Journal of Plant Process and Function, Vol. 14, No. 70, Year 2026 **page: 79-89**
https://doi.org/10.22034/14.70.79

Research Article

Evaluation of lipoxygenase and glutathione peroxidase genes along with some biochemical traits under salt stress in wild and mutant cultivars of Hashemi rice

Hourieh Najafi[1], Saeid Navabpour[1*], Ahad Yamchi[1] and Elahe Tavakol[2]

[1] Department of Plant Breeding and Plant Biotechnology, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2] Department of Plant Production and Genetics, Faculty of Plant Production and Genetics, Shiraz University, Shiraz, Iran

Abstract

Salinity is an unfavorable environmental factor for plants that affects their growth and fertility. Rice, the second most produced grain in the world after wheat, is relatively sensitive to salt stress. To assess the response of the Hashemi rice genotype and its advanced mutant line under stress, root samples were used for biochemical analysis. In this study, an advanced mutant line of Hashemi rice (tolerant to salinity stress) resulting from irradiation with gamma rays and its wild line (variety Hashmi - sensitive to salt stress) were exposed to salt stress in chopped plots. The experiment was conducted using a completely randomized block design with three replications, utilizing a hydroponic solution. The main factor in the experimental design was salinity treatment (0, 100, and 150 mM sodium chloride) and sampling time, with two levels. The sub-factor included genotypes. The results showed that while the amount of H_2O_2 increased significantly in both genotypes when exposed to NaCl, it was higher in the wild genotype. The activity of antioxidant enzymes was significantly increased in the advanced mutant line. Additionally, gene expression increased at different concentrations of salinity stress, with higher values observed in the rice mutant line compared to its wild line. Overall, these findings are valuable for identifying and introducing genes specifically involved in salt stress tolerance in rice roots during the seedling stage for use in breeding programs.

Keywords: Stress, Antioxidant genes, Mutant genotype

Introduction

Rice (*Oryza sativa*) is a valuable crop that serves as the staple food for nearly half of the world's population. However, abiotic stresses such as salinity and drought, also known as osmotic stress, can significantly reduce its yield (Hasanuzzaman *et al.*, 2019). Biotic and abiotic stresses are key factors that limit the growth and yield of rice, with projections indicating that by 2050, over 50% of the world's agricultural land will be affected by drought and salinity stress (Chauhan *et al.*, 2017). Drought and salinity are the primary abiotic stresses that rice plants encounter from germination to harvest (Bhat *et al.,* 2020). Salinity poses a serious threat to crops, underscoring the need to identify tolerant genetic resources and effective strategies (Beyzavi *et al.*, 2021). Despite the identification of various rice varieties with differing levels of salinity tolerance, rice remains one of the most sensitive grains to this stress (Chen *et al.*, 2021). Rice is classified as a sensitive plant to salinity stress during both vegetative and reproductive growth stages (Zhang *et al.*, 2019). According to literature, in electrical conductivities (EC) higher than 3, for each increase of one unit of salinity, 12% of rice yield is reduced. In EC equal to 6, a 50% decrease in yield has been reported (Chen *et al.*, 2021). Rice cultivation holds special significance in Iran, but the increasing salinity of agricultural soils has posed challenges to its production (Nabiollahi *et al.*, 2017). Developing cultivars that are tolerant to salinity stress is a crucial aspect of breeding programs (Kordrostami *et al.*, 2017).

Received: Oct. 02, 2024; Revised: Feb. 14, 2025; Accepted: Mar. 04, 2025; Published Online: Feb. 28, 2026
*Corresponding Author: s.navabpour@yahoo.com

The plant defense system can be evaluated at three levels: Morphological, biochemical, and molecular. Among the molecular and biochemical factors involved in this defense system are various transcription factors, receptors, and antioxidant enzymes (Upadhyay *et al.,* 2019). Additionally, salinity-induced oxidative stress leads to the production of significant levels of reactive oxygen species (ROS), which cause lipid peroxidation and interfere with membrane stability under stressful conditions (Chunthaburee *et al.,* 2016). The free radical removal system is one of the mechanisms involved in tolerance to all kinds of stresses. The production speed, production rate, stability, and performance power of enzymatic and non-enzymatic antioxidants are among the most important parameters effective in maintaining cellular homeostasis in various organisms, especially plants (Riyazuddin *et al.,* 2020). Lipoxygenase (LOX), belonging to the family of non-heme iron dioxygenases, has been identified in many living organisms, including plants, animals, fungi, and bacteria (Banthiya, 2017). This enzyme has the ability to oxidize unsaturated fatty acids such as linolenic acid and linoleic acid, producing hydroxy unsaturated fatty acids with its catalytic reaction (Wennman *et al.,* 2016). It also converts other hydroperoxides to water and corresponding alcohols using reduced thioredoxin or other reducing equivalents as electron donors (Bela *et al.,* 2015). Glutathione peroxidase (GPX) is found both inside the cell (cytosol, vacuole) and in the cell wall and outside the cell. It is a key enzyme in removing hydrogen peroxide radicals (Roychoudhury, 2014). In addition to hydrogen peroxide, this enzyme also acts to break down lipid peroxides into their corresponding alcohols, which are mainly located in the mitochondria and sometimes in the cytosol (Ighodaro *et al*., 2017). In *Arabidopsis thaliana*, eight members of the GPX gene family were identified through extensive research, with some highly regulated under salt stress (Milla *et al.,* 2003). Further investigations of biochemical and genetic mechanisms related to salinity tolerance are crucial for addressing this global problem (Zeeshan *et al.,* 2020). Considering these complications, using isogenic or mutant lines with the same genetic background but showing different tolerance to salinity can be a suitable method to improve salinity tolerance in plants (Kiani *et al.,* 2017). The development of salt-resistant cultivars may be the most effective approach for rice cultivation in salt-damaged lands (Tahjib al-Araf *et al.,* 2017). In this study, the difference between the mutant line and the wild in terms of traits related to salt stress tolerance was investigated. The evaluation of *LOX* and *GPX* genes, as well as some biochemical traits under salt stress, was also conducted. This was done in order to identify the resistant bases of the plant using these enzymes and genes.

Materials and methods

Cultivation and sampling: In this study, rice seeds of the Hashemi genotype were used as the sensitive wild, along with advanced mutant lines (7th generation) obtained from the Rasht-Kishor Rice Research Institute in 2023. The mutant line was created through gamma irradiation from a Cobalt 60 source and has been identified as a tolerant line to salt stress in field evaluations with 8 decisiemens salinity. The research was conducted using a split-plot based on a randomized complete blocks design with three replications through a hydroponic solution. The seeds were treated with a 10% calcium hypochlorite solution for cleaning, then placed in a dark incubator at 23°C for six days to germinate. The germinated seeds were then transferred to Yoshida culture medium (Yoshida *et al.,* 1976). The nutrient solution was changed every three days, and the acidity was adjusted to 5.7 with sodium hydroxide (NaOH) solution. After six days of growth in the normal culture medium, salt stress was applied by adding 100 and 150 mM NaCl for an additional six days. Samples were taken at three and six days after the salt stress application from both normal and saline environments, specifically from the root tissue, and immediately frozen in liquid nitrogen at -196°C for biochemical trait measurements.

Measurement of enzymes and biochemical traits: The activity of the LOX enzyme was measured using the method of Minguez-Mosquera, (1993), In this method, the reaction mixture contained 25 µL of 100 mM linoleic acid as the substrate, 2.775 mL of 100 mM acetic acid buffer (pH = 5.5), and 200 µL of enzyme extract. The reaction was carried out for 1 minute at 30°C. Subsequently, the absorbance of the reaction was measured at 234 nm.

While the amount of GPX was measured following the method of Griffith (1980). To measure the amount of glutathione peroxidase, first, 0.5 g of plant leaves were ground completely in a porcelain mortar containing 2 ml of 2% metaphosphoric acid. The resulting homogeneous solution was transferred to a centrifuge tube and centrifuged at 10,000 g for 10 minutes at 4°C in a refrigerated centrifuge. Then, 100 µL of the supernatant solution was added to a test tube containing 700 µL of NADPH, 100 µL of dithiobis, and 100 µL of distilled water. After 3-4 minutes, 10 µL of glutathione was added, and the absorbance was read at 412 nm.

Malondialdehyde, an indicator of lipid peroxidation, was measured based on the method of Heath and Packer (1968). To measure the concentration of sodium and potassium, plant samples were dried in an oven for 48 hours at 72°C, powdered, and then processed. The powdered tissue was mixed with 100 mg of 500 mM nitric acid and left at room temperature for 24 hours before being extracted at 85°C for two hours. The solution was then filtered, and sodium and potassium ions were measured using the film photometer method (Williams and Twain, 1960).

Gene expression study: RNA extraction was performed using P-Biosol extraction buffer from Bioflex (Tokyo, Japan) (Fig. 1). The quality of the extracted RNA was assessed through electrophoresis on a 1.5% agarose gel. Subsequently, cDNA synthesis was carried out using the method proposed by the Fermentase Company, and the synthesized cDNA was tested with actin housekeeping gene primers using PCR.

[DOI: DOI: 10.22034/14.70.79]

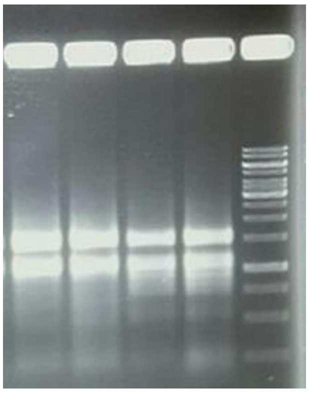

Figure 1. RNA extracted from rice roots of both wild and mutant genotypes under saline conditions

Table 1. Specifications of the primers used

Initiator name	Primer sequence	The length of the reaction product	Melting temperature (C°)	Accession number at NCBI
Actin, FOR	5′-TCCCGAGTATTGTTGGTCGT-3′	176	58	AF111812
Actin, REV	5′-TCCATGTCATCCCAGTTGCT-3′		58	
FOR *LOX*	5′-GGTGTTCTGCGAGGTGATGA-3′	180	60	X16099
REV *LOX*	5′-AGGGTGGTGCTTCAGCTTGT-3′		60	
FOR *OsGPX1*	5′-AAGCGGAAAAGACGTGAACC-3′	180	59	AY100689
REV *OsGPX1*	5′-TCCTGCCCTCCAAACTGATT-3		59	

The expression pattern of *LOX* and *GPX* genes was evaluated using the iQ5 device from Bio-Rad and the Cyber Biopars kit (Gorgan University of Agricultural Sciences and Natural Resources) for real-time evaluation. The housekeeping gene actin was used for data normalization. Primers were designed based on information from the NCBI website and Primer 3 software, with sequence lengths between 132 and 187 bp and melting point temperatures between 51.4 and 60°C. Data analysis was conducted using REST software (Table 1).

Data analysis: The data obtained from biochemical traits was analyzed using SAS statistical software. The comparison of average data was conducted using the LSD test method, and graphs were created using Excel software.

The relative expression of the studied genes was calculated using the following formula (Pfaffle *et al.*, 2002).

$$Ratio = (E\ target)^{\Delta CPtarget(conttol-sample)} / (E\ ref)^{\Delta CPref\ (control-sample)}$$

In this equation, the ratio of the expression level of a target gene is calculated based on the PCR for the target and reference genes and the difference (Δ) of the crossing point (CP) of an unknown sample versus the control (CP control-sample Δ). Evaluation of gene expression was done by Excel and REST software. In this experiment, the samples were measured with respect to a housekeeping gene, which here was *Actin* and had the same expression in all stages of plant growth and under all conditions. At the end of the reaction and after receiving the charts, the information was transferred to REST software, and data analysis was done.

Results and discussion

The analysis of variance for elements measured in roots at the seedling stage showed that the three-way effects of genotype × time × stress were not significant for sodium concentration but were significant for other traits (Table 2).

The results of analysis of variance also showed that the triple effect of genotype × time × stress was not significant in the case of malondialdehyde (MDA), but it was significant for LOX and GPX enzymes at the level of 1% and for hydrogen peroxide at the level of 5%. (Table 3).

Sodium and potassium and their ratio: The concentration of sodium ions in the roots of wild and mutant rice seedlings increased after exposure to salt stress, with a significantly lower increase observed in the mutant genotype compared to the wild (Fig. 2). Conversely, the amount of potassium ions in the roots decreased during salinity stress in both genotypes (Fig. 3). Additionally, the ratio of sodium to potassium in wild and mutant genotypes increased in the initial sampling under 100 µM treatment and then decreased as stress concentration increased. In the second sampling, under both 100 and 150 mM treatments, the sodium to potassium ratio increased in both genotypes, with no significant difference observed between the levels (Fig. 4).

The decrease in potassium concentration in the tissue may be attributed to the competition between sodium and potassium on the cell membrane, hindering the transport of these ions in the xylem and the release of potassium outside the root (Khan and Panda, 2008). Various plant species have shown an increase in sodium ion accumulation, a decrease in potassium ion

[DOI: DOI: 10.22034/14.70.79]

Table 2. Variance analysis of the concentration of sodium and potassium elements and the ratio of sodium to potassium in the roots of the wild and mutant rice genotypes under stress conditions

Sources of variation	df	Root		
		Sodium / Potassium	Potassium	Sodium
Block	2	0.001	19.53	7/03
Stress	1	4.81^{**}	3900.34^{**}	1019.84^{**}
Time	1	0.11^{**}	1327.59^{**}	555.84^{**}
Time × stress	1	0.04^{**}	776.34^{**}	2.34^{ns}
Error 1	6	0.0003	7.78	1.61
Genotype	1	0.061^{**}	3139.59^{**}	433.5^{**}
Genotype × Stress	1	0.044^{**}	615.09^{**}	294^{**}
Genotype × Time	1	0.006^{**}	348.84^{**}	18.37^{ns}
Genotyp × Time × Stress	1	0.003^{*}	207.09^{**}	9.37^{ns}
Error 2	8	0.001	21.75	25.5

n.s, * and **: non-significance, significance at 5% and 1% levels respectively

Table 3. Variance analysis of the traits studied in the root of the wild and mutant rice genotypes under salt stress conditions

Sources of variation	df	Root			
		Glutathione peroxidase	Lipooxygenase	Malondialdehyde	Hydrogen peroxide
Block	2	0.57	0.45	0.015	0.0002
Stress	1	60.96^{**}	196.65^{**}	771.23^{**}	1.80^{**}
Time	1	$0.11^{n.s}$	1.65^{**}	50.02^{**}	0.039^{**}
Stress × Time	1	17.59^{**}	0.37^{**}	7.09^{**}	0.005^{**}
Error 1	6	0.33	0.31	0.44	0.0003
Genotype	1	68.85^{**}	139.68^{**}	233.43^{*}	0.058^{**}
Stress × Genotype	1	49.16^{**}	47.04^{**}	16.08^{**}	0.002^{**}
Time × Genotype	1	107.73^{**}	19.44^{**}	1.14^{*}	0.0002^{ns}
Stress × Time × Genotyp	1	56.27^{**}	4.59^{**}	0.075^{ns}	0.009^{*}
Error 2	8	0.17	0/07	0.19	0.0006

n.s, * and **: non-significance, significance at 5% and 1% levels respectively

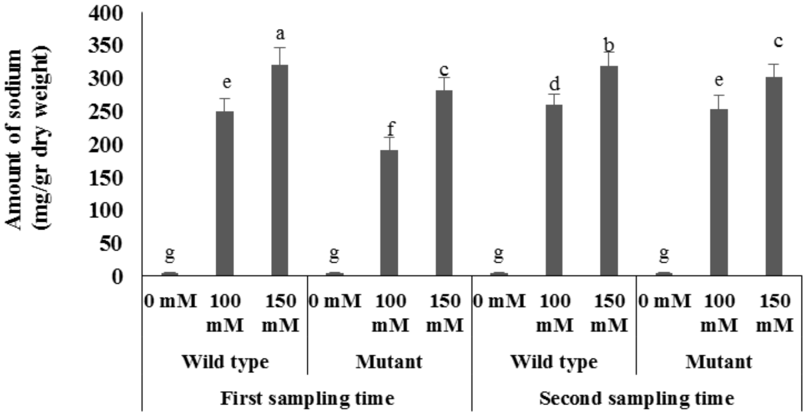

Figure 2. Sodium concentration in roots of wild and mutant rice genotypes under salinity stress conditions

absorption, and an imbalance of these ions during salt stress. It can be suggested that the higher concentration of potassium ions and the elevated potassium to sodium ratio in the mutant leaf exposed to salt stress may be an adaptive response to maintain high potassium levels in stomatal cells during salt stress (Fallah, 2015).

Plants accumulate various compatible solutes in the cytoplasm to enhance their hyperosmotic balance and protect cells from water stress caused by salinity. This process helps maintain the osmotic potential of Na^+ and Cl^- in the vacuole (Islam *et al.,* 2016). Improved salt tolerance in rice is closely related to maintaining a low Na:K ratio, which is achieved through mechanisms such as salt removal, salt dilution, leaf-to-leaf division, salt reabsorption, and Na^+ partitioning (Omisun *et al.*, 2018). During salinity stress, Na^+ and K^+ ions compete for entry into plant cells. When potassium absorption surpasses that of sodium ions, the plant exhibits greater resistance to salinity stress, resulting in reduced sodium toxicity (Chen *et al.*, 2015). Fallah (2015) demonstrated

[DOI: DOI: 10.22034/14.70.79]

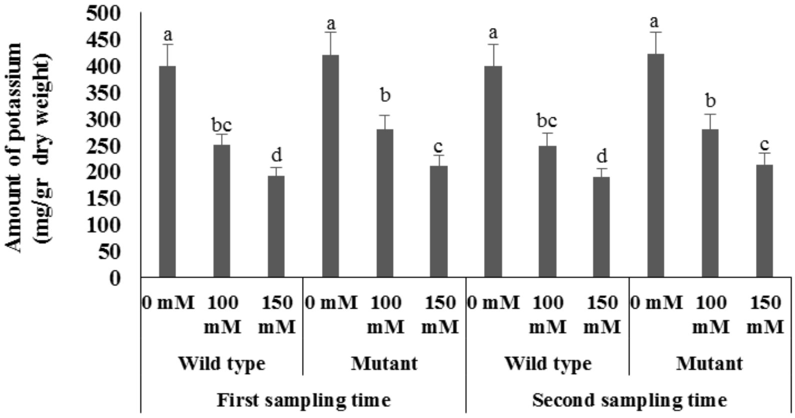

Figure 3. Potassium concentration in roots of wild and mutant rice genotypes under salt stress conditions

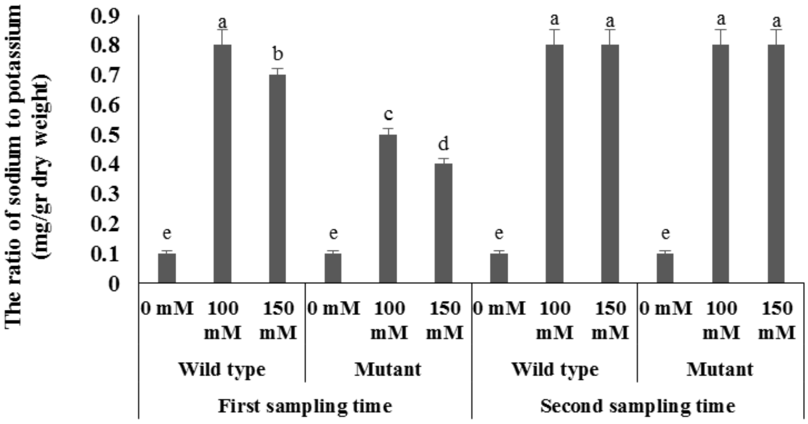

Figure 4. Sodium ions to potassium concentration in roots of wild and mutant rice genotypes under salinity stress conditions

that under salinity stress, sodium tends to accumulate in the roots rather than transferring to the leaves in rice seedlings. Tolerant cultivars show more effective strategies for coping with salt stress.

Hydrogen peroxide and lipid peroxidation: Salt stress resulted in this study; salt stress was applied, resulting in an increase in the amount of hydrogen peroxide. The mean comparison results indicated that during the first and second samplings, the levels of H_2O_2 and MDA in the wild genotype under normal and stress conditions were higher than those in the mutant genotype (Figs. 5 and 6).

Oxidative stress caused by salinity leads to the production of a significant level of ROS in rice, which causes lipid peroxidation and interferes with membrane stability under stressful conditions (Chunthaburee *et al*., 2016), and Lin *et al.* (2016) demonstrated the impact of salinity on H_2O_2 production. The level of lipid peroxidation is correlated with the accumulation of MDA. This study showed the highest amount of MDA in response to salinity stress, indicating the highest level of lipid peroxidation occurred six days after exposure to salinity stress in the rice roots of the wild genotype compared to the mutant (Figs. 5 and 6). These findings suggest that the damage caused by ROS on the plasma membrane was more pronounced in the wild genotype. Mutant genotypes exhibited lower levels of MDA in shoots and roots compared to their wild type counterparts. The results indicate that mutants possess a greater ability to withstand salt stress-induced damage to the cell membrane than wild genotypes. Chutipaijit *et al.* (2009) found that rice seedlings with lower levels of MDA displayed increased tolerance to salt stress. In other reports, plants suffering from salt stress, such as barley (Kiani *et al*., 2017) and wheat (Nouri *et al*., 2015), have shown that the level of malondialdehyde can be used as an oxidative stress marker. The results show that salt stress leads to an increase in the accumulation of toxic compounds such as hydrogen peroxide in rice plants, which leads to the induction of oxidative stress and damage to biological macromolecules, including membrane lipids. The increase in malondialdehyde levels also confirms this (Ghorbani *et al*., 2019). Increased accumulation of hydrogen peroxide damaging biological membranes under salinity stress was also reported by Ghorbani *et al.* (2018) and Xu *et al.* (2022). Therefore, one of the main reasons for the reduction in growth and development of plants under salt stress is the induction of oxidative stress and damage to the plant's vital

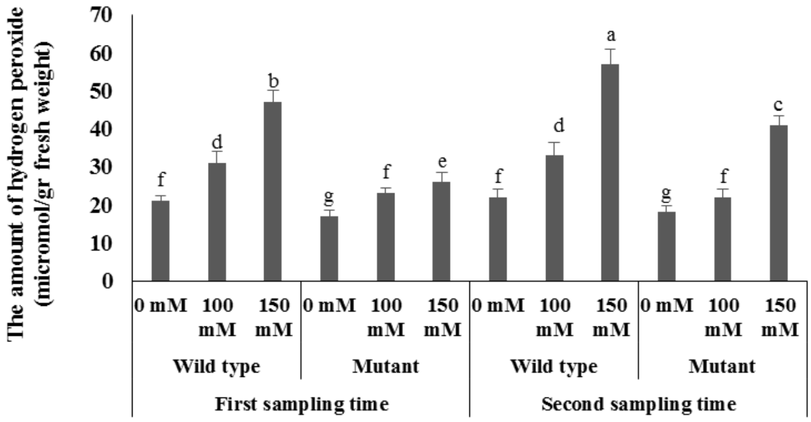

Figure 5. Hydrogen peroxide concentration in roots of wild and mutant rice genotypes under salt stress conditions

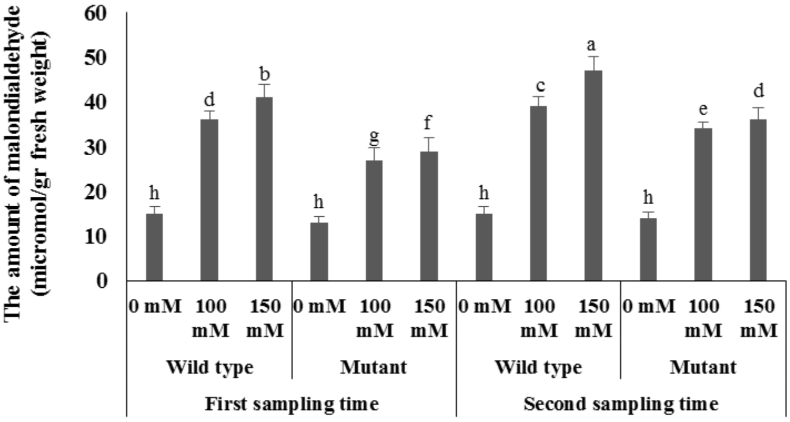

Figure 6. Malondialdehyde concentration in roots of wild and mutant rice genotypes under salt stress conditions

processes. Salinity stress destroys ionic homeostasis by increasing sodium accumulation and decreasing potassium accumulation, creating ionic toxicity in plants, which inhibits their growth and development (Ghorbani *et al.*, 2023). Chen *et al.* (2020) reported that salinity prevents the growth of flax plants by increasing sodium accumulation and decreasing potassium absorption. Our results showed that salt stress increased sodium accumulation in roots and leaves. While decreasing potassium accumulation in roots and leaves. This caused a decrease in the potassium to sodium ratio in roots and leaves, which is consistent with the results obtained by Liu *et al.* (2022) and Jini and Joseph (2017). Considering the induction of oxidative stress and the increase in MDA levels in plants under salt stress, the disruption of potassium to sodium homeostasis can be partly caused by the induction of oxidative stress and damage to the structure and function of plasma membranes.

Antioxidant enzymes: The results of the mean comparison showed a significant increase in the amount of LOX enzyme in the studied mutant compared to its wild type after being affected by stress. The mutant variety exhibited the highest amount of enzyme under stress, reaching 150 ml in both sampling stages. The free radical removal system is a mechanism involved in tolerance to various stresses. The production speed, rate, stability, and performance power of enzymatic and non-enzymatic antioxidants are crucial approaches in maintaining cellular homeostasis in different organisms, particularly plants (Riyazuddin *et al.*, 2020). Additionally, it was observed that stressful conditions significantly increase the activity of LOX, an important lipid-related enzyme in plant cells (Lim *et al.*, 2015).

In this study, the levels of oxidative stress, lipid peroxidation, H_2O_2, and LOX activity increased under salt stress. The rise in lipid peroxidation and H_2O_2 levels during salt stress is attributed to the increased production of ROS, which the plant is able to eliminate through defense mechanisms, indicating that the mutant plant has developed sufficient defenses against oxidative stress. LOX, a key enzyme in this process, plays a crucial role. Additionally, the intermediate and final products of the LOX pathway can activate protein kinases, transmit signals, and facilitate transduction, making LOX activity a valuable molecular marker for plant response to abiotic stresses (Babenko *et al.*, 2017).

Therefore, this enzyme is synthesized to protect the

[DOI: DOI: 10.22034/14.70.79]

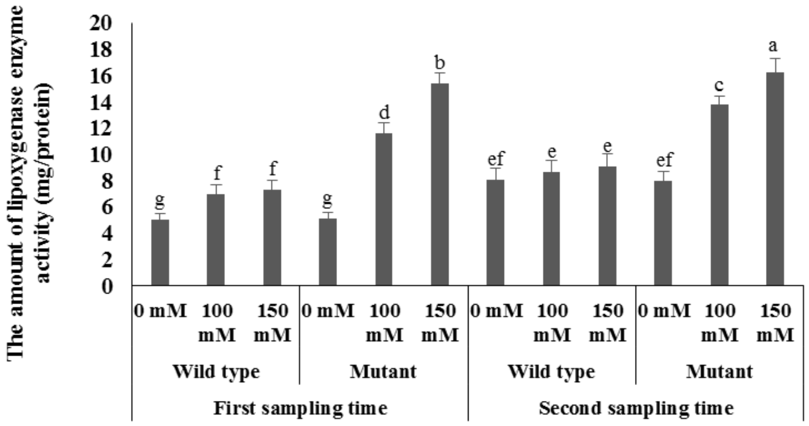

Figure 7. Lipoxygenase enzyme in roots of wild and mutant rice genotypes under salt stress conditions

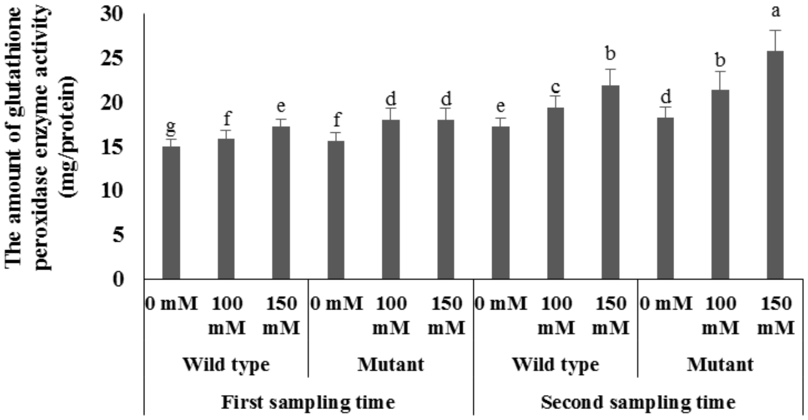

Figure 8. Glutathione peroxidase enzyme in roots of wild and mutant rice genotypes under salt stress conditions

plant from oxidative damage. From an enzymology perspective, LOXs are linoleate: oxygen oxidoreductases, functioning as multifunctional enzymes involved in lipid peroxidation, peroxidase, hydrogen peroxide, and leukotriene synthesis reactions (Li *et al.,* 2014). LOX can oxidize unsaturated fatty acids like linolenic acid and linoleic acid, generating hydroxy unsaturated fatty acids through its catalytic activity (Wennman *et al.*, 2016). In a study on potato plants, an increase in lipoxygenase activity was also reported under salt stress (Daneshmand, 2014). Salt treatment increased the activity of antioxidant enzymes in wheat (Talaat and Todorova, 2022).

The results of the mean comparison of the amount of glutathione peroxidase enzyme showed that as stress increased, the amount of enzyme increased in both varieties under treatment. This increase was more pronounced in the mutant variety, particularly during the second stage of sampling. The highest amount of GPX enzyme recorded was 25.77 mg/protein, which occurred under the treatment of 150 ml in the mutant variety during the second sampling (Fig. 8).

To cope with oxidative stress induced by environmental factors, plants possess an antioxidant defense system that enhances plant adaptation efficiently (Isayenkov and Maathuis, 2019). In plants, GPX is a non-heme-containing peroxidase that plays a crucial role in catalyzing H_2O_2 and other hydroperoxides into water and corresponding alcohols using reduced thioredoxin or other reducing equivalents as electron donors (Bela *et al.,* 2015). Mutation of APX1/2 in rice (double mutation for cytosolic APXs) has been shown to increase the amount of H_2O_2 (Bonifacio *et al.,* 2011). There is a direct relationship between the activity of glutathione peroxidase and the detoxification of lipid hydroperoxides and other reactive molecules in various species under different stress conditions, particularly salinity (Dixon and Edwards, 2010). These enzymes employ different mechanisms to neutralize H_2O_2 under stress conditions, prompting plants to activate these enzymatic antioxidant systems to prevent the excessive accumulation of ROS under stress conditions (Hassan *et al.,* 2017).

Expression of genes: The study on lipoxygenase gene expression revealed that applying salt stress increased gene expression in both sampling stages. The highest expression value of gene 23 was observed in the mutant cultivar under the treatment of 150 ml during the second sampling, and the difference with the levels of other treatments was significant. *LOX* have various functions and are involved in growth, development, seed germination, fruit ripening, aging, cell death, and the synthesis of abscisic acid and jasmonic acid hormones, as well as response to biotic and abiotic stresses (Wang *et al.,* 2019; Viswanath *et al.,* 2020). This increase in expression began 2 hours after salt treatment. The expression level of *7SiLOX* significantly increased 4 days after stress and peaked at 6 days in variety under stress treatment (Zhang *et al.,* 2021). In a study conducted by Shaban *et al*. (2018), increased expression of *LOX-9* gene was reported in cotton plants under drought and salinity stress conditions. These results are consistent with the findings of the present study.

In the analysis of the *GPX* gene under salt stress, it was observed that in the first sampling with 100 ml of salt treatment, there was a significant increase in gene expression in both cultivars compared to the control sample. In the treatment of 150 ml, the highest expression of the *GPX* gene was observed in the mutant variety during the second sampling. Under salt stress, the increase in intracellular levels of ROS causes significant damage to cellular structures and affects the expression of a number of genes, such as *SOD* and *GPX* (Gill and Tuteja, 2010). It has been shown that a number of proteins are induced by abiotic stresses, indicating the complexity of biochemical and molecular responses of plants to stress (Arefian *et al.,* 2014). In different plant species, several genes encoding *GPX* have been identified and characterized. The effective role of *GPXs* in protecting cells against oxidative damage by acting as efficient ROS scavengers under various abiotic stresses, such as salinity, heavy metals, cold, and drought, has been documented (Zhang *et al.,* 2019). *GPX* plays an important role in maintaining the health of cells in stressful environmental conditions by eliminating superoxide radicals, catalyzing their conversion to O_2, and reducing H_2O_2 and organic H_2O_2 to water and alcohols using reduced glutathione (GSH) (Alharby *et al.,* 2016). An increase in *GPX* mRNA expression was observed in tomatoes under saline conditions (Srinineng *et al.,* 2015). The increase in mRNA levels of *SOD* and *GPX* genes can also result from increased stability of transcriptional mRNAs (Soydam *et al.,* 2013).

The salt tolerance of transgenic rice was significantly improved by overexpressing the *NnGPX* gene transformed from lotus (Diao *et al.,* 2014). In *Lotus japonicus*, *GPXs* have been found to be major scavengers of ROS in nodules, as overexpression of LjGPX1 and LjGPXs3 effectively provides protection against salt stress-induced oxidative damage, especially in nodules (Matamoros *et al.,* 2015). Additionally, the *GPX* gene family was recently characterized in rapeseed, with 25 *GPX* genes identified, and their roles in multiple abiotic stress responses and hormonal signaling have been documented (Li *et al.,* 2021).

Conclusion

The results of this experiment confirm that soil salinity has a significant adverse effect on growth and also causes ROS production and removal inside the cell. Further studies should be conducted to find the possible mechanism at the molecular level in cereal crops in saline environments. The results also showed that the mutants studied had much better inhibition of hydrogen peroxide by increasing the activity of the antioxidant enzyme system at salinity levels compared to their wild as a result, they had the lowest amount of hydrogen peroxide and lipid peroxidation. Therefore, the mutant line studied showed resistance to salinity by reducing the amount of H_2O_2 and lipid peroxidation and inducing an increase in the activity of the antioxidant enzymes studied compared to its wild. The results showed that in the wild, the inhibitory systems are limited compared to the mutants, and therefore it is unable to cope with salinity stress due to the inefficiency of its antioxidant defense systems; as a result, higher amounts of H_2O_2 and lipid peroxidation were observed. From the above results, it can be concluded that the increase in the activity of antioxidant enzymes during salt stress is important for protection against ROS. As a result, investigating the effects of salt stress with the help of this type of enzyme can lead to the identification of resistant roots of a plant, because there is a good relationship between tolerance to environmental stresses and changes in the concentration of these enzymes in plants. Considering that the production of any substance in cells is under the control of genes, it is possible to produce salt-resistant plants more quickly by identifying the genes responsible for the production of these substances and transferring them to other plants. Considering the superiority of the mutant genotype in most of the traits studied, it can be concluded that mutation, as a useful tool in plant breeding programs, is capable of providing heritable diversity for selection. In order to improve salt tolerance in rice cultivars, it is suggested that the use of more advanced techniques such as genome editing (CRISPR) to identify and modify key genes can lead to enhanced capabilities to cope with salt stress. Also, studying the effect of salt stress under different cultivation and climatic conditions can help to better understand the diversity of responses and have better choices for breeding. According to the findings of this study, breeding and production of more salt-tolerant rice cultivars can be considered an effective approach to increase productivity in saline and salt-producing lands. These findings and perspectives can help develop effective strategies in rice cultivation in areas under salt stress.

References

Alharby, H. F., Metwali, E. M., Fuller, M. P., & Aldhebiani, A. Y. (2016). The alteration of mRNA expression of SOD and GPX genes, and proteins in tomato (*Lycopersicon esculentum* Mill) under stress of NaCl and/or ZnO nanoparticles. *Saudi Journal of Biological Sciences*, *23*(6), 773-781. https://DOI:10.1016/j.sjbs.2016.04.012

Arefian, M., Vessal, S., & Bagheri, A. (2014). Biochemical changes in response to salinity in chickpea (*Cicer arietinum* L.) during early stages of seedling growth. *Journal of Animal and Plant Sciences*, *24*(6), 1849-1857.

Babenko, L., Shcherbatiuk, M. M., Skaterna, T., & Kosakivska, I. V. (2017). Lipoxygenases and their metabolites in formation of plant stress tolerance. *The Ukrainian Biochem Journal*, *89*(1), 5-21. https://DOI:10.15407/ubj89.01.005

Banthiya, S. (2017). Recombinant expression and characterization of a prokaryotic lipoxygenase from *Pseudomonas aeruginosa*. Investigations into the biological role of this enzyme. (Doctoral dissertation).

Bela, K., Horvath, E., Galle, A., Szabados, L., Tari, I., & Csiszar, J. (2015). Plant glutathione peroxidases: Emerging role of the antioxidant enzymes in plant development and stress responses. *Journal of Plant Physiology*, 176, 192-201. https://DOI:org/10.1016/j.jplph.2014.12.014

Beyzavi, F., Baghzadeh, A., Mirzaei, S., Maleki, M., & Mozafari, H. (2021). Investigation of some biochemical traits of tolerant and sensitive wheat cultivars (*Triticum bioticum*) under salinity stress. *Journal of Crop Breeding*, *36*, 216 - 234 (In Persian). https://DOI: 10.52547/jcb.12.36.216

Bhat, J. A., Deshmukh, R., Zhao, T., Patil, G., Deokar, A., Shinde, S., & Chaudhary, J. (2020). Harnessing high-throughput phenotyping and genotyping for enhanced drought tolerance in crop plants. *Journal of Biotechnology*, *324*, 248-260. https://DOI: 10.1016/j.jbiotec.2020.11.010

Bonifacio, A., Martins, M. O., Ribeiro, C. W., Fontenele, A. V., Carvalho, F. E., Margis-Pinheiro, M. A. R. C. I. A., & Silveira, J. A. (2011). Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. *Plant, Cell and Environment*, *34*(10), 1705-1722. https://DOI: 10.1111/j.1365-3040.2011.02366.x

Chauhan, B. S., Jabran, K., & Mahajan, G. (2017). Rice Production Worldwide. Springer. https://DOI:10.1007/978-3-319-47516-5

Chen, G., Hu, Q., Luo, L., Yang, T., Zhang, S., Hu, Y., & Xu, G. (2015). Rice potassium transporter Oshak1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant, Cell and Environment*, *38*, 2747-2765. https://DOI: 10.1111/pce.12585

Chen, L., Liu, L., Lu, B., Ma, T., Jiang, D., Li, J., Zhang, K., Sun, H., Zhang, Y., Bai, Z., & Li, C. (2020). Exogenous melatonin promotes seed germination and osmotic regulation under salt stress in cotton (*Gossypium hirsutum* L.). *PLoS ONE*, *15*, e0228241. https://DOI:10.1371/journal.pone.0228241

Chen, T., Shabala, S., Niu, Y., Chen, Z. H., Shabala, L., Meinke, H., Venkataraman, G., Pareek, A., Xu, J., & Zhou, M. (2021). Molecular mechanisms of salinity tolerance in rice. *The Crop Journal*, *9*(3), 506-520. https://DOI:org/10.1016/j.cj.2021.03.005

Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W., & Theerakulpisut, P. (2016). Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi Journal of Biological Sciences*, *23*, 46. https://DOI: 10.1016/j.sjbs.2015.05.013

Chutipaijit, S., Cha-Um, S., & Sompornpailin, K. (2009). Differential accumulations of proline and flavonoids in indica rice varieties against salinity. *Pakistan Journal of Botany*, *41*(5), 2497-2506.

Daneshmand, F. (2014). The effect of ascorbic acid in reducing oxidative stress resulting from salinity stress in potatoes. *Plant Research Journal (Iranian Biology Journal) (Scientific)*, *27*(3), 417-426.

Diao, Y., Xu, H., Li, G., Yu, A., Yu, X., Hu, W., Zheng, X., Li, S., Wang, Y., & Hu, Z. (2014). Cloning a glutathione peroxidase gene from *Nelumbo nucifera* and enhanced salt tolerance by overexpressing in rice. *Molecular Biology Reports*, *41*, 4919-4927. https://DOI: 10.1007/s11033-014-3358-4

Dixon, D. P, & Edwards, R. (2010). Glutathione transferases. *The Arabidopsis Book/American Society of Plant Biologists*, 8. https://DOI: 10.1199/tab.0131

Fallah, A. (2015). Investigation of some physiological mechanisms associated with salt stress tolerance in Iranian rice cultivars. *Deputy of Rice Research Institute of Iran (Amol), 21*, 25-29. (In Persian).

Ghorbani, A., Pishkar, L., Saravi, K. V., & Chen, M. X. (2023). Melatonin-mediated endogenous nitric oxide coordinately boosts stability through proline and nitrogen metabolism, antioxidant capacity, and Na^+/K^+ transporters in tomato under NaCl stress. *Frontier in Plant Science*, *14*, 1135943. https://DOI: org/10.3389/fpls.2023.1135943

Ghorbani, A., Razavi, S. M., Ghasemi Omran, V., & Pirdeshti, H. (2019). Effects of endophyte fungi symbiosis on some physiological parameters of tomato plants under 10 day long salinity stress. *Journal of Plant Process and Function*, *7*(27), 193-208. https://DOI: 20.1001.1.23222727.1397.7.27.16.7

Ghorbani, A., Razavi, S. M., Ghasemi Omran, V. O., & Pirdashti, H. (2018). *Piriformospora indica* alleviates salinity by boosting redox poise and antioxidative potential of tomato. *Russian Journal of Plant Physiology*, *65*, 898-907. https://DOI:10.1134/S1021443718060079

Gill, S. S, & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*, 909-930. https://DOI: 10.1016/j.plaphy.2010.08.016

Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry*, *106*(1), 207-212. https://DOI: 10.1016/0003-2697(80)90139-6

Hasanuzzaman, M., Fujita, M., Kamrun, N., & Biswas, J. K. (2019). Advances in Rice Research for Abiotic Stress Tolerance. Woodhead Publishing, Elsevier Inc. https://DOI:org/10.1016/C2017-0-01486-6

Hassan, M. A., Chaura, J., Maria, P., Torres, M. P. D., Boscaiu, M. V., & icente, M. (2017). Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. *An Official Journal of the Annals of Botany, 9,* plx009. https://DOI: 10.1093/aobpla/plx009

Heath, R. L, & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*(1), 189-198. https://DOI: 10.1016/0003-9861(68)90654-1

Isayenkov, S. V, & Maathuis, F. J. (2019). Plant salinity stress: Many unanswered questions remain. *Frontiers in Plant Science*, *10,* 80. https://DOI: 10.3389/fpls.2019.00080

Islam, F., Ali, B., Wang, J., Farooq, M. A., Gill, R. A., Ali, S., Wang, D., & Zhou, W. (2016). Combined herbicide and saline stress differentially modulates hormonal regulation and antioxidant defense system in *Oryza sativa* cultivars. *Plant Physiology and Biochemistry*, *107*, 82-95. https://DOI: 10.1016/j.plaphy.2016.05.027

Jini, D, & Joseph, B. (2017). Physiological mechanism of salicylic acid for alleviation of salt stress in rice. *Rice Science*, *24*(2), 97-108. https://DOI:org/10.1016/j.rsci.2016.07.007

Khan, M. H, & Panda, S. K. (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum*, *30*(1), p.81. https://DOI:10.1007/s11738-007-0093-7

Kiani, D., Soltanloo, H., Ramezanpour, S. S., Nasrolahnezhad Qumi, A. A., Yamchi, A., Zaynali Nezhad, Kh., & Tavakol, E. (2017). A barley mutant with improved salt tolerance through ion homeostasis and ROS scavenging under salt stress. *Acta Physiologiae Plantarum, 39*, 90. https://DOI:org/10.1007/s11738-017-2359-z

Kordrostami, M., Rabiei, B., & Kumleh, H. H. (2017). Different physiobiochemical and transcriptomic reactions of rice (*Oryza sativa* L.) cultivars differing in terms of salt sensitivity under salinity stress. *Environmental Science and Pollution Research*, *24*, 7184. https://DOI: 10.1007/s11356-017-8411-0

Li, M., Li, L., Dunwell, J. M., Qiao, X., Liu, X., & Zhang, S. (2014). Characterization of the lipoxygenase (*LOX*) gene family in the Chinese white pear (*Pyrus bretschneideri*) and comparison with other members of the Rosaceae. *BMC Genomics*, *15*(1), 1-12. https://DOI: 10.1186/1471-2164-15-444

Li, W., Huai, X., Li, P., Raza, A., Mubarik, M. S., Habib, M., Fiaz, S., Zhang, B., Pan, J., & Khan, R. S. A. (2021). Genome-wide characterization of glutathione peroxidase (GPX) gene family in rapeseed (*Brassica napus* L.) revealed their role in multiple abiotic stress response and hormone signaling. *Antioxidants*, *10*, 1481. https://DOI: org/10.3390/antiox10091481

Lim, C. W., Han, S. W., Hwang, I. S., Kim, D. S., Hwang, B. K., & Lee, S. C. (2015). The pepper lipoxygenase CaLOX1 plays a role in osmotic, drought and high salinity stress response. *Plant and Cell Physiology*, *56*(5), 930-942. https://DOI:org/10.1093/pcp/pcv020

Lin, K. C., Jwo, W. S., Chandrika, N. N. P., Wu, T. M., Lai, M. H., Wang, C. S., & Hong, C. Y. (2016). A rice mutant defective in antioxidant-defense system and sodium homeostasis possesses increased sensitivity to salt stress. *Biologia Plantarum*, *60*(1), 86-94. https://DOI:10.1007/s10535-015-0561-7

Liu, Z., Ma, C., Hou, L., Wu, X., Wang, D., Zhang, L., & Liu, P. (2022). Exogenous SA affects rice seed germination under salt stress by regulating Na^+/K^+ balance and endogenous GAs and ABA homeostasis. *International Journal of Molecular Sciences*, *23*, 3293. https://DOI: 10.3390/ijms23063293

Matamoros, M. A., Saiz, A., Penuelas, M., Bustos-Sanmamed, P., Mulet, J. M., Barja, M. V., Rouhier, N., Moore, M., James, E. K., & Dietz, K. J. (2015). Function of glutathione peroxidases in legume root nodules. *Journal of the Society of Experimental Botany*, *66*, 2979-2990. https://DOI:10.1093/jxb/erv066

Minguez-Mosquera, M. I., Jaren-Galan, M., & Garrido-Fernandez, J. (1993). Lipoxygenase activity during pepper ripening and processing of paprika. *Phytochemistry*, *32*(5), pp.1103-1108. https://DOI:org/10.1016/S0031-9422(00)95073-8

Milla, M. A. R., Maurer, A., Huete, A. R., & Gustafson, J. P. (2003). Glutathione peroxidase genes in Arabidopsis are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. *Plant Journal*, *36*, 602-615. https://DOI:10.1046/j.1365-313x.2003.01901.x

Nabiollahi, K., Taghizadeh-Mehrjardi, R., Kerry, R., & Moradian, S. (2017). Assessment of soil quality indices for salt-affected agricultural land in Kurdistan Province. *Iran Ecological Indicators*, *83,* 482-494. https://DOI:org/10.1016/j.ecolind.2017.08.001

Nouri, H., Navabpour, S., Yamchi, A., & Zeyaee, F. (2015). Differential response of wild and advanced mutant lines of wheat (*Triticum aestivum* L. cv. Tabasi) genotypes in antioxidant activity to salinity stress at seedling stage. *International Journal of Biosciences (IJB)*, *6*(4), 133-147. https://DOI:10.12692/ijb/6.4.133-147

Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies.

Medicina, *53*(6), 365-374. https://DOI:1016/j.medici.2018.02.001

Omisun, T., Sahoo, S., Saha, B., & Panda, S. K. (2018). Relative salinity tolerance of rice cultivars native to North East India: A physiological, biochemical and molecular perspective. *Protoplasma*, *255*, 193-202. https://DOI:10.1007/s00709-017-1142-8

Pfaffl, M. W., Horgan, G. W., & Dempfle, L. (2002). Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research*, *30*(9), 36. https://DOI:10.1093/nar/30.9.e36

Riyazuddin, R., Verma, R., Singh, K., Nisha, N., Keisham, M., Bhati, K. K., Kim, S. T., & Gupta, R. (2020). Ethylene: A master regulator of salinity stress tolerance in plants. *Biomolecules*, *10*(6), 959. DOI:org/10.3390/biom10060959

Roychoudhury, K. D. A. A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Sciences*, *13*. https://DOI:10.3389/fenvs.2014.00053

Shaban, M., Ahmed, M. M., Sun, H., Ullah, A., & Zhu, L. F. (2018). Genome-wide identification of lipoxygenase gene family in cotton and functional characterization in response to abiotic stresses. *BMC Genom*, *19*, 599. https://DOI:10.1186/s12864-018-4985-2

Soydam, S., Buyuk, I., & Aras, S. (2013). Relationships among lipid peroxidation, SOD enzyme activity, and SOD gene expression profile in *Lycopersicum esculentum* L. exposed to cold stress. *Genetics and Molecular Research, 12*, 3220-3229. https://DOI:10.4238/2013.August.29.6

Srinineng, K., Salsavoey, T., & Karnchanatat, A. (2015). Effect of salinity stress on antoxidantive enzyme activities in tomato in vitro. *Pakistan Journal of Botany*, *47*, 1-10.

Tahjib-Ul-Arif, M., Roy, P. R., & Al Mamun Sohag, A. (2017). Exogenous calcium supplementation improves salinity tolerance in brri dhan28; A salt-susceptible high-yielding *Oryza sativa* cultivar. *Journal of Crop Science and Biotechnology*, *21*, 383-394. https://DOI:10.1007/s12892-018-0098-0

Talaat, N. B, & Todorova, D. (2022). Antioxidant machinery and glyoxalase system regulation confers salt stress tolerance to wheat (*Triticum aestivum* L.) plants treated with melatonin and salicylic acid. *Journal of Soil Science and Plant Nutrition*, *22*, 3527-3540. https://DOI:10.1007/s42729-022-00907-8

Upadhyay, R. K., Handa, A. K., & Mattoo, A. K. (2019). Transcript abundance patterns of 9-and 13- lipoxygenase subfamily gene members in response to abiotic stresses (heat, cold, drought or salt) in tomato (*Solanum lycopersicum* L.) highlights member-specific dynamics relevant to each stress. *Genes*, *10*(9), 683. https://DOI:10.3390/genes10090683

Viswanath, K. K., Varakumar, P., Pamuru, R. R., Basha, S. J., Mehta, S., & Rao, A. D. (2020). Plant lipoxygenases and their role in plant physiology. *Journal of Plant Biology*, *63*(2), 83-95. https://DOI:10.1007/s12374-020-09241-x

Wang, J., Hu, T., Wang, W., Hu, H., Wei, Q., Wei, X., & Bao, C. (2019). Bioinformatics analysis of the lipoxygenase gene family in radish (*Raphanus sativus*) and functional characterization in response to abiotic and biotic stresses. *International Journal of Molecular Sciences*, *20*(23), 6095. https://DOI:10.3390/ijms20236095

Wennman, A., Oliw, E. H., Karkehabadi, S., & Chen, Y. (2016). Crystal structure of manganese lipoxygenase of the rice blast fungus Magnaporthe oryzae. *Journal of Biological Chemistry*, *291*(15), 8130-8139. https://DOI:10.1074/jbc.M115.707380

Williams, V, & Twine, S. (1960). Flame photometric method for sodium, potassium and calcium. *Modern Methods of Plant Analysis*, *5*, 3-5. https://DOI:10.1074/jbc.M115.707380

Xu, L., Chen, H., Zhang, T., Deng, Y., Yan, J., & Wang, L. (2022). Salicylic acid improves the salt tolerance capacity of *Saponaria officinalis* by modulating its photosynthetic rate, osmoprotectants, antioxidant levels, and ion homeostasis. *Agronomy*, *12*, 1443. https://DOI:org/10.3390/agronomy12061443

Yoshida, S., Forne, D. A., Cock, J. H., & Gomez, K. A. (1976). Laboratory Manual for Physiological Studies of Rice. 3rd Ed. International Rice Research Institute, Manila, Philipine.

Zeeshan, M., Lu, M., & Sehar, S. (2020). Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. *Agronomy*, *10*, 127. https://DOI:10.3390/agronomy10010127

Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., & Luo, L. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular Breeding*, *39*, 1-10. https://DOI:org/10.1007/s11032-019-0954-y

Zhang, Q., Zhao, Y., Zhang, J., Li, X., Ma, F., Duan, M., & Li, H. (2021). The responses of the lipoxygenase gene family to salt and drought stress in foxtail millet (*Setaria italica*). *Life*, *11*(11), 1169. https://DOI:10.3390/life11111169