Exogenous Hemin enhances the antioxidant defense system of rice by regulating the AsA-GSH cycle under NaCl stress

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Abiotic stress caused by soil salinization remains a major global challenge that threatens and severely impacts worldwide crop growth causing yield reduction. Rice is an important economic crop affected by salt stress. In this study, we aimed to investigate the damage of salt stress on the leaf physiology of two rice varieties (HuanghuaZhan, HHZ and XiangliangYou 900, XLY900) and the regulatory mechanism of Hemin to maintain seedling growth under the imposed stress. Therefore, at the three leave and one heart, leaves were foliar sprayed with 5 μmol·L^{-1} Hemin or 25 μmol·L^{-1} ZnPP (Zinc protoporphyrin IX) followed by 50 mmol·L^{-1} NaCl stress. The findings revealed that salt stress increased antioxidant enzyme activity and decreased the content of nonenzymatic antioxidants such as ascorbate (AsA) and glutathione (GSH). Furthermore, the content of osmoregulatory substances like soluble proteins and proline was raised. Moreover, salt stress increased reactive oxygen species (ROS) content in leaves of two varieties of rice. However, spraying Hemin increased the activities of antioxidants such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and accelerated AsA-GSH cycling to remove excess ROS. In summary, Hemin reduced the effect of salt stress on the physiological characteristics of rice leaves due to improved antioxidant defense mechanisms that impeded lipid peroxidation. Thus, Hemin was demonstrated to lessen the damage caused by salt stress.

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Abstract

Abiotic stress caused by soil salinization remains a major global challenge that threatens and severely impacts worldwide crop growth causing yield reduction. Rice is an important economic crop affected by salt stress. In this study, we aimed to investigate the damage of salt stress on the leaf physiology of two rice varieties (HuanghuaZhan, HHZ and XiangliangYou 900, XLY900) and the regulatory mechanism of Hemin to maintain seedling growth under the imposed stress. Therefore, at the three leave and one heart, leaves were foliar sprayed with 5 μmol·L^{-1} Hemin or 25 μmol·L^{-1} ZnPP (Zinc protoporphyrin IX) followed by 50 mmol·L^{-1} NaCl stress. The findings revealed that salt stress increased antioxidant enzyme activity and decreased the content of nonenzymatic antioxidants such as ascorbate (AsA) and glutathione (GSH). Furthermore, the content of osmoregulatory substances like soluble proteins and proline was raised. Moreover, salt stress increased reactive oxygen species (ROS) content in leaves of two varieties of rice. However, spraying Hemin increased the activities of antioxidants such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and accelerated AsA-GSH cycling to remove excess ROS. In summary, Hemin reduced the effect of salt stress on the physiological characteristics of rice leaves due to improved antioxidant defense mechanisms that impeded lipid peroxidation. Thus, Hemin was demonstrated to lessen the damage caused by salt stress.

Keywords: Hemin, Rice, AsA-GSH cycle, Enzymatic defense system

Introduction

In the background of global warming, soil salinization has accelerated due to various factors such as seawater back-up, over-exploitation of groundwater and the over development of arable land (Alkharabsheh et al., 2021). Saline land accounts for about one-fifth of the cultivated land and one-third of the irrigated farmland on the planet, and the area is increasing at an even faster rate (Mukhopadhyay et al., 2021). Salinity stress is one of the most widespread and severe abiotic stresses globally. It has destructive effects on plant growth and physiological and biochemical processes and causes a decrease in grain production. According to current data, the yield loss caused by salt stress accounts for about 20% of global yield (Ding et al., 2021).

With salt stress increasing soil osmotic pressure, plant roots fail to absorb water and nutrients, which causes delayed growth and development or even death (Liu et al., 2022). In addition, salt stress induces excessive production of reactive oxygen species (ROS) in plant cells. ROS is weakly stable and easily causes oxidative stress to cells. The excessive ROS enhances cell membrane lipid peroxidation and disrupts membrane system stability, which results in the expansion of cell membrane permeability and extravasation of intracellular materials (Seleiman et al., 2020; Hasanuzzaman et al., 2020). Researches showed that ROS could break down proteins, damage DNA structure and cause lipid peroxidation. (Chandrakar et al., 2017; Lin et al., 2020). This disturbed the normal growth and physiological metabolic activities of plants. To avoid ROS accumulation, plants use antioxidant enzymes and non-enzymatic antioxidants to scavenge excess ROS (Alisofi et al., 2020). Among them, antioxidant enzymes include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Non-enzymatic antioxidants include ascorbic acid and glutathione, which act as co-factors for different enzymes and participate in various metabolic processes (Hasanuzzaman et al., 2020). In addition, plants accumulate osmoregulatory substances to maintain the balance of inside and outside cell osmosis. There are two categories of osmoregulatory substances: inorganic ions (Na^+); and organic substances, including proline and soluble proteins (Athar et al., 2022). Under salt stress, plants balance the osmotic pressure between the plant and the external environment by conducting selective uptake of ions and promoting the accumulation of phase-soluble solutes.

Rice, a gramineous crop, has a long history of cultivation and consumption in China. The consumer demand for rice in China is the most in the world, and more than half of the population eats rice as a major food (Huang et al., 2022; Zuo et al., 2022). However, salt stress has become one of the major abiotic stresses which limits rice production. Many studies showed that the seedling stage was an essential stage of plant development and was closely related to the later development of tillers and spikelets. However, this stage is susceptible to the impact of salt stress (Zeng et al., 2001). Therefore, way to improve the salt tolerance of rice seedlings has become an urgent problem at present.

Plant growth regulators are a group of synthetic compounds with phytohormonal activity that improve the tolerance to abiotic stresses by affecting the expression of endogenous hormones in crops. Hemin is a small molecule with a porphyrin structure, consisting of nitrogen atoms on four pyrrole rings in a porphyrin ligated to a ferrous ion. In recent years, Hemin has been used more frequently in different crops for its natural, non-polluting, low cost, and high safety features. Hemin acts as a substrate and promoter of heme oxygenase 1 (HO-1), an initiator and rate-limiting enzyme for Hemin degradation, and has a specific inhibitor, zinc protoporphyrin (ZnPP). Hemin triggered salt acclimation in wheat by increasing HO-1 expression, while ZnPP, an inhibitor, was shown to decreased the salt tolerance of wheat (Xie et al., 2011). Under salt stress, Hemin increased proline and soluble protein content, enhanced antioxidant enzyme activities such as SOD, CAT, and APX, and alleviated oxidative damage in *Cassia obtusifolia* L (sickle senna) (Zhang et al., 2012). In addition, under zinc (Zn), lead (Pb), and chromium (Cr) metal stress, Hemin activated the activities of various antioxidant enzymes (SOD, GR, and APX) in rice seedlings, improved the content of AsA and GSH, and reduced heavy metal accumulation.

At present, there are fewer studies on the mitigation of salt stress by Hemin on rice seedings, and based on the mitigation effect of Hemin on stresses such as heavy metal stress of *Medicago sativa* L (alfalfa) (Fu et al., 2011), low-temperature stress of *Conyza blini* (bear gall grass) (Zheng et al., 2021), and salt stress in *Brassica juncea* L (mustard) (Verma et al., 2015). Furthermore, spraying plant growth regulators can improve the resistance of rice seedlings during the critical period before transplanting, which is essential for the subsequent transplanting of rice seedlings on saline land. Hence, in this study, we used two rice varieties, Huang Huazhan and Xiang Liangyou 900, to research the impacts of foliar spraying with Hemin at the three-leaf-one-heart stage on the growth and ROS metabolism (antioxidant enzymes and non-enzymatic antioxidants) of rice seedlings under salt stress, which aimed to reveal the mechanism of hemin in enhancing the salt tolerance of rice, and to provide theoretical basis and technical guidance for the cultivation of saline rice.

Materials and methods

Plant materials

The experiment was carried out in 2022 at Binhai Agricultural College of Guangdong Ocean University. To ensure the results are more universal, we selected the conventional rice variety Huanghuazhan (HHZ) and hybrid rice variety Xiangliangyou900 (XLY900). Hemin was provided from Shanghai Changdeduo Agricultural Technology Co., Ltd.

Experiment design

Seeds were selected for uniformity of size and color, sterilized with 3% H_2O_2 for about 15 min, and then rinsed 3-5 times with distilled water. These seeds were soaked and germinated for 24 hours under dark conditions at 30℃. Sixty-five seeds were sown into pots containing 3 kg of test soil with 1:3 sand to latosol content. The plastic pot sizes were 19 cm for the upper diameter, 14 cm for the lower diameter, and 17 cm for the height, without holes of the pots. Regular water irrigation was performed until the three leaf and one heart stage (about 18 days after planting). Rice leaves were foliar sprayed with 5 μmol·L^{-1} Hemin and 25 μmol·L^{-1} ZnPP alone or in combination, and plants were exposed to 25 mmol·L^{-1} NaCl stress twice at two 24 h intervals which resulted in the salt concentration in the soil reaching 50 mmol·L^{-1} at 48 h after spraying. In subsequent experiments, concentrations were maintained by measuring soil conductivity (EC=5.0±0.5 dS·m^{-1}). Each variety had five treatments: (1) normal water (CK); (2) 50 mmol·L^{-1}

NaCl (S); (3) Hemin + 50 mmol·L^{-1} NaCl (SH); (4) ZnPP + 50 mmol·L^{-1} NaCl (SZ); and (5) Hemin + ZnPP + 50 mmol·L^{-1} NaCl (SZH). Each treatment had 25 pots. The plant samples were harvested at 3, 5, 7, and 9 d after NaCl stress application for morphological parameters and physiological were collected and frozen at -80 ℃ for biochemical analysis.

Morphological measurements

Plant height was measured with a ruler, stem diameter was measured with vernier, shoot fresh and dry weight were measured by a caliper electronic analytical balance. The shoots were dried for 30 min at 105 ℃ and 72 h at 85 ℃.

Measurement of electrolyte leakage (EL), malonaldehyde (MDA) and Hydrogen peroxide (H_2O_2) content

Electrolyte leakage (EL) was determined as described by Yu et al. (2021). The measurement of malonaldehyde (MDA) content was carried out according to the method outlined by Ahmad et al. (2016). The frozen leaf sample (0.5g) was extracted in 10 mL phosphate buffer (0.05 mmol·L^{-1} PBS, pH 7.8) and centrifuged at 6,000 rpm for 20 min. One milliliter of the supernatant was added to 2 mL of 0.6% TBA, then boiled at 100 ℃ for 15 min. The mixture was cooled quickly with cold water and centrifuged at 4,000 rpm for 20 min. The absorption value was determined at 450 nm, 532 nm and 600 nm. The H_2O_2 content was using the method described by Rasheed et al. (2022). More specifically, 0.5g of the frozen sample was ground into homogenate in 5 mL of TCA and centrifuged at 19,000 rpm for 20 min. Five hundred microliters of supernatant was added to 0.5 mL PBS (10 mmol·L^{-1} Ph 7.0) and 1 mL KI (1 mol·L^{-1}), then the reaction mixture was incubated at 28 ℃ for 1 h in the dark. The absorbance values were recorded at 410 nm.

Histochemical Detection of Hydrogen Peroxide and Superoxide Anion

The histochemical staining of hydrogen peroxide (H_2O_2) and superoxide radicle ($O_2^{\cdot-}$) was measured by Zhang et al. (2009) and Sudhakar et al. (2015). On the 3rd day of stress, the second leaf of CK, S, SH, SZ and SZH treatments of both varieties were taken in a solution containing nitrogen blue tetrazolium (NBT) and 3,3'-diaminobenzidine (DAB) for staining. The leaves were vacuumed and then kept at room temperature and dark conditions for 24 h until brown and blue spots appeared, respectively. Poured off the staining solution, then added 95% ethanol to extract the chlorophyll in a water bath at 80°C. Ethanol was added continuously until the leaves did not contain chlorophyll which became visible and photographed.

Measurement of the activities of SOD, POD, and CAT

The frozen leaf sample was extracted in 10 mL PBS (50 mmol·L^{-1} pH 7.8) at 4℃, centrifuged at 12,000 rpm and 4 ℃ for 20 min. The supernatant was used to determine SOD (EC 1.15.1.1), POD (EC 1.11.1.7), and CAT (EC 1.11.1.6) (Habib et al., 2021). SOD activity was carried out according to the method by Lu et al. (2022). The supernatant was mixed with 14.5 mmol·L^{-1} methionine solution, 3 mmol·L^{-1} EDTA-Na_2 solution, 60 μmol·L^{-1} riboflavin solution, and 2.25 mmol·L^{-1} NBT solution. One unit of SOD activity was defined as the amount of enzyme that would inhibiting 50% of NBT photoreduction. POD was determined following the method outlined by Kenawy et al. (2022). The supernatant was mixed with PBS (pH 6.0), guaiacol, and 30% H_2O_2. The absorbance was measured at 470 nm. CAT was determined by the decreased absorbance rate of H_2O_2 at 240 nm, outlined by Basilio-Apolinar et al. (2021).

Measurement of AsA-GSH cycle products and substrate content

The procedure outlined by Costa et al. (2002) and Yan et al. (2021) was followed to measure the contents of AsA and total AsA. More specifically, the frozen leaf sample was extracted in 5% TCA and centrifuged at 12,000 rpm and 4℃ for 15 min. The supernatant fluid was then moved to a calibration tube. The supernatant was used to determine the content of AsA and total AsA. For AsA, the supernatant was mixed with a reaction solution containing 5% TCA, ethanol, H_3PO_4-ethanol, BP-ethanol, and $FeCl_3$-ethanol. The reaction was carried out at 30°C for 90 min. The absorbance was assayed at 534 nm. For total AsA, it was similar to the AsA assay. Still, it was first reacted with dithiothreitol (DTT)-ethanol solution and Na_2HPO_4-NaOH solution for 10 min. Then, 20% TCA was added and mixed with the above reaction solution. The absorbance was assayed at 540 nm. Dehydroascorbate (DHA) content was calculated based on the difference between total AsA and reduced AsA.

The glutathione (GSH) and oxidized glutathione (GSSG) content was determined according to the method described by Kaya et al. (2023). Namely, 0.5 g frozen sample was ground into homogenate in 5 mL of 5% metaphosphoric acid and centrifuged at 20,000 x g for 20 min. The supernatant was used to determine the content of total glutathione (GSH+GSSG) and oxidized glutathione (GSSG). The supernatant was mixed homogeneously with the reaction solution, which contained 5% sulfosalicylic acid, 1.84 mol·L^{-1} triethanolamine, 25°C water bath for 1h. Then added 50 mmol·L^{-1} phosphate buffer, 10 mmol·L^{-1} NADPH, 12.5 mmol·L^{-1} DTNB, kept warm at 25°C for 10min, and added 50 U glutathione reductase (GR). The absorbance value of (GSH+GSSG) was measured at 412 nm. Besides adding the reaction solution, which contained 5% sulfosalicylic acid, 1.84 mol·L^{-1} triethanolamine and 2-vinylpyridine (2-VP), the subsequent steps were kept consistent with the determination of (GSH+GSSG) content. The GSSG absorbance value was measured at 412 nm. The GSH content was then calculated from the GSH+GSSG content reduced the GSSG content.

Measurement of the critical enzyme indexes of the AsA-GSH cycle

0.5 g of the frozen leaf sample was placed in a mortar, ground into a powder with 50 mmol·L^{-1} sodium phosphate buffer solution (pH7.8), and loaded into a centrifuge tube. The centrifuge tube was centrifuged at 12,000 x g for 20 min. The resulting solution was used to measure the levels of ascorbate peroxidase (APX) (EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2).

The APX activity was determined according to the method described by Sharifi et al. (2021). The assay mixture contained 0.1 mL of enzyme extract, 2.6 mL EDTA-Na_2 (0.1 mmol·L^{-1}), 0.15 mL AsA (5 mmol·L^{-1}) and 20 mmol·L^{-1} H_2O_2. The absorbance was assayed at 290 nm. (E=2.8 mM^{-1} cm^{-1}). MDHAR activity was measured using the method described by Hasanuzzaman et al. (2011). The reaction mixture consisted of 25 mmol·L^{-1} sodium phosphate buffer solution (pH7.8), 7.5 mmol·L^{-1} AsA, 2 mmol·L^{-1} NADPH, 50 U AsA oxidase (EC 1.10.3.3), and enzyme extract. The absorbance was assayed at 340 nm. (E=6.2 mM^{-1} cm^{-1}). DHAR activity was determined using the method described by Shan and Liu (2017). DHAR was assayed in a mixed solution containing 25 mmol·L^{-1} sodium phosphate buffer solution (pH7.8), 20 mmol·L^{-1} GSH, 10 mmol·L^{-1} DHA, and enzyme extract. The absorbance was assayed at 340 nm. (E=14 mM^{-1} cm^{-1}). GR activity was done according to Keles and Oncel (2002). GR (EC 1.6.4.2) was assayed in a mixed solution containing 25 mmol·L^{-1} sodium phosphate buffer solution (pH 7.8), 2 mmol·L^{-1} EDTA, 10 mmol·L^{-1} GSSG, 24 mmol·L^{-1} NADPH, and enzyme extract. The absorbance was assayed at 340 nm. (E=6.2 mM^{-1} cm^{-1}).

Measurement of soluble protein and proline content

Soluble protein content was determined according to the method described by Tian et al. (2022). The absorbance value was measured at 595 nm using Coomassie brilliant blue. Proline content was carried out according to the method by Liu et al. (2020). The frozen sample (0.5g) was ground in 5 mL of 3% sulfosalicylic acid and then centrifuged at 3,000 x g for 10 min. 2 mL of the supernatant was added to 2 mL acetic acid and 2 mL acidic ninhydrin, boiling water bath at 100 °C for 30 min. After cooling, 4 mL of toluene was added and measured absorbance at 520nm.

Statistical analysis

The data was analyzed by Microsoft Excel 2019 and SPSS 25.0. The figures were drawn in Origin 2021. Duncan test ($p < 0.05$) was used to evaluate the difference within treatments, and the significant differences among different materials were determined.

Result

The morphological parameters of rice seedlings

There was significant inhibition of rice growth under NaCl stress, which showed a remarkable decrease in plant height, stem base width, shoot fresh weight, and shoot dry weight (Table 1 and Table 2). From days 3 to 9, in comparison to CK, the plant height, stem diameter, shoot fresh weight and shoot dry weight of HHZ under NaCl stress significantly decreased by 13.48%-16.58%, 23.08%-28.95%, 29.67%-32.41% and 21.14%%-23.34% respectively. Similarly, in XLY900, the above indicators decreased by 10.67%-13.98%, 17.43%-23.08%, 27.24%-30.71% and 18.22%-22.15% respectively. Exogenous Hemin alleviated the inhibition of rice seedling growth by NaCl stress (Fig. 1). From days 3 to 9, in comparison to the NaCl treatment, the plant height, stem diameter, shoot fresh weight and shoot dry weight of HHZ with SH treatment were significantly higher by 9.62%-12.38%, 20.00%-32.10%, 18.63%-27.43%, and 11.96%-15.84%, respectively. Similarly, in XLY900, the above indicators were increased by 5.33%-8.01%, 15.56%-24.14%, 15.85%-26.58%, and 12.78%-14.26%, respectively. This finding suggested that the hemin effectively mitigated the inhibitory effect of NaCl stress on the growth of rice seedlings. Hemin promoted a higher growth of HHZ seedlings. In contrast to the NaCl treatment, ZnPP treatment did not lead to an increase in plant height, stem base width, shoot fresh weight, or shoot dry weight for both rice varieties. The addition of Hemin reversed the inhibition caused by ZnPP and enhanced the growth of rice seedlings. From days 3 to 9, the plant height of both HHZ and XLY900 was notably elevated in the SZH treatment, exhibiting a rise of 5.15% to 7.16% and 2.54% to 4.92%, respectively, as compared to the SZ treatment; the stem base width was enhanced by 7.59%-12.20% and 7.78%-15.91%, respectively; shoot fresh weight was increased by 9.12%-19.43% and 9.86%-16.19%, respectively and shoot dry weight was increased by 8.56%-10.66%, 6.70%-10.32%, respectively.

The membrane damage and ROS accumulation in rice seedlings

Compared to the CK, EL, MDA, and H_2O_2 contents in two rice varieties gradually increased with the increased period of NaCl stress treatment (Fig. 2). Compared to CK, the El of HHZ and XLY900 under NaCl stress significantly increased by 16.26%-126.50% and 35.25%-71.98% from days 3 through to 9, respectively. After NaCl treatment, there was a significant rise in the MDA and H_2O_2 content of HHZ in the S treatment. This increase ranged from 31.79% to 51.73% for MDA and 13.92% to 30.29% for H_2O_2 during the period from day 3 to 9, as compared to CK. In the NaCl treatment of XLY900, the content of MDA and H_2O_2 was significantly increased by 22.25%-40.52% and 20.26%-25.09%, compared with CK, from days 3 to 9, respectively. The H_2O_2 and MDA contents of HHZ were higher than that of XLY900 on 9 d after NaCl stress, showing that NaCl stress was more harmful to HHZ, which was more sensitive to NaCl stress than XLY900. Compared with NaCl treatment, spraying Hemin effectively reduced EL and the MDA and H_2O_2 contents of both rice varieties.

In contrast to the NaCl treatment, the EL of both HHZ and XLY900 exhibited a noticeable decrease in the SH treatment, including reductions of 9.64% to 28.20% and 8.78% to 18.41%, respectively. The MDA and H_2O_2 content in the SH treatment of HHZ compared to the NaCl treatment decreased by 15.20%-20.28% and 11.59%-18.14%, respectively. Similarly, in the SH treatment of XLY900, MDA and H_2O_2 content decreased by 8.30%-16.52% and 5.97%-15.72% compared to the NaCl treatment from day 3 to 9, respectively. Electron leakage, MDA, and H_2O_2 remained high in both varieties under ZnPP treatment. Throughout the stress period, the SZH treatment led to a reduction in EL, MDA content, and H_2O_2 content of both HHZ and XLY900, when compared to the SZ treatment. On day 3 and 9, compared to SZ treatment, EL of HHZ exhibited noticeable decreases of 9.21% and 10.43%, respectively, in SZH treatment. From days 3 to 9, compared to SZ treatment, the EL of XLY900 with SZH treatment dramatically declined by 6.09%-9.01%. From days 3 to 9, compared with SZ treatment, the MDA and H_2O_2 content were decreased by 6.51%-7.15% and 3.51%-10.99% in HHZ with SZH treatment, were reduced by 1.44%-7.71% and 1.22%-9.71% in XLY900 with SZH treatment, respectively.

The histochemical localization of reactive oxygen species in rice leaves

The distribution of H_2O_2 and superoxide anion ($O_2{\cdot}^-$) were localized and expressed visually by histochemical localization of HHZ and XLY900 rice leaves. H_2O_2 was stained with dark brown spots and $O_2{\cdot}^-$ was stained with dark blue spots (Fig. 3). Compared to CK, dark brown and dark blue spots were significantly increased in rice leaves of both varieties under NaCl stress. Compared to the NaCl treatment, dark brown and dark blue spots on leaves were significantly decreased in HHZ and XLY900 with SH treatments, which indicated that foliar spraying of Hemin could potentially reduce the accumulation and distribution of H_2O_2 and $O_2{\cdot}^-$. ZnPP treatment failed to lower the accumulation of ROS in the leaves, and dark brown spots and dark blue spots remained at a higher abundance. There was a reduced accumulation of ROS with the combination of ZnPP and Hemin. Compared to the ZnPP treatment, the number of dark brown spots and dark blue spots decreased in HHZ and XLY900 with SZH treatments.

The superoxide dismutase, peroxidase and catalase activity in rice seedlings

With the extension of exposure time, the SOD and POD activities in the NaCl treatment of HHZ showed an upward and downward trend, respectively, and CAT activity showed an increased trend compared to CK (Fig. 4). Compared to CK, the SOD, POD, and CAT activities in the NaCl treatment of XLY900 showed an upward trend with the prolonged time of NaCl stress. The SOD and POD activities in NaCl treatment of HHZ reached the maximum at 3 d of NaCl stress, which were significantly increased, by 13.82% and 13.64%, respectively, and CAT activities increased by 11.45%-21.71% from 3 to 9 d of NaCl stress compared to CK. In comparison to CK, the SOD, POD, and CAT activities of XLY900 under NaCl stress increased by 7.30%-26.63%, 6.64%-14.26%, and 15.97%-24.76% respectively, from days 3 through to 9. The application of exogenous Hemin boosted the SOD, POD, and CAT activities of SH treatment in two rice varieties. Compared to NaCl treatment, the SOD, POD, and CAT activities of HHZ with SH treatment were increased by 4.41%-17.66%, 6.48%-12.67%, and 6.43%-17.33%, respectively, from days 3 through to 9. In comparison to NaCl treatment, the SOD and CAT activities of XLY900 with SH treatment were increased, by 5.53%-27.47% and 10.54%-18.12%, from day 3 to 9, respectively, while POD activity increased by 4.53%-9.20% except for the day 5. Compared with the NaCl treatment, the ZnPP treatment did not enhance the enzyme activity under the stress but lowered the enzyme activity. For example, compared to NaCl treatments, on day 3, the CAT activity in SZ treatment of HHZ was significantly decreased by 6.54%; on day 5, the SOD activity in SZ treatment of XLY900 was significantly reduced by 11.12%. The combination with Hemin relieved the adverse effects of ZnPP and improved the above enzyme activities. Compared with the SZ treatment, the SZH treatment of HHZ showed SOD activity increased by 3.10%-13.12% from day 3 through to 9; POD activity was significantly enhanced by 8.05% on day 9; CAT activity was significantly raised by 11.52% on day 3. Compared with the SZ treatment, the SZH treatment of XLY900 showed SOD activity markedly increased by 15.79% and 22.93% on day 3 and 5, respectively; POD activity significantly enhanced by 7.47%-8.07% from day 5 to 9; CAT activity was significantly raised by 13.67% and 12.48% on day 5 and 7, respectively.

The assessment of the non-enzymatic antioxidants of the AsA-GSH cycle in rice leaves in rice leaves

As the period of NaCl stress was extended, the AsA content decreased and the DHA and AsA+DHA content increased in the leaves of HHZ and XLY900 (Fig. 5). From day 5 through to 9, compared to CK, the AsA content in the NaCl treatment of HHZ and XLY900 decreased by 2.16%-15.52% and 4.62%-14.26%, significantly different between treatments and control. In comparison to CK, the DHA and AsA+DHA content of HHZ under NaCl stress increased by 21.16%-60.17% and 4.47%-34.18%; for XLY900, the assessed parameters increased by 57.73%-67.58% and 10.39%-32.46%, from day 3 to 9, respectively. The application of exogenous Hemin further boosted the AsA content and diminished the accumulation of DHA and AsA+DHA. Compared to the NaCl treatment, the AsA content in SH treatment of HHZ and XLY900 increased, by 4.63%-15.54% and 5.46%-10.44% from day 5 through to 9, respectively, significantly different between NaCl treatments and control. In comparison to NaCl treatment, the DHA and AsA+DHA content in SH treatment of HHZ decreased by 15.53%-30.23% and 5.06%-19.87% from day 3 to 9, respectively; for XLY900, the assessed parameters decreased by 19.87%-29.67% and 5.43%-12.57%. Under NaCl stress, ZnPP treatment mainly raised DHA and AsA+DHA contents in the leaves of the two assessed rice varieties. In comparison to NaCl treatments, on day 7, the DHA and AsA+DHA content in the SZ treatment of HHZ were significantly increased by 15.00% and 8.49%; on day 9, the DHA content in the SZ treatment of XLY900 was significantly increased by 8.00%. In the combination of ZnPP and Hemin, the AsA content was higher, and the DHA and AsA+DHA contents were lower in both rice varieties compared to the ZnPP treatment. In comparison to SZ treatments, on day 5 and 9, the AsA content in the SZH treatment of HHZ was significantly increased by 11.53% and 3.22%, respectively; on day 5 and 7, the AsA content in the SZ treatment of XLY900 was significantly increased by 7.15% and 9.09%, respectively. In comparison to SZ treatment, the DHA and AsA+DHA content in SZH treatment of HHZ decreased by 12.39%-26.77% and 2.81%-14.35% from day 3 to 9, respectively. Similarly, the assessed parameters of XLY900 decreased by 8.08%-16.27% and 1.72%-7.34%, respectively.

It can be seen from Fig. 6 that, with the extension of the period of stress, the contents of GSH and GSH+GSSG in NaCl treatment leaves of both HHZ and XLY900 decreased; GSSG in NaCl treatment leaves of both HHZ and XLY900 increased. On day 3, 5, and 9, and compared to the CK, the GSH content in the NaCl treatment of HHZ significantly decreased 5.83%, 8.27% and 2.28%, respectively; in XLY900, the GSH content significantly decreased 3.49%, 7.17% and 8.68%, respectively. From days 3 to 9, and when compared to the control, the GSSG content in the NaCl treatment of HHZ and XLY900 significantly increased by 7.25%-22.36% and 8.20%-16.87%, respectively. On day 3, and day 5, compared to CK, the GSH+GSSG content in the NaCl treatment of HHZ significantly decreased 4.63% and 6.96%, respectively; On day 5 and day 9 in XLY900, the GSH content significantly decreased 5.22% and 6.93%, respectively. The Hemin further boosted the content of GSH and GSH+GSSG and reduceded the accumulation of DHA. Compared to the NaCl treatment, the GSH content in SH treatment of HHZ and XLY900 increased, by 1.96%-14.31% and 3.60%-8.69% from day 5 through to 9, respectively. In comparison to NaCl treatment, the GSSG content in SH treatment of HHZ and decreased by 8.57%-22.36% and 5.74%-6.35% from day 3 to 9, respectively. Under NaCl stress, ZnPP treatment mainly raised GSSG content in the leaves. In comparison to NaCl treatments, on day 3 and day 7, the GSSG content in the SZ treatment of XLY900 were significantly increased by 4.71% and 8.34%. In the combination of ZnPP and Hemin, the GSH and GSH+GSSG content were higher, and the GSSG content was lower in both rice varieties compared to the ZnPP treatment. Compared to the SZ treatment, the GSH content in SZH treatment of HHZ and XLY900 increased, by 1.77%-10.55% and 1.80%-8.16% from day 3 through to 9, respectively. Compared to the SZ treatment, the GSSG content in SZH treatment of HHZ and XLY900 decreased, by 4.90%-5.82% and 6.71%-8.33% from day 3 through to 9, respectively. In comparison to SZ treatments, on day 3, 5 and 7, the GSSG content in the SZH treatment of HHZ were significantly increased by 4.90%, 9.11% and 3.22%, respectively; on day 3 and 9, the GSSG content in the SZH treatment of XLY900 was significantly increased by 6.41% and 4.34%, respectively.

The AsA-GSH cycle enzymatic activities in rice leaves

As shown in Figure 7, APX, MDHAR, DHAR, and GR activities were increased along with the period of stress treatment. Compared with CK, during the stress period, the activities of the above four enzymes in the NaCl treatment of HHZ were markedly enhanced by 11.00%-18.88%, 14.95%-54.23%, 23.19%-56.82% and 12.22%-27.96% respectively. Similarly, in XLY900, the assessed parameters were significantly increased 18.82%-21.21%, 29.84%-51.15%, 19.62%-46.87% and 10.48%-13.56%, respectively. The use of Hemin further improved the activity of APX, MDHAR, DHAR, and DHAR. Compared with NaCl treatment, from day 3 to 9, the activities of APX, MDHAR, DHAR, and GR in the SH treatment of HHZ were enhanced by 15.18%-25.33%, 19.95%-58.63%, 7.10%-33.25% and 8.65%-14.11%, while in SH treatment of XLY900 were increased 17.76%-26.90%, 11.84%–50.44%, 15.92%-24.11% and 7.47%-12.26%, respectively. However, with the use of ZnPP the activity of APX, MDHAR, DHAR, and GR was diminished. On day 3, in comparison to NaCl treatment, the APX activity of HHZ in SZ treatment was significantly decreased by 17.03%. On day 9, in comparison to CK, the GR activity of HHZ and XLY900 in SZ treatment was significantly decreased, by 7.14% and 6.46%, respectively. The combination of ZnPP with Hemin increased the above enzyme activities. In HHZ with SZH treatment, compared with SZ treatment, the APX activity was significantly increased by 11.41% and 21.15% on day 3 and day 7, respectively; the MDHAR activity was markedly increased by 21.78%-38.70%, from day 3 to 9; the DHAR activity was dramatically increased by 9.98%-29.65%, from day 3 to 7; the GR activity was remarkably increased by 13.47%, and 8.81%, on day 7, and day 9, respectively. In XLY900 with SZH treatment, compared with SZ treatment, the APX activity significantly increased by 21.60% and 29.99% on day 5 and day 9, respectively. Similarly, The MDHAR activity was markedly increased by 32.81% and 20.13% on day 5, and day 7, respectively. The DHAR activity was dramatically increased by 14.37%-16.89%, from day 5 to 9. The GR activity was remarkably increased by 7.18%-9.02%, from day 5 to 9.

The content of osmoregulatory substances in rice leaves

The applied salt stress caused a significant increase in proline content in the leaves of HHZ and XLY900 (Fig. 8 a and b). Compared to CK, the proline content of HHZ under NaCl stress was significantly increased by 34.95%-65.34%, from days 3 to 9. From days 3 to 9, compared to CK, the proline content of XLY900 with NaCl treatment dramatically increased by 18.95%-54.16%. Under NaCl stress, the proline content increased to a greater degree in HHZ than in XLY900. Hemin treatment further enhanced the proline content in the leaves of the two assessed rice varieties. Compared to NaCl treatment, the proline content of HHZ and XLY900 with SH treatment significantly increased by 8.38%-27.10%, and 15.02%-24.35%, respectively, from days 3 to 9. Proline content of rice leaves was not elevated by ZnPP treatment. For example, on day 3, compared to NaCl treatment, the proline content of XLY900 with SZ treatment decreased by 8.64%. In combination with ZnPP and Hemin, the proline content was enhanced. Compared to SZ treatment, the proline content of HHZ with SZH treatment had a maximum improvement of 26.87% on day 9, and XLY900 with SZH treatment had a maximum growth of 26.51% on day 7. The soluble protein content of HHZ markedly increased in the early stage (3 d) and then decreased in the later stage (5-9 d) compared with CK under NaCl stress (Fig.8 c and d). The soluble protein content in XLY900 increased during the stress period with the difference reaching significant levels at all four-time points. Applying of Hemin enhanced soluble protein content in the leaves of two rice varieties. Compared with the salt stress treatment, soluble protein content noticeably increased by 2.75%, in SH treatment of HHZ, on day 9, while significantly elevated by 3.93%, and 1.17%, respectively in XLY900. Spraying ZnPP did not increase soluble protein content. For example, compared with NaCl treatment, soluble protein content significantly decreased by 3.20%, in SH treatment of HHZ, on day 3. When ZnPP was combined with Hemin, soluble protein content was enhanced. On day 3, soluble protein content was increased by 3.02% and 3.21%, respectively, of HHZ and XLY900 in SZH treatment.

Discussion

Globally, salt stress is the most prevalent abiotic stress that limits crop growth and development. Research has shown that salt stress impedes the growth of several crops, such as wheat (Ashraf et al., 2023), sorghum (Liu et al., 2023), and soybean (Feng et al., 2021). Excessive salt interferes with normal biological and physiological processes to negatively impact plant growth (Talubaghi et al., 2022), such as reduced plant height, narrowed stem base width and diminished biomass. This study was similar to the above studies. Under salt stress, the seedling growth of both HHZ and XLY900 was significantly inhibited, and all the morphological indexes were decreased (Table 1 and Table 2). Foliar spraying of Hemin positively regulated various morphological indicators and promoted aboveground growth and biomass accumulation in rice seedlings. Liu et al. (2021) showed that Hemin improved the growth of maize seedlings and increased biomass accumulation under drought stress. Furthermore, Hemin degraded in plants to produce CO, which alleviated the inhibition of wheat growth by NaCl stress (Ling et al., 2009). Exogenous ZnPP was unable to promote rice growth under salt stress in this study, which was consistent with the research of Cao et al. (2011).

ROS can be used at low concentrations as a secondary messengers or signaling molecules (Antoniou et al., 2016). Plants generate and remove ROS in dynamic balances under normal growth conditions. Under abiotic stress conditions, ROS are surged, which in large quantities are destructive, leading to changes in the structure of DNA, proteins and enzymes, ultimately resulting in programmed cell death (Gill and Tuteja 2010; Singh et al., 2019). MDA is one of the membrane lipid peroxidation products whose content can reflect the level of ROS and the degree of membrane lipid peroxidation. EL can evaluate cell membrane permeability; larger represents stronger osmotic cell membranes (Ben Youssef et al., 2021). In this experiment, the findings showed that salt stress caused higher leaf EL, increased MDA and H_2O_2 contents in two rice varieties and that the results were positively correlated with stress duration (Fig. 2). Compared with XLY900, HHZ had a much stronger increase in the above three indexes, indicating that HHZ was more intensely exposed to the stress. This was similar to the findings of previous study (Chen et al., 2022). The localization of H_2O_2 and $O_2{\cdot^-}$ in leaves was measured by histochemical methods. Salt stress induced the accumulation of H_2O_2 and $O_2{\cdot^-}$ in the leaves of HHZ and XLY900 compared with CK (Fig. 3). This is in conformity with the findings of Jabeen et al. (2020) who worked on cultivated rice under salt stress. Hemin has the ability to mitigate the damage caused by stress in plants, reducing ROS accumulation, MDA content and cell membrane permeability (Chen et al., 2009; Cui et al., 2012). The results of this experiment were in agreement with these previous findings. Foliar spraying of Hemin effectively diminished EL levels, H_2O_2 and MDA content (Fig. 2), reduced H_2O_2 and $O_2{\cdot^-}$ accumulation (Fig. 3), and alleviated the damage of salt stress to the cell membranes. Exogenous

ZnPP could not scavenge excess ROS and maintain cell membrane stability. When ZnPP was combined with Hemin, it scavenged part of the ROS and alleviated oxidative damage, this finding is in agreement with Zhang et al. (2012).

Facing stress, plants activate antioxidant defense systems to minimize damage caused by oxidative stress. Among them, antioxidant enzymes mainly include SOD, CAT, and POD. SOD represents the first barrier for plants to resist ROS damage caused by abiotic stresses and catalyzes the transformation of $O_2^{\cdot-}$ to O_2 (Karuppanapandian and Kim 2013). CAT eliminates H_2O_2 with minimal energy consumption and very high conversion rates for large-scale scavenging of ROS (Zamocky et al., 2012). POD has a strong affinity for H_2O_2 and is used for the fine tuning modulation of H_2O_2 (Abogadallah 2010). In this study, compared with CK, SOD and POD activities of NaCl treatment in HHZ was firstly increased and then decreased, and CAT activity was increased (Fig. 4 a, c and e); SOD, POD and CAT activities of XLY900 were on an upward trend (Fig. 4 b, d and f). This indicates that in the short term of salt stress, rice eliminates ROS by increasing the activity of antioxidant enzymes; in the long term of salt stress, rice accumulates more ROS, which can't be scavenged in time by antioxidant enzymes. This is consistent with the results of previous findings (Vaidyanathan et al., 2003; Seckin et al., 2009 and Kumari et al., 2023).

Foliar spraying of Hemin enhanced SOD, POD and CAT activities in leaves of the two rice varieties under assessment when exposed to salt stress (Fig. 4). This demonstrates that exogenous Hemin stimulates the antioxidant enzyme system in rice and facilitates the increase of enzyme activity, which avoids oxidative damage and ensures normal plant growth. The inhibitor ZnPP was unable to increase the activities of antioxidant enzymes or even inhibited them. ZnPP combined with Hemin mitigated the inhibitory effect caused by ZnPP, with improved SOD, POD and CAT activities (Fig. 4). Based on a previous study (Zhang et al., 2012), it is hypothesized that Hemin enhances antioxidant enzyme activity in rice leaves by promoting HO expression and thereby increasing the antioxidant enzyme activity, while ZnPP acts as an inhibitor of HO hindering its expression which restricts the increase in antioxidant enzyme activity.

The AsA-GSH cycle is an essential ROS scavenging mechanism, and mainly consists of the antioxidant enzymes APX, MDHAR, DHAR, and GR and the nonenzymatic antioxidants AsA and GSH, which can alleviate the oxidative damage caused by salt stress (Wang et al., 2022). As part of the cycling process: AsA is catalyzed by APX, which converts H_2O_2 to H_2O and is oxidized to MDHA. MDHA is converted to AsA by reduction-oxidation reaction with MDHAR or to DHA by a non-enzymatic disproportionation reaction. DHAR catalyzes DHA and GSH to produce AsA and GSSG, while GSSG can be restored to GSH by GR (Nahar et al., 2015; Tan et al., 2022). AsA and GSH act as nonenzymatic antioxidants and assist other antioxidant enzymes in scavenging ROS. In this study, salt stress decreased AsA content and increased DHA and AsA+DHA content in rice leaves (Fig. 5), indicating that APX activity enhancement decreased the AsA content. Foliar spraying of Hemin significantly improved AsA content and diminished DHA and AsA+DHA content. This finding suggests that the increase in MDHAR and DHAR activities causes an increase in AsA content and a decrease in DHA content. Under salt stress, GSH and GSH+GSSG content decreased, and GSSG content increased, while exogenous Hemin treatment increased GSH and GSH+GSSG content and decreased GSSG content in rice leaves (Fig. 6). This shows that the enhanced GR activity facilitated the conversion of GSSG to GSH and maintained a high level of reduction state GSH, which was in agreement with the research of Piao et al. (2022). These indicated that Hemin sustained cellular reduction force at a high level, which resisted oxidative damage. In addition, in this experiment, salt stress increased APX, MDHAR, DHAR, and GR activities in two rice varieties compared with the control (Fig. 7). It indicated that salinity stress increased the H_2O_2 content of rice leaves, which prompted APX to accelerate the scavenging of H_2O_2; while the increased activities of MDHAR, DHAR and GR were beneficial to the resistance of a leaf to oxidative damage, which was a stress response to excess H_2O_2. Foliar spraying of Hemin further induced the activities of APX, MDHAR, DHAR, and GR (Fig. 7). Previous studies have suggested that this might be possible by upregulating the transcription of genes for enzymes related to the metabolism of the degradation products CO and GSH, which could increase the enzyme activity to help plants mitigate the oxidative damage caused by the stresses (Zhang et al., 2016). ZnPP cannot be degraded to CO_2 in plants and reduces endogenous CO_2 production by blocking HO expression; thus, could not enhance the activities of MDHAR, DHAR, and GR under salt stress. Moreover, Hemin induced HO gene expression and enhanced gene expression of critical enzymes in the AsA-GSH cycle, while ZnPP prevented HO expression and even strengthened the inhibitory effect of NaCl stress on the AsA-GSH cycle in rice seedlings (Cui et al., 2012). These results reflect that Hemin improved the efficiency of ROS scavenging in rice leaves, which maintained cell membrane stability and enhanced the resistance of rice.

Although saline soils contain water, plants cannot absorb the water, mainly because the soil has a high level of ions that increase the osmotic pressure of the external environment, which prevents plant cells from absorbing water or even leading to the loss of water content. Therefore, plants ensure water absorption by increasing osmoregulatory substances and decreasing the difference in osmotic potential between the inside and outside cells. The important osmoregulatory substances, soluble proteins, and proline have different physiological functions in maintaining osmotic balance in plants. Soluble proteins can help the bound water in plant cells and maintain the stability of the cell structure (Hao et al., 2021). Proline is a potential non-enzymatic antioxidant that functions as a scavenger of single-linear oxygen molecules and hydroxyl radicals; thus, proline prevents lipid peroxidation of cell membranes and avoids exposure of plants to ROS-induced oxidative damage (Szabados and Savoure 2010). In this study, we found that with the increase of NaCl stress exposure, soluble protein content initially increased and then decreased in HHZ, while it continuously increased in XLY900 (Fig. 7). A previous study have shown that salt stress disrupts the protein synthesis pathway at later stages, accelerating its catabolism, generating large amounts of amino acids, and ultimately reducing protein content (Alisofi et al., 2020). This could be the reason for the decrease in soluble protein content in HHZ leaves. The soluble protein content in XLY900 leaves was enriched to relieve the difference in osmotic potential. The two rice varieties exposed to salt stress had significantly increased proline content. Compared with XLY900, salt stress caused HHZ to produce much more proline (Fig. 8 c and d). This was similar to the results of Gao et al. (2016), in which salt-sensitive varieties had high proline content in content when exposed to stress. Foliar spraying of Hemin promoted the accumulation of osmoregulatory substances in rice leaves, which significantly increased soluble protein and proline contents.

However, in the ZnPP treatment, the content of osmoregulatory substances was reduced instead of increased. Similar results were reported by Zhao et al. (2022). Together, these results indicate indicates that Hemin induces a large accumulation of proline and soluble proteins, which is beneficial for the absorption of water and the maintenance of cellular osmotic pressure in rice leaves under salt stress.

Conclusions

During the seedling stage, the activity of antioxidant enzymes and the contents of non-enzymatic antioxidants initially rose in response to salt stress. This response effectively countered the accumulation of reactive oxygen species (ROS) induced by the stress. However, with prolonged exposure to stress, the enzyme activity continued to increase while the content of the antioxidants decreased, failing to adequately alleviate the stress in a timely manner. The accumulated ROS and membrane lipid peroxides exacerbated the damage caused by the imposed stress, eventually leading to a decrease in growth. The application of Hemin through foliar treatment additionally enhanced the antioxidant enzymes activity and elevated the non-enzymatic antioxidants contents, which contributed to an overall improvement in the antioxidant capacity of rice, resulting in a reduction of membrane lipid peroxidation. The consistent functionality of the AsA-GSH cycle was ensured, consequently enhancing the resistance of rice to the imposed stress.

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Competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Fengyan Meng conceived and designed the study performed the experiments, analyzed the data, authored or reviewed drafts of the article, interpreted the results and improve manuscript, and approved the final draft.
Naijie Feng conceived and designed the experiment performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
Dianfeng Zheng analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Meiling Liu carried out experiments, prepared figures and/or tables, and approved the final draft.
Hang Zhou reviewed drafts of the article, and approved the final draft.
Rongjun Zhang performed the experiments, prepared figures and/or tables, and approved the final draft.
Xixin Huang analyzed the data, prepared figures and/or tables, and approved the final draft.
Anqi Huang analyzed the data, prepared figures and/or tables, and approved the final draft.

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Table 1(on next page)

Effects of exogenous Hemin on the morphological indexes of rice seedlings under NaCl stress

Notes: Data in this table is mean±standard error of at least three replicates. According to Duncan's multiple range tests, different letters indicate significant difference at the five percent significant level Within each column.

Table 1

Effects of exogenous Hemin on the morphological indexes of rice seedlings under NaCl stress

morphological indexes	Varieties	Treatments	NaCl stress time (d)			
			3	5	7	9
Plant height (cm)	HHZ	CK	31.67±0.20a	32.63±0.19a	33.57±0.07a	33.63±0.09a
		S	27.13±0.71c	27.93±0.13d	28.00±0.00d	29.10±0.21d
		SH	29.83±0.19b	30.97±0.17b	31.47±0.03b	31.90±0.15b
		SZ	27.00±1.00c	27.63±0.57d	27.90±0.57d	28.50±0.32d
		SZH	28.93±0.03b	29.57±0.03c	29.80±0.30c	29.97±0.09c
	XLY900	CK	32.90±0.31a	33.43±0.18a	33.80±0.46a	34.67±0.03a
		S	28.30±0.06d	29.40±0.12d	29.70±0.06d	30.97±0.27d
		SH	30.57±0.03b	30.97±0.23b	31.33±0.18b	33.17±0.33b
		SZ	28.33±0.03d	29.50±0.06d	29.50±0.06d	30.50±0.06d
		SZH	29.10±0.10c	30.25±0.14c	30.53±0.03c	32.00±0.00c
Stem diameter (mm)	HHZ	CK	3.27±0.06a	3.47±0.06a	3.63±0.06a	3.80±0.10a
		S	2.43±0.00d	2.67±0.12d	2.63±0.06d	2.70±0.12d
		SH	3.07±0.06b	3.20±0.00b	3.40±0.00b	3.57±0.06b
		SZ	2.63±0.15d	2.67±0.12d	2.73±0.06d	2.87±0.21d
		SZH	2.83±0.06c	2.93±0.06c	3.07±0.06c	3.20±0.00c
	XLY900	CK	3.53±0.03a	3.63±0.03a	3.77±0.03a	3.90±0.00a
		S	2.87±0.03d	3.00±0.00d	2.90±0.06d	3.00±0.00d
		SH	3.37±0.03b	3.47±0.09b	3.60±0.00b	3.63±0.03b
		SZ	2.93±0.07d	3.00±0.06d	2.93±0.03d	3.10±0.06d
		SZH	3.20±0.00c	3.23±0.03c	3.40±0.00c	3.47±0.03c

Notes: Data in this table is mean±standard error of at least three replicates. According to Duncan's multiple range tests, different letters indicate significant difference at the five percent significant level Within each column.

Table 2(on next page)

Effects of exogenous Hemin on the biomass of rice seedlings under NaCl stress

Notes: Data in this table is mean±standard error of at least three replicates. According to Duncan's multiple range tests, different letters indicate significant difference at the five percent significant level Within each column.

Table 2

Effects of exogenous Hemin on the biomass of rice seedlings under NaCl stress

morphological indexes	Varieties	Treatments	NaCl stress time (d)			
			3	5	7	9
Shoot fresh weight (g)	HHZ	CK	0.4771±0.0060a	0.4742±0.0109a	0.4829±0.0009a	0.5147±0.0041a
		S	0.3225±0.0092d	0.3335±0.0025d	0.3467±0.0043d	0.3617±0.0007d
		SH	0.4109±0.0094b	0.3956±0.0029b	0.4270±0.0043b	0.4430±0.0029b
		SZ	0.3169±0.0032d	0.3399±0.0056d	0.3398±0.0024d	0.3610±0.0091d
		SZH	0.3557±0.0109c	0.3709±0.0040c	0.4058±0.0018c	0.4180±0.0068c
	XLY900	CK	0.5147±0.0021a	0.5110±0.0262a	0.5225±0.0053a	0.5477±0.0098a
		S	0.3566±0.0101d	0.3577±0.0068c	0.3718±0.0051c	0.3985±0.0014d
		SH	0.4385±0.0076b	0.4528±0.0195b	0.4546±0.0318b	0.4617±0.0160b
		SZ	0.3623±0.0033d	0.3602±0.0220c	0.3732±0.0132c	0.3923±0.0094d
		SZH	0.4034±0.0057c	0.4185±0.0088b	0.4260±0.0016b	0.4310±0.0025c
Shoot dry weight (g)	HHZ	CK	0.0938±0.0014a	0.0966±0.0022a	0.1004±0.0007a	0.1016±0.0015a
		S	0.0740±0.0023c	0.0761±0.0025c	0.0770±0.0007c	0.0783±0.0013d
		SH	0.0840±0.0012b	0.0852±0.0013b	0.0870±0.0015b	0.0907±0.0013b
		SZ	0.0725±0.0024c	0.0751±0.0004c	0.0767±0.0014c	0.0779±0.0011d
		SZH	0.0824±0.0006b	0.0831±0.0015b	0.0841±0.0012b	0.0846±0.0015c
	XLY900	CK	0.0933±0.0023a	0.1016±0.0017a	0.1044±0.0005a	0.1096±0.0045a
		S	0.0817±0.0017d	0.0825±0.0015d	0.0818±0.0008cd	0.0853±0.0000cd
		SH	0.0933±0.0006b	0.0943±0.0003b	0.0934±0.0029b	0.0962±0.0009b
		SZ	0.0804±0.0023d	0.0816±0.0009d	0.0802±0.0043d	0.0811±0.0010d
		SZH	0.0876±0.0006c	0.0871±0.0015c	0.0880±0.0000bc	0.0895±0.0006bc

Notes: Data in this table is mean ± standard error of at least three replicates. According to Duncan's multiple range tests, different letters indicate significant difference at the five percent significant level Within each column.

Figure 1

Figure 1. Effect of Hemin on growth of rice seedlings under NaCl (9 d) in HHZ (a) and XLY900 (b).

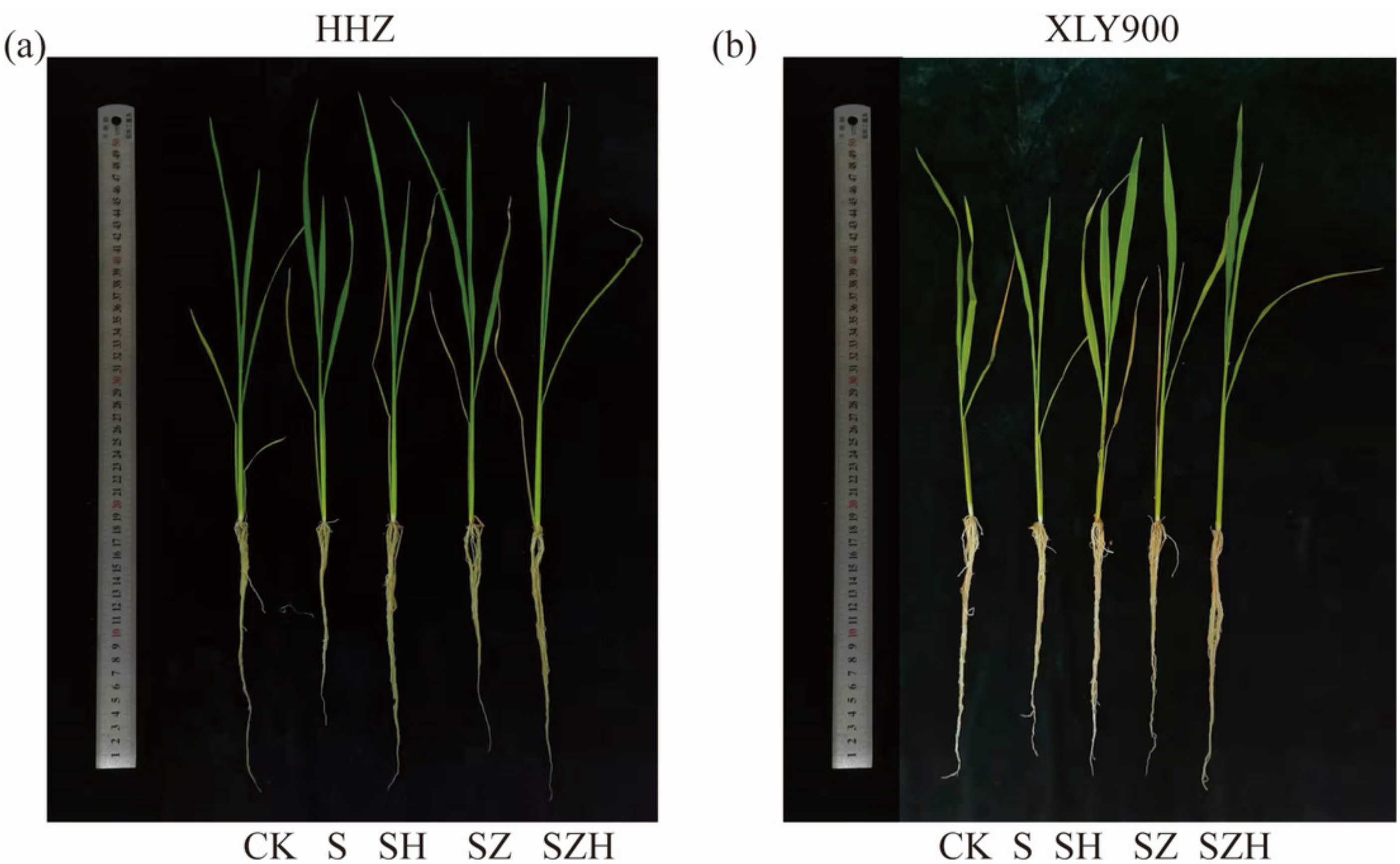

Figure 2

Figure 2. Effect of Hemin on membrane damage and ROS accumulation of rice seedlings under NaCl.

Electrolyte leakage in HHZ (a) and XLY900 (b); MDA in HHZ (c) and XLY900 (d) and H_2O_2 in HHZ (e) and XLY900 (f). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan's test.

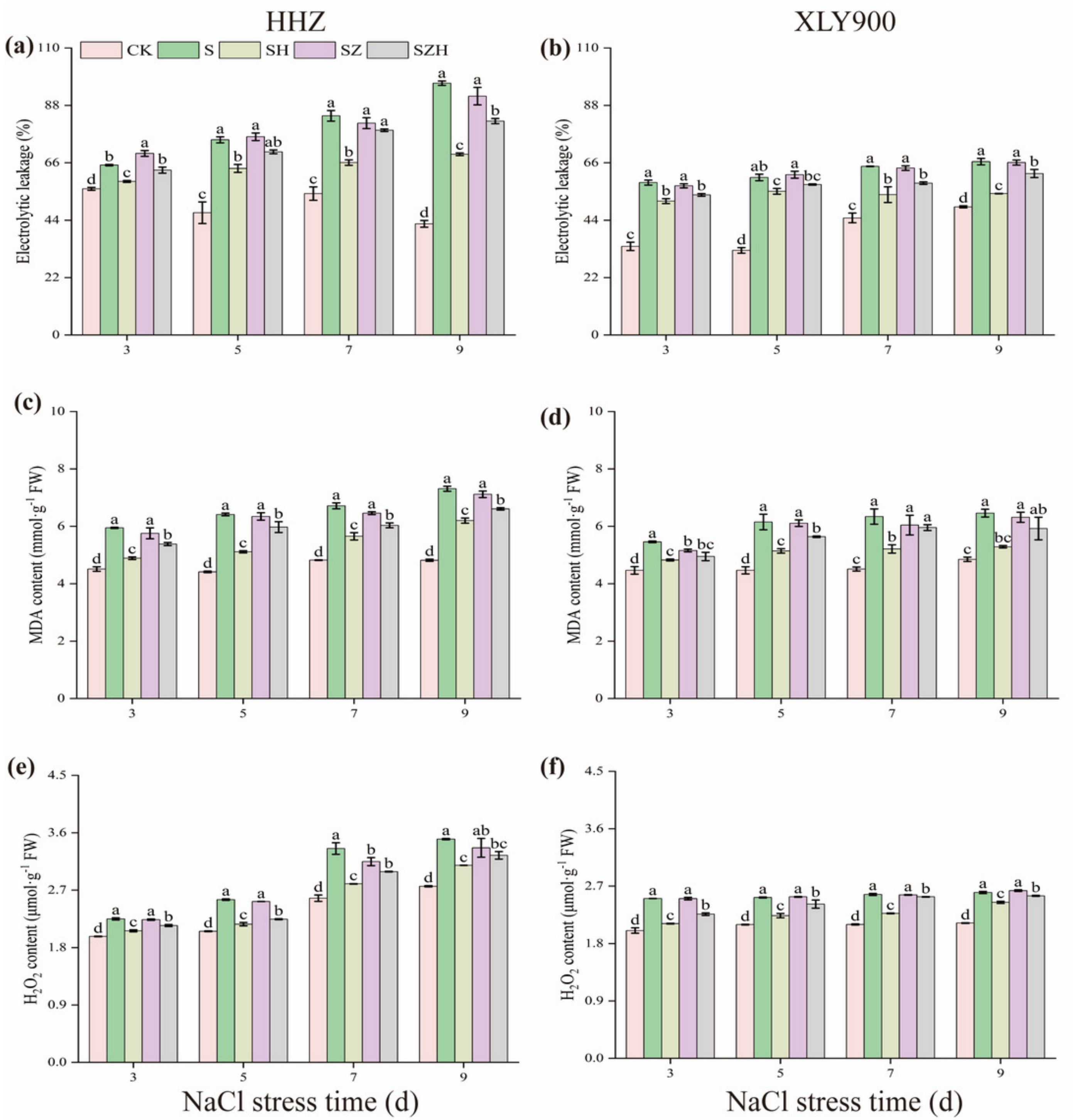
HHZ
XLY900
(a)
(b)
(c)
(d)
(e)
(f)
CK
S
SH
SZ
SZH
Electrolytic leakage (%)
MDA content (mmol·g⁻¹ FW)
H₂O₂ content (μmol·g⁻¹ FW)
NaCl stress time (d)
NaCl stress time (d)

Figure 3

Figure 3. Effect of Hemin on histochemical localization of H_2O_2 and $O_2 \cdot^-$ on rice leaves under NaCl stress (3 d).

H_2O_2 in HHZ (a) and XLY900 (b) and $O_2 \cdot^-$ in HHZ (c) and XLY900 (d).

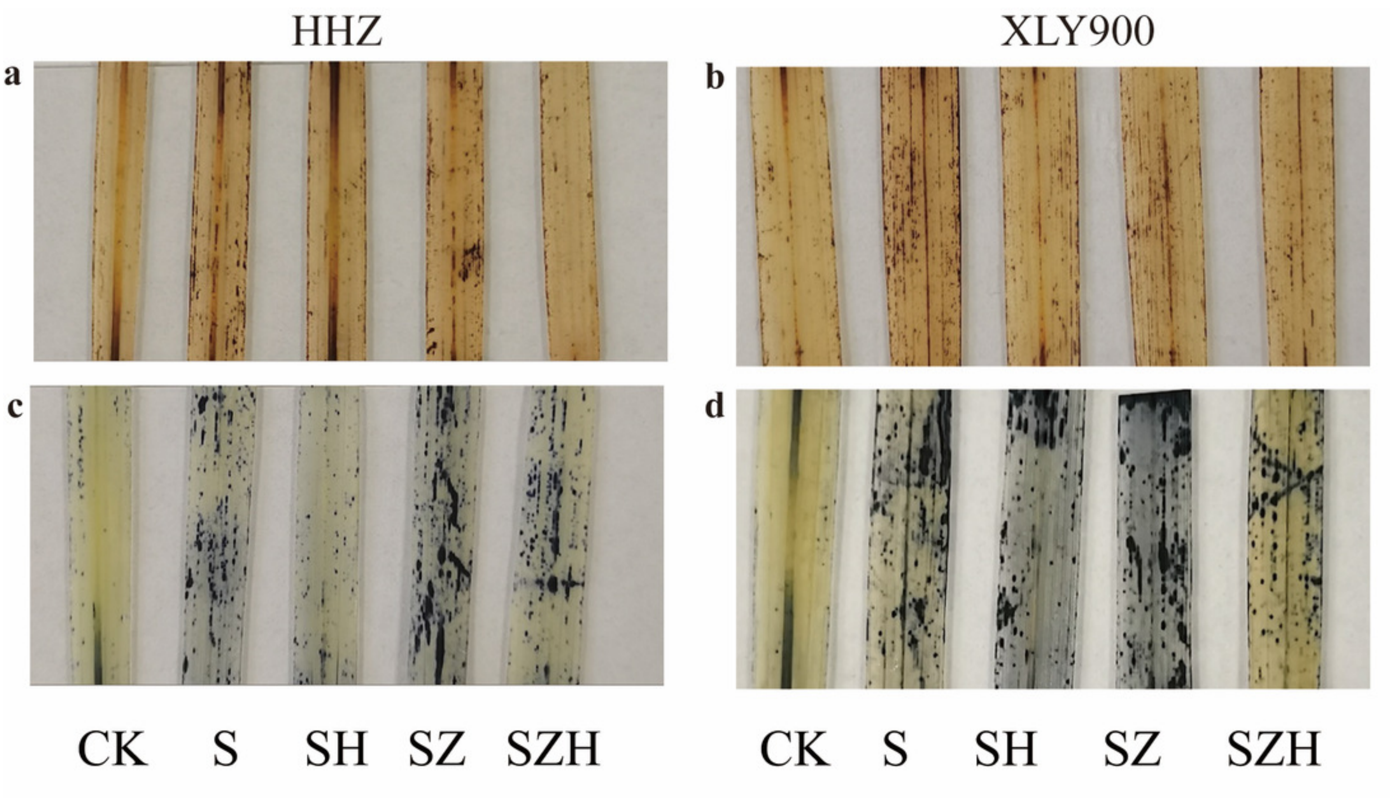

Figure 4

Figure 4. Effect of Hemin on SOD, POD, and CAT activity of rice seedlings under NaCl stress.

SOD in HHZ (a) and XLY900 (b); POD in HHZ (c) and XLY900 (d) and CAT in HHZ (e) and XLY900 (f). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan's test.

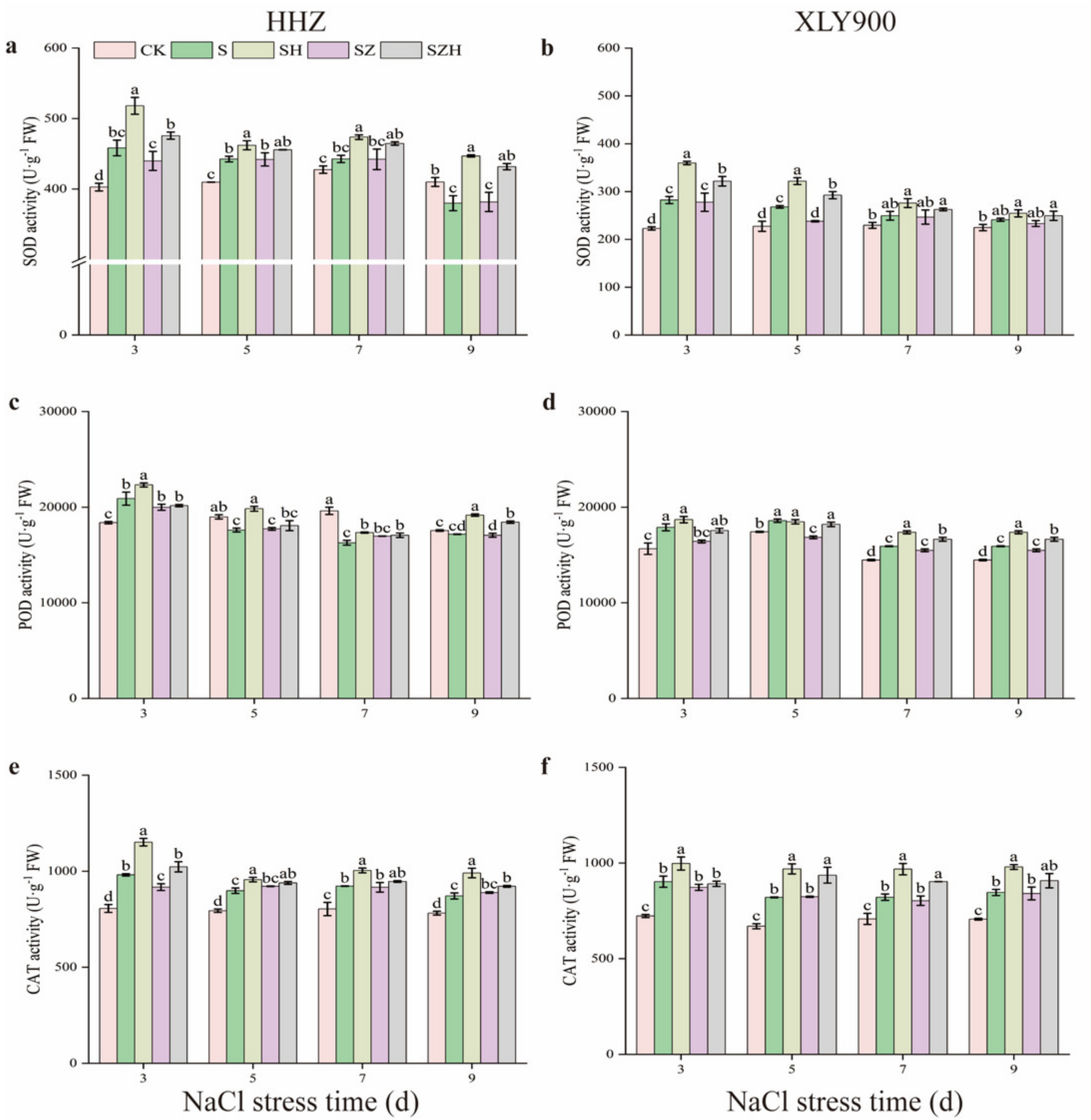
HHZ
XLY900
a
b
c
d
e
f
CK
S
SH
SZ
SZH
SOD activity (U·g-1 FW)
POD activity (U·g-1 FW)
CAT activity (U·g-1 FW)
NaCl stress time (d)

Figure 5

Figure 5. Effect of Hemin on ascorbic acid content of rice seedlings under NaCl stress.

AsA in HHZ (a) and XLY900 (b); DHA in HHZ (c) and XLY900 (d) and AsA+DHA in HHZ (e) and XLY900 (f). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan’s test.

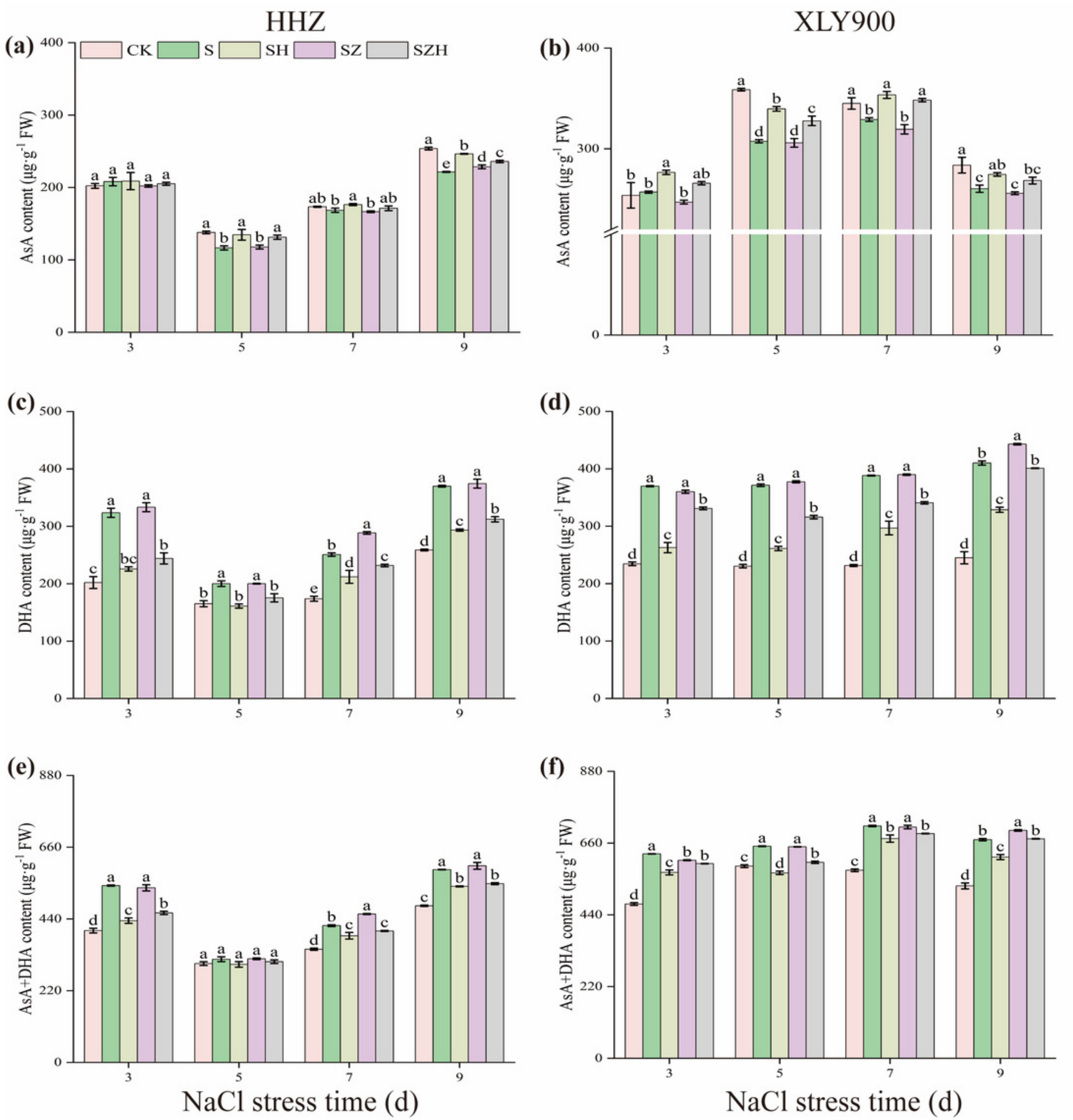
HHZ
XLY900
(a)
(b)
(c)
(d)
(e)
(f)
CK
S
SH
SZ
SZH
AsA content (μg·g⁻¹ FW)
DHA content (μg·g⁻¹ FW)
AsA+DHA content (μg·g⁻¹ FW)
NaCl stress time (d)
NaCl stress time (d)

Figure 6

Figure 6. Effect of Hemin on glutathione content of rice seedlings under NaCl stress.

GSH in HHZ (a) and XLY900 (b); GSSG in HHZ (c) and XLY900 (d) and GSH+GSSG in HHZ (e) and XLY900 (f). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan's test.

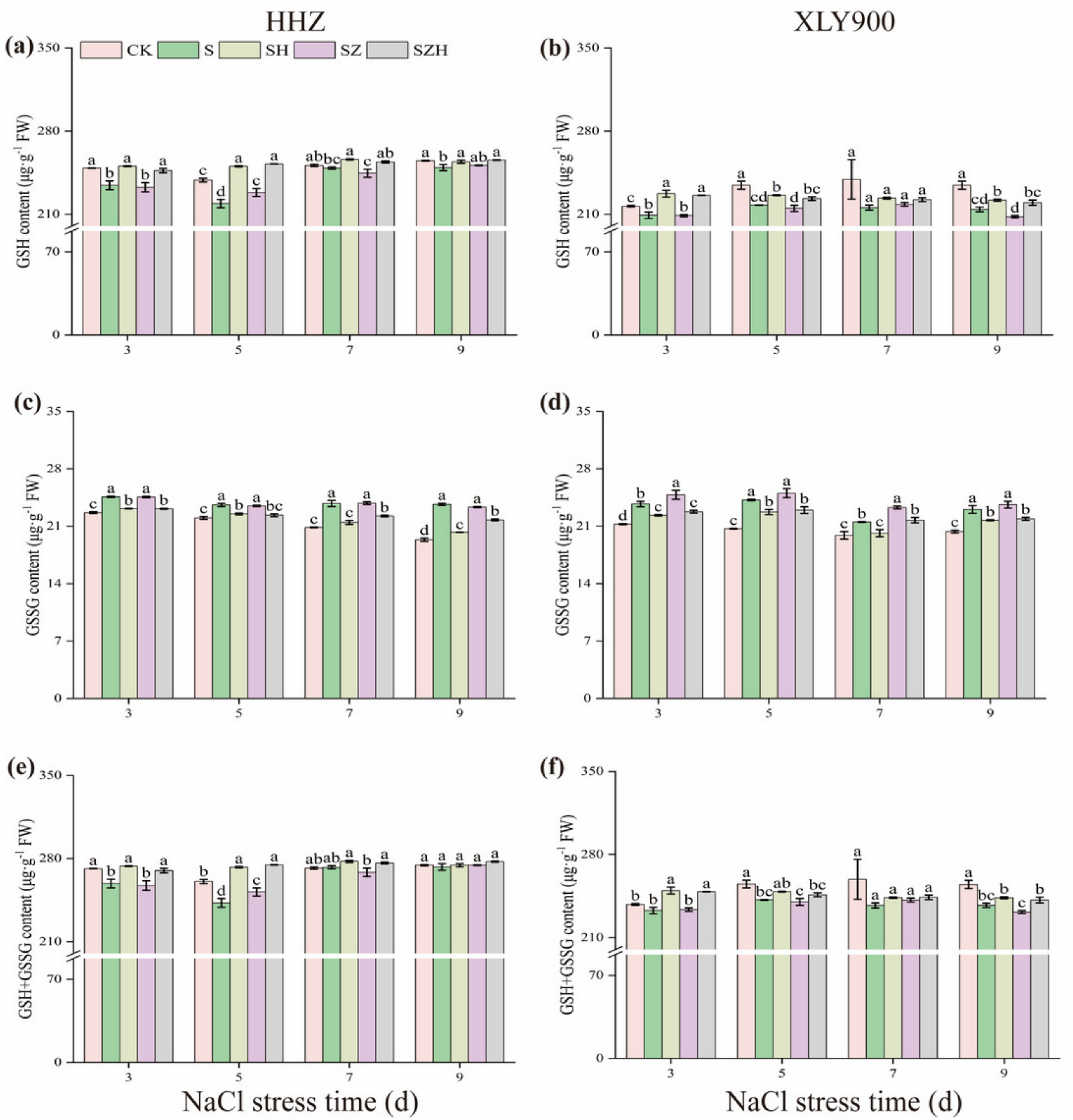
HHZ
XLY900
(a)
(b)
(c)
(d)
(e)
(f)
CK
S
SH
SZ
SZH
GSH content (μg·g-1 FW)
GSSG content (μg·g-1 FW)
GSH+GSSG content (μg·g-1 FW)
NaCl stress time (d)

Figure 7

Figure 7. Effect of Hemin on key enzyme activities in the AsA-GSH defense system of rice seedlings under NaCl stress.

APX in HHZ (a) and XLY900 (b); MDHAR in HHZ (c) and XLY900 (d) DHAR in HHZ (e) and XLY900 (f) and GR in HHZ (g) and XLY900 (h). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan’s test.

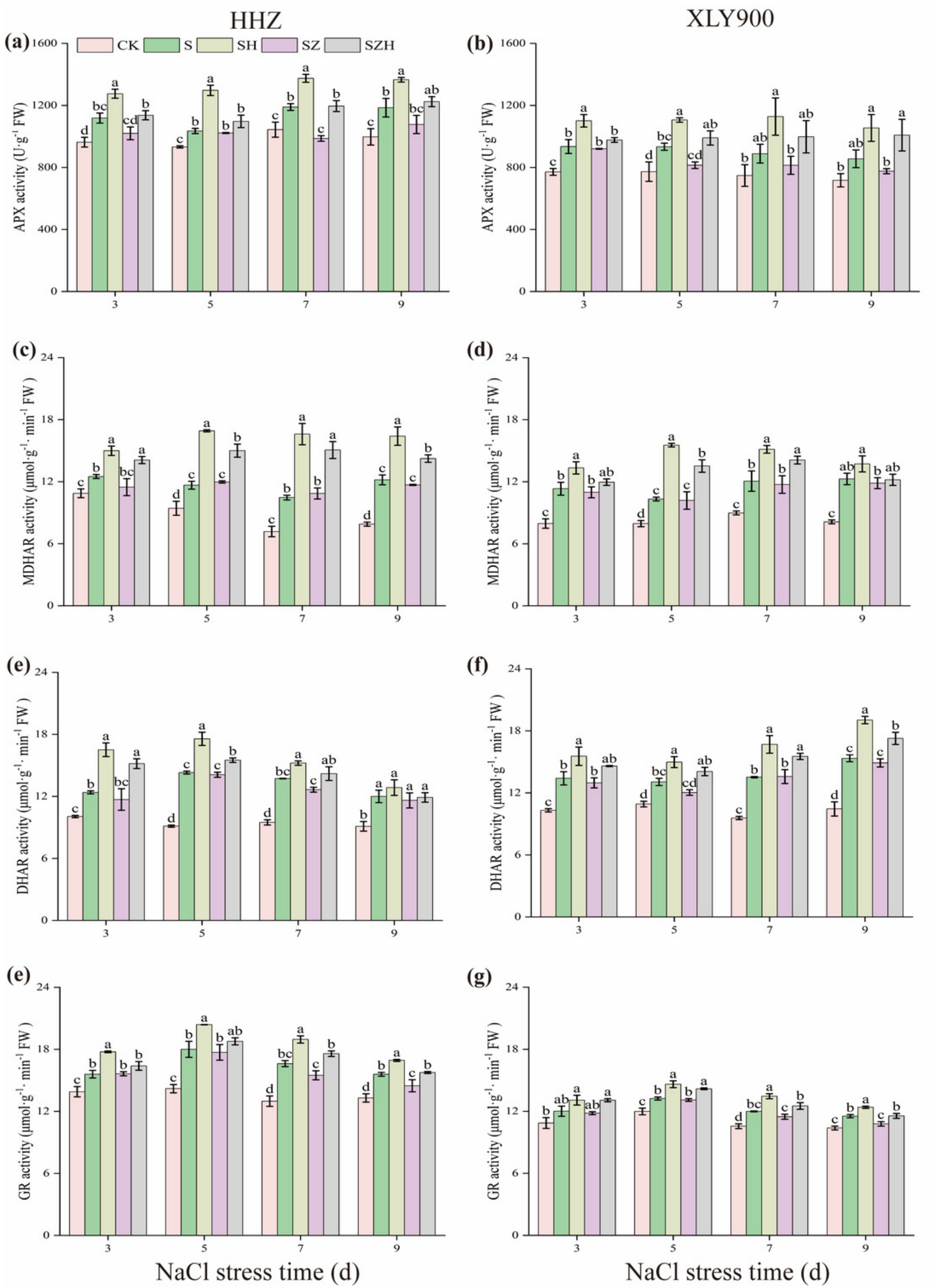
HHZ
XLY900
(a)
(b)
CK
S
SH
SZ
SZH
APX activity (U·g⁻¹ FW)
(c)
(d)
MDHAR activity (μmol·g⁻¹· min⁻¹ FW)
(e)
(f)
DHAR activity (μmol·g⁻¹· min⁻¹ FW)
(e)
(g)
GR activity (μmol·g⁻¹· min⁻¹ FW)
NaCl stress time (d)
NaCl stress time (d)

Figure 8

Figure 8. Effect of Hemin on osmoregulatory substances of rice seedlings under NaCl stress.

Proline content in HHZ (a) and XLY900 (b); soluble protein content in HHZ (c) and XLY900 (d). Values are the means±SD of three replicate samples. Different letters in the data column indicate significant differences ($p<0.05$) according to Duncan's test.