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Foliar-applied arginine alleviates physiological and oxidative responses to salinity in rice seedlings

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Abstract

Salinity stress substantially limits rice productivity by disrupting redox homeostasis, membrane stability and photosynthetic function. This investigation assessed the effectiveness of foliar-applied arginine (Arg; 1 g L^{-1}) in alleviating salinity-induced oxidative and physiological disturbances in rice seedlings. **A randomized complete block design (RCBD)** greenhouse pot experiment was conducted under three salinity levels salinity treatments (0, 2000 and 4000 ppm) prepared using diluted seawater, with or without Arg application. Salinity treatment led to increases in $O_2^{\bullet-}$, H_2O_2, TBARs and total phenols along with decreases in membrane stability, stomatal conductance, transpiration rate and net photosynthesis. This response was associated with increased SOD, CAT, APX and POD activities. Foliar application of Arg reduced ROS accumulation and lipid peroxidation while enhancing antioxidant enzyme activities, thereby promoting improved membrane stability and gas exchange. The influence of Arg application were more pronounced under higher salinity levels, particularly in terms of the measured oxidative and gas-exchange responses. These findings suggest that foliar Arg application can alleviate selected physiological and biochemical disturbances associated with salinity in rice seedlings. However, further investigations assessing plant growth, biomass, ion homeostasis and yield are needed to assess whether these benefits translate into broader improvements in salinity tolerance.

Keywords: Antioxidant enzymes, *Oryza sativa*, Phenolic compounds, Reactive oxygen species.

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Introduction

Abiotic stress, such as drought and salinity, are critical environmental constraints that impair plant growth and crop productivity, posing substantial challenges to global food security, particularly under a changing climate (Zhang *et al*., 2022; Salehi *et al*., 2023). Of these environmental stresses, soil salinity is an increasingly important agricultural problem because excessive salt accumulation disrupts water and ion homeostasis, induces oxidative stress and impairs several physio-biochemical processes in plants. Rice (*Oryza sativa* L.), a major staple crop globally, is in particular vulnerable to salinity, especially during early developmental stages. Therefore, insight into the physio-biochemical responses of rice seedlings to salinity and identifying approaches that alleviate salinity-induced disturbances are important for improving crop performance under saline conditions.

Salinity-induced ionic imbalance, especially the impairment of Na^+ and K^+ homeostasis, is well established as an important component of plant adaptive responses to salinity stress (SS) (Hussain *et al*., 2021; Almeida *et al*., 2017). Earlier investigations have also reported that exogenous arginine application can influence ion accumulation and antioxidant responses in salt-stressed plants, including wheat (Li *et al*., 2024) and lentil (Nahar, *et al*., 2025). However, because Na^+, K^+ and Cl^- concentrations were not measured in the present rice experiment, the contribution of altered ionic homeostasis to the observed effects of Arg cannot be established. Therefore, the current findings should be interpreted in terms of the measured oxidative, membrane-stability and gas-exchange responses, while any potential effects of Arg on ion regulation remain a hypothesis for future investigation.

Plant cells have an antioxidant mechanism that is responsible for redox homeostasis via the action of enzyme and non-enzymatic antioxidants. Among these enzymes are the superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR). Phenolic compounds make up the non-enzyme antioxidants. Antioxidant metabolism, membrane stability and stomatal physiology are very essential in developing salinity tolerance in rice. However, under extreme salinity conditions, these antioxidant defenses might not be enough to protect against oxidative damage. Thus, there is a need for eco-friendly measures to enhance plant defense responses (Li *et al*., 2024; Sackey *et al*., 2025).

L-arginine is a naturally occurring amino acid participating in nitrogen metabolism and serves as a precursor for several compounds associated with plant stress responses, including nitric oxide and polyamines. Previous studies have suggested that exogenous arginine may

influence antioxidant defense and physiological responses under adverse environmental conditions (Corpas *et al*., 2021; Kolbert *et al*., 2021; Mishra *et al*., 2021; Sun *et al*., 2021). In the present study, the effects of foliar Arg application were evaluated in rice seedlings under SS by assessing ROS (reactive oxygen species) accumulation, antioxidant enzyme activities (AOEA), membrane stability and gas-exchange parameters. Focusing on these measured traits allows the effects of Arg to be interpreted in relation to the oxidative and physiological responses directly assessed in this experiment, without assuming changes in unmeasured processes such as ion homeostasis or chloroplast protection.

While the beneficial impacts of exogenous arginine under abiotic stress have been documented across diverse plant species, its effects on oxidative status, membrane stability and gas-exchange responses in salt-stressed rice seedlings warrant further investigation. Thus, this study evaluated the influence of foliar arginine application on rice seedlings under SS conditions, focusing on ROS accumulation, AOEA, membrane stability and gas-exchange parameters. The findings provide insight into the physiological and biochemical responses associated with arginine application under SS and may help guide future research on its potential use in rice production under saline conditions.

MATERIALS AND METHODS

A pot experiment was conducted under greenhouse conditions during the 2026 growing season at the Department of Rice Pathology, Plant Pathology Research Institute, Agricultural Research Center (ARC), Kafr El-Sheikh, Egypt. The experiment was arranged in a randomized complete block design (RCBD) consisting of five blocks, with six treatment combinations and one replicate of each treatment within each block, resulting in a total of 30 experimental units. Each experimental unit consisted of a plastic pot (20 cm in diameter) filled with clay soil and sown with 50 seeds of rice (*Oryza sativa* L.) cv. Sakha 177. The pots were maintained under continuously flooded conditions, with a water layer approximately 4 cm above the soil surface throughout the experimental period. Representative seedlings were randomly selected from each pot for physiological and biochemical assessments. Each pot was considered an independent experimental unit, whereas seedlings sampled from the same pot were regarded as subsamples rather than independent biological replicates.

Three levels of irrigation-water salinity were imposed: freshwater (S0) and saline water at concentrations of 2000 ppm (S1) and 4000 ppm (S2). Each salinity level was combined with either the presence or absence of foliar arginine application, resulting in six treatment

combinations. The electrical conductivity (EC) of the irrigation water was monitored periodically throughout the experimental period. The measured EC values corresponding to S1 and S2 were 3.13 and 6.25 dS m^{-1}, respectively. Periodic EC measurements were performed to monitor fluctuations in irrigation-water salinity during the experiment. The seawater used for preparing the saline irrigation solutions had an approximate pH of 8.1. The reported concentrations of Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and K^+ in the irrigation water were 10.75, 19.1, 47, 1.54, and 53 mg L^{-1}, respectively.

The experiment evaluated the influence of saline irrigation water and foliar-applied arginine on rice seedlings using six treatment combinations. Irrigation treatments consisted of freshwater (S0) and two saline-water treatments prepared by diluting seawater, designated S1 and S2, with nominal salt concentrations of 2000 and 4000 ppm, respectively. Salinity treatments were initiated at 7 days after sowing (DAS) and maintained throughout the experimental period. L-arginine was applied as a foliar spray at 1 g L^{-1} at 15 DAS. The six treatments were freshwater irrigation without arginine (S0), freshwater irrigation with arginine (S0 + Arg), 2000-ppm saline-water irrigation without arginine (S1), 2000-ppm saline-water irrigation with arginine (S1 + Arg), 4000-ppm saline-water irrigation without arginine (S2) and 4000-ppm saline-water irrigation with arginine (S2 + Arg). Plants in the non-arginine treatments received foliar applications of distilled water under identical conditions to those used for arginine application, serving as spray controls. Physiological and biochemical traits were evaluated at 15 days after arginine foliar application.

Hydrogen peroxide and superoxide anion

Hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) contents were quantified as described by Sergiev et al. (1997) and Achary et al. (2012), respectively, with modifications. For H_2O_2 determination, fresh leaf tissue (0.5 g) was homogenized in 1% (w/v) trichloroacetic acid and the resulting homogenate was processed to obtain the extract. Hydrogen peroxide was quantified using the reaction of the extract with KI (potassium iodide) and absorbance was determined at 390 nm. Superoxide anion content was measured using the nitroblue tetrazolium (NBT) reduction assay. Briefly, fresh leaf tissue (0.15 g) was extracted and the absorbance of the reaction mixture was recorded at 530 nm. Superoxide anion content was computed using an extinction coefficient of 12.8 mM^{-1} cm^{-1}. The contents of both ROS were expressed on a fresh-weight basis.

Antioxidant enzymes activity

Antioxidant enzyme extracts were prepared by homogenizing frozen rice leaf samples in ice-cold 0.1 M Tris–HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% (v/v) 2-mercaptoethanol. The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C and the resulting supernatants were collected as crude enzyme extracts. Catalase, POD, SOD and APX activities were determined according to Aebi (1984), Maehly (1954), Beauchamp and Fridovich (1971) and Nakano and Asada (1981), respectively. The POD assay procedure, including the substrate, reaction conditions, wavelength, reaction time and activity calculation, was specified according to the protocol actually used. Soluble protein concentration was measured using the Bradford (1976) method and enzyme activities were reported as units per mg of soluble protein (U mg^{-1} protein).

Gas exchange parameters

Gas exchange including stomatal conductance (SC), transpiration rate (TR) and net photosynthetic rate (NP) were measured using a LI-6400XT portable photosynthesis system equipped with an infrared gas analyzer (IRGA; LI-COR Biosciences, USA), following the manufacturer's instructions and Krishnan et al. (2011). Assessments were conducted on fully expanded upper leaves of randomly selected rice plants. Gas-exchange measurements were conducted 09:00 and 12:00 h under ambient greenhouse conditions, including photosynthetic photon flux density (PPFD; 1200 µmol photons m^{-2} s^{-1}), reference CO_2 concentration (400 µmol mol^{-1}), leaf temperature (24 °C) and relative humidity (60%) or vapor pressure deficit (VPD; 1.1 kPa). The chamber settings and leaf acclimation period were [specify actual conditions]. All measurements were performed at the same time of day, as far as practicable, using consistent chamber settings across treatments. SC, TR and NP were expressed as mol H_2O m^{-2} s^{-1}, mmol H_2O m^{-2} s^{-1} and g m^{-2} w^{-1}, respectively.

Thiobarbituric acid-reactive substances

Thiobarbituric acid-reactive substances (TBARS) accumulation was determined using the thiobarbituric acid (TBA) assay, following Du and Bramlage (1992). Fresh leaf tissue was homogenized in 80% (v/v) ethanol and the extract was reacted with TBA reagent and incubated at 95 °C for 25 min. After cooling, the absorbance was measured at 440, 532 and 600 nm using a UV–Vis spectrophotometer. TBARs content was estimated using the extinction coefficient of 157 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight (FW). The measured TBARs content

was interpreted as an indicator of lipid peroxidation-associated oxidative injury rather than as a specific measurement of malondialdehyde (MDA) or direct evidence of membrane damage.

Membrane stability and total phenolic content

The membrane stability index was assessed based on the method following by Lutts et al. (1996). MSI was computed as (MSI=[1-(C_1/C_2)]\times100), where (C_1) and (C_2) represent the electrical conductivity readings obtained during the first and second measurement stages, respectively, as specified in the assay protocol. The results were presented as a percentage (%).

Total phenolic content was determined using the Folin–Ciocalteu method according to Bessada et al. (2016). The absorbance of the reaction mixture was assessed at 765 nm. A calibration curve was prepared and the resulting calibration equation was used to calculate the phenolic content of the samples. Results were expressed as mg gallic acid equivalents g^{-1} fresh weight. The calibration equation and the sample dilution factor were used in calculating the final values.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) and treatment means were compared using Duncan's multiple range test at the 5% significance level ($P \leq 0.05$) according to (Duncan 1955). The analysis was conducted to assess differences among the experimental treatments. The results are presented as means ± [SD/SE], as appropriate.

RESULTS

Superoxide and hydrogen peroxide content

Figure 1 shows that salt stress significantly increased ROS deposition in rice seedlings, with $O_2^{\bullet-}$ and H_2O_2 contents rising progressively with salinity intensity. Compared with S0, $O_2^{\bullet-}$ increased by 101.4% and 218.8%, while H_2O_2 increased by 56.2% and 168.6% under S1 (2000 ppm) and S2 (4000 ppm), respectively. Foliar arginine (1 g L^{-1}) significantly reduced both ROS under salinity stress conditions (SS). Relative to untreated plants, arginine decreased $O_2^{\bullet-}$ by 11.7%, 29.2% and 40.2% and H_2O_2 by 24.8%, 20.5% and 38.6% under S0, S1 and S2, respectively, indicating an effective mitigation of salinity-induced OS.

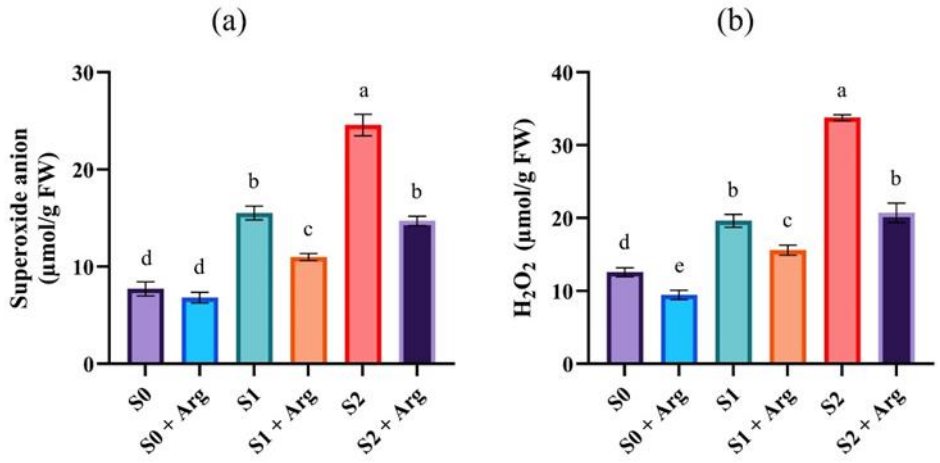


Figure 1. Effects of foliar-applied arginine (1 g L^{-1}) on the formation of $O_2^{\bullet -}$ (a) and H_2O_2 (b) in seedling of rice grown across different salinity levels (for four weeks. Values are means ± SE (n = 5). Means sharing the same letter are not significantly different, whereas distinct letters demonstrate significant differences across treatments following Duncan's multiple range test at $P \leq 0.05$ following ANOVA. S0: freshwater control, S1: 2000 ppm diluted seawater, S2: 4000 ppm diluted seawater.

Antioxidant enzymes activity

Figure 2 illustrates that salinity stress (SS) significantly increased the activities of SOD, CAT, POD, and APX, with the magnitude of these increases generally becoming more pronounced as salinity intensity increased. Compared with the freshwater control (S0), the activities of these enzymes increased by 9.1%, 16.9%, 34.7%, and 34.9% under S1, respectively, and by 15.7%, 37.2%, 42.0%, and 56.0% under S2. Foliar application of arginine (1 g L^{-1}) further enhanced antioxidant enzyme activities under saline conditions. Under S1, arginine treatment increased SOD, CAT, POD, and APX activities by 2.0%, 19.1%, 13.2%, and 15.2%, respectively, relative to the corresponding untreated plants. These increases were more pronounced under S2, reaching 15.6%, 56.0%, 37.2%, and 42.0%, respectively. Collectively, these findings indicate that exogenous arginine enhanced the activities of key antioxidant enzymes, particularly under severe salinity stress, suggesting an improved enzymatic component of the plant defense response to salinity-induced oxidative stress.

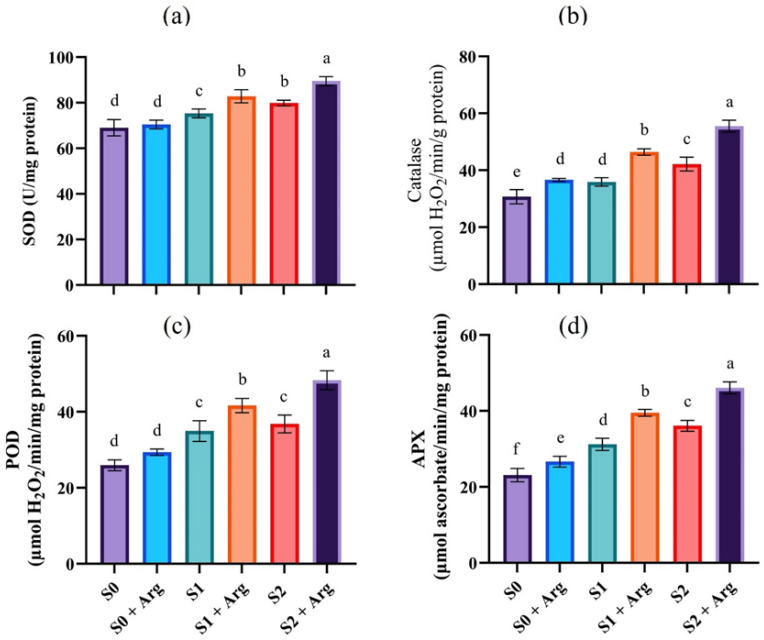


Figure 2. Effects of foliar-applied arginine (1 g L^{-1}) on antioxidant enzyme activities, particularly SOD (a), CAT (b), POD (c) and APX (d) in seedlings of rice exposed to different salinity levels for four weeks. Values are expressed as means ± SE (n = 5). Treatment means denoted by different alphabetical superscripts differ significantly as outlined by Duncan's multiple range test at $P \leq 0.05$ after ANOVA. S0: freshwater control, S1: 2000 ppm diluted seawater, S2: 4000 ppm diluted seawater.

TBARs content and membrane stability index

Foliar application of arginine (1 g L^{-1}) reduced thiobarbituric acid-reactive substances (TBARs) accumulation and improved membrane stability under both control and saline conditions. Compared with the corresponding untreated plants, arginine application reduced TBARs content by 9.1%, 21.1% and 33.2% under S0, S1 and S2, respectively, while increasing the membrane stability index (MSI) by 2.9%, 16.9% and 31.8%, respectively. The greatest reduction in TBARs content and the largest increase in MSI were observed under the highest salinity level (S2). These results indicate reduced TBARs accumulation and improved membrane stability, without directly demonstrating structural membrane protection.

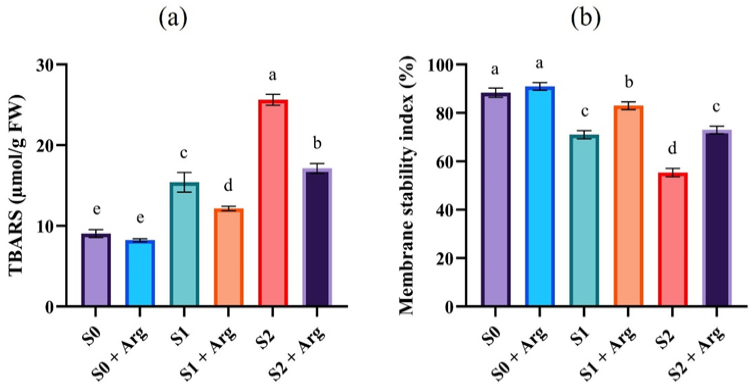


Figure 3. Effects of foliar-applied arginine (1 g L^{-1}) on thiobarbituric acid-reactive substances content (TBARS; a) and membrane stability index (MSI; b) in rice exposed to different salinity levels for four weeks. Values are shown as means ± SE (n = 5). Various letters denote significant differences between treatment means, as assessed using Duncan's multiple range test at $P \leq 0.05$ following ANOVA. S0: freshwater control, S1: 2000 ppm diluted seawater, S2: 4000 ppm diluted seawater.

Gas exchange

Figure 4 shows that SS significantly reduced SC, TR and NP in rice seedlings in comparing with the freshwater control (S0). The reduction in Transpiration rate (TR) and nete photosynthesis (NP) was insignificant under normal conditions (S0). The magnitude of these reductions increased with salinity intensity. Stomatal conductance decreased by 27.4% and 52.8% under S1 (2000 ppm) and S2 (4000 ppm), respectively. Similarly, transpiration rate declined by 31.2% and 51.1%, while net photosynthetic rate decreased by 30.1% and 54.9% under S1 and S2, respectively.

Foliar application of arginine (1 g L^{-1}) substantially reduced the adverse impacts of salinity and promoted gas exchange characteristics under control and saline conditions. Relative to the corresponding untreated plants, arginine increased SC by 19.0%, 23.3% and 59.7% under S0, S1 and S2, respectively. Likewise, NP increased by 2.4%, 26.6% and 59.5% under the respective treatments. Under saline conditions, arginine also enhanced TR by 24.4% and 44.5% under S1 and S2, respectively. Overall, the greatest improvement in gas exchange parameters was recorded in arginine-treated plants exposed to the highest salinity level (S2).

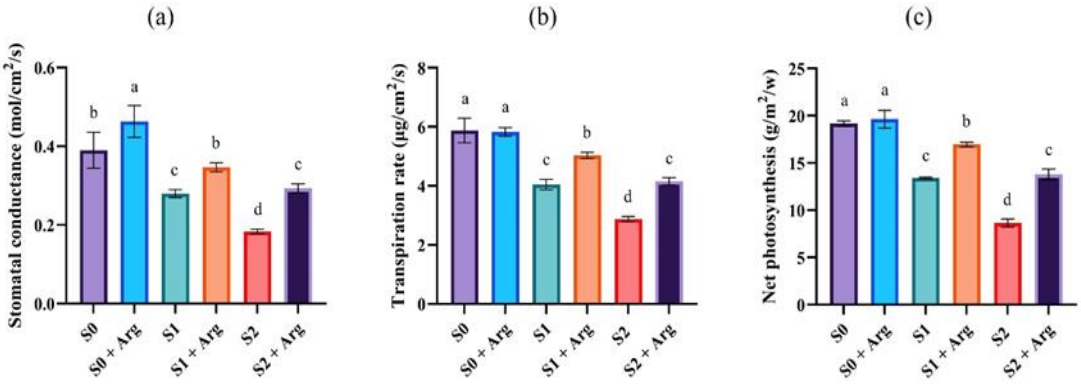


Figure 4. Effects of foliar-applied arginine (1 g L^{-1}) on stomatal conductance (a), transpiration rate (b) and net photosynthesis (c) in rice exposed to different salinity levels for four weeks. Values are given as means ± SE (n = 5). Statistical differences among treatment means are indicated by distinct letters using Duncan's multiple range test conducted after ANOVA at $P \leq 0.05$. S0: freshwater control, S1: 2000 ppm diluted seawater, S2: 4000 ppm diluted seawater.

Total phenols content

Figure 5 shows that SS significantly increased the total phenolic compounds (TPC) content in rice seedlings relative to the freshwater control (S0). Salinity increased total phenolic content (TPC) by 34.9% under S1 and 52.2% under S2 compared with the control. Arginine application further increased TPC by 8.7%, 20.2% and 18.6% under S0, S1 and S2, respectively, relative to the corresponding untreated plants. The greatest stimulatory effect of arginine on TPC accumulation was observed under the moderate salinity level (S1).

Salinity increased total phenolic content (TPC) by 34.90% under S1 and 52.16% under S2 compared with the control. Arginine application further increased TPC by 8.69%, 20.22% and 18.63% under S0, S1 and S2, respectively, relative to the corresponding untreated plants.

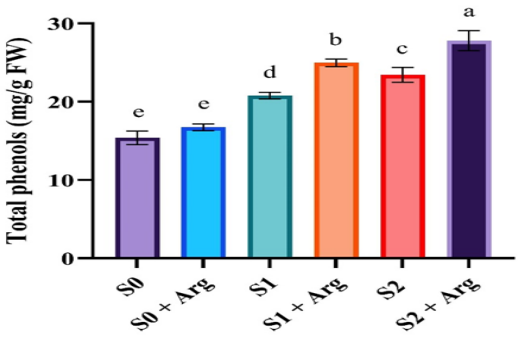


Figure 5. Effects of foliar-applied arginine (1 g L^{-1}) on total phenols content in rice subjected to different salinity levels for four weeks. Data are reported as means ± SE (n = 5). Statistical differences within treatment means are indicated by distinct letters following Duncan's multiple range test conducted after ANOVA at $P \leq 0.05$. S0: freshwater control, S1: 2000 ppm diluted seawater, S2: 4000 ppm diluted seawater.

DISCUSSION

In the current study, SS was found to significantly affect the physiological responses of rice seedlings due to excessive ROS generation, membrane lipid peroxidation and gas exchange disorder. The adverse impacts were increased with rising levels of salinity and OS proved to be a very important factor responsible for growth restriction caused by salinity. In the current investigation, salinity was associated with increased H_2O_2 and $O_2^{\bullet -}$ accumulation, higher TBARs content, lower MSI and reduced gas-exchange parameters in rice seedlings. Salinity also increased the activities of the AOEA and total phenolic content (TPC). Compared with the corresponding untreated plants, arginine-treated plants generally exhibited lower ROS and TBARs levels, higher MSI and AOEA and improved gas-exchange parameters. These findings demonstrate concurrent changes in oxidative-status indicators, antioxidant responses, membrane stability, phenolic content and gas exchange. However, the experimental measurements do not establish direct causal relationships among these responses.

Salinity increased H_2O_2 and $O_2^{\bullet -}$ contents in rice seedlings, with greater increases at the higher salinity level. These changes are consistent with an altered oxidative status under SS conditions. Previous experimental studies have shown that salinity can affect cellular redox processes and ROS accumulation in plants (Li et al., 2024; Jiang et al., 2024; Sackey et al., 2025). However, because ROS production rates and the activity of ROS-generating organelles were not assessed in this study, the observed increases are viewed as greater ROS accumulation rather than definitive evidence of increased ROS production through a specific cellular pathway.

Salinity increased TBARs content and decreased the membrane stability index (MSI), with larger changes under the higher salinity level. These concurrent responses are consistent with increased lipid peroxidation-associated products and reduced membrane stability under saline conditions. Although ROS accumulation may contribute to lipid peroxidation, the present experiment did not directly test the causal relationship between ROS accumulation, TBARs content and MSI. Accordingly, the observed changes are interpreted as associated indicators of oxidative status and MSI rather than direct proof of structural membrane damage (Li et al., 2024; Sackey et al., 2025; Su et al., 2025).

Salinity increased the activities of SOD, CAT, POD and APX, indicating an antioxidant enzymatic response to the imposed stress. SOD catalyzes the dismutation of superoxide anion to H_2O_2, whereas CAT and APX contribute to H_2O_2 removal. The simultaneous increases in AOEA and ROS contents indicate that enzyme induction occurred alongside continued ROS

accumulation. Therefore, increased enzyme activity should be interpreted as an antioxidant response and not, by itself, as evidence of effective ROS control or complete restoration of redox homeostasis. Comparable findings have been documented in experimental studies of salt-stressed plants (Jiang et al., 2024; Li et al., 2024; Sackey et al., 2025).

Salinity reduced SC, TR and NP with greater reductions at the higher salinity level. These changes indicate impaired gas-exchange performance under the experimental salinity conditions. Reduced stomatal conductance may limit CO_2 diffusion into leaves, while non-stomatal constraints may also promote lower photosynthesis under SS conditions. However, because chloroplast integrity, photosynthetic electron transport and the relative contributions of stomatal and non-stomatal limitations were not directly assessed, these mechanisms remain possible explanations rather than demonstrated causes of the observed responses (Jiang et al., 2024; Li et al., 2024; Sackey et al., 2025).

Salinity increased total phenolic content (TPC), indicating a change in the overall phenolic content of rice leaves. However, the TPC assay does not identify individual phenolic compounds or directly establish their antioxidant activity. Salinity increased total phenolic content (TPC), with a greater increase under the higher salinity level. Arginine application further increased TPC relative to the corresponding untreated plants. These changes indicate alterations in the total phenolic content of rice leaves under the tested conditions. Although individual phenolic compounds may contribute to plant stress responses, the TPC assay used in this study does not identify individual compounds or directly measure their antioxidant activity. Therefore, the observed increase in TPC should not be regarded as direct evidence of improved overall non-enzymatic antioxidant capacity. Its physiological significance requires further investigation using compound-specific analyses and appropriate functional assays (Sharma et al., 2019; Zagoskina et al., 2023; Li et al., 2024).

Leaf spray with arginine significantly decreased $O_2^{\bullet-}$ and H_2O_2 accumulation and further promoted the activity of SOD, CAT, POD and APX under both control and salinity conditions. This suggests that arginine enhanced the ROS detoxification and redox balance. Arginine is an intermediate in the synthesis of nitric oxide, polyamines, proline and other nitrogen-containing metabolites for stress tolerance. Foliar use of arginine decreased H_2O_2 and $O_2^{\bullet-}$ accumulation and improved the activities of SOD, CAT, POD and APX relative to the corresponding control. These results demonstrate that arginine application was linked to changes in ROS generation and AOEA. Arginine is also involved in metabolic pathways related to nitric oxide, polyamines, proline and other nitrogen-containing compounds, which have been investigated

in plant stress responses (Corpas *et al*., 2021; Kolbert *et al*., 2021; Mishra *et al*., 2021; Sun *et al*., 2021; Jiang et al., 2024). However, NO production, NO-dependent signaling and these associated metabolic pathways were not assessed in this study. Their involvement in the responses observed here should therefore be considered a plausible hypothesis based on previous research, rather than a mechanism demonstrated by the current experiment.

Arginine-treated plants showed lower thiobarbituric acid-reactive substances (TBARs) content and higher MSI than the corresponding untreated plants. These changes indicate reduced accumulation of TBARs and improved membrane stability under the tested conditions. Although membrane stability is physiologically important for cellular function, the present measurements do not directly demonstrate structural membrane protection or establish that the changes in MSI were caused by reduced ROS accumulation. Similar changes in oxidative-status indicators and membrane stability have been reported in previous studies of salt-stressed crops (Jiang *et al*., 2024; Nahar *et al*., 2025).

Improvement in SC, TR and NP was also observed in arginine treatment, suggesting that gas exchange improved in salt conditions. Arginine application increased SC, TR and NP relative to the corresponding untreated plants, indicating improved gas-exchange performance under the tested conditions. These changes occurred alongside lower ROS and TBARs contents, higher AOEA and increased MSI. Nevertheless, the present experimental design did not establish causal relationships among these responses. Although previous experimental studies have investigated possible roles of NO signaling, antioxidant metabolism and membrane stability in regulating plant gas exchange under stress, these mechanisms were not directly tested in the current study (Corpas et al., 2021; Kolbert et al., 2021; Sun et al., 2021; Jiang et al., 2024). The contribution of these pathways to the observed gas-exchange responses therefore remains to be determined.

Salinity increased total phenolic content (TPC), indicating a change in the overall phenolic content of rice leaves. However, the TPC assay does not identify individual phenolic compounds or directly establish their antioxidant activity. Arginine application increased TPC relative to the corresponding untreated plants across the tested salinity levels. This response indicates an increase in total phenolic content but does not, by itself, demonstrate enhanced nonenzymatic antioxidant capacity. The combination of enhancement of phenolics and AOE implies that arginine acts on various levels for ROS scavenging instead of a single pathway. Such a comprehensive approach would ensure better ROS scavenging and thereby minimize oxidative damage under SS conditions.

Arginine application also increased TPC, particularly under saline conditions. This response indicates a change in leaf phenolic content, but it does not establish increased antioxidant activity or enhanced overall non-enzymatic antioxidant capacity. The concurrent changes in TPC and AOEA suggest that several biochemical responses occurred following arginine application; however, their individual contributions to ROS regulation cannot be determined from the present measurements.

Overall, the findings demonstrate that salinity stress (SS) substantially affected ROS accumulation, antioxidant enzyme activities (AOEA), TBARS content, membrane stability index (MSI), total phenolic content (TPC), and gas-exchange parameters in rice seedlings. Foliar arginine application modulated these responses, as evidenced by reduced ROS accumulation and T thiobarbituric acid-reactive substances (BARS) content, accompanied by increased AOEA, MSI, and gas-exchange parameters compared with the corresponding untreated plants. Collectively, these results suggest that arginine application is associated with improved physiological and biochemical status in rice seedlings exposed to salinity stress. Nevertheless, the present study did not establish causal relationships among the measured responses or directly investigate the involvement of nitric oxide (NO) signaling and ion homeostasis. Further investigations incorporating these aspects are therefore warranted to elucidate the physiological and molecular processes potentially underlying the observed responses.

Figure 6 Schematic representation of the physio-biochemical processes responsible for the beneficial effects of application of foliar arginine (1 g L^{-1}; 15 days post sowing) in rice seedlings under SS (2000 and 4000 ppm). Salt stress leads to disruption of homeostasis in cells mainly due to an increase in ROS content, mainly H_2O_2 and $O_2^{\bullet -}$, causing oxidative injury, instability in membrane structure and gas exchange processes, such as SC, TR and NP rates. Exogenously applied arginine reduces the effect by increasing antioxidants activities, *i.e.*, SOD, CAT, POD and APX, thus ensuring detoxification and redox homeostasis. Arginine also increases phenol content and thereby enhances non-enzymatic antioxidant protection, in addition to membrane stability and gas exchange properties.

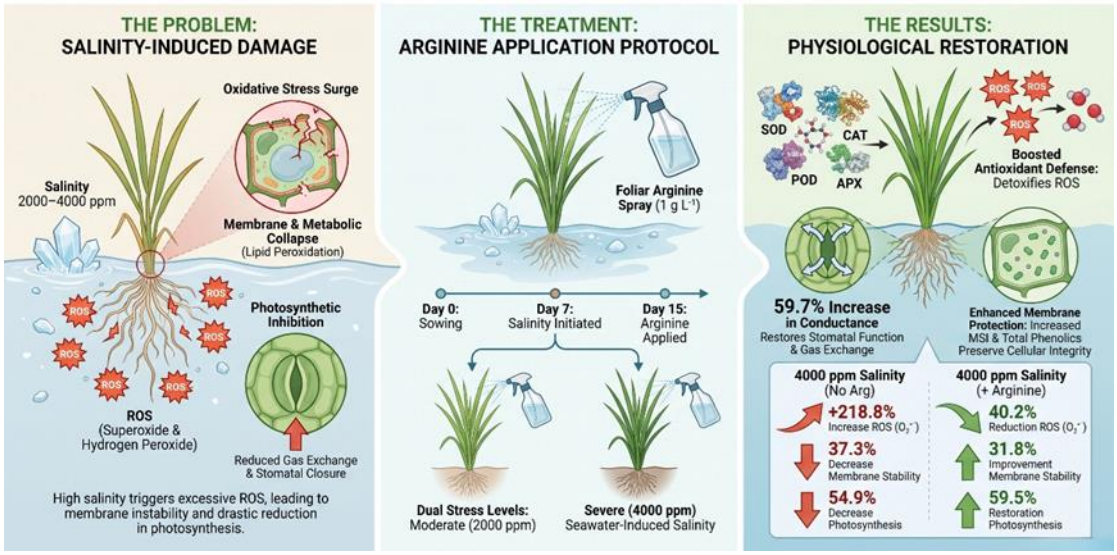


Figure 6. Schematic summary of the measured physiological and biochemical responses to foliar-applied arginine (1 g L^{-1}) in rice seedlings under SS (2000 and 4000 ppm). The measured responses include ROS accumulation (H_2O_2 and $O_2^{\bullet-}$), antioxidant enzyme activities (SOD, CAT, POD and APX), thiobarbituric acid-reactive substances (TBARs) content, membrane stability index (MSI), total phenolic content (TPC) and gas-exchange parameters (stomatal conductance, transpiration rate and net photosynthetic rate). The diagram summarizes the observed treatment-associated changes and distinguishes them from proposed mechanisms that were not directly examined in this study.

CONCLUSIONS

The findings of this study show that foliar spraying of arginine can alleviate salinity-induced damage in rice seedlings by modulating key physio-biochemical responses. Salinity stress increased ROS accumulation and lipid peroxidation, impaired membrane stability and adversely affected stomatal function and photosynthetic performance, despite the activation of antioxidant enzymes and changes in leaf total phenolic content. Arginine application mitigated these adverse effects by reducing ROS accumulation and lipid peroxidation, improving membrane stability and enhancing stomatal conductance and gas exchange. These responses were accompanied by improved antioxidant enzyme activities and increased leaf total phenolic content, indicating an overall improvement in the physiological status of salt-stressed seedlings. Collectively, the results support the potential of foliar-applied arginine as a promising approach to improving rice seedling tolerance to salinity, although further investigations under field conditions are needed to establish its agronomic effectiveness and practical applicability.

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