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Effects of exogenous Uniconazole (S3307) on oxidative damage and carbon metabolism of rice under salt stress

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Abstract

Background Salt stress significantly suppresses rice growth. Uniconazole (S3307) is recognized for its potential to enhance plant stress tolerance. Nevertheless, the mechanisms through which S3307 induces salt tolerance in rice by modulating the carbon metabolism pathway are not fully understood. In this study, at the one-leaf-one-heart stage, the foliage of rice HD961 and 9311 was treated with 10 mg·L^{-1} S3307, followed by a 0.6% (102.56 mmol·L^{-1}) NaCl treatment 24 h later.

Results The results demonstrated that salt stress markedly suppressed the growth of rice aboveground and underground, reduced the net photosynthetic rate (P_n), and ultimately led to a decline in yield. However, salt stress increased the activities of peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) and enhanced sucrose metabolism simultaneously of rice leaves. However, compared to salt stress, foliar spraying of S3307 under salt stress increased rice biomass accumulation, enhanced photosynthetic efficiency, reduced malondialdehyde (MDA) content, and further enhanced the activities of superoxide dismutase (SOD), POD, CAT, and APX. Meanwhile, the application of S3307 effectively further promoted the accumulation of sucrose, glucose, and soluble sugar (SS) in rice leaves under salt stress. It also enhanced the activities of key enzymes in glycolysis, namely hexokinase (HK) and pyruvate kinase (PK), and facilitated the accumulation of α-ketoglutaric acid (α-KG), citric acid (CA), and pyruvate (PA). Meanwhile, it increased the effective panicle number (EPN), grains per panicle, yield per panicle and theoretical yield of rice.

Conclusion Therefore, S3307 can mitigate the damage caused by salt stress and enhance yield and rice resistance by improving photosynthetic characteristics, strengthening the antioxidant system, and promoting physiological activities in carbon metabolism pathways such as Carbohydrate, glycolysis (EMP) and the tricarboxylic acid (TCA) cycle.

Keywords Rice, S3307, Salt stress, Antioxidant enzymes, Carbohydrate, Glycolysis, Tricarboxylic acid cycle, Yield

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Background

Rice (*Oryza sativa* L.) is regarded as one of the most economically and nutritionally significant food crops, yet it exhibits high sensitivity to salt stress [1]. In China, saline-alkali lands are widely distributed, with the total area ranking third globally, and the extent of saline soils continues to expand [2]. Salt stress adversely affects various aspects of rice, including agronomic traits, physiological and biochemical activities, and energy metabolism. Generally, rice is more susceptible to salt stress during the seedling and reproductive growth stages [3], with seedling-stage tolerance being critical for ensuring optimal crop performance in field conditions. Excessive accumulation of NaCl in saline soils leads to the overproduction of reactive oxygen species (ROS) in plants, resulting in osmotic stress, ion toxicity, and oxidative stress. These factors collectively inhibit plant growth and can even lead to plant death. Salt stress impacts crops through a two-phase mechanism: the initial phase involves rapid osmotic stress triggered by root exposure to salt, while the second phase is marked by the accumulation of salt ions (mainly Na^+ and Cl^-) in the leaves [4, 5]. Additionally, salt stress induces secondary effects such as oxidative stress and nutrient imbalance [6]. Research has shown that salt stress severely suppresses the aboveground growth of plants, reduces photosynthetic pigment content, inhibits photosynthesis, diminishes antioxidant capacity, and increases the accumulation of malondialdehyde (MDA), ultimately reducing biomass accumulation in plants [7]. Carbon metabolism serves as the central pathway for energy and material cycling. Glycolysis pathway (EMP) and the tricarboxylic acid (TCA) cycle are two key metabolic pathways involved in carbohydrate metabolism, generating energy to sustain essential life processes. Carbon metabolites, such as sucrose, constitute the primary material foundation for grain filling. Salt stress disrupts carbon translocation to seeds, resulting in yield loss [8]. Their stability under salt stress directly impacts the plant's overall metabolic equilibrium. Research has demonstrated that salt stress disrupts plant carbon metabolism, including sugar metabolism, the EMP pathway, the TCA cycle, and related biosynthetic pathways [9]. An experiment on salt-stress treatment of gumbo (*Abelmoschus esculentus*) seeds revealed that salt stress promoted sugar accumulation, reduced starch levels, and led to a significant decline in seed germination rates [10]. It is evident that salt stress inhibits plant growth through multiple mechanisms, ultimately constraining crop development and yield. Therefore, it is essential to select appropriate methods to mitigate salt stress and gain a deeper understanding of its underlying mechanisms, thereby alleviating the global food crisis.

Previous studies have demonstrated that plant growth regulators can improve the tolerance of plants to abiotic stresses [11, 12]. Uniconazole (S3307) is a plant growth retardant that can effectively improve the yield and resistance of crops [13, 14]. S3307 inhibits the biosynthesis of gibberellins (GA) and abscisic acid (ABA) in plants [15]. Additionally, it modulates endogenous hormone levels, enhances the accumulation of photosynthetic pigments, improves photosynthetic efficiency, increases antioxidant capacity, and promotes starch accumulation [16], thereby improving crop quality and yield [17]. Studies by Keshavarz and Khodabin et al. [18] have shown that foliar application of S3307 enhances drought stress resistance in bean (*Phaseolus vulgaris* L.) seedlings by increasing soluble sugar (SS) accumulation, upregulating the activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), and reducing membrane lipid peroxidation, ultimately increasing yield. Treatment with S3307 alleviates drought stress in soybeans by increasing chlorophyll content and photosynthetic rate, reducing lipid peroxidation levels, and enhancing foliar SS content [19]. Furthermore, the foliar application of S3307 on mung beans enhanced the activities of antioxidant enzymes, including ascorbate peroxidase (APX) and peroxidase (POD), as well as the net photosynthetic rate (P_n). This further enhanced sucrose synthesis and upregulated the expression levels photosynthetic genes, thereby improving cold tolerance and increasing mung bean yield [14]. Research indicates that drought stress reduces the P_n and carbon fixation capacity of plants, thereby impairing carbon assimilation and metabolism [20]. Energy supply pathways, such as the EMP pathway and the TCA cycle, are crucial for enhancing plant salt tolerance [21]. Research has shown that changes in the EMP pathway and key enzymes and substances in the TCA cycle in respiratory metabolism are highly correlated with plant salt tolerance [22]. Research findings by Wang et al. [23] indicate that exogenous application of γ-aminobutyric acid increases organic acids content in maize leaves under salt stress, promotes the TCA cycle, and ultimately enhances salt tolerance. Therefore, the study of S3307's role in alleviating salt stress in rice is supported by specific scientific evidence.

Previous studies have shown that the positive effects of S3307 on plant stress have been explored in many crops, primarily focusing on its role in alleviating stress through the antioxidant system. However, few studies have systematically explored the effect of S3307 on carbon metabolism in rice under salt stress. Therefore, the objective of this study is to explore the effects of foliar spray of S3307 on the morphology, photosynthetic pigments, photosynthesis, antioxidant system, and carbon metabolism-related substance content and enzyme activity of rice under salt stress. This research aims to provide a theoretical foundation for the application of plant growth regulators to cultivate salt-tolerant rice seedlings. It also

holds significant importance for advancing the understanding of plant growth and development mechanisms and carbon cycling pathways.

Results

Effects of exogenous S3307 on the morphology of rice seedlings under salt stress

The effects of salt and S3307 treatments on the morphological growth of rice seedlings are shown in Fig. 1. The results indicate that under salt stress, morphological parameters such as plant height, stem diameter, leaf area, root fresh weight, and root dry weight of both rice materials were reduced. Compared to the CK, the plant height of HD961 and 9311 under S treatment decreased by 4.02% and 0.67%, respectively, while the stem diameter significantly decreased by 17.50% and 14.29%, respectively. The leaf area of HD961 and 9311 significantly decreased by 4.66% and 10.90%, the shoot fresh weight significantly decreased by 8.55% and 8.21%, the shoot dry weight decreased by 5.34% and 4.99%, the root fresh weight decreased by 4.49% and 6.83%, and the root dry weight significantly decreased by 15.98% and 13.75%, respectively. The growth-related parameters of both rice materials were influenced by S3307. Compared to the S treatment, the US treatment reduced the plant height of HD961 and 9311 by 6.88% and 2.04%, respectively, while significantly increasing the stem diameter by 9.09% and 9.52%, respectively. The leaf area of HD961 and 9311 significantly increased by 4.45% and 9.01%, the root fresh weight increased by 1.34% and 8.36%, and the root dry weight significantly increased by 9.78% and 13.04%, respectively. As shown in Figs. 2 and 3, salt stress significantly inhibited the growth and development of both the shoot and root systems in rice. In contrast, the US treatment markedly alleviated these inhibitory effects. These findings demonstrate that S3307 treatment mitigates the detrimental effects of salt stress on the morphological growth of rice and promotes root growth, exhibiting a "top-control and bottom-promotion" effect.

Effects of exogenous S3307 on photosynthetic pigment content in rice seedlings under salt stress

Under salt stress, the chlorophyll content in rice leaves increased (Fig. 4). Compared to CK, the S treatment significantly increased the contents of Chl a, Chl b, Car, and Total Chl in the leaves of both HD961 and 9311. In HD961, these increases were 48.24%, 57.30%, 24.63%, and 50.29%, respectively. Similarly, in 9311, the increases were 8.61%, 11.61%, 5.12%, and 7.66%, respectively. Compared to the S treatment, the US treatment significantly enhanced the chlorophyll content in rice leaves. In HD961, the contents of Chl a, Chl b, Car, and Total Chl increased by 7.64%, 9.15%, 5.45%, and 8.99%, respectively. For 9311, these increases were 7.10%, 7.96%, 5.46%, and 6.70%, respectively. These results indicate that exogenous application of S3307 promotes the synthesis of photosynthetic pigments in rice under salt stress, thereby protecting the photosynthetic structures.

Effects of exogenous S3307 on photosynthetic parameters in rice seedlings under salt stress

Salt stress reduced the P_n, G_s, C_i, T_r, and AMC in both HD961 and 9311 rice varieties (Fig. 5). Compared to CK, the S treatment significantly decreased P_n, G_s, C_i, T_r, and AMC in HD961 by 32.82%, 48.20%, 12.64%, 57.48%, and 23.19%, respectively. Similarly, in 9311, these parameters decreased by 44.19%, 74.04%, 12.85%, 80.39%, and 36.02%, respectively. Conversely, the Ls significantly increased in both HD961 and 9311 by 109.26% and 76.86%, respectively. The application of exogenous S3307 alleviated the inhibitory effects of salt stress on photosynthesis in both rice materials (Fig. 5). Compared to the S treatment, the US treatment significantly increased P_n, G_s, C_i, T_r, and AMC in HD961 by 25.71%, 28.46%, 5.73%, 58.82%, and 19.04%, respectively. In 9311, these increases were 35.61%, 152.10%, 10.79%, 318.61%, and 22.65%, respectively. Meanwhile, the Ls levels in both HD961 and 9311 significantly decreased by 20.69% and 31.80%, respectively.

Effects of exogenous S3307 on membrane damage and osmolyte content in rice seedlings under salt stress

As shown in Fig. 6, the MDA content in rice leaves under salt stress gradually increased with prolonged treatment duration. Compared to CK, the S treatment elevated the MDA content in the leaves of both HD961 and 9311. Specifically, the MDA content in HD961 increased by 8.43-19.4%, while that in 9311 significantly increased by 8.81-24.40%. In contrast, the US treatment reduced the MDA content in HD961 leaves by 6.13-6.61% compared to the S treatment. The same parameter in 9311 was significantly reduced by 12.38-13.61%. These results indicate that exogenous application of S3307 effectively mitigates membrane damage in rice under salt stress.

Compared to CK, the S treatment reduced the SP content in the leaves of HD961 and 9311 at the two-leaf-one-heart stage. Specifically, SP content in HD961 significantly decreased by 3.49%, while in 9311, it decreased by 1.92%. Salt stress also reduced the SP content in 9311 leaves at the four-leaf-one-heart stage, although the effect was not statistically significant. In contrast, the US treatment significantly increased the SP content in both HD961 and 9311, with increases of 7.05-8.99% and 3.80-23.68%, respectively, compared to the S treatment. This demonstrates that exogenous S3307 effectively alleviates osmotic stress in rice under salt stress, promotes the biosynthesis of osmotic adjustment

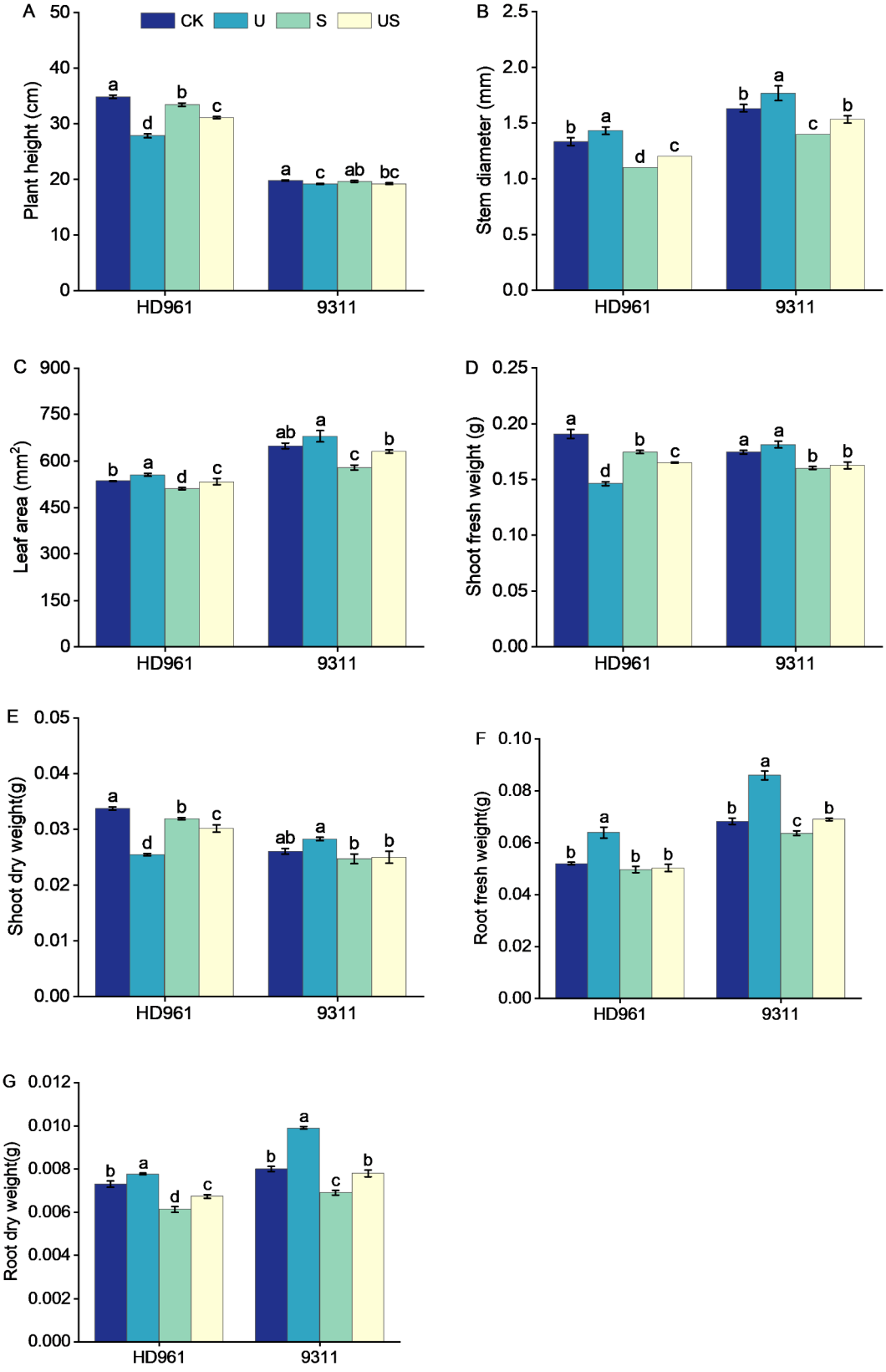

Fig. 1 Effects of exogenous S3307 on the morphology of rice seedlings under salt stress. (**A**) Plant height; (**B**) Stem diameter; (**C**) Leaf area; (**D**) Shoot fresh weight; (**E**) Shoot dry weight; (**F**) Root fresh weight; (**G**) Root dry weight. (**A**)-(**G**) represent morphological data at the two-leaf-one-heart stage of rice. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

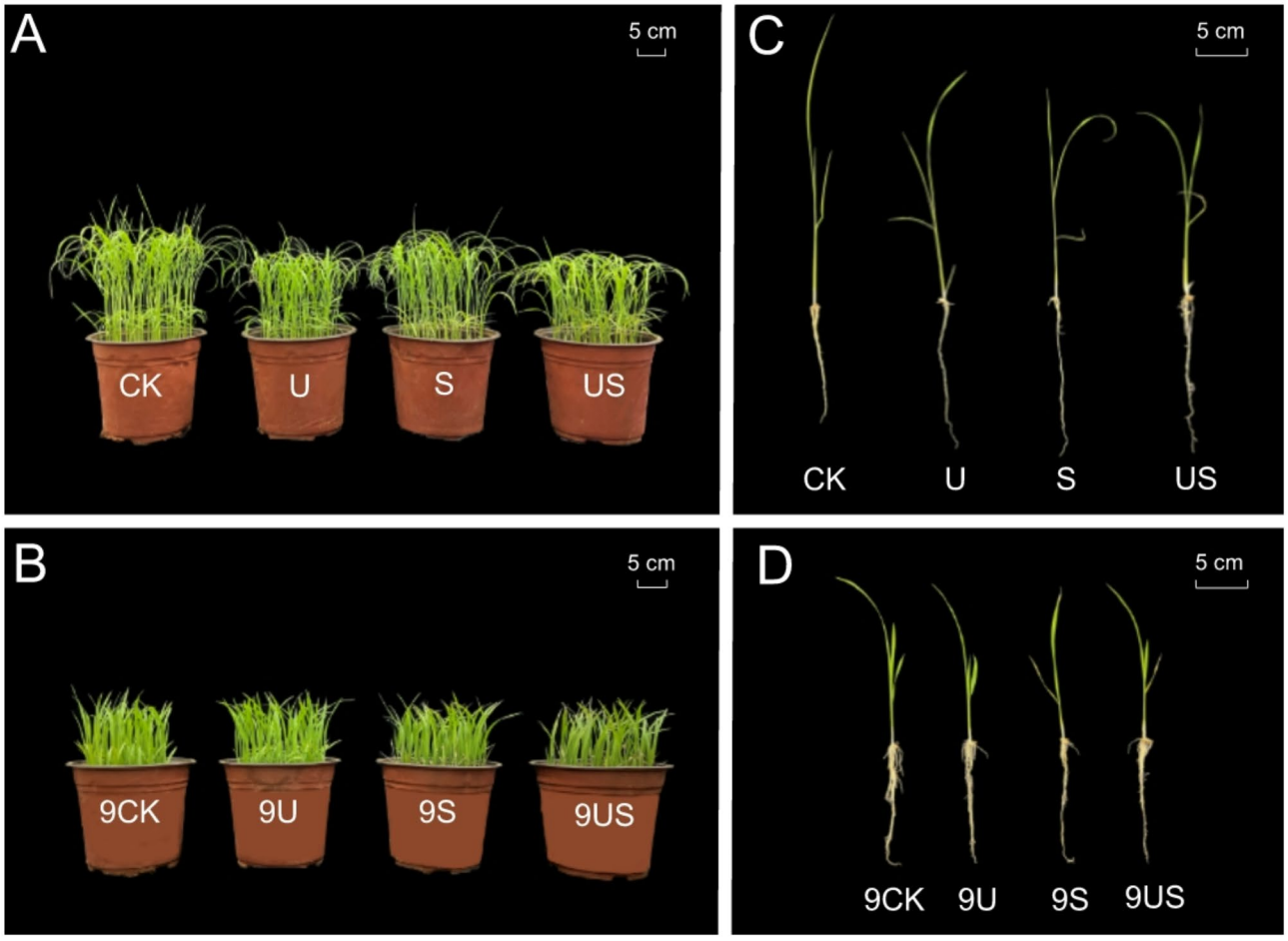

Fig. 2 Effects of exogenous S3307 on the growth of rice seedlings under salt stress. (**A**) and (**B**) show the growth morphology of individual pots for HD961 and 9311 under different treatments, respectively. (**C**) and (**D**) show the growth morphology of individual seedlings for HD961 and 9311 under different treatments, respectively. (**A**), (**B**), (**C**), and (**D**) were photographed at the two-leaf-one-heart stage of rice

substances in rice leaves, and helps maintain intracellular osmotic pressure.

Effects of exogenous S3307 on antioxidant enzyme activities in rice seedlings under salt stress

As shown in Fig. 7, salt stress reduced the activity of SOD in rice leaves while increasing the activities of POD, CAT, and APX. Compared to the CK, the S treatment decreased SOD activity in HD961 leaves by 0.98%, while the same parameter in 9311 was significantly reduced by 6.88%. In contrast, the S treatment increased the activities of POD, CAT, and APX in HD961 leaves by 5.16%, 11.47%, and 13.96%, respectively. The same parameters in 9311 increased by 8.52%, 64.46%, and 17.58%, respectively. Compared to the S treatment, the US treatment enhanced the activities of SOD, POD, CAT, and APX in the leaves of both HD961 and 9311. Specifically, these activities increased by 1.46%, 24.85%, 7.32%, and 18.74% in HD961, and by 1.74%, 21.83%, 18.01%, and 31.82% in 9311. These results indicate that salt stress stimulates the activity of antioxidant enzymes in rice leaves, while exogenous application of S3307 further enhances their activity.

Effect of exogenous S3307 on carbohydrate content in rice under salt stress

As shown in Fig. 8, salt stress increased the contents of sucrose, SS, and glucose in the leaves of both rice materials during the seedling stage (two-leaf-one-heart and four-leaf-one-heart stages) but decreased the sucrose and SS contents in the leaves at the full heading stage. Compared to CK, the S treatment increased the sucrose, SS, and glucose con-tents in the leaves of HD961 during the seedling stage by 10.25-17.50%, 4.26-12.78%, and 20.64-35.83%, respectively. In 9311, the sucrose and SS contents increased by 19.17-22.39% and 13.10-32.06%, respectively, during the seedling stage. Compared to CK, the S treatment reduced the sucrose content in HD961 leaves at the full heading stage by 68.40%, while increasing the soluble sugar content by 3.70%. Exogenous application

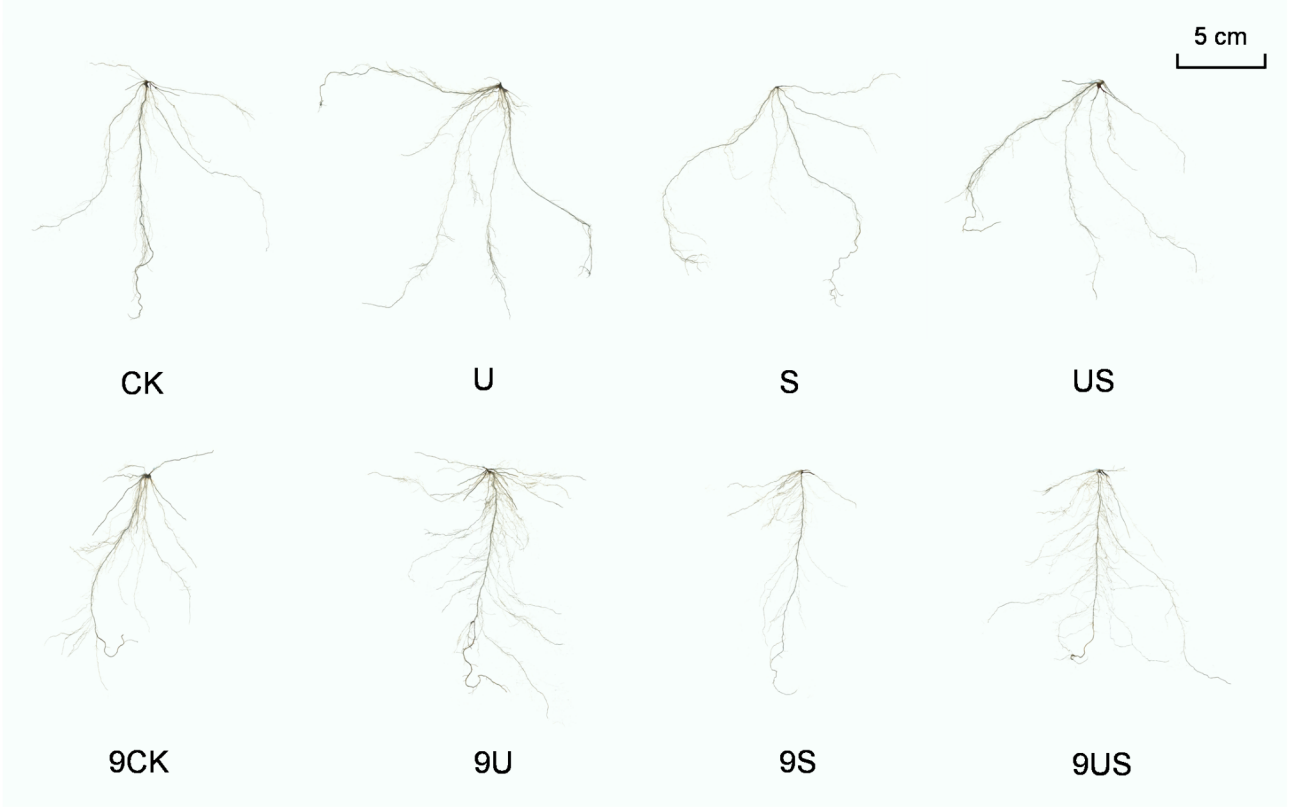

Fig. 3 Effects of exogenous S3307 on root growth of rice seedlings under salt stress

of S3307 enhanced the sucrose, SS, and glucose contents in both rice materials. Compared to the S treatment, the US treatment increased the carbohydrate contents in the leaves of both materials during the seedling and full heading stages. In HD961 and 9311, the sucrose contents in seedling leaves increased by 11.05-15.04% and 9.77-12.84%, respectively, while the SS contents increased by 9.79-15.65% and 8.51-20.53%, respectively. The glucose contents increased by 4.38-21.01% and 15.60-20.75%, respectively. Additionally, the sucrose and SS contents in HD961 leaves at the full heading stage significantly increased by 201.41% and 32.58%, respectively.

Effect of exogenous S3307 on enzyme activities related to starch metabolism in rice under salt stress

As shown in Fig. 9, salt stress increased the activities of β-AL and TAL in the leaves of both rice materials. Compared to CK, the S treatment significantly reduced the α-AL activity in HD961 leaves during the seedling stage (two-leaf-one-heart and four-leaf-one-heart stages) by 17.81-27.69%. The same parameter was significantly reduced by 14.35-35.31% in 9311 during the seedling stage. Salt stress increased the β-AL activity in HD961 and 9311 seedlings by 87.80-255.69% and 22.99-286.28%, respectively, and increased the TAL activity by 86.32-255.99% and 22.54-264.67%, respectively. The same parameters increased by 24.71% and 24.88%, respectively, in HD961 at the full heading stage. Exogenous S3307 treatment reduced the activities of starch metabolism-related enzymes in the leaves of HD961 and 9311. Compared to the S treatment, the US treatment decreased the α-AL activity in HD961 and 9311 seedling leaves by 6.62-13.94% and 9.93-12.24%, respectively, and reduced the TAL by 29.97-35.83% and 7.25-20.16%, respectively. In HD961 leaves at the full heading stage, the activities of α-AL, β-AL, and TAL decreased by 1.90%, 26.77%, and 15.74%, respectively. These results indicate that salt stress accelerates starch hydrolysis in rice leaves, and the application of exogenous S3307 alleviates this hydrolysis.

Effect of exogenous S3307 on the activities of key enzymes in glycolytic pathway in rice seedlings under salt stress

Figure 10 illustrate the activities of HK and pyruvate PK in rice leaves under different treatments. Compared to CK, the S treatment significantly increased HK activity in the leaves of both HD961 and 9311, with increases of 29.22% and 44.44%, respectively. In contrast, PK activity in the leaves of both rice materials significantly decreased, with reductions of 8.79% and 18.48%, respectively. Exogenous application of S3307 enhanced the activities of both HK and PK in rice leaves under salt stress. Compared to the S treatment, US treatment

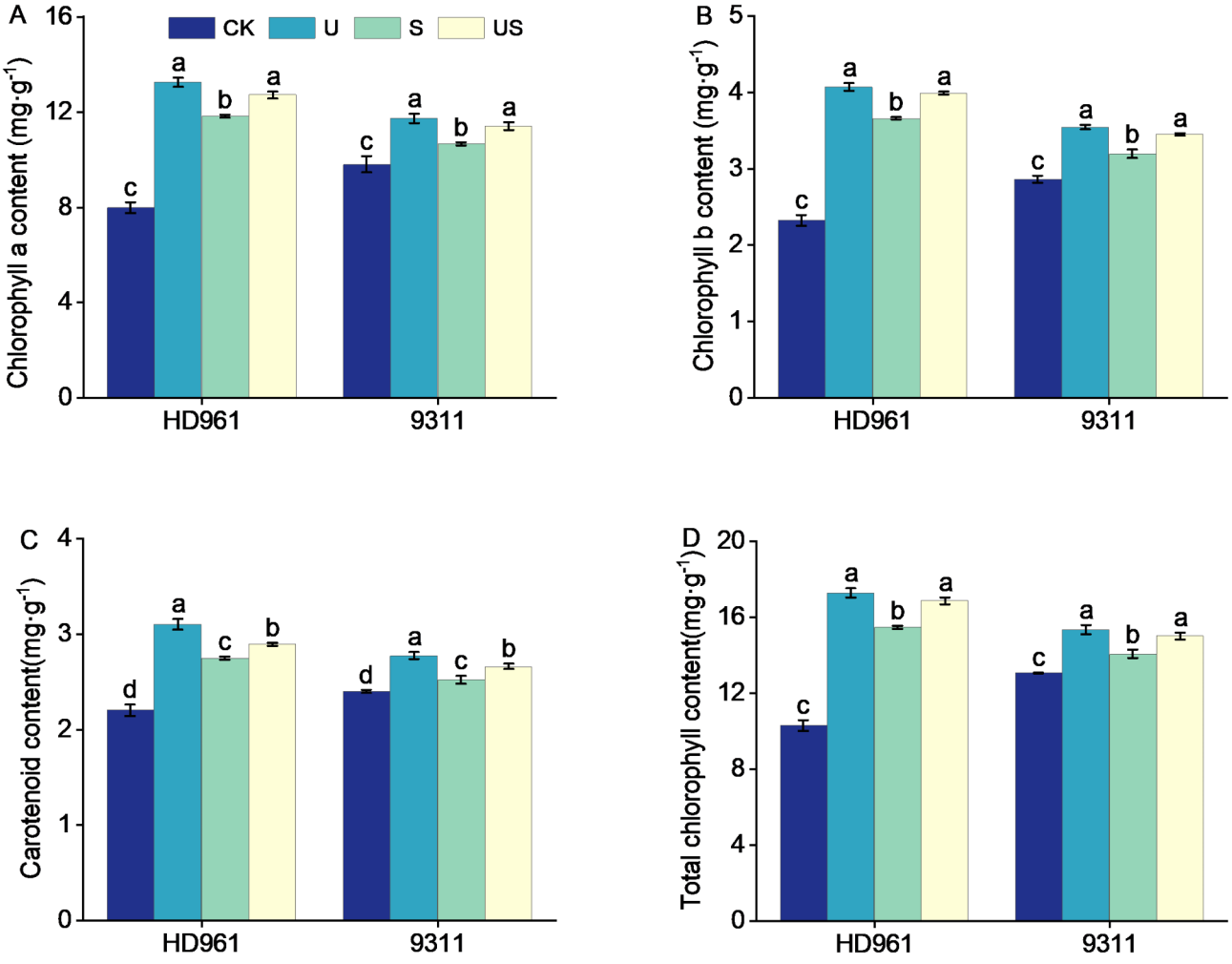

Fig. 4 Effects of exogenous S3307 on photosynthetic pigment content in rice seedlings under salt stress. (**A**) Chlorophyll a content; (**B**) Chlorophyll b content; (**C**) Carotenoid content; (**D**) Total chlorophyll content. (**A**)-(**D**) represent data at the two-leaf-one-heart stage of rice. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

increased HK activity in HD961 and 9311 leaves by 5.08% and 13.46%, respectively, while PK activity significantly increased by 11.70% and 36.92%, respectively.

Effects of exogenous S3307 on organic acid metabolism in rice seedlings under salt stress

Figure 11 shows the contents of PA, CA, and α-KG in rice leaves under different treatments. Compared to CK, the S treatment increased the PA content in HD961 seedling leaves by 35.33%. Over time, the CA content in HD961 and 9311 leaves showed a trend of decreasing first and then increasing. Compared to CK, the CA content in HD961 and 9311 under the S treatment decreased by 19.26% and 6.82%, respectively, at the two-leaf-one-heart stage. But increased by 22.43% and 6.96%, respectively, at the four-leaf-one-heart stage. The changes in α-KG content followed a similar trend to that of CA content. Exogenous S3307 treatment increased the organic acid content in the leaves of both rice materials. Compared to the S treatment, the US treatment significantly increased the PA content in HD961 and 9311 seedling leaves by 7.88-19.92% and 11.76-46.63%, respectively, the CA content by 6.78-17.33% and 8.91-41.30%, respectively, and the α-KG content by 20.58-59.52% and 22.21-37.43%, respectively. This indicates that exogenous S3307 treatment promotes the operation of the TCA cycle in rice leaves under salt stress, providing energy for rice growth.

Effects of exogenous S3307 on the yield of rice HD961 under salt stress

As shown in Fig. 12, salt stress severely inhibits the yield of HD961 in rice. Compared to CK, the S treatment significantly reduced the EPN by 21.95%, grains per panicle by 13.34%, yield per panicle by 19.20%, and theoretical yield by 23.09% in HD961. In contrast, compared to the S treatment, the US treatment significantly increased

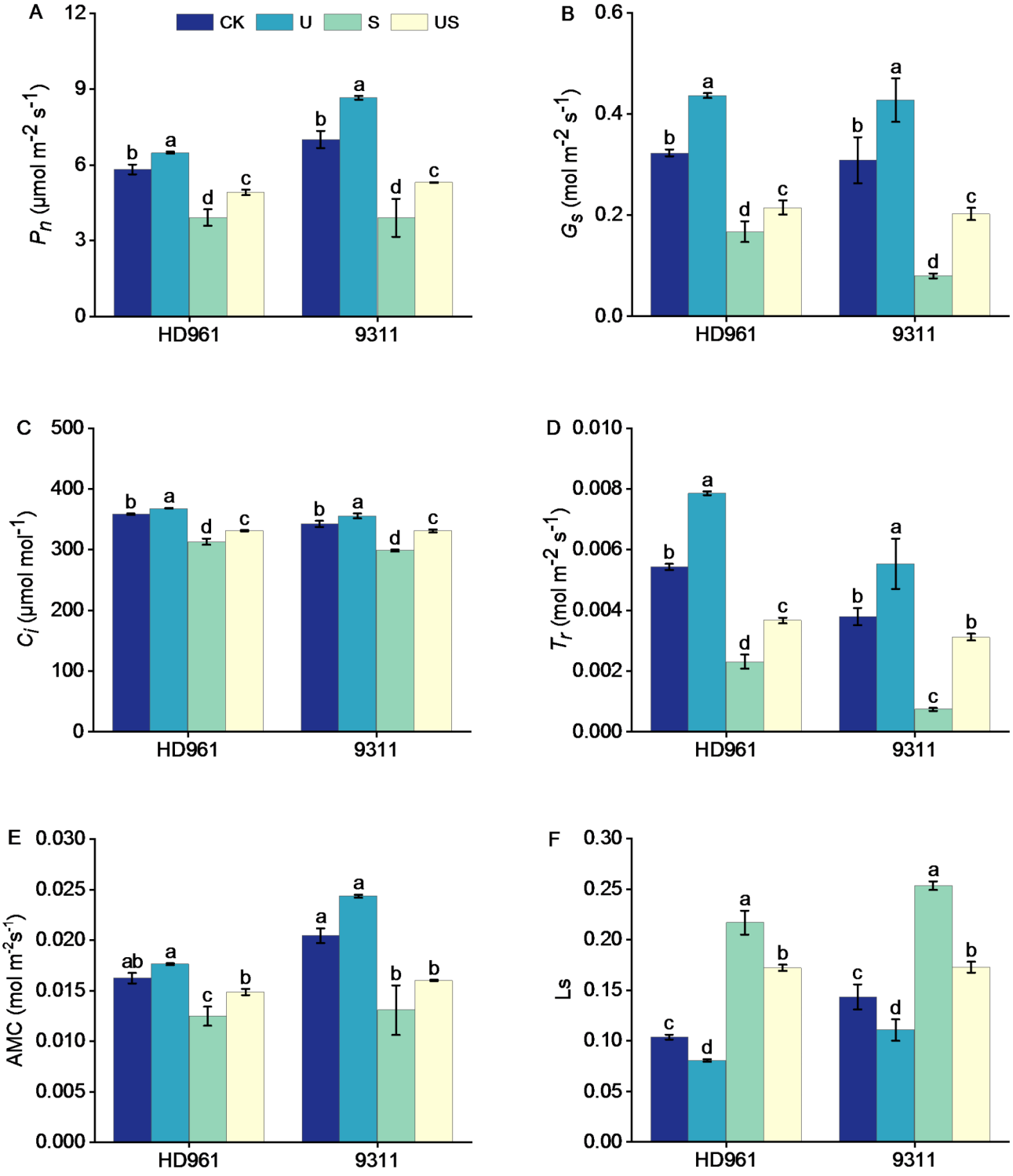

Fig. 5 Effects of exogenous S3307 on photosynthetic parameters in rice seedlings under salt stress. (**A**) P_n; (**B**) G_s; (**C**) C_i; (**D**) T_r; (**E**) AMC; (**F**) Ls. (**A**)-(**F**) represent data at the four-leaf-one-heart stage of rice. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

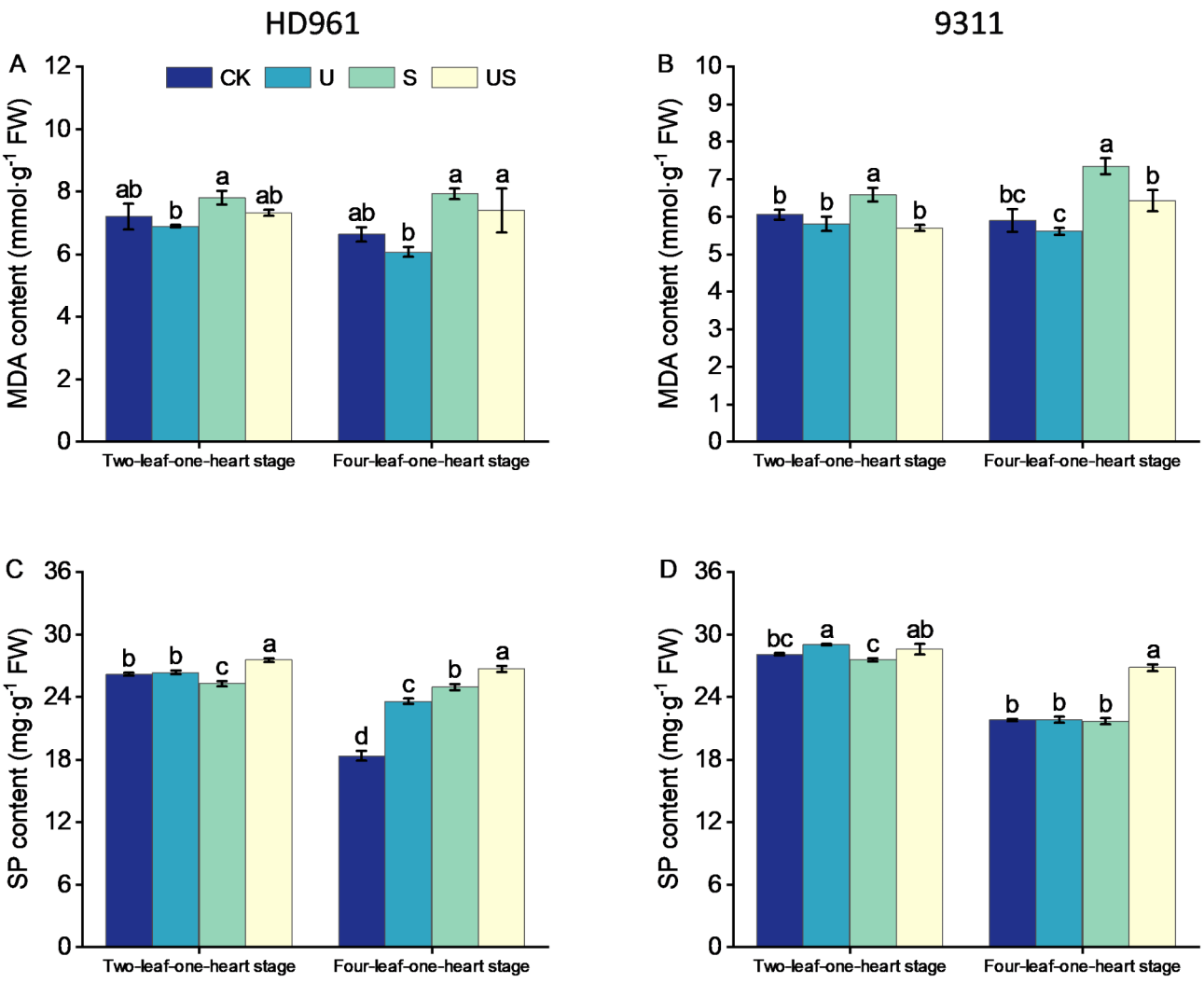

Fig. 6 Effects of exogenous S3307 on membrane damage and osmotic adjustment substance content in rice seedlings under salt stress. (**A**) and (**B**) show MDA content in the leaves of HD961 and 9311, respectively; (**C**) and (**D**) show SP content in the leaves of HD961 and 9311, respectively. Data represent the mean ± standard error (SE) of three replicates. Different lowercase letters above the bars indicate significant differences according to Duncan's test ($p < 0.05$)

the EPN by 18.75%, grains per panicle by 11.01%, yield per panicle by 11.58%, and theoretical yield by 18.33%. These results indicate that exogenous S3307 significantly enhances the EPN and improves the yield of HD961 under salt stress.

Discussion

Salt stress is one of the major abiotic stresses limiting crop growth and development. Excessive salt content disrupts normal physiological and biochemical activities in plants, adversely affecting their growth and development [24]. Numerous studies have shown that salt stress severely inhibits the growth of both aboveground and underground parts of rice and reduces biomass accumulation [24]. This study demonstrates that salt stress decreases plant height, stem diameter, and leaf area in rice HD961 and 9311, ultimately reducing the accumulation of both aboveground and underground dry and fresh weights. This is likely due to the high-salinity environment hindering water absorption by root cells, inducing osmotic stress, and impairing cell elongation and division [25]. Compared to salt stress, the US treatment reduces plant height, increases stem diameter and leaf area, and enhances the accumulation of underground biomass in HD961 and 9311. Du et al. [26] demonstrate that exogenous S3307 inhibits aboveground biomass accumulation in HD961 and 9311 while promoting root growth and biomass accumulation. These findings align with our results. In contrast to HD961, the US treatment increases aboveground biomass accumulation in 9311 at the two-leaf-one-heart stage. We speculate that this difference may be related to rice materials growth patterns and the duration of salt stress exposure. Therefore, the application of S3307 mitigated the detrimental effects of

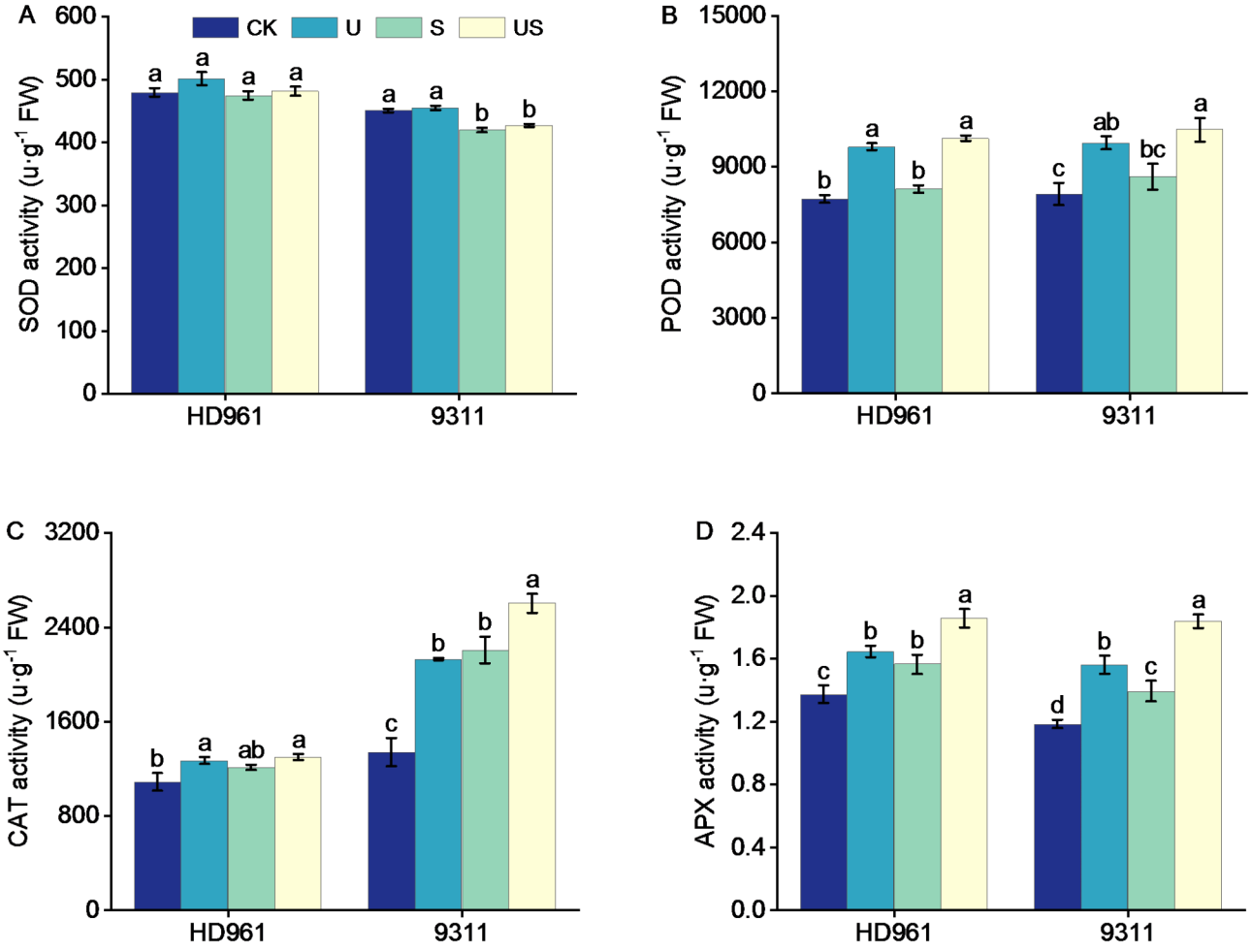

Fig. 7 Effects of exogenous S3307 on the activities of antioxidant enzymes in rice seedlings under salt stress. (**A**) SOD activity; (**B**) POD activity; (**C**) CAT activity; (**D**) APX activity. (**A**)-(**D**) represent data from the two-leaf-one-heart stage. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters above the bars indicate significant differences according to Duncan's test ($p < 0.05$)

salt stress on the morphological development of rice. It effectively reduced plant height, promoted root growth, enhanced lodging resistance, and ultimately improved salt tolerance.

Chlorophyll, an essential photosynthetic pigment, is critical for light energy utilization and conversion. Its content fluctuates in response to environmental changes, thereby influencing photosynthetic efficiency [27]. This study reveals that salt stress significantly increases the contents of Chl a, Chl b, Car, and Total Chl in the leaves of rice HD961 and 9311. This may be due to salt stress causing leaf water loss, hindering leaf expansion and growth, and creating a concentration effect, leading to an increase in chlorophyll content [28]. This may also represent an initial stress response in rice under salt stress, where increased photosynthetic pigment content enhances light capture capacity, maintains normal photosynthetic function, and ensures carbohydrate synthesis, thereby providing energy and material resources for plant growth. This adaptive response helps rice seedlings maintain higher photosynthetic efficiency, which is crucial for their survival under salt stress conditions. However, as rice grows to the four-leaf-one-heart stage, photosynthetic parameters (P_n, C_i, T_r, G_s, Ls, and AMC) decrease. This indicates that prolonged salt stress causes severe damage to the photosynthetic system of rice. The reduction in G_s limits its CO_2 supply, thereby affecting the carbon assimilation process of photosynthesis and leading to a decrease in P_n. Even though photosynthetic pigments increase initially, they cannot effectively sustain photosynthetic efficiency in the long term. A study by Hu et al. [29] demonstrates that the application of S3307 under low-temperature stress increases chlorophyll content in mung bean leaves. Our results are similar to their findings, showing that exogenous S3307 improves photosynthetic pigment content in rice, delays leaf senescence [30], and alleviates salt stress-induced damage to chloroplast structure and pigment-protein complexes. This may be attributed to the application of exogenous S3307 stabilizing the activities of antioxidant enzymes and carotenoids in rice under salt

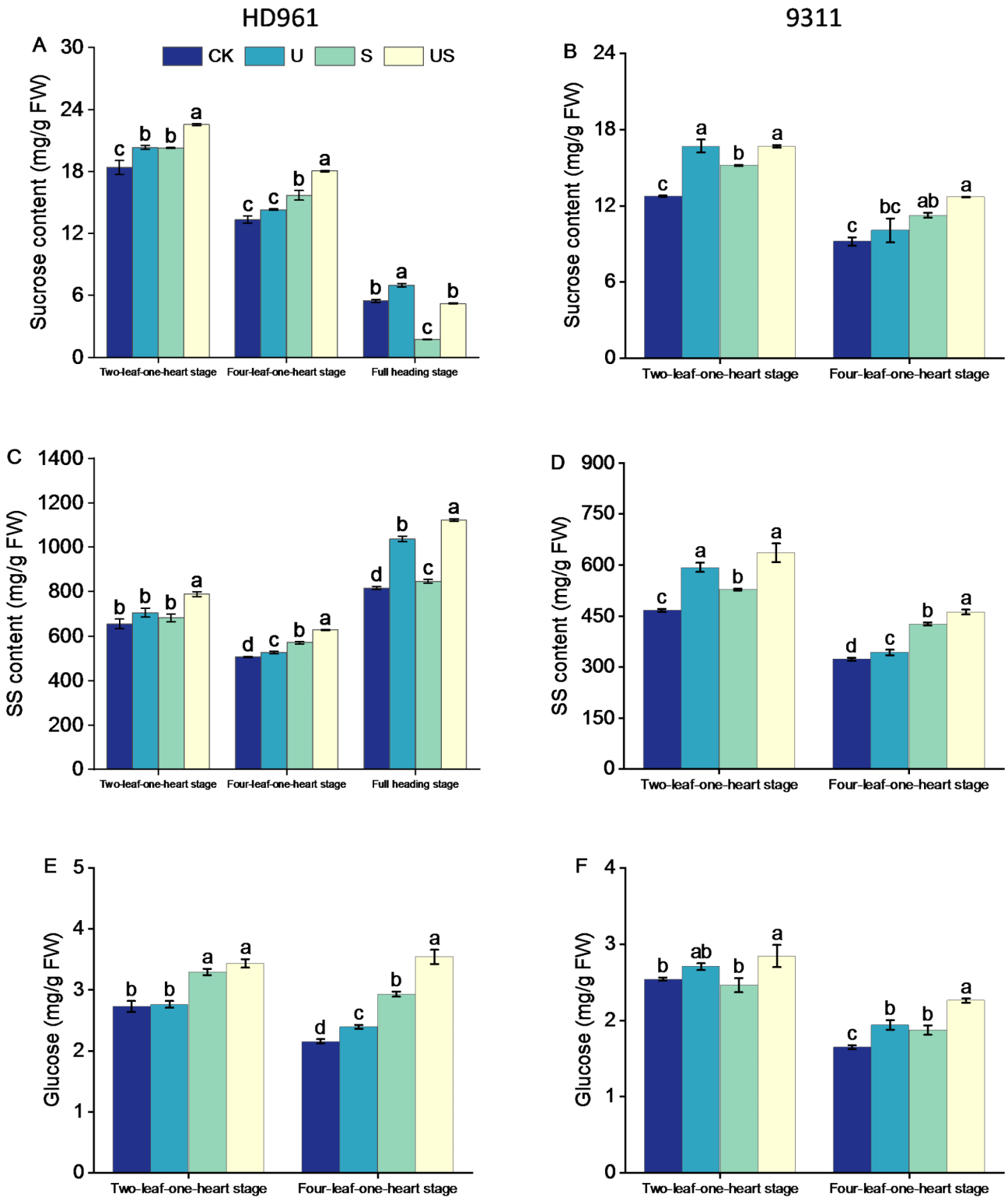

Fig. 8 Effects of exogenous S3307 on carbohydrate content in rice under salt stress. (**A**) and (**B**) show sucrose content in the leaves of HD961 and 9311, respectively; (**C**) and (**D**) show soluble sugar content in the leaves of HD961 and 9311, respectively; (**E**) and (**F**) show glucose content in the leaves of HD961 and 9311, respectively. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

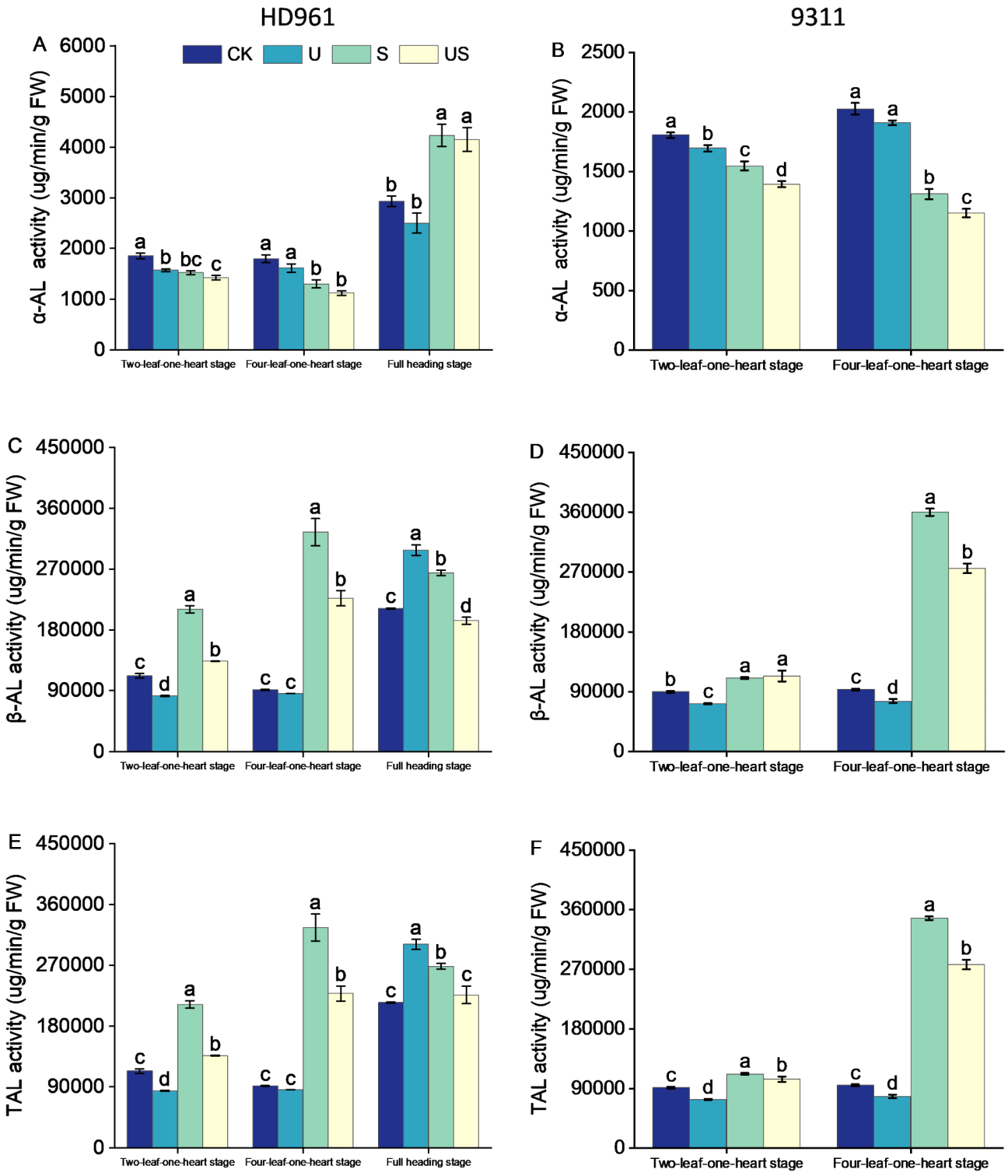

Fig. 9 Effects of exogenous S3307 on starch metabolism in rice under salt stress. (**A**) and (**B**) show α-AL activity in the leaves of HD961 and 9311, respectively; (**C**) and (**D**) show β-AL activity in the leaves of HD961 and 9311, respectively; (**E**) and (**F**) show TAL activity in the leaves of HD961 and 9311, respectively. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

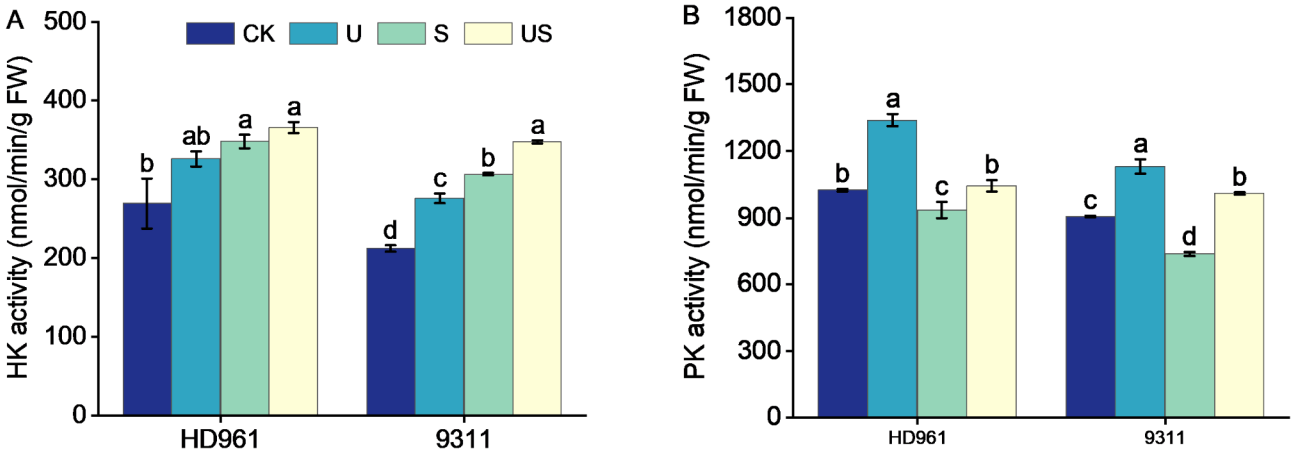

Fig. 10 Effects of exogenous S3307 on the activities of carbon metabolism-related enzymes in rice under salt stress. (**A**) HK activity; (**B**) PK activity. (**A**)-(**B**) represent data from the two-leaf-one-heart stage. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters above the bars indicate significant differences ac-cording to Duncan's test ($p < 0.05$)

stress, resulting in decreased ROS levels and protection of chloroplast membrane structure [31].

Maintaining photosynthetic capacity is a critical approach to enhance plant salt tolerance [32]. Under salt stress, the P_n in plants is influenced by both stomatal and non-stomatal limiting factors [33]. This study demonstrates that S treatment significantly decreases C_i, T_r, G_s, and AMC, while significantly increasing Ls. This may result from salt stress-induced damage to the chloroplast membrane system, causing chloroplast degradation and a subsequent decline in P_n [34]., and it indicating that the reduction in P_n is primarily caused by stomatal limiting factors [35]. Under salt stress conditions, plants often accumulate a large amount of SS and starch, leading to feedback inhibition of photosynthesis and severely impairing its normal function, which hinders plant growth [36]. Zhang et al. [37] report that exogenous S3307 treatment increases the photosynthetic rate in soybean under water deficits, delays leaf senescence, protects chloroplast structures, and maintains photosynthetic activity. This is similar to the findings of this study. Additionally, compared to the S treatment, the US treatment also increases the content of organic acids in rice leaves, which play a crucial role in the carbon fixation process of photosynthesis. The results demonstrate that S3307 enhances chlorophyll content and gas exchange capacity in rice under salt stress, promoting efficient photosynthesis. This enables rice to fix more CO_2 and synthesize more photosynthetic products, thereby improving stress tolerance and increasing yield.

Previous studies have found that salt stress-induced damage in rice is associated with the excessive accumulation of ROS and MDA [38]. MDA is a product of membrane lipid peroxidation, and the change in its content is the key indicator of oxidative damage to cell membranes [39]. Plants possess an antioxidant defense system that eliminates excess ROS generated under salt stress, including antioxidant enzymes such as SOD, POD, CAT, and APX [40]. The results of this study indicate that salt stress reduces SOD activity at the two-leaf-one-heart stage in rice while enhancing the activities of antioxidant enzymes such as POD, CAT, and APX, and promoting the accumulation of MDA. The increase in MDA content suggests that salt stress induces oxidative stress in rice cells, leading to severe damage to the cell membrane system. Earlier studies have reported that salt stress can promote increased antioxidant enzyme activity in plants [41, 42]. The reduction in SOD activity, the first line of defense in the antioxidant system, implies an insufficient antioxidant capacity in rice during the initial stages of salt stress. However, the increase in POD, CAT, and APX activities reflects a compensatory mechanism. We hypothesize that rice may reduce SOD activity to enhance the activities of POD, CAT, and APX, thereby alleviating oxidative damage caused by salt stress. The results indicate that, compared to S treatment, foliar application of S3307 increases the activities of SOD, POD, CAT, and APX in rice leaves under salt stress while reducing MDA accumulation, similar to the findings from previous studies on mung bean resistance to low-temperature stress [13]. Notably, the CAT activity in 9311 is significantly higher than in HD961. This may be due to the fact that 9311 is not as salt-tolerant as HD961, resulting in higher ROS production and consequently greater CAT activity. These findings suggest that S3307 enhances the antioxidant capacity of rice under salt stress, reduces MDA accumulation, ensures the normal progression of other physiological activities, and mitigates salt stress-induced damage.

Carbohydrates play a vital role in plants, serving as the primary energy substrates for sustaining life activities [43]. Among the components of carbohydrates,

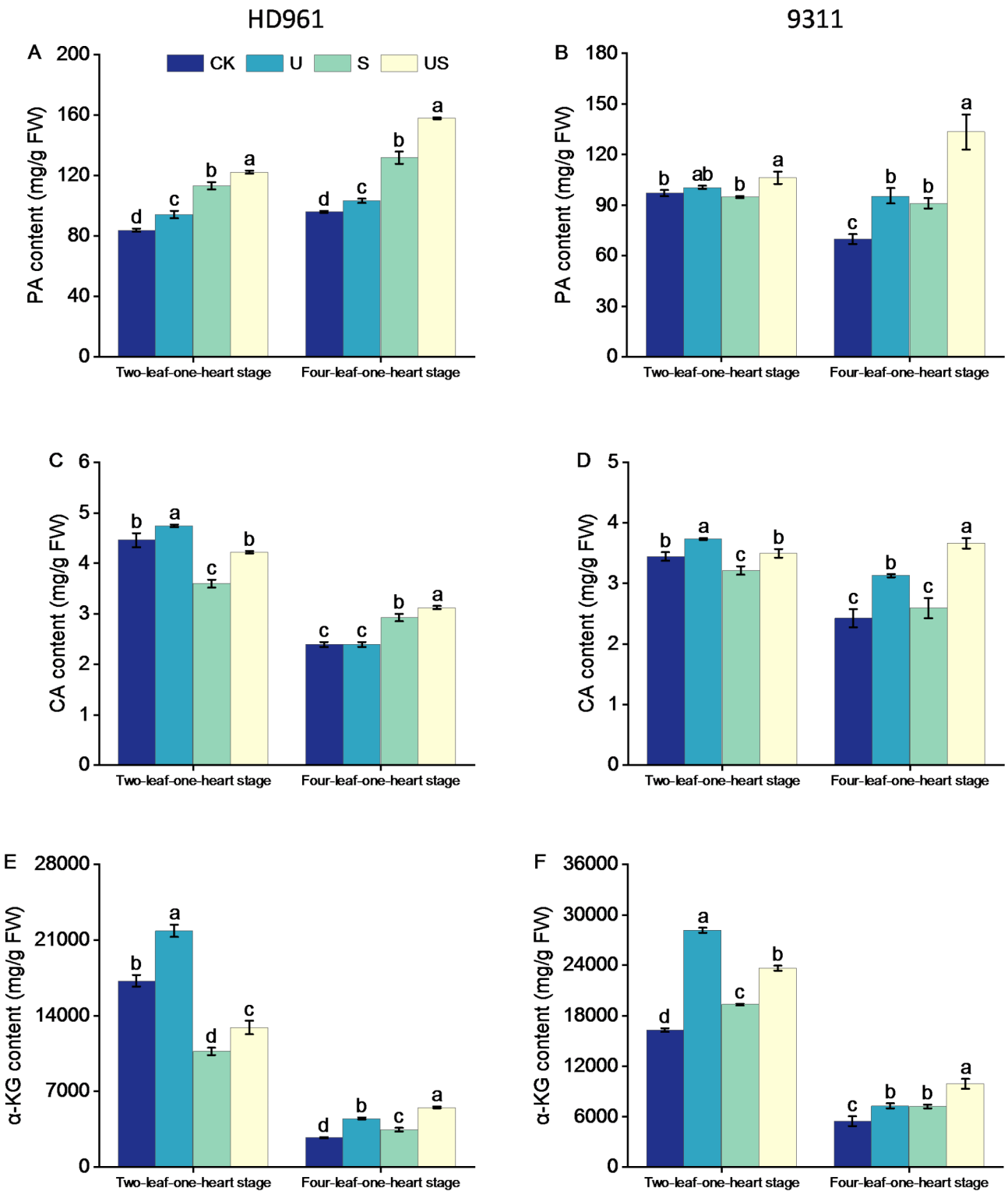

Fig. 11 Effects of exogenous S3307 on organic acid metabolism in rice under salt stress. (**A**) and (**B**) show PA content in the leaves of HD961 and 9311, respectively; (**C**) and (**D**) show CA content in the leaves of HD961 and 9311, respectively; (**E**) and (**F**) show α-KG content in the leaves of HD961 and 9311, respectively. Data are presented as the mean ± standard error (SE) of three replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

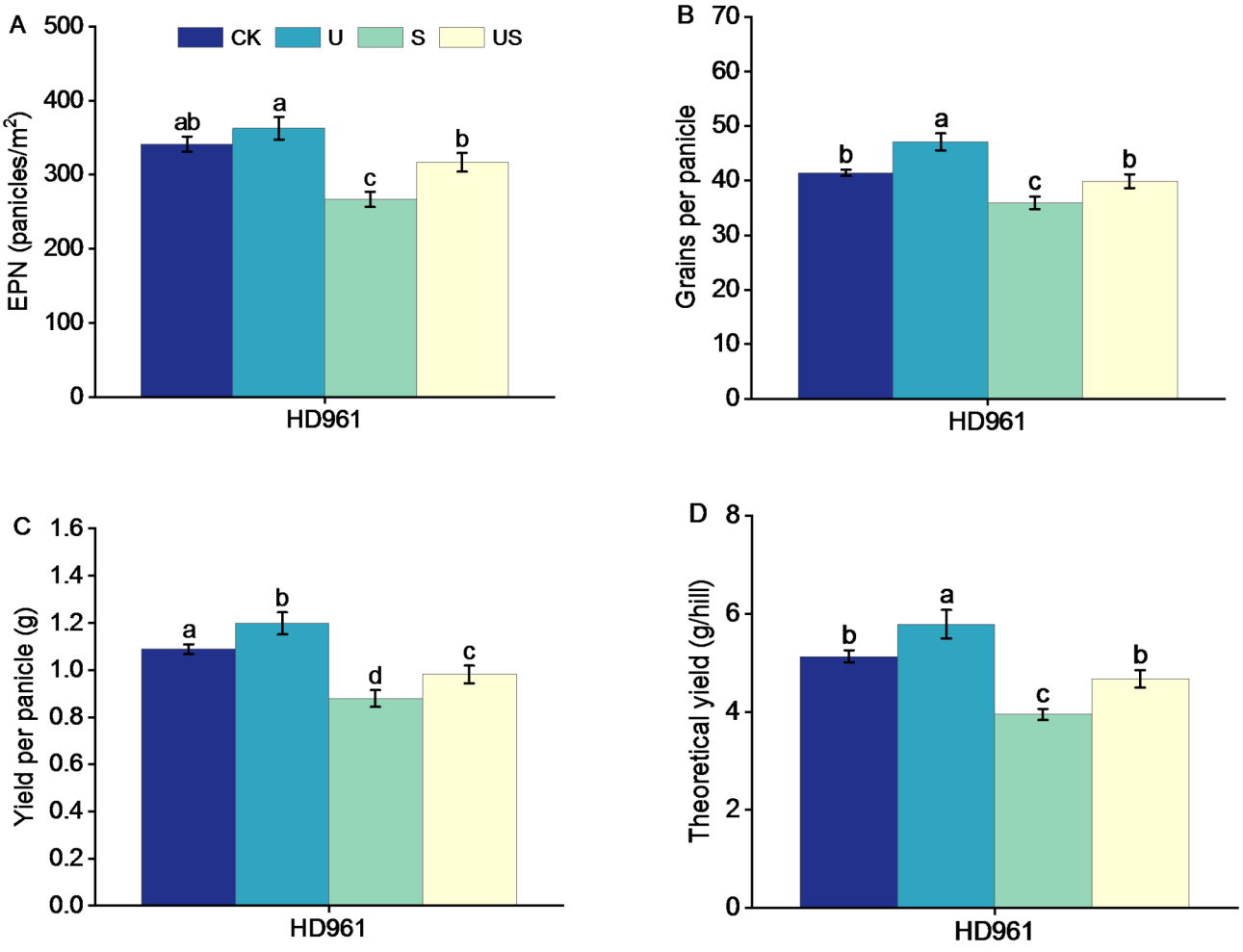

Fig. 12 Effects of exogenous S3307 on the yield of rice variety HD961 under salt stress. (**A**) Effective Panicle Number (EPN); (**B**) Grains per Panicle; (**C**) Yield per Panicle; (**D**) Theoretical Yield. Data are presented as the mean ± standard error (SE) of 16 replicates. Different lowercase letters in the data columns indicate significant differences according to Duncan's test ($p < 0.05$)

sucrose, SS, glucose, and starch are indispensable and critical components [44]. Under abiotic stress conditions, starch and sucrose metabolism can help plants cope with adverse conditions by providing energy and carbon storage. Simultaneously, soluble carbohydrates act as "bridge metabolites" between source and sink and can buffer the damage caused by stress to plants through osmoregulation. In this study, we found that the accumulation levels of carbohydrates such as sucrose, SS, and glucose in rice leaves increase under salt stress compared to CK. This suggests that, in response to salt stress, the normal growth of rice is restricted, stimulating protective mechanisms in the leaves that lead to the storage of substantial carbohydrates to alleviate the damage caused by salt stress. The full heading stage is a critical period in the reproductive growth phase of rice, during which plants transport large amounts of carbohydrates (such as sucrose) to reproductive organs, resulting in a decline in sucrose content. Previous studies also indicate that SS content is associated with plant stress resistance, and its increase can effectively alleviate osmotic stress and enhance resistance [45]. However, at the full heading stage, the sucrose content in the leaves of rice HD961 decreases, while the SS content increases. This indicates that carbohydrate allocation and utilization in rice vary significantly across different growth stages, enabling adaptation to the energy requirements and physiological regulation under salt stress conditions. Under salt stress, the decline in α-AL activity in rice leaves may result from reduced endogenous gibberellin levels [46] In contrast, the increase in β-AL and TAL activities facilitates the breakdown of starch into SS and sucrose, enhancing intracellular osmotic adjustment and aiding rice adaptation to saline conditions [47]. Additionally, compared to salt stress, the US treatment increases the SP and SS content in rice leaves under salt stress, maintaining intracellular osmotic pressure balance and mitigating salt stress-induced damage. These findings align with the

results of Xu et al. [30] on the application of S3307 in maize under saline-alkali conditions. Additionally, exogenous S3307 application increases the contents of sucrose, SS, and glucose in rice leaves under salt stress. This may be attributed to the enhanced photosynthesis induced by S3307 under salt stress, which supplied ample substrates for carbohydrate metabolism, thereby facilitating carbohydrate accumulation. However, it is noteworthy that, compared to the S treatment, the US treatment reduces the activities of α-AL, β-AL, and TAL. This indicates that the starch decomposition and conversion process is suppressed, resulting in the accumulation of more photosynthetic products as soluble sugars (SS) rather than being largely converted into starch. This may represent an adaptive regulation in rice under US treatment to facilitate the rapid utilization of carbohydrates for energy, addressing the physiological pressure imposed by salt stress. Studies have demonstrated that S3307 enhances the synthesis and metabolism of sucrose and starch under salt stress, promoting the synthesis, loading, and export of assimilates at the "source" end. This increases the accumulation of assimilates in source organs, providing a material foundation for "sink" formation, thereby improving rice salt tolerance and yield [48].

The EMP pathway and TCA cycle play extremely important roles in maintaining various physiological functions and delivering energy to different organelles [49]. PA serves as a key link between the EMP pathway and the TCA cycle. Organic acids, which form the material basis of the TCA cycle and EMP pathway, are essential for plant development [50]. Under salt stress, PK activity is reduced in both HD961 and 9311; however, PA content decreases in the leaves of HD961 but increases in those of 9311. This discrepancy may be attributed to the limited metabolic regulatory capacity of salt-sensitive rice varieties [51], leading to the accumulation of PA. Additionally, we observe that salt stress enhances HK activity in rice leaves and increases glucose content. This may be attributed to the plant's adjustment of metabolic pathways under salt stress. This process diverts more carbon sources toward the synthesis of other stress-related metabolites such as proline and SS, leading to glucose accumulation. The results demonstrate that exogenous application of S3307 under salt stress increases the activity of HK and PK, ensuring the efficient operation of the EMP pathway. This generates substantial PA, which enters the TCA cycle to produce abundant energy, increases the proportion of energy sources in sugar metabolism. Simultaneously, the increase in glucose content provided abundant substrate for the catalytic reaction of HK. The enhanced activity of HK, in turn, ensured the efficient entry of glucose into the EMP pathway for further breakdown and utilization. This synergistic interaction accelerates the EMP pathway, leading to increased production of PA. The elevated PA levels enhance the operation of the TCA cycle, promoting the accumulation of organic acids (α-KG and CA), which alleviates osmotic stress and compensates for charge imbalance under salt stress. These findings align with the results reported by Wang et al. [23]. These results indicate that, under salt stress, S3307 effectively enhances the EMP pathway and TCA cycle in rice. Through this process, rice is able to generate more energy (ATP) and intermediate metabolites, providing sufficient energy and carbon skeletons for plant growth and development, thereby improving rice yield and salt tolerance.

This study revealed that rice yield (EPN, grains per panicle, and yield per panicle) was significantly inhibited under salt stress. A possible explanation is that salt stress suppressed the growth and development of rice panicles, leading to an increased incidence of panicle sterility and a higher proportion of sterile florets, ultimately reducing yield [52]. However, the application of S3307 significantly increased rice yield under salt stress. This may be attributed to the improved photosynthetic efficiency, reduced oxidative damage in cells, and enhanced physiological activity of carbon metabolism induced by S3307 treatment. These optimized and improved physiological processes were ultimately reflected in yield-related traits, enhancing rice yield under salt stress.

Conclusions

Salt stress inhibits shoot and root growth in rice seedlings, increases MDA accumulation in leaves, and suppresses photosynthetic activity. Concurrently, it activates protective mechanisms in rice, enhancing antioxidant enzyme activity and promoting carbohydrate accumulation. Foliar spraying of S3307 improves the morphological traits of rice seedlings under salt stress and stimulates root development. Additionally, S3307 reduces MDA accumulation under salt stress, enhances photosynthetic efficiency and antioxidant enzyme activity, and promotes the accumulation of soluble proteins, soluble sugars, and antioxidants in rice. Ultimately, it increases the activity of key enzymes in the EMP pathway, leading to the accumulation of PA, which facilitates the efficient operation of the EMP pathway and the TCA cycle, providing carbon skeletons and energy for rice growth, thereby enhancing salt tolerance (Fig. 13). This study is based on pot experiments and therefore has certain limitations. The findings of this study further elucidate the potential mechanisms by which exogenous S3307 alleviates salt stress in rice, laying the foundation for improving rice yield in saline-alkali soils.

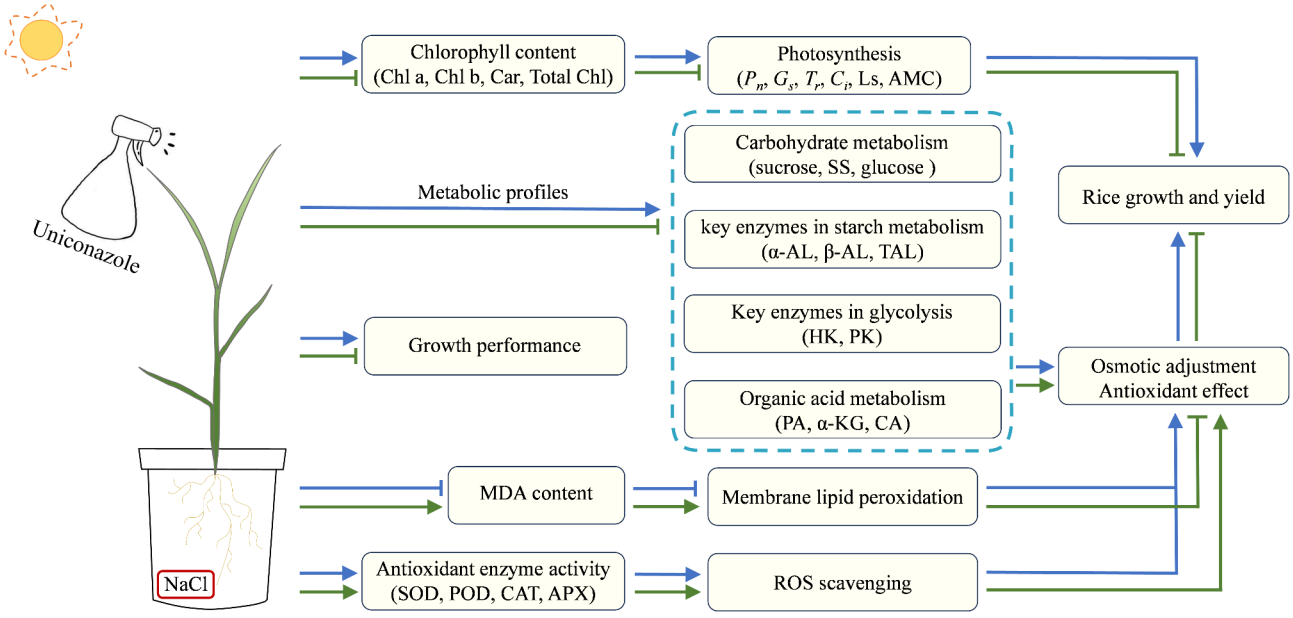

Fig. 13 Model of S3307-induced responses in rice under salt stress. Blue rep-resents S3307 + salt stress; green represents salt stress. Arrows indicate promotion, while blunt-end lines indicate inhibition

Materials and methods

Test materials

This experiment was conducted in 2024 in a greenhouse at Guangdong Ocean University in Zhanjiang, Guangdong Province, using pot culture. The indoor humidity was maintained at 80 ± 5%, with an average daytime temperature of 33 ± 2 °C and an average nighttime temperature of 30 ± 2 °C. The tested materials, HD961 and 9311, were provided by the Germplasm Resource Bank of the Coastal Agriculture College at Guangdong Ocean University. The regulator S3307 was supplied by the South China Center of the Seawater Rice Innovation Team at Guangdong Ocean University (Zhanjiang, China). The experimental soil was filled into perforated pots with an upper diameter of 20.5 cm, a lower diameter of 15 cm, and a height of 17 cm, with each pot containing 3.2 kg of soil. The experimental soil was a mixture of latosol and sand in a volume ratio of 3:1.

Experimental design

Seedling test design

Healthy and plump rice seeds were selected and sterilized with 3% H_2O_2 for 10 min, followed by rinsing with distilled water 5 times. The rinsed seeds were placed in a constant-temperature incubator at 30 °C and soaked in distilled water for 24 h in the dark for germination. One day before sowing, each pot was thoroughly irrigated with 1 L of nutrient solution (urea: 0.146 g·L^{-1}, potassium chloride: 0.125 g·L^{-1}, diammonium phosphate: 0.200 g·L^{-1}). Uniformly germinated rice seeds were then sown in the pots, with 69 seeds per pot spaced 1 cm apart.

Four treatments were set up for each of the two rice, with three replicates per treatment. Each replicate consisted of 15 pots. At the one-leaf-one-heart stage (A fully expanded leaf and a heart leaf), seedlings with uniform growth were selected for treatment. Around 18:00, a 10 mg·L^{-1} S3307 solution or distilled water was sprayed onto both the adaxial and abaxial surfaces of the rice leaves until the leaves were thoroughly moistened but without dripping. After 24 h, 0.6% (102.56 mmol·L^{-1}) NaCl solution or water was applied. Subsequently, every two days, a quantified amount of 0.6% NaCl solution or distilled water was irrigated at three times the water-holding capacity, allowing approximately 2/3 of the solution to drain out so as to flush out the accumulated salt and maintain a constant salt concentration. The experimental treatments were designated as follows: (1) CK: 0% NaCl + spraying with water; (2) U: 0% NaCl + spraying with 10 mg·L^{-1} S3307; (3) S: 0.6% NaCl + spraying with water; (4) US: 0.6% NaCl + spraying with 10 mg·L^{-1} S3307. Samples were collected at the two-leaf-one-heart and four-leaf-one-heart stages (Four fully expanded leaf and a heart leaf) of rice growth for the determination of physiological indicators (stored in a -40 °C freezer for testing).

Yield test design

Two days before transplanting the rice seedlings (HD961), 4 L of clean water or saline water was added to corresponding treatment plastic bucket (cultivation substrate: air-dried latosol, 8.5 kg per bucket, diameter 30 cm, height 22 cm). Once the water level stabilized, a marking line (2 cm) was drawn, and water was

replenished periodically to maintain the water layer. Base fertilizer (urea: 0.499 g, diammonium phosphate: 0.445 g, potassium chloride: 0.712 g) was applied 1 day before transplanting and thoroughly mixed. When the rice seedlings reached the four-leaf-one-heart stage (Same experimental design as that in the seedling stage), uniformly grown seedlings were selected for transplanting, with three hills per bucket and two seedlings per hill. Seven days after transplanting, tillering fertilizer (urea: 0.552 g) was applied, and panicle fertilizer (urea: 0.552 g, potassium chloride: 0.182 g) was applied when the penultimate leaf was 11–15 cm unfolded. A completely randomized design was adopted, with four replicates per treatment and each replicate consisting of 5 buckets of rice. At the full heading stage, flag leaves were collected, flash-frozen in liquid nitrogen, and stored at -40 °C for the determination of physiological indicators.

Measurement items and methods

Determination of morphological indexes

At the two-leaf-one-heart stage of rice growth, 20 seedlings with uniform growth were selected. The plant height was measured using a ruler, the stem diameter was measured using a vernier caliper, and the leaf area was measured using a leaf area meter (YX-1241). The aboveground and underground parts of the rice seedlings were separated, and after blotting excess moisture with filter paper, the fresh weight was measured using an electronic balance. The seedlings were then placed in an oven at 105 °C for 30 min, and the dry weight was measured after drying to a constant weight at 75 °C.

Determination of chlorophyll content

The chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car), and total chlorophyll (Total Chl) content in rice seedlings were determined using the method proposed by Ko-lomeichuk [53]. Approximately 0.1 g of freshly chopped leaf tissue was weighed and placed into a test tube containing 10 mL of 95% ethanol and soaked in the dark for 24 h. The concentrations of Chl a, Chl b, and Car were measured using spectrophotometry at wavelengths of 665 nm, 649 nm, and 470 nm, respectively.

Chl a ($mg{\cdot}g^{-1}$ FW) = 13.95 A_{665} – 6.88 A_{649},

Chl b ($mg{\cdot}g^{-1}$ FW) = 24.96 A_{649} – 7.32 A_{665},

Car ($mg{\cdot}g^{-1}$ FW) = (1000 A_{470} – 2.05 Chl a – 114.8 Chl b)/ 245,

Total Chl ($mg{\cdot}g^{-1}$ FW) = Chl a + Chl b.

Determination of photosynthetic parameters

Photosynthetic parameters were measured at the five-leaf stage of rice (approximately 25 days after sowing). On a cloudless sunny morning between 8:30 and 11:00, a Li-6800 portable photosynthesis system (Li-Cor, Inc., Lincoln, USA) was used to measure the middle section of the youngest fully expanded leaf of rice seedlings. Five plants per treatment were measured, and the net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) were determined, respectively. In the photo-synthesis instrument, the concentration of CO_2 in the leaf chamber was 400 μmol mol^{-1}, the light intensity was 1000 μmol m^{-2} s^{-1}, the leaf temperature was 32 ± 1 ℃, and the relative humidity was between 60 – 65%. The apparent mesophyll conductance (AMC) and stomatal limitation value (Ls) were calculated using the following equations [54]:

$AMC = P_n/C_i$.

Ls = (Ca – C_i)/Ca.

Ca: Atmospheric CO_2 Concentrations, C_i: Intercellular CO_2 Concentration.

Determination of membrane damage substances

The MDA content was determined according to the method provided by Ahmad et al. [55], measuring the absorbance of the supernatant at 450 nm, 532 nm, and 600 nm.

Determination of osmoregulatory substance content

The soluble protein (SP) content was determined using the Thomas Brilliant Blue G-250 method described by Luo Q et al. [56], measuring the absorbance of the supernatant at 595 nm.

Determination of antioxidant enzyme activity

Frozen leaf samples (0.5 g) were homogenized in 10 mL of phosphate buffer solution (PBS, pH 7.8) and then centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was collected for the determination of SOD, CAT, POD, and APX activities. SOD activity was determined according to the method described by Lu et al. [57]. POD activity was measured following the method of Kenawy et al. [58], recording the absorbance change of the supernatant at 470 nm every 30 s for a total of 4 readings. CAT activity was determined based on the method of Basilio-Apolinar et al. [59], measuring the absorbance of the supernatant at 240 nm every 30 s for 4 readings. APX activity was assayed ac-cording to the method of Wei et al. [60], monitoring the absorbance change at 600 nm every 30 s at 20 °C for a total of 4 readings.

Determination of physiological indexes related to carbon metabolism

The contents of sucrose and soluble sugars (SS) were determined according to the method proposed by Du et al. [61]. The activities of α-amylase (α-AL), β-amylase (β-AL), total amylase (TAL), hexokinase (HK), and pyruvate kinase (PK), as well as the contents of glucose, α-ketoglutarate (α-KG), citric acid (CA), and pyruvic acid (PA), were measured using biochemical kits from Suzhou Geruisi Biotechnology Co., Ltd., following the provided instructions.

Determination of Yield - related indicators

When HD961 was mature, 20 representative plants without disease and insect pests were randomly selected for each treatment, and the effective panicle number (EPN), grains per panicle, yield per panicle, and theoretical yield of HD961 were calculated.

Theoretical yield = panicles per hill × grains per panicle × 1000-grain weight /1000.

Statistical analysis

Data were processed using Microsoft Excel 2016. One-way analysis of variance (ANOVA) was performed using SPSS 26.0 software (SPSS, Inc., Chicago, IL, USA). Multiple comparisons were conducted using Duncan's test. The least significant difference (LSD) test at the $P < 0.05$ level (LSD 0.05) was used to compare means. Graphs were generated using Origin 2021 software. All data in this experiment are presented as the mean ± standard error (SE) of multiple replicates.

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Author contributions
X.D. was responsible for the methodology, data curation, investigation, formal analysis, writing the original draft. N.F. contributed to the methodology and conceptualization, acquisition of funding and administration of the project. D.Z. aided in the conceptualization and acquisition of funding. Y.L. contributed to the investigation. H.Z. aided in the investigation. J.L. and X.Y. contributed to the investigation and formal analysis. J.H. helped with the formal analysis. W.M. aided in the formal analysis. All authors contributed to the manuscript preparation, writing and revision and approved the submitted version.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable. All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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