



## EXPLORING THE RESPONSE MECHANISMS OF RICE TO SALINITY STRESS

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(Received, 15<sup>th</sup> January 2023, Revised 2<sup>nd</sup> January 2024, Published 5<sup>th</sup> January 2024)

**Abstract** The world's agricultural productivity has been on the decline due to salinity, which is a significant abiotic element. To find a solution to this problem, researchers have been concentrating their efforts on the enzymes and biochemical pathways involved in salt tolerance. The ultimate objective is to develop crops that are resistant to salt. Developments in molecular biology have facilitated the production of salt-tolerant cultivars by conventional breeding techniques. A significant amount of salt can inhibit the growth of rice (*Oryza sativa* L.), a major food crop in many nations. This is especially true during the early stages of plant development. Rice's physiological, molecular, and biochemical reactions to excessive salinity have been the subject of significant exploration and investigation. The possible applications and implications of salinity tolerance are also discussed in this article, as well as the approaches that can be used to locate plants that are tolerant of salt.

[Citation: Abbas, A., Arshad, A., Rehman, AU., Bukhari, M.S., Zaman S. (2024). Exploring the response mechanisms of rice to salinity stress. Bull. Biol. All. Sci. Res. 9: 58. doi: <https://doi.org/10.54112/bbasr.v2024i1.58>]

**Keywords:** Salinity; Traditional; Molecular Biology; Physiological; Molecular; Screening of rice

### Introduction

Poaceae is the family to which rice (*Oryza sativa* L) belongs. Rice has n = 12 chromosomes as its fundamental number (Mohapatra et al., 2022; Shchapova, 2012). The species has the option of being tetraploid or diploid. Regarding this, *Oryza glaberrima* L. and *Oryza sativa* L. The two species are diploid (2n=24). Asian rice (*Oryza sativa* L) is the first agricultural crop with a fully sequenced genome. Rice is an important food crop because it is a staple in many countries. Over three billion people depend on it for 50–80% of their calories (Mohidem et al., 2022). About one-third of the total carbohydrate supply is produced by it. It offers the significant quantity of zinc and niacin that is advised. Because rice has a very high digestion, it has the highest biological protein (88%). Almost 90% of the land in Asia is planted to this crop, the world's second-most important crop after wheat. The crop is used for various purposes in India, including religious ceremonies, flour, rice bran oil, snacks, and brewed drinks. The crop's therapeutic benefits extend the list further (Ahmed, 2021; McHugh, 2021). Throughout history, rice farming has been the main industry in India. Approximately 650 million tons of crops are produced by rice on 156 million hectares of land worldwide (Anand et al.,

2022). This food crop is grown throughout 149.15 million hectares worldwide, producing around 550.19 million tons in India over a 44.6-million-hectare area. Rice production in India is second in the world behind China in land area and quantity. Rice is essential to India's economy and industry, accounting for 23% of global rice production and 45% of food grain production (Fahad et al., 2019; Samal et al., 2022). Nonetheless, given the growing population, there is an immediate need to boost agricultural output to maintain the country's food supply and a livelihood security framework. India is a prominent rice supplier specializing in basmati rice (Sharma et al., 2020). But to maintain the country's food and livelihood security system, there is an urgent need to enhance agricultural output due to the growing population. India is a prominent rice supplier specializing in basmati rice (Sofi et al., 2020).

### Salinity stress

Salinity reduces crop productivity (Majeed et al., 2019). Mg<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>3-</sup> are abundant in saline soils. High K<sup>+</sup>, CO<sub>3</sub><sup>2-</sup>, and NO<sub>3</sub><sup>3-</sup> levels are also common. The soil is saline when its electrical conductivity (EC) is 4 dS/m (Anjum et al., 2023; Chandel et al., 2022; Sakai et al., 2020), corresponding to 40 mM NaCl, and its osmotic pressure is 0.2 MPa. High EC can significantly

diminish agricultural yields. Saline soils are pH 7-8.5 (Thorat et al., 2018; Yadav, 2022). Low precipitation and excessive evaporation in dry and semi-arid environments prevent salts from leaching from the soil profile, causing salt buildup. This is a common reason for dry and semi-arid salinity (Meena et al., 2022). Furthermore, the process has been further enhanced by the weathering of the parent rocks (Hussain et al., 2018; Li et al., 2022). Due to seawater overflow, salinity is a well-known natural phenomenon near seashores. However, two of the several well-known human causes of salinity are irrigation and land clearance (Kumar and Sharma, 2020). Salinity has been a possible danger to over 900 million hectares of land, or around 20% of all cultivated land and half of all irrigated land worldwide salt (Zaman et al., 2018). Approximately one billion hectares of land are impacted. The scenario describes India's 8.4 million hectares of salinized soil (Sharma and Singh, 2019). Even though irrigated land makes up just 15% of all agricultural land in India, it produces roughly one-third of global consumption and is twice as efficient as rain-fed land. Given the information above and the current situation, salt-tolerant genotypes must be developed soon.

#### **Salinity Controls Production: An Important Environmental Limitation**

Agricultural soil salinity is a major cause of global agricultural production loss. Around 6% of the Earth's surface, 800 million hectares of land have been damaged by salt. Of 1500 million acres of dry land cultivation, secondary salinity has destroyed 32 million hectares (2%). High salt levels damage 45 million hectares (20%) of irrigated land, totalling 230 million (Munawar et al., 2020). Ancient humans faced soil salt as an abiotic stress before cultivation. Modern irrigation and farming have exacerbated the devastation of this abiotic stressor (Tessema et al., 2022). The effects of saltiness on plants greatly affect the output and effectiveness of farming. Two kinds of stress are caused by salinity: osmotic stress (which starts with ionic stress and is harmful because of high ionic concentration) and higher osmotic potential in the rhizosphere because of high salt concentration (Arif et al., 2020; Hussain et al., 2019). High salt levels can slow plant growth due to higher  $\text{Na}^+$  levels (Safdar et al., 2019), cause flowers to bloom later, lower fertility, and cause grains to be lost, which can make it hard for rice panicles to form (Hu et al., 2021; Parida et al., 2022). Low salt levels also cause  $\text{P}^{3-}$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  levels to drop (Ahmed et al., 2023) and stop photosynthesis from happening. Because salinity is a polygenic trait, plants respond to it differently. This makes responses unpredictable. Most plants have developed ways to handle and control the amount of NaCl present. NaCl is very soluble and is found in many things. To do this, either the roots can effectively keep out  $\text{Na}^+$  and  $\text{Cl}^-$  or the roots can selectively take them in (Ali et al., 2015; Ali et al., 2016; Ali et al., 2014; Rodríguez Coca et al., 2023).

The exclusion principle helped generate halophytes, which thrive in extremely salty soils. Once salt concentration hits 450 mM, plants like *Hordeum marinum* reject sodium and chloride ions. *Triplex halimus*, another halophyte, contains trichomes on its surface that tolerate salt. These hairs help the plant store salts and protect leaf tissues from salt stress. Barley is the most salt-tolerant, followed by bread and durum wheat, and rice is the most vulnerable. Interestingly, dicots react differently to salt than monocots. Scientists have discovered salt tolerance mechanisms by comparing wild-type and halophytic plant responses. The wild-type plant *Arabidopsis* ceases growing at 100 mM salt, yet its halophytic cousin, the *Ilungella*, thrives with little effect. This shows these plants' selective salt tolerance.

#### **High salinity affects rice growth**

Rice is grown in 114 countries worldwide and is a crop of significant economic importance (Asibi et al., 2019). Abiotic and biotic stressors, however, have the potential to lower production. This issue will worsen in awareness of the growing world population and the shortage of food sources (Teshome et al., 2020). Salt is more harmful to rice throughout its vegetative and reproductive stages (Gerona et al., 2019; Teshome, 2020). Due to gene additive effects, rice genotypes vary in salt tolerance (Chattopadhyay et al., 2020; Gerona, 2019). Rice is more robust during reproductive and grain-filling than germination and vegetative stages (Santanoo et al., 2023). Salinity induction at lower levels can improve rice's salt tolerance (Ganie et al., 2019). After drought, salt has become the second largest stressor and rice production hurdle (Fahad et al., 2019). Salinity affects rice development and yields under field circumstances, and several paddy germplasm have been tested for salt resistance (Ravikiran et al., 2018).

#### **Salinity affects rice growth**

Numerous morpho-physiological research has been completed so far to create salt-tolerant rice cultivars. This strategy's primary goal was to increase genetic diversity between the parents' genetics. Plants react to salt randomly and organically. According to reports, rice is sensitive throughout the seedling and reproductive phases of the crop (Gerona et al., 2019; Wang et al., 2019; Wu et al., 2019), which has led to a decrease in crop production and output. Regarding rice, salinity has been discovered to cause physiological and metabolic alterations that impede development and reduce production (Arif et al., 2020). True salt-tolerant lines have been evaluated using plant height, dry weight, leaf damage, and  $\text{Na}^+/\text{K}^+$  ratio to examine the physiological effects of salt accumulation (Chaurasia et al., 2020; Wangsawang et al., 2021). Salinity has various effects on rice, including inhibiting germination, making it harder to establish crop areas, developing leaf areas, reducing the amount of dry matter produced, delaying seed set, and sterility (Hussain et al., 2018). The effects of salinity on seedling growth and grain production

factors, including tiller and spikelet numbers, have been extensively studied. High salinity consistently reduces grain yield. A comprehensive study of varying floodwater salinity across rice types showed the importance of salt tolerance in rice productivity. Further research has indicated that floodwater electrical conductivity (EC) can reduce yield by up to 80% and germination rate by roughly 50% for the most vulnerable rice cultivar, depending on salt exposure. Additionally, independent of the season, salt decreased the number of spikelets per panicle, a rise in sterility, 1000 grain weight, and phase of development (Paik et al., 2020; Radanielson et al., 2018).

### **Morpho-physiological change**

Rice's diverse reaction should be used to study its salt stress tolerance. A complete examination of rice's salinity response must include the physiological processes that activate its defense mechanisms under stress. The osmotic effect, which decreases osmotic potential, causes stress, followed by the ionic effect, which causes ion toxicity. Rice's mitochondria and chloroplasts are particularly vulnerable, according to a study. Thus, chlorophyll content, fluorescence (Fv/Fm), and membrane permeability can help explain how salt inhibits photosynthetic efficiency. Studies have also found that salt stress reduces rice leaf area and structure under greenhouse or in vitro environments. Ultrastructural investigations show salt disrupts photosynthetic activities by expanding thylakoid and damaging leaf chloroplasts. Salinity also affects mesophyll tissue, harming vascular bundles.

Understanding the harmful ionic effect of salt on plants requires evaluating the crop plant's reaction at later stages. The plant reduces the majority of the harmful effects of sodium salt buildup by the following methods: (a) selective ion absorption, (b) salt exclusion, and (c) control of the K<sup>+</sup>/Na<sup>+</sup> ratio (Iqra et al., 2020a; Iqra et al., 2020b; Jam et al., 2023; Mazhar et al., 2020; Wang et al., 2022). A study of the ultra-structure of the roots revealed how saline penetrates rice and how the crop plant responds by increasing the pace. According to [69], vacuolation and vesiculation reduce the mucilage produced in treated plants compared to control. It has been proposed that there is a significant association between the amount of sodium present, the K<sup>+</sup>/Na<sup>+</sup> ratio, seedling development, and grain yield under salt stress (Naseem et al., 2020; Pour-Aboughadareh et al., 2021; Saddiq et al., 2020; Sarwar et al., 2022; Sarwar et al., 2021).

Morpho-physiological evaluations were done to determine the salt tolerance of cultivars. Tiller count, leaf area, panicle length, root length, biomass, dry weight, and RGR were measured. Relative water content (RWC) in leaves can indicate the presence of osmoprotectant, which protects cells from salt stress-induced dehydration. Prakash et al. (2019) found that native landraces may include salt tolerance genes.

Hence, the Salt Tolerance Index (STI) should be considered when morpho-biochemically evaluating them. Thus, these landraces may be useful for study and development. Therefore, by evaluating various cultivars at the morpho-physiological level, it was possible to develop a thorough understanding of the various physiological mechanisms the crop plant uses to respond to salt stress. Still, this analysis could not shed light on the precise defense mechanisms, pathways, and components directly or indirectly involved in the process (Ghafoor et al., 2020; Iqbal et al., 2021; Khadka et al., 2020).

### **Biochemical cellular reaction**

Two main ways salinity impacts agricultural plants. When salt concentration rises, plants' osmotic potential rises, and their water potential falls (Okon, 2019). This can reduce water availability and damage plants. To combat this, plants accumulate huge amounts of low-molecular-weight inorganic ions or organic solutes through osmotic adjustment (Polash et al., 2019). Examples are low-molecular-weight sugars, organic acids, polyols, proteins, amides, amino acids, and quaternary ammonium compounds. Osmolytes help plants adjust to salinity changes and stay hydrated. Second, ionic stress occurs when salt accumulation is deadly, harming plants. Understanding these two mechanisms is essential to reducing salinity's negative effects on crops. Salt stress promotes proline assimilation in higher plants (Torre-González et al., 2018). Anee, et al. (2019) found rice with increased proline, active participation in osmotic adjustment, membrane and enzyme protection, and energy and nitrogen delivery after salt exposure. Several plants respond to salt stress by using soluble carbohydrates and starch as osmotic agents, such as Karalija et al. (2018) reporting a rise in sugar levels in shoots, and Prathap et al. (2019) discovered an increase in starch in rice roots. Optimizing primary metabolism store resources aids osmotic compensation. Syeed et al. (2021) found that rice's glycine betaine accumulation reduces salt's negative effects.

It has been reported that these chemicals actively modify osmotic processes, strengthen cellular macromolecules, and store nitrogen. The elimination of reactive oxygen species, the detoxification of cells, and the preservation of the appropriate pH levels within cells depend on their presence. Alterations in protein levels or accumulation are yet another strategy for countering the effects of salt. According to Alkharabsheh et al.'s research from 2021, exposure to salinity can result in the formation of new proteins or an increase in the production of certain proteins already present in the plant, increasing the concentration of such proteins. The proteins found in plants grown in salty settings serve as a sort of nitrogen storage when the conditions are not stressful. In addition, the creation of proteins is essential for regulating osmotic pressure. The levels of soluble protein-intolerant rice seedlings were much higher

than those of sensitive rice seedlings, and there was a positive association between the two.

### **Precise and Directed Molecular Response and Cell Communication**

To choose breeding lines that can tolerate salt, efforts have been made to understand salinity tolerance at both the molecular and genetic levels. Research that was done in the past on the mechanisms of salinity tolerance in rice has demonstrated that this abiotic stress is complicated and can result in a variety of responses in plants that span both the same species and distinct varieties (Thorat et al., 2018). To screen for salt tolerance using molecular markers, genetic diversity was evaluated in many genotypes using RFLP, SSLP mapping, and RAPD and SSR analysis techniques. Both of these methods were utilized to screen for salt tolerance. Traditional techniques, including insertional mutagenesis and positional cloning, were utilized to acquire a more comprehensive understanding of the inheritance of salinity in rice (Panini et al., 2021). Several genes in rice, including de novo genes, salt, and catalase, are elevated in response to high salt levels, according to research that was conducted in the past (Singh et al., 2018). Osmotic stress, also known as Osmo-sensing, is something that plants can sense, enabling them to adapt to salinity stress effectively. Although physiological mechanisms can help explain alterations at the cellular or tissue level, the knowledge of the signaling pathway caused by this stress is mostly based on the control and regulation imposed by genetic programming. In addition, research on *Arabidopsis thaliana* mutants uncovered several regulatory genes and pathways involved in salinity tolerance. These include the Plantago thaliana Histidine kinase (Keisham et al., 2018), Ca<sup>+</sup> dependent protein kinases (CDPKs) (Wen et al., 2020), Map kinases (MAP kinases) (Bhatt et al., 2020), and the SOS pathway (Ali et al., 2023). Because salinity tolerance is a polygenic trait, researchers have also concentrated on locating quantitative trait loci (QTLs) commonly related to this characteristic. In a population of F8 recombinant inbred lines (RIL) derived from the Pokkali X IR29 cross, a substantial quantitative trait locus (QTL) referred to as "salt" was discovered on chromosome I by the utilization of AFLP markers.

Rice QTL research has found and mapped several salinity-induced QTLs. These include qRL-7 for root length, qDWRO-9a and qDWRO-9b for dry weight root, and qBI-1a and qBI-1b for biomass, with strong QTLs on chromosomes 1 and 2 for shoot growth (Thorat et al., 2018). Chen et al. (2021) found individual QTLs related to sodium and potassium uptake and selectivity. Along with other studies, Yang et al. (2021) revealed eight QTLs for each of the three shoot qualities and five root traits within five chromosomal areas. An F<sub>2</sub> mapping population from a Sadri/FL478 hybrid yielded 35 QTLs, with the largest QTL clusters mapped in chromosomes 2, 4,

and 6 for several characteristics under salt stress. The discovery of the QTL for salt tolerance opened up new avenues for research on salinity and how plants respond to subsequent stress. Research is being done to construct and identify several alleles that may or may not be related to the corresponding salinity QTLs (Ali et al., 2017; Asif et al., 2020; Farooq et al., 2021; Sayed et al., 2021; Tahir et al., 2020).

### **Rice salt-stress defense**

Plants use three primary methods to avoid salt stress. First, they modify their osmotic equilibrium to tolerate stress. Second, selective absorption and molecular regulation exclude sodium ions from leaves. Finally, compartmentalization lets plants endure high salt or chloride levels. High salt levels hinder photosynthesis, causing plants oxidative, osmotic, and ionic stress. Plants use xanthophyll pigments and transfer electrons to oxygen acceptors instead of water to fight this, which can produce reactive oxygen species. To mitigate this response, plants additionally upregulate catalase, ascorbate peroxidase, superoxide dismutase, and peroxidases. The plant's antioxidant defense system relies on non-enzymatic antioxidants such as ascorbate and glutathione and enzymes like glutathione reductase. The duration between ROS production and antioxidant scavenging determines this system's efficiency. Studies reveal salt-tolerant rice cultivars have stronger antioxidant enzyme activity, especially catalase, than salt-susceptible variants. Others have seen a decrease in catalase activity with salt exposure, suggesting distinct oxidative responses in rice. These contradicting results suggest more research on antioxidants and salt stress defense in plants.

### **Salinity stress intolerance screening**

The susceptibility of plants to salinity throughout development impacts their salt tolerance. Research shows that rice plants are more sensitive to salt during early growth but less sensitive during reproduction (Singh et al., 2021). Proper screening is needed to assess how rice germplasms react to salt. Both sensitive phases of paddy can be screened. Screening seedlings is easy and fast with clear instructions. This can be done in a lab or field. However, soil variability, climate, and other environmental factors might affect the plant's physiological activities, making field screens difficult. Salt tolerance is complicated by temperature and humidity, which alter evapotranspiration and ion transport (Resende et al., 2019). Thus, lab screenings have many advantages over field screenings. Many physiological, pharmacological, and molecular factors affect plant growth and development. Early attempts to find salinity-tolerant plants used agronomic features. Early experiments produced salt-resistant genotypes using classic selection and breeding methods. Morphological screening assessed physiological and agronomic parameters. Root length, shoot length, plant biomass, and shoot Na<sup>+</sup>/K<sup>+</sup> ratio are good salinity tolerance indicators for morphological



screening ([Huqe et al., 2021](#); [Pour-Aboughadareh, 2021](#)). Morphological screening should start 10 days after exposure to a saline solution to accurately identify tolerant and susceptible genotypes ([Afzal et al., 2023](#)).

Since the environment significantly impacts agronomic parameters, assessing salt tolerance based on them may not be profitable. Many scientists use biochemical screening to understand salt tolerance mechanisms and levels better. This has provided insights and markers on cellular, tissue, and whole-plant salt tolerance ([Morton et al., 2019](#); [Rasel et al., 2021](#); [Saradadevi, 2021](#)). With this understanding, salt stress biochemical pathways and plant defense systems can be identified. In particular, rice molecular screening has revealed its salt stress response and identified salt-tolerant genes ([Gul et al., 2022](#); [Sampangi-Ramaiah et al., 2020](#)). [Saradadevi et al. \(2021\)](#) identified a salt QTL, enabling genetics studies.

### Conclusion

After drought, salinity is the second most destructive abiotic stressor that affects rice productivity and output worldwide. The primary food crop that is grown in many nations worldwide. The demand for rice is rising with the world's growing population. As a glycophyte, rice is sensitive to salinity by nature and responds broadly and vividly to the negative consequences of excessive salt buildup. Rice uses a plant defense mechanism to reduce salt toxicity's physiological, biochemical, and molecular effects. Here is an extensive analysis of the numerous studies conducted to understand the relationship between salt and crop response. It has been extremely difficult to mention the precise method of mitigating stress because of its polygenic character. Recent discoveries of metabolic pathways, enzyme complexes, regulatory genes, and QTLs have illuminated the processes during this abiotic stress. Accruing information about the crop's reaction to salt is crucial given salinity's disastrous impact on this essential food crop. This will support ongoing efforts to increase saltwater tolerance and create salinity-tolerant animals. Many studies are being carried out worldwide to comprehend salinity and the intricate processes of rice to mitigate the same. In this context, it is crucial to summarize all the research to comprehend the relationship between salt and rice. To better understand the reaction and, consequently, the plant defense against salt as a stress, the key contributions and discoveries achieved in this field are briefly summarized in this study.

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