Journal of Plant Process and Function, Vol. 14, No. 73, Year 2026 **page: 45-55**

Research Article

Study of gene expression in a mutant rice cultivar under salt stress

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Abstract

Salinity stress dramatically impacts growth and production in rice. Expanding the genetic diversity of rice varieties through artificial mutation should "lead to discovering" new salt tolerant varieties. In this study, a potential salt-tolerant advanced mutant was compared to its high-quality background, Hashemi, to identify its improved salinity response mechanism. Therefore, various effective biochemical criteria related to salinity tolerance and the expression of two genes encoding superoxide dismutase (*SOD*) and glutathione (*GR*) were evaluated under 100 mM and 150 mM salinity, in comparison to no stress condition at 3 and 5 days after the application of stress. A significant difference was identified in proline, glycine betaine, soluble sugars, and soluble protein content between the wild-type and the mutant. Proline content was significantly higher in the mutant under both salinity levels and time points in comparison to the wild-type background .The mutant Hashemi rice showed greater tolerance to salt stress compared to wild-type Hashemi, making it a more dynamic cultivar. This greater resistance to salt stress in the mutant in comparison to its wild-type background was at least partially attributed to its increased ability to produce proline and glycine betaine, as well as heightened expression of antioxidant genes (*SOD* and *GR*) in two sampling stages. These traits help the mutant variety to survive and grow better under salt stress conditions. Selection of this variety in breeding programs could lead to the development of varieties with high tolerance and better performance in saline areas and help create effective strategies for rice cultivation in these areas, especially under the impact of climate change. The results of these findings indicate a significant survival and growth advantage for the mutant variety in saline environmental conditions. Therefore, selecting this variety in breeding programs can result in the creation of more tolerant and better-performing rice varieties in areas with salt stress. This is especially important for enhancing the sustainability of rice cultivation in saline and critical areas by combining mutant traits with local genotypes to improve yield and efficiency of saline water use. Additionally, combining metabolic traits (such as proline and glycine betaine) and the expression of antioxidant genes like *SOD* and *GR* in optimized lines can further improve salt tolerance.

Keywords: Rice, Abiotic stress, Salinity, Gene expression

Introduction

Soil salinity is a growing global concern, with detrimental effects on plant development leading to reduced crop productivity. A decade ago, it was estimated that 20% of irrigated land was affected by soil salinity, and if left unchecked, saline soils could encompass more than 50% of the total global irrigated area by 2050 (Singh, 2022). Among cereal crops, rice is the most sensitive to salinity, with approximately one-third of rice-producing areas globally being impacted by salinity. The growth and yield of rice are severely limited under saline conditions, with the responses of rice genotypes varying depending on their growth stages. Rice shows tolerance to salinity during germination and vegetative stages but sensitivity during early seedling and reproductive stages. Salinity stress leads to significant reductions in plant growth, tiller number, biomass accumulation, panicle number, florets

Received: Jun. 17, 2025; Revised: Sep. 29, 2026; Accepted: Dec. 08, 2026; Published Online: Jul. 27, 2026
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per panicle, grain filling percentage, and grain yield. These reductions are primarily caused by ion toxicity, nutrient deficiency, and oxidative stress induced by salinity conditions (Xu *et al*., 2024). Excessive salinity not only hampers the plant's ability to absorb water and nutrients (Gong, 2021) but also triggers a chain of stress responses, including osmotic pressure response, ionic imbalance, and oxidative stress (Liu *et al*., 2022). Salt tolerance in rice: Physiological responses and molecular mechanisms. Plants grown in high salinity undergo various morphophysiological and biochemical changes due to excess ions and osmotic stressors. Specifically, Na^+ and Cl^- toxicity slow down metabolic processes, leading to premature senescence and cell death (Balasubramaniam *et al*., 2023). Under salinity stress, rice plants accumulate high levels of Na^+ ions, generating reactive oxygen species (ROS) that damage nucleic acids and proteins, causing lipid peroxidation, reduced chlorophyll synthesis, and altered enzyme activities in leaves. This ultimately results in reduced plant growth and yield loss (Ahmed *et al*., 2021). Plants utilize the biosynthesis and accumulation of osmolytes as a crucial defense mechanism against osmotic stress and oxidative damage from various stimuli (Ghosh *et al*., 2021). Osmolytes like Pro, GB, and soluble sugars are accumulated by plants to protect themselves from osmotic stress caused by different stressors (Wu, 2018). The damage caused by ROS under salinity can be mitigated through two pathways. One is the antioxidant enzyme system, which boosts the activity of SOD, POD, CAT, etc. (Gerona *et al*., 2019). The other pathway involves the non-enzymatic system, which produces osmoregulatory substances like proline, soluble protein, MDA, etc. (Pongprayoon *et al*., 2023). Superoxide dismutase (SOD), a key enzyme in the antioxidant system of plants, has the ability to convert superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Xu *et al*., 2024). This process serves as the first line of defense for plants against oxidative stress caused by the accumulation of free radicals under saline conditions. Therefore, measuring SOD gene expression can indicate the plant's ability to reduce oxidative damage and maintain oxidative balance under stress conditions (Jhanani *et al*., 2024). Glutathione, a cysteine-containing tripeptide, acts as a protective antioxidant in plants. This compound plays a crucial role in reducing free radicals and decomposing hydrogen peroxide. Assessing the expression and activity of glutathione can offer insight into the plant's defense potential against salt stress (Fang *et al*., 2023). Osmotic compounds like proline and glycine betaine assist plants in retaining water and balancing their osmotic pressure under saline conditions. These compounds stabilize proteins and maintain membrane structure during stressful conditions (Alamri *et al*., 2020). Taking into account the information provided, the objective of this study is to investigate the role and function of SOD and glutathione reductase (GR) genes, in addition to osmotic compounds, in the defense mechanisms of wild and mutant Hashemi rice cultivars against salinity. The ultimate goal is to enhance salinity resistance by enhancing biochemical-genetic characteristics and implementing breeding and crop management tactics.

Materials and methods

In this study, Hashemi genotype rice seeds were used as the susceptible wild-type (Siyeh Chehre *et al*., 2020), and advanced mutant lines (seventh generation) were obtained from the Rice Research Institute of Iran-Rasht. The mutant line was created by gamma irradiation from a cobalt-60 source and was introduced as a line tolerant to salt stress in field evaluations with a salinity of 8 dS (Ebadi, 2018). The experiment was conducted as a factorial split design with three replications. The main factor was the factorial combination of salinity stress and sampling time, and the subfactor was genotype. This was carried out using a hydroponic solution. Seeds were sterilized with a 10% calcium hypochlorite solution and placed in a dark incubator at 23°C for six days for germination. Identically germinated seeds were transferred to Yoshida culture medium (Yoshida *et al*., 1971). The nutrient solution was replaced every three days, and the pH of the solution was adjusted to 7.5 with sodium hydroxide (NaOH) solution. After six days of growth in the normal culture medium, salinity stress was applied by adding 100 and 150 mM sodium chloride for five days. The first sampling was done three days, and the second sampling was done five days after the application of salinity stress from both normal and saline media, and leaf tissue samples were immediately frozen in liquid nitrogen and stored at -80°C for biochemical trait measurements.

RNA extraction: For RNA extraction, one milliliter of BIOZOL buffer was poured into a 1.5 mL tube, and 0.1 g of the sample crushed in liquid nitrogen was added to it. The sample was then shaken by hand and placed at room temperature for 10 to 15 minutes. Next, 200 µL of chloroform was added to the liquid and placed on ice for 15 minutes. The samples were centrifuged for 15 minutes at 4°C and 12,000 rpm. The upper phase of the tube, which contained a clear liquid, was transferred to another tube in an amount of 500 µL. The same amount of cold isopropanol was added, and after shaking 4-5 times, it was placed in a -20 freezer for 20 to 30 minutes. After removing the sample from the freezer, it was centrifuged again for 10 minutes at 4°C at 12,000 rpm. The clear top of the tube was removed, and the pellet formed was separated. 1 ml of ethanol was added to it, and the tubes were centrifuged for 5 minutes at 4°C at 12,000 rpm. The upper phase was removed again, and the RNA pellet was dried at room temperature. Finally, 50 µl of distilled water was added to the pellet. The extracted RNA was transferred to a -80 freezer for storage. The quantity and quality of the extracted RNA were determined using a spectrophotometer and electrophoresis on a 1.5% agarose gel, respectively.

Table 1. Specifications of the primers used

Initiator name	Primer sequence	Length of reaction product	Melting temperature (C°)	NCBI accession number
Actin, FOR	5′-AGCAACTGGGATGACATGGA-3′	176	58	AF-111812
Actin, REV	5′-GCRACATACATRGCWGGSAC-3′		58	
GR, FOR	5′- TCTCAGAGGGACTTCTCTACT -3′	195	60	D78136
GR, REV	5′- AGGCAGTGGTACTCACATGGT -3′		60	
SOD, FOR	5'-CTGGGAATCAATGCAGCCAG-3'	189	58	GI-1002276304
SOD, REV	5'-CAAGACAAGCCAAACCCAGC-3'		60	

cDNA synthesis: cDNA synthesis was then performed using the method suggested by Fermentase Company. The synthesized cDNA was tested using PCR with actin housekeeping gene primers. To make cDNA, each RNA sample was poured into a new tube after being treated with DNase. To each tube containing 11 µL of the contents, 1 µL of oligo dt primer and 0.5 µL of ddH_2O were added until the final volume reached 12.5 µL. The tube was then placed at 65°C for five minutes. Next, four µL of cDNA synthesis buffer x5, 0.5 µL of RNase inhibitor, and two µL of dNTP mixture with a concentration of 10 µM were added, and finally, one µL of reverse transcriptase enzyme was added until the volume of the solution reached 20 µL. The RT reaction was performed for five minutes at 25°C, 60 minutes at 42°C, and 10 minutes at 70°C.

To evaluate the expression pattern of *SOD* and *GR* genes, the iQ5 device from Bio-Rad and the Cyber Biopars kit (Gorgan University of Agricultural Sciences and Natural Resources) were used, which are capable of performing real-time evaluations. To normalize the data, the housekeeping gene actin, which has consistent expression in all treatments, was used. The primers were designed based on information available on the NCBI website and using the Primer 3 software, considering desired characteristics for use in the QRT-PCR method. Information about the primers is provided in Table 1. After the reaction was completed, the data was transferred to REST software for analysis.

Proline Content: To measure proline content, 0.2 g of root tissue is homogenized in 10 ml of 3% sulfosalicylic acid and then centrifuged at 15,000 g for five minutes. Two ml of the supernatant is mixed with two ml of glacial acetic acid and two ml of ninhydrin, then heated at 100°C for 60 minutes. The reaction is terminated by placing it on ice, and extraction is performed with toluene. The absorption spectrum is read at 520 nm. The proline content is calculated based on a standard curve of different proline concentrations (Bates *et al*., 1973).

Glycine betaine: Glycine betaine was measured using the method described by Grieve and Grattan (1983) as follows: 0.5 g of dried plant leaves were ground, and the resulting powder was mixed with 20 ml of double distilled water. The solution was then shaken and left at 25°C for 48 hours. The samples were filtered and stored in the freezer until the next analysis. Next, the test tubes were taken out of the freezer, and the diluted extract was mixed with an equal amount of two normal sulfuric acid. 0.5 ml of the diluted solution was added to test tubes and placed in ice water for one hour. Then, 0.2 ml of cold potassium iodide-iodine reagent was slowly added and mixed by vortexing. The solutions were then refrigerated at zero to four degrees Celsius for 16 hours. Afterward, the samples were transferred to centrifuge tubes and centrifuged at 10,000 rpm for 15 minutes at zero degrees Celsius. The upper phase of the solution was separated using a micropipette, and 9 ml of 1,2-dichloroethane was added. After vortexing for one minute, the absorbance was read at a wavelength of 365 nm.

Total soluble sugars: The measurement of soluble carbohydrates was conducted following the method outlined by Allen (1989). A mixture of 0.1 g of each plant sample was combined with liquid nitrogen and 80% ethanol in a 15 mL Falcon tube, then vortexed and centrifuged at 3000 rpm to separate the phases and eliminate the sugars. All liquids were dried in an oven at 44–47°C, then transferred to distilled water for 48–72 hours. Sediments and pigments were removed using buffers and centrifugation to obtain a sugar solution. Subsequently, the Long-Stark reaction was carried out with phenol-sulfuric acid, and the transmittance reading was taken with a spectrophotometer at 485 nm to determine the amount of soluble sugars using the glucose standard curve.

Measurement of soluble protein: A total of 100 microliters of enzyme extract was transferred into a test tube, and five milliliters of Bradford reagent was added. After two minutes, the absorbance is measured at 595 nm. Based on the standard curve, the protein concentration is calculated in mg/ml (Bradford, 1976).

Statistical methods: All statistical analyses of biochemical data in this study were performed using SAS 9.1 statistical software. Data means were compared using the LSD method, and graphs were created using Excel 2019 software.

Results and discussion

The amount of osmolytes examined in this study (proline, glycine betaine, soluble protein, and soluble sugar) showed a significant difference. The three-way interaction factor of stress at the time of sampling in genotype caused this difference, which was significant

Table 2. Analysis of variance of proline, glycine betaine, soluble sugar, and soluble protein in leaves of wild-type and mutant rice genotypes under salt stress conditions

SOV	df	Soluble protein	Soluble sugar	Glycine betaine	Proline
Block	2	0.0007	0.08	0.0002	0.003
Stress	1	3.59**	4761.35**	0.46**	5.81**
Time	1	0.04ns	581.73**	0.0004ns	3.62**
Stress × Time	1	0.35**	877.28**	0.002ns	3.43**
Error 1	6	0.001	2.32	0.004	0.009
Variety	1	3.54**	1042.53**	0.44**	0.92**
Variety × Stress	1	2.62**	2942.63**	0.17**	0.005ns
Variety × Time	1	0.94**	121.53**	0.023**	2.06**
Variety×Time× Stress	1	0.500**	455.96**	0.041**	1.53**
Error 2	8	0.01	4.13	0.0009	0.008
CV (%)	-	3.76	1.49	6.92	2.05

ns and ** are non-significant and significant at the 1% level, respectively

at the one percent level (Table 2).

The results of the mean comparison showed an increasing trend in the amount of proline in the mutant compared to the wild-type under stress conditions at both sampling stages. The highest amount of proline in the second sampling (5 days after applying salt stress) was obtained in the mutant variety, showing a significant difference from other stress levels and similar conditions in the 3-day sample. This indicates the plant's long-term response to stress and the mutant variety's better ability to manage stress by using proline as a protective metabolite. In plant stress physiology, the accumulation of compatible solutes is generally considered a factor in maintaining osmotic balance in cells. For example, proline accumulation in plants increases salt tolerance. As salinity increased from 0 mM (control) to 100 mM, proline significantly decreased in the Hashemi wild-type (sensitive) but continued to increase in the mutant (resistant) variety with increasing salinity (Figure 1). This observation, along with a previous report, suggests that the stressed Hashemi wild-type did not rely on osmotic regulation as the primary mechanism to cope with salt stress (Thomson *et al*., 2010). In a study, the salt-tolerant field rice Pokkali accumulated less free proline during the first 48 hours of NaCl treatment compared to JC 2304. Meanwhile, the salt-sensitive variety Nipponbare showed unchanged proline levels during this period of salt treatment (Nguyen *et al*., 2021). In field rice *O. sativa*, the accumulation of free proline under salt stress varies between cultivars and growth stages. Several studies have shown that salt-tolerant rice cultivars accumulate more proline than salt-sensitive rice under salt stress conditions (Abdelaziz *et al*., 2018). Proline serves as a source of nitrogen and carbon for plants under severe stress, enhancing plant stress tolerance. Therefore, proline is considered the most effective regulator of osmotic pressure in plants under salt stress. Under stress conditions, proline plays a crucial role in maintaining membrane structure, establishing osmotic adaptation, and preserving enzyme structure in the cell (Masarmi *et al*., 2023). Genotypes that produce more proline may exhibit higher resistance to stress (Mwadzingeni *et al*., 2016). Forlani *et al*. (2019) studied seventeen Italian rice cultivars at the seedling stage and found a correlation between proline content and salt stress tolerance.

The results of the comparison of the averages for soluble sugar osmolytes showed a significant increase in the mutant under salinity stress compared to the wild-type, with the highest levels observed in the sample taken 3 days after stress. The highest amount of soluble sugar was recorded at 150 mM after 3 days of stress (Figure 2), indicating that salinity stress can stimulate an increase in soluble sugars. During the early stages of salt stress, enzymes involved in sugar metabolism pathways, such as sucrose and gluconeogenic enzymes, may be affected, leading to an increase in enzymatic activity and greater production of soluble sugars (Sangwongchai *et al*., 2022). These changes in increased sugar levels can help maintain hydraulic balance, provide energy, and aid in coping with oxidative stress (Kaur *et al*., 2024). These characteristics enable the mutant cultivar to perform better under saline conditions and enhance plant performance. This increase in soluble sugars may be seen as an adaptive mechanism to counteract the negative effects of salinity. Cultivars with better salt tolerance are able to manage their resources more effectively (Xu *et al*., 2024). Most plants synthesize and accumulate osmolytes in response to drought and salinity stress. The accumulation of soluble sugars is crucial for enhancing osmolytes and providing energy for various cellular processes and metabolites (Saleh and Madani, 2015). Plants under salt stress accumulate sugars in their tissues for osmotic regulation, osmotic protection, and carbon storage. Adaptive metabolites like soluble sugars increase in response to stress to regulate osmotic balance and prevent a decrease in leaf relative water content (Garcia-Caparros and Teresa Lao, 2018). The accumulation of sugars under salt stress conditions serves as an adaptive mechanism to improve salt tolerance in plants (Karimian and Samiei, 2021). The accumulation of soluble sugars and proline under stress conditions helps regulate cell osmolarity, preserving biological molecules and membranes (Nanda and Agrawal, 2018).

A significant increase in glycine betaine due to salt

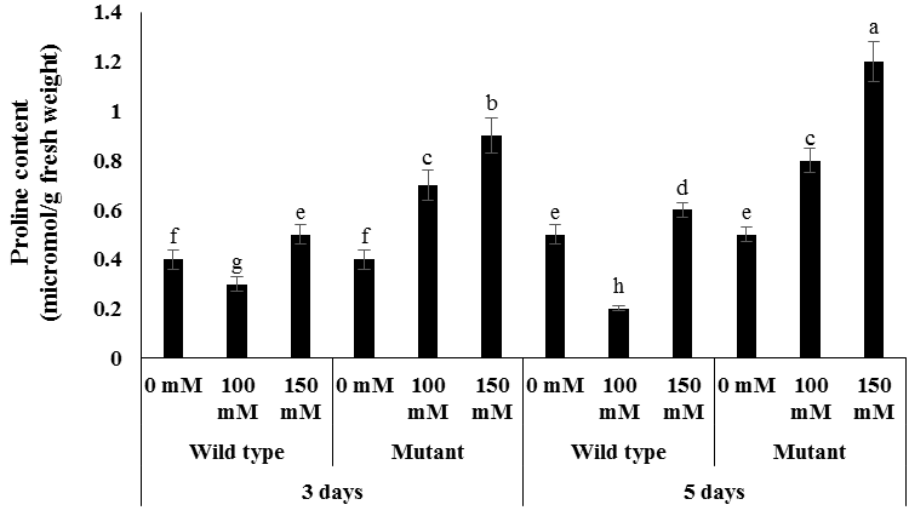

Figure 1. Proline content in wild-type and mutant rice cultivars under different levels of salt stress, 3 and 5 days after applying the salinity stress. Means that share the same letters are not statistically significantly different from each other (LSD = 0.05).

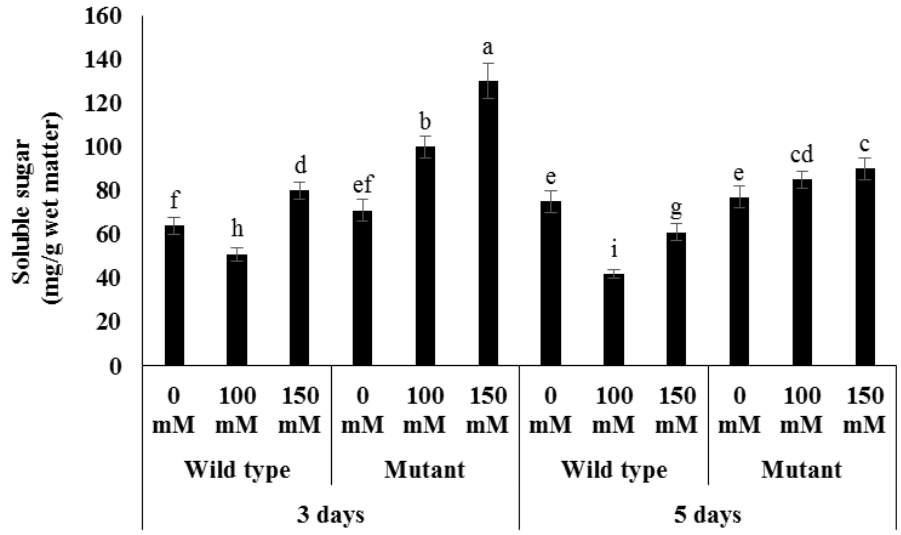

Figure 2. Soluble sugar content in wild-type and mutant rice cultivars under different levels of salt stress 3 and 5 days after applying the salinity stress .Means that share the same letters are not statistically significantly different from each other (LSD = 0.05).

stress was observed in mutant rice seedlings. The highest level of glycine betaine was recorded at 150 µM salt stress after 6 days in the mutant cultivar, showing a notable difference compared to other treatments (Figure 3). The lowest amount of glycine betaine was also obtained in the control in the first sampling in the wild-type. This elevated glycine betaine content may indicate improved osmotic regulation in the mutant genotype, resulting in enhanced salt stress tolerance (Ahmed *et al*., 2021). When rice plants face salt stress, the concentration of glycine betaine naturally rises. This osmolyte plays a crucial role in cell protection and minimizing damage from salt stress. The increase in glycine betaine helps manage osmotic potential, enabling better water utilization from the soil. This can aid in maintaining cell rigidity and preventing wilting (Hafez *et al*., 2021). Glycine betaine can enhance physiological functions like photosynthesis and respiration. Through increased glycine betaine levels, plants may perform better under salt stress conditions. Glycine betaine helps stabilize proteins and cell membranes, preventing their degradation under stress. Additionally, it can influence enzyme activities and regulate metabolism during stressful conditions. Acting as an antioxidant, glycine betaine can shield plants from free radical damage that escalates due to salt stress (Rhaman *et al*., 2024). Several studies have demonstrated the significant roles of proline and glycine

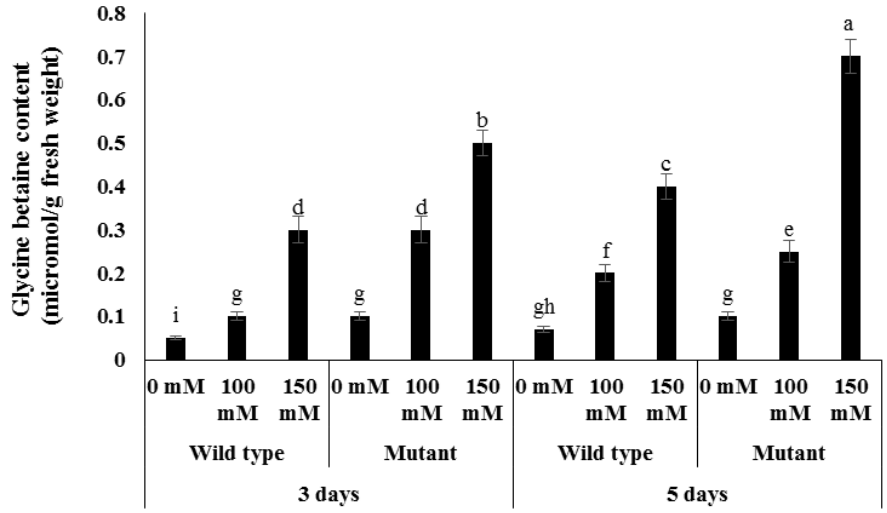

Figure 3. Glycine betaine content in wild-type and mutant rice cultivars under different levels of salt stress, 3 and 5 days after applying the salinity stress. Means that share the same letters are not statistically significantly different from each other (LSD = 0.05).

betaine as adaptive osmolytes in plant responses to abiotic stressors such as salinity, drought, heavy metals, and low temperatures (Khatun *et al*., 2020; Bai *et al*., 2022). Proline and glycine betaine have been shown to positively impact the reduction of abiotic stresses in various crops, including rice (Tania *et al*., 2021) and wheat (Mamedi *et al*., 2022).

Salinity stress increased the total leaf protein concentration in the mutant compared to its wild-type, and this increase was observed more in the mutant cultivar 5 days after stress (Figure 4). The mutation can lead to changes in the structure and function of enzymes, which may contribute to the production of more soluble proteins in response to salinity. These enzymes may be involved in various metabolic processes related to stress tolerance (Jing *et al*., 2021). There are conflicting reports on the effect of salinity stress on the amount of total soluble protein in different plants and different organs. Salinity stress can have significant effects on soluble proteins in rice. Salinity stress can cause the activation or repression of some genes that contribute to the production of specific proteins related to salt resistance. Some proteins, such as thermal proteins (thermal inducers) and stimulatory proteins, are increased in response to salt stress and help protect cells. Proteins may undergo structural changes due to salt stress, which affects their function. Some soluble proteins help regulate plant osmotic pressure, allowing the plant to better respond to stress conditions (Yang *et al*., 2024). The results obtained in the present study are consistent with the findings of Demir and Kocacaliskan (2002). Who believe that increased proline concentration is the cause of increased protein levels under salt stress conditions. Sugars also interact with proteins and membranes through hydrogen bonding, which is why they prevent protein degradation.

Gene expression study: Examination of superoxide dismutase gene expression in rice leaves under salinity treatment showed that gene expression increased with increasing stress. This increase in expression was greater 5 days after stress, and in the mutant cultivar, higher expression was seen in both sampling stages. The highest amount of superoxide dismutase gene expression was obtained under 150 µM salinity treatment in the 5-day sampling of the Mutan cultivar, and the difference with other treatments was significant (Figure 5).

The response of different genes to stress factors varies greatly depending on the severity of the stress and its duration. Also, based on a review of the sources, the evaluation time does not show a consistent trend (Gill and Tuteja, 2010; Hasanuzzaman *et al*., 2013). Superoxide dismutase acts as the first line of defense in scavenging reactive oxygen species (ROS) and plays a vital role in the physiological and biochemical processes of plants to manage environmental stresses. Findings have shown that SOD catalyzes superoxide radicals, converting them into oxygen and hydrogen peroxide, thus protecting plant cells against oxidative damage (Su *et al*., 2021). Mutations in the *SOD* gene and protein structure may lead to changes that can increase or decrease the efficiency of the enzyme. Mutations that increase the enzyme activity can lead to greater production of hydrogen peroxide and ultimately aid in the scavenging of free radicals under saline conditions (Huo *et al*., 2022). Recently, several studies have shown that the transcript levels of plant SOD genes respond to various environmental cues and help plants cope with harsh environmental conditions. For example, increased SOD activity helps plants resist salinity and drought stress in Brassica juncea plants (Alamri *et al*., 2020), temperature-induced oxidative damage in *Acutodesmus dimorphus* (Chokshi *et al*., 2020), and cold stress altered the expression of *Cu/ZnSOD* genes in tomato plants

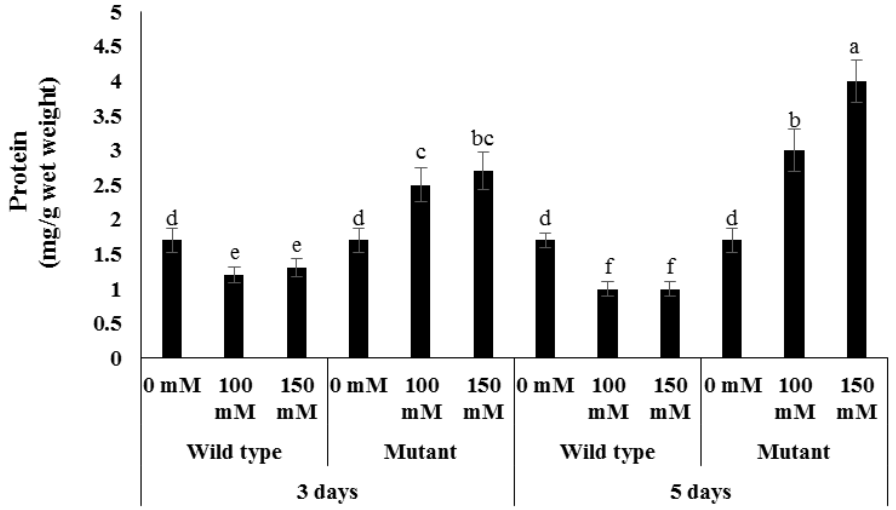

Figure 4. Soluble protein content in wild-type and mutant rice cultivars under different levels of salt stress, 3 and 5 days after applying the salinity stress. Means that share the same letters are not statistically significantly different from each other (LSD = 0.05).

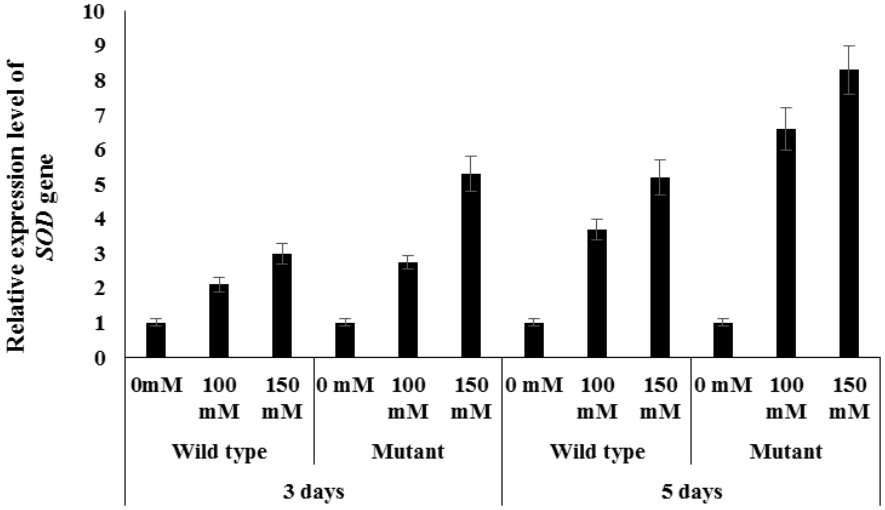

Figure 5. The relative expression of the *SOD* gene in wild-type and mutant rice cultivars under different levels of salt stress, 3 and 5 days after applying the salinity stress.

(Liu *et al*., 2020).

Examination of the expression level of the glutathione reductase gene showed that with increasing salinity stress, the gene expression level increased in both sampling stages. However, in the first sampling 3 days after stress, gene expression was higher. This increase in expression in the mutant cultivar under 150 mM treatment was greater than in the wild- type and showed a significant difference with other treatments (Figure 6). Mutations can cause changes in the structure of the *GR* protein, which may affect the activity of the enzyme and lead to an increase in its efficiency in response to salinity stress (Wang *et al*., 2023). This could be one of the reasons for the increase in *GR* in the mutant cultivar compared to the wild type. Excessive salt accumulation in plants can lead to cellular oxidative stress, osmotic stress, and ion toxicity, all of which have a negative impact on the capacity of plants to grow, develop, and produce (Hoque *et al*., 2022). Glutathione reductase plays an important role in the scavenging of hydrogen peroxide and the maintenance of reduced glutathione (Rahantaniaina *et al*., 2017). Glutathione exists in reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) states. Cellular oxidative stress is measured by the ratio of reduced glutathione to oxidized glutathione inside cells. An increase in the ratio of GSSG to GSH indicates greater oxidative stress (Lu, 2013). Glutathione reductase regulates the GSSG/GSH ratio and provides *GSH* to *GPX* and *DHAR*, which convert H_2O_2 to O_2 and H, respectively, and

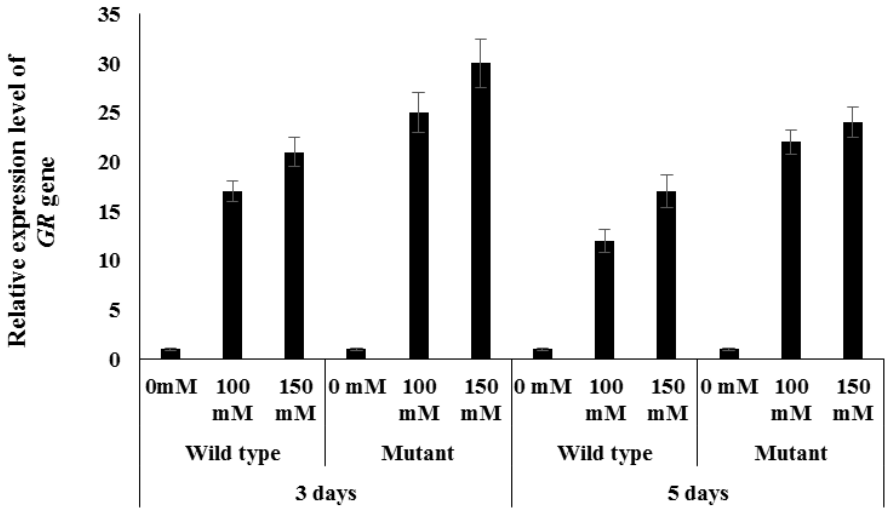

Figure 6. The relative expression of the *GR* gene in wild-type and mutant rice cultivars under different levels of salt stress, 3 and 5 days after applying the salinity stress

reduce oxidized ascorbate (Bhabak and Mugesh, 2010). Research results show that oxidative stress, pathogens, metals, cold, drought, and salinity increase the transcription of the glutathione gene (Gao *et al*., 2016). In an experiment, increasing the amount of hydrogen peroxide and cold stress increased the expression of the glutathione gene in rice (Passaia *et al*., 2013). Lu *et al*. (2013) also showed that the amount of GR increases under stress conditions in wheat plants and in flag leaves compared to spikes. Broadbent *et al*. (1995) observed that in transgenic tobacco plants, increasing the expression of the glutathione reductase gene increased tolerance to salt stress.

Conclusion

The results revealed that salinity stress significantly impacts gene expression diversity and biochemical changes in both rice varieties. Under salinity stress conditions, the increased expression of *SOD* and *GR* genes indicates the plant's effort to cope with oxidative stress and maintain internal balance. This response was more pronounced in the mutant Hashemi rice, potentially due to genetic alterations and enhanced defense mechanisms. Biochemical analyses demonstrated heightened levels of osmoprotectant and stress-related metabolic changes in both rice varieties. Mutant Hashemi rice ultimately exhibited higher levels of protective compounds (proline and glycine betaine) compared to wild-type Hashemi rice, attributed to mutations in certain genes within the mutant variety that increased the expression of genes related to antioxidant enzymes and protective proteins against salinity stress. This enhancement can mitigate oxidative damage and improve plant performance under adverse conditions, giving this cultivar a significant survival advantage over non-mutated cultivars due to its superior ability to manage oxidative stress. It is recommended to focus on genetic and biochemical traits associated with salt stress tolerance in rice breeding programs. The robust stress response displayed by the mutant Hashemi offers a valuable genetic resource for developing rice varieties capable of withstanding high salinity conditions. Additionally, the temporal gene expression patterns identified in this study offer crucial timing information for stress adaptation mechanisms, enabling breeders to optimize their breeding programs. The study underscores the potential of utilizing the identified biomarkers as early detection tools for salt stress tolerance in breeding programs. By pinpointing specific gene expression changes linked to salt stress, breeders can screen rice plants at an early stage and select those with desired genetic traits for further breeding. Moreover, conducting additional experiments to explore the interaction of other genes and metabolic pathways under salt stress can significantly enhance our understanding of this phenomenon. Lastly, emphasizing the significance of different plant tissues in responding to environmental stresses and the pivotal role of antioxidant genes can pave the way for developing optimal cultivation strategies and water resource management in salt stress-prone areas.

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