EFFECT OF FOLIAR SODIUM SELENATE ON LEAF SENESCENCE OF FRAGRANT RICE IN SOUTH CHINA

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Abstract. Selenium (Se) is an essential element to humans, animals and plants, but little is known about roles of Se in yield and antioxidant enzyme activities of rice. In this study, sodium selenate with 10 (T1), 30 (T2) and 50 (T3) μmol L^{-1} concentrations and distilled water (CK) were sprayed onto rice cultivars, or more precisely *Meixiangzhan-2* at rupturing stage during the experiments in South China, 2017. Treatments were arranged in a split-plot design with three replications. The result showed that spraying 10, 30 and 50 μmol L^{-1} sodium selenate at rupturing stage could improve the activities of oxidant enzymes such as peroxidase (POD), superoxide (SOD) and catalase (CAT) and lower the malonaldehyde (MDA) concentrations at filling stage. Furthermore, Se applications could enhance the chlorophyll content at middle and late phase of filling stage and grain yield at maturity. Therefore, Se applications could alleviate the detrimental effects of rice leaf senescence by regulating the activity of enzymatic antioxidants and also increasing chlorophyll content at the filling stage which will be helpful to sustain growth and yield formation in rice production.
Keywords: *aromatic rice, selenate, chlorophyll, antioxidant enzyme, malonaldehyde,*

Introduction

Rice is one of the most important crops, and a staple food all over the world and its production is an essential part of Chinese food security. A previous study showed that the world population may rise to 10 billion people, which means the need for rice will increase dramatically in next few decades (Krishnan et al., 2007). Therefore, it would be a challenging task to ensure the stabilization of rice yield, and exogenous plant growth regulators might come in handy.

A previous study showed that about 90% of the dry matter formed during the growth and development of crops came from photosynthetic assimilates (Petridis et al., 2018). Normally, the photosynthesis of rice will decrease with the leaf senescence at filling stage. The senescence of plant leaves is a complex and highly ordered process accompanied by the degradation of macromolecular materials, which is an important manifestation of plant

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adaptation to the environment in the process of evolution (Zhang et al., 2018; Li et al., 2018; Toscano et al., 2018). Early researches on plant leaf senescence mainly focused on the morphological and anatomical features at the organ level, the changes of physiological and metabolic features of senescence and the index of classification of senescence types (Osborne, 1959, 1962; Shaw and Srivastava, 1964; Jacobs et al., 1962). From early 1960s to late 1980s, plant aging was studied at tissue, cellular, and subcellular levels (Mae, 1990; Davies and Grierson, 1989; Davies et al., 1990; Adams et al., 1990). Since the late 1980s, with the wide application of modern molecular biology technologies such as in vitro translation technology, cDNA difference display, differential subhybridization, antisense RNA technology and functional genome research in the field of aging biology, the molecular mechanism of aging in plant leaves has been gradually revealed (Hafsi et al., 2000; Morris et al., 2000). Many researchers studied the mechanism of plant aging from different perspectives, and put forward some hypotheses about the mechanism of plant aging, including gene regulation theory, light-carbon imbalance theory, nutrition imbalance theory, hormone balance theory and free radical damage theory (May et al., 2017; Diao and Wang, 2018).

Selenium (Se) is an essential element to humans, animals and plant. Se deficiency had been associated with poor immune function, increased risk of mortality, and cognitive decline (Yang et al., 2018; Ishizawa et al., 2018). The research of Lee even revealed that Se could be a cancer-protective agent (Lee, 1996). Plants varied considerably in their physiological response to Se because some plant species growing on seleniferous soils were able to accumulate very high concentrations of Se, but some were Se non-accumulators and Se-sensitive (Terry et al., 2000). Early investigations had evidenced that Se application could against toxic elements such as arsenic, antimony, mercury and copper (Terry et al., 2000). Moreover, the study of Wang (Wang et al., 2012) even generated a selenium-enriched rice with higher yield and bioavailability by using selenite fertilization. Therefore, Se might have potential to be an exogenous regulator which could help to ensure the food security in rice production.

Present study was conducted in Guangdong province (major rice producing province in South China) with the hypothesis that exogenous sodium selenate application in rupturing stage could delay the leaf senescence of rice during the filling stage.

Materials and Methods

Plant materials and growing condition

The fragrant rice cultivar, *Meixiangzhan-2*, having 111-114 days of growth period was in planted in late season of 2017 in Guangzhou, Guangdong, China (23°13’ N, 113°81’ E). The experimental sites had subtropical-monsoon type climate. 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 seeds were sow in July, transplanted in August and harvested in November. The experimental soil was sandy loam with of 20.12% organic matter content, 1.408% total N, 1.068% total P, and 15.767% total K. This region has subtropical-monsoonal type of climate with mean annual air temperatures of 26.6°C, mean annual maximum and minimum air temperatures of 14°C in November and 37°C in August during the experiment, respectively. ‘Special biological organic fertilizer (Dao Feng Xiang)’ manufactured by Guangzhou Huayuan Agricultural Ltd, China comprised of N+ P2O5+K2O ≥74%, active living bacteria ≥20 million g^{-1}, and organic matter ≥10% was applied at 900 kg ha^{-1} with 60% as basal dose and 40% at tillering.

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Treatments and plant sampling

Experimental treatments were as described:

- CK: Overhead sprinkle with double distilled water at rupturing stage of rice.
- T1: Overhead sprinkle with 10µmol L^{-1} sodium selenate at rupturing stage of rice.
- T2: Overhead sprinkle with 30µmol L^{-1} sodium selenate at rupturing stage of rice.
- T3: Overhead sprinkle with 50µmol L^{-1} sodium selenate at rupturing stage of rice.

A special Knapsack Electric sprayer (3WBD-Qianfeng Agricultural machinery, Yangjiang, Guangdong, China) with 0.2–0.5 mPa pressure and 16–18 L capacity fitted with a special windproof atomizing spray nozzle was used for sprinkle. The thirty fresh flag leaves were sampled from the rice at heading stage, 7, 14, and 21 days after heading stage and at maturity (Maturity was 28 days after the heading stage). The leaves were washed with double distilled water and stored at -80°C for physio-biochemical analysis. The measurements were repeated in triplicate and averaged.

Grain Yield

At maturity stage, the rice grains were harvested from six unit sampling area ($1m^2$) in each plot and then threshed by machine. The harvested grains were sundried and weighted in order to determinate the grain yield.

Estimation 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 (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.

The peroxidase (POD EC1.11.1.7) activity was estiamated after the reaction which the solution was including enzyme extract (50 µl), 1 ml of 0.3% H_2O_2, 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% H_2O_2 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).

Determination of Chlorophyll contents

The contents of total chlorophyll, chlorophyll a, chlorophyll b and Carotenoid were determined by the methods of Anjum (Anjum, 2016). Grinding leaf sample (about 0.1 g) was placed in 15 ml centrifuge tube along with 95% absolute ethyl alcohol (10 ml) and then kept at dark condition until the color of sample transformed turn into white. Then, the liquor was read at 665, 652, 649 and 470 nm.

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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).

Result

Chlorophyll content

Figure 1 showed the effect of different concentration of sodium selenate on chlorophyll content in flag leaves of rice at different time intervals during the filling stage and heading stage. T2 treatment increased significantly the chlorophyll content in rice flag leaves at whole filling stage. At HS and 7d AH, the highest total chlorophyll content was recorded in T2 while the trend of chlorophyll content at 21d AH and 28d AH was recorded as: T3 > T2 > T1 > CK. Similar conditions were also found in Chlorophyll a, Chlorophyll b and carotenoid content.

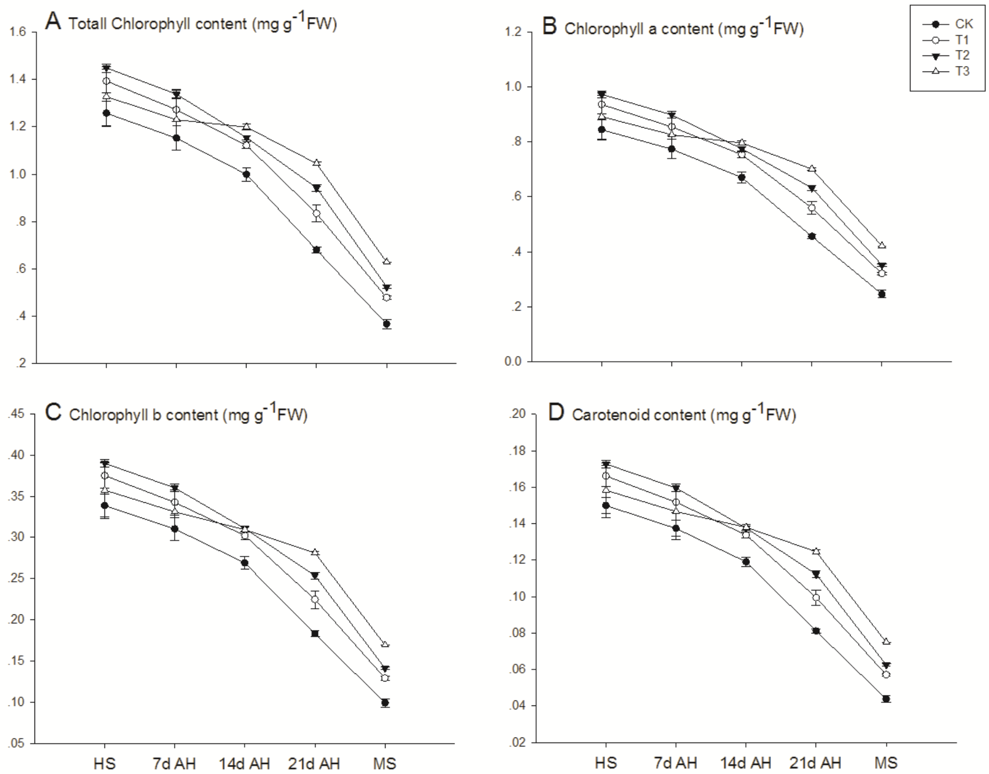

Figure 1. *Effect of exogenous selenium selenate on chlorophyll content during the filling stage.* ***A****: Total Chlorophyll conten;* ***B****: Chlorophyll a content;* ***C****: Chlorophyll b content;* ***D****: Carotenoid content;* ***HS****: heading stage;* ***7d AH****: 7 days after heading;* ***14d AH****: 14 days after heading;* ***21d AH****: 21 days after heading;* ***MS****: maturity. Capped points represent S.E. of three replicates and significance between treatments at (P≤ 0.05) according to least significant difference (LSD) test. The same as below*

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Anti-oxidant enzyme activities and MDA contents

As shown in *Figure 2*, different foliar of selenium selenate affected activities of antioxidant enzymes in rice leaves differently. For SOD activity, both T1 and T2 increased the SOD activities significantly during the early and middle phase of filling stage (HS, 7 and 14d AH). There was no remarkable difference between CK and Se3 at HS and 7d AH whilst the SOD activity of T3 at 14d AH was significantly higher than CK and at 21d AH and maturity, the highest activity was recorded in T3; For POD activity, compared to CK, Se1 treatment increased significantly the POD activity at HS, 7 and 14d AH. At 21d AH and maturity, the POD activity in T2 and T3 were significantly higher than CK while the highest activity was recorded in T3. In addition, there was no significant difference between CK and T1 in POD activity at maturity; For CAT activity, At HS and 7d AH, the highest CAT activity was recorded in T1 and there was no significant difference between CK and T3. However, at 14d AH, the CAT activity of T1, T2 and T3 were significantly higher than CK. At maturity, the highest CAT activity was recorded in T3 while there was no remarkable difference between CK and T1 at maturity; For MDA content, at early phase of filling stage (HS and 7d AH), T1 treatment reduced MDA content significantly compared with CK. At 14 and 21d AH, MDA contents of all Se treatments (T1, T2 and T3) were lower than CK. At maturity, there was no remarkable difference between CK and T1 while T3 remained the lowest level content.

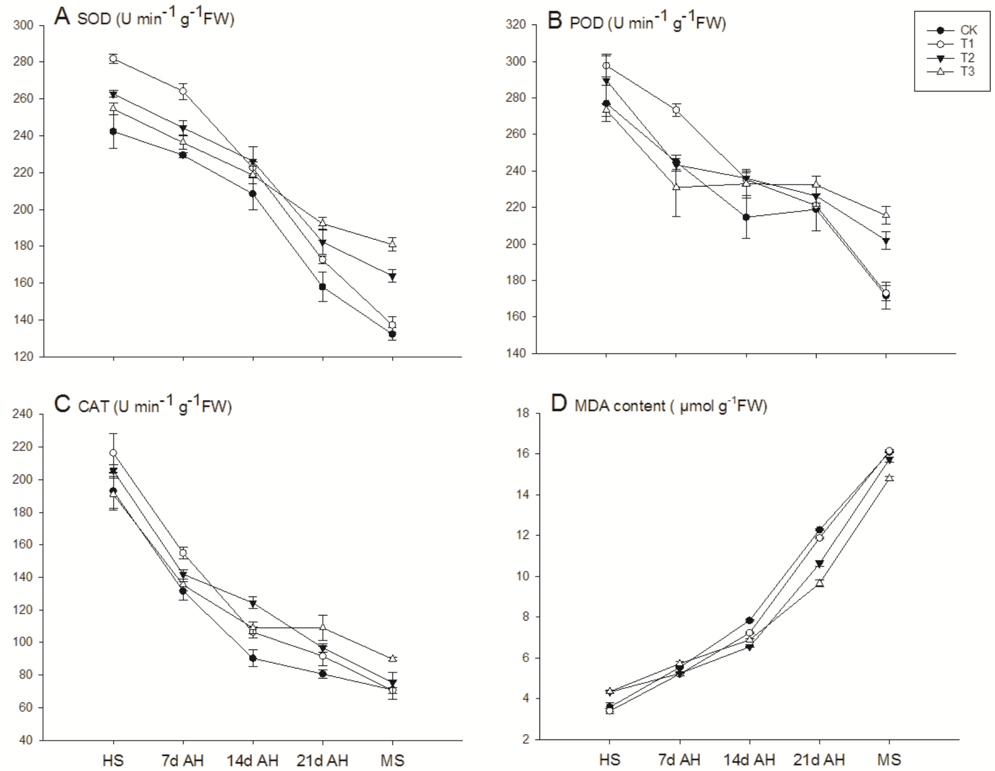

***Figure 2.** Effect of exogenous selenium selenate on antioxidant enzyme activity and MDA content during the filling stage. **A**: SOD activity; **B**: POD activity; **C**: CAT activity; **D**: MDA content*

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Grain yield

Overall, foliar application of selenium selenate at rupturing stage can affected rice grain yield (*Figure 3*). Compared with CK, there was significant difference between CK and T2 while there was no remarkable difference among CK, T1 and T3.

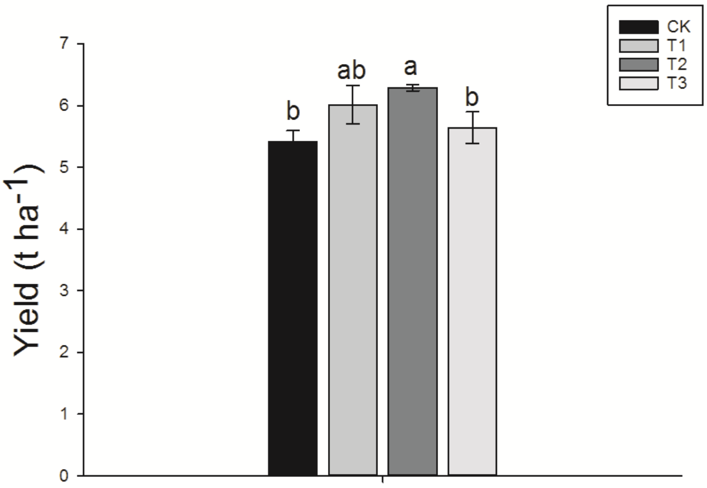

Figure 3. *Effect of exogenous selenium selenate on rice yield. Capped bars represent S.E. of three replicates. Means sharing a common letter don't differ significantly at (P≤ 0.05) according to least significant difference (LSD) test*

Discussion

Se plays important roles in plant growth and development (Nawaz et al., 2014). The most important biological function of Se in plant is a consist element of the glutathione peroxidase system (GSH-PX), which was involved in the REDOX reaction in the body, scavenging free radicals, and reducing the body peroxidation damage caused by biofilm (Qiang et al., 2011). The study of Xin et al. (2004) revealed that Se improved the activity of GSH-PX and the oxidizing ability of rice roots while reducing the MDA content under ferrous stress. Yu et al. (2013) demonstrated that Se application significantly enhanced the antioxidant capacity of wheat, corn, soybean and rape while maintain the normal growth of rice. In our study, there were some noticeable effects on rice antioxidant system with foliar application of Se. We observed that Se treatments regulated the antioxidant enzymatic activities at filling stage in terms of POD, CAT and SOD while decreasing the lipid per-oxidation (MDA concentration). This result agrees with the find of Ríos (Ríos et al., 2009) who found that application of selenite at low rate could induce higher increases in activities of enzymes that detoxify H_2O_2, especially glutathione (GSH) peroxidase and SOD. SOD, POD and CAT are key antioxidant enzymes which aid cells to remove the harmful oxygen species. Our result suggested that Se application was able to partially alleviate the detrimental effects of rice leaf senescence by enhancing the activity of antioxidant enzymes which can help in sustaining rice growth at filling stage and yield formation.

The grain yield of rice is one of the most important goals in rice production. There were significant effects on grain yield by spraying exogenous sodium selenate. Boldrin et al. (2012) studied that the applications of both selenite and selenate increased rice

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yield. A previous study (You et al., 1996) also showed that the sodium selenite and selenium-antaining humie acid compound fertilizer increased the yield and selenium content of grain in these crops with low cost and high profit. In present study, foliar sodium selenate at 30µmol L^{-1} increased the grain yield significantly. Our result agreed with the investigation of (Mengxing et al., 2016) who demonstrated that selenium application at appropriate concentration can remarkably increase the seed-setting rate and grain weight and finally enhance the grain yield of aromatic rice.

Furthermore, we observed that the chlorophyll contents under Se treatments were remained at higher level at filling stage. It might because the improvement of activities of antioxidant enzymes delayed the leaf senescence and it also might be the reason why the Se applications improved the grain yield. The study of Cao et al. (2002) revealed that there exited a significant positive correlation between photosynthesis at filling stage and grain yield. The chlorophyll plays an important role in photosynthesis process for absorbing and converting light energy (Wu et al., 2000). Hence, possible that foliar sodium selenate could be a useful application in increasing grain yield and ensuring the stability of rice production.

Conclusions

Spraying sodium selenate with 10, 30 and 50 µmol L^{-1} at rupturing stage could improve the activities of oxidant enzymes such as POD, SOD and CAT and lowered the MDA concentrations at filling stage. Furthermore, Se applications could enhance the chlorophyll content and the grain yield. Whether or not this would be sufficient to apply in rice production should require more detail and long-term investigation in different rice genotypes.

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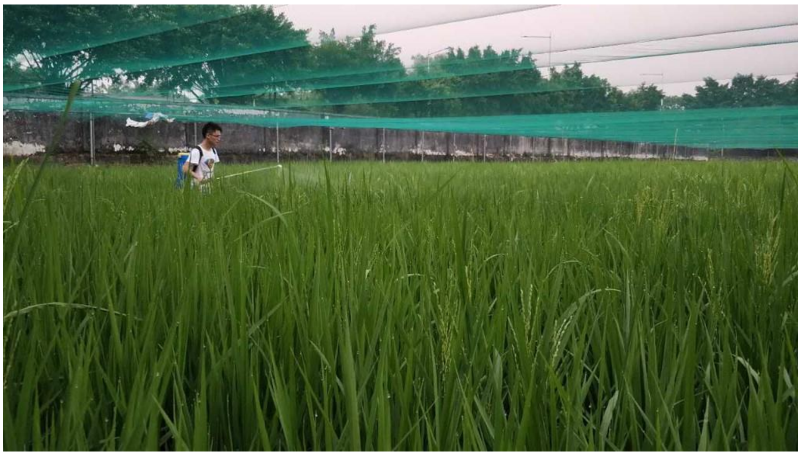
APPENDIX



Appendix 1. *Photo of the experiment*

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