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Effects of salicylic acid on growth, physiology, and gene expression in rice seedlings under salt and drought stress

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ABSTRACT

Salt and drought stress has been an important factor limiting agricultural production, and SA is an important phenolic involved in stress response, but the function of SA in response to dual salt and drought stress in rice is not clear. In this study, the effects and mechanisms of exogenous SA-triggered in rice adaptation to dual salt and drought stress were investigated by detecting physiological and biochemical indexes and the expression of salt and drought tolerance genes. The results showed that the application of SA could significantly increase the antioxidant enzyme activities of rice seedlings under salt and drought stress, thereby reducing the contents of rice H_2O_2 and MDA and maintaining the growth of rice seedlings. Moreover, the expression of genes involved in the response of abiotic stress, such as *OsDREB2A, OsSAPK8, OsSAPK10* and *OsMYB2*, were up-regulated under salt and drought treatment, and SA application could further enhance the expression of those genes like *OsDREB2A* and *OsSAPK8*, suggesting that SA might regulate antioxidant enzyme activity via inducing the expression of salt and drought tolerance genes and enhancing the salt and drought tolerance of rice. The results will enrich the knowledge of the function of SA and provide a reference for studying the mechanism of SA in the salt and drought resistance of rice, and breeding new rice germplasm with improved salt and drought resistance.

1. Introduction

Rice is the main source of food for at least 65 % of the population in China. Maintaining high and stable rice yields plays an extremely important role in China's food security. Growing rice requires large amounts of fresh water, and most cultivars are salt-sensitive glycophytes. Therefore, rice production has always been severely constrained by salt and drought stress. According to report by which agency, the area of salinized land in China has reached 9.91×10^7 hectares, which is mainly distributed in northern arid and semi-arid areas as well as coastal areas of China. In addition, overirrigation has intensified secondary soil salinization in the main rice production areas, such as the Yangtze River Basin (Kaushal et al., 2016). Global crop yield reductions caused by salt and drought stress account for more than 50 % of total crop production each year (Ibrahim et al., 2019). Soil salinization and drought stress have become major constraints to agricultural production (Wen et al., 2020).

Salt stress and drought stress often occur simultaneously in natural environments. It is relatively costly to increase crop yields through salinized soil improvement and increased irrigation. Improving the salt and drought tolerance of crops is an effective way to increase crop yields (Meng et al., 2023). Previous studies showed that the melatonin treatment reduces salt and drought stress damage by increasing chlorophyll contents, inhibiting excessive production of oxidative stress markers, and promoting plant growth in rice (Khan et al., 2024). Nitric oxide can improve plant salt tolerance by crosstalk with calcium, hydrogen peroxide, hormone signaling, and mediated post-translational modifications (Gupta et al., 2020; Mariyam et al., 2023; Jindal et al., 2023). Light emitting diodes (LED) enhances salt tolerance by altering the circadian clock in *Spinacia oleracea* (Vajjiravel et al., 2024). In addition, some nanomaterials also play an important role in plant stress resistance, such as AgNPs, CuO-NPs and ZnO-NPs, TiO_2-NPs and ZnO-NPs (R. Khan et al., 2022; Kumar et al., 2023; Mariyam et al., 2024; Kim et al., 2024a; 2024b). However, there are currently no particular effective methods to enhance salt and drought tolerance of rice.

Salicylic acid (SA) is a small-molecule phenolic compound

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commonly found in plants, and moderate application does not harm the plant itself (Hashempour et al., 2014). SA plays an important role in plant development, for instance the induction of plant flowering, root growth, seed germination, and ion uptake (Bagautdinova et al., 2022; Liu et al., 2022). Furthermore, SA also involved in the regulation of plant responses to biotic or abiotic stresses, such as heat, heavy metals toxicity, and pathogen invasion (Sedaghat et al., 2017; Agnihotri et al., 2018). Previous studies have shown that the external application of SA significantly reduces cadmium uptake in Cd-stressed barley (Metwally et al., 2003). Similarly, SA alleviates chromium (VI) toxicity through restricting its uptake, improving photosynthesis and augmenting antioxidant defense in *Solanum lycopersicum* L(Gupta et al., 2021). In addition, previous research found that the exogenous addition of SA changed the expression of genes related to Na^+ and K^+ transport, reducing the accumulation of Na^+ in rice seeds, and maintaining the balance of Na^+/K^+ under salt stress (Wang et al., 2015). Exogenous SA improves salt tolerance of mustard through increase in ascorbate-glutathione metabolism and S assimilation (Nazar et al., 2015). Similarly, SA also alleviates the effects of drought stress in pepper, wheat, rice etc (Khalvandi et al., 2021; Urmi et al., 2023; Zhang et al., 2023; Gao et al., 2023; Torun et al., 2024).

The function of SA in plant adaptations to single stresses has been investigated in previous studies. However, there are few study on the functional mechanism of SA in the adaptation to dual stresses. The purpose of this study is to investigate the role of SA in rice adaptation to salt and drought stresses, and to explore the possible molecular mechanisms, providing a theoretical basis and a genetic target for the cultivation of new rice varieties with salt tolerance and drought resistance.

2. Materials and methods

2.1. Plant materials and plant growth conditions

Healthy and uniform sized rice seeds from the NIP (japonica) and 9311 (indica) were selected, and then sterilized and germinated in a constant-temperature incubator at 28 °C for 3 days. After germination, rice seedlings were planted in a 96-hole hydroponic box and cultured in an artificial climate chamber.

2.2. Stress treatments

Treatments were performed when rice plants grew to the two-leaf-one-heart stage, and the treatment groups were set up as follows: (1) Control: no stress; (2) SP: 15 % PEG-6000 + 120 mM NaCl; (3) SP-SA1: 15 % PEG-6000 + 120 mM NaCl + 0.1 mM SA; (4) SP-SA2: 15 % PEG-6000 + 120 mM NaCl + 0.2 mM SA; and (5) SP-SA3: 15 % PEG-6000 + 120 mM NaCl + 0.3 mM SA. All treatments were set up in triplicates. After 5 days of treatment, photographs were taken, and the growth parameters as well as the physiological and biochemical indexes were determined.

For quantitative real-time polymerase chain reaction (qRT-PCR), the shoots of two-leaf-one-heart stage seedlings were harvested 5 h after all treatments, immediately frozen in liquid nitrogen, and stored at −80 °C.

2.3. Measurement of growth indexes

Rice seedlings with different treatments were randomly selected, and the mean values of root length, plant height were calculated after measuring the root length and plant height. Fifteen rice plants were randomly selected from each treatment group and divided into three groups. The fresh weight of each group was measured with an electronic scale, and the dry weight of seedlings was determined after drying seedlings at 60 °C until constant weight obtained.

2.4. Measurement of physiological indexes

Photosynthetic pigments were determined using the acetone-ethanol leaching method. Concisely, 0.15–0.2 g of fresh rice leaves was immersed in 10 mL of a mixture of acetone and anhydrous ethanol (95 % acetone: anhydrous ethanol = 2:1). After incubation at 26 °C for 48 h in the dark, the supernatant was collected to determine the optical density values of chlorophyll a, and chlorophyll *b* at two wavelengths of 470 nm, 645 nm on an ultraviolet (UV) spectrophotometer. The chlorophyll content was calculated using Arnon's formula: $C = (A \times V \times 1000) / (E \times l)$ (Arnon et al., 1949). In the formula, C denotes the chlorophyll content in g/L; A denotes the absorbance value of the sample; V denotes the volume of the sample in mL; E denotes the molar absorbance coefficient of chlorophyll in L/(mol.cm); and 1 denotes the optical range length in cm.

2.5. Measurement of biochemical indexes

Rice seedling leaf samples weighing 0.1–0.2 g were harvested and ground with liquid nitrogen, and then transferred to eppendorf tubes for the measurements of antioxidant enzyme activities. The malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline (Pro) contents as well as the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities were measured using kits obtained from Beijing Solarbio Science and Technology Co. (Beijing, China). Five biological replicates were tested for each indicator.

2.6. Expression analysis of rice adversity stress-related genes

2.6.1. Plant total RNA extraction

The total RNA was extracted from rice shoots using the RNA prep Pure Plant Total RNA Extraction Kit (Item No. DP432, TIANGEN, Beijing, China).

2.6.2. Reverse-transcription

The genomic DNA was removed and cDNA was synthesized through the reverse transcription of total RNA from each sample using the Hifair® II 1st Strand cDNA Synthesis Kit (Item No.: 11123ES60, YEASEN), reverse transcription program: 25 °C for 5 min, 42 °C for 30 min, and 85 °C for 5 min. The cDNA products were diluted and used for qRT-PCR or stored at −80 °C.

2.6.3. qRT-PCR primers

The related salt and drought stress response genes were selected in the website (https://pubmed.ncbi.nlm.nih.gov/), and the cDNA sequences of the genes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). Primer Premier 5.0 software was utilized to design primers. Primer sequences with Tm values of 58–62 °C and GC values of 40–60 % were selected. The specificity was verified using the Ensembl Plants website (http://plants.ensembl.org/Oryza_sativa/Tools/Blast?db=core), and the internal reference gene was rice ubiquitinase gene *UBQ5*. The primer sequences are shown in Supplementary Material 1.

2.6.4. Real-Time pcr of genes and data analysis

Fluorescence quantitative analysis was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad, USA) and the Hieff® qPCR SYBR® Green Master Mix (No Rox) kit (YEASEN). The relative expression levels were normalized to *OsUBQ5* for quantification using the $2^{-\Delta\Delta CT}$ method (Livak et al., 2001). Four biological replicates were used in all reactions. The reaction process was as follows: 95 °C for 5 min, 95 °C for 10 s, and 60 °C for 30 s. The reaction was cycled 40 times.

2.7. Data analysis

Excel software was used to organize the data, and SPSS 20.0 was used to perform the statistic analysis. One-way analysis of variance (ANOVA)

and Duncan's method for multiple comparisons were used to execute the statistical analyses, with significant differences defined as $P < 0.05$. Data are expressed as the mean ± standard deviation. Graphs were made using GraphPad Prism 9 software.

3. Results

3.1. Effects of different concentrations of exogenous SA on the growth of rice seedlings under salt and drought stress

The phenotypic observation of salt and drought stress treatments showed that the growth of both rice varieties, NIP and 9311, was inhibited to different degrees compared with the control (Fig. 1A). The root length, plant height, and shoot and root dry weights of NIP and 9311 rice plants were significantly reduced compared with the control. Treatments with different concentrations of SA significantly improved the root length, plant height, and biomass accumulation of rice seedlings under salt–drought stress. Compared with the salt–drought stress treatment groups, the root lengths of NIP rice in the SP-SA1, SP-SA2, and SP-SA3 groups were 1.09, 1.11, and 1.10 times longer than that of SP, respectively, and the plant heights were increased by 21.8 %, 28.6 %, and 28.1 % (Fig. 1B); the dry weight of the aboveground part of the plant increased by 68.3 %, 73.8 %, and 74.5 %, respectively, and the dry weight of the belowground part increased by 103.9 %, 86.0 %, and 58.8 %, respectively (Fig. 1C). Similarly, 9311 showed the consistent behaviors compared to NIP under different treatments. The results indicated that the application of SA alleviated the inhibition of rice growth by salt and drought stress.

3.2. Effects of different concentrations of exogenous SA on the chlorophyll content of rice seedlings under salt and drought stress

Compared with the control, the chlorophyll contents of NIP and 9311 seedlings decreased by 66.90 % and 72.9 %, respectively, under salt-drought stress (Fig. 2C). Compared with SP, the chlorophyll contents of NIP increased by 68.17 %, 99.42 %, and 26.58 %, and that of 9311 increased by 240.7 %, 190.9 %, and 230.2 % in the SP-SA1, SP-SA2, and SP-SA3 treatments, respectively (Fig. 2C). This indicated that SA was able to alleviate the negative effects of salt and drought stress on chlorophyll degradation in rice seedlings, and the alleviation effect was more obvious in 9311.

3.3. Effects of different concentrations of exogenous SA on the MDA, H_2O_2, and Pro contents of rice seedlings under salt and drought stress

The MDA, H_2O_2, and Pro-contents in NIP were significantly increased by 289.4 %, 152.2 %, and 364.2 %, respectively, and by 200.0 %, 55.2 %, and 2113.5 % in 9311 under salt and drought stress compared with the control (Fig. 3A–C). Under salt–drought stress conditions, the application of 0.1 mM, 0.2 mM, and 0.3 mM exogenous SA significantly reduced the MDA and H_2O_2 contents and increased the Pro-content in NIP and 9311. In NIP, the application of 0.3 mM exogenous SA had the most significant effect on the reduction of MDA (Fig. 3A), and the application of 0.2 mM exogenous SA had the most significant effect on the reduction of H_2O_2 content (Fig. 3B), while the application of 0.1 mM exogenous SA had the most significant effect on the elevation of Pro-content (Fig. 3C). In 9311, the application of 0.1 mM exogenous SA caused the most significant reduction of MDA content (Fig. 3D) and elevation of Pro-content (Fig. 3F), while the different concentrations of

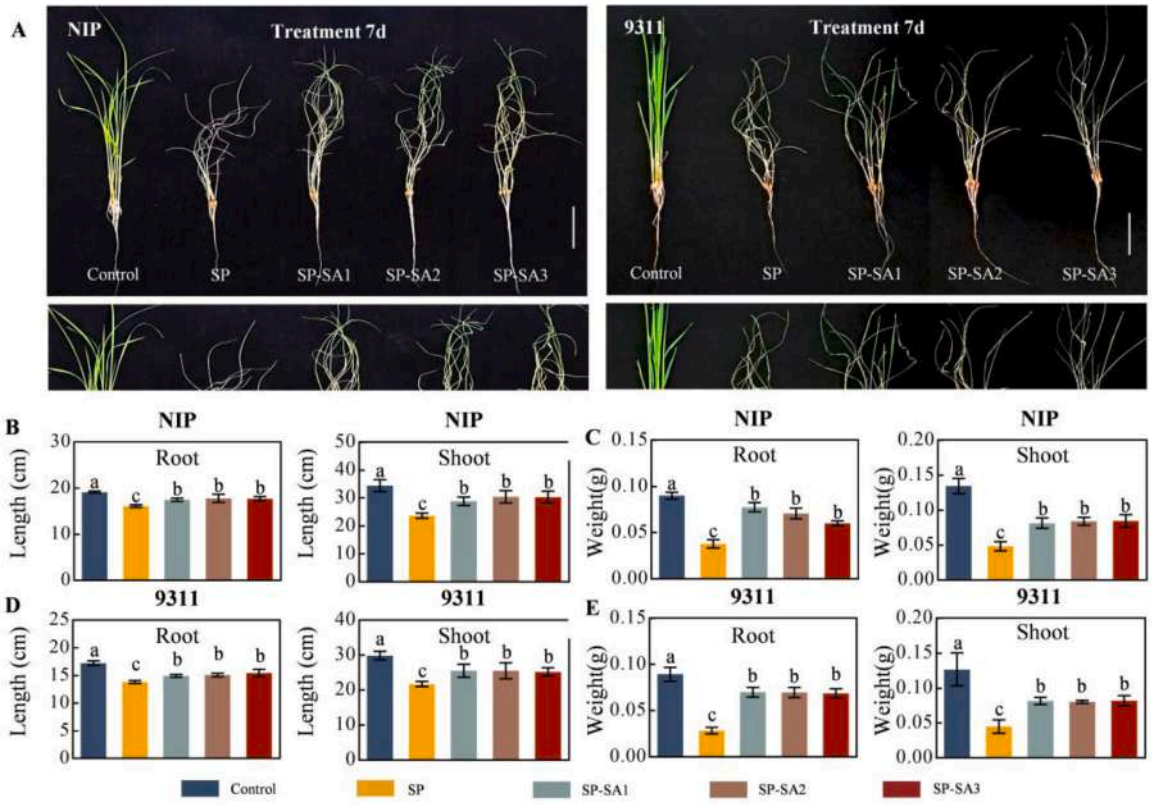

Fig. 1. SA positively regulates rice seedling growth under salt and drought stress.
A: Plant morphologies of NIP and 9311 seedlings after treatments; B: Root and shoot length of NIP; C: Root and shoot dry weight of NIP; D: Root and shoot length of 9311; E: Root and shoot dry weight of 9311. SP: 120 mM NaCl+15 % PEG, SP-SA1: 120 mM NaCl+15 % PEG+0.1 mM SA, SP-SA2: 120 mM NaCl+15 % PEG+0.2 mM SA, and SP-SA3: 120 mM NaCl+15 % PEG+0.3 mM SA. Vertical bars indicate mean ± *S*.E. for $n = 15$. Means with different letters are significantly different at $p \leq 0.05$ according to the Duncan's studentized range.

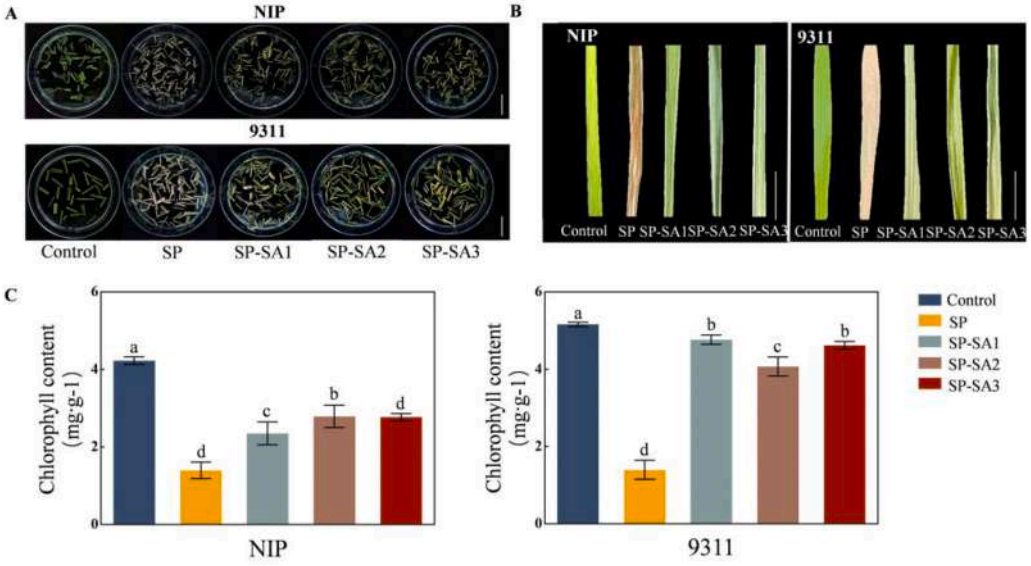

Fig. 2. SA increases chlorophyll content in rice seedlings under salt and drought stress.
A: Phenogram of rice seedling leaves after treatments; B: Localized phenogram of rice seedling leaves after treatments; C: Chlorophyll content of NIP and 9311. Vertical bars indicate mean ± *S*.E. for $n = 5$. Means with different letters are significantly different at $p \leq 0.05$ according to the Duncan's studentized range.

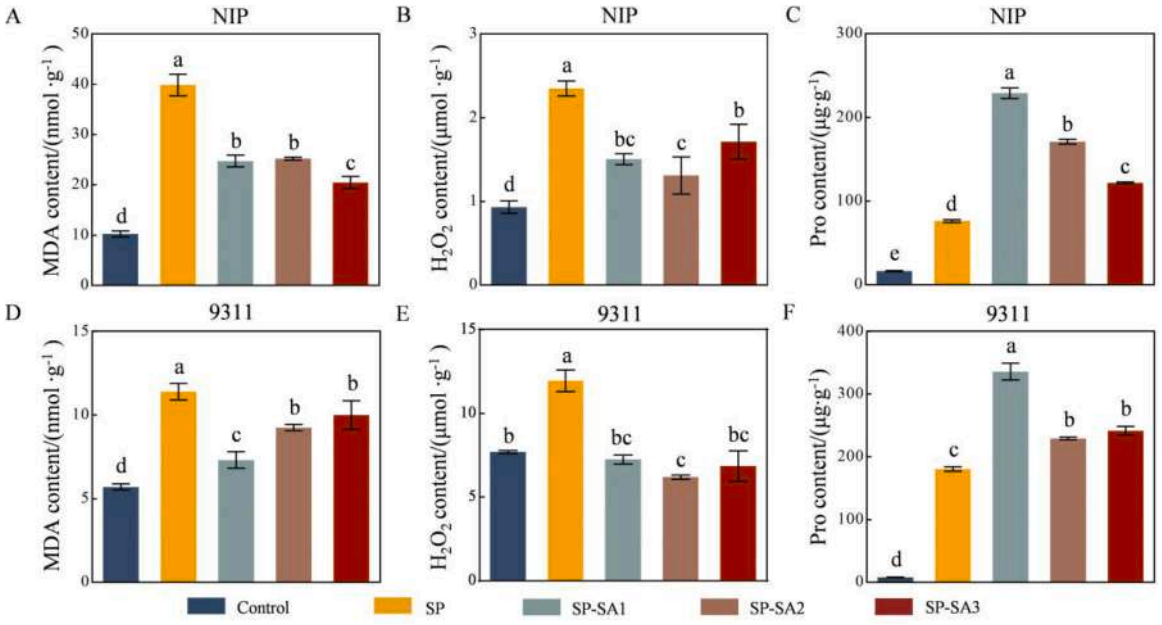

Fig. 3. Effect of SA on the MDA, H_2O_2, and Pro-contents of rice seedlings under salt and drought stress.
A: MDA content of NIP; B: H_2O_2 content of NIP; C: Pro-content of NIP; D: MDA content of 9311; E: H_2O_2 content of 9311; F: Pro-content of 9311. Vertical bars indicate mean ± *S*.E. for $n = 5$. Means with different letters are significantly different at $p \leq 0.05$ according to the Duncan's studentized range.

exogenous SA had no effect on the H_2O_2 content (Fig. 3E).

3.4. *Effects of different concentrations of exogenous SA on the antioxidant enzyme activities of rice seedlings under salt and drought stress*

Compared with the control, the activities of POD, CAT, and SOD were significantly increased by 134.2 %, 161.4 %, and 319.5 % in NIP under salt and drought stress treatments, respectively (Fig. 4A–C), while those of 9311 were increased by 199.5 %, 123.9 %, and 149.3 % (Fig. 4D–F). The enzyme activities of 9311 were higher than those of NIP in all groups of treatments. Under salt and drought stress treatments, the application of 0.1 mM, 0.2 mM, and 0.3 mM SA significantly increased the activity of POD, SOD, and CAT in both NIP and 9311 (Fig. 4C). The activities of the three antioxidant enzymes showed a gradual increase in both rice varieties with the increase of the concentration gradient after SA treatment under salt and drought stress. These results indicate that salt and drought stress can stimulate the enhancement of antioxidant enzyme activities in rice, and the application of SA can increase the antioxidant enzyme activities, thereby, resulting in the activation of the antioxidant system in rice.

3.5. *Effects of different concentrations of exogenous SA on the expression of resistance genes in rice seedlings under salt and drought stress*

The gene expression analysis showed that *OsDREB2A, OsSAPK8, OsSAPK10* and *OsMYB2* exhibited a significant increase by 277 %, 105

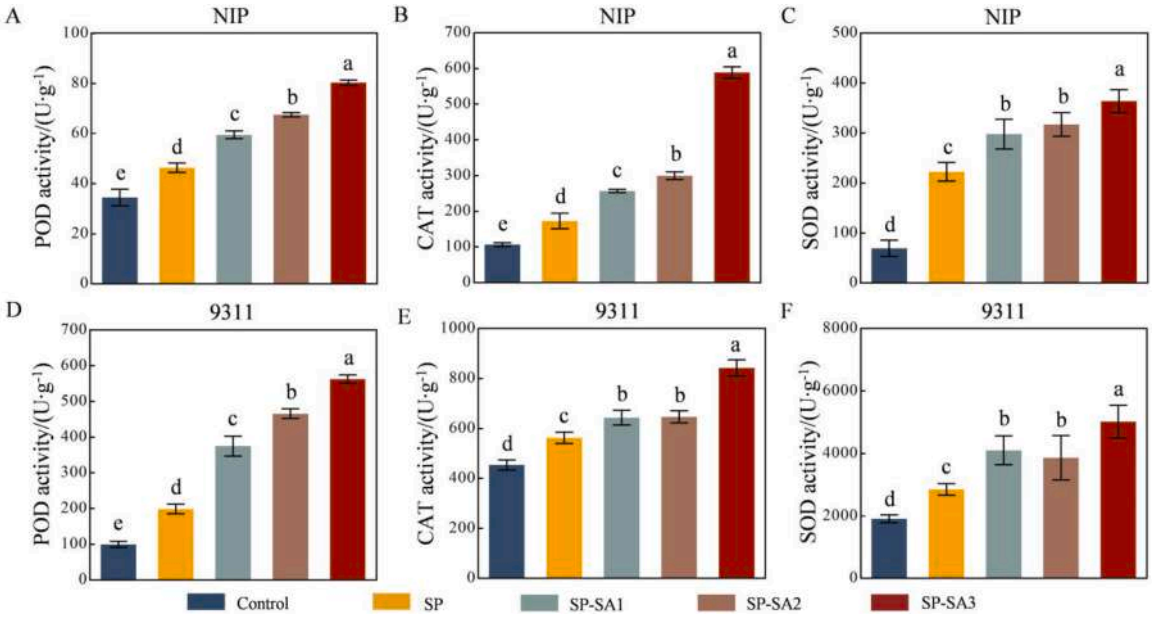

Fig. 4. SA increase antioxidant enzyme activities in rice seedlings under salt and drought stress.
A: POD activity of NIP; B: CAT activity of NIP; C: SOD activity of NIP; D: POD activity of 9311; E: CAT activity of 9311; F: SOD activity of 9311. Vertical bars indicate mean ± *S*.E. for $n = 5$. Means with different letters are significantly different at $p \leq 0.05$ according to the Duncan's studentized range.

%, 73 % and 4 % in NIP, respectively, and by 277 %, 89 %, 564 % and 8 % in 9311 under salt and drought stress (SP) compared with control, (Fig. 5). In NIP, *OsDREB2A* was up-regulated 30 %, 23 %, and 210 %, respectively, after the application of 0.1 mM (SP-SA1), 0.2 mM (SP-SA2) and 0.3 mM SA (SP-SA3), in comparison to the expression in SP, and SP-SA3 had the most significant effect. Similarly, compared with SP, the gene expression of *OsMYB2* was increased by 34 %, 30 % and 58 % in SP-SA1, SP-SA2, SP-SA3. Compared with SP, the gene expression levels of *OsSAPK8* and *OsSAPK10* were increased by 39 % and 27 % in SP-SA3, respectively. In 9311, compared with SP, the gene expression of *OsDREB2A* was up-regulated by 51 %, 33 % and 33 % in SP-SA1, SP-SA2 and SP-SA3, respectively, and the *OsSAPK8* was increased by 57 % and 86 % in SP-SA2 and SP-SA3, respectively. Similarly, the gene expression of *OsSAPK10* and *OsMYB2* were increased by 22 % and 4 % in SP-SA1, respectively. The results suggest that the application of exogenous SA may enhance the stress tolerance of rice through enhancing the expression of salt and drought-tolerant genes.

4. Discussion

Salt stress and drought stress, two major negative factors that inhibit crop growth and development, often occur simultaneously in natural environments, and pose a great challenge to global agricultural production (Bashir et al., 2019). The biomass of both rice varieties was significantly reduced under salt and drought stress, with a more pronounced inhibitory effect on roots. This was similar to the previous

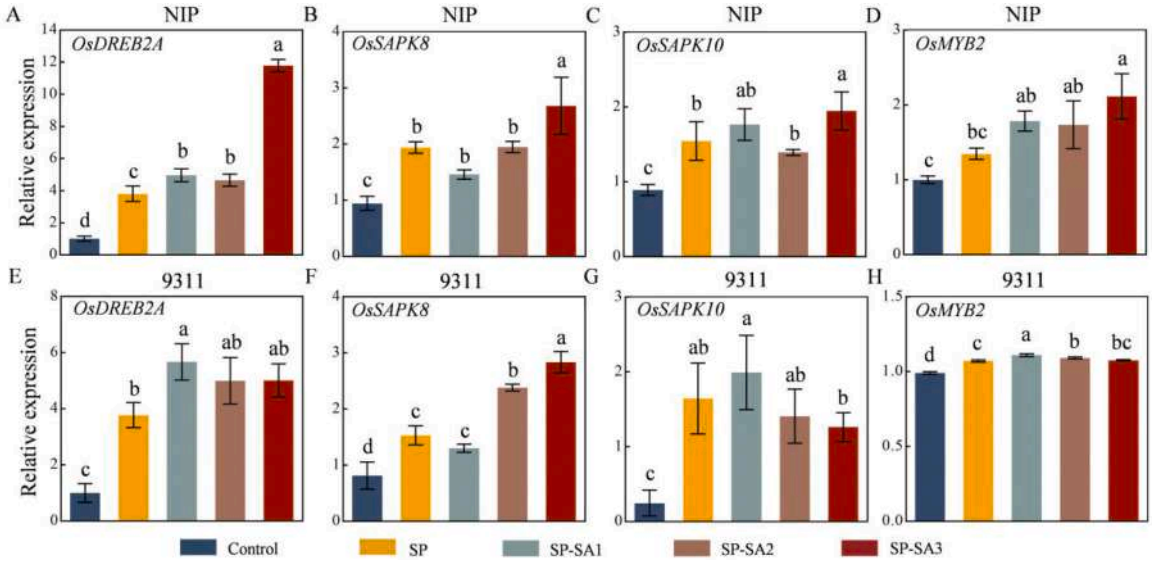

Fig. 5. Effect of SA on the expression of stress-responsive genes in rice seedlings under salt and drought stress.
A-D: Expression analysis of *OsDREB2A, OsSAPK8, OsSAPK10, and OsMYB2* under treatments using qRT-PCR in NIP; E-H: Expression analysis of *OsDREB2A, OsSAPK8, OsSAPK10*, and *OsMYB2* under treatments using qRT-PCR in 9311. *OsUBQ5* was used as an internal reference. Vertical bars indicate mean ± *S*.E. for $n = 4$. Means with different letters are significantly different at $p \leq 0.05$ according to the Duncan's studentized range.

findings, where plant growth and biomass were significantly reduced under salt–drought stress, which may have been due to the reduction in leaf area, an imbalance in plant water status, and a decrease in the production of photoassimilates (Bashir et al., 2019; Movahhedi-Dehnavi et al., 2019). The results showed that the application of exogenous SA promoted both root and stem growth under salt and drought stress, which was consistent with the results of a previous study on linseed (Movahhedi-Dehnavi et al., 2019).

SA is an endogenous signaling molecule that is used to activate plant growth and plant defense responses to biotic and abiotic stresses (Khan et al., 2012). Potential mechanisms through which SA induces tolerance in plants under abiotic stresses include inducing the accumulation of osmotic substances, maintaining intracellular oxidative homeostasis, and improving the regulation of mineral nutrient uptake (Khan et al., 2015). Exogenous SA can mitigate the adverse effects of abiotic stress on plant cell growth through enhancing photosynthesis and attenuating intracellular oxidative damage (Sedaghat et al., 2017). In this study, the chlorophyll content determination indicated that the mitigation effect of SA on 9311 may be better than NIP. The chlorophyll content was significantly reduced under salt and drought stress, while the application of SA could increase the chlorophyll contents suggesting that the application of SA could alleviate the negative effects of salt and drought stress on photosynthesis. It has been reported that the protective effect of SA on chlorophyll in rice seedlings under salt and drought stress may be related to the fact that SA improves photosynthetic capacity through regulating stomatal conductance, which protects and enriches photosynthetic performance and photosynthetic pigment content (Assche et al., 2010; Kaur et al., 2017; Li et al., 2019; Lu et al., 2018). In addition, SA can effectively alleviate plant stress in response to reactive oxygen species (ROS), stimulate the antioxidant defense system of plants, and protect photosynthetic organs from damage by excess ROS (Huang et al., 2022).

Reactive molecules of ROS include superoxide anion (O^{2-}), hydroxyl radical (-OH), and hydrogen peroxide (H_2O_2), while MDA is a product of lipid peroxidation and is formed by the ROS-induced catabolism of polyunsaturated lipids. Both ROS and MDA are unavoidable products of normal plant growth and metabolism (Saleem et al., 2021). It has been shown that under abiotic stress, excessive ROS accumulation occurs in plants, leading to lipid peroxidation and the production of MDA, which results in the impairment of cell membrane stability, the photosynthetic system, and chlorophyll *b*iosynthesis (Noreen et al., 2016; Samanta et al., 2024). To mitigate or prevent ROS-induced oxidative damage, plants have evolved mechanisms to scavenge these toxic and reactive species through antioxidant oxidation by enzymatic and nonenzymatic systems (Choudhary et al., 2024). In this study, the MDA and H_2O_2 contents of the two rice cultivars increased significantly under salt and drought stress, whereas the MDA and H_2O_2 contents decreased significantly after the application of SA, indicating that exogenous SA protected the membrane lipid system. SA as a signaling molecule involved in the regulation of plant antioxidant enzyme systems. Yang et al. (2022) showed that exogenous SA treatment can increase the activity of antioxidant enzymes and the osmoregulatory substances contents in winter jujube (Yang et al., 2022). In the present study, the application of SA resulted in a sustained increase in rice SOD, POD, and CAT enzyme activities, which was consistent with the previous studies. The results of the present study suggest that exogenous SA may reduce the accumulation of H_2O_2 and MDA in rice seedlings under abiotic stress through stimulating the activities of antioxidant enzymes, such as SOD, POD, and CAT, to alleviate the membrane lipid peroxidation damage in rice seedlings.

Osmoregulatory substances play an important role in plant adaptation to salinity and drought adversity (Rong et al., 2011). In this study, the application of SA significantly increased the Proline content of both rice varieties under salt–drought stress, and the results showed that the accumulation of proline content was more significant under the 0.1 mM SA treatment than the 0.2 mM and 0.3 mM SA treatments in both rice varieties. Rajabi et al. found that the exogenous application of SA increased the activity of Pro-synthases such as Pro-and glutamyl kinase, which ultimately increased the Pro-content of cells (Rajabi et al., 2022), which well supports the findings of the present study.

Previous reports showed that SA had different effects on different varieties, which may be due to the difference in active oxygen scavenging capacity, antioxidant capacity of different varieties of plants, and has been found in rice, wheat and mung bean etc. (Loutfy et al., 2012; Sharma et al., 2017; Yadav et al., 2020; Liu et al., 2022; Urmi et al., 2023). Nipponbare is more sensitive to salt stress than Huidao5, and exogenous application of SA significantly improved germination of Nipponbare, however, different concentrations of SA had no significant effect on the germination rate of Huaidao 5 under salt stress (Liu et al., 2022). Similarly, the effect of SA on drought-tolerant rice varieties BRRI dhan66 and drought-susceptible rice varieties BRRI dhan75 was different (Urmi et al., 2023). In this study, application of 0.3 mM exogenous SA had the most significant effect on reducing MDA content in NIP, while application of 0.1 mM exogenous SA had the most significant effect on reducing MDA content in 9311 (Fig. 3), indicating that NIP and 9311 May have different optimal SA concentrations resistant to salt stress.

Salt and drought tolerance in rice are polygenic controlled traits. Previous studies have explored many genes related to salt and drought stress in rice. For example, the OsDREB2A can regulate the expression of genes related to soluble sugar synthesis. Transgenic plants overexpressing *OsDREB2A* were found to have higher survival rates under severe drought and salt stress conditions (Cui et al., 2011). OsSAPK8 is a positive regulator in response to drought and salt stress, and mutants of *OsSAPK8* are less tolerant to low temperature, high salt stress, and drought stress during vegetative growth phase (Zhong et al., 2020). OsSAPK10 phosphorylates and activates *OsbZIP86*, and rice plants overexpressing *OsbZIP86* exhibit enhanced drought tolerance (Gao et al., 2022). In addition, *OsMYB2* plays a regulatory role in rice tolerance to salt and drought stress (Yang et al., 2012). In this experiment, the expression of the above four genes was investigated, and it was found that the expression of the *OsDREB2A, OsSAPK8, OsSAPK10*, and *OsMYB2* genes was significantly increased in both rice varieties under salt and drought stress. SA up-regulated the expression of the *OsDREB2A, OsSAPK8, OsSAPK10*, and *OsMYB2* genes under salt and drought stress.

5. Conclusion

SA is widely involved in plant stress response, but its function in rice adaptation to salt-drought dual stress is less studied. Our study showed that the salt and drought stresses led to oxidative damage through generation of ROS and membrane damage due to lipid peroxidation, which resulting in delayed growth and development of rice. The application of exogenous SA could significantly reduce the salt and drought damage, mainly through the following points. First, increasing the activity of plant antioxidant enzymes such as SOD, POD, and CAT to reduce ROS contents and cell membrane damage; second, increasing the content of osmotic substances such as proline to avoid damage to the photosynthetic system; and third, increasing the expression of stress-responsive genes However, the molecular mechanism of SA to enhance salt and drought tolerance of rice is still unclear, which need to be further investigated. It will be beneficial in providing theoretical basis and genetic manipulation target for breeding salt and drought-resistant rice varieties.

Data availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information Files.

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CRediT authorship contribution statement

Liqing Shan: Writing – original draft, Methodology, Investigation. **Yating Xu:** Formal analysis, Data curation. **Dan Wu:** Data curation. **Jiayi Hu:** Formal analysis. **Tongyuan Yu:** Formal analysis, Data curation. **Cong Dang:** Methodology, Investigation. **Yunxia Fang:** Methodology, Formal analysis. **Xiaoqin Zhang:** Investigation, Funding acquisition. **Quanxiang Tian:** Writing – review & editing, Supervision, Funding acquisition. **Dawei Xue:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100413.

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