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 depicted 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.
H₂ 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 H₂ 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 H₂ 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°C 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 °C 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°C 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$_2$O$_2$, 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$_2$O$_2$. 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 and 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                                      |
| 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                | **                                    | ns                                | **                                  | **                                               |
| Variety × NaCl   | *                 | ns                                    | ns                                | ns                                  | ns                                               |
| Variety × HRW    | **                | **                                    | ns                                | *                                   | ns                                               |
| NaCl × HRW       | **                | **                                    | 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   | **      | **   | **  | *             | ns            | **         | **                   |
| CAT_R U g⁻¹ FW   | *       | **   | *   | *             | ns            | *          | ns                   |
| MDA_S µmol g⁻¹ FW| *       | **   | ns  | **            | **            | **         | *                    |
| MDA_R µmol 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).

| SDW  | PC1     | PC2     | PC3     | PC4     | PC5     |
|------|---------|---------|---------|---------|---------|
| RDW  | -0.0028 | 0.0008  | 0.0210  | -0.0121 | 0.0148  |
| TDW  | -0.0028 | 0.0004  | 0.0195  | -0.0115 | 0.0144  |
| DWPH | -0.0001 | 0.0000  | 0.0009  | -0.0008 | 0.0005  |
| CAT  | 0.0248  | -0.0726 | 0.1855  | 0.0519  | -0.9775 |
| CAT  | -0.0001 | 0.0000  | 0.0023  | -0.0026 | -0.0012 |
| POD  | 0.1590  | -0.9481 | -0.1658 | -0.2178 | 0.0312  |
| POD  | 0.9866  | 0.1587  | 0.0315  | 0.0081  | 0.0196  |
| SOD  | 0.0161  | -0.2658 | 0.5308  | 0.7879  | 0.1627  |
| SOD  | -0.0219 | -0.0095 | 0.8089  | -0.5733 | 0.1229  |
| MDA  | 0.0008  | -0.0013 | -0.0148 | 0.0008  | 0.0315  |
| MDA  | -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 H₂, 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 H₂ 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 (H₂O₂) 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 H₂.

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.

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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.
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-1 of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the box mark the median, and whiskers below and above the box indicate the 10th and 90th percentiles, respectively.
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-1 of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the box marks 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-1 of NaCl. The box boundaries indicate the 25th and 75th percentiles; the black line in the box mark 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.