# Hydrogen rich water (HRW) induces plant growth and physiological attributes in fragrant rice varieties under salt stress

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# Abstract

Hydrogen is an important molecule, exerting antioxidant ability in plants and animals through antioxidant enzymes, which can be dissolved in water. Previous studies have showed that application of hydrogen rich water (HRW), containing a high concentration of hydrogen, plays an important role in enhancing drought tolerance and alleviating the metal stress in plants. However, the effects of HRW on plant growth and physiological attributes in fragrant rice varieties under salt stress are still unclear. A pot experiment was conducted with two fragrant rice varieties i.e. Yuxiangyouzhan and Xiangyaxiangzhan to study the effects of HRW treatments i.e. foliar application of HRW (F-HRW) and irrigation application of HRW (I-HRW) on plant growth and physiological attributes under two NaCl levels (0 mmol L -1 and 150 mmol L -1 ). The results depicte d that, compared with without HRW treatment (CK), the F-HRW and I-HRW treatments significantly increased the dry weight per unit seedling height by 12.64% and 22.99%, while decreased the plant height by 3.92% and 2.97% respectively of two fragrant rice varieties under salt stress. Moreover, compared with CK treatment, the activities of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) were enhanced by F-HRW and I-HRW treatments in NaCl-stressed fragrant rice cultivars and opposite results were observed for MDA content. In crux, our findings conclude that application of HRW modulates the plant growth and physiological attributes in salt-stressed fragrant rice cultivars.


# 1. Introduction

Adverse environmental conditions, especially high temperature significantly affects the crop growth, yield and quality of rice (Kong *et al.*, 2017). Similarly, low temperature at panicle initiation may lead the rice to low panicle sterility (Sipaseuth *et al.*, 2007) and higher $CO_2$ concentration reduces the nutritional contents in crops (McGrath *et al.*, 2013). Also, the water stress (waterflooding) resulted in decreasing the yield and quality of crops (Foster *et al.*, 2017). Moreover, heavy metal pollution caused damages in crop plants and affected human health (Cui *et al.*, 2014; Edelstein *et al.*, 2018). Further, salty in field lead to inhibit plant growth and reduced grain yield and quality (Wani *et al.*, 2013; Cheng *et al.*, 2019; Thu *et al.*, 2019). Therefore, studies are needed to mitigate the adverse environmental effects on crop growth and other physiological attributes.

Several adverse environmental conditions affect the plant growth and other physiological attributes; however, the salt stress is one of the critical circumstances for normal plant growth (Zhang *et al.*, 2011; Cheng *et al.*, 2019; Frukh *et al.*, 2020). Previous study suggested that crops seeds sown in the soil of NaCl significantly affected and decreased the crop yield (Wani *et al.*, 2013; Thu *et al.*, 2015), while the NaCl with low concentrations could positively affect plant growth, showing that plant height, fresh weight and dry weight of plant shoot could be increased due to salinity (Qados *et al.*, 2011). Many studies have suggested that salt stress had various adverse effects on plants growth and development. Zhang *et al.* (2019) reported that salt stress could dramatically not only decrease the emergence rate of rice, but also inhibit the plant biomass and delay the growth process of rice seedlings. Wani *et al.* (2013) suggested that the length, fresh and dry mass of the shoot and root of crops showed a marked decrease on being

subjected to different levels of NaCl applied through soil, and the damage increased with increasing concentration. Al Hassan *et al*. (2015) showed that salt treatment could induce the inhibition of plant growth through decreasing the mean fresh and dry weights of plant leaves. The antioxidant enzyme activities were important indications of oxidation resistance of crops, which can be produced by crops and alleviate the damage of reactive oxygen species (ROS). Wani *et al*. (2013) suggested that antioxidant enzyme activities could be increased in response to the concentrations of NaCl in the soil. Huang *et al*. (2006) reported that the activities of SOD, POD and CAT increased due to the salt stress process. Crops could increase ROS production as a result of suffering abiotic stress conditions and MDA is a representative parameter of ROS. Wang *et al*. (2019) suggested that MDA content of rice seedlings roots after exposure to NaCl stress could be improved, whilst the appearance was opposite in rice seedlings shoot. Al Hassan *et al*. (2015) revealed that salt stress treatment caused secondary oxidative stress in the plants, therefore a significant increase in MDA contents was detected. The dry mass of the shoot and root of crops presented a distinct decrease on being suffered to NaCl (Huang *et al*., 2006; Wani *et al*., 2013), which might be related to the disturbance in metabolic activities affected by the decrease in water adsorption and/or disturbance in water balance (Abdallah *et al*., 2016).

Besides, NaCl stress also significantly effects the plant chlorophyll synthesis. The plants cultivated in the soil with NaCl showed significantly lower SPAD values of chlorophyll and reduced the chlorophyll 'a' and chlorophyll 'b' contents in crop growth period than unstressed control treatments (Qados *et al*., 2011; Wani *et al*., 2013). Huang *et al*. (2006) reported that wheat plants with 300 mmol/L NaCl treatment showed a significant reduction in chlorophyll contents even after four days of NaCl treatment. There are also some useful purposes on plant growth. An increase was observed in the protein content in crops due to the impact of salinity stress (Qados *et al*., 2011). Siddiqui *et al*. (2010) suggested fresh weight and dry weight of plants were significantly higher with the application nitrogen under NaCl treatment. Rice is a salt stress-sensitive crop, the total Na and Cl content in the rice seedlings were dramatically increased with increasing salt stress (Zhang *et al*., 2019). However, the accumulation of $Na^+$ and $Cl^-$ could result in hyper ionic, hyperosmotic and oxidative stress, affecting germination, plant growth and reproductive development, ultimately caused the death of the plant (Tripathi *et al*., 2017; Yang and Guo, 2018). Nevertheless, how to relieve the salt stress of rice is extremely necessary for normal crop growth and development.

There have been reported that many approaches could be employed to alleviate the salt stress. The application of salt-tolerant cultivars is beneficial to alleviate salt stress with the ability to restrict $Na^+$ accumulation in leaves under salt stress (Mekawy *et al*., 2015). Shannon *et al*. (1998) assessed the salt tolerance in rice cultivars in California and established a salt tolerance evaluation. Besides, exogenous application of other matters to alleviate salt stress is feasible. Zhang *et al*. (2019) reported that application of biochar into soil could significantly improve the physicochemical properties of the soil under salt stress and greatly decrease exchangeable $Na^+$ and $Cl^-$ contents in the soil, proving a better soil environment for rice seedlings under salt stress. Additionally, the application of nitrogen and silicon could alleviate the adverse effects of salt stress and adjust the plants to perform normally by detoxifying the

ROS (Siddiqui *et al*., 2010; Torabi *et al*., 2015). Athar *et al*. (2008) reported that exogenously applied ascorbic acid alleviated the salt stress in wheat via improving photosynthetic capacity of wheat against salt-induced oxidative stress and maintaining ion homeostasis. Therefore, further studies to find how to alleviate salt stress is still needed.

$H_2$ has been regarded as a widely-used and anti-stress molecule which can alleviate oxygen damage by modulating antioxidant enzymes (Cui *et al*., 2014). Many studies have reported that $H_2$ protected plants growth against stressed environments, such as salinity (Xu *et al*., 2013) and drought (Zeng *et al*., 2013). The use of HRW was characterized as a safe and available way to mimic the physiological functions of endogenous $H_2$ in plants. There were many positive effects on the application of HRW. Through alleviating growth stunt, decreasing Hg accumulation and modulation of oxidative stress, exogenously applied HRW could attenuate Hg toxicity in alfalfa seedlings (Cui *et al*., 2014). Xu *et al*. (2013) revealed that exogenous HRW could improve germination of rice seeds and alleviate seedlings growth inhibition under salt stress. Guan *et al*. (2019) reported that HRW could regulate plant antioxidant defense by decreasing ROS levels, which led to the improvement of seedlings growth. Additionally, HRW exerted its protective effects by regulating antioxidative enzymes, including CAT, SOD, POD and APX (Xu *et al*., 2013; Su *et al*., 2014). Therefore, it seems viable to apply HRW to modulate the rice growth under salt stress.

Therefore, this study applied HRW to fragrant rice varieties and analyzed growth parameters, antioxidant enzymes activities and MDA contents through the methods of F-HRW and I-HRW.

## 2. Materials And Methods

# 2.1 Plant materials and growth conditions

Seeds of two fragrant rice cultivars i.e. Yuxiangyouzhan (YX), Xiangyaxiangzhan (XY), were collected from the College of Agriculture, South China Agricultural University, Guangzhou, China. These two fragrant rice varieties are widely cultivated in South China due to their aroma. A pot experiment was performed in College of Agriculture, South China Agricultural University, Guangzhou, China on the 8th September 2018. The seeds of both rice cultivars were germinated at 28°C for 48 h. Then the seeds were sowed in 20cm × 50 cm box containing four-liter of Kimura nutrient solution, the uniform seedlings was used in each pot for experimental treatment.

# 2.2 Treatments and sampling

Two levels of NaCl were applied in this study, i.e. 0 mmol $L^{-1}$ of NaCl (NaCl 0) and 150 mmol $L^{-1}$ of NaCl (NaCl 150). Three application methods of HRW were used in this experiment, i.e. without application of HRW (CK), foliar application of HRW (F-HRW) and root application of HRW (I-HRW). Briefly, this experiment was comprised of six treatments, i.e. CK × NaCl 0, CK × NaCl 150, F-HRW × NaCl 0, F-HRW × NaCl 150, I-HRW × NaCl 0 and I-HRW × NaCl 150. All the treatments were applied 17 days after sowing seeds, and sampled three days later. The morphological and physiological indexes, including plant height,

shoot dry weight, root dry weight, total dry weight, dry weight per unit seedling weight, antioxidant enzymes (SOD, POD and CAT) and MDA contents were measured after sampling.

## 2.3 Preparation of HRW

Purified hydrogen gas (99.99%, v/v) generated by a hydrogen gas generator (SPH-300A, Beijing zhonghuipu Co Ltd., Beijing, China), was bubbled into 4000 mL Kimura nutrient solution at a rate of 150 ml $min^{-1}$ for 20 min, a sufficient duration to saturate the solution with $H_2$ (Bernardi *et al.*, 2008). The concentration of $H_2$ in the solution was 500 ppm measured by concentration Merer (ENH-1000, TRVSTLEX)

## 2.4 Determination of plant height, dry weight and dry weight per unit seedling height

The plant height was measured from the base of the intact plant stem to the top of the highest leaf at maturity stage. After three days of treatments, plants were harvested from the boxes and taken to the laboratory for further analysis. Plant samples were separated into leaves, stems, sheath and panicles and oven dried at 80℃ to constant weight to determine shoot dry weight, root dry weight and total dry weight of rice seedlings, the dry weight per unit seedling height was calculated (Mo *et al.*, 2015).

## 2.5 Determination of antioxidant enzymes (SOD, POD, CAT) activities and MDA contents

The rice seedlings were harvested and separated into roots and shoots and then stored at -80 ℃ until the determination of SOD, POD, CAT activities and MDA contents. (Li et al., 2019).

The crude enzymes were extracted according to the methods described by Lee and Lee (2000) with some modifications. Briefly, fresh root and shoot samples were homogenized with 5 mL (pH 7.8) sodium phosphate buffer (PBS), and centrifuged at 8000 rpm at 4℃ for 15 min to get the crude enzyme extracts.

The SOD activity was estimated by using nitro tetrazolium (NBT) previously described by MacAdam et al. (1992). 0.05 mL of supernatant enzyme extract was added to the reaction mixture containing 1.5 mL of pH7.8 sodium phosphate buffer, 0.3 mL of 130 mM methionine buffer, 0.3 mL of 750 μmol/L NBT buffer, 0.3 mL of 100 μmol/L EDTA-$Na_2$ buffer and 0.3 mL of 20 μmol/L riboflavin. The absorption value was measured at 560 nm. The SOD activity was expressed as U $g^{-1}$ FW.

The POD activity was measured according to previous procedure (MacAdam et al., 1992). The POD activity was estimated through the reaction mixture comprising of 0.05 mL enzyme extract, 1.0 mL of 0.3% $H_2O_2$, 0.95 mL of 0.2% guaiacol and 1 mL of 50 mM PBS. Absorption was measured at 470 nm during 2 min with the records every 30 seconds. The POD activity was expressed as U $g^{-1}$ FW.

The CAT activity was estimated by adding the supernatant enzyme extract (0.05 mL) to the reaction mixture containing 1.95 mL of ultrapure water and 1.0 mL of 0.3% $H_2O_2$. The reaction immediately

started after adding the enzyme solution and shaking well. The absorption value was measured at 240 nm every 30 sec in 2 minutes. An absorbance change of 0.01 was regarded as one unit(U) of CAT activity. The CAT activity was expressed as U $g^{-1}$ FW.

The MDA content was measured through mixing 0.75 mL supernatant enzyme extract and the 1.0 mL thiobarbituric acid (TBA). The mixture was heated in a boiling water bath for 30 min, then cooled to room temperature and centrifuged the mixture for 15 min. The absorption was measured at 532 nm, 600 nm and 450 nm, to calculate the MDA contents in root and shoot tissues of rice seedlings. The MDA content was expressed as μmol $g^{-1}$ FW.

# 2.6 Statistical analysis ant plotting

The data and relationships among the data were analyzed by using analytical software, Statistix version 8. Means among treatments were compared based on the least significant difference test (LSD) at the 0.05 probability level.

## 3. Result

# 3.1 Plant height, dry weight and dry weight per unit seedling height

Significant HRW effect on shoot dry weight, root dry weight, total dry weight and dry weight per unit seedling height was observed. Compared with CK treatment, F-HRW and I-HRW significantly increased shoot dry weight (8.54% and 17.59%), total dry weight (7.70% and 16.99%) and dry weight per unit seedling height (12.64% and 22.99%) in two fragrant varieties under two NaCl levels. I-HRW treatment significantly increased root dry weight by 6.55% as compared to CK treatment. F-HRW treatment significantly reduced plant height by 3.92% when compared to CK treatment. Yuxiangyouzhan showed significantly higher plant height, shoot dry weight, total dry weight and dry weight per unit seedling height than Xiangyaxiangzhan across three HRW treatments and two NaCl levels. NaCl 150 treatment significantly decreased shoot dry weight and dry weight per unit seedling height in two varieties under three HRW treatments. Variety × NaCl significantly affected plant height. Variety × HRW significantly affected plant height, shoot dry weight and total dry weight. Variety × HRW significantly affected plant height and dry weight per unit seeding height (Table 1).

Table 1
Effect of HRW on plant height, dry weight and dry weight per unit seedling height

| **Treatment** | | **Plant height (cm)** | **Shoot dry weight (mg plant$^{-1}$)** | **Root dry weight (mg plant$^{-1}$)** | **Total dry weight (mg plant$^{-1}$)** | **Dry weight per unit seedling height (mg cm$^{-1}$)** |
|---|---|---|---|---|---|---|
| CK | | | | | | |
| Yuxiangyouzhan | NaCl 0 | 20.49 ± 0.53 | 20.29 ± 0.38 | 3.35 ± 0.13 | 22.90 ± 1.15 | 0.99 ± 0.02 |
| | NaCl 150 | 18.38 ± 0.41 | 18.88 ± 0.45 | 3.63 ± 0.08 | 22.50 ± 0.46 | 1.03 ± 0.02 |
| Xiangyaxiangzhan | NaCl 0 | 16.85 ± 0.31 | 12.11 ± 0.36 | 3.20 ± 0.14 | 15.15 ± 0.38 | 0.72 ± 0.03 |
| | NaCl 150 | 15.72 ± 0.35 | 11.50 ± 0.24 | 3.28 ± 0.09 | 14.78 ± 0.27 | 0.73 ± 0.03 |
| | Mean | **17.86 ± 0.40a** | **15.69 ± 0.36c** | **3.36 ± 0.11b** | **18.83 ± 0.56c** | **0.87 ± 0.02c** |
| F-HRW | | | | | | |
| Yuxiangyouzhan | NaCl 0 | 17.72 ± 0.19 | 20.48 ± 0.28 | 3.30 ± 0.11 | 23.78 ± 0.37 | 1.16 ± 0.00 |
| | NaCl 150 | 18.68 ± 0.44 | 20.18 ± 1.20 | 3.03 ± 0.13 | 23.20 ± 1.31 | 1.08 ± 0.04 |
| Xiangyaxiangzhan | NaCl 0 | 15.79 ± 0.37 | 13.40 ± 0.33 | 3.40 ± 0.22 | 16.80 ± 0.47 | 0.85 ± 0.02 |
| | NaCl 150 | 16.44 ± 0.22 | 14.08 ± 0.11 | 3.25 ± 0.03 | 17.33 ± 0.13 | 0.86 ± 0.01 |
| | Mean | **17.16 ± 0.31b** | **17.03 ± 0.48b** | **3.24 ± 0.12b** | **20.28 ± 0.57b** | **0.98 ± 0.02b** |
| I-HRW | | | | | | |
| Yuxiangyouzhan | NaCl 0 | 16.30 ± 0.49 | 20.45 ± 0.71 | 3.45 ± 0.10 | 23.90 ± 0.70 | 1.26 ± 0.08 |

Lower-case letter indicates comparisons among the treatments; ns: nonsignificant at 0.05 probability level; * and **: significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant at P > 0.05 level.

CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L$^{-1}$ and 150 mmol L$^{-1}$of NaCl.

| Treatment | | Plant height (cm) | Shoot dry weight (mg plant$^{-1}$) | Root dry weight (mg plant$^{-1}$) | Total dry weight (mg plant$^{-1}$) | Dry weight per unit seedling height (mg cm$^{-1}$) |
|---|---|---|---|---|---|---|
| | NaCl 150 | 18.78 ± 0.34 | 20.80 ± 0.49 | 3.63 ± 0.23 | 24.43 ± 0.43 | 1.11 ± 0.03 |
| Xiangyaxiangzhan | NaCl 0 | 17.83 ± 0.33 | 18.58 ± 0.79 | 3.73 ± 0.45 | 22.30 ± 0.82 | 1.04 ± 0.06 |
| | NaCl 150 | 16.40 ± 0.57 | 13.98 ± 1.36 | 3.53 ± 0.09 | 17.50 ± 1.35 | 0.85 ± 0.05 |
| | Mean | **17.33 ± 0.46ab** | **18.45 ± 1.20a** | **3.58 ± 0.15a** | **22.03 ± 1.21a** | **1.07 ± 0.04a** |
| ANOVA | | | | | | |
| | Variety | ** | ** | ns | ** | ** |
| | NaCl | ns | * | ns | ns | * |
| | HRW | ns | ** | ** | ** | ** |
| | Variety × NaCl | * | ns | ns | ns | ns |
| | Variety × HRW | ** | ** | ns | * | ns |
| | NaCl × HRW | ** | ns | ns | ns | ** |
| | Variety × NaCl × HRW | ** | * | ns | * | ns |
| Lower-case letter indicates comparisons among the treatments; ns: nonsignificant at 0.05 probability level; * and **: significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant at P > 0.05 level. | | | | | | |
| CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L$^{-1}$ and 150 mmol L$^{-1}$of NaCl. | | | | | | |

## 3.2 Antioxidant enzymes and MDA content

Significant HRW effect on SOD activity in shoot was observed. Compared with CK treatment, F-HRW and I-HRW significantly increased SOD activity in shoot (50.67% and 25.32%) in two rice varieties under two NaCl levels. Yuxiangyouzhan showed significantly higher in SOD activity in root than Xiangyaxiangzhan across three HRW treatments and two NaCl levels. NaCl 150 treatment significantly decreased SOD activity in root in two varieties under three HRW treatments. Variety × HRW and Variety × NaCl × HRW significantly affected SOD activity in shoot (Fig. 1, Table 2).

Table 2
The ANOVA analysis of SOD, POD, CAT and MDA

| Parameters | Variety | NaCl | HRW | Variety × NaCl | Variety × HRW | NaCl × HRW | Variety × NaCl × HRW |
|---|---|---|---|---|---|---|---|
| SOD_S $U\ g^{-1}$ FW | ns | ns | ** | ns | ns | ** | ** |
| SOD_R $U\ g^{-1}$ FW | ** | * | ns | ns | ns | ns | ns |
| POD _S $U\ g^{-1}$ FW | ** | ** | ** | ** | ns | ** | ** |
| POD _R $U\ g^{-1}$ FW | ns | ns | ** | * | ns | ns | ns |
| CAT _S $U\ g^{-1}$ FW | ** | ** | ** | * | ** | ** | ** |
| CAT_R $U\ g^{-1}$ FW | * | ** | * | * | ns | * | ns |
| MDA _S $\mu mol\ g^{-1}$ FW | * | ** | ns | ** | ** | ** | * |
| MDA _R $\mu mol\ g^{-1}$ FW | ns | ** | ns | ns | * | ** | ** |

* and **: significant at the 0.05 and 0.01 probability levels, respectively; ns: nonsignificant at $P > 0.05$ level.

SOD_S: SOD activity in shoot; SOD_R: SOD activity in root; POD_S: POD activity in shoot; POD_R: POD activity in root; CAT_S: CAT activity in shoot; CAT_R: CAT activity in root; MDA_S: MDA content in shoot; MDA_R: MDA content in root; CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root.

Significant HRW effect on POD activity in shoot and POD activity in root was observed. Compared with CK treatment, F-HRW and I-HRW significantly increased POD activity in shoot (34.62% and 19.05%) and POD activity in root in two rice varieties under two NaCl levels. Yuxiangyouzhan showed significantly higher in POD activity in shoot across three HRW treatments and two NaCl levels. NaCl 150 treatment significantly increased POD activity in shoot in two varieties under three HRW treatments. Variety × NaCl, NaCl × HRW and Variety × NaCl × HRW significantly affected POD activity in shoot (Fig. 2, Table 2).

Significant HRW effect on CAT activity in shoot and CAT activity in root was observed. Compared with CK treatment, F-HRW and I-HRW significantly increased CAT activity in shoot (92.59% and 63.58%) and CAT activity in root (33.94% and 71.00%) in two rice varieties under two NaCl levels. Yuxiangyouzhan showed

significantly higher in CAT activity in shoot and CAT activity in root than Xiangyaxiangzhan across three HRW treatments and two NaCl levels. NaCl 150 treatment significantly decreased CAT activity in shoot and CAT activity in root in two varieties under three HRW treatments. Variety × NaCl, Variety × HRW and NaCl × HRW significantly affected CAT activity in shoot. Variety × NaCl and NaCl × HRW significantly affected CAT activity in root (Fig. 3, Table 2).

NaCl 150 treatment significantly increased MDA content in shoot and MDA content in root in two varieties under three HRW treatments. Xiangyaxiangzhan showed significantly higher in MDA content in shoot than Yuxiangyouzhan across three HRW treatments and two NaCl levels. Variety × NaCl, Variety × HRW and NaCl × HRW significantly affected MDA content in shoot. Variety × HRW and NaCl × HRW significantly affected MDA content in root (Fig. 4, Table 2).

# 3.3 Correlation analysis

The dry weight per unit seedling height showed significantly positive correlation with shoot dry weight in fragrant varieties. Significantly positive correlation between total dry weight and shoot dry weight, dry weight per unit seedling height, SOD activity in root, CAT activity in root was showed in fragrant cultivars. The correlation comparison was shoot dry weight > dry weight per unit seedling height > SOD activity in root > CAT activity in root (Fig. 5).

# 3.4 PCA analysis

The PCA analysis of the investigated parameters revealed that PC1, PC2, PC3, PC4 and PC5 accounted for 64.90%, 28.60%, 3.80%, 2.00% and 0.70%, respectively (Fig. 6).

The PCA analysis revealed that the POD activity in sprout, POD activity in radicle were detected with high loading value for PC1; the CAT activity, POD activity and SOD activity in radicle/sprout were detected with high loading value for PC2, PC3, PC4 and PC5 (Table 3).

Table 3

PCA loadings for the parameters

| | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| SDW | -0.0028 | 0.0008 | 0.0210 | -0.0121 | 0.0148 |
| RDW | -0.0001 | -0.0001 | -0.0001 | 0.0000 | -0.0007 |
| TDW | -0.0028 | 0.0004 | 0.0195 | -0.0115 | 0.0144 |
| DWPH | -0.0001 | 0.0000 | 0.0009 | -0.0008 | 0.0005 |
| CAT_S | 0.0248 | -0.0726 | 0.1855 | 0.0519 | -0.9775 |
| CAT_R | -0.0001 | 0.0000 | 0.0023 | -0.0026 | -0.0012 |
| POD_S | 0.1590 | -0.9481 | -0.1658 | -0.2178 | 0.0312 |
| POD_R | 0.9866 | 0.1587 | 0.0315 | 0.0081 | 0.0196 |
| SOD_S | 0.0161 | -0.2658 | 0.5308 | 0.7879 | 0.1627 |
| SOD_R | -0.0219 | -0.0095 | 0.8089 | -0.5733 | 0.1229 |
| MDA_S | 0.0008 | -0.0013 | -0.0148 | 0.0008 | 0.0315 |
| MDA_R | -0.0001 | -0.0005 | 0.0019 | -0.0005 | 0.0077 |

SDW: Shoot dry weight; RDW: Root dry weight; TDW: Total dry weight; DWPH: Dry weight per unit seedling height; SOD_S: SOD activity in shoot; SOD_R: SOD activity in root; POD_S: POD activity in shoot; POD_R: POD activity in root; CAT_S: CAT activity in shoot; CAT_R: CAT activity in root; MDA_S: MDA content in shoot; MDA_R: MDA content in root.

# 4. Discussion

The application of hydrogen is an approach that could improve antioxidant ability in both plants and animals through the enhancement of antioxidant enzymes (SOD, POD, CAT) activities (Liu *et al*., 2010; Lin *et al*., 2014). Su *et al*. (2018) illustrated that application of hydrogen improved antioxidant defense, which was confirmed by the histochemical staining for ROS production, lipid peroxidation, representative antioxidant enzyme activities and transcripts in stressed plants. Xie et al. (2012) reported that the salt stress was alleviated by applying hydrogen in Arabidopsis seedlings.

HRW containing higher concentration of hydrogen and the application of HRW could be used to regulate plant growth under adverse environment (Su *et al*., 2018). Previous study has reported that applied HRW not only could increase the production of $H_2$, but also reduced the stomatal aperture which resulted in the enhancing drought tolerance in Arabidopsis (Su *et al*., 2018). Additionally, different irrigation methods had different impacts on plant growth and development (Liang *et al*., 2016). Therefore, the application of HRW through different methods i.e. F-HRW treatment and I-HRW treatment were adopted in this study.

There have been reported that HRW had many effective impacts on alleviating environmental stress. For example, HRW could enhance the Arabidopsis drought tolerance through significantly improving the intracellular $H_2$ production and reducing the stomatal aperture (Su *et al.*, 2018). In addition, Jin *et al.* (2016) confirmed that seedlings pretreated by HRW fast accumulated hydrogen peroxide ($H_2O_2$) in extensive amounts and showed more tolerance to drought stress. Cui *et al.* (2014) reported that exogenously application of HRW alleviated Hg toxicity in alfalfa seedlings through attenuating growth stunt, reducing Hg accumulation and improving oxidative-stress tolerance in plant.

Previous studies (Bailly 2004; Wahid et al. 2007) have proved that NaCl stress not only inhibits seed germination and seedling growth, but also affects redox homeostasis at the same time. However, the application of HRW improved the rice seeds germination and alleviated the seedling growth inhibition caused by salt stress (Xu *et al.*, 2013). Therefore, exogenous HRW application might be a nice method to alleviate salt stress and improve the agricultural productivity. In this study, 500 ppm HRW was used with 150 mmol $L^{-1}$ NaCl treatment to study how the HRW induced plant growth and physiological attributes in NaCl-stressed fragrant rice varieties.

Guan *et al.* (2019) concluded that antioxidative ability of the sprouted black barley was improved after the HRW treatment and HRW treatment could also regulated antioxidative ability in animal (Liu et al.2010; Xie et al. 2010). Xu *et al.* (2013) suggested that HRW exerted its protective effects through some antioxidative enzymes, such SOD and CAT, which are consistent with the results in this study (Fig. 1, Fig. 3). Xu *et al.* (2013) reported that pretreatment with 50% concentration of HRW distinctly increased the activity of SOD in plants plus NaCl treatment. In this study, SOD activity significantly improved by 50.67% and 25.32% under F-HRW and I-HRW treatments respectively, in plant shoot, compared with CK treatment in fragrant rice cultivars (Fig. 1). Additionally, POD activity was significantly improved under F-HRW and I-HRW treatments by 34.62% and 19.05% in shoot tissues, compared with CK treatment (Fig. 2). And the POD activity in fragrant rice root was significantly improved by 21.39% with I-HRW treatment than the CK treatment (Fig. 2). Xu *et al.* (2013) reported that CAT activity was increased with 50% or 100% concentrated HRW under salt stress. In this study, F-HRW and I-HRW treatments improved CAT activity by 92.59% and 63.58% in shoots, and by 33.94% and 71.00% in plant roots respectively than the samples treated with CK treatment (Fig. 3). Liu *et al.* (2011) showed that MDA content could be attenuated by HRW treatment in the rats and oxidative damage was alleviated by decreasing the level of MDA in rat's lung tissues. In this study, the content of MDA in fragrant rice was decreased in fragrant rice shoot (4.78% and 6.79%) due to F-HRW and I-HRW treatment, compared with CK treatment (Fig. 4). Liu *et al.* (2010) suggested that higher MDA contents were associated with the decrease in the activities of antioxidant enzymes (SOD and CAT) in the liver of rats. Interestingly, we observed that the reduction in the MDA contents was accompanied by the increase of antioxidant enzymes SOD, POD and CAT in fragrant varieties in this study (Fig. 1, Fig. 3, Fig. 4).

Recent study has showed that 50% and 100% concentration of HRW could increase seed germination in the presence of NaCl exposure and HRW with 50% concentration significantly increased the root length while it did not affect shoot length under NaCl stress (Xu *et al.*, 2013). Conversely, we observed that the

plant height was respectively decreased by 3.90% and 2.97% under F-HRW and I-HRW treatments than CK treatment (Table 1). Lin et al. (2014) suggested that the number and length of adventitious roots showed increasement to a different degree with the application of HRW with 10%-100% concentration. In this study, the shoot dry weight of fragrant varieties was improved under F-HRW and I-HRW treatments (8.54% and 17.59%) than CK treatment, the root dry weight of rice was increased 6.55% treated by I-HRW treatment than CK treatment (Table 1). Compared with CK treatment, the total dry weight of fragrant rice treated by F-HRW and I-HRW treatments was increased (7.70% and 16.99%) (Table 1) and the similar results have been reported by Su *et al*. (2018) who indicated that the inhibition of osmotic stress in fresh weight and dry weight in alfalfa seedlings root and shoot could significantly be alleviated through the application of HRW containing 0.39 mM $H_2$.

In general, application of HRW under NaCl stress improved the total dry weight in fragrant varieties than CK treatment. F-HRW treatment significantly increased shoot dry weight, dry weight per unit seedling height and antioxidant enzymes activities, including SOD, POD, CAT in salt-stressed fragrant rice cultivars, compared with CK treatment. I-HRW treatment remarkably increased root dry weight and antioxidant enzymes activities, including CAT and POD in fragrant rice varieties under salt stress than CK treatment (Fig. 7). And the POD activity in rice shoot and root played the most important role in PCA loading for parameters (Table 2). Overall, these results revealed that the application of HRW could induce plant growth and physiological attributes in fragrant rice under salt stress.

In sum, the F-HRW and I-HRW treatments substantially improved the plant growth and physiological attributes in salt-stressed fragrant rice varieties. The activities of antioxidant enzymes, including SOD, POD and CAT in fragrant rice were increased under salt stress, associated with the decrease of MDA content with F-HRW and I-HRW treatments. Generally, the shoot dry weight, root dry weight and total dry weight were improved through F-HRW and I-HRW treatments, compared with CK treatment. In addition, the plant height and dry weight per unit seedling height were significantly improved due to HRW treatments under NaCl stress. However, further studies are still needed to illustrate the metabolic and molecular basis of HRW induced plant growth and physiological attributes in fragrant rice under salt stress.

# Declarations

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### Author contributions

ZM conceived and supervised the project. XF, LM and RG contributed to do the pot experiments. XF, LM, YL, XY and JZ performed the sampling and observation of the rice material. XF, LM and HT analyzed the data. XF, LM, RG, ZM and MI wrote the first draft. XT is the leader of our research team. All authors made a substantial, direct and intellectual contribution to this work. All of the authors approve the final version of the manuscript.

### Availability of data and materials

All data generated or analyzed during this study are included in this manuscript. Please turn to the corresponding author for all other requests.

### Ethics approval and consent to participate

Our institutional review board approved this study and all biological samples were collected under the approval of the Department of Crop Science and Technology, College of Agriculture, South China Agricultural University.

### Consent for publication

Not applicable.

### Conflict of interest

All the authors declare that there are no conflicts of interest.

## References

Abdallah, MM-S., Abdelgawad, Z.A, El-Bassiouny, HMS., 2016. Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. *South African Journal of Botany*, 103, 275-282.

Al, Hassan.M., Martinez, Fuertes.M., Ramos, Sanchez.F.J., Vicente, O., Boscaiu, M., 2015. Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43, 1-11.

Athar, H., Khan, A., Ashraf, M., 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany*, 63, 224-231.

Bailly, C., 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research*, 14, 93–107.

Bernardi, C., Chiesa, L.M., Soncin, S., Passero, E., Biondi, P.A., 2008. Determination of carbon monoxide in tuna by gas chromatography with micro-thermal conductivity detector. *Journal of Chromatographic Science*, 46, 392–394.

Buchholz, B.M., Kaczorowski, D.J., Sugimoto, R., Yang, R., Wang, Y., Billiar, T.R., McCurry, K.R., Bauer, A.J., Nakao, A., 2008. Hydrogen inhalation ameliorates oxidative stress in transplantation induced intestinal graft injury. *American Journal of Transplantation*, 8, 2015-2024.

Cheng, Z.B., Chen, Y., Zhang, F.H., 2019. Effect of cropping systems after abandoned salinized farmland reclamation on soil bacterial communities in arid northwest China. *Soil and Tillage Research*, 187, 204-213.

Cui, W.T., Fang, P., Zhu, K.K., Mao, Y., Gao, C.Y., Xie, Y.J., Wang, J., Shen, W.B., 2014 Hydrogen-rich water confers plant tolerance to mercury toxicity in alfalfa seedlings. *Ecotoxicology and environmental safety*, 105, 103-111.

Edelstein, M., Ben-Hur, M., 2018. Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Scientia Horticulturae*, 234, 431-444.

Foster, T., Brozović, N., Butler, A.P., Neale, C.M.U., Raes, D., Steduto, P., Fereres, E., Hsiao, T.C., 2017. AquaCrop-OS: An open source version of FAO's crop water productivity model. *Agricultural water management*, 181, 18-22.

Frukh, A., Siddiqi, T.O., Khan, M.I.R., Ahmad, A., 2020. Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. *Plant Physiology and Biochemistry*, 146, 55-70.

Guan, Q., Ding, X.W., Jiang, R., Ouyang, P.L., Gui, J., Feng, L., Yang, L., Song, L.H., 2019. Effects of hydrogen-rich water on the nutrient composition and antioxidative characteristics of sprouted black barley. *Food Chemistry*, 299, 125095.

Huang, B.K., Xu, S., Xuan, W., Li, M., Cao, Z.Y., Liu, K.L., Ling, T.F., Shen, W.B., 2006. Carbon monoxide alleviates salt-induced oxidative damage in wheat seedling leaves. *Journal of Integrative Plant Biology*, 48, 249-254.

Jin, Q.J., Zhu, K.K., Cui, W.T., Li, L.N., Shen, W.B., 2016. Hydrogen-modulated stomatal sensitivity to abscisic acid and drought tolerance via the regulation of apoplastic pH in Medicago sativa. *Journal of Plant Growth Regulation*, 35, 565–573.

Kong, L.L., Ashraf, U., Cheng, S., Rao, G.S., Mo, Z.W., Tian, H., Tang, X.R., 2017. Short-term water management at early filling stage improves early-season rice performance under high temperature stress in South China. *European journal of agronomy*, 90, 117-126.

Lee, D.H., Lee, C.B., 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Science*, 159, 75–85.

Li, S.Y., Jiang, H.L., Wang, J.J., Wang, Y.D., Pan, S.G., Tian, H., Duan, M.Y., Wang, S.L., Tang, X.R., Mo, Z.W., 2019. Responses of plant growth, physiological, gas exchange parameters of super and non-super rice to rhizosphere temperature at the tillering stage. *Scientific Reports*, 9, 1-17.

Liang, Y.F., Li, F.S., Nong, M.L., Luo, H., Zhang, J.H., 2016. Microbial activity in paddy soil and water-use efficiency of rice as affected by irrigation method and nitrogen level. *Communications in Soil Science and Plant Analysis*, 47, 19-31.

Lin, Y.T., Zhang, W., Qi, F., Cui, W.T., Xie, Y.J., Shen, W.B., 2014. Hydrogen-rich water regulates cucumber adventitious root development in a heme oxygenase-1/carbon monoxide-dependent manner. *Journal of Plant Physiology*, 171, 1-8.

Liu, Q., Shen, W.F., Sun, H.Y., Fan, D.F., Nakao, A., Cai, J.M., Yan, G., Zhou, W.P., Shen, R.X., Yang, J.M., Sun, X.J., 2010. Hydrogen-rich saline protects against liver injury in rats with obstructive jaundice. *Liver International*, 30, 958-968.

Liu, S.L., Liu, K., Sun, Q., Liu, W.W., Xu, W.G., Denoble, P., Tao, H.Y., Sun, X.J., 2011. Consumption of hydrogen water reduces paraquat-induced acute lung injury in rats. *Biomed Research International*, 2011, 1-7.

MacAdam, J.W., Nelson, C.J., Sharp, R.E., 1992. Peroxidase activity in the leaf elongation zone of tall fescue. *Plant Physiology*, 99, 872–878.

McGrath, J.M., Lobell, D.B., 2013. Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO2 concentrations. *Plant, Cell & Environment*, 36, 697-705.

Mekawy, A.M.M., Assaha, D.V., Yahagi, H., Tada, Y., Ueda, A., Saneoka, H., 2015. Growth, physiological adaptation, and gene expression analysis of two Egyptian rice cultivars under salt stress. *Plant Physiology and Biochemistry*, 87, 17-25.

Qados, AMA., 2011. Effect of salt stress on plant growth and metabolism of bean plant Vicia faba (L.). *Journal of the Saudi Society of Agricultural Sciences*, 10, 7-15.

Shannon, M.C., Rhoades, J.D, Draper, J.H., Scardaci, S.C., Spyres, M.D., 1998. Assessment of salt tolerance in rice cultivars in response to salinity problems in California. *Crop Science*, 38, 394-398.

Siddiqui, M.H., Mohammad, F., Khan, M.N., Al-Whaibi, M.H., Bahkali, A.H., 2010. Nitrogen in relation to photosynthetic capacity and accumulation of osmoprotectant and nutrients in Brassica genotypes grown under salt stress. *Agricultural Sciences in China*, 9, 671-680.

Sipaseuth., Basnayake, J., Fukai, S., Farrell, T.C., Senthonghae, M., Sengkeo., Phamixay, S., Linquist, B., Chanphengsay, M., 2007. Opportunities to increasing dry season rice productivity in low temperature affected areas. *Field Crops Research*, 102, 87-97.

Su, J.C., Zhang, Y.H., Nie, Y., Cheng, D., Wang, R., Hu, H.L., Chen, J., Zhang, J.F., Du, Y.W., Shen, W.B., 2018. Hydrogen-induced osmotic tolerance is associated with nitric oxide-mediated proline accumulation and reestablishment of redox balance in alfalfa seedlings. *Environmental and Experimental Botany*, 147, 249-260.

Su, N.N., Wu, Q., Liu, Y.Y., Cai, J.T., Shen, W.B., Xia, K., Cu, J., 2014. Hydrogen-rich water reestablishes ROS homeostasis but exerts differential effects on anthocyanin synthesis in two varieties of radish sprouts under UV-A irradiation. *Journal of Agricultural and Food Chemistry*, 62, 6454-6462.

Thu, H.P.T., Thu, T.N., Thao, N.D.N., Minh, K.L., Tan, K.D., 2019. Evaluate the effects of salt stress on physico-chemical characteristics in the germination of rice (Oryza sativa L.) in response to methyl salicylate (MeSA). *Biocatalysis and Agricultural Biotechnology*, 23, 101470.

Torabi, F., Majd, A., Enteshari, S., 2015. The effect of silicon on alleviation of salt stress in borage (Borago officinalis L.). *Soil Science and Plant Nutrition*, 61, 788-798.

Tripathi, D.K., Singh, S., Gaure, S., Singh, S., Yadav, V., Liu, S.L., Singh, V.P., Sharma, S., Srivastava, P., Prasad, S.M., Dubey, N.K., Chauhan, D.K., Sahi, S., 2017. Acquisition and homeostasis of iron in higher plants and their probable role in abiotic stress tolerance. *Frontiers in Environment Science*, 5, 1-8.

Wahid, A., Perveen, M., Gelani, S., Basra, S.M.A., 2007. Pretreatment of seed with H2O2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology*, 164, 283–294.

Wang, Y.Y., Wang, L.Q., Ma, C.X., Wang, K.X., Hao, Y., Chen, Q., Mo, Y., Rui, Y.K., 2019. Effects of cerium oxide on rice seedlings as affected by co-exposure of cadmium and salt. *Environmental Pollution*, 252, 1087-1096.

Wani, A.S., Ahmad, A., Hayat, S., Fariduddin, Q., 2013. Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of Brassica juncea. *Saudi Journal of Biological Sciences*, 20, 183-193.

Xie, Y.J., Mao, Y., Lai, D.W., Zhang, W., Shen, W.B., 2012. $H_2$ enhances Arabidopsis salt tolerance by manipulating ZAT10/12-mediated antioxidant defence and controlling sodium exclusion. *PLoS ONE*, 7, e49800.

Xie, K.L., Yu, Y.H., Pei, Y.P., Hou, L.C., Chen, S.Y., Xiong, L., Wang, G.L., 2010. Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock*, 34, 90-97.

Xu, S., Zhu, S.S., Jiang, Y.L., Wang, N., Wang, R., Shen, W.B., Yang, J., 2013. Hydrogen-rich water alleviates salt stress in rice during seed germination. *Plant and Soil*, 370, 47-57.

Yang, Y.Q., Guo, Y., 2018. Unraveling salt stress signaling in plants. *Journal of Integrative Plant Biology*, 60, 796–804.

Zeng, J.Q., Zhang, M.Y., Sun, X.J., 2013. Molecular hydrogen is involved in phytohormone signaling and stress responses in plants. *PLoS ONE*, 8, e71038.

Zhang, H.J., Dong, H.Z., Li, W.J., Zhang, D.M., 2011. Effects of soil salinity and plant density on yield and leaf senescence of field-grown cotton. *Journal of Agronomy and Crop Science*, 198, 27–37.

Zhang, J.H., Bai, Z.G., Huang, J., Hussain, S., Zhao, F.T., Zhu, C.Q., Zhu, L.F., Cao, X.C., Jin, Q.Y., 2019. Biochar alleviated the salt stress of induced saline paddy soil and improved the biochemical characteristics of rice seedlings differing in salt tolerance. *Soil and Tillage Research*, 195, 104372.

# Figures

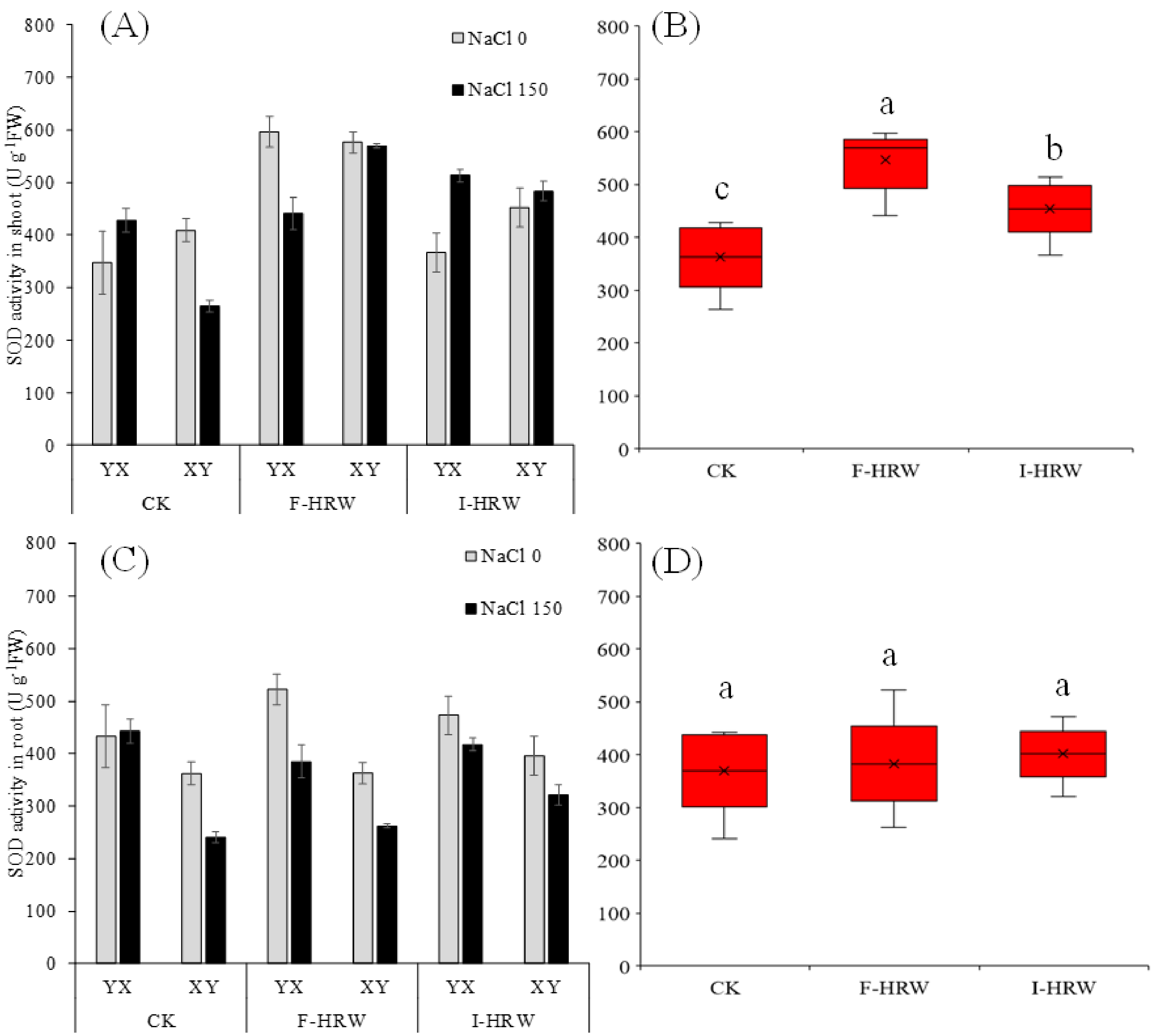
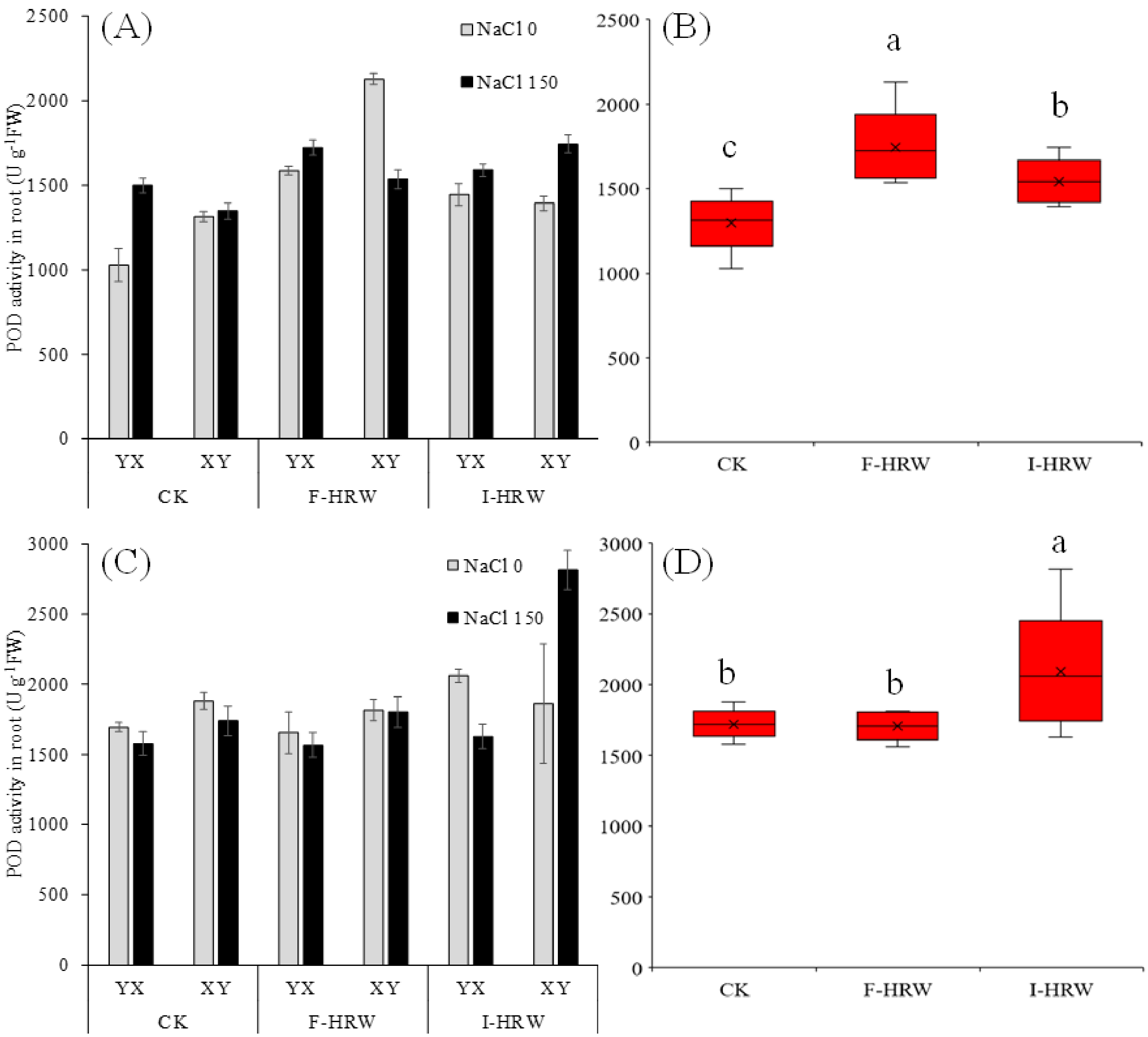



Figure 1

Effect of HRW on SOD activity in shoot and root Vertical bars represent mean value. Capped bars represent SD. Lower-case letter indicates comparisons among the treatments. CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L-1 and 150 mmol L-1 of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the boxmark the median, and whiskers below and above the box indicate the 10th and 90th percentiles, respectively.




## Figure 2

Effect of HRW on POD activity in shoot and root Vertical bars represent mean value. Capped bars represent SD. Lower-case letter indicates comparisons among the treatments. CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L-1 and 150 mmol L-1of NaCl. The box boundaries indicate the 25th and 75th percentiles;

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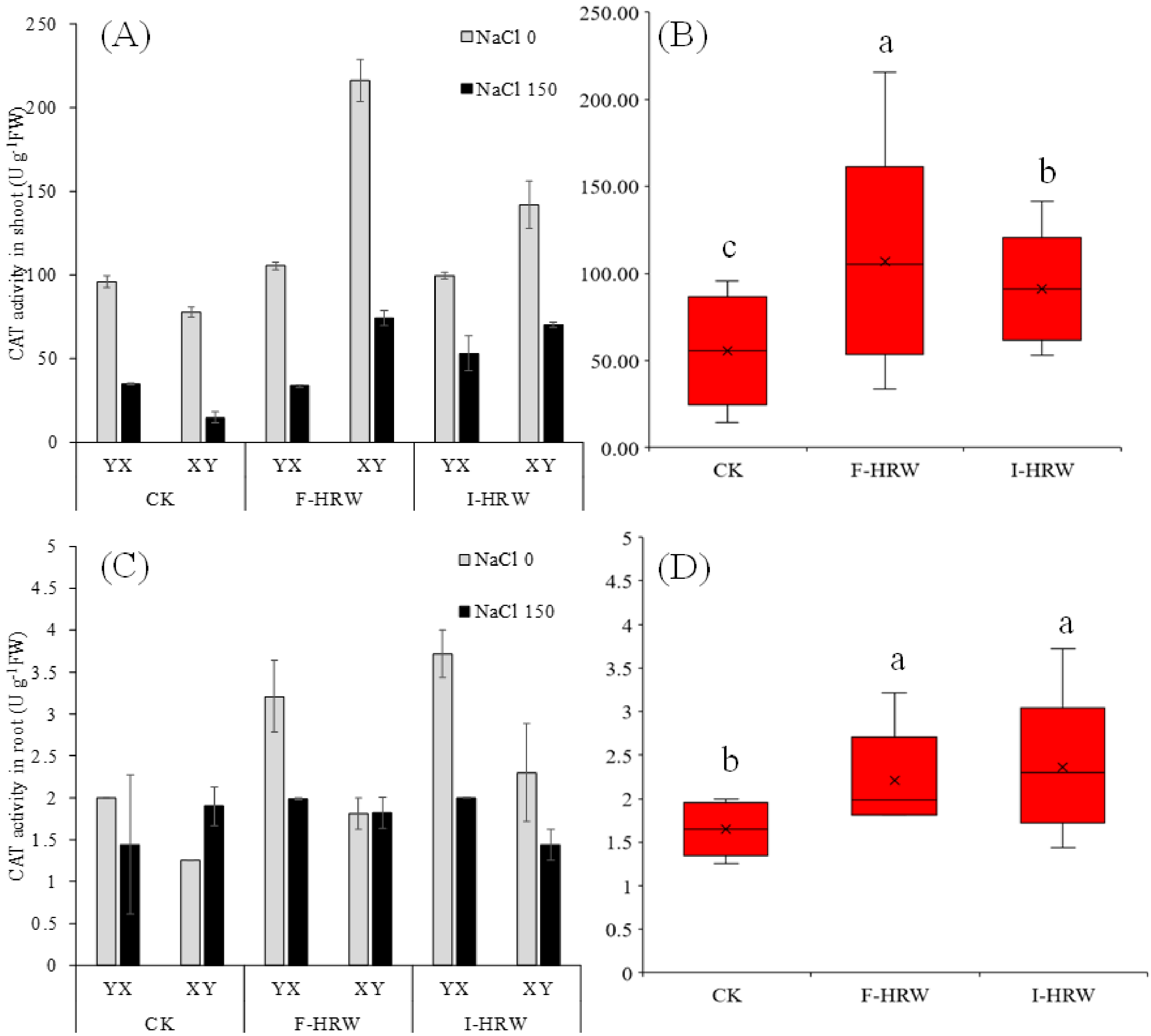



## Figure 3

Effect of HRW on CAT activity in shoot and root Vertical bars represent mean value. Capped bars represent SD. Lower-case letter indicates comparisons among the treatments. CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L-1 and 150 mmol L-1of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the boxmark the median, and whiskers below and above the box indicate the 10th and 90th percentiles, respectively.

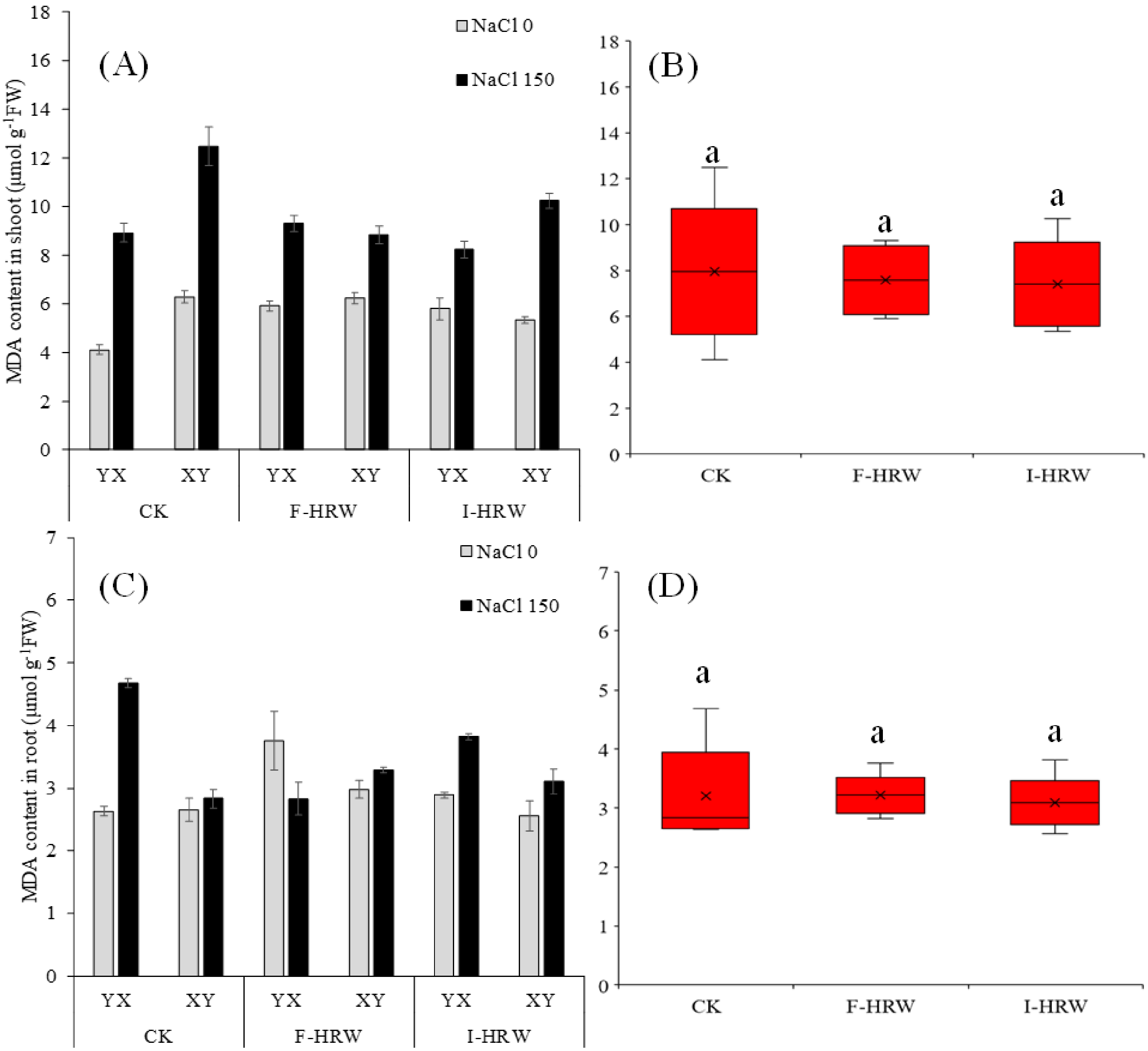



**Figure 4**

Effect of HRW on MDA content in shoot and root Vertical bars represent mean value. Capped bars represent SD. Lower-case letter indicates comparisons among the treatments. CK: without HRW; F-HRW: HRW was sprayed to the shoot of the seedling; I-HRW: HRW was watered to the root; NaCl 0: and NaCl 150: 0 mmol L-1 and 150 mmol L-1of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the boxmark the median, and whiskers below and above the box indicate the 10th and 90th percentiles, respectively.

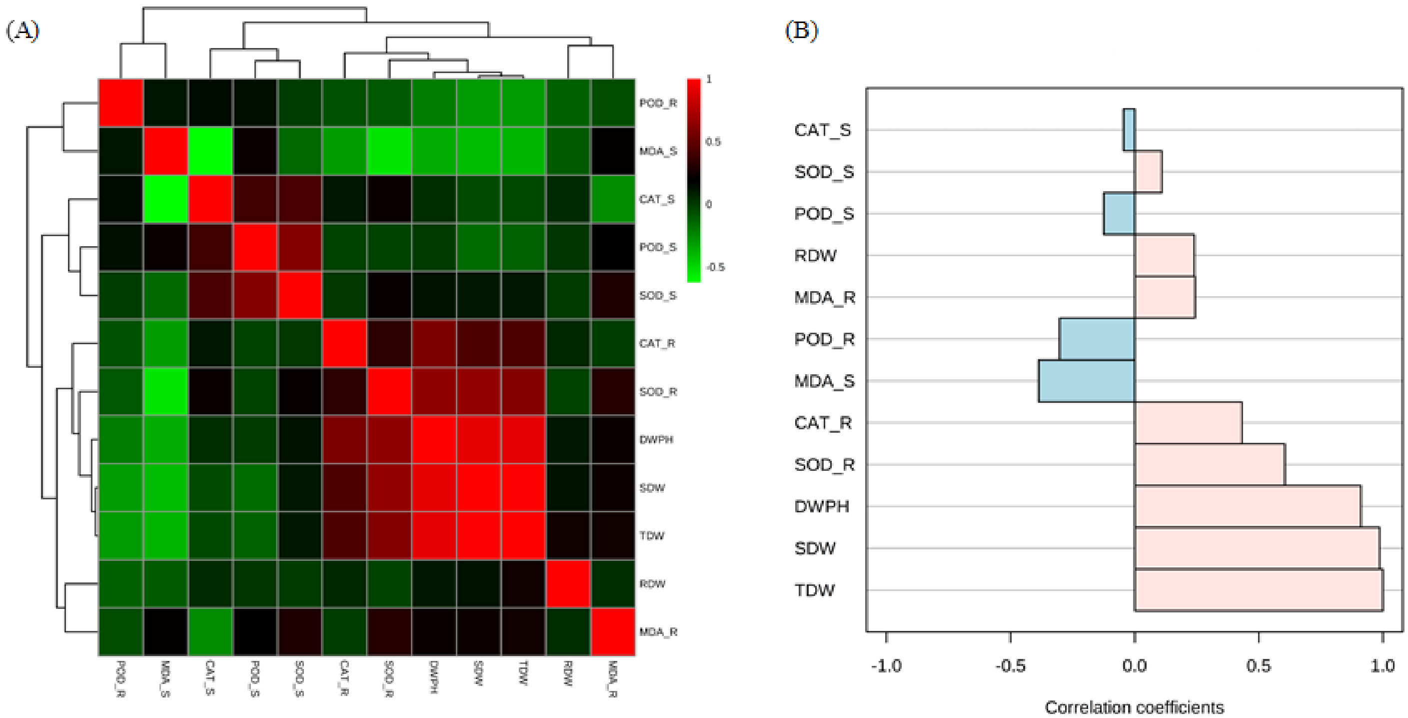



**Figure 5**

The correlation analysis of the investigated parameters. PH: plant height; SDW: Shoot dry weight; RDW: Root dry weight; TDW: Total dry weight; DWPH: Dry weight per unit seedling height; SOD_S: SOD activity in shoot; SOD_R: SOD activity in root; POD_S: POD activity in shoot; POD_R: POD activity in root; CAT_S: CAT activity in shoot; CAT_R: CAT activity in root; MDA_S: MDA content in shoot; MDA_R: MDA content in root.

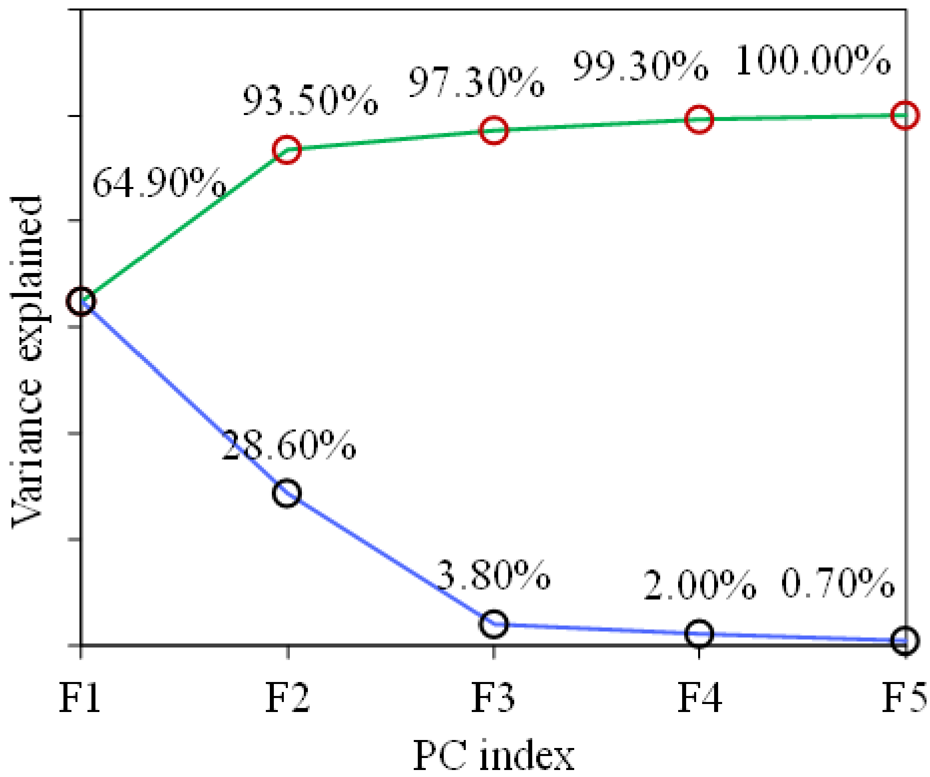
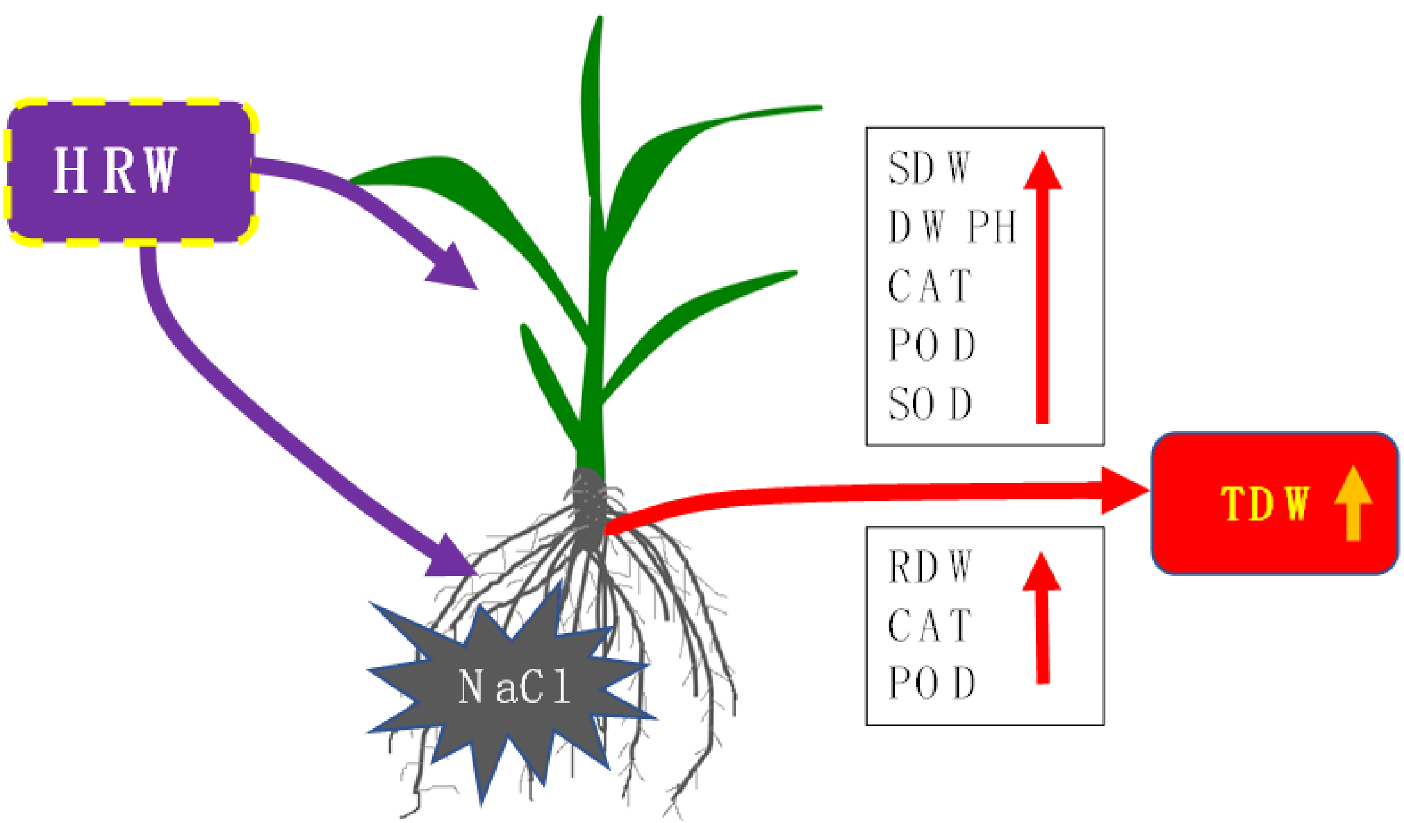



**Figure 6**

PCA analyses of the investigated parameters



## Figure 7

HRW regulated seedling grow under salt stress SDW: Shoot dry weight; RDW: Root dry weight; TDW: Total dry weight; DWPH: Dry weight per unit seedling height; SOD: SOD activity in fragrant rice; POD: POD activity in fragrant rice; CAT: CAT activity in fragrant rice.