Effect of salt stress on different tiller positions in rice and the regulatory effect of Prohexadione calcium (#89342)

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Effect of salt stress on different tiller positions in rice and the regulatory effect of Prohexadione calcium

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Soil salinization has led to a sharp decline in crop yields, which has long inhibited the production of crops such as rice (*Oryza sativa* L.). Prohexadione calcium (Pro-Ca) can improve the resistance to crop failure by controlling plant height, but its effect on different tiller positions at the tillering stage of rice under salt stress is not known. In this study, we investigated the differential effects of salt stress on the physiological characteristics of the main stem and different tiller parts of rice plants, as well as the role of Pro-Ca in alleviating salt stress.The experimental results showed that the number of tillers and the number of leaves of the main stem were significantly reduced under salt stress conditions in rice, the content of malondialdehyde (MDA) and H_2O_2 in the leaves and stems of each tiller position were significantly elevated, and the percentage of tillers that were reduced or elevated was higher than that of the main stem in comparison with the respective control. Foliarspraying Pro-Ca under NaCl stress could effectively alleviate the effects of salt stress on the growth of rice tillers at the tillering stage, increase the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), in the leaves and stems of the tillers, and effectively alleviate the damage caused by salt stress on the cell membrane of rice tillers at the tillering stage, and the mitigating effect of calcium regulating cyclic acid was more significant in the mitigating effect of the tillers under the conditions of salt stress. Overall, the damage caused by salt stress on rice tillers was stronger than that on the main stem under the same conditions. Foliarspraying Pro-Ca could improve the antioxidant capacity of rice under salt stress, and effectively alleviate the damage caused by salt stress to each tiller position of rice.

Effect of salt stress on different tiller positions in rice and the regulatory effect of Prohexadione calcium

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Abstract

Soil salinization has led to a sharp decline in crop yields, which has long inhibited the production of crops such as rice (*Oryza sativa* L.) . Prohexadione calcium (Pro-Ca) can improve the resistance to crop failure by controlling plant height, but its effect on different tiller positions at the tillering stage of rice under salt stress is not known. In this study, we investigated the differential effects of salt stress on the physiological characteristics of the main stem and different tiller parts of rice plants, as well as the role of Pro-Ca in alleviating salt stress.The experimental results showed that the number of tillers and the number of leaves of the main stem were significantly reduced under salt stress conditions in rice, the content of malondialdehyde (MDA) and H_2O_2 in the leaves and stems of each tiller position were significantly elevated, and the percentage of tillers that were reduced or elevated was higher than that of the main stem in comparison with the respective control. Foliar spraying Pro-Ca under NaCl stress could effectively alleviate the effects of salt stress on the growth of rice tillers at the tillering stage, increase the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), in the leaves and stems of the tillers, and effectively alleviate the damage caused by salt stress on the cell membrane of rice tillers at the tillering stage, and the mitigating effect of calcium regulating cyclic acid was more significant in the mitigating effect of the tillers under the conditions of salt stress.

Overall, the damage caused by salt stress on rice tillers was stronger than that on the main stem under the same conditions. Foliar spraying Pro-Ca could improve the antioxidant capacity of rice under salt stress, and effectively alleviate the damage caused by salt stress to each tiller position of rice.

Introduction

In recent years, soil salinization has become a worldwide problem due to rising sea levels and increasing salinized land areas due to global warming (Ahmed et al., 2021), severely reducing global crop yields and agricultural production (Munns & Tester, 2008; Dai et al., 2022). Research on improving crop salt tolerance and effectively mitigating the damage caused by salt stress on yield has become a key research concern (Endo et al., 2011). Salt stress acts as one of the abiotic factors affecting the growth of most plants. When soil salinity exceeds the threshold level of plant salt tolerance, it can negatively affect plant growth characteristics and yield, and even cause plant death (Darwish et al., 2009). In arid regions, high temperatures and water scarcity often lead to salinity problems, when soil salinity increases, the water potential of the soil solution falls below that of the plant root cells, making root uptake difficult and causing osmotic stress, which leads to the closure of the plant stomata and inhibits CO_2 uptake, thus weakening photosynthesis and causing nutrient deficiencies, and the accumulation of Na^+ and Cl^- in cells affects the uptake and transport of minerals and inhibits enzyme activity in cells leading to dehydration of plant cells. In addition, salt stress can lead to the accumulation of reactive oxygen species (ROS) and the destruction of cellular structures and biomolecules (Dai et al., 2022), limiting the growth of major crops, including rice (Hussain et al., 2018). Prolonged exposure to harsh environments has caused crops to evolve a range of salt tolerance mechanisms that, within certain limits, allow them to competitively obtain water from the soil and maintain nutrient balances in the body in response to ionic stresses thereby surviving adverse soil conditions (Hoang et al., 2016).

Rice (*Oryza sativa* L.), a moderately salt-sensitive crop (Joseph et al., 2010), is severely affected by salt stress in its growth and development (Zhang et al., 2012). Salt stress has a significant negative effect on rice development and yield, and this effect varies according to developmental stage, degree and duration of stress, and variety. It has shown that the effects of salt stress on rice germination and emergence are mainly characterized by a reduction in germination rate, germination speed, and germination energy, leading to a reduction in shoot length, root length, and dry weight of rice (Taratima et al., 2022). Salt stress on the seedling period is mainly manifested in the damage to leaves and root system (Chang et al., 2019). Salt stress affects rice tillering mainly by reducing tillering capacity and delaying the reproductive process, and the duration of delay is positively correlated with the degree of salt stress, and primary and secondary tillers are more affected than the main stem. In addition, salt stress reduces soil fertility and causes nutrient imbalance, and salinity stress inhibits nutrient uptake by the root system, ultimately leading to reduced tillering or tiller death due to nutrient deficits (Ruan et al., 2008). Salt stress in the formation of young spikes and spiking and flowering stage of rice is mainly manifested in the following ways: yellowing of leaves, delayed spiking, prolonged spiking period, increase in the number of degradation of glumes, shorter spike lengths, decrease in the number of solid grains, less full grains, more black rotting of roots in the late stage, early senescence, and ultimately affecting the yield of rice (Chang et al., 2019).

The development of tillers as branches arising from the base of the rice stem, which includes the formation and growth of axillary buds, is an important component of the ideal architecture (Zhang et al., 2023), a key agronomic trait that affects rice yield and quality, and a key morphological trait for plant survival and competition. Tillering is significantly affected by various environmental factors such as drought and soil nutrient deficiencies (Zha et al., 2022), and previous studies have shown that tiller formation is related to plant hormones (Dun et al., 2009; Leyser, 2009; Beveridge & Kyozuka, 2010). Excessive tillering increases the number of ineffective tillers and induces resource competition between the main stem and tillers, resulting in collapse (Zheng et al., 2017; Lynch et al., 2017). Primary stems and primary tillers contribute more to crop yield than secondary tillers due to asymmetric competitive advantages under stress conditions, and these advantages are associated with increased leaf number. Transportation of water and nutrients between the primary stem and tiller through the vascular bundles at the tiller nodes is essential for tiller development and survival (Yang et al., 2022).

Plant growth regulators, as organic compounds with growth and development regulating effects similar to those of natural plant hormones, regulate plant growth and development by triggering many physiological and metabolic processes (Kaya et al., 2023; Zhao et al., 2023). The formation of the endogenous plant hormone gibberellin requires hydroxylase enzymes to catalyze a series of hydroxylation reactions, and these hydroxylases require 2-ketoglutarate as a coenzyme. Prohexadione calcium (Pro-Ca) mimics the structure of the coenzymes and competitively inhibits their activity, thereby inhibiting the synthesis of active gibberellins. Among these hydroxylation reactions, the reaction pathway for the formation of GA1 is the most sensitive to Pro-Ca, whereas the pathway for the formation of GA4 is not involved in the β-hydroxylation reaction, so that Pro-Ca selectively inhibits the synthesis of gibberellin GA1. GA1 is mainly found in the nutrient organs, controlling the elongation and growth of stems and leaves, while GA4 is mainly found in the reproductive organs, controlling flower bud differentiation and hot grain development. Pro-Ca is an ideal dwarfing agent because of its strong synthetic activity in inhibiting GA1. Pro-Ca inhibits active gibberellin synthesis while protecting the activity of both surviving gibberellins, so Pro-Ca has dual activity on gibberellin metabolism (Kim et al., 2010; Ilias & Rajapakse, 2005). Pro-Ca has been shown by previous authors to have specific regulatory effects on rice, apple, strawberry, etc. (Kim et al., 2010; Kim et al., 2007; Lee et al., 1998). In summary, the hazards of salt stress, the characteristics of tillering, and the mechanism of action of Pro-Ca acid have been investigated by previous researchers, and our previous studies demonstrated that calcium switched acid can alleviate the damage caused by NaCl to the antioxidant capacity, photosynthetic properties, and cell membranes during the tillering stage in rice (Zhang et al., 2023; Zhang et al., 2023; Huang et al. 2023), but further explorations are needed regarding the differential effects of salt stress on main stem and tiller and the regulatory role of Pro-Ca.

In this study, we aimed to investigate the differential effects of salt stress on rice main stems and tillers and the regulatory role of calcium switched acid by comparing the relevant morphology building indexes, antioxidant enzyme activities, membrane damage indexes, and soluble protein contents in leaves and stems of rice main stems, first tillers and second tillers at the tillering stage under different treatments.

Materials and Methods

Materials and reagents

Huanghuazhan (conventional rice) was provided by Longping Seed Co. Ltd (Hunan, China), and Xiangliangyou900 (hybrid rice) was provided by Nianfeng Seed Science and Technology Co. Ltd (Hunan, China).

The original solution of the test regulator 5% Pro-Ca used in this experiment was provided by Sichuan Runer Technology Co. Ltd (Chengdu, Sichuan).

Experimental designs

Full and uniform rice seeds were selected, sterilized with 3% H_2O_2 for 15 min and then washed repeatedly with distilled water, distilled water was added until the seeds were submerged, and the seeds were soaked for 24 h at 30°C, after which they were germinated under dark conditions for 24 h. The experiment was selected to be carried out in the daylight linkage greenhouse of the College of Coastal Agriculture, Guangdong Ocean University, and the germinated seeds were uniformly sown on the rice-planting trays (specifications of 28~30 cm × 58~60 cm), about 5-8 seeds in each hole, and the soil used for seedling was a 3:1 mixture of brick red soil and nutrient soil.

After transplanting cultivation using caliber × bottom diameter × height of 19 × 15 × 18 cm plastic pots, each pot containing 3 kg of sun-dried soil, before transplanting a fixed amount of each pot to add 1 L of water, to be stabilized when the water surface line marking, and regularly replenish water to maintain the water layer. When the seedlings in the seedling tray were three leaves and one heart, the seedlings with consistent growth were selected and transplanted, and the depth of transplanting was about 1.5 cm, with 3 holes in each bucket and 1 plant in each hole. After the end of greening and before tillering, select the evening of sunny weather at about 16:00 to carry out regulator treatment through foliar spraying, about 10ml per pot, to ensure that the front and back of the leaf spraying evenly, in order to ensure its normal absorption. The regulator treatment was followed by a 0.3% salt treatment 48 h later. Tagging and tracking marking of tiller occurrence. Tillers were labeled with secondary and leaf positions. Ensure spatial distance between seedlings at each sampling to prevent competition between individuals due to different spatial size.

The experiment was set up with eight treatments, Xiangliangyou900 variety included four treatments as follows: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg·L^{-1} Pro-Ca), XPro-Ca+S (100 mg·L^{-1} Pro-Ca + 0.3% NaCl), and Huanghuazhan variety included four treatments as follows: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg·L^{-1} Pro-Ca), and ZPro-Ca+S (100 mg·L^{-1} Pro-Ca+0.3% NaCl), with four replications per treatment. The leaves and stems of the main stem, the first tiller, and the second tiller were taken every 7 d (7 d, 14 d, 21 d, 28 d, and 35 d after salt treatment) for the determination of related indexes.

Determination of morphological indices

Morphological indexes such as plant height, root length, and number of tillers were measured directly by using vernier calipers to measure the intersection of stem and root to determine the stem base width, and by using a leaf area meter (YX-1241) to measure the inverted two leaves and inverted three leaves of each tiller position, and by using the conventional drying method, the samples of each part of each treatment were killed for several and a half times for 30 min in an oven at 105°C, and then dried at 80°C to a constant weight and then determined the dry weight.

Determination of antioxidative enzyme activities

At 7 d, 14 d, 21 d, 28 d, and 35 d after NaCl stress, rice leaves and stems at different tiller positions were rapidly frozen in liquid nitrogen and then stored in -80°C. 0.5 g of the samples were ground in liquid nitrogen, and then 10 ml of pre-cooled phosphate buffer (0.05 mM PBS, pH 7.8) was added, ground to homogenate, and then centrifuged at 6000 × g for 20 min at 4°C, and the supernatant was aspirated and set aside in a 4°C refrigerator.

3 ml of reaction solution (PBS pH 6.0 + guaiacol) was mixed with 40 μL of supernatant. The absorbance was recorded every 30 s for 4 times and the dynamic absorbance was measured at 470 nm using a spectrophotometer (GENESYS 180 UV-Vis, Thermo Sci) to determine the peroxidase (POD) activity.

0.1 ml of supernatant was taken and mixed with 2.9 ml of reaction solution (PBS pH 7.0 + 30% H_2O_2) and the absorbance at 240 nm was measured and recorded every 30 s for 4 times using a spectrophotometer (GENESYS 180 UV-Vis, Thermo Sci). Catalase (CAT) activity was calculated according to the method provided by Aebi (1984).

Superoxide dismutase (SOD) activity was determined using the nitro blue tetrazolium (NBT) method (Giannopolitis & Ries, 1977). 0.1 ml of supernatant was added to 2.9 ml of reaction mixture (2.61 ml meet + 0.097 ml EDTA-Na_2 + 0.097 ml NBT + 0.097 ml riboflavin) and irradiated for 20 min at 4000 lux light at 25°C. At the end of the reaction the absorbance at 560 nm was measured using the solvent in the unilluminated cuvette as a control tube, and the total activity of SOD was calculated.

0.1 ml of supernatant was mixed with the reaction solution (2.6 ml EDTA-Na_2 + 0.15 ml AsA + 0.15 ml H_2O_2) and the absorbance at 290 nm was determined by spectrophotometer recording every 30 s for 4 times. Ascorbic acid peroxidase (APX) activity level was calculated according to the method described by Nakano & Asada (1981).

Determination of membrane damage index

Malondialdehyde (MDA) content was determined by TBA method (Guo et al., 2018). 10 ml of phosphate buffer (0.05 mM PBS, pH 7.8) was added to 0.5 g of sample and ground, then centrifuged at 10,000 × g for 10 min at 4°C. 1 ml of the supernatant was taken and mixed with 2 ml of 0.6% TBA (thiobarbituric acid) in a centrifuge tube. The mixture was boiled in a boiling water bath for 15 min and then centrifuged at 10000 × g and 25 °C for 10 min. The absorbance of the supernatant was measured spectrophotometrically at 450 nm, 532 nm, and 600 nm, respectively.

0.5 g of the sample was taken, 5 ml of 0.1% TCA solution was added, ground in liquid nitrogen and centrifuged at 10,000 × g for 10 min. Then, 0.5 ml of the supernatant was added to 0.5 ml of 10 mM PBS buffer and 1 ml of KI solution, and the reaction was carried out in the dark at 28°C for 1 h. The H2O2 content of the sample was determined by spectrophotometric (GENESYS 180 UV-Vis, Thermo Sci) at 390 nm to determine the H2O2 content by measuring the absorbance (Jessup et al., 2018).

Subcellular localization staining for O_2^- and H_2O_2 in plant leaf tissues was performed with reference to Romero Puertas et al. (2004).

Histochemical staining of leaf cell death was referred to Martina Schraudner et al. (1998).

Determination of the soluble protein content

To determine the soluble protein content, the method of Bradford (1976) was used with the Caumas Brilliant Blue G-250 staining method, 0.5 g of the sample was added to 10 ml of 0.05 mol/L pre-cooled phosphate buffer (pH 7.8) and ground in liquid nitrogen, and centrifuged at 12,000 × g at 4°C for 20 min, and the supernatant was the crude protein extract. The protein content was determined by adding 1 ml of enzyme solution to 5 ml of koammas brilliant blue solution and then shaking well, and the absorbance value at 595 nm was measured after 2 min of reaction.

Statistical analyses

Using Excel 2016 statistics and analyzed using SPSS 25.0, one-way (one-way ANOVA) and Duncan's method were used for ANOVA and multiple comparisons, and the results were expressed as mean (X) ± standard error (SE). Origin 2018 software was used to make graphs, and different lowercase letters indicated significant differences between treatments ($P < 0.05$).

Results

Effect of salt stress on morphological indexes at the tillering stage of rice and regulation by Pro-Ca

From the experimental results, it was found that salt stress negatively affected the tillering ability of both rice varieties (Fig. 1a and b), and the number of tillers decreased by 30.0%~44.43% and 12.52%~33.35% in Xiangliangyou900 and Huanghuazhan, respectively, from the 7th to the 35th days (Fig. 1c and d). The number of main stem leaves of Xiangliangyou900 decreased by 9.09%, 5.72%, 15.00%, and 2.04% on the 14th, 21st, 28th, and 35th days, respectively, and that of Huanghuazhan decreased by 6.90%, 21.05%, 11.11%, 12.20%, and 8.16% on the 7th, 14th, 21st, 28th, and 35th days, respectively, after NaCl stress (Fig. 1e and f).

The plant heights of Xiangliangyou900 and Huanghuazhan were reduced by 4.07%~11.60% and 10.31%~28.10%, respectively, compared with the control from the 7th to the 35th days after salt stress (Table 1). The first tiller length of both varieties was reduced by 2.34% to 29.22% and 13.02% to 38.46%, respectively, compared with the CK from the 7th to the 35th days after salt stress (Table 1). Compared with the main stem and the first tiller, the effect of NaCl stress on the length of the second tiller was more significant, and the length of the second tiller of Xiangliangyou900 and Huanghuazhan was reduced by 12.70%~31.86% and 19.60%~39.38% from the 7th to the 35th d after salt stress, respectively (Table 1). The stem base width of the main stem of Xiangliangyou900 was reduced by 16.45% to 25.68% from 7th to 35th days after salt stress compared with the control, and that of Huanghuazhan was reduced by 17.41% to 38.99% from 7th to the 35th days after NaCl stress (Table 1). Salt stress also significantly reduced the stem base width of the first and second tillers of both varieties. Compared with the CK treatment, the stem base width of the first tiller was reduced by 16.42%~44.02% and 7.74%~43.86%, and the stem base width of the second tiller was reduced by 13.63%~36.32% and 26.62%~43.46%, respectively, in Xiangliangyou900 and Huanghuazhan from the 7th to the 35th days after the salt stress (Table 1).

The main stem leaf area of Xiangliangyou900 was reduced by 19.83% to 41.70% from the 7th to the 35th days after salt stress compared with the control, and the main stem leaf area of

Huanghuazhan was reduced by 29.74% to 45.76% from the 7th to the 35th days (Table 2). Compared with the control, the leaf area of the first tiller decreased by 36.83% to 60.23% and 30.66% to 73.22%, and the leaf area of the second tiller decreased by 30.63% to 43.52% and 43.26% to 65.35%, respectively, in Xiangliangyou900 and Huanghuazhan from the 7th to the 35th days after salt stress (Table 2). In addition, compared with the CK, the root length of Xiangliangyou900 decreased by 25.73% to 48.08% from the 7th to the 35th days after salt stress, and the root length of Huanghuazhan decreased by 15.75% to 34% from the 7th to the 35th days after NaCl stress, which were significant differences (Table 2).

As shown in Table 4, the aboveground dry weight of the main stems of Xiangliangyou900 and Huanghuazhan decreased by 27.53% to 62.62% and 29.77% to 52.86%, respectively, compared with the control from the 7th to the 35th d after NaCl stress. Compared with CK, the dry weight of the first tiller decreased by 9.86% to 63.22% and 30.39% to 65.68%, and the dry weight of the second tiller decreased by 8.44% to 37.46% and 20.99% to 65.03%, respectively, in Xiangliangyou900 and Huanghuazhan from the 7th to the 35th days after salt stress (Table 3). In addition, the root dry weight of Xiangliangyou900 decreased by 34.32% to 70.88% from the 7th to the 35th days after NaCl stress compared with the control, and which of Huanghuazhan decreased by 36.33% to 70.21% (Table 3).

We can see from Fig. 1 that exogenous foliar application of Pro-Ca effectively alleviated the inhibitory effect of NaCl stress on the growth parameters of the two varieties. Foliar application of Pro-Ca under NaCl stress increased the number of tillers by 14.32% to 59.99% and 42.86% to 100.04% in Xiangliangyou 900 and Huanghuazhan, respectively, from the 7th to the 35th days. Compared with S treatment, the number of main stem leaves of both rice varieties increased significantly in Pro-Ca+S treatment, where the number of main stem leaves of Xiangliangyou900 increased by 4.16%, 23.33%, 3.03%, 23.53%, and 6.25%, respectively, and the number of main stem leaves of Huanghuazhan increased by 11.11%, 10.00%, 15.62%, 19.44% and 17.78%, respectively (Fig. 1e and f).

As shown in Table 1, compared with the control, foliar spraying Pro-Ca significantly reduced the plant height of the two rice varieties, in which the plant height of Xiangliangyou900 was reduced by 5.00%~15.00% and Huanghuazhan's plant height was reduced by 3.51%~20.42% from the 7th to the 35th days. The spraying of Pro-Ca reduced the first tiller length of Xiangliangyou900 and Huanghuazhan by 8.77%~30.02% and 9.90%~26.17% from the 7th to the 35th days, respectively, and the second tiller length of the two varieties was reduced by 6.91%~22.69% and 6.52%~38.96% (Table 1). Compared with the S treatment, foliar spraying Pro-Ca under NaCl stress significantly alleviated the stem base width of each tiller position in both rice, in which the main stem basal width, first tiller basal width and second tiller basal width of Xiangliangyou900 were increased by 15.24%~44.07%, 30.87%~45.30%, and 10.58%~48.32%, respectively, from the 7th to the 35th days, the stem base width of each tiller position increased by 23.84%~47.56%, 11.19%~76.13%, and 36.28%~76.96%, respectively (Table 1). Compared with S treatment, foliar spraying Pro-Ca under NaCl stress increased the leaf area of main stem, first tiller leaf area and second tiller leaf area of Xianglaingyou900 by 30.50%~65.47%, 30.11%~165.73%, and 38.19%~115.18% from the 7th to the 35th days, respectively, and the leaf area of each tiller position of Huanghuazhan increased by 19.78%~81.97%, 74.45%~329.32%, and 69.65%~204.06%, respectively (Table 2). The root lengths of Xiangliangyou900 and Huanghuazhan under Pro-Ca+S treatment increased by 13.43% to 52.29% and 8.37% to 26.09%, respectively, from the 7th to the 35th days compared with that of S treatment, and the differences were significant (Table 2). As shown in Table 3, compared with NaCl, the main stem dry weight, first tiller dry weight and second tiller dry weight of Pro-Ca+S treatment of Xiangliangyou900 increased by 35.75%~137.61%, 2.51%~152.69%, and 28.29%~54.31%, respectively, and the dry weights of each tiller position of Huanghuazhan increased by 21.60%~88.90%, 61.33%~174.87%, and 61.22%~205.01%, respectively. Compared with the S treatment, foliar spraying of Pro-Ca alleviated the suppression of below-ground biomass by NaCl stress, and the root dry weight of Xiangliangyou900 increased by 23.64%~61.74% and that of Huanghuazhan increased by 39.22%~139.70% from the 7th to the 35th days (Table 3).

Effect of salt stress on antioxidant enzymes in rice leaves at each tiller position at tillering stage and regulation by Pro-Ca

Compared with the control, the SOD activity of the main stem leaves of Xiangliangyou900 increased by 6.88% to 31.25% from the 7th to the 35th days after salt treatment, and the SOD activity of the main stem leaves of Huanghuazhan decreased by 10.86% and 9.81% on the 7th and 14th days, respectively, and increased by 13.00% to 24.32% from the 21st to the 35th days (Fig. 2a and b). The SOD activity of the first tiller leaves of Xiangliangyou900 decreased by 12.55% and 9.39% on the 7th and 14th days after salt treatment, respectively, and that of the first tiller leaves of Huanghuazhan decreased by 14.48% on the 7th day, and did not show any significant difference compared with the control in the following days (Fig. 2c and d). Compared with the control, the SOD activity of the second tiller leaves of Xiangliangyou900 was significantly increased by 22.51% on the 7th day but did not change significantly from the 14th to the 35th days after NaCl treatment, however, the SOD activity of the second tiller leaves of Huanghuazhan decreased by 3.17% to 32.22% from the 7th to the 35th days(Fig. 2e and f).

As can be seen from Fig. 3a and b, compared with the control, salt stress reduced the CAT activity of main stem leaves of Xiangliangyou900 by 9.03% to 9.63% from the 7th to the 28th days, and that of Huanghuazhan main stem leaves by 1.89% to 16.99% from the 14th to the 35th days. Meanwhile, as shown in Fig. 3, NaCl stress reduced the CAT activity of the second tiller leaves of Xiangliangyou900 by 0.50% to 32.15% from the 7th to the 35th days, respectively, and that of Huanghuazhan's first tiller leaves by 3.90% to 8.54% from the 21st to 35th days after salt stress, and that of the second tiller leaves by 0.78% to 5.62%.

NaCl stress reduced the POD activity of main stem leaves of Xiangliangyou900 by 2.83% and 13.67% on the 7th and 14th days, respectively (Fig. 4a), and that of Huanghuazhan by 13.01% and 6.55% , respectively, compared with the control (Fig. 4b). In addition, salt stress reduced the POD activity of the first tiller leaves of Xiangliangyou900 by 10.81% and 8.82% on the 7th and 14th days, and increased it by 5.12% and 24.40% on the 21st and 28th days, respectively, compared with the CK (Fig. 4c). The POD activity of the first tiller leaves of Huanghuazhan was reduced by 5.71% to 10.71% from the 7th to the 21stdays and increased by 77.45% and 66.59% at the 28th and 35th days, respectively (Fig. 4d). Under salt stress, the POD activities of the second tiller leaves of Xiangliangyou900 and Huanghuazhan were reduced by 3.69%~44.75% and 4.67%~26.11%, respectively, compared with the control from the 7th to the 35th days (Fig. 4e and f).

The results in Fig. 5a and b showed that NaCl stress increased increased the APX activity of main stem leaves of Xiangliangyou900 by 9.02% to 89.09% from the 14th to the 35th days, and decreased the APX activity of Huanghuazhan's by 56.24% and 7.31% on the 7th and 14th days, respectively, and increased it by 8.55% to 16.30% from the 21st to 35th days. Compared with the CK treatment, the APX activity of the first tiller leaves of Xiangliangyou900 and Huanghuazhan decreased by 12.80%~54.26% and 3.38%~50.04%, respectively, from the 7th to the 35th days after NaCl stress (Fig. 5c and d), and that of the second tiller leaves of the two varieties decreased by 5.62%~34.90% and 2.74%~33.33%, respectively (Fig. 5e and f).

Under Pro-Ca+S treatment, the SOD activity of main stem leaves of Xiangliangyou900 significantly increased by 3.08% to 18.45% from the 7th to the 35th days, and that of Huanghuazhan was only increased by 4.76% and 0.09% on the 7th and 14th days, respectively (Fig. 2a and b). The SOD activity of the first tiller leaves of both varieties were increased by 1.93% to 21.78% and 4.14% to 31.35% from the 7th to the 35th days after spraying with Pro-Ca compared to S treatment, respectively (Fig. 2c and d). In addition, it can be seen from Fig. 2e and f that under salt stress, spraying Pro-Ca increased the SOD activity of the second tiller leaves of Xiangliangyou900 by 11.64% and 10.13% on the 28th and 35th days, respectively, and that of Huanghuazhan by 5.16%~23.86% from the 21st to the 35th days.

Compared with salt stress, spraying Pro-Ca before salt stress had no significant effect on the CAT activity of leaves of all tillers of Xiangliangyou900, in which, it increased the CAT activity of leaves of the main stem of Huanghuazhan by 6.18%~17.17% from the 14th to the 28th days, respectively. Compared with the S treatment, spraying Pro-Ca increased the CAT activity of the first tiller leaves of Huanghuazhan by 2.37%~7.12% from the 14th to the 28th days, respectively (Fig. 3).

As can be seen in Fig. 4, the POD activity of the main stem leaves of Xiangtwoyou 900 under Pro-Ca+S treatment increased by 2.38% to 51.79% from the 7th to the 28th days, and that of the main stem leaves of Huanghuazhan increased by 8.27% to 26.63% from the 7th to the 21st days, as compared with that of S treatment. The POD activities of the first tiller and second tiller leaves of Xiangliangyou 900 under Pro-Ca+S treatment increased by 0.45% to 12.84% and 0.83% to 23.74%, respectively, from the 7th to the 35th days (Fig. 4c and e). In addition, the spraying of Pro-Ca increased the POD activity of the first tiller leaves of Huanghuazhan under salt stress by 4.84% to 26.07% from the 7th to the 28th days after salt stress, and that of the second tiller leaves by 9.82% to 34.71% from the 7th to the 35th days (Fig. 4d and f).

Compared with NaCl stress alone, spraying Pro-Ca under NaCl stress increased the APX activity of main stem leaves of Xiangliangyou900 by 8.48% to 38.46% from the 7th to the 35th days (Fig. 5a). The APX activity of main stem leaves of Huanghuazhan increased by 1.10% to 57.35% from the 7th to the 35th days, but not significantly (Fig. 5b). Spraying Pro-Ca under NaCl stress increased the APX activity of the first tiller leaves of Xiangliangyou900 and Huanghuazhan by 6.15%~79.71% and 19.02%~103.47%, respectively, from the 7th to the 35th days (Fig. 5c and d). The APX activity of the second tiller leaves of Xiangliangyou900 was increased by 2.38%~40.31% from the 14th to the 35th days (Fig. 5e). The activity of the second tiller leaves of Huanghuazhan was increased by 0.15%~51.94% from the 7th to the 28th days (Fig. 5f).

Effect of salt stress on antioxidant enzymes in rice stems at each tiller position at tillering stage and regulation by Pro-Ca

Compared with the CK treatment, the SOD activity of the main stem of Xiangliangyou900 decreased by 7.64% to 30.56% from the 7th to the 35th days after NaCl treatment, and that of the main stem of Huanghuazhan decreased by 1.43% to 13.72% from the 21st to the 35th days (Fig.

6a and b). The SOD activity in the stem of the first tiller of Xiangliangyou900 decreased by 3.81% to 11.08% from the 7th to the 35th days after NaCl treatment, and that in the stem of the second tiller decreased by 1.88% to 32.36% from the 7th to the 28th days (Fig. 6c and e). NaCl stress reduced the SOD activity in the stem of the first tiller of Huanghuazhan by 4.98% to 52.21% from the 21st to the 35th days, and reduced the SOD activity in the stem of the second tiller by 3.27% to 26.61% from the 7th to the 35th days, respectively (Fig. 6d and f).

Fig. 7 shows that NaCl stress reduced the main stem CAT activity of Xiangliangyou900 by 5.85% to 25.60% from the 7th to the 28th days. The CAT activity of Huanghuazhan decreased by 6.67% and 26.94% on the 14th and 35th days, respectively, and increased but not significantly at the 7th, 21st,and 28th days. The CAT activity of the first tiller stems of Xiangliangyou900 decreased by 3.10% to 26.99% from the 21st to the 35th days, and that of Huanghuazhan decreased by 3.14%, 3.27%, and 45.44% at the 7th, 21st, and 35th, respectively (Fig. 7c and d). NaCl stress reduced the CAT activity in the stem of the second tiller of Xiangliangyou900 by 8.69% to 38.70% from the 7th to the 28th days after NaCl stress, and that of Huanghuazhan by 10.25% to 37.52% from the 7th to the 35th days (Fig. 7e and f).

Compared with their respective CKs, the POD activity of main stem of Xiangliangyou900 under NaCl stress increased by 5.02% to 44.88% from the 7th to the 35th days, and that of Huanghuazhan increased by 7.39% to 11.37% from the 7th to the 21st days, and decreased by 18.49% and 35.13% at the 28th and 35th, respectively (Fig. 8a and b). The POD activity of the first tiller stems of Xiangliangyou900 decreased by 5.51%% to 45.94% in 7~35 days under salt stress (Fig. 8c). The POD activity in the stem of the first tiller of Huanghuazhan decreased by 2.64% to 10.67% from the 7th to the 28th days, and increased by 4.11% at the 28th day (Fig. 8d). Compared with the control, salt stress reduced the POD activity in the stem of the second tiller by 2.48% to 25.92% and 2.68% to 25.68% in the stem of the second tiller of Xiangliangyou900 and Huanghuazhan, respectively, from the 7th to the 35th days (Fig. 8e and f).

The main stem APX activity of Xiangliangyou900 decreased by 12.12% to 39.98% from the 7th to the 35th days after NaCl stress, and that of Huanghuazhan decreased by 2.65% to

34.28%, respectively (Fig. 9a and b). The APX activity in the stem of the first and second tillers of both varieties decreased by 2.57% to 49.67% and 5.29% to 64.09%, 21.47% to 63.03% and 21.35% to 34.96%, respectively, from the 7th to the 35th days after salt stress (Fig. 9).

Compared with the S treatment, spraying Pro-Ca under salt stress increased the SOD activity of the main stem of Xiangliangyou900 by 13.66% to 65.88% from the 7th to the 28th days, and that of the main stem of Huanghuazhan by 2.05% to 7.96% from the 21st to the 35th days (Fig. 6a and b), the SOD activities in the stem of the first tiller increased by 6.34%~51.90% and 6.91%~97.69% from the 7th to the 35th days in Xiangliangyou900 and Huanghuazhan, respectively (Fig. 6c and d). Spraying Pro-Ca under salt stress increased the SOD activity of the second tiller stems of Xiangliangyou900 by 2.68% to 123.51% from the 14th to the 35th days, and that of the second tiller stems of Huanghuazhan by 3.27% to 26.61% from the 7th to the 35th days (Fig. 6e and f).

Compared with the S treatment, the CAT activities of main stems sprayed with Pro-Ca Xiangliangyou900 and Huanghuazhan under salt stress increased by 5.72% to 92.16% and 6.04% to 40.09% from the 7th to the 35th days, respectively (Fig. 7a and b). Foliar spraying of Pro-Ca also effectively increased the CAT activity in the first tiller stems of two rice varieties under salt stress, which was increased by 0.83%~30.00% at the 7~28 days in Xiangliangyou900 and by 3.59%~95.57% at the 7~35 days in Huanghuazhan (Fig. 7c and d). CAT activity in the second tiller stems of both varieties was increased by 0.93% to 66.28% and 7.57% to 65.13% under Pro-Ca+S treatment compared to S, respectively (Fig. 7e and f).

Compared with the S treatment alone, spraying Pro-Ca under salt stress increased the main stem POD activity by 5.89% to 51.39% and 4.76% to 33.32% from the 7th to the 35th days in Xiangliangyou900 and Huanghuazhan, respectively. The POD activity in the first and second tiller stems of both varieties increased by 0.18% to 24.07% and 13.49% to 98.03%, 14.51% to 31.29% and 4.15% to 33.22%, respectively, from the 7th to the 35th days (Fig. 8).

Compared with salt stress alone, spraying Pro-Ca under NaCl stress increased the main stem APX activity of Xiangliangyou900 by 11.43% to 87.63% from the 7th to the 35th days, and that of Huanghuazhan by 10.98% to 31.58%, respectively (Fig. 9a and b). It increased the APX activity of the first and second tiller stems of both varieties by 0.85% to 103.18% and 10.74% to 99.20%, 4.11% to 34.69% and 18.49% to 83.24%, respectively, from the 7th to the 35th days (Fig. 9).

Effect of salt stress on membrane damage index in rice leaves at each tiller position at tillering stage and regulation by Pro-Ca

Salt stress significantly increased the MDA content of leaves of two rice varieties compared with the control (Fig. 10). Among them, the MDA contents of main stem, first tiller and second tiller leaves of Xiangliangyou900 increased by 13.04%~54.61%, 0.58%~79.40%, and 3.33%~39.59%, respectively, from the 7th to the 35th days after salt stress (Fig. 10a, c, and e), the MDA content of the leaves of each tiller position of Huanghuazhan increased by 10.62% to 127.93%, 13.25% to 75.94%, and 6.01% to 64.67%, respectively, from the 7th to the 35th days after salt stress (Fig. 10b, d, and f). In addition, as seen in Fig. 11, salt stress increased the H_2O_2 content of the main stem leaves of Xiangliangyou900 and Huanghuazhan by 4.57%~38.51% and 3.88%~21.84% at 7~35 days, and the H_2O_2 content of the first tiller leaves of two kinds of rice by 5.24%~39.80% and 0.44%~41.06%. The H_2O_2 content of the second tiller leaves of the two varieties was increased by 16.46%~28.88% and 8.37%~58.75% at 7~35 days, respectively (Fig. 11).

Compared with S treatment, foliar spraying of Pro-Ca before salt stress decreased the MDA content of main stem leaves of Xiangliangyou900 and Huanghuazhan by 0.53%~27.59% and 8.65%~25.00%, respectively (Fig. 10a and b), and that of the first tiller leaves of the two varieties under the same conditions by 17.24%~26.80% and 2.39%~29.12%, respectively (Fig. 10c and d), from the 7th to the 35th days. The MDA contents of second tiller leaves of the two varieties under the same conditions were decreased by 0.09%~34.98% and 1.60%~26.93%, respectively (Fig. 10e and f). Spraying Pro-Ca before salt stress reduced the H_2O_2 content of main stem leaves of Xiangliangyou900 and Huanghuazhan by 0.63%~22.87% and 5.91%~42.28%, respectively, from the 7th to the 35th days (Fig. 11a and b). Spraying Pro-Ca reduced the H_2O_2 content of the first tiller leaves of Xiangliangyou900 by 4.03%~31.42% and that of Huanghuazhan by 7.56%~26.04%, respectively at 7~35 days (Fig. 11c and d), and reduced the H_2O_2 content of the second tiller leaves of both rice varieties by 5.49%~29.10% and 8.23%~34.97% (Fig. 11e and f). The staining test revealed that there were more spots on the leaves of both rice varieties under salt stress, and the spots on the tiller leaves were larger in area and darker in color. In contrast, the area of spots on the leaves of the treatments sprayed with Pro-Ca before salt stress decreased and became lighter in color (Fig. 12).

Effect of salt stress on membrane damage index in rice stems at each tiller position at tillering stage and regulation by Pro-Ca

Compared with the control, NaCl stress increased the MDA content of the main stem of Xiangliangyou900 and Huanghuazhan by 14.85%~115.59% and 39.38%~95.94%, respectively (Fig. 13a and b), and that of the first tiller stems of Xiangliangyou900 and Huanghuazhan by 4.98%~90.72% and 9.55%~72.13%, respectively, from the 7th to the 35th days after salt stress (Fig. 13c and d), and the MDA content of the second tiller stems of the two varieties increased by 22.21%~156.61% and 7.98%~61.68%, respectively (Fig. 13e and f). NaCl stress at 0.3% also significantly increased the H_2O_2 content in the stems of both rice varieties (Fig. 14). Among them, salt stress increased the H_2O_2 content of the main stem of Xiangliangyou900 by 1.28% to 6.12% from the 7th to the 35th days, and increased the H_2O_2 content of the main stem of Huanghuazhan by 7.61% to 24.09% from the 7th to the 28th days (Fig. 14a and b). Salt stress increased the H_2O_2 content of the first tiller stem of Xiangliangyou900 by 17.28%, 14.83%, and 8.49% at the 7th, 14th, and 35th days, respectively, and that of Huanghuazhan by 0.63%~9.16% at 14~35 days (Fig. 14c and d). In addition, NaCl stress increased the H_2O_2 content in the stem of the second tiller of Xiangliangyou900 by 1.37%~18.04% from the 7th to the 35th days, and there was no significant change on the 28th day, that of Huanghuazhan increased the content by 2.67% and 28.36% on the at the 7th and 21st days (Fig. 14e and f).

Compared with S treatment, spraying Pro-Ca before salt stress caused a significant decrease in MDA content, in which the MDA content of the main stems of Xiangliangyou900 and Huanghuazhan decreased by 2.53% to 48.87% and 14.94% to 35.39%, respectively, from the 7th to the 35th days (Fig. 13a and b). The MDA content of the first tiller stems of the two varieties sprayed with Pro-Ca decreased by 3.79%~46.45% and 5.39%~27.28%, respectively, from the 7th to the 35th days after salt stress (Fig. 13c and d), compared with S treatment, spraying Pro-Ca decreased the MDA content of the second tiller stems of Xiangliangyou900 and Huanghuazhan by 1.70%~66.09% and 0.32%~37.09% from the 7th to the 35th days, respectively (Fig. 13e and f). Foliar spraying of Pro-Ca before salt stress reduced H_2O_2 in the main stem of Xiangliangyou900 by 7.07%~12.62% from the 7th to the 28th days, and that of Huanghuazhan by 2.79%~14.74% from the 7th to the 35th days (Fig. 14a and b), and reduced the H_2O_2 content in the stem of the first tiller of Xiangliangyou900 and Huanghuazhan by 9.58%~15.60% and 3.20%~12.45% from the 7th to the 35th days respectively (Fig. 14c and d). Compared with salt stress, spraying Pro-Ca reduced the H_2O_2 content in the stem of the second tiller of the two rice varieties by 1.35%~33.05% and 7.92%~24.15%, respectively, from the 7th to the 35th days (Fig. 14e and f).

Effect of salt stress on soluble protein content in rice leaves at each tiller position at tillering stage and regulation by Pro-Ca

As can be seen from Fig. 15, the soluble protein content of the main stem leaves of Xiangliangyou900 increased by 0.88% to 2.59% from the 7th to the 28th days after salt stress, and the soluble protein content of the main stem leaves of Huanghuazhan decreased by 1.59% and 2.87% on the 7th and 14th days after salt stress, respectively, and increased by 0.44% to 2.41% from the 21st to 35th d. NaCl stress increased the soluble protein content of the first tiller leaves of Xiangliangyou900 by 0.61% and 5.33% on the 21st and 28th days, respectively, and decreased the soluble protein content of the first tiller leaves of Huanghuazhan by 2.06% to 4.22% from the 7th to the 28th days after NaCl stress (Fig. 15c and d). The soluble protein content of the second tiller leaves of Xiangtwoyou 900 increased by 3.27% and 0.45% on the 7th and 21st after NaCl stress, and decreased that of Huanghuazhan by 0.31% to 4.63% from the 7th to the 35th days (Fig.

15e and f).

Compared with the S treatment, the soluble protein content of the main stem leaves of Xiangliangyou900 increased by 0.21% to 4.67% from the 7th to the 35th days in Pro-Ca+S treatment, and that of the main stem leaves of Huanghuazhan increased by 1.28% to 1.90% from the 7th to the 21st days (Fig. 15a and b). In addition, spraying Pro-Ca under salt stress increased the soluble protein content of the first tiller leaves of Xiangliangyou900 by 4.30%, 0.25%, and 1.94% at the 7th, 14th, and 35th, respectively, and that of Huanghuazhan by 0.57% to 4.64% from the 7th to the 35th days (Fig. 15c and d). Foliar spraying of Pro-Ca increased the soluble protein content of the second tiller leaves of Xiangliangyou900 by 0.55% to 4.38% from the 14th to the 35th days and Huanghuazhan by 0.16% to 5.15% from the 7th to the 35th days after salt stress (Fig. 15e and f).

Effect of salt stress on soluble protein content in rice stems at each tiller position at tillering stage and regulation by Pro-Ca

As can be seen from the Fig. 16a and b, the soluble protein content of the main stem of Xiangliangyou900 increased by 1.92% to 9.30% from the 14th to the 35th days after salt stress, and the soluble protein content of the main stem of Huanghuazhan increased by 1.03% to 7.41% from the 7th to the 28th days. Salt stress reduced the soluble protein content of the first tiller stems of Xiangliangyou900 by 0.31%, 5.92%, and 3.83% at the 7th, 28th, and 35th days, and reduced the soluble protein content of the first tiller stems of Huanghuazhan by 1.48% and 1.12% at the 21st and 35th days, respectively (Fig. 16c and d). The soluble protein content of the second tiller stems of Xiangliangyou900 decreased by 0.88% to 12.45% from the 7th to the 35th days after salt stress, and there was no significant difference at the 14th day, while that of the second tiller stems of Huanghuazhan decreased by 4.81% to 12.49% from the 14th to the 35th days (Fig. 16e and f).

Spraying Pro-Ca under salt stress conditions increased the soluble protein content of the main stems of Xiangliangyou900 and Huanghuazhan by 0.97%~9.13% and 2.05%~15.38%, respectively, from the 7th to the 35th days after NaCl stress (Fig. 16a and b). Compared with the S treatment, the soluble protein content of the first tiller stems of Xiangliang900 under Pro-Ca+S treatment increased by 0.90% to 14.89% from the 7th to the 35th days, but there was no significant difference at the 21st day, and that of the first tiller stems of Huanghuazhan under Pro-Ca+S treatment increased by 3.89% to 22.64% from the 7th to the 35th days (Fig. 16c and d). Foliar spraying of Pro-Ca under NaCl stress increased the soluble protein content of the second tiller stems of Xiangliangyou900 and Huanghuazhan by 8.09% to 26.44% and 2.11% to 17.37%, respectively, from the 7th to the 35th days after salt stress (Fig. 16e and f).

Discussion

Salt stress is one of the major abiotic stresses leading to significant inhibition of crop growth and development. High levels of salt stress lead to an imbalance of internal ions in cells (Apel K & Hirt H., 2004). High concentrations of salt stress also increase the production of reactive oxygen species (ROS), such as mono-linear oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Zushi et al., 2009). Plants have also evolved complex mechanisms to counteract salt stress-induced oxidative stress, including antioxidant enzymes as well as non-enzymatic antioxidants, including SOD, CAT, and POD, which play an important role in scavenging ROS (Zou et al., 2015). Salt stress damage to crops has been validated in several crops (Kousar et al., 2021; Jung et al., 2017; Feng et al., 2023). Jian et al. (2022) demonstrated that growth rate was reduced after 1 day of 100 mM NaCl stress in rice, and more severe wilting symptoms appeared on the tips of rice plant leaves after 15 day. 'IR29' was particularly damaged, which contained high levels of ROS. Liu et al. (2022) in wheat (*Triticum aestivum* L.) also demonstrated a significant reduction in plant height and root length of wheat seedlings under saline stress. The results of this experiment showed that salt stress reduced the tiller number of Xiangliangyou900 and Huanghuazhan, delayed the development of the number of leaves in the main stem, and reduced the related morphological indexes such as plant height, stem base width and leaf area, and increased the indexes of membrane damage of leaves and stems in each tiller position of rice, which is in agreement with the findings obtained by Xu et al. (2008) and

Rasheed et al. (2014) in naked oat (*Avena nuda* L.) and canola (*Brassica napus* L.). It indicates that salt stress caused damage to growth and development of rice at the tillering stage.

In addition, by comparing the percentage reduction of morphological indicators such as plant height, stem base width, and leaf area at 7~35 days for each tiller position, it was found that the same concentration of salt stress damaged the morphological indicators of rice primary tillers to a stronger extent than the main stem. The main stem leaves showed stronger antioxidant capacity under salt stress through increased SOD, POD, and APX activities, CAT activity was reduced in all tillers under salt stress, and the main stem stem showed greater salt tolerance than the tillers under salt stress by increasing SOD, CAT, and POD enzyme activities and by a weaker reduction in APX. We hypothesized that this phenomenon might be due to a weaker degree of stress in the main stem on the one hand, and a greater tolerance in the main stem relative to the tillers on the other hand. Yang et al. (2022) showed that because tillers differentiate later than the main stem, the main stem always has an advantage in growth and development. Increased stress duration increases this dominance and asymmetric competition for C and N between the main stem and tillers reduces seed yield (Tilley et al., 2017). Therefore, we hypothesize that the leaf tiller co-extension law leads to a prior advantage of temperature and light resource utilization in the main stem and the first and second tillers, which contributes to the increase of physiological activity and the accumulation of nutrients.

Foliar spraying of Pro-Ca under salt stress effectively mitigated the damage of salt stress on morphogenesis at the tillering stage of rice, about which has been demonstrated in our previous studies (Zhang et al., 2023; Zhang et al., 2023; Huang et al., 2023), In addition to similar conclusions with previous experiments, the results of this experiment showed that foliar spraying of Pro-Ca had a stronger effect on tiller morphogenesis than on the main stem. Pro-Ca effectively alleviated the oxidative damage caused by salt stress on rice leaves. Foliar spraying of Pro-Ca increased the SOD and APX activities of tiller leaves and stems to a stronger extent than that of the main stems, but the effect on CAT activity of main stem leaves was more pronounced; moreover, Pro-Ca showed a better modulation in the alleviation of membrane damage and in the increase of soluble protein content. This may be related to the spraying site of Pro-Ca. In this experiment, the treatment was carried out at the seedling stage, the sampling period was at the tillering stage, and the tillers measured were developed from the leaf axils of the leaves that were sprayed with Pro-Ca in the previous period, so the regulator may have a better regulatory effect on the site it was sprayed to. In previous experiments, it was found that even though oat T1 and T2 tillers were not directly applied with ethylene and chlormequat chloride in the experiment, it still resulted in slow stem growth. This may be due to the conversion from chlormequat chloride-treated to untreated parts of the plant, an effect also demonstrated in wheat (Kang et al., 2010; Peltonen-Sainio et al., 2003).

Conclusion

This experiment demonstrated that salt stress inhibited the growth of leaves and stems of rice tillers at the tillering stage, and that exogenous spraying of Pro-Ca effectively alleviated the oxidative damage caused by salt stress on the tillers, and the effect on the tillers was stronger than that on the main stems under the same conditions. This experiment also provides new insights into the differential effects of salt stress on rice tillers and the regulatory effects of Pro-Ca.

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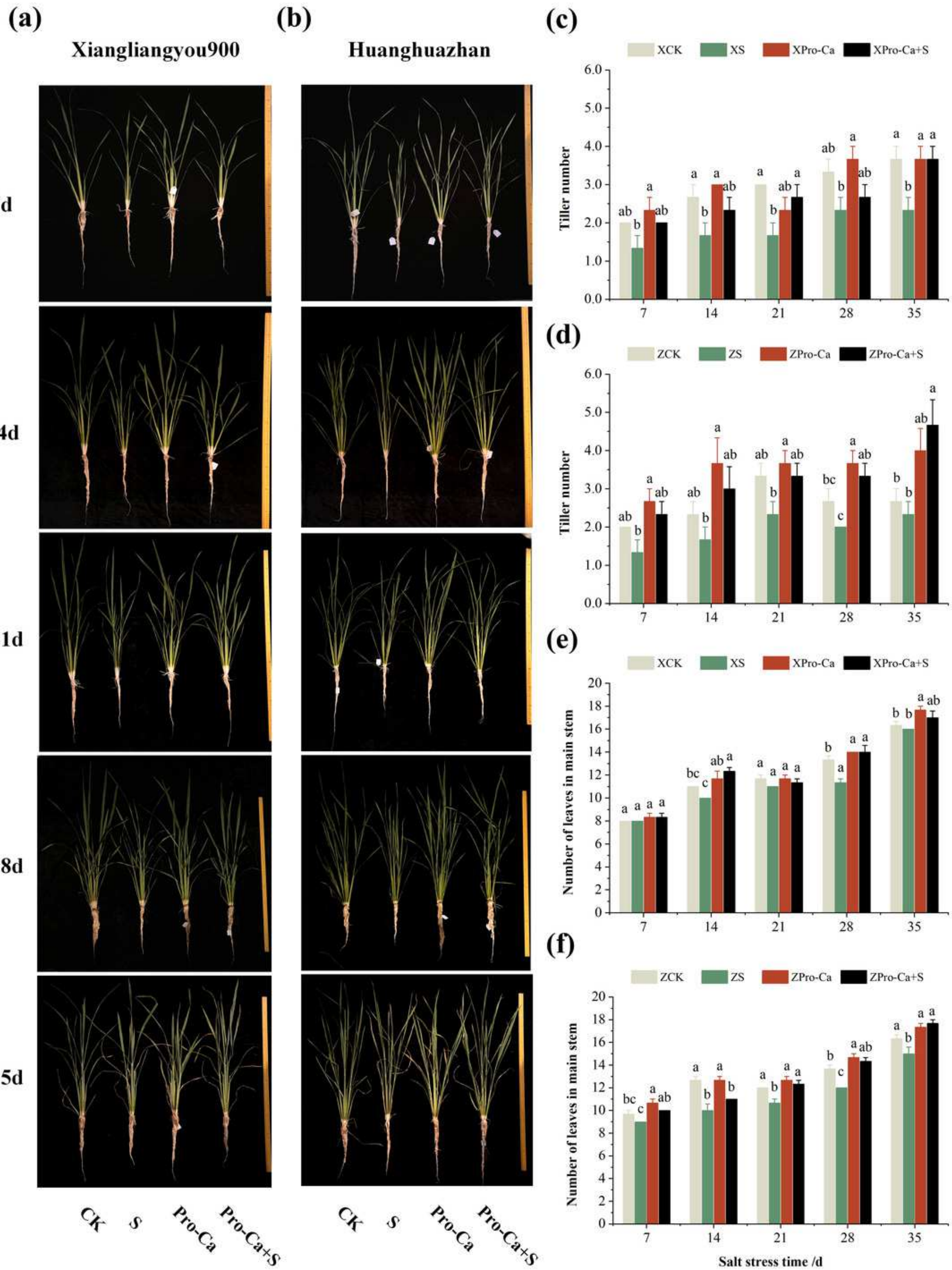
Figure 1

Effects of Pro-Ca on rice growth under salt stress.

(a-b) Plant growth. Rice morphology after 7, 14, 21, 28, and 35d of salt stress. Figure (a) shows the form of Xiangliangyou900, and figure (b) shows Huanghuazhan.(c-d) Tiller numbers of Xiangliangyou900 and Huanghuazhan in the main stem between different treatments.(e-f) Leaf numbers in the main stem. Comparison of tiller numbers and leaf numbers in the main stem between different treatments. Values are means ± SD (n = 3) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan's test). Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L Pro-Ca), XPro-Ca+S (100 mg•L Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L Pro-Ca), ZPro-Ca+S (100 mg•L Pro-Ca + 0.3% NaCl).



(a)
Xiangliangyou900
(b)
Huanghuazhan
7d
14d
21d
28d
35d
CK
S
Pro-Ca
Pro-Ca+S
(c)
XCK
XS
XPro-Ca
XPro-Ca+S
Tiller number
(d)
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
Tiller number
(e)
XCK
XS
XPro-Ca
XPro-Ca+S
Number of leaves in main stem
(f)
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
Number of leaves in main stem
Salt stress time /d

Figure 2

Effect of Pro-Ca on SOD activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

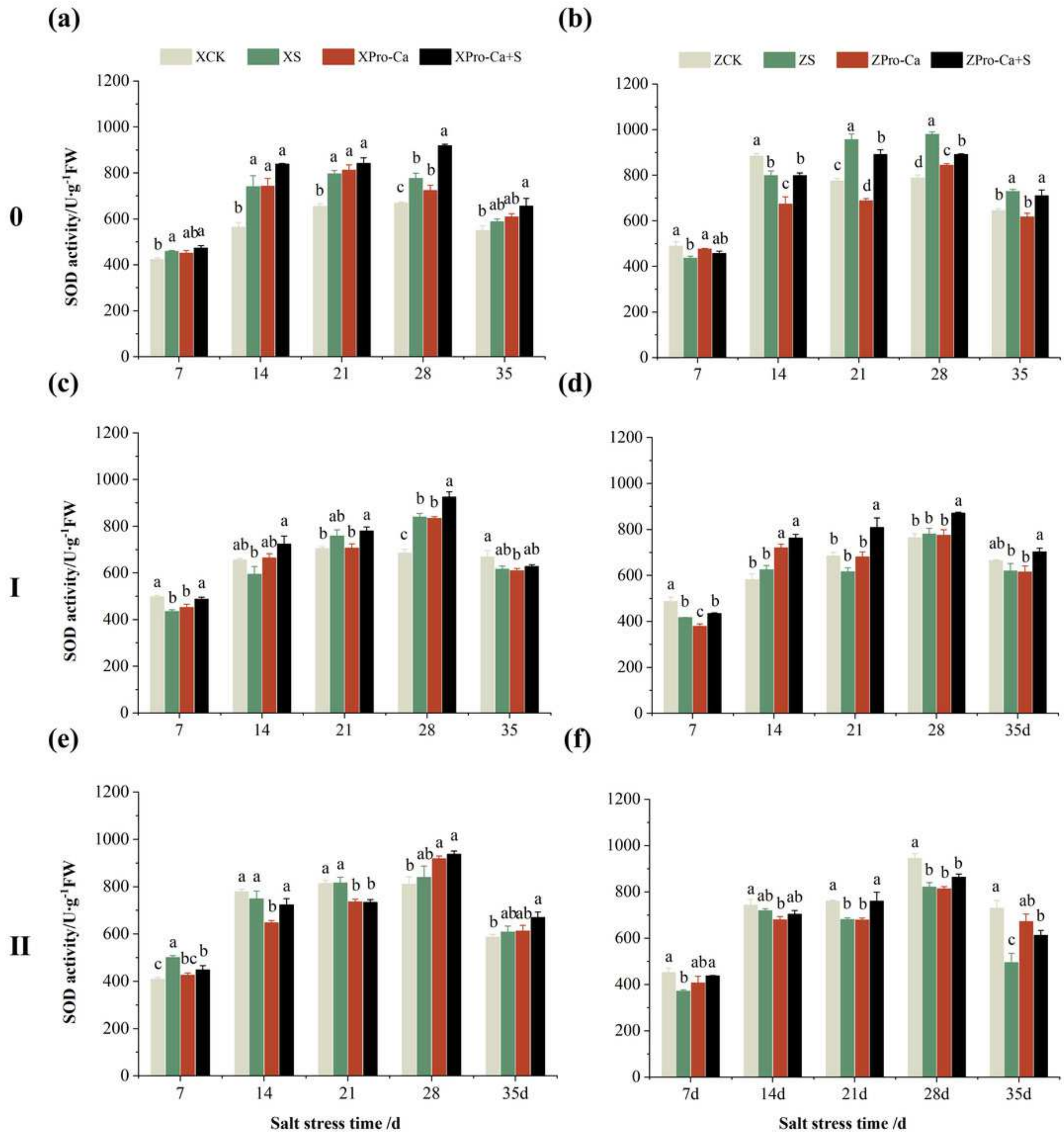

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
(c)
(d)
(e)
(f)
SOD activity/U·g⁻¹FW
Salt stress time /d
7
14
21
28
35
35d
7d
14d
21d
28d

Figure 3

Effect of Pro-Ca on CAT activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan’s new multiple range test ($P < 0.05$) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

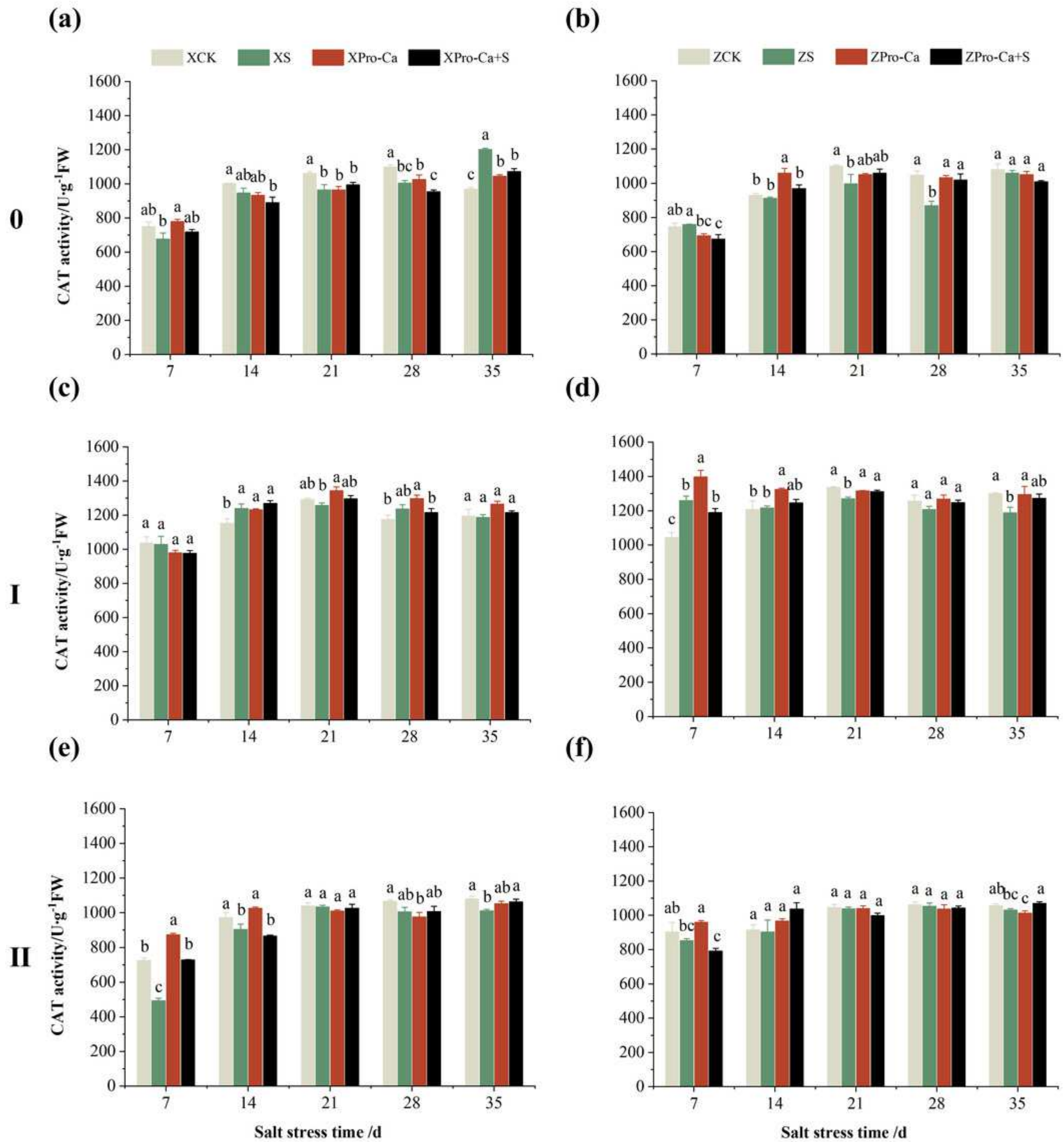

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
(c)
(d)
I
(e)
(f)
II
CAT activity/U·g-1FW
Salt stress time /d

Figure 4

Effect of Pro-Ca on POD activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan's new multiple range test ($P < 0.05$) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

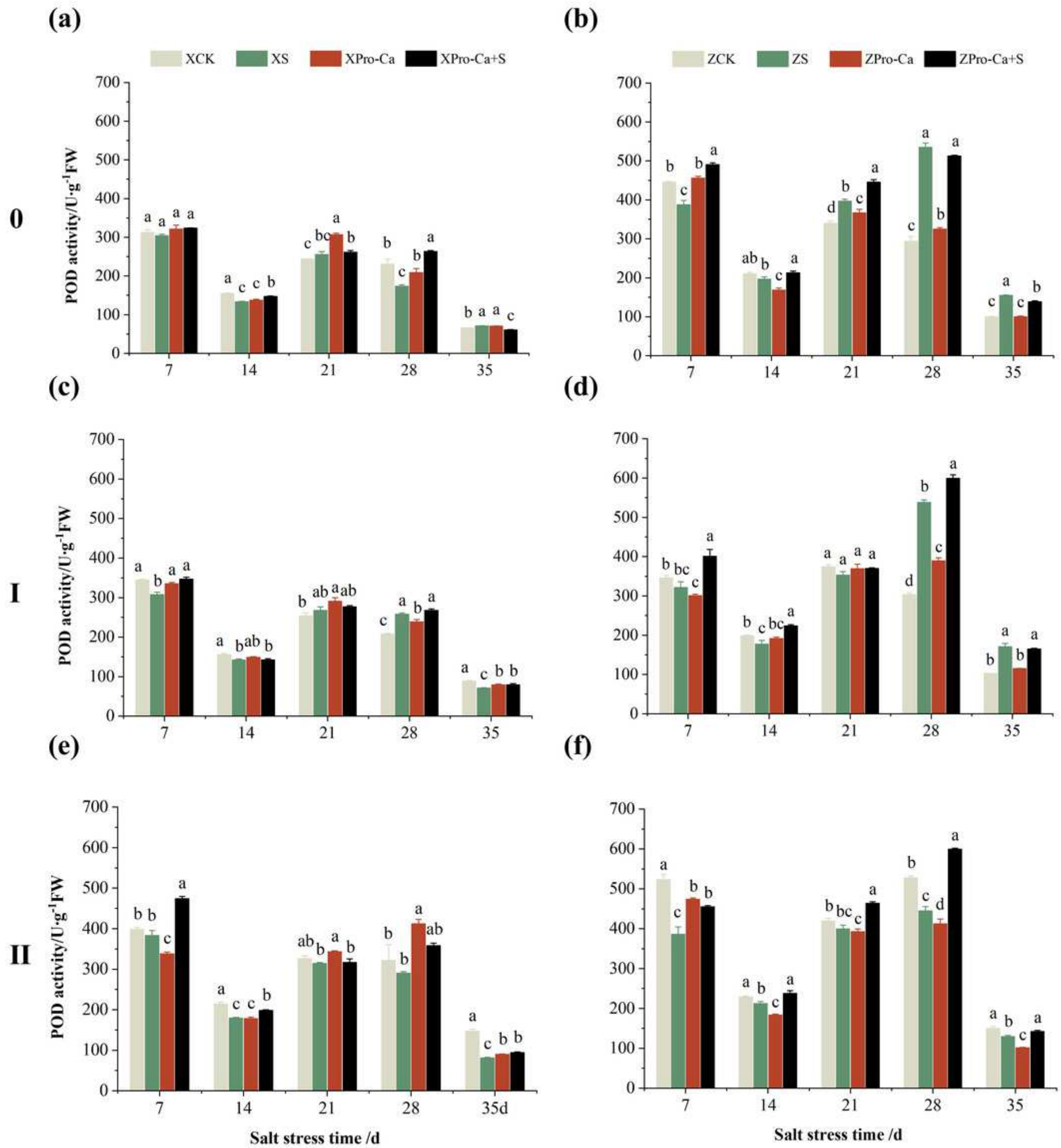

(a)
(b)
(c)
(d)
(e)
(f)
0
I
II
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
POD activity/U·g-1FW
Salt stress time /d
7
14
21
28
35
35d

Figure 5

Effect of Pro-Ca on APX activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan’s new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

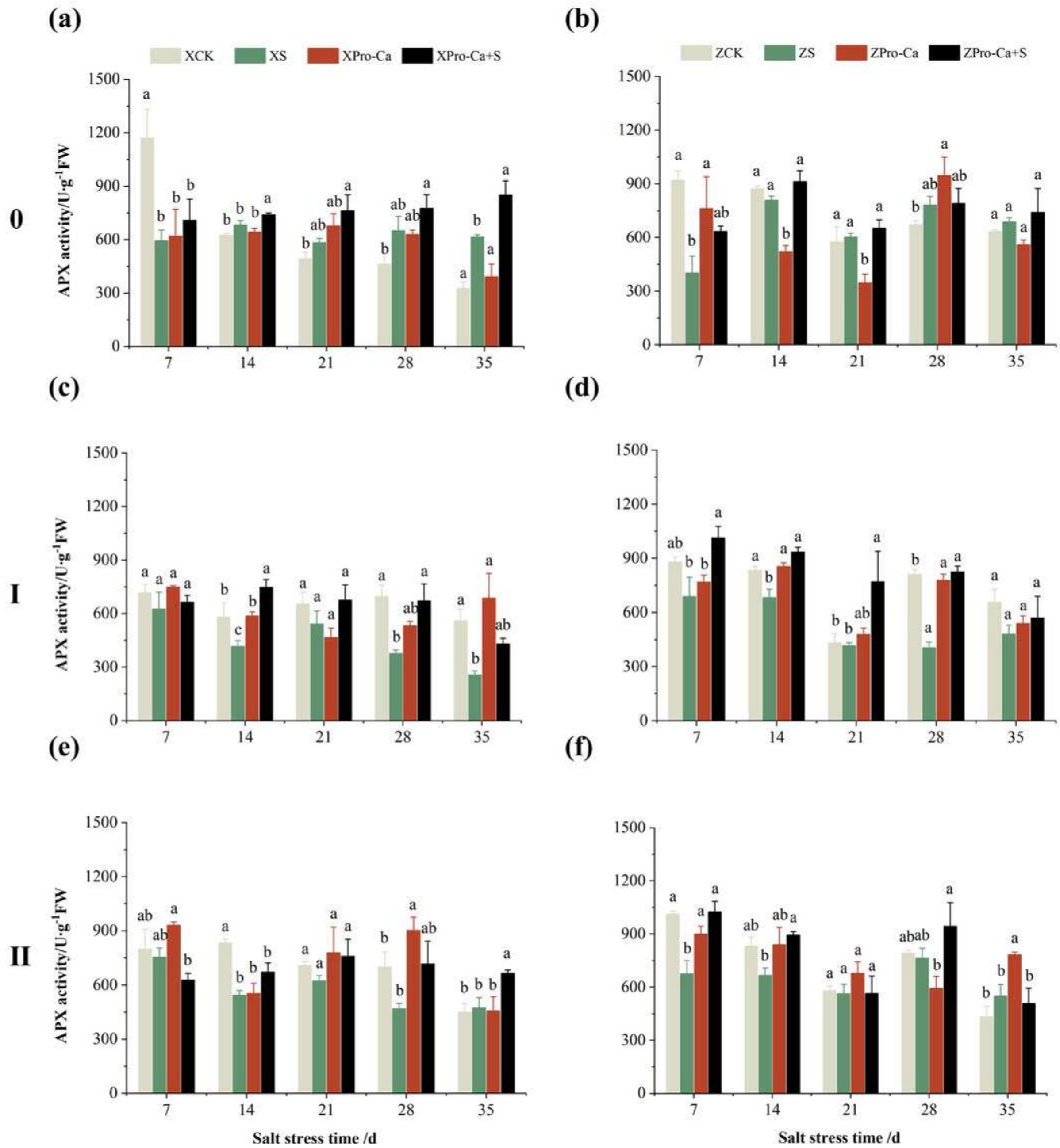

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
(c)
(d)
(e)
(f)
APX activity/U·g⁻¹FW
Salt stress time /d

Figure 6

Effect of Pro-Ca on SOD activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test ($P < 0.05$) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

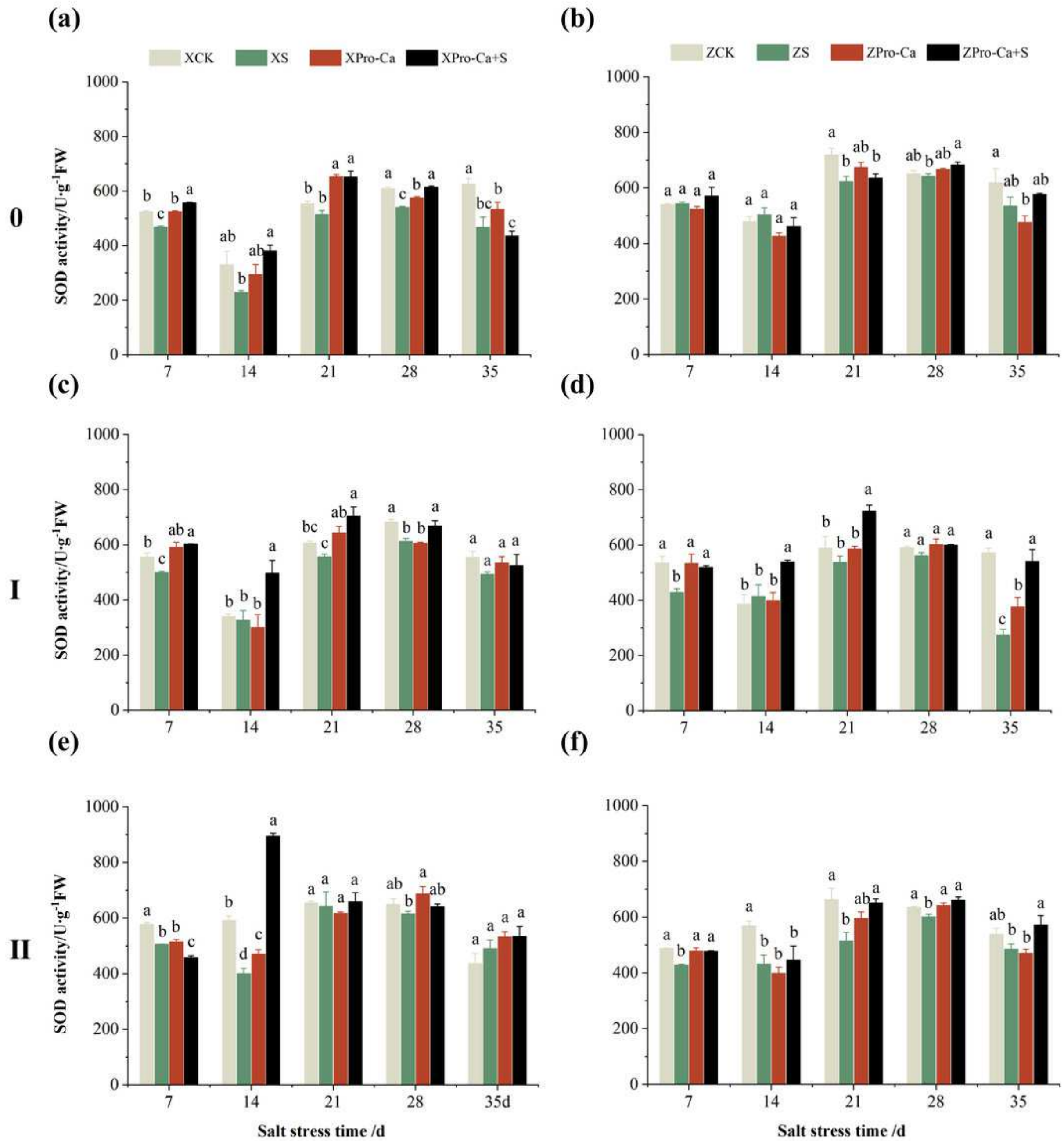

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
(c)
(d)
(e)
(f)
SOD activity/U·g⁻¹FW
Salt stress time /d

Figure 7

Effect of Pro-Ca on CAT activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan’s new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

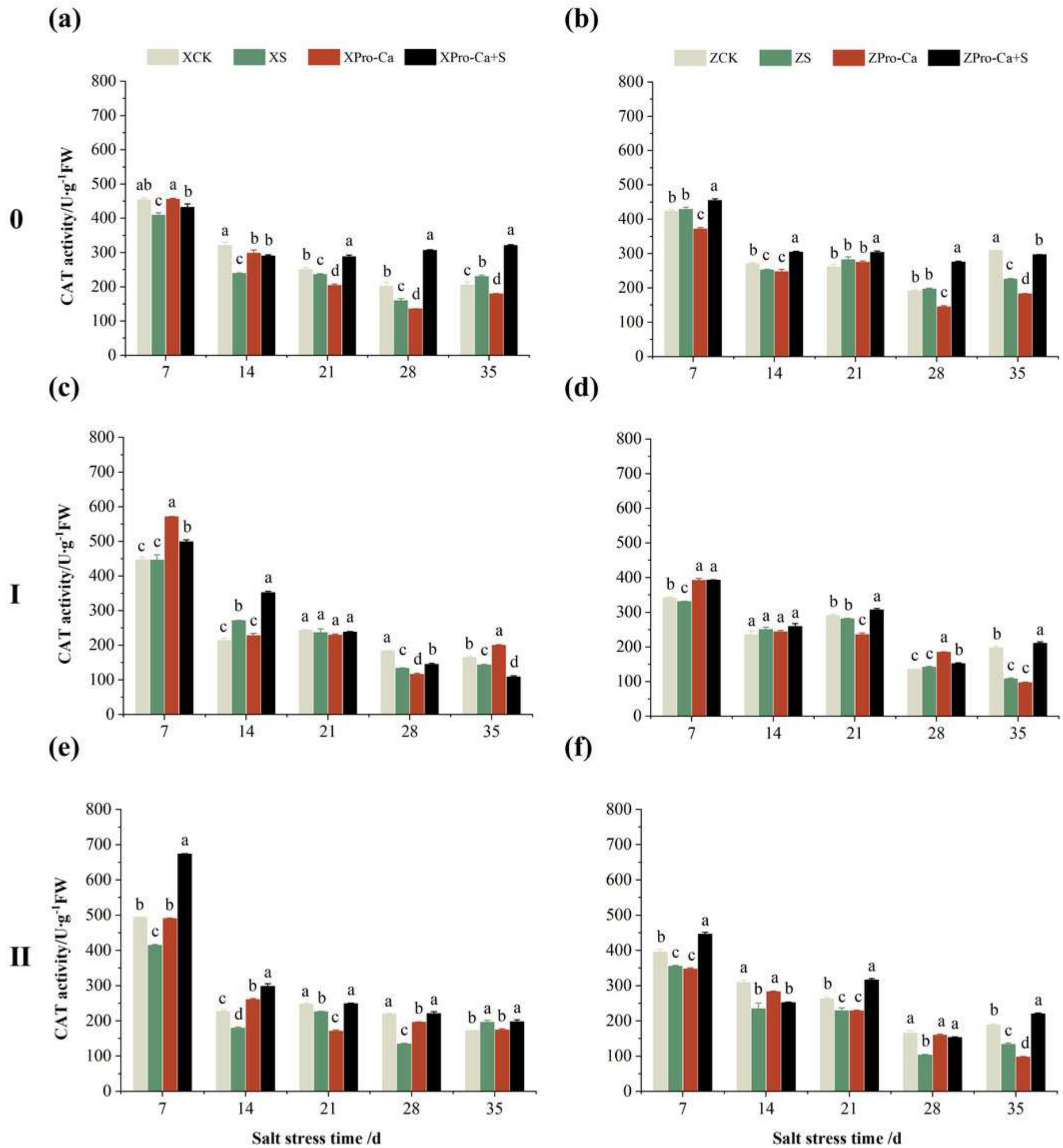

(a)
(b)
(c)
(d)
(e)
(f)
XCK XS XPro-Ca XPro-Ca+S
ZCK ZS ZPro-Ca ZPro-Ca+S
0
I
II
CAT activity/U·g-1FW
Salt stress time /d
Salt stress time /d

Figure 8

Effect of Pro-Ca on POD activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test ($P < 0.05$) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

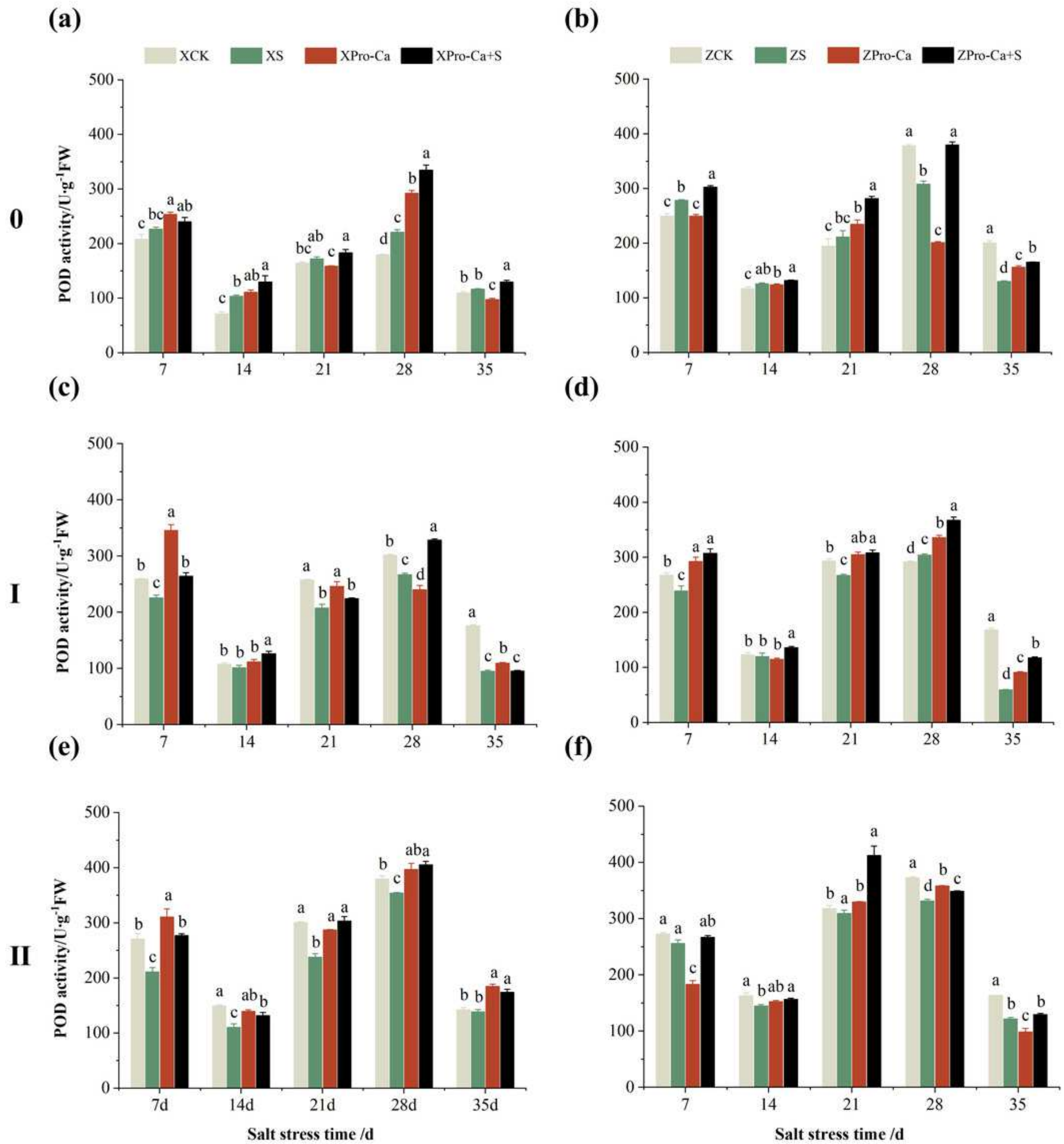
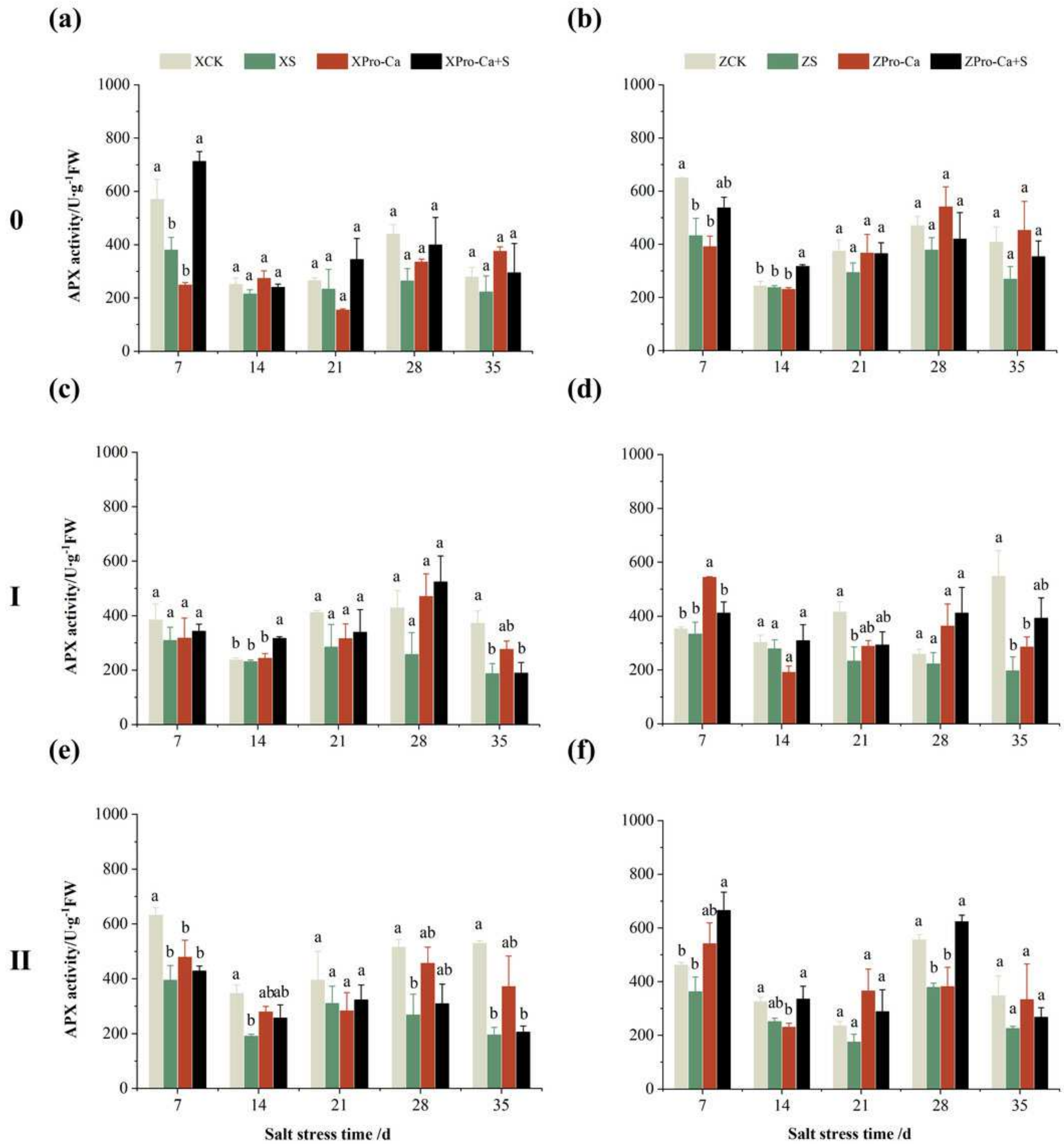

(a)
(b)
XCK XS XPro-Ca XPro-Ca+S
ZCK ZS ZPro-Ca ZPro-Ca+S
0
(c)
(d)
I
(e)
(f)
II
POD activity/U·g-1FW
Salt stress time /d

Figure 9

Effect of Pro-Ca on APX activity of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).


(a)
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XPro-Ca+S
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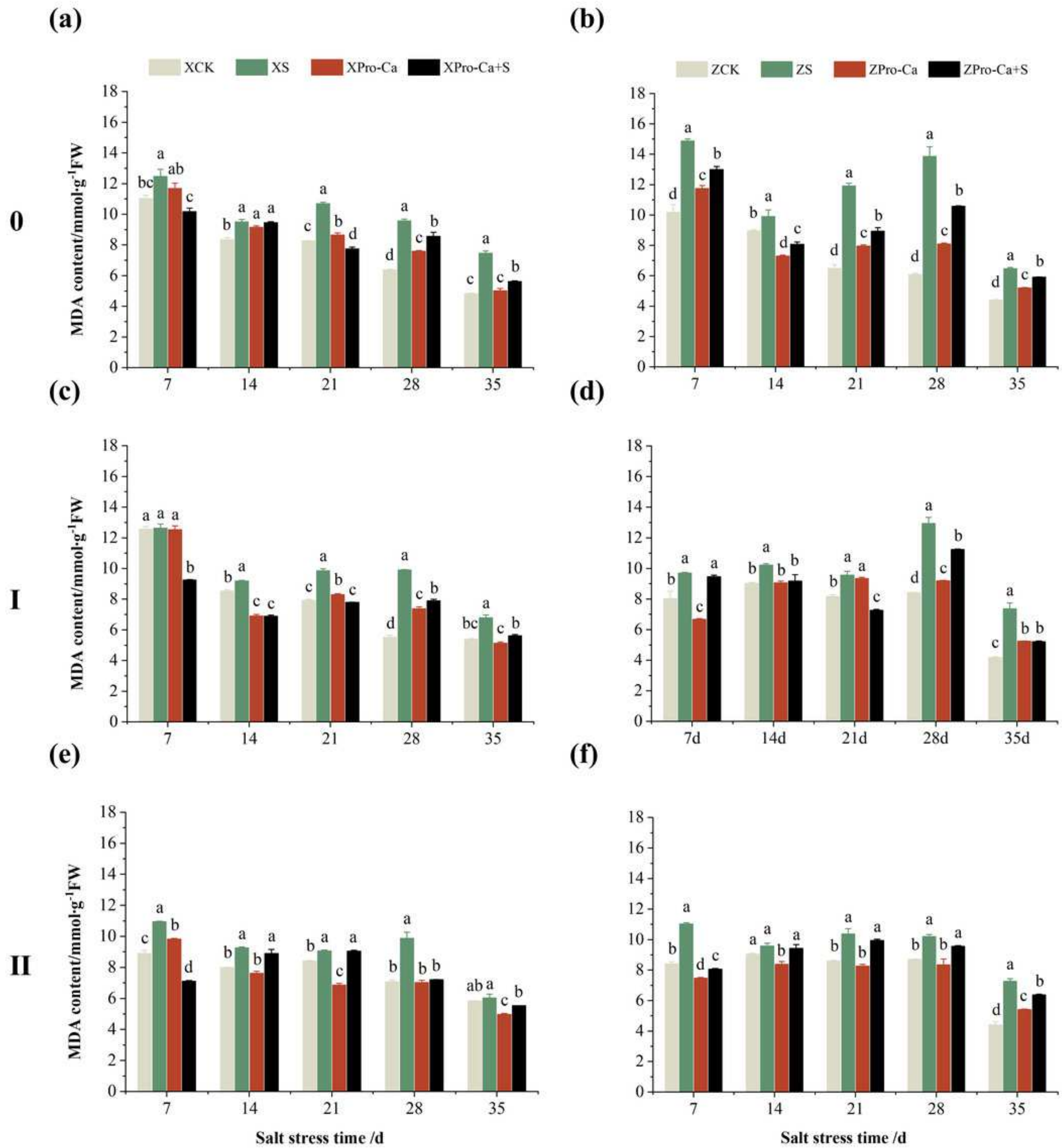
ZS
ZPro-Ca
ZPro-Ca+S
APX activity/U·g-1FW
Salt stress time /d

Figure 10

Effect of Pro-Ca on MDA content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (P < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).



(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
(c)
(d)
I
(e)
(f)
II
MDA content/mmol·g⁻¹FW
Salt stress time /d

Figure 11

Effect of Pro-Ca on H_2O_2 content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (P < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

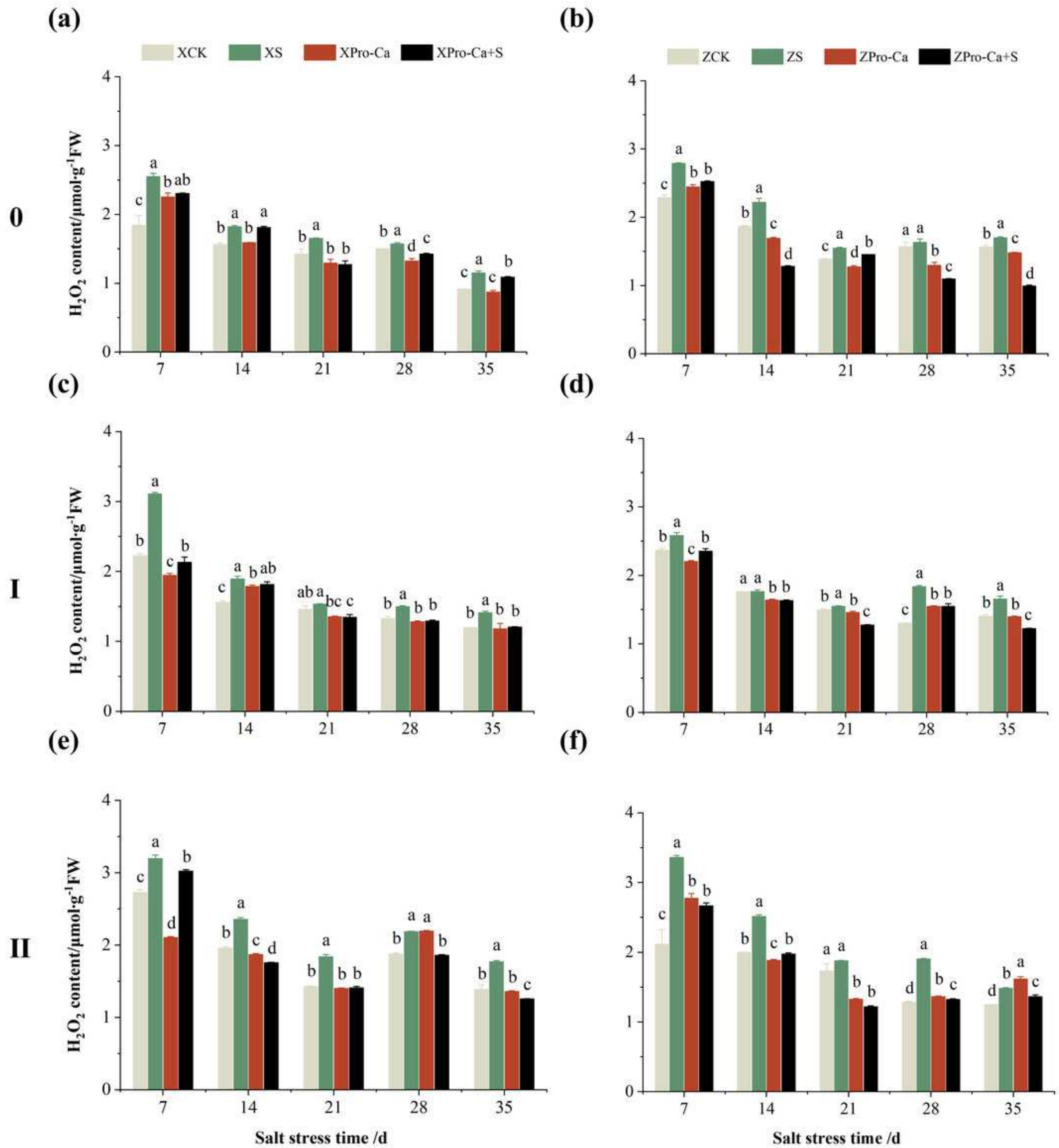

(a)
(b)
(c)
(d)
(e)
(f)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
H2O2 content/μmol·g-1FW
Salt stress time /d

Figure 12

Effect of Pro-Ca on O_2^-, H_2O_2 content and cellular activity of rice main stem leaves at each tiller position under salt stress.

The spot area represents the degree of stress, and the larger staining area indicates that the more severe stress of leaves.

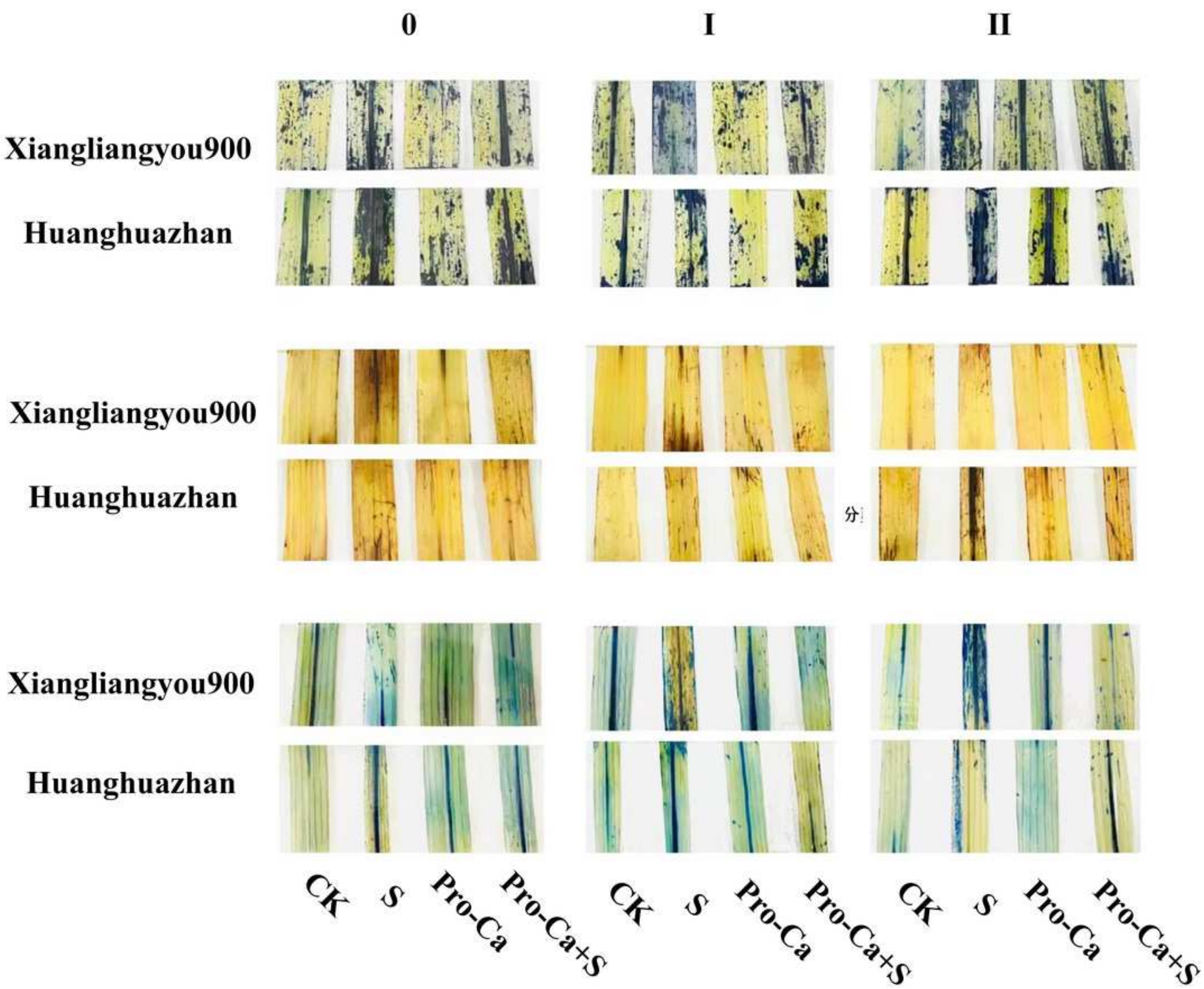

0
I
II
Xiangliangyou900
Huanghuazhan
Xiangliangyou900
Huanghuazhan
Xiangliangyou900
Huanghuazhan
CK
S
Pro-Ca
Pro-Ca+S
CK
S
Pro-Ca
Pro-Ca+S
CK
S
Pro-Ca
Pro-Ca+S

Figure 13

Effect of Pro-Ca on MDA content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

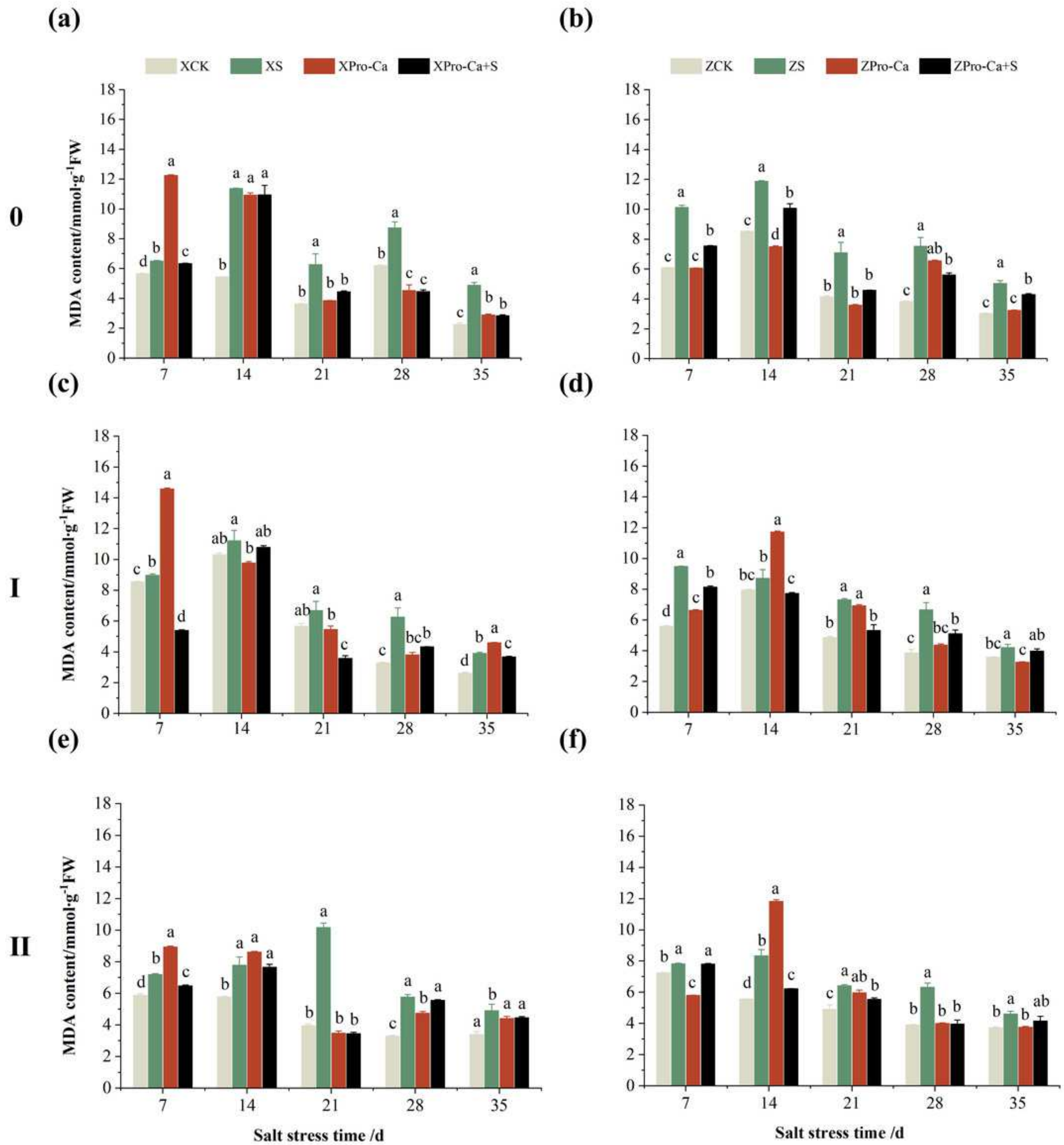

(a)
(b)
(c)
(d)
(e)
(f)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
MDA content/mmol·g-1FW
Salt stress time /d

Figure 14

Effect of Pro-Ca on H_2O_2 content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

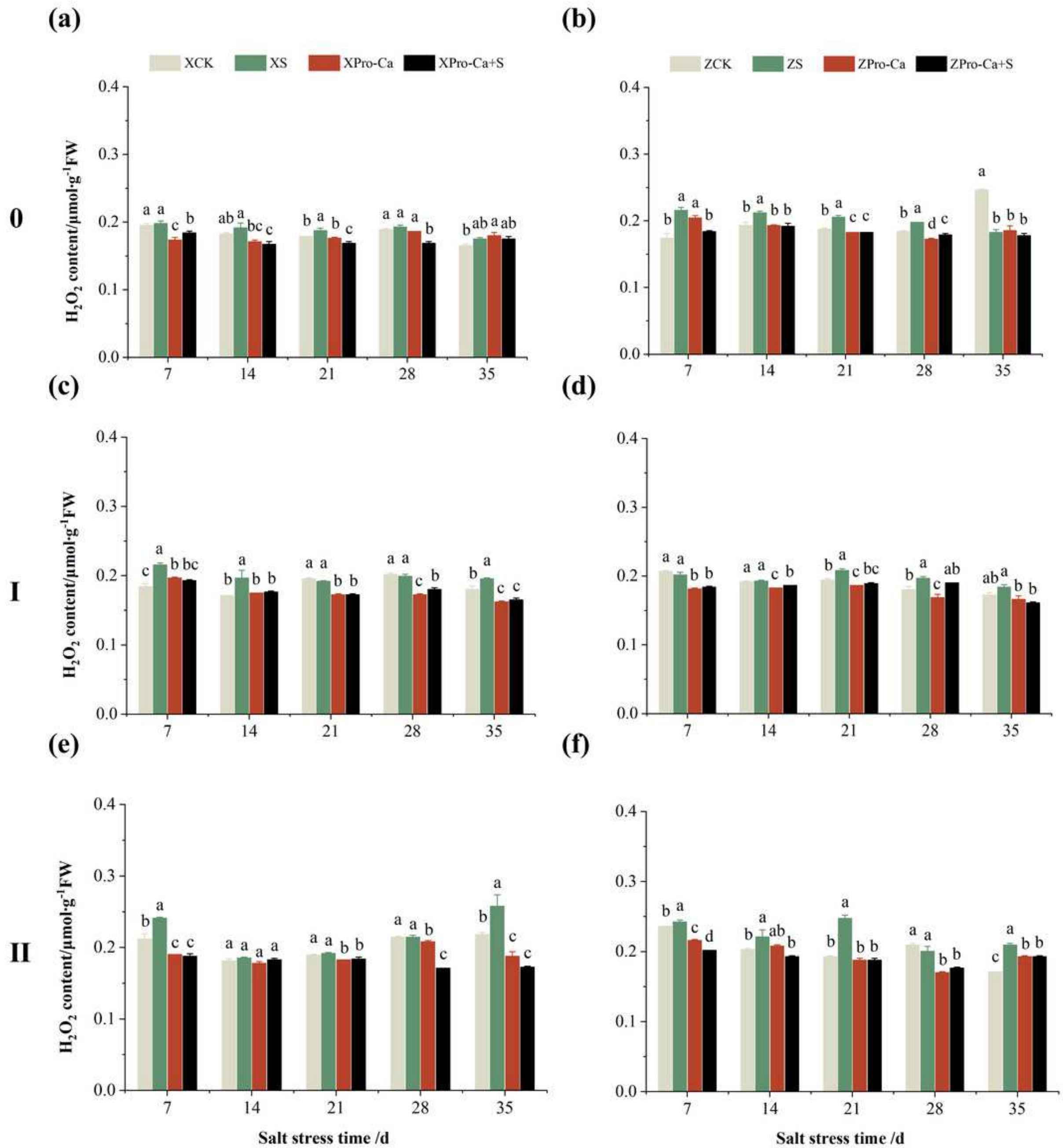

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
(c)
(d)
(e)
(f)
H_2O_2 content/μmol·g^{-1}FW
Salt stress time /d
7
14
21
28
35
0.0
0.1
0.2
0.3
0.4

Figure 15

Effect of Pro-Ca on the soluble protein content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) leaves under salt stress.

The different letters are significant differences according to Duncan's new multiple range test (*P* < 0.05) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

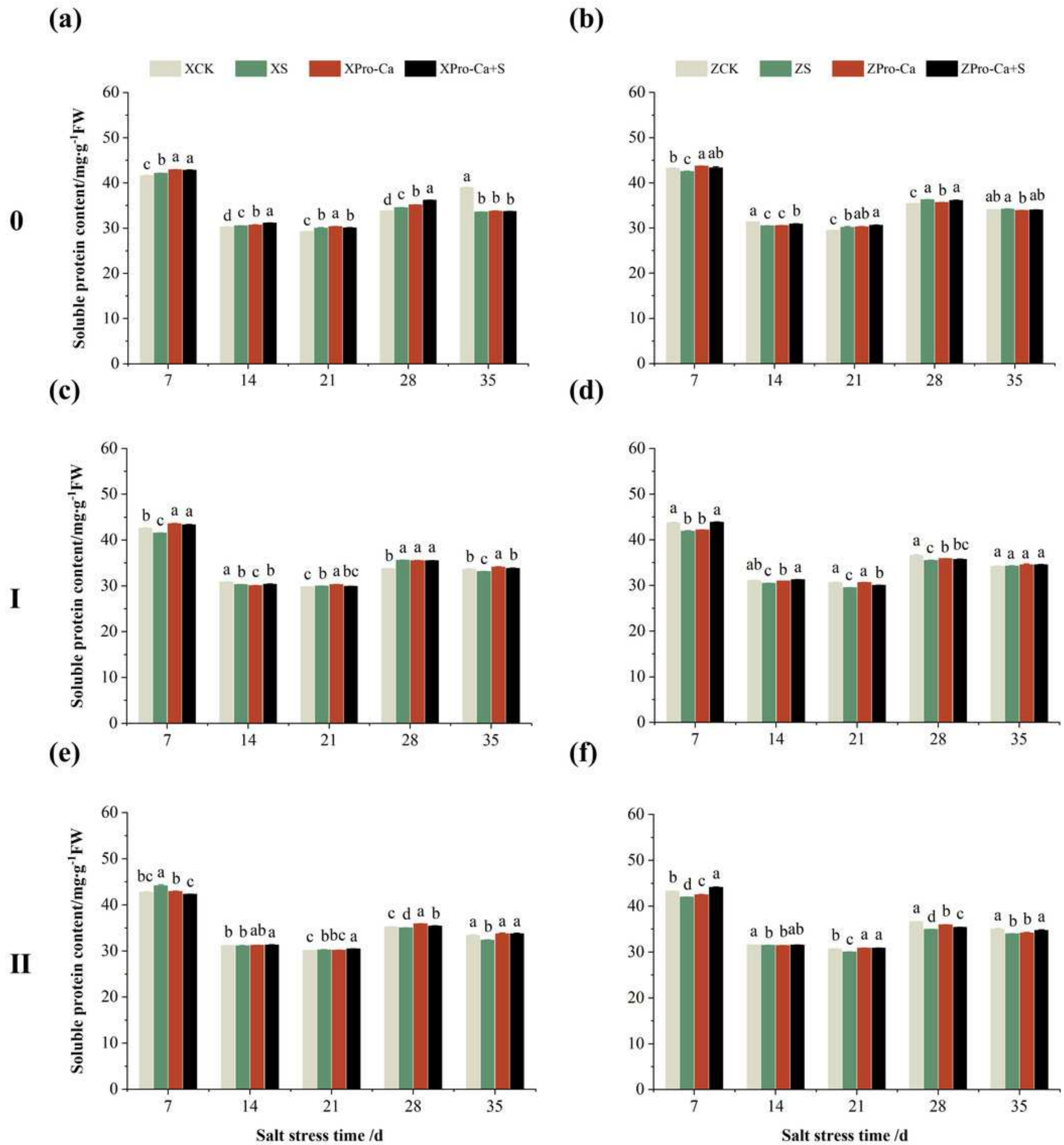

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
I
II
(c)
(d)
(e)
(f)
Soluble protein content/mg·g-1FW
Salt stress time /d
7
14
21
28
35

Figure 16

Effect of Pro-Ca on the soluble protein content of rice main stem (a, b), first tiller (c, d), and second tiller (e, f) stems under salt stress.

The different letters are significant differences according to Duncan's new multiple range test ($P < 0.05$) based on one-way ANOVA. Xiangliangyou900: XCK (distilled water), XS (0.3% NaCl), XPro-Ca (100 mg•L^{-1} Pro-Ca), XPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl), Huanghuazhan: ZCK (distilled water), ZS (0.3% NaCl), ZPro-Ca (100 mg•L^{-1} Pro-Ca), ZPro-Ca+S (100 mg•L^{-1} Pro-Ca + 0.3% NaCl).

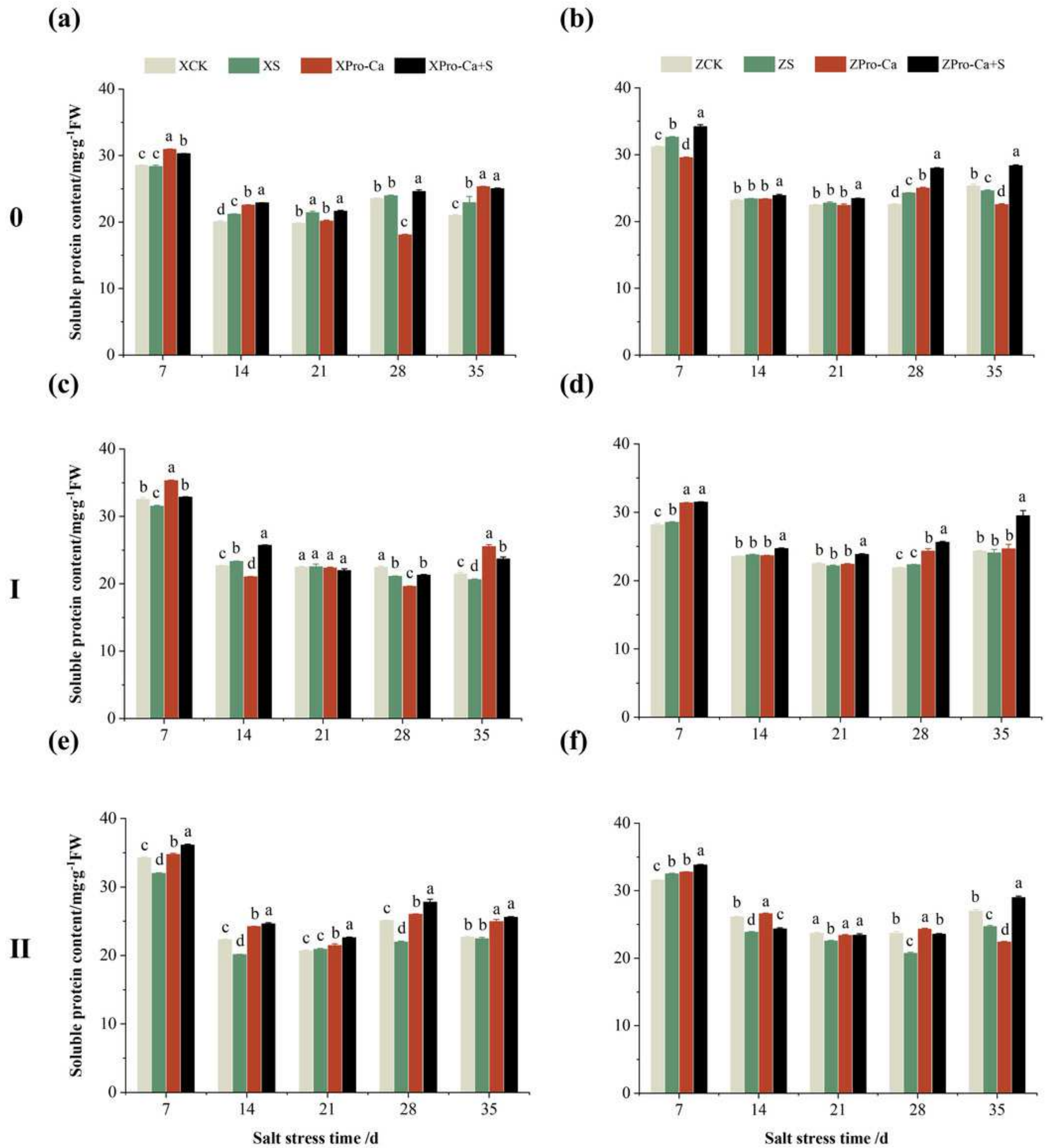

(a)
(b)
XCK
XS
XPro-Ca
XPro-Ca+S
ZCK
ZS
ZPro-Ca
ZPro-Ca+S
0
(c)
(d)
I
(e)
(f)
II
Soluble protein content/mg·g⁻¹FW
Salt stress time /d
Salt stress time /d

Table 1 (on next page)

Effects of Pro-Ca on plant height and stem base width of rice main stem, first tiller, and second tiller stems at the tillering stage under salt stress.

Values described are the means±SE (n = 3). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).

Table 1

Effects of Pro-Ca on plant height and stem base width of rice main stem, first tiller, and second tiller stems at the tillering stage under salt stress.

Time/ d	Treatment	Plant height/ cm			Stem base width/ mm		
		0	I	II	0	I	II
7	XCK	56.4±0.4a	41.3±0.9a	36.5±1.2a	9.4±0.6b	8.0±0.2a	5.2±0.1b
	XS	52.4±0.9b	29.2±2.7b	27.3±1.9c	7.1±0.2c	5.6±0.4b	4.5±0.3b
	XPro-Ca	48.7±0.9c	28.9±0.4b	31.7±0.7b	11.2±0.5a	8.3±0.2a	6.2±0.2a
	XPro-Ca+S	51.2±0.4b	29.9±2.0b	35.0±1.0ab	9.4±0.3b	7.3±0.6a	6.2±0.3a
	ZCK	63.2±2.7a	42.9±2.7a	45.2±5.0a	9.2±0.4a	7.9±0.5b	6.4±0.1a
	ZS	45.5±1.6c	26.4±1.7c	27.4±1.9b	6.3±0.2b	5.2±0.2c	3.6±0.3b
	ZPro-Ca	51.6±0.6b	34.1±2.3b	31.6±2.3b	9.7±1.2a	8.9±0.1a	6.3±0.1a
	ZPro-Ca+S	56.5±1.3b	36.5±1.2ab	35.2±2.0ab	8.5±0.1a	7.2±0.2b	6.0±0.4a
14	XCK	66.5±2.1a	46.9±1.0a	43.6±1.0a	10.8±0.7a	6.9±0.4a	6.7±0.3a
	XS	58.2±0.2b	45.8±1.6a	29.7±1.5c	8.1±0.2b	5.8±0.1b	4.6±0.5b
	XPro-Ca	56.5±0.7b	41.7±1.0b	33.7±3.9bc	12.4±0.5a	7.3±0.4a	6.7±0.1a
	XPro-Ca+S	52.3±0.3c	32.6±0.9c	37.4±0.6ab	11.7±0.9a	7.7±0.1a	6.8±0.1a
	ZCK	67.3±1.9a	56.3±2.0a	51.8±2.6a	9.9±0.4a	8.0±0.6a	6.8±0.2a
	ZS	53.9±2.5b	43.7±1.0b	36.8±1.8bc	6.5±0.1b	4.6±0.4b	4.3±0.9b
	ZPro-Ca	53.5±1.4b	41.6±2.8b	31.6±2.8c	9.2±0.2a	9.6±0.2a	6.9±0.1a
	ZPro-Ca+S	51.8±3.5b	44.2±2.1b	41.7±0.4b	9.6±0.6a	8.2±0.5a	6.8±0.3a
21	XCK	83.3±1.5a	58.1±5.4a	49.0±0.8a	12.6±0.2a	11.4±0.7a	7.8±0.6a
	XS	73.7±1.2bc	48.0±2.6ab	38.0±1.0c	10.5±0.2b	6.4±0.5c	5.0±0.6b
	XPro-Ca	79.2±1.6ab	43.5±2.8b	44.8±0.7b	12.7±0.3a	9.8±0.8ab	7.6±0.2a
	XPro-Ca+S	71.0±2.6c	41.7±4.8b	41.5±2.1bc	12.1±0.7a	9.2±0.5b	7.4±0.2a
	ZCK	76.0±0.6a	64.0±6.1a	56.6±1.0a	10.5±0.3a	10.0±1.0a	7.9±0.2a
	ZS	68.2±2.2b	55.7±0.3a	41.5±1.5b	7.8±0.3b	5.6±0.5b	5.2±0.2c
	ZPro-Ca	73.3±3.2ab	57.7±3.3a	51.1±2.1a	10.9±0.8a	11.1±0.3a	8.3±0.2a
	ZPro-Ca+S	72.3±0.9ab	41.5±4.3b	50.5±2.6a	9.7±0.3a	8.9±0.6a	7.3±0.1b

Table 1 continued

28	XCK	86.8±1.9a	71.6±3.9a	51.6±2.2a	16.0±0.1b	12.1±1.2a	8.5±0.5a
	XS	83.2±1.5ab	58.8±2.8bc	45.0±1.2bc	11.9±0.5c	8.2±0.7a	5.9±0.6b
	XPro-Ca	81.4±1.7b	62.8±1.3b	48.1±1.6ab	17.6±0.5a	13.2±0.6a	7.9±0.2a
	XPro-Ca+S	80.5±0.4b	52.0±1.4c	42.3±0.6c	15.3±0.5b	11.9±2.6a	7.5±0.0a
	ZCK	88.2±1.4a	73.5±2.1a	61.3±0.9a	14.5±0.5a	10.6±0.8a	8.4±0.1b
	ZS	70.5±0.3c	58.4±1.4c	44.9±2.9b	8.9±0.5c	6.1±0.4b	5.5±0.2c
	ZPro-Ca	76.9±3.2b	65.6±2.0b	57.3±3.5a	12.4±0.5b	10.7±0.3a	8.8±0.3b
	ZPro-Ca+S	74.2±0.7bc	70.3±1.4ab	56.0±3.5a	12.1±0.8b	9.6±0.2a	9.7±0.1a
35	XCK	86.2±2.6ab	70.0±3.9a	58.8±1.0a	19.8±1.0a	13.4±0.9a	9.9±0.5a
	XS	80.3±1.6b	64.4±0.8ab	51.3±0.2bc	15.6±0.4b	9.5±0.6b	7.5±0.3b
	XPro-Ca	87.3±1.3a	63.8±2.4ab	52.8±0.4b	20.0±1.1a	15.8±0.7a	10.0±0.9a
	XPro-Ca+S	82.0±2.0ab	55.8±4.9b	49.2±1.4c	18.9±0.3a	13.4±1.1a	8.3±0.0ab
	ZCK	90.0±0.3a	84.0±2.0a	75.8±2.0a	14.9±0.8a	10.3±0.2b	9.3±0.1a
	ZS	80.2±0.6b	70.3±6.6b	61.0±5.1b	12.3±0.5b	9.5±0.3b	6.8±0.2b
	ZPro-Ca	76.3±0.5bc	68.8±1.9b	61.9±2.8b	15.8±0.5a	13.3±0.9a	9.8±0.5a
	ZPro-Ca+S	75.1±2.4c	69.6±2.1b	63.8±0.2b	15.5±0.3a	10.6±0.2b	9.3±0.1a

Values described are the means ± SE (n = 3). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).

Table 2(on next page)

Effects of Pro-Ca on root length of rice main stem and leaf area per stem of rice main stem, first tiller, and second tiller stems at the tillering stage under salt stress.

Values are the means±SE (n = 3). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).

Table 2

Effects of Pro-Ca on root length of rice main stem and leaf area per stem of rice main stem, first tiller, and second tiller stems at the tillering stage under salt stress.

Time/ d	Treatment	Root length/ cm	Leaf area per stem/ cm^2		
			0	I	II
7	XCK	32.2±0.2a	5370.7±726.7ab	1817.1±158.7b	1297.3±249.1b
	XS	16.7±2.1c	4305.7±149.7b	974.4±196.4c	863.1±169.3b
	XPro-Ca	32.3±0.6a	6739.5±461.0a	2828.0±171.5a	995.5±141.1b
	XPro-Ca+S	25.4±0.6b	6116.9±293.6a	2589.2±117.2a	1857.3±57.1a
	ZCK	28.0±0.4a	4316.7±701.1a	2139.5±320.7a	1812.1±29.7a
	ZS	20.2±0.4c	2503.7±181.5b	573.0±47.9b	628.0±114.9b
	ZPro-Ca	28.3±0.7a	4958.6±249.8a	2751.1±330.2a	1735.9±327.2a
	ZPro-Ca+S	24.1±0.6b	4556.0±449.6a	2459.9±198.9a	1909.4±183.4a
14	XCK	34.0±0.7a	8768.8±265.8ab	4157.0±342.1a	2850.5±19.0a
	XS	23.3±1.0d	5765.6±533.7c	2625.8±151.3b	1610.0±138.4b
	XPro-Ca	30.4±0.1b	9411.7±502.4a	3800.7±95.0a	2544.2±169.6a
	XPro-Ca+S	26.5±0.9c	7524.2±54.7b	3416.4±357.4ab	2659.9±94.3a
	ZCK	31.6±0.5a	4542.4±699.9bc	3800.3±49.7b	2476.3±155.0b
	ZS	24.0±0.5c	3191.6±310.9c	1884.3±108.6c	1335.7±146.5c
	ZPro-Ca	30.4±0.2a	7599.9±557.3a	5933.6±537.9a	4297.4±297.2a
	ZPro-Ca+S	29.1±0.2b	5725.1±198.5b	3424.3±163.0b	2854.3±261.0b
21	XCK	36.9±0.9a	13073.6±31.9b	9086.0±1632.7a	3487.7±242.7b
	XS	26.3±0.3c	8518.4±824.1c	4431.7±473.8b	2343.2±156.9c
	XPro-Ca	35.1±0.7a	16377.4±1268.1a	9106.6±655.3a	4243.2±107.5ab
	XPro-Ca+S	31.1±0.4b	13157.8±1026.7b	7390.5±261.9ab	4539.4±405.0a
	ZCK	31.3±0.3a	11510.1±169.6ab	8480.1±214.0b	4263.7±158.3b
	ZS	25.9±0.3c	6699.3±469.0c	3349.3±104.3c	2419.2±134.3c
	ZPro-Ca	31.9±0.4a	12499.1±591.7a	9743.8±430.3a	5790.4±431.2a
	ZPro-Ca+S	28.1±0.5b	10685.9±115.6b	8323.6±267.9b	5174.6±300.1ab

Table 2 continued

28	XCK	38.9±0.6a	19356.5±214.9a	10053.9±1078.9a	4877.8±232.1a
	XS	28.9±0.5c	11285.3±1893.9b	4555.5±186.5c	3383.9±246.4b
	XPro-Ca	35.5±0.2b	21134.8±1004.4a	11031.7±344.0a	5339.8±348.8a
	XPro-Ca+S	35.7±0.6b	18674.2±442.4a	7893.0±501.8b	4676.2±44.4a
	ZCK	34.3±1.0a	13441.2±333.7a	8997.6±286.3b	6685.3±605.7a
	ZS	27.6±0.6b	7290.5±219.1c	4316.1±337.7c	3482.6±238.6b
	ZPro-Ca	34.9±0.4a	12883.3±750.9a	9499.3±462.4b	7186.9±142.7a
	ZPro-Ca+S	34.8±0.3a	10919.9±694.5b	10803.5±466.6a	6794.2±293.9a
35	XCK	41.9±0.7a	22136.7±1754.2a	15401.6±3314.5ab	7314.4±1013.4a
	XS	30.0±0.4b	14410.6±860.6b	6125.3±374.9c	4154.9±161.7a
	XPro-Ca	39.7±0.7a	22637.8±649.4a	18446.2±1148.1a	7497.9±1618.8a
	XPro-Ca+S	41.0±1.0a	20386.9±589.8a	11051.6±981.7bc	5948.2±318.4a
	ZCK	36.4±0.6a	17411.4±454.4a	11695.5±378.4b	9364.4±773.9a
	ZS	30.7±0.3b	10297.9±287.1d	8110.1±300.1c	4612.9±130.9b
	ZPro-Ca	38.3±1.2a	14462.8±419.4b	12506.7±509.8b	8107.0±497.7a
	ZPro-Ca+S	37.8±0.5a	12335.2±361.4c	14148.4±387.9a	7825.9±929.4a

Values are the means ± SE (n = 3). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).

Table 3(on next page)

Effects of Pro-Ca on dry weight per stem of rice main stem, first tiller, and second tiller stems and root dry weight of rice main stem at the tillering stage under salt stress.

Values are the means±SE ($n = 3$). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).

Table 3

Effects of Pro-Ca on dry weight per stem of rice main stem, first tiller, and second tiller stems and root dry weight of rice main stem at the tillering stage under salt stress.

Time/ d	Treatment	Dry weight per stem/ g			Root dry weight/ g
		0	I	II	
7	XCK	0.470±0.017a	0.168±0.004a	0.103±0.005b	0.195±0.007a
	XS	0.340±0.015b	0.062±0.011b	0.065±0.006c	0.118±0.007d
	XPro-Ca	0.478±0.045a	0.178±0.003a	0.133±0.005a	0.170±0.005b
	XPro-Ca+S	0.466±0.011a	0.156±0.015a	0.094±0.012b	0.146±0.004c
	ZCK	0.426±0.053a	0.168±0.023b	0.129±0.004a	0.154±0.005b
	ZS	0.202±0.005b	0.058±0.004c	0.045±0.008b	0.098±0.002c
	ZPro-Ca	0.392±0.011a	0.212±0.009a	0.143±0.012a	0.189±0.007a
	ZPro-Ca+S	0.365±0.006a	0.159±0.008b	0.138±0.008a	0.139±0.013b
14	XCK	0.626±0.020a	0.295±0.019b	0.198±0.002a	0.268±0.011ab
	XS	0.392±0.040b	0.188±0.004b	0.124±0.006c	0.140±0.000c
	XPro-Ca	0.573±0.028a	0.440±0.074a	0.206±0.014a	0.318±0.053a
	XPro-Ca+S	0.532±0.016a	0.239±0.012b	0.168±0.002b	0.191±0.004bc
	ZCK	0.464±0.017a	0.352±0.033a	0.191±0.013b	0.235±0.008b
	ZS	0.305±0.027b	0.148±0.012c	0.101±0.004c	0.112±0.001c
	ZPro-Ca	0.552±0.026a	0.415±0.032a	0.262±0.010a	0.311±0.010a
	ZPro-Ca+S	0.468±0.050a	0.245±0.007b	0.236±0.024ab	0.229±0.009b
21	XCK	1.145±0.066a	0.382±0.003c	0.199±0.003b	0.405±0.020b
	XS	0.638±0.065b	0.345±0.029c	0.182±0.008b	0.266±0.007d
	XPro-Ca	1.194±0.078a	0.579±0.024a	0.250±0.020a	0.266±0.007d
	XPro-Ca+S	1.249±0.030a	0.460±0.018b	0.259±0.016a	0.266±0.007d
	ZCK	0.988±0.049a	0.650±0.019a	0.291±0.012b	0.331±0.021c
	ZS	0.565±0.005c	0.256±0.014b	0.222±0.020c	0.206±0.009d
	ZPro-Ca	1.008±0.062a	0.681±0.047a	0.367±0.012a	0.596±0.013a
	ZPro-Ca+S	0.791±0.037b	0.598±0.031a	0.359±0.018a	0.493±0.035b

Table 3 continued

28	XCK	2.160±0.134a	0.943±0.041b	0.344±0.009a	1.469±0.073a
	XS	0.896±0.023c	0.617±0.033c	0.224±0.002b	0.428±0.046d
	XPro-Ca	1.828±0.070b	1.373±0.117a	0.326±0.030a	1.278±0.013b
	XPro-Ca+S	1.571±0.045b	0.633±0.029c	0.346±0.022a	0.601±0.048c
	ZCK	1.387±0.121a	0.767±0.051b	0.376±0.013b	0.954±0.012a
	ZS	0.654±0.019b	0.400±0.008c	0.278±0.011b	0.284±0.011d
	ZPro-Ca	1.467±0.114a	0.948±0.047a	0.689±0.030a	0.695±0.019b
	ZPro-Ca+S	1.235±0.126a	0.908±0.076ab	0.824±0.077a	0.631±0.020c
35	XCK	2.817±0.131a	1.765±0.056a	0.446±0.042ab	1.938±0.081ab
	XS	1.053±0.009c	0.725±0.011b	0.350±0.013b	1.014±0.075c
	XPro-Ca	2.802±0.072a	1.547±0.258a	0.603±0.086a	2.153±0.219a
	XPro-Ca+S	2.502±0.099b	1.432±0.039a	0.449±0.036ab	1.639±0.037b
	ZCK	2.243±0.104ab	1.265±0.139b	0.544±0.076b	1.446±0.059a
	ZS	1.575±0.054c	0.881±0.056c	0.429±0.012b	0.551±0.019c
	ZPro-Ca	2.321±0.144a	1.539±0.024a	1.341±0.052a	0.842±0.079b
	ZPro-Ca+S	1.915±0.121bc	1.421±0.018ab	1.212±0.017a	0.767±0.035b

Values are the means±SE (n = 3). Different letters denote significant difference from Duncan's LSD test ($p < 0.05$).