EXCESSIVE FERTILIZATION RESULTED IN DECREASED ANTIOXIDANT PERFORMANCE OF THREE VARIETIES OF SUPER RICE

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(Received 28th Oct 2018; accepted 11th Jan 2019)

Abstract. Fertilizer application is an important part in super rice production. In order to investigate the effect of fertilizer amounts on the antioxidant performance of the super rice, three super hybrid rice cultivars, *YLiangyou-1173*, *YLiangyou-911* and *Chaoyou-1000*, were used in the experiment. The fertilizer amounts were set as: (F1)1050 kg N ha^{-1}; (F2) 1200 kg N ha^{-1}; (F3) 1350 kg N ha^{-1}; (F4) 1500 kg N ha^{-1}; (F5) 1650 kg N ha^{-1}. The results showed that the activities of antioxidant enzymes were decreased when excessive fertilizers such as F4 and F5 were applied. Compared with F1, F4 and F5 significantly reduced the activity of superoxide (SOD, EC 1.15.1.1), guaiacol type peroxidase (POD EC1.11.1.7) and catalase (CAT, EC1.11.1.6). Furthermore, there was no remarkable significant difference related to the yield among F2, F3, F4 and F5.
Keywords: *rice, superoxide, catalase, peroxidase, yield, fertilizer*

Introduction

Rice (*Oryza sativa* L.) has been cultivated in China for a very long time and its production has played an important role in Chinese food security (Ren et al., 2017). Many experts had done many researches in different fields to improve to improve rice yield and quality (Wang and Zhang, 2017; Wu et al., 2018; Jiang et al., 2018). Generating rice varieties with high yield potential is a main method to improve rice yield. In 1996, China's ministry of agriculture proposed the super hybrid rice cultivation program which was led by Yuan Longping, the "father of hybrid rice", in 2000, the super hybrid rice varieties reached the first-stage single rice yield standard, that is, the yield per hectare exceeded 10.5 tons and in 2004, the output of super hybrid rice reached the second stage (Schmalzer, 2017). Normally, in order to improve the potential of yield and increase the final yield of super rice, researchers and farmers would apply as much fertilizer as possible and try to found the correlation between rice yield and fertilizing amount. However, there was no much report about the effect of fertilizer amount on rice physiological property especially antioxidant property.

The antioxidant system is very important when rice faces the environment stress such as heat stress, chilling, heavy metal stress and it also has relationship with rice yield and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_18891898

quality. When rice faces the environmental stress including UV stress and pathogen invasion, the reactive oxygen species (ROS) will be produced and accumulated while oxidative stress is induced. Then, a lot of cellular reactions will be produced in by various enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). The formation of ROS is prevented and decreased by an antioxidant system which including ROS-interacting enzymes such as SOD, CAT and POD (Blokhina, 2003). The study of Kong (Kong et al., 2017) showed that the malondialdehyde (MDA) content would increase when rice suffered from high temperature stress at filling stage and the soluble protein, POD, SOD, CAT and free proline would work together to quench the ROS and decrease the MDA concentration. Pan (Pan et al., 2013) demonstrated that spraying paclobutrazol (PBZ) and 6-Benzylaminopurine (6-BA) at heading stage could not only increase POD and SOD activities but also improve the rice yield and grain quality.

Many researches had proved that fertilizer application is a key part in rice production (Shakoor et al., 2018; Liu et al., 2018). The study of Li et al. (2016) showed that manganese (Mn) fertilizer could enhance enzyme activity involved in 2-acetyl-1-pyrroline (2-AP) biosynthesis in fragrant rice while regulating the grain yield. The investigation of Pan et al. (2017) revealed that mechanized deep placement of nitrogen fertilizer in direct-seeded rice improved rice yield by promoting the fertilizer uptake of rice and increasing the utilization rate. Fertilizer application is the other source of rice nutrition beside photosynthesis and it also could affect the rice growth environment such as soil properties (Sun et al., 2018; Ghaley et al., 2018). However, the relationship between fertilizer amount and rice antioxidant property is still unknown to us.

Therefore, the study was conducted in Guangzhou, Guangdong (major rice producing province in South China) in early season in 2018 in order to investigate the effect of different amount of fertilizer on antioxidant performance of three super rice cultivars.

Materials and methods

Plant materials and growing condition

Three super hybrid rice cultivars, *YLiangyou-1173, YLiangyou-911* and *Chaoyou-1000*, well-known and widely grown in South China, were in planted in the early season in Guangzhou (23°16’ N, 113°23’ E) during 2018. Before sowing, the seeds were soaked in water for 24 h, germinated in manual climatic box for the next 24 h, shade dried and the germinated seeds were sown in PVC trays for nursery raising. 20-day-old seedlings were transplanted to the field at the planting distance (30 cm × 12 cm). The experimental soil in Guangzhou was sandy loam with of 24.65% organic matter content, 1.560% total N, 0.956% total P, and 18.460% total K.

Treatments and plant sampling

Five amounts of commercial compound fertilizer were applied at the experiment and set as below:

F1: 1050 kg ha^{-1} commercial compound fertilizer (pure nitrogen 131.25 kg ha^{-1}, pure phosphate 63 kg ha^{-1}, pure potassium 105 kg ha^{-1}, organic fertilizer 210 kg ha^{-1});
F2: 1200 kg ha^{-1} commercial compound fertilizer (pure nitrogen 150 kg ha^{-1}, pure phosphate 72 kg ha^{-1}, pure potassium 120 kg ha^{-1}, organic fertilizer 240 kg ha^{-1});
F3: 1350 kg ha^{-1} commercial compound fertilizer (pure nitrogen 168.75 kg ha^{-1}, pure phosphate 81 kg ha^{-1}, pure potassium 135 kg ha^{-1}, organic fertilizer 270 kg ha^{-1});

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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F4: 1500 kg ha^{-1} commercial compound fertilizer (pure nitrogen 187.50 kg ha^{-1}, pure phosphate 90 kg ha^{-1}, pure potassium 150 kg ha^{-1}, organic fertilizer 300 kg ha^{-1});
F5: 1650 kg ha^{-1} commercial compound fertilizer (pure nitrogen 206.25 kg ha^{-1}, pure phosphate 99 kg ha^{-1}, pure potassium 165 kg ha^{-1}, organic fertilizer 330 kg ha^{-1}).

The fertilizer was invented by Tang Xiangru (CN 101955394 A). The special fertilizer is a mixture of nitrogen (N) fertilizer, phosphate (P) fertilizer, potassium (K) fertilizer and biological organic fertilizer (12.50% N, 6.00% P, 10.00% K and 20.00% organic matter content). Normally, the local farmers in Guangdong were used to applied 1500 kg ha^{-1}. The treatments were arranged in randomized complete block design (RCBD) in triplicate in each year with net plot size of 20 m^2.

The fresh flag leaves were separated and collected from the rice plants at tillering stage, heading stage and maturity stage, respectively. The sample was washed with double distilled water and stored at -80 °C for physio-biochemical analysis.

Measurement of malondialdehyde (MDA) and anti-oxidants responses

The MDA content and activities of POD, SOD and CAT were determined according to the methods described by Kong et al. (2017). MDA reacted with thiobarbituric acid (TBA) and the absorbance was read at the 532 nm, 600 nm, and 450 nm. The content of the reaction solution was calculated as: MDA content (μmol/L) = 6.45(OD532 −OD600) − 0.56OD450, while the final result was expressed as μmol/g FW.

Guaiacol type peroxidase (POD EC1.11.1.7) activity was estimated after the reaction which the solution was including enzyme extract (50 μl), 1 ml of 0.3% H2O2, 0.95 ml of 0.2% guaiacol, and 1 ml of 50 mM l−1 sodium phosphate buffer (pH 7.0) while One POD unit of enzyme activity was expressed as the absorbance increase because of guaiacol oxidation by 0.01 (U/g FW). The superoxide (SOD, EC 1.15.1.1) activity was measured by using nitro blue tetrazolium (NBT). 0.05 ml of enzyme extract was added into the reaction mixture which contained 1.75 ml of sodium phosphatebuffer (pH 7.8), 0.3 ml of 130 mM methionine buffer, 0.3 ml of 750 μmol L^{-1} NBT buffer, 0.3 ml of 100 μmol L^{-1} EDTA-Na 2 buffer and 0.3 ml of 20 μmol L^{-1} lactoflavin. After reaction, the absorbance was recorded at 560 nm. One unit of SOD activity is equal to the volume of extract needed to cause 50% inhibition of the color reaction. Catalase (CAT, EC1.11.1.6) activity was estimated by adding an aliquot of enzyme extract (50 μl) to the reaction solution containing 1 ml of 0.3% H2O2 and 1.95 ml of sodium phosphate buffer and then the absorbance was read at 240 nm. One CAT unit of enzyme activity was defined as the absorbance decrease by 0.01 (U/g FW).

Estimation of rice yield

At maturity stage, the rice grains were harvested from ten unit sampling area (1.00 m^2) in each plot and then threshed by machine. The harvested grains were sundried and weighed in order to determinate the grain yield.

Statistical analysis

Data were analyzed using statistical software ‘Statistix 8.1’(Analytical Software, Tallahassee, FL, USA) while differences amongst means were separated by using least significant difference (LSD) test at 5% probability level. Graphical representation was conducted via Sigma Plot 14.0 (Systat Software Inc., California, USA).

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Results

SOD activity

As shown in *Figure 1*, there were some differences on SOD activity among different fertilizer amounts. For *YLiangyou-1173*, there was no remarkable difference between F1 and F2. However, F4 and F5 had lower SOD activity than F1 at tillering stage, heading stage and maturity stage. The trend of SOD activity at tillering stage and maturity stage was recorded as: F1 = F2 > F3 > F4 > F5. For *YLiangyou-911*, the activity decreased with the increase of fertilizer amount. The lowest activity was recorded in F5 while the highest was recorded in F1 at tillering stage and heading stage. The trend at maturity was recorded as: F1 > F2 = F3 > F4 = F5. For *Chaoyou-1000*, at tillering stage, the trend of SOD activity was recorded as: F1 = F2 > F3 > F4 = F5, at heading stage, highest activity was recorded in F1 and the lowest was observed in F5 while in maturity stage, there was no significant difference among F1, F2 and F3 while F4 and F5 were significantly lower than F1.

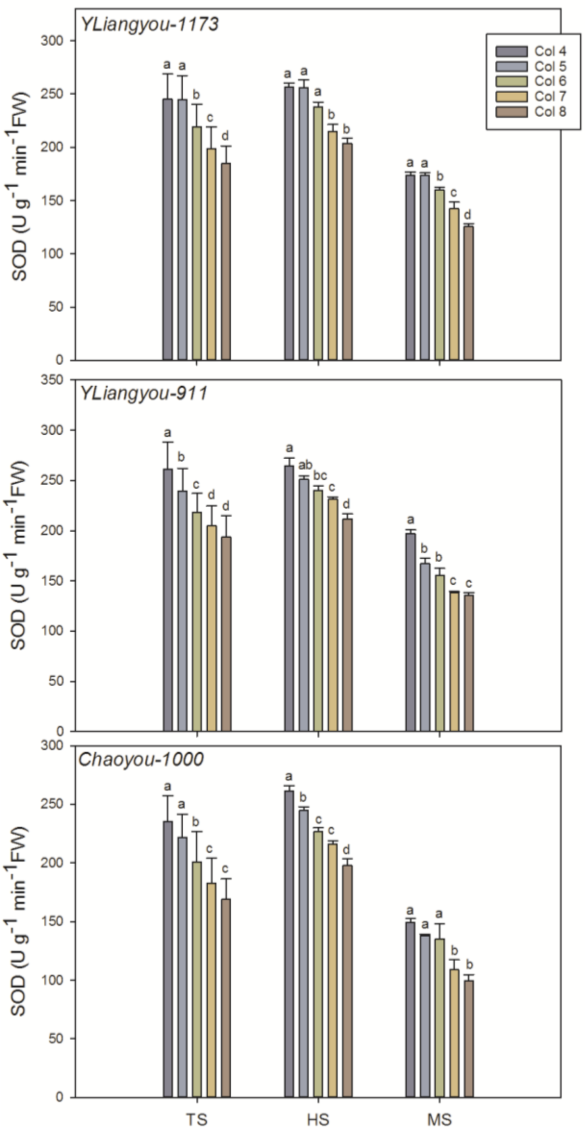

Figure 1. *Effect of fertilizer amounts on SOD activity. TS: tillering stage; HS: heading stage; MS: maturity stage. Means sharing a common letter do not differ significantly (at P < 0.05) according to least significant difference (LSD) test for both the seasons*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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CAT activity

As shown in *Figure 2*, fertilizer amounts affected CAT activity significantly. For *YLiangyou-1173*, the trends of CAT activity at tillering stage, heading stage and maturity stage were recorded as: F1 = F2 > F3 > F4 = F5, F1 = F2 ≥ F3 ≥ F4 = F5, F1 = F2 = F3 ≥ F4 ≥ F5, respectively. For *YLiangyou-911*, at tillering stage, the highest CAT activity was recorded in F1 and there was no remarkable difference among F3, F4 and F5. At heading stage, there was no significant difference among F1, F2, F3 and F4 while F5 had lower activity than F1. At maturity stage, compared with F1, F2, F3, F4 and F5 all significantly reduced the CAT activity while there was no remarkable difference among F2, F3, F4 and F5. For *Chaoyou-1000*, F3, F4 and F5 all had lower activity than F1 at tillering stage and heading stage, however, at maturity, there was no significant difference among all fertilizer treatments.

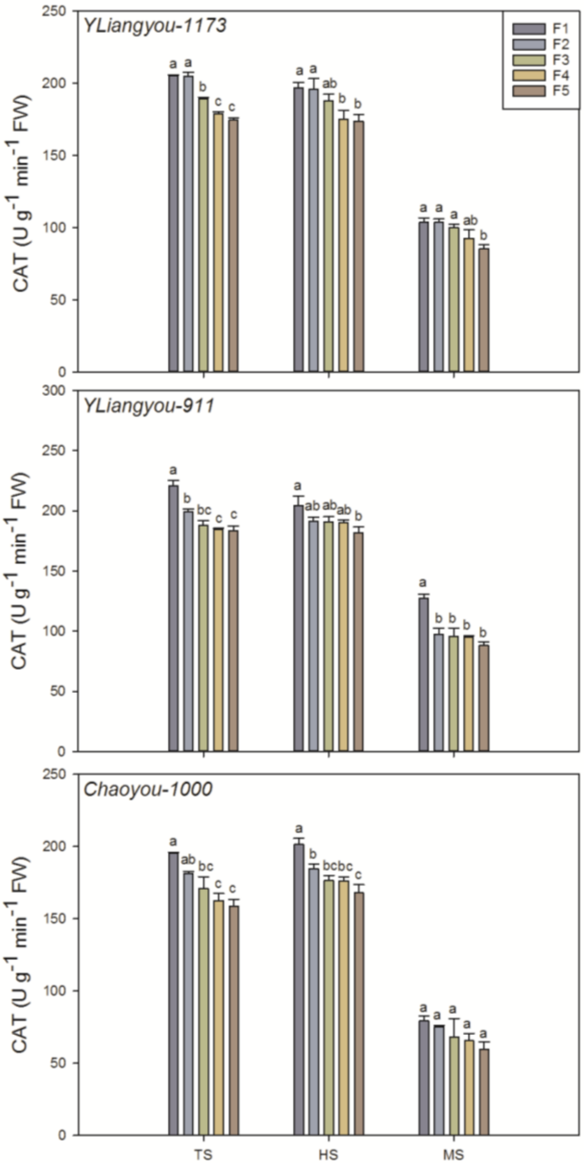

Figure 2. *Effect of fertilizer amounts on CAT activity. TS: tillering stage; HS: heading stage; MS: maturity stage. Means sharing a common letter do not differ significantly (at P < 0.05) according to least significant difference (LSD) test for both the seasons*

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POD activity

As shown in *Figure 3*, fertilizer amounts affected POD activity significantly. For *YLiangyou-1173*, compared with F1, F4 and F5 reduced the POD activity significantly and there was no significant difference between F2 and F1 at heading stage and maturity stage. For *YLiangyou-911*, there was no remarkable difference between F1 and F2 at all three growth stage. However, F3, F4, F5 had lower activity than F1 at all three growth stage. For *Chaoyou-1000*, compared with F1, F4 and F5 significantly decreased the CAT activity at all growth stage. Furthermore, there was no remarkable difference among F1, F2 and F3 at maturity stage.

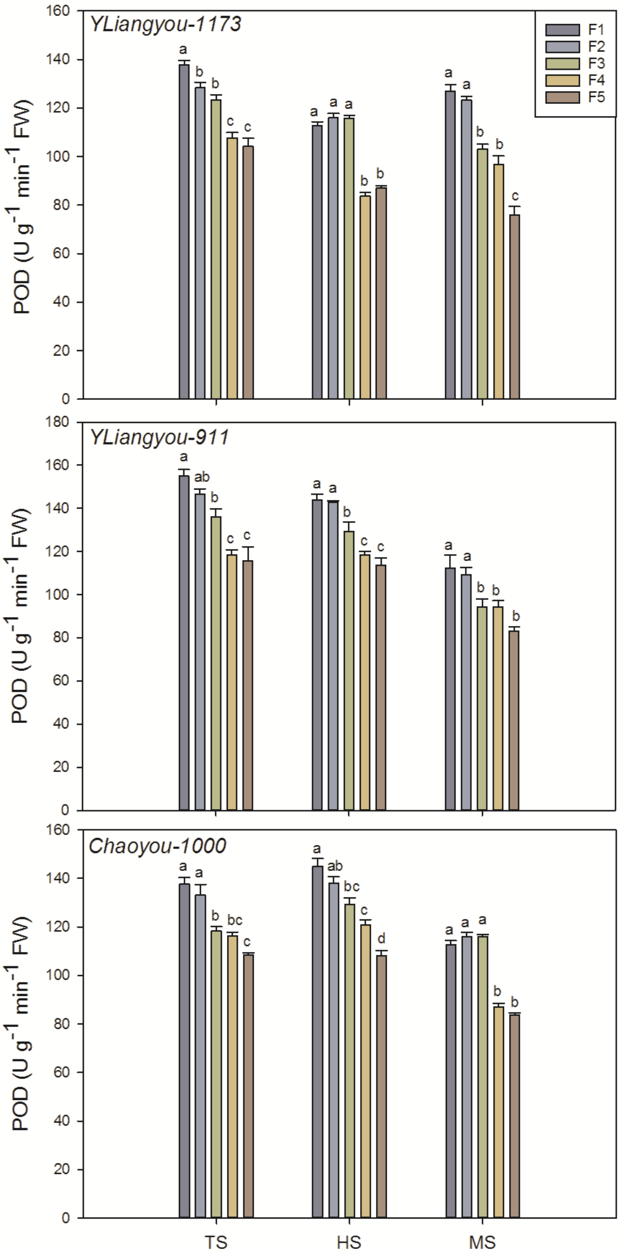

Figure 3. *Effect of fertilizer amounts on POD activity. TS: tillering stage; HS: heading stage; MS: maturity stage. Means sharing a common letter do not differ significantly (at P < 0.05) according to least significant difference (LSD) test for both the seasons*

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MDA content

As shown in *Figure 4*, fertilizer amounts affected MDA concentration significantly. For *YLiangyou-1173*, the highest MDA content was recorded in F5 and the lowest was recorded in F2. For *YLiangyou-911*, at tillering stage, there was no significant difference among F1, F2, F3 and F4, at heading stage, the highest content was recorded in F5 while there was no remarkable difference among F1, F2 and F3, at maturity stage, there was no significant difference among all fertilizer treatments. For *Chaoyou-1000*, the trend of MDA content was recorded as: F5 > F1 > F4 > F3 > F2 at tillering stage, at heading stage, the highest content was recorded in F5 while the lowest was recorded in F2 and F3. At maturity, the highest concentration was recorded in both F5 and F4 while the lowest was recorded in F1.

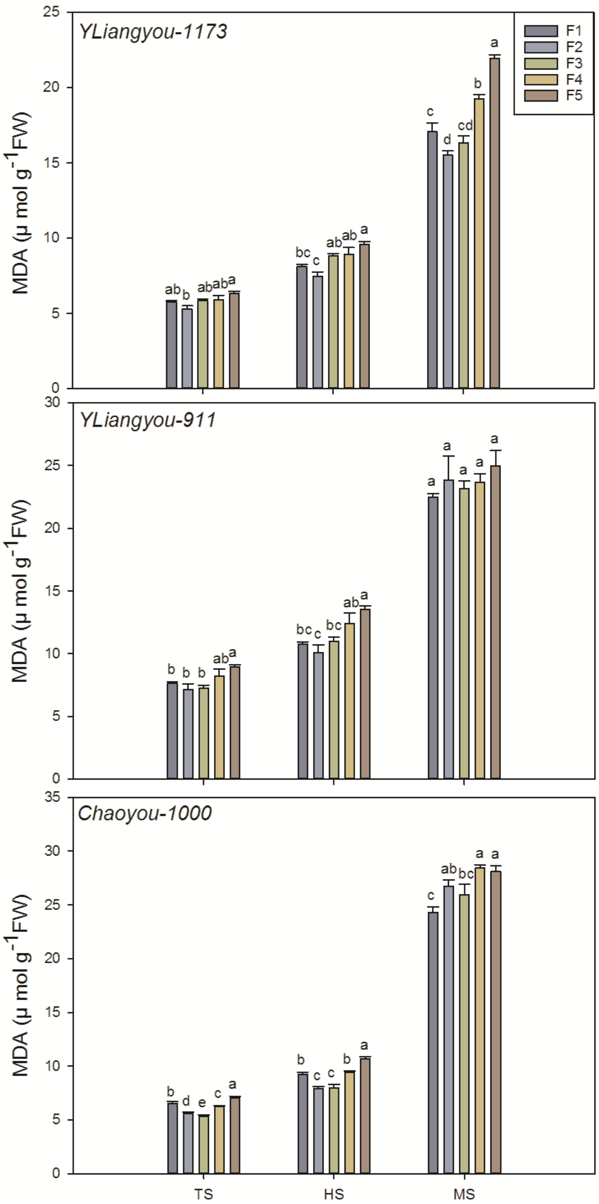

Figure 4. *Effect of fertilizer amounts on MDA content. TS: tillering stage; HS: heading stage; MS: maturity stage. Means sharing a common letter do not differ significantly (at P < 0.05) according to least significant difference (LSD) test for both the seasons*

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Yield

As shown in *Figure 5,* fertilizer amounts affected grain yield significantly. For *YLiangyou-1173*, F2, F3, F4 and F5 had higher yield than F1 but there was no remarkable difference among F2, F3, F4 and F5. For *YLiangyou-911*, with the increase of fertilizer application, the yield increased first and then decreased but there was no significant difference between two adjacent fertilizer gradients. For *Chaoyou-1000*, the trend of was recorded as: F5 = F4 = F3 ≥ F2 ≥ F1.

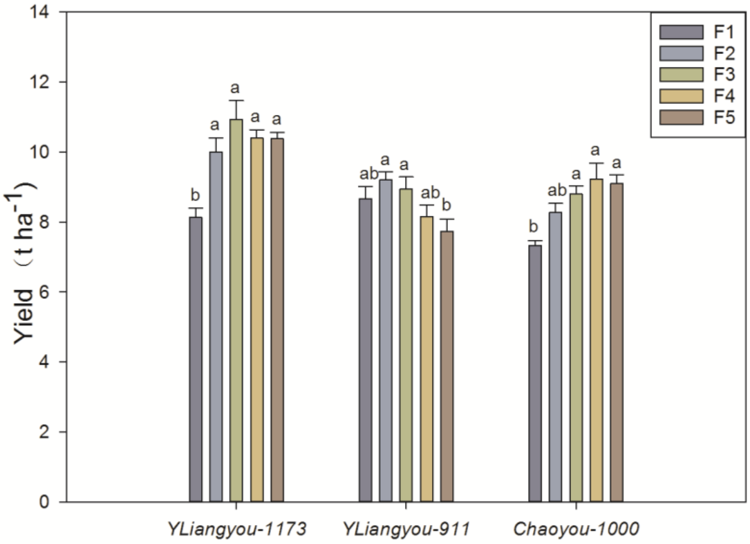

Figure 5. *Effect of fertilizer amounts on yield. Means sharing a common letter do not differ significantly (at P < 0.05) according to least significant difference (LSD) test for both the seasons*

Discussion

The effect of fertilizer on rice had been reported by many researchers. Mo et al. (2017) demonstrated that Silicon (Si) fertilizer had ability to regulate the fragrant rice yield and the content of 2-AP. The research of Sikar et al. (2008) showed that 80 kg hm^{-2} nitrogen improved grain quality significantly and the nitrogen utilization efficiency is connected to the locations and rice genotypes. Previous study (Mahajan et al., 2010) also revealed that high nitrogen application could cause the lodging in rice. In our study, we observed that different amounts of fertilizer influenced the super rice antioxidant system in terms of POD, SOD, CAT and MDA. The result showed that F4 and F5 treatments reduced the enzymes activity significantly which indicated excessive fertilization might improve rice growth, but would reduce the antioxidant capacity.

Antioxidant capacity which involves antioxidant enzymes such as POD, SOD and CAT is a key indicator to measure the ability of rice to resist environmental stress (Almeselmani et al., 2006). Normally, when rice faces environmental stress such as heat damage, chilling, heavy metal stress or extreme weather, the reactive oxygen species (ROS) concentration would increase quickly and cause a number of reactions (ZhangWei and Peng, 2018). For example, the peroxidation of unsaturated fatty acid in plant membranes induced by ROS, resulting in a decrease in the content of unsaturated fatty acid, an increase in MDA concentration and a decreased in membrane fluidity (El-Shintinawy, 2000), then, POD, SOD and CAT would play an important part in

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quenching the ROS and lowering the MDA content. In our study, the results showed that the activity of antioxidant enzymes including SOD, CAT and POD would decrease if too much fertilizer was applied in super rice production. The decrease in antioxidant enzymes activity meant that the reduction of rice ability to quench the ROS and decreased MDA. Then, the risk of environmental stress and disease would be increased.

The harm of excessive fertilization had been reported in previous studies. For example, the study of Zhong and Zu (1997) revealed that supplying with excessive phosphate (P) fertilizer in early stage could not increase yield more than that applied with adequate P, due to the reduction in the valid grain percentage and weight per thousand grains. Similar phenomenon also was found in present study by showing that both F4 and F5 did not have a higher yield than F3. The investigation of Peng et al. (2010) showed that excessive nitrogen application to rice in China could cause environmental pollution, increase the cost of rice farming, reduce grain yield and contribute to global warming. From the perspective of antioxidant performance of rice, this experiment proved again that the importance of moderate fertilization and the harm of excessive fertilization.

Conclusion

Excessive fertilization could cause the decrement of activity of antioxidant enzymes such as SOD, POD and CAT, thus reduced antioxidant capacity of super rice. In rice production, people should pay more attention on rational application of fertilizer and for revealing the mechanism of how fertilizer amounts affecting rice antioxidant system, much work should be done at molecular and physiological level.

Acknowledgements. This study was supported by National Natural Science Foundation of China (31271646), National Key R&D Program of China (2016YFD0700301), the World Bank Loan Agricultural Pollution Control Project in Guangdong (0724-1510A08N3684), the Technology System of Modern Agricultural Industry in Guangdong (2017LM1098) and Student's Platform for Innovation and Entrepreneurship Training Program (201810564029). The authors declare no conflict of interests.

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