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Exogenous selenium alleviates salt stress in rice seedlings by improving growth and antioxidant defenses

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ABSTRACT

Salt stress severely affects the growth and physiological characteristics during the early growth stages of rice (*Oryza sativa* L.). This study aimed to investigate the potential impact of exogenous selenium (Se) in mitigating the adverse effects of salt stress during rice germination and seedling establishment. At the germination stage, seeds were incubated for nine days under either non-stress (control) or 100 mM NaCl stress conditions, with or without Se supplementation. At the seedling stage, rice plants were grown hydroponically in nutrient solution under control or 100 mM NaCl conditions, again with or without Se. The results revealed that NaCl-stressed rice plants markedly compromised germination indices and plumule growth and reduced photosynthetic pigment contents. Under salt stress, rice plants also exhibited enhanced H_2O_2 accumulation, elevated lipid peroxidation measured as malondialdehyde (MDA) level, and increased activities of antioxidant enzymes - peroxidase (POD) and ascorbate peroxidase (APX). In contrast, exogenous Se application significantly improved germination indices, and seedling growth under salt stress. Selenium treatment also enhanced chlorophyll and carotenoid synthesis and boosted antioxidant enzyme activities while suppressing the accumulation of H_2O_2, thereby protecting against oxidative damage. Furthermore, exogenous Se alleviated membrane injury under saline stress, as reflected by significantly lower MDA levels in stressed seedlings. Finally, these findings suggest that exogenous Se enhances salt stress tolerance in rice by strengthening antioxidant defense responses and reducing oxidative damage during early growth stages.

1. Introduction

Soil salinization is one of the major abiotic stresses adversely affecting crop production. Salinity of arable land is an escalating problem worldwide, which confines crop productivity by affecting plant growth and restricting land use (Safdar et al., 2019; Ondrasek et al., 2022). Soil salinization is the result of both natural processes and human activities (Safdar et al., 2019; Ondrasek et al., 2022; Atta et al., 2023). Climate change and inappropriate agronomic practices are progressively increasing the salt-affected areas which in turn cause threat to food security (Ondrasek et al., 2022). If it continues, it is projected to result in the loss of 50% of cultivable land by 2050 (Atta et al., 2023). Therefore, the utilization of salt-affected land for crop production is crucial to meet expanding food demand (Safdar et al., 2019).

For sustainable food production and security, exploration of effective methods and strategies to improve salt tolerance of crops, and thereby bring saline soil under cultivation, is vital. Soil salinity adversely impacts plant development by altering metabolic activities, disrupting ionic balance, triggering osmotic and oxidative stress, etc. (Safdar et al., 2019; Ondrasek et al., 2022). Several studies have reported that exogenous application of potential substances, also called biostimulants, can play a role in reducing salt stress damage and enhancing salt tolerance via activating various metabolic processes (Nephali et al., 2020; Quamruzzaman et al., 2021; Lakhdar et al., 2023; Jiang et al., 2024). Specific nutrients (macro- or micronutrient) have also been applied for alleviating the adverse effects of salinity stress (Shahverdi et al., 2018; Kamran et al., 2019; Rajput et al., 2021; Tsivileva, 2025). It has been reported that selenium (Se), an essential micronutrient for humans and animals, exerts beneficial impacts on plants grown under stress conditions (Kamran et al., 2019). Selenium has been categorized as a beneficial (non-essential) element for plants, which plays a significant role in regulating biochemical processes (Sieprawska et al., 2015; Kamran et al., 2019;

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Tsivileva, 2025). However, Se exhibits both beneficial and toxic responses to plants, depending on plant species and Se concentration. Se supplementation at lower dosages acts as an antioxidant and gives protection to plants from oxidative damage by activating the antioxidative mechanisms (Kamran et al., 2019; Tsivileva, 2025).

Many studies have demonstrated that Se may act as a protective agent against several abiotic stresses, such as heavy metals (Sardar et al., 2022; Jia et al., 2023), drought (Nawaz et al., 2015; Rady et al., 2020), high temperature (Djanaguiraman et al., 2010) and salinity (Jiang et al., 2017; Rasool et al., 2023; Fatahiyan et al., 2025). Exogenous supplementation of Se has been exhibited significant improvement in the growth and development of several crops under salt stress conditions by increasing photosynthetic pigments levels, accumulating osmoprotectants, activating enzymatic and non-enzymatic defense mechanisms, reducing reactive oxygen species (ROS) levels, and regulating Na^+ transporters responsible for ion homeostasis (Rasool et al., 2023; Hussain et al., 2023a, 2023b; Zafar et al., 2024; Mishra et al., 2025; Fatahiyan et al., 2025).

For safeguarding global food security and sustainability, the persistent supply of rice (*Oryza sativa* L.) is crucial as it is a staple diet for more than half of the world's population (Hashim et al., 2024). Globally, over 21% of caloric needs of a human is met by rice, whereas in Southeast Asia, it encompasses around 76% of calorific intake (Mohidem et al., 2022). Globally, rice production is substantially threatened by several abiotic stresses, including salinity (Kumar et al., 2022; Mishra et al., 2025). Global warming, climate change, and rising sea levels are predicted to worsen the negative effects of soil salinization on rice productivity onwards (Kumar et al., 2022; Mishra et al., 2025). In saline soil, rice performs poorly than other major cereal crops such as wheat and maize, therefore, it is classified as a salt-sensitive crop (Kumar et al., 2022; Munns and Tester, 2008). Soil salinity adversely affects seedling growth, panicle initiation, spikelets per panicle, and ultimately grain yield in rice (Mishra et al., 2025; Sackey et al., 2025). Reduced tiller number, leaf area, and biomass, delayed flowering, and lower seed set also occur in rice crops due to salinity (Sackey et al., 2025). Therefore, for coping with hostile saline soils and sustaining rice production, exploration and implementation of effective tactics such as improved agricultural practices, use of biostimulants, genetic enhancement, etc., are crucial.

Taking the above-mentioned points into view, this research was designed to investigate the impact of exogenous selenium on rice seedlings under high salinity. Growth and biochemical responses of salt-stressed rice after application of exogenous selenium were assessed to inspect the potential contribution of selenium in improving the tolerance of plants to salt stress.

2. Materials and Methods

2.1. Experimental location and material

The experiment was conducted at the laboratory of the Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh, during the period from October 2019 to March 2020. Seeds of BRRI dhan-29, collected from the Bangladesh Rice Research Institute, Gazipur, were used for experiments.

2.2. Germination of seeds

Seeds were surface sterilized by soaking in 2.5% sodium hypochlorite + 2.0% Tween-20 solution for 15 minutes, followed by washing with distilled water (dH_2O) for four times. Then the seeds were soaked in dH_2O and kept in the dark at 25°C. After 24 hours of imbibition, seeds were placed on petri dishes and divided into four treatment groups (Fig. 1A). In the first group, seeds were placed in a petri dish containing dH_2O-soaked paper, and this petri dish was marked as control (C). In the second group, seeds were kept in a petri dish where 10 mL of 100 mM NaCl solution was added to soak the paper and marked as NaCl group. The third group was marked as NaCl+Se, where a solution of 100 mM NaCl with 1 µM selenium (Se) (in the form of Sodium selenate; Na_2SeO_4) was supplied to the seeds. In the case of the fourth group, seeds were supplemented with only Se solution and therefore, designated as Se group. All treatment conditions were replicated thrice. Afterwards, the petri dishes were kept in the dark at 25°C for seed germination. When the radicle reached a length of 2 mm, a seed was considered germinated.

2.3. Measurement of germination indices

The number of germinated seeds was recorded from the 4th day after incubation (DAI) up to 9th DAI (Fig. 1A). Moreover, the radicle length (RaL) and plumule length (PL) were recorded on the 9th DAI. By using the germination counts, several germination indices such as final germination percent (FGP) and germination energy (GE) were calculated as described by Tahjib-Ul-Arif et al. (2018), Adilu and Gebre (2021), and Alhammad et al. (2023). The formula for FGP and GE is as follows:

$$\mathrm{GE}(\%) = \frac{\text{Total number of seeds germinated on 4th day}}{\text{Total number of seeds placed in a Petri dish}} \times 100$$

$$\mathrm{FGP}(\%) = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds placed in a Petri dish}} \times 100$$

2.4. Growing rice seedlings and application of treatments

For experimenting at the seedling stage, rice seedlings were grown hydroponically using Hoagland nutrient solution (Hoagland and Arnon, 1950). After sterilization as described earlier, the sterilized seeds were imbibed in dH_2O for 24 hours and then incubated in the dark for germination at 25°C. When germinated, seeds were transferred to plastic pots filled with distilled water and allowed to grow for 3 days. From the 4th day of sowing, distilled water in the plastic pots was replaced with Hoagland nutrient solution.

After 14 days of sowing, rice seedlings were divided and exposed to four different treatment conditions (Fig. 1B). For salinity stress, rice seedlings were supplied with the nutrient solution supplemented with 100 mM NaCl (marked as NaCl group). The control (C) plants were continued to grow in nutrient solution only. For salinity with selenium treatment (NaCl+Se), seedlings were exposed to the nutrient solution containing 100 mM NaCl as well as sprayed with 1 µM selenium. In the case of Se alone treatment (Se), only foliar spray of 1 µM selenium was carried out in the seedlings. For each treatment, there were three independent replicates. After 8 days of growth under the above conditions (Fig. 1B), samples were collected and different biochemical parameters were measured.

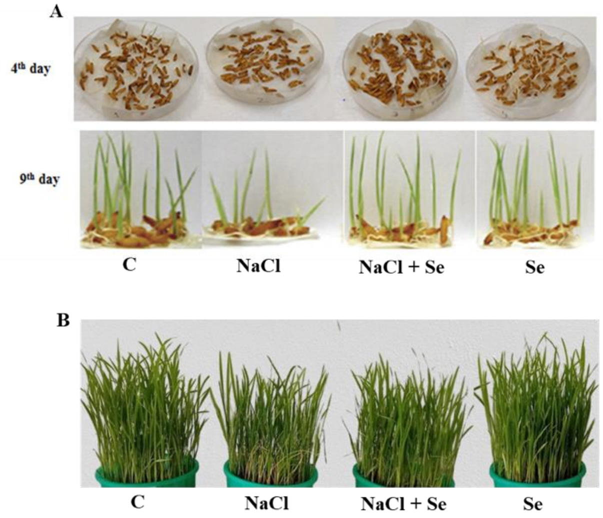

Figure 1. Effect of selenium on (A) seed germination at 4th and 9th day of incubation and (B) growth of rice seedlings at 22nd day under normal or salt stress conditions.

C = Control, distilled water; ***NaCl*** = 100 mM NaCl; ***NaCl+Se*** = 100 mM NaCl + 1 µM Selenium; ***Se*** = 1 µM Selenium.

2.5. Determination of chlorophyll content

To determine the chlorophyll content, a method developed by Coombs et al. (1985) was followed. The fresh leaf sample of 0.05 g was dipped into a screw-capped test tube containing 80% acetone and preserved in the dark for 7-10 days. The absorbance readings were recorded at 645 and 663 nm using a UV/Vis spectrophotometer (Shimadzu, UV-1201; Japan) and the result was expressed as mg g^{-1} fresh weight (F.W.) of leaf.

2.6. Determination of hydrogen peroxide (H_2O_2) and malondialdehyde activity

Hydrogen peroxide was measured as described by Velikova et al. (2000). Freshly collected leaves (0.1 g) were homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 4 °C. Then 0.5 mL of resultant supernatant was mixed with 0.5 mL of phosphate buffer (10 mM) and 1 mL of potassium iodide (1M) and incubated in the dark for 1 hour. The absorbance of the resulting solution was read at 390 nm using a UV/Vis spectrophotometer, and the H_2O_2 content was calculated from the extinction coefficient of 0.28 $\mu M^{-1}cm^{-1}$.

The MDA content was determined by following the method of Zhang and Huang (2013). The supernatant of leaf extract mediated by 0.1% TCA was mixed with 20% TCA solution containing 0.5% thiobarbituric acid (TBA). This mixture was then boiled for 15 min at 95 ºC, followed by cooling and centrifugation at 4 ºC. The absorbance of the supernatant was recorded at 532 nm using a UV/Vis spectrophotometer, and the MDA content was expressed as nmol MDA g^{-1} FW.

2.7. Determination of antioxidant enzyme activities

For enzyme extraction, fresh leaf samples (0.05 g) were homogenized in 3 mL of 50 mM potassium phosphate buffer (PB) (pH 8.0). The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatant was used for the determination of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) enzyme activities. The activity of CAT (EC: 1.11.1.6) was measured according to Aebi (1984). The reaction mixture was made by mixing 0.7 mL of PB, 0.1 mL of EDTA, and 0.1 mL of H_2O_2. In the assay of POD (EC: 1.11.1.7) and APX (EC: 1.11.1.11) activities, the method described by Nakano and Asada (1981) was followed. In case of POD activity, the reaction mixture was prepared with 0.6 mL of 50 mM PB, 0.1 mL of EDTA, 0.1 mL of H_2O_2 and 0.1 mL of guaiacol. For assaying APX activity, 0.6 mL of PB, 0.1 mL of EDTA, 0.1 mL of H_2O_2 and 0.1 mL of ascorbate were added in the reaction mixture. The changes in absorbances were recorded immediately after adding 0.1 mL of enzyme extract at 30s intervals for two minutes. The absorbances were read at 240 nm, 470 nm, and 290 nm for assaying CAT, POD and APX activities, respectively, using a UV/Vis spectrophotometer.

2.8. Statistical analysis

A one-way analysis of variance (ANOVA) was performed using the statistical software R (version 4.3.1). All the data presented in the figures and tables are means with standard errors (n = 3). Different letters denote statistically significant differences between treatments at $p < 0.05$, obtained from Fisher LSD Test.

3. RESULTS

3.1. Germination of seeds and growth of seedlings

In the present study, salt stress (100 mM NaCl) imposed adverse effects on seed germination (Fig. 1A & Table 1). However, Se application showed a protective role in improving germination indices under salinity.

Table 1. Effects of exogenous selenium (Se) on final germination percentage (FGP), germination energy (GE), plumule length (PL), and the radicle length (RaL) of rice seeds under normal or salt stress conditions

Treatment	Germination indices		Growth parameters	
	FGP (%)	GE (%)	PL (cm)	RaL (cm)
C	95.3 ± 1.20^a	64.7 ± 1.20^a	5.35 ± 0.08^a	3.88 ± 0.08^b
NaCl	69.7 ± 1.20^d	29.7 ± 0.88^d	3.86 ± 0.06^c	5.22 ± 0.11^a
NaCl+Se	74.7 ± 0.88^c	40.0 ± 1.53^c	5.10 ± 0.1^b	3.15 ± 0.03^c
Se	91.7 ± 0.88^b	60.0 ± 1.53^b	5.30 ± 0.01^{ab}	5.25 ± 0.03^a

Data presented are means ± standard errors (n = 3). One-way ANOVA was conducted. Different letters in each column represent significant differences at $P < 0.05$ obtained from Fisher's LSD Test. *C* = Control; *NaCl* = 100 mM NaCl; *NaCl+Se* = 100 mM NaCl + 1 µM Selenium; *Se* = 1 µM Selenium.

Under salt stress conditions, FGP, GE, and PL decreased significantly by 26.9%, 54.1% and 27.9%, respectively, but RaL increased by 34.5% compared to non-stressed control conditions (Table 1). However, exogenous Se application in saline conditions showed a significant elevation in FGP, GE, and PL by 7.2%, 34.7%, and 32.1%, respectively, while it resulted in a significant decrement in RaL (by 39.7%) relative to only salt conditions. On the other hand, when only Se was applied, FGP, GE, and PL diminished by 3.8%, 7.3% and 0.9%, respectively, but RaL enhanced by 35.3% compared to control conditions (Table 1).

3.2. Chlorophyll and carotenoid contents

The exposure of rice plants to NaCl stress severely affected different photosynthetic pigments, such as chlorophyll and carotenoid contents (Fig. 2). Chlorophyll contents in rice leaves exhibited a significant reduction in response to salt stress (Fig. 2A-C). Compared to control groups, salt treatment decreased chl *a*, chl *b* and total chl contents by 32.2%, 11.8%, and 26.1%, respectively (Fig. 2A-C). However, when seedlings were treated with Se in the presence of NaCl, an increment in chl *a*, chl *b* and total chl contents was observed by 27.3%, 14.8% and 22.8%, respectively, compared to seedlings treated only with NaCl. On the other hand, application of Se in the absence of NaCl did not cause any significant alteration in chlorophyll contents in comparison with control groups (Fig. 2A-C).

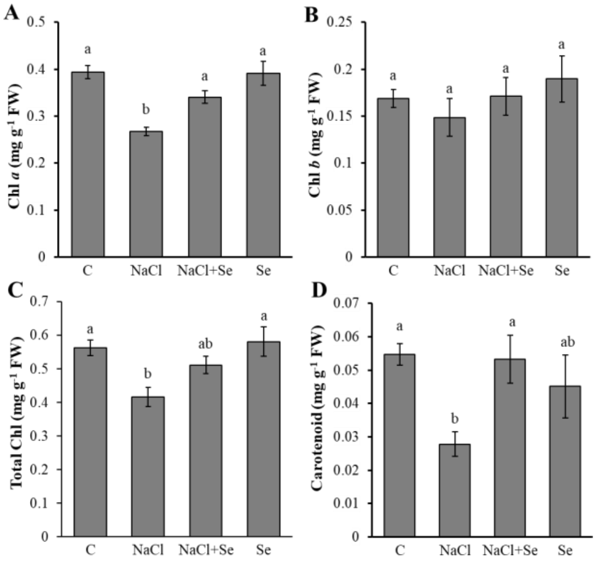

Figure 2. Effect of selenium on (A) chlorophyll *a,* (B) chlorophyll *b*, (C) total chlorophyll, and (D) carotenoid content in the leaves of rice seedlings grown under normal or salt stress conditions. The bar indicates the mean ± SE of three replicates. The letter on top of each bar denotes statistically significant differences at $p< 0.05$ obtained from Fisher's LSD test.

C = Control; ***NaCl*** = 100 mM NaCl; ***NaCl+Se*** = 100 mM NaCl + 1 μM Selenium; ***Se*** = 1 μM Selenium.

A similar response was also exhibited in the case of carotenoid in rice seedlings, where salt treatment decreased the carotenoid content by 49.1% in comparison with controls (Fig. 2D). Nevertheless, NaCl+Se combined treatment enhanced the carotenoid content by 89.3% compared to NaCl only treatment. Besides, Se only treatment caused a slight decline (18.2%) in carotenoid content compared to controls (Fig. 2D).

3.3. H_2O_2 and MDA contents

To examine whether exogenous Se mitigates salt-induced excess ROS accumulation and membrane damage in rice seedlings, H_2O_2 and MDA contents were determined (Fig. 3). The results showed that salt stress enhanced the accumulation of H_2O_2 and MDA by 17.4% and 23.5%, respectively, compared to control conditions (Fig. 3A-B). However, the application of Se in salt conditions exhibited a significant reduction in H_2O_2 and MDA contents by 11.8% and 17.1%, respectively, compared to only salt treatment. In contrast, selenium without salt treatment did not exhibit any significant changes in H_2O_2 and MDA contents compared to control plants (Fig. 3A-B).

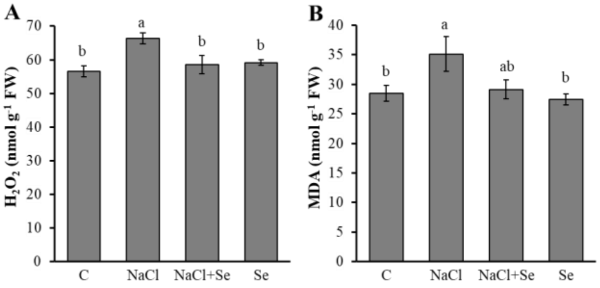

Figure 3. Effect of exogenous selenium on (A) hydrogen peroxide (H_2O_2) and (B) malondialdehyde (MDA) content in the leaves of rice seedlings grown under normal or salt stress conditions. The bar indicates the mean ± SE of three replicates. The letter on top of each bar denotes statistically significant differences at $p< 0.05$ obtained from Fisher's LSD test.

C = Control; ***NaCl*** = 100 mM NaCl; ***NaCl+Se*** = 100 mM NaCl + 1 μM Selenium; ***Se*** = 1 μM Selenium.

The activities of antioxidant enzymes in rice leaves

To investigate the changes in antioxidant defense responses in salt-stressed rice after selenium supplementation, the activities of different antioxidant enzymes such as CAT, APX, and POD were measured (Fig. 4). In this study, CAT activity reduced by 26.5%, while POD and APX activities increased by 29.8% and 14.9%, respectively, in salt-stressed seedlings compared to non-stressed controls (Fig. 4A-C). However, supplementation of Se under salt stress increased CAT activity by 101.9% and further elevated POD and APX activities by 104.1% and 18.0%, respectively, compared to saline condition without Se. Supplementation of Se under non-stress conditions also increased CAT activity by 42.7%, while POD and APX activities remained unaffected compared to controls (Fig. 4A-C).

4. Discussion

High salt concentrations in the soil diminish the accessibility of water and nutrients to roots, hinder their uptake, and lead to water and nutrient deficits in plant tissues (Zafar et al., 2024; Safdar et al., 2019). Due to both osmotic and ionic stress induced by high salinity, cell expansion and overall plant growth are reduced (Zafar et al., 2024; Safdar et al., 2019). High salinity also exerts antagonistic effects on seed germination which is a crucial step in crop establishment (Safdar et al., 2019; Atta et al., 2023). In the current experiment, seed germination and seedling growth (plumule length) were significantly inhibited in response to salt stress (Table 1), which corresponds to the earlier reports on rice, rapeseed, turnip, safflower, etc. (Tahjib-Ul-Arif et al., 2018; El-Badri et al., 2022; Hussain et al., 2023a, 2023b; Fatahiyan et al., 2025). Reduced and delayed seed germination under high salinity may be attributed to diminished water absorption and decreased α-amylase activity, the enzymes for breaking down of starch to provide energy to the growing embryo (Liu et al., 2018; Atta et al., 2023). However, seed germination and plumule growth were observed to enhance under salt stress when Se was applied (Table 1).

The ameliorating effect of Se on seed germination and seedling length under high salinity has also been reported on other plants, e.g., rapeseed, turnip, safflower, etc. (El-Badri et al., 2022; Hussain et al., 2023a, 2023b; Fatahiyan et al., 2025). Thus, the findings indicated that germination rate and plant growth in response to salt stress can be restored by selenium application.

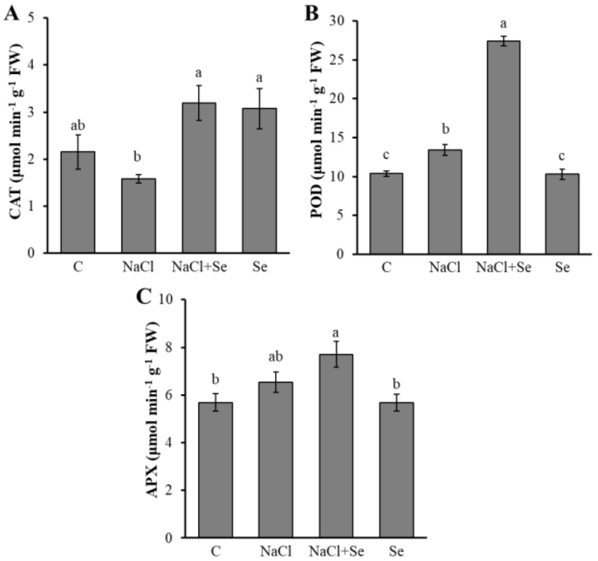

Figure 4. Effects of exogenous Se on the activities of (A) catalase (CAT), (B) peroxidase (POD), and (C) ascorbate peroxidase (APX) enzymes in the leaves of rice grown under normal or salt stress conditions. The bar indicates the mean ± SE of three replicates. The letter on top of each bar denotes statistically significant differences at $p< 0.05$ obtained from Fisher's LSD test.

C = Control; ***NaCl*** = 100 mM NaCl; ***NaCl+Se*** = 100 mM NaCl + 1 µM Selenium; ***Se*** = 1 µM Selenium.

Remarkably, in our study, an increase in radicle length was observed due to salt exposure, but in the presence of Se, radicle growth was compromised (Table 1). These findings indicated more susceptibility of shoot growth than root to salt stress. This could probably happen due to reallocating photosynthetic assimilates in the root part (Upreti and Murti, 2010). Improved root growth in response to salinity was reported in the halophyte (Rubinigg et al., 2003, 2004). Besides, in grape, the root length was observed to increase up to 100 mM NaCl stress (Upreti and Murti, 2010). Amplified radicle length observed in our study under 100 mM NaCl salinity may be an adaptive strategy against osmotic stress for seeking water, as suggested by Upreti and Murti (2010). Selenium has been reported to have antioxidative property and participate in antioxidant defense systems (Khan et al., 2023). Se boosts antioxidative activities, thereby protects plants from oxidative damage and promotes plant development (Kamran et al., 2019; Khan et al., 2023; Tsivileva, 2025).

When encountering stress conditions, an excessive amount of ROS is produced in plants, which disrupts cell membranes, degrades proteins and chloroplasts, and prevents photosynthesis (Gupta & Gupta, 2017; Kamran et al., 2019; Khan et al., 2023). Photosynthetic pigments-chlorophylls and carotenoids, play pivotal roles in harvesting light and driving the mechanism of photosynthesis and hence improving plant growth as well as biomass production (Simkin et al., 2022). It has been reported that salinity-induced oxidative and ionic stress suppresses photosynthetic capacity of plants by impairing the biosynthesis of photosynthetic pigments, accelerating the degradation of pigments and thylakoid membrane of chloroplast, by restricting CO_2 supply via reduction in stomatal conductance, etc. (Safdar et al., 2019; Muhammad et al., 2021). Our study also showed that salinity stress reduced chlorophyll and carotenoid contents, which were then enhanced by Se application (Fig. 2A-D), reflecting a defensive effect of Se on the photosynthetic efficacy of plants under salt stress. Similar findings have been reported for Se application on other plants (El-Badri et al., 2022; Rasool et al., 2023; Hussain et al., 2023a; Zafar et al., 2024; Fatahiyan et al., 2025; Mishra et al., 2025). In the leaves of Lycium chinense, chlorophyll and carotenoid contents have displayed a significant positive correlation and have enhanced with Se enrichment (Dong et al., 2013). In Cucumis melo plants exposed to As-contaminated soil, iron oxide nanoparticles (IONPs) and Se treatment improved plant growth by regulating expression of Chl biosynthesis related genes i.e. chlorophyll synthase (CHLG) and protochlorophyllide oxidoreductase (POR) (Shah et al., 2022). According to Khan et al. (2023), an accurate Se level can increase the concentration of photosynthetic pigments e.g., chlorophyll and carotenoids, and in turn can lessen ROS-induced damage to chloroplasts.

Oxygen radicals and their derivatives are called ROS which include hydrogen peroxide, superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^\bullet OH$), etc. (Muhammad et al., 2021; Hasanuzzaman et al., 2021; Atta et al., 2023). Under normal conditions, ROS generation is a part of cellular metabolic pathways such as respiration, photosynthesis and photorespiration, while over production of ROS is a general phenomenon under abiotic stresses (Muhammad et al., 2021; Hasanuzzaman et al., 2021). Different cell organelles such as chloroplasts, mitochondria, peroxisomes are major cites for ROS production (Muhammad et al., 2021; Hasanuzzaman et al., 2021; Atta et al., 2023). The ROS-induced oxidative stress under salinity causes damage in cell membrane lipids, resulting in lipid peroxidation, which can be quantified by the final product, malondialdehyde (MDA) (Kamran et al., 2019; Hasanuzzaman et al., 2021). In the current study, the rice plants treated with salt stress displayed a marked elevation in H_2O_2 and MDA contents compared to controls (Fig 3A-B), indicating oxidative damage of membrane lipids due to ROS accumulation. Nevertheless, exogenous application of Se relieved salinity-induced oxidative stress, as supported by the reduced MDA and H_2O_2 contents in rice seedling (Fig. 3A-B). Our results are also supported by other reports on turnip, wheat, safflower, etc. (Hussain et al., 2023a, 2023b; Zafar et al., 2024; Fatahiyan et al., 2025). At lower dosages, Se acts positively as an antioxidant, controls ROS levels, and thus protects plants from lipid peroxidation and cell membrane injury (Hawrylak-Nowak, 2009; Kamran et al., 2019; Khan et al., 2023; Tsivileva, 2025). Therefore, it could be concluded that Se plays a role in diminishing ROS levels and preventing membrane damage under salt stress.

Under salinity, Se supplementation mediates ROS detoxification likely by boosting both enzymatic and non-enzymatic antioxidants, and thus improves salinity tolerance of plants (Kamran et al., 2019; Tsivileva, 2025). Antioxidant defense machineries are indispensable for plants to deal with oxidative stress. A variety of enzymatic (e.g. APX, POD and CAT) and non-enzymatic (e.g. carotenoids) scavengers counteract salinity-induced ROS accumulation (Rao et al., 2025). CAT and APX, two heme-containing enzymes, are vital enzymes associated with the direct detoxification of H_2O_2 into water and oxygen molecules (Anjum et al., 2016; Li, 2023; Rao et al., 2025). CATs, which are generated in peroxisomes, degrade H_2O_2 without any reductants. On the other hand, APXs use ascorbate as a specific electron donor for catalyzing the conversion of H_2O_2 into H_2O in various subcellular compartments including chloroplasts, peroxisomes, mitochondria and cytosol (Anjum et al., 2016; Li, 2023; Rao et al., 2025). Moreover, APX has a higher affinity for H_2O_2 than does CAT (Anjum et al., 2016; Li, 2023; Rao et al., 2025). The POD, located in the cell wall, apoplast, and vacuole, catalyzes the oxidation of phenolic substrates using H_2O_2 as an electron acceptor and thus scavenges the H_2O_2 (Rajput et al., 2021b). In our study, CAT exhibited a slight decline, while POD and APX showed an increase in their activities in response to salinity compared to control plants (Fig. 4A-C). Boosted activities of antioxidant enzymes after exposure to salt stress were also observed in several studies (Mishra et al., 2025; Jiang et al., 2017; Nie et al., 2023; Rasool et al., 2023). Again, in the present study, when rice seedlings were supplemented with Se, further enrichment in antioxidant activities was found (Fig. 4A-C). This outcome corroborated with the earlier findings of Rasool et al. (2023), Hussain et al. (2023a), Hussain et al. (2023b) and Nie et al. (2023). Increased antioxidant enzyme activities under salt stress after Se application indicated that Se can positively stimulate antioxidant defense mechanisms to detoxify the ROS. The detoxification of ROS, therefore, offered membrane stability and pigment protection, and helped rice seedlings to maintain normal growth.

5. Conclusion

The present study focused on the influence of selenium on seed germination, growth of seedlings, synthesis of photosynthetic pigments, and antioxidant defense responses of rice plants subjected to salinity stress. Salinity stress adversely impaired seed germination and growth at the early stage of rice seedlings. Salt-stressed rice plants exhibited enhanced levels of H_2O_2, MDA, and antioxidant enzyme activities, the indicators of oxidative stress. However, supplementation of Se contributed in mitigating the detrimental effects of salt stress on seed germination and growth of rice at the early stage. Improved synthesis of photosynthetic pigments, boosted antioxidant enzyme activities, lesser hydrogen peroxide accumulation and diminished lipid peroxidation in rice plants subjected to salt stress supplied with Se directed to the antioxidative role of Se. Thus, the findings of our study demonstrated the potential role of selenium in improving the performance of rice at the early growth stage under saline soil and addressed its efficacy for climate-resilient agriculture.

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References

Adilu, G. S., Gebre, Y. G. 2021. Effect of salinity on seed germination of some tomato (*Lycopersicon esculentum* mill.) varieties. Journal of Aridland Agriculture, 7, 76-82.

Alhammad, B. A., Ahmad, A., Seleiman, M. F., Tola, E. 2023. Seed priming with nanoparticles and 24-epibrassinolide improved seed germination and enzymatic performance of *Zea mays* L. in salt-stressed soil. Plants, 12(4), 690.

Anjum, N.A., Sharma, P., Gill, S.S., Hasanuzzaman, M., Khan, E.A., Kachhap, K., Mohamed, A.A., Thangavel, P., Devi, G.D., Vasudhevan, P., Sofo, A. 2016. Catalase and ascorbate peroxidase—representative H_2O_2-detoxifying heme enzymes in plants. Environmental science and pollution research, *23*(19), pp.19002-19029.

Atta, K., Mondal, S., Gorai, S., Singh, A.P., Kumari, A., Ghosh, T., Roy, A., Hembram, S., Gaikwad, D.J., Mondal, S., Bhattacharya, S. 2023. Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Frontiers in Plant Science, 14, p.1241736.

Coombs J, Hind G, Leegood R.C., Tieszen L.L., Vonshak A. 1985. Analytical techniques, Techniques in Bioproductivity and Photosynthesis (Second Edition). Elsevier, pp. 219-228.

Cortleven, A., Schmülling, T. 2015. Regulation of chloroplast development and function by cytokinin. Journal of experimental botany, 66(16), 4999-5013.

Djanaguiraman M., Prasad P.V., Seppanen, M. 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiology and Biochemistry**,** 48(12), 999-1007.

Dong, J.Z., Wang, Y., Wang, S.H., Yin, L.P., Xu, G.J., Zheng, C., Lei, C., Zhang, M.Z. 2013. Selenium increases chlorogenic acid, chlorophyll and carotenoids of *Lycium chinense* leaves. Journal of the Science of Food and Agriculture, 93(2), pp.310-315.

El-Badri, A.M., Hashem, A.M., Batool, M., Sherif, A., Nishawy, E., Ayaad, M., Hassan, H.M., Elrewainy, I.M., Wang, J., Kuai, J., Wang, B. 2022. Comparative efficacy of bio-selenium nanoparticles and sodium selenite on morpho-physiochemical attributes under normal and salt stress conditions, besides selenium detoxification pathways in *Brassica napus* L. Journal of Nanobiotechnology, 20(1), p.163.

Fatahiyan, F., Najafi, F., Shirkhani, Z. 2025. Enhancing salt stress tolerance in *Carthamus tinctorius* L. through selenium soil treatment: anatomical, biochemical, and physiological insights. BMC Plant Biology, 25(1), 100.

Gupta, M., Gupta, S. 2017. An overview of selenium uptake, metabolism, and toxicity in plants. Frontiers in plant science, *7*, 2074.

Hasanuzzaman, M., Raihan, M.R.H., Masud, A.A.C., Rahman, K., Nowroz, F., Rahman, M., Nahar, K., Fujita, M. 2021. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. International Journal of Molecular Sciences, 22(17), p.9326.

Hashim, N., Ali, M. M., Mahadi, M. R., Abdullah, A. F., Wayayok, A., Kassim, M. S. M., Jamaluddin, A. 2024. Smart farming for sustainable rice production: an insight into application, challenge, and future prospect. Rice Science, 31(1), 47-61.

Hawrylak-Nowak, B. 2009. Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biological trace element research, 132(1), 259-269.

Hoagland D.R. and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station. Circular 347, pp. 1-32.

Hussain, S., Ahmed, S., Akram, W., Li, G., Yasin, N. A. 2023a. Selenium seed priming enhanced the growth of salt-stressed *Brassica rapa* L. through improving plant nutrition and the antioxidant system. Frontiers in Plant Science, 13, 1050359.

Hussain, S., Ahmed, S., Yasin, N. A., Akram, W., Sardar, R., Ahmad, A., Li, G. 2023b. *In vitro* and *in silico* study of salt stress resilience in *Brassica rapa* through selenium seed priming. South African Journal of Botany, 160, 504-515.

Jia, K., Zhan, Z., Wang, B., Wang, W., Wei, W., Li, D., Huang, W., Xu, Z. 2023. Exogenous selenium enhances cadmium stress tolerance by improving physiological characteristics of cabbage (*Brassica oleracea* L. var. *capitata*) seedlings. Horticulturae, 9(9), p.1016.

Jiang, C., Zu, C., Lu, D., Zheng, Q., Shen, J., Wang, H., Li, D. 2017. Effect of exogenous selenium supply on photosynthesis, Na^+ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. Scientific reports, 7(1), 42039.

Jiang, S., Lan, Z., Zhang, Y., Kang, X., Zhao, L., Wu, X., Gao, H. 2024. Mechanisms by which exogenous substances enhance plant salt tolerance through the modulation of ion membrane transport and reactive oxygen species metabolism. Antioxidants, 13(9), 1050.

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M.S., Saleem, M.H., Adil, M., Heidari, P., Chen, J.T. 2019. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. International journal of molecular sciences, 21(1), p.148.

Khan, Z., Thounaojam, T. C., Chowdhury, D., Upadhyaya, H. 2023. The role of selenium and nano selenium on physiological responses in plant: a review. Plant Growth Regulation, 100(2), 409-433.

Kumar, P., Choudhary, M., Halder, T., Prakash, N.R., Singh, V., V, V.T., Sheoran, S., T, R.K., Longmei, N., Rakshit, S., Siddique, K.H. 2022. Salinity stress tolerance and omics approaches: revisiting the progress and achievements in major cereal crops. Heredity, 128(6), pp.497-518.

Lakhdar, A., Trigui, M., Montemurro, F. 2023. An overview of biostimulants' effects in saline soils. Agronomy, 13(8), 2092.

Li, S. 2023. Novel insight into functions of ascorbate peroxidase in higher plants: more than a simple antioxidant enzyme. Redox Biol 64: 102789.

Liu, L., Xia, W., Li, H., Zeng, H., Wei, B., Han, S., Yin, C. 2018. Salinity inhibits rice seed germination by reducing α-amylase activity via decreased bioactive gibberellin content. Frontiers in Plant Science, 9, 275.

Mishra, M., Afzal, S., Yadav, R., Singh, N. K., Zarbakhsh, S. 2025. Salinity stress amelioration through selenium and zinc oxide nanoparticles in rice. Scientific Reports, 15(1), 27554.

Mohidem, N. A., Hashim, N., Shamsudin, R., Che Man, H. 2022. Rice for food security: Revisiting its production, diversity, rice milling process and nutrient content. Agriculture, 12(6), 741.

Muhammad, I., Shalmani, A., Ali, M., Yang, Q. H., Ahmad, H., Li, F. B. 2021. Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. Frontiers in plant science, 11, 615942.

Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59, 651-681.

Nawaz, F., Ashraf, M. Y., Ahmad, R., Waraich, E. A., Shabbir, R. N., Bukhari, M. A. 2015. Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. Food Chemistry, 175, 350-357.

Nephali, L., Piater, L. A., Dubery, I. A., Patterson, V., Huyser, J., Burgess, K., Tugizimana, F. 2020. Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. Metabolites, 10(12), 505.

Nie, M., Ning, N., Liang, D., Zhang, H., Li, S., Li, S., Fan, X., Zhang, Y. 2023. Seed priming with selenite enhances germination and seedling growth of Sorghum [*Sorghum bicolor* (L.) Moench] under salt stress. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science, 73(1), pp.42-53.

Ondrasek, G., Rathod, S., Manohara, K.K., Gireesh, C., Anantha, M.S., Sakhare, A.S., Parmar, B., Yadav, B.K., Bandumula, N., Raihan, F., Zielińska-Chmielewska, A. 2022. Salt stress in plants and mitigation approaches. Plants, 11(6), 717.

Quamruzzaman, M., Manik, S. N., Shabala, S., Zhou, M. 2021. Improving performance of salt-grown crops by exogenous application of plant growth regulators. Biomolecules, 11(6), 788.

Rady, M. M., Belal, H. E., Gadallah, F. M., Semida, W. M. 2020. Selenium application in two methods promotes drought tolerance in *Solanum lycopersicum* plant by inducing the antioxidant defense system. Scientia Horticulturae, 266, 109290.

Rajput, V.D., Harish, Singh, R.K., Verma, K.K., Sharma, L., Quiroz-Figueroa, F.R., Meena, M., Gour, V.S., Minkina, T., Sushkova, S., Mandzhieva, S. 2021b. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology, 10(4), p.267.

Rajput, V.D., Minkina, T., Kumari, A., Harish, Singh, V.K., Verma, K.K., Mandzhieva, S., Sushkova, S., Srivastava, S. Keswani, C. 2021a. Coping with the challenges of abiotic stress in plants: New dimensions in the field application of nanoparticles. Plants, 10(6), p.1221.

Rao, M.J., Duan, M., Zhou, C., Jiao, J., Cheng, P., Yang, L., Wei, W., Shen, Q., Ji, P., Yang, Y., Conteh, O. 2025. Antioxidant defense system in plants: Reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. Horticulturae, 11(5), p.477.

Rasool, A., Hafiz Shah, W., Padder, S. A., Tahir, I., Alharby, H. F., Hakeem, K. R., ul Rehman, R. 2023. Exogenous selenium treatment alleviates salinity stress in Proso Millet (*Panicum miliaceum* L.) by enhancing the antioxidant defence system and regulation of ionic channels. Plant Growth Regulation, 100(2), 479-494.

Rubinigg, M., Posthumus, F., Ferschke, M., Elzenga, J. T. M., Stulen, I. 2003. Effects of NaCl salinity on ^{15}N-nitrate fluxes and specific root length in the halophyte *Plantago maritima* L. Plant and Soil, 250(2), 201-213.

Rubinigg, M., Wenisch, J., Elzenga, J. T. M., Stulen, I. 2004. NaCl salinity affects lateral root development in *Plantago maritima*. Functional plant biology, 31(8), 775-780

Sackey, O. K., Feng, N., Mohammed, Y. Z., Dzou, C. F., Zheng, D., Zhao, L., Shen, X. 2025. A comprehensive review on rice responses and tolerance to salt stress. Frontiers in Plant Science, 16, 1561280.

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., Hussan, M.U., Sarwar, M.I. 2019. A review: Impact of salinity on plant growth. Nature and Science 17(1):34-40.

Sardar, R., Ahmed, S., Shah, A. A., Yasin, N. A. 2022. Selenium nanoparticles reduced cadmium uptake, regulated nutritional homeostasis and antioxidative system in *Coriandrum sativum* grown in cadmium toxic conditions. Chemosphere, 287, 132332.

Shah, A.A., Yasin, N.A., Mudassir, M., Ramzan, M., Hussain, I., Siddiqui, M.H., Ali, H.M., Shabbir, Z., Ali, A., Ahmed, S., Kumar, R. 2022. Iron oxide nanoparticles and selenium supplementation improve growth and photosynthesis by modulating antioxidant system and gene expression of chlorophyll synthase (CHLG) and protochlorophyllide oxidoreductase (POR) in arsenic-stressed *Cucumis melo*. Environmental Pollution, 307, p.119413

Shahverdi, M. A., Omidi, H., & Tabatabaei, S. J. (2018). Plant growth and steviol glycosides as affected by foliar application of selenium, boron, and iron under NaCl stress in *Stevia rebaudiana* Bertoni. Industrial crops and products, 125, 408-415.

Sieprawska, A., Kornas, A., Filek, M. 2015. Involvement of selenium in protective mechanisms of plants under environmental stress conditions-review. Acta Biologica Cracoviensia. Series Botanica, 57(1).

Simkin, A. J., Kapoor, L., Doss, C. G. P., Hofmann, T. A., Lawson, T., Ramamoorthy, S. 2022. The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. Photosynthesis Research, 152(1), 23-42.

Tahjib-Ul-Arif, M., Roy, P.R., Sohag, A.A.M., Afrin S., Rady, M.M., Hossain, M.A. 2018. Exogenous calcium supplementation improves salinity tolerance in BRRI Dhan 28; a Salt-Susceptible high-yielding *Oryza sativa* Cultivar. Journal of Crop Science and Biotechnology **21,** 383-94.

Tsivileva, O. 2025. Selenium-containing nanoformulations capable of alleviating abiotic stress in plants. International Journal of Molecular Sciences, 26(4), 1697.

Upreti, K. K., Murti, G. S. R. 2010. Response of grape rootstocks to salinity: changes in root growth, polyamines and abscisic acid. Biologia Plantarum, 54(4), 730-734.

Velikova, V., Yordanov, I., Edreva, A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science, 151(1): 59-66.

Zafar, S., Hasnain, Z., Danish, S., Battaglia, M. L., Fahad, S., Ansari, M. J., Alharbi, S. A. 2024. Modulations of wheat growth by selenium nanoparticles under salinity stress. BMC Plant Biology, 24(1), 35.

Zhang Z., Huang, R. 2013. Analysis of malondialdehyde, chlorophyll proline, soluble sugar, and glutathione content in *Arabidopsis* seedling. Bio-protocol, 3(14): e817

Zhao, Y., Xu, W., Wang, L., Han, S., Zhang, Y., Liu, Q., Liu, B., Zhao, X. 2022. A maize necrotic leaf mutant caused by defect of coproporphyrinogen III oxidase in the porphyrin pathway. Genes, 13(2), p.272.