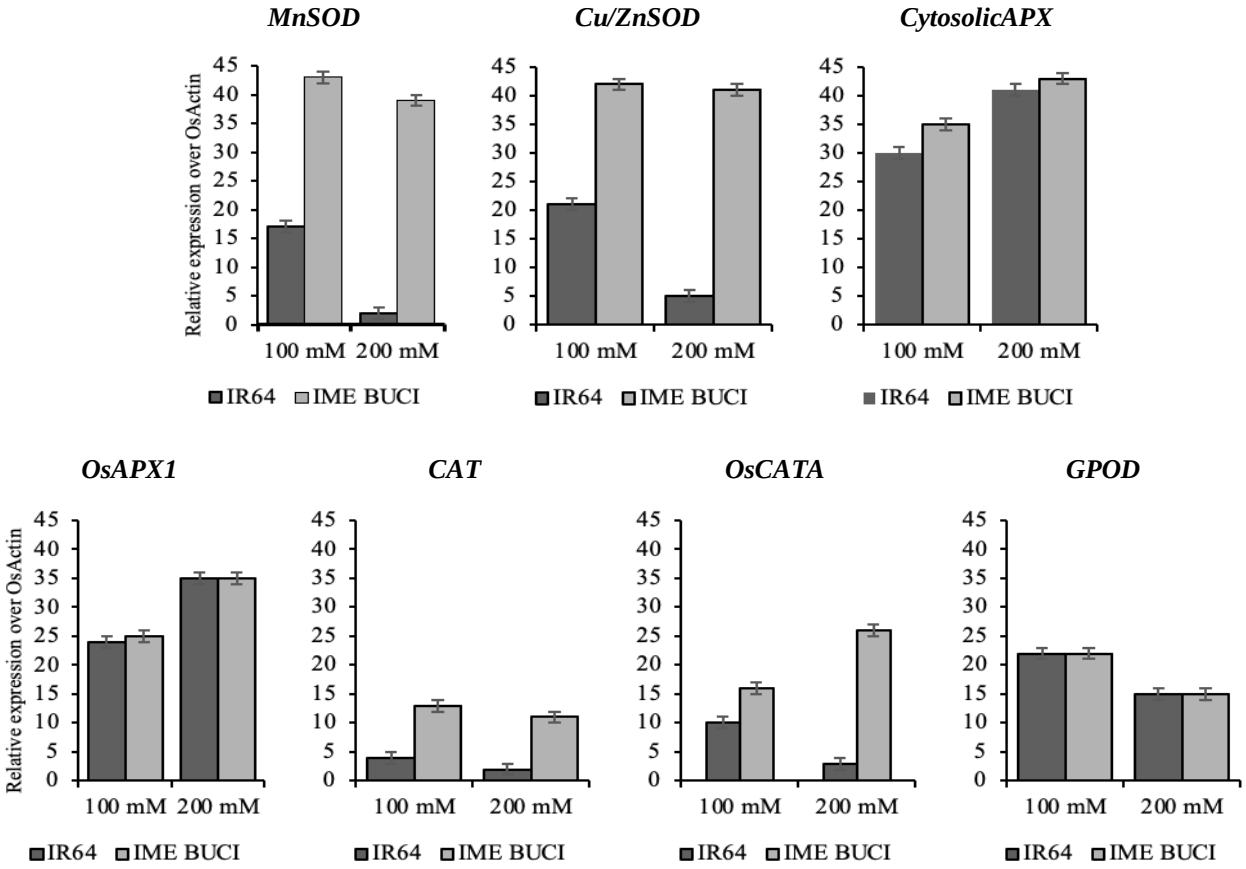

Figure 4. Gene expression profiles after 9 days of treatment with 100mM NaCl and 200 mM NaCl. Relative expression of *Mn-SOD*, *Cu/ZnSOD*, *Cytosolic APX*, *OsAPX1*, *CAT*, *OsCATA*, and *GPOD* were performed from total RNA samples for RT-PCR analysis. OsActin was used as a reference gene and RNA samples were collected from leaves of rice plants

Ascorbate Peroxidase (APX) is one of the most important enzymatic antioxidants in the AsA-GSH cycle to detoxify ROS under stress conditions and has a greater affinity for H_2O_2 to scavenge than CAT (Hossen et al. 2022). APX is located in the cytosol, mitochondria, peroxisomes, chloroplasts, and stroma and thylakoids (Das and Roychoudhury 2014). CAT requires a reducing agent in the form of ascorbic acid to catalyze the conversion of H_2O_2 to H_2O and O_2. The gene expression results shown in the study showed the increased enzyme activity in the salinity stress treatment. This indicates that the salinity stress treatment was able to show the activity of the cytosolic antioxidant genes *APX* and *OsAPX1*. There are several isoforms of APX, one of which is cytosolic APX or cAPX. The role of the cytosolic APX is to respond optimally to the content of H_2O_2 in the cytosol so that it can be converted into H_2O. The cytosolic APX isoform is specific for AsA as an electron donor and less sensitive to AsA depletion than other APX isoforms (Sharma et al. 2012). Rice plants during stress conditions will activate the *OsAPX1* gene which functions to code for APX to convert H_2O_2 into H_2O and O_2. Deficiency of cytosolic APX1 causes a constitutively higher increase in H_2O_2 and induces the expression of many other genes under moderate light stress (Davletova et al. 2005).

Catalase (CAT) is an antioxidant gene that has the same function as APX, that is catalyzing the conversion of H_2O_2 to H_2O and O_2 but does not require a reducing agent. CAT is produced in the cytosol, chloroplasts and mitochondria (Das and Roychoudhury 2014). In the results of gene expression seen in the study showed the increased of enzyme activity in the salinity stress treatment, this indicates that the administration of salinity stress treatment was able to show the activity of the *CAT* and *OsCATA* antioxidant genes. CAT can convert about 6 million molecules of hydrogen peroxide into water and oxygen every minute (Kurutas 2016). CAT is frequently active in the liver and erythrocytes but is also found in all tissues (Sies 2015). Rice plants during stress conditions will activate the *OsCATA* gene which functions to code for CAT to convert H_2O_2 into H_2O and O_2. *OsCATA* is more expressed in young leaves and seeds.

Guaiacol Peroxidase (GPOD) is an antioxidant that can catalyze H_2O_2 products into H_2O. GPOD is located in the cell wall, apoplast, and vacuole (Hasanuzzaman et al. 2020). GPOD is found in many plants, animals and microbes. In the results of gene expression seen in the study showed the increased enzyme activity in the salinity stress treatment, this indicates that the administration of salinity stress treatment was able to show the activity of the *GPOD*

antioxidant gene. GPOD requires reducing agents in the form of phenolic compounds to catalyze the conversion of H_2O_2 to H_2O and O_2. GPOD activity acts as a biomarker for oxidative reactions under sub-lethal metal toxicity in plants (Sharma et al. 2012).

The level of salinity concentration gradient during the germination phase of rice affects the decrease in potency and relative germination index. The level of salinity concentration gradient in the rice germination phase also molecularly affects the increase in the production of ROS content in plants so that it has an impact on high levels of cell damage causes the induction of MDA values, and activates the work of resistance genes in rice plants as a self-defense response.

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