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Deciphering plant responses to Salt stress: Growth inhibition, Chlorophyll degradation and Antioxidant imbalance

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Abstract

Salinity is a key environmental stressor that adversely affects growth and development of plants. Effect of increasing NaCl concentrations (0-200mM) on germination, growth of plants, with a focus on antioxidant defense mechanisms has been investigated for rice plants. Significant declines in germination percentage, shoot length, and root length on increasing of salinity. Seed germination dropped from 92% in control plants to 40% at 200 mM concentration of NaCl. The quantities of chlorophyll-a and chlorophyll-b also dropped significantly indicating impaired photosynthetic capacity, while carotenoids exhibited resilience at moderate level of NaCl concentrations but declined at higher levels. Proline accumulation increased significantly with rising salinity. The study further shows that relative water content (RWC) declined as salt concentration increased, however malondialdehyde (MDA) levels increases indicating enhanced oxidative stress. Antioxidant enzyme analysis revealed that superoxide dismutase (SOD) activity rises with salinity, while catalase (CAT) activity decreased, highlighting an imbalance in the plant's capacity to detoxify ROS. Investigations provides important insights into the physiological and biochemical responses of plants to salinity stress and suggests possible strategies for enhancing salt tolerance in rice through antioxidant defense.

Key words : Salinity, rice, antioxidant, chlorophyll, germination.

Majorly 50% of the world's populations depend on rice as it is one of the primary food crops mostly in Asia, where it is crucial to food security. However, rice is highly susceptible to salinity, which is a growing concern in regions where soil salinization is increasing due to factors such as improper irrigation practices, seawater intrusion, and climate change[24]. It is estimated that nearly 20% of the world's irrigated lands are affected by salinity, directly threatening rice production[21]. The understanding of physiological and biochemical mechanisms underlying rice's response to salt stress is essential for developing salt-tolerant varieties and ensuring sustainable rice production in saline-prone areas[19].

The first drought-tolerant rice variety IR-64 DRT-1 (DRR DHAN-42) was created by marker assisted selection and introduced in 2014. Under drought stress, it yields a lot during the grain filling and reproductive phases. The QTLs, qDTY 2.2 and qDTY 4.1 genes have been introgressed for yield under stress in IR-64 DRT-1, a near-isogenic line of IR 64. The IRRI-India STRASA program backcrossed many times before intermating the QTLs for yield under drought stress. Under drought settings, DRR Dhan 42 yields more than IR 64, while under normal conditions; it yields about the same as IR 64 (https://www,icar-iirr.org>index.php>institute-research).

Salt stress affects plants through two primary mechanisms: osmotic stress and ionic toxicity[19]. Osmotic stress occurs when high concentrations of NaCl in the soil reduce plant's capacity to absorb water, leads to drought-like conditions. Ionic toxicity arises as a result of the accumulation of sodium (Na) and chloride (Cl) ions in plant tissues, which disrupts ion homeostasis, inhibits enzymatic activities, and leads to oxidative damage[21]. In rice seedlings, the accumulation of Naz in chloroplasts disrupts the photosynthetic apparatus, leading to a reduction in chlorophyll content and overall photosynthetic efficiency[24]. This degradation of chlorophyll is one of the key markers of salt stress, as it directly impacts plant development and productivity. The significance of using IR64 as a model for studying salt stress lies in its wide cultivation and genetic background, which is often used as a reference point for evaluating new rice varieties[14]. Despite its sensitivity to salinity, the of study of its physiological and biochemical responses under salt stress conditions can serve as a baseline for comparing other rice varieties and related species.

In this context, the objective of this research is to investigate how IR64 DRT-1 physiological and biochemical reactions to varying degrees of salt stress are interrelated. Through examination of different indices such as root and shoot growth, chlorophyll degradation, stress enzyme activities, and sodium localization, exploration seeks to identify potential biomarkers for salt tolerance. The results of this investigation will not only advance knowledge of IR64 DRT-1 salt stress responses but also provide valuable insights for developing resilient rice varieties and related crops that can thrive in saline-prone environments.

Seed germination and Growth monitoring:

Seeds of the rice variety IR-64 DRT-1 were collected from Krishi Vigyan Kendra (KVK), Indira Gandhi National Tribal

University (IGNTU), Amarkantak, Madhya Pradesh. This research work was carried out in Department of Botany, IGNTU, Amarkantak (M.P.). The NaCl solutions was prepared at concentrations of 25mM, 50mM, 75mM, 100mM, 125mM, 150mM, 175mM, and 200mM. Distilled water was used as a control. Dehusked IR64 rice seeds were surface sterilized by immersing them in 70% ethanol for 1 minute, followed by a wash with 5% sodium hypochlorite for 10 minutes. The seeds were then thoroughly rinsed with distilled water to ensure complete removal of sterilizing agents. For the germination setup, sterile filter paper was placed in Petri plates and an equal number of seeds were added to each dish. Each filter paper was moistened with sterile water. The seeds were spaced evenly to avoid overlapping. The Petri plates were incubated in a growth chamber at 25°C. Seed germination and growth were monitored on daily basis.

Measurement of Root and Shoot length:

After the incubation period of 14 days seeds were germinated and seedlings exhibiting visible root and shoot. Each seedling was gently removed from the Petriplate using forceps to avoid damage to the delicate roots and shoots. The root length was measured from the base of the seed, where the root emerged and up to the tip of the longest root. A ruler or digital caliper was used to measure the root length to the nearest millimeter (mm), and the length was recorded for each seedling. Similarly, the shoot length was measured from the base of the seed, where the shoot emerged and up to the tip of the highest shoot leaf. The shoot length was also measured to the nearest millimeter using a ruler or caliper and recorded for each seedling. A minimum of 10 seedlings from each plate per treatment were measured to ensure statistical significance.

Salt localization :

After the treatment period, root and shoot samples from each treatment group, including the control, were harvested. The samples were thoroughly washed with de-ionized water to remove any surface contaminants, including external salt residues. The washed samples were then dried at 70°C in an oven for 48 hours. The dried root and shoot samples were ground into fine powder using a mortar and pestle. About 0.5 g of each powdered sample was weighed and transferred into a digestion flask. To each sample, 10 mL of a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 4:1 ratio was added. The samples were digested at 150°C on a hot plate until a clear solution was obtained. Once digestion was complete, the solution was allowed to cool to room temperature. The digested sample was filtered through Whatman filter paper into a volumetric flask, and the volume was adjusted to 50 mL with de-ionized water. The prepared samples were then analyzed using an atomic absorption spectrophotometer (AAS) to quantify the concentration of sodium ($Naz^{}$) and other salts. The results were expressed as milligrams of sodium per gram of dry weight (mg/g DW).

Measurement of Biochemical substances:

For the estimation of photosynthetic capacity chlorophyll a, chlorophyll b, and carotenoids experiment was conducted following the method as suggested by Arnon[3].

To quantify proline accumulation method of Bates *et al*.[7] was used with some modifications. Proline serves as an osmoprotectant under salt-induced osmotic stress. The glycine betaine (GB) concentration in shoots and roots was measured according to the protocol of Grieve and Grattan[12]. Accumulation of glycine betaine acts as an osmoprotectant that helps to maintain cellular osmotic balance under salt stress conditions. The relative water retention ability under salt stress was measured by the method of Barrs and Weatherley[6]. To measure the extent of oxidative membrane damage caused by salt-induced reactive oxygen species (ROS) Lipid peroxidation was measured as malondialdehyde (MDA) content, using the method of Heath and Packer[13]. Superoxide dismutase (SOD) activity was measured based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), following the method as described by Beauchamp[8]. Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide (H_2O_2), following the method as described by Aebi[1].

Antioxidant activity :

The antioxidant activity of plant extracts was evaluated using the DPPH free radical scavenging method of Mensor *et al*.[17]. Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 80% methanol. The homogenate was centrifuged at 12,000 rpm for 15 minutes, and the supernatant was used for the DPPH assay. A volume of 1 mL of the extract was mixed with 3 mL of 0.1 mM DPPH solution in methanol. After incubation in dark for 30 minutes at room temperature absorbance was measured at 517 nm, and the percentage of DPPH scavenging activity was calculated. FRAP assay Antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay method as described by Benzie and Strain[9].

Effect of salinity on growth parameters :

This study provides an in-depth analysis of the effects of increasing salt concentrations on plant development, physiological parameters, and antioxidant defense mechanisms. The data showed in Table-1 indicate that increasing NaCl concentrations have a detrimental effect on plant germination and growth. Under control conditions, plants exhibited optimal growth with a germination rate of 92%, along with shoot (6.2cm) and root (5.7cm) lengths. However, as salinity increased, the growth parameters progressively declined. At 200 mM concentration of NaCl, the germination rate dropped to 40%, with shorter shoot (2.8cm) and root (3.2cm) lengths. This reduction in growth can primarily be attributed to osmotic stress, which limits water uptake by lowering the soil's osmotic potential. Munns and Tester (2008) explained that reduced water availability under salt stress condition hinders cell expansion, especially in shoots and roots, which directly impacts growth of plant. Additionally, salinity leads to ionic stress due to the accumulation of Na^+ and Cl^- ions, which can cause cellular toxicity and further inhibit growth. The results are consistent with findings of Almansouri *et al*.[2], who reported that high salinity negatively affects seed metabolism, enzyme activity, and water uptake during germination, leading to reduced germination rates. Interestingly, the root and shoot (R/S) ratio increased under severe salt stress, particularly at 175 mM and 200 mM concentration

of NaCl. This suggests that plants prioritize root development over shoot growth in response to salinity, likely as an adaptive mechanism to maintain water uptake under stressful conditions. Jaleel *et al*.[15] observed a similar pattern, where the R/S ratio increased under salt stress, indicating that root growth is less sensitive to salinity than shoot growth. This adaptation allows plants to maximize water absorption while minimizing water loss through transpiration.

Effect of salinity on pigments :

The results (Table-2) demonstrate that increasing NaCl concentrations significantly impact the levels of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids. Chlorophyll a, which plays a central role in photosynthesis, showed a consistent decline from 1.213 mg/g FW in control plants to 0.753 mg/g FW at 200 mM concentration of NaCl. Similarly, chlorophyll b levels decreased from 0.560 mg/g FW under control conditions to 0.113 mg/g FW at 200 mM concentration of NaCl. These reductions reflect the damage caused by oxidative stress and ionic imbalance, which disrupts chlorophyll biosynthesis and degrades pigment molecules and similar results were reported by Ashraf and Harris[5], showing that high NaCl levels interfere with the photosynthetic apparatus, primarily by damaging chloroplast structures. Unlike chlorophyll, carotenoid levels followed a different pattern. While carotenoid content decreased at higher NaCl concentrations, it remained relatively stable at moderate salinity levels (50 mM to 75 mM), suggesting that carotenoids play a protective role under these conditions by scavenging reactive oxygen species (ROS). Ashraf and Harris[5] found that carotenoids act as antioxidants, helping plants to mitigate oxidative stress under moderate salinity. However, at severe stress levels (175 mM and 200 mM), carotenoid levels dropped to 1.0 mg/g FW, indicating that the plant's antioxidant capacity may have been inundated by excessive ROS. At moderate NaCl stress levels such as 100 mM, a slight increase or maintenance of chlorophyll content may occur compared to both lower (75 mM) and higher (125 mM) concentrations. This counter intuitive result can be attributed to the activation of stress-responsive mechanisms, a phenomenon known as hormesis, wherein low to moderate levels of stress trigger beneficial adaptive responses. Under such conditions, plants can initiate the production of antioxidant enzymes, osmoprotectants like proline, and ion transporters, which collectively mitigate the damaging effects of salt stress and help to maintain chlorophyll synthesis and stability. It is possible that the plants exposed to 100 mM NaCl were in a slightly better physiological state or possessed greater inherent tolerance, which allowed them to retain more chlorophyll. Natural differences in metabolic response or micro-environmental factors during experimentation can influence such variations in pigment content. Another contributing factor is the osmotic adjustment phase, where plants accumulate compatible solutes such as sugars, proline, and glycine betaine. These compounds stabilize chloroplast membranes and protect the photosynthetic machinery, thereby supporting chlorophyll retention. Furthermore, enzymes involved in chlorophyll biosynthesis, like chlorophyll synthase, may still be partially active at 100 mM NaCl but inhibited at higher stress levels, such as 125 mM concentration of NaCl. In contrast, at 75 mM concentration of NaCl, the plant may be in the initial transition phase of

stress exposure, where adaptive responses are not yet fully activated, leading to a decline in chlorophyll. At 125 mM, the salt stress becomes more severe, surpassing the plant's tolerance threshold, and results in significant damage to the chloroplasts and photosynthetic system. This is especially evident in the drastic reduction of chlorophyll b content, which is generally more sensitive to environmental stress than chlorophyll a. For example, in the observed data: at 75 mM NaCl concentration, chlorophyll a and b levels dropped to 0.870 ± 0.017 and 0.323 ± 0.011, respectively, indicating increased stress. However, at 100 mM, there was a slight rise to 0.876 ± 0.015 (Chl a) and 0.346 ± 0.015 (Chl b), suggesting temporary adaptation. At 125 mM, the values fell further to 0.840 ± 0.020 and 0.333 ± 0.005, signifying the onset of pigment degradation and structural damage due to overwhelming stress[5].

Accumulation of NaCl in roots and shoots of plants :

The results (Figure 1) on salt accumulation in plants highlight the strategy for coping with salinity by differentially distributing salts between shoots and roots. In the control set, shoot salt concentrations was low (9.2 ppm), but as NaCl levels increased, salt accumulation in the shoots rose sharply, reaching 30 ppm at 200 mM concentration of NaCl. This suggests that the plant sequesters salts in its shoots as a mechanism to avoid toxicity in more sensitive tissues, such as roots and meristematic regions. Zhu[25] explained that plants often compartmentalize salts in vacuoles to maintain osmotic balance while minimizing ion toxicity. Interestingly, salt accumulation in the roots followed a different pattern. While root salt concentrations initially decreased at 50 mM and rose sharply beyond 100 mM, reaching 12.0 ppm at 200 mM concentration of NaCl. This suggests that the root's ability to exclude salts becomes overwhelmed under severe salinity. Verbruggen and Hermans[23] reported similar findings, noting that root exclusion mechanisms can fail under high salt concentrations, leading to greater ion accumulation and potential damage to root cell membranes.

Table-1. Effect of NaCl concentration on germination %, shoot length, root length and the root and shoot (r/s) ratio of seedlings (SD-±)

NaCl Concentration	Germination %	Shoot Length (cm)	Root (cm)	Root/ shoot
Control	92± 0.037	6.2 ± 0.20	5.7 ± 0.14	0.91
25Mm	88 ± 0.06	5.4 ± 0.24	4.8 ± 0.18	0.88
50mM	80 ± 0.21	5.2 ± 0.28	4.5 ± 0.17	0.86
75mM	72 ± 0.32	4.5 ± 0.34	4.3 ± 0.22	0.95
100mM	68 ± 1.40	4.1 ± 0.39	3.8 ± 0.25	0.92
125mM	64± 0.05	4.0 ± 0.44	3.6 ± 0.3	0.9
150mM	60 ± 0.06	3.8 ± 0.49	3.4 ± 0.35	0.89
175mM	52 ± 1.0	3.0 ± 0.58	3.3 ± 0.4	1.1
200mM	40 ± 0.42	2.8 ± 0.68	3.2 ± 0.45	1.14

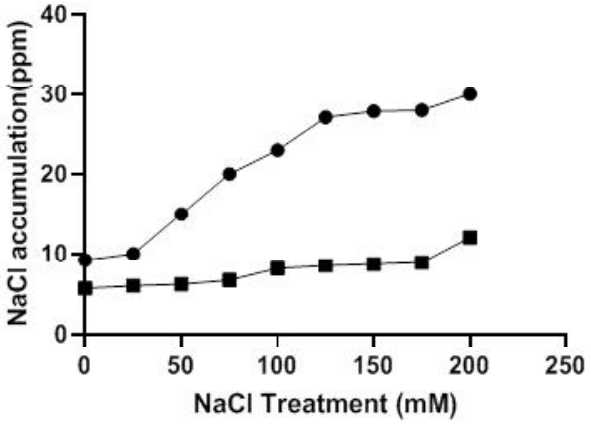

Figure 1. Effect of NaCl concentrations on the salt present in shoot and root (SD-±).

Table-2. Effect of NaCl concentrations on Cholorophyll a, Cholorophyll band carotenoids (SD-±)

NaCl Concentration (mM)	Chlorophyll a (mg/gFW)	Cholorophyll b (mg/gFW)	Carotenoid (mg/gFW)
Control	1.213 ± 0.035	0.560 ± 0.020	5.90± 0.104
25mM	1.206 ± 0.023	0.553± 0.011	3.09 ± 0.072
50mM	0.873 ± 0.015	0.335 ± 0.005	3.0 ± 0.052
75mM	0.870 ± 0.017	0.323 ± 0.011	2.19 ± 0.065
100mM	0.876 ± 0.015	0.346 ± 0.015	1.29 ± 0.055
125mM	0.840 ± 0.020	0.333 ± 0.005	1.25 ± 1.0
150mM	0.826 ± 0.015	0.313 ± 0.005	1.22 ± 0.034
175mM	0.776 ± 0.015	0.310± 0.010	1.0 ± 0.037
200mM	0.753 ± 0.015	0.113 ± 0.005	1.0 ± 0.015

Table-3. Effect of NaCl concentrations on proline, betain, Relative water content (RWC) and Malondialdehyde (MDA) (SD-±).

NaCl Concen-tration (mM)	Proline (mmol/L)	Betaine (µg)	Relative Water Content (RWC%)	MDA Content (n mol/g)
Control	0.97±0.011	130.27 ±0.46	91.24 ± 0.030	11.29± 0.18
25mM	4.70±0.005	132.65±0.31	90.1 ± 0.005	14.24± 0.03
50mM	4.93±0.036	136.75±0.26	89.1±0.03	14.27 ± 0.01
75mM	5.60±0.005	143.06±0.02	82.16 ± 0.11	17.37± 0.03
100mM	5.97±0.026	146.33±0.19	80.1 ± 0.005	17.39 ± 0.05
125mM	6.04 ±0.047	175.85±0.42	72.45± 0.37	20.39± 0.02
150mM	7.46 ±0.045	179.26±0.015	70.2 ± 0.18	24.53 ± 0.21
175mM	8.24±0.161	185.25±0.66	65.53± 0.05	30.53 ± 0.005
200mM	9.53±0.057	199.7±1.35	60.2± 0.032	32.40 ± 0.30

Effect of salinity on proline :

The results (Table-3) indicate a remarkable increase in proline accumulation as NaCl concentrations rise, with levels reaching to 9.53 mmol/L at 200 mM concentration of NaCl. Proline is a well-known osmoprotectant, helping plants stabilize proteins and membranes under osmotic stress. Its accumulation under salt stress reflects the plant's adaptive response to mitigate the effects of dehydration and ion toxicity. Ashraf and Foolad[4] emphasized that proline plays a critical role in maintaining cellular osmotic balance and reducing oxidative stress by scavenging ROS. This increase in proline content is consistent with the findings which demonstrated that proline biosynthesis is upregulated in response to salt stress, contributing to enhance stress tolerance. Moreover, Verbruggen and Hermans[23] found that proline accumulation correlates with increased ROS scavenging capacity, further highlighting its protective role under salinity.

Effect of salinity on glycine betaine :

The results are given in (Table-3) indicates that the accumulation of Glycine Betaine was significantly higher after imposing salt concentrations. As the NaCl concentration raises from 25mM to 200mM the glycine betaine increases from lower concentrations to higher that is at 25mM it was 130.27 µg to 199.7 µg at 200mM clearly indicating that the increased level of betain is helping the plant to contribute to the increase of the Na^+ flux from the cytoplasm to the vacuole and also known to modify the membrane potentiality. In addition to osmoregulation glycine betaine stabilizes the oxygen evolving activity of photosystem-II protein complex ultimately improve the plant growth parameters by protecting against dissociation of regulatory extrinsic proteins[16]. Also they reported that in rice seedlings, tolerance to salt stress is enhanced by the accumulation of glycine betaine even if the levels of glycine betaine are lower in the chloroplast[22].

Effect of relative water content on salinity:

As NaCl concentrations increased, relative water content (RWC) decreased while malondialdehyde (MDA) a marker of lipid peroxidation increased. This indicates that salt stress simultaneously induces water loss and oxidative damage in plants. RWC declined from 91.24% in the control to 60.2% at 200 mM NaCl, reflecting the reduced ability of plants to retain water under osmotic stress. Munns (2002) also noted that high salinity reduces water availability, leading to dehydration and impaired growth.

Effect of salinity on Lipid Peroxidation:

Malondialdehyde (MDA) level rise from 11.29 nmol/g in control plants to 32.40 nmol/g at 200 mM NaCl, suggesting increased oxidative stress due to the accumulation of ROS. Parida and Das[20] observed a similar rise in MDA levels in salt-stressed plants, linking it to oxidative damage and membrane lipid peroxidation. The simultaneous reduction in RWC and increase in MDA levels highlight the dual impact of salt stress on both water retention and oxidative stress management. The antioxidant response, as indicated by DPPH, FRAP activity (Figure 2), superoxide dismutase (SOD), and catalase (CAT) activity (Figure 3) showed differential responses towards increasing NaCl concentrations.

Effect of salinity on DPPH :

DPPH activity, which measures the plant's ability to scavenge free radicals, increased significantly from 22.84% in the control sets to 57.41% at 200 mM NaCl. This rise reflects the plant's upregulation of antioxidant mechanisms in response to salt-induced oxidative stress. Similarly an increased DPPH activity was observed in salt-stressed plants, linking it to enhanced antioxidant production availability of plant. FRAP indicating antioxidant response under salt stress; the antioxidant activity was found remarkably effected[18].

Effect of salinity on Ferric reducing power assay:

The FRAP (Ferric Reducing Antioxidant Power) results revealed that on increasing the NaCl concentrations FRAP percent enhanced till 125 mM concentrations of NaCl however on further increasing of NaCl concentrations decline in the FRAP was reported. The FRAP is a widely used as an indicator of total antioxidant capacity in plant tissues, reflecting the plant's ability to scavenge and neutralize reactive oxygen species (ROS). In IR-64 DRT1, the FRAP values peaked between 125 and 150 mM NaCl, correspondingly FRAP

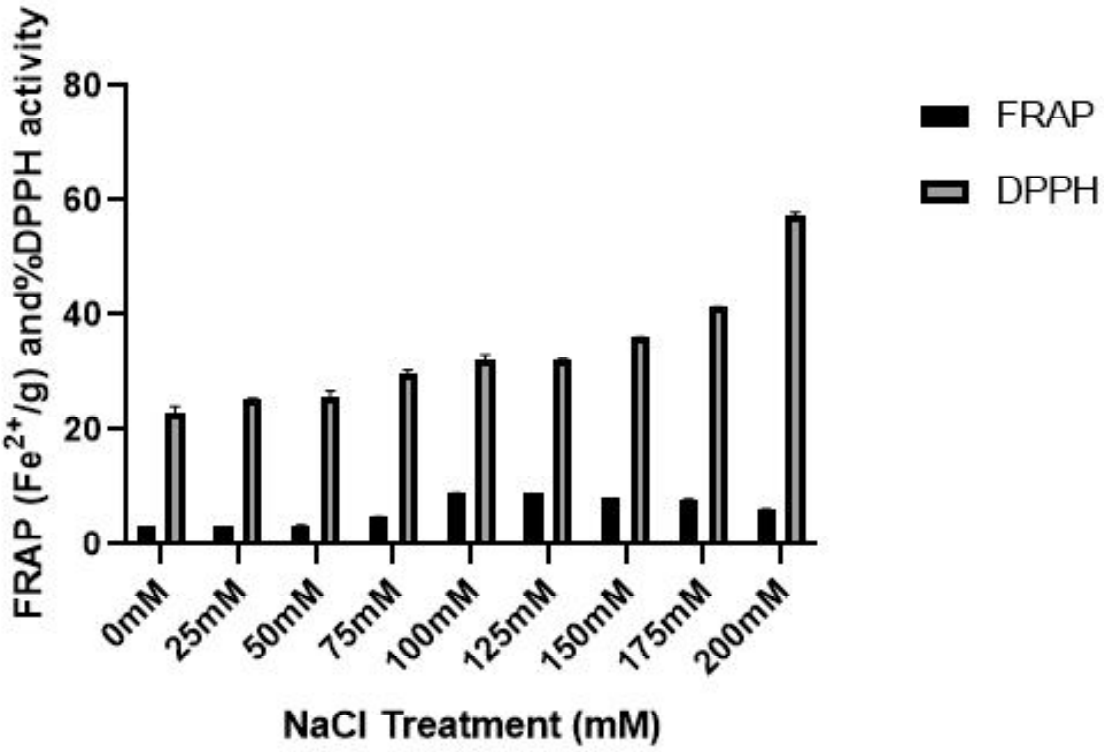

Figure 2. Effect of NaCl concentration on DPPH activity (%) and FRAP(Fe^{2+}/g).

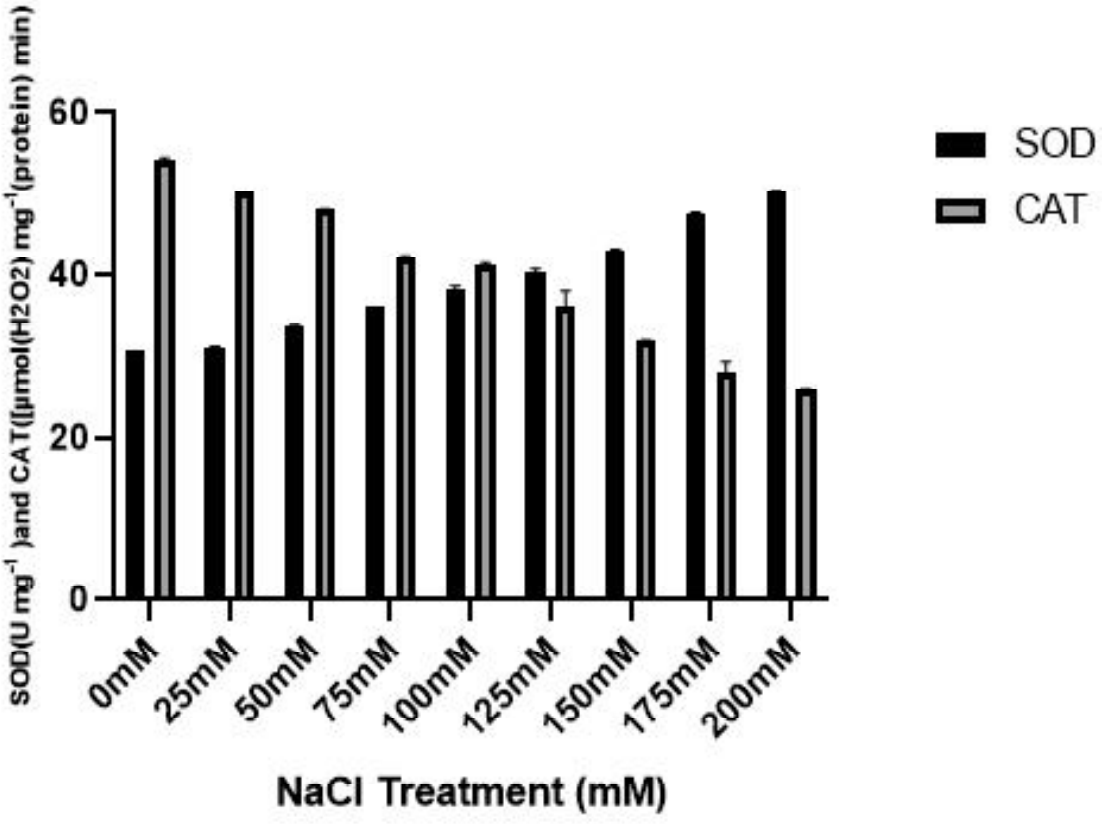

Figure 3. Effect of NaCl concentration on superoxidase dismutase (SOD) activity and Catalase (CAT) activity.

values reaching 9.15 and 8.14 Fe^{2+} /g respectively. This increase is associated with the upregulation of the plant's antioxidant defense system, including key enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic molecules like glutathione, ascorbate, and phenolics. However, beyond 150 mM NaCl, the FRAP values begin to decline, dropping to 7.74 and 6.2 Fe^{2+} /g at 175 and 200 mM, respectively. This decline suggests that the stress level at the higher salinity concentrations become too severe, resulting in the overproduction of ROS that overwhelms the plant's antioxidant machinery. Under such extreme conditions, enzyme activity may be inhibited due to oxidative damage, denaturation, and energy depletion (*e.g.*, limited ATP and NADPH availability), leading to reduced antioxidant biosynthesis and repair mechanisms. Consequently, the decrease in FRAP values at 175 and 200 mM NaCl reflects a breakdown of the plant's oxidative stress defense system, pointing to metabolic exhaustion and potential cellular damage[9].

Effect of salinity on superoxidase mutase:

The SOD activity also increased steadily with rising NaCl concentrations, reaching 50.4 U mg^{-1} at 200 mM NaCl. SOD is critical for converting superoxide radicals into hydrogen peroxide, a less harmful molecule. Gill and Tuteja[11] noted that SOD is often the first line of defense against oxidative stress, and its upregulation in this study indicates the plant's effort to neutralize ROS.

Effect of salinity on Catalase :

The catalase (CAT) activity showed the reverse trend, decreasing from 53.2 µmol H_2O_2 mg^{-1} in control plants to 25.93 µmol H_2O_2 mg^{-1} at 200 mM NaCl. The decline in CAT activity suggests that while SOD is effectively converting superoxide radicals into hydrogen peroxide, the plant's ability to detoxify hydrogen peroxide is impaired under severe stress. De[10] reported similar findings in maize, where CAT activity decreased under high salinity due to the accumulation of ROS. The imbalance between SOD and CAT activities likely contributes to the observed increase in oxidative stress, as excess hydrogen peroxide is not adequately detoxified.

The present study unveils the complex physiological and biochemical responses of IR-64 DRT1, a newly developed drought-tolerant rice variety, under varying levels of salt stress, providing important insights into its cross-tolerance mechanisms. Although IR-64 DRT1 demonstrated promising activation of adaptive responses, such as proline accumulation and elevated superoxide dismutase (SOD) activity, its declining catalase (CAT) levels at higher NaCl concentrations indicate that antioxidant defenses are insufficient under severe salinity. The findings highlight the possibility of strengthening salt tolerance by improving ROS detoxification, especially by increasing CAT activity. Crucially, proline's dual-functionality as an osmoprotectant and ROS stabilizer highlights a crucial adaptive characteristic deserving of further research. This paper bridges the information gap between tolerances to various abiotic stressors by focusing on the reaction of a drought-resilient cultivar to salt. In light of growing climatic unpredictability, our results pave the way for the breeding or engineering of rice cultivars with improved multi-stress tolerance.

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