

LOW-CONCENTRATION SODIUM SELENITE APPLICATIONS IMPROVE OXIDATION RESISTANCE OF FILLING-STAGE RICE

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Abstract. Effects of sodium selenite treatments on rice were investigated using two known cultivars *Xiangyaxiangzhan* and *Meixiangzhan2* cultivated at two sites of Guangdong Province, China. Sodium selenite solutions at 10 (Se1), 30 (Se2) and 50 (Se3) $\mu\text{mol}\cdot\text{L}^{-1}$ were sprinkled to the rupturing-stage rice separately, with a control set with double distilled water instead. For *Xiangyaxiangzhan*, comparing with control, 6.91, 24.81 and 25.00% higher SOD, POD and CAT activity were recorded in Se1. For *Meixiangzhan2*, 2.10, 9.44 and 10.00% higher SOD, POD and CAT activity were recorded in Se1. Moreover, high-concentration sodium selenite (Se3) depressed the antioxidant enzyme activities and caused MDA over-production in *Xiangyaxiangzhan*. In conclusion, sodium selenite low-concentration applications could be an exogenous regulator to improve rice oxidation resistance in rice production.

Keywords: sodium selenite, rice, yield, antioxidant enzymes, malondialdehyde

Introduction

As a staple food in most Asian nations, rice contributes to 35%–75% of caloric intake for more than three billion people around the globe, which means rice production is an important part of the global food security system. The world population may increase to 10 billion, which intensifies the demand of rice in the next few decades (Krishnan et al., 2007). However, many uncertain factors could affect the rice yield and quality. Normally, rice is widely grown where the rainfall during the growing season is 50-3000 mm. Paddy fields are often under inundated condition for long time and produce abundant reduced toxic matter, such as ferrous ion and organic acids (XinLiu and Song, 2004), which could negatively affect rice growth. However, the concerns over the effects of global climate change and heavy metal pollution on crop growth and yield have been increased markedly (Krishnan et al., 2007). The stresses of environment pollution and climate changes would cause great loss in rice yield and quality, if the stress tolerance of rice plants cannot be strengthened. Hence, cultivation techniques should be improved in order to ensure the food security novel genetic approaches, of which exogenous regulators might be necessary in rice production.

Selenium (Se) is an essential element to humans, animals and plants. Se deficiency is associated with poor immune function, increased risk of mortality, and cognitive decline, while Se sufficiency or supplementation has antiviral effects and reduces the risk of autoimmune thyroid disease. Se could be a cancer-protective agent (Gladyshev et al., 1999). Moreover, the physiological response to Se varies considerably among plants because some plant species growing on seleniferous soils are tolerant and could accumulate abundant Se, but most plants are Se nonaccumulators and Se-sensitive (Terry et al., 2000). It has been proved that Se helps to resist toxic elements such as arsenic, antimony, mercury and copper (Gotsis, 1982; Srivastava et al., 2009). The study of Hu et al. (2014) showed that Se fertilizer significantly decreases the accumulation of cadmium and lead in rice tissues. Moreover, Proteomics analysis suggests Se treatment could highly affect the biological processes of rice seedlings, such as primary metabolism, photosynthesis and redox homeostasis (Wang et al., 2012). Thus, Se might be a potential exogenous regulator that helps to ensure the food security in rice production.

As is well-known, Se not only is a component element of glutathione peroxidase and thioredoxin reductase, but also is the key component of the catalyzing active center. Se could enhance the activity of glutathione peroxidase and hence the resisting oxidation, scavenge abundant free radicals and protect membranes from the injury of lipid peroxidation (Xin et al., 2004). Suitable Se supply promotes the growth of rice seedlings, but excessive Se injures rice plants and reduces biomass, especially in the roots (Liu et al., 2004).

Hence, this study was conducted in Guangdong (a major rice-producing province in South China) with the hypothesis that exogenous sodium selenite application in the rupturing stage could enhance the rice antioxidant capacity during the filling stage.

Materials and Methods

Plant materials and growing condition

Two rice cultivars, *Xiangyaxiangzhan* and *Meixiangzhan2* with a growth period of 111-114 days, which were planted widely in South China, were used as materials and planted at late season in both Guangzhou (23°16' N, 113°23' E) and Zengcheng (23°13' N, 113°81' E), Guangdong, China. Both experimental sites enjoyed a subtropical monsoon climate with mean annual air temperatures of 22.4°C and mean annual precipitation of 2680.9 mm. Before sowing, the seeds were soaked in water for 24 h, germinated in manual climatic boxes for another 24 h, and shade-dried, followed by sowing in polyvinyl chloride trays for nursery raising. Then the 20-day-old seedlings were transplanted to the fields at the planting distance of 30 × 12 cm². Rice seedlings were transplanted in July and harvested in October. The experimental soil in Guangzhou was sandy loam containing 25.65% organic matter, 1.360% total N, 0.956% total P, and 17.460% total K, while that in Zengcheng was sandy loam with 20.12% organic matter, 1.408% total N, 1.068% total P, and 15.767% total K.

Treatments and plant sampling

Four treatments were as set: Overhead sprinkle with 0, 10, 30 and 50 µmol·L⁻¹ sodium selenite at the rupturing stage of rice, which were marked as CK (double distilled water instead), Se1, Se2 and Se3, respectively. 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 treatments were arranged in randomized complete block design (RCBD) in triplicate in each year with net plot size of 36 m². Fresh leaves were separated and collected from the rice plants after 15 days at the heading stage, washed with double distilled water and stored at -80°C for physio-biochemical analysis.

Yield and yield-related traits

At the maturity stage, the rice grains were harvested from six unit sampling areas (1.75 m²) in each plot and then threshed. The harvested grains were sun-dried and weighed in order to determine the grain yield. Twenty hills of rice from different locations in each plot were sampled for estimation of average effective panicle number per hill. At flowering, 30 panicles were randomly marked and then sampled from each treatment and weighed. Seed-setting rate was measured as: $\text{Panicle Weight}_2 - \text{Panicle Weight}_1 / \text{Time}_2 - \text{Time}_1$ (Kong et al., 2017). Then representative plants from three hills were taken to estimate the yield-related traits.

Estimation of malondialdehyde (MDA) and anti-oxidant responses

The MDA content and activities of peroxidase (POD), superoxide (SOD) and catalase (CAT) were detected according to the methods of Kong et al. (2017). After MDA reacted with thiobarbituric acid, the absorbance was read at the 532, 600 and 450 nm. The MDA content in the reaction solution was calculated as: $\text{MDA content } (\mu\text{mol/L}) = 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56\text{OD}_{450}$, and finally expressed as $\mu\text{mol/g FW}$.

POD (EC 1.11.1.7) activity was estimated after the reaction in the solution 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 (SPB, pH 7.0). One POD unit of enzyme activity was expressed as the absorbance increase by 0.01 (U/g FW) due to guaiacol oxidation. SOD (EC 1.15.1.1) activity was measured by using nitro blue tetrazolium (NBT). In brief, 0.05 ml of an enzyme extract was added into the reaction mixture which contained 1.75 ml of SPB (pH 7.8), 0.3 ml of 130 mM methionine buffer, 0.3 ml of 750 $\mu\text{mol}\cdot\text{L}^{-1}$ NBT buffer, 0.3 ml of 100 $\mu\text{mol}\cdot\text{L}^{-1}$ ethylene diamine tetraacetic acid (EDTA)-2Na buffer and 0.3 ml of 20 $\mu\text{mol}\cdot\text{L}^{-1}$ lactoflavin. After the reaction, the absorbance was recorded at 560 nm. One unit of SOD activity was equal to the volume of the extract needed to cause 50% inhibition of the color reaction. CAT (EC 1.11.1.6) 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 SPB 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).

Detection of Chlorophyll contents

The contents of total chlorophyll (total Chl), chlorophyll a (Chl a) and chlorophyll b (Chl b) were detected by the methods of Anjum (2016). A ground leaf sample (about 0.1 g) was placed in a 15 ml centrifuge tube along with 95% absolute ethyl alcohol (10 ml) and then kept at dark until the sample turned white. Then Chl a, Chl b and total Chl contents were estimated at 645, 652 and 663 nm respectively on an ultraviolet-visible spectrophotometer.

Statistical analysis

Data were analyzed on Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) while differences among 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).

Results

MDA content

Sodium selenite applications significantly affected the MDA contents in rice leaves at the filling stage (Figure 1). For *Xiangyaxiangzhan* in both Guangzhou and Zengcheng, MDA contents under Se1 treatment were significantly lower compared with CK, Se2 or Se3, while the MDA contents increased with the increment of sodium selenite concentration. For *Meixiangzhan2*, MDA contents in Guangzhou were not remarkably different among CK, Se1 and Se3, but significantly decreased after the Se2 treatment. In Zengcheng, however, both Se1 and Se2 treatments lowered the MDA contents compared with CK and the trend was Se2 < Se1 < CK < Se3.

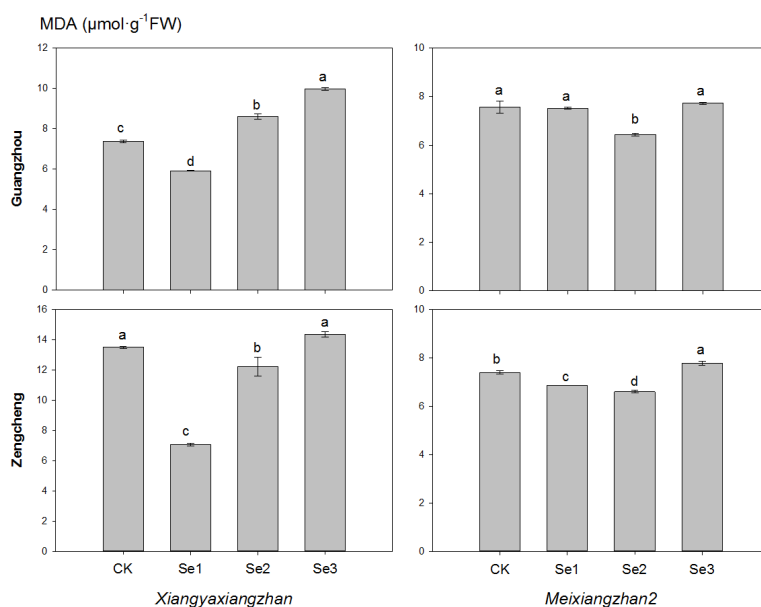


Figure 1. Effects of sodium selenite application on MDA contents. Means sharing a common letter don't differ significantly at ($P \leq 0.05$) according to least significant difference (LSD) test. The same as below

Anti-oxidant responses

Sodium selenite applications into *Xiangyaxiangzhan* regulated the anti-oxidant system in terms of POD, SOD and CAT activities and soluble protein contents (Figure 2). For example, POD activity was maximized in Se1 while did not significantly differ among CK, Se2 and Se3. For *Meixiangzhan2*, POD activity was the highest in Se2 and

changed in the trend of $CK < Se3 < Se1 < Se2$. SOD activity was significantly higher in Se1 treatment and was lowest in Se3 for *Xiangyaxiangzhan*. For *Meixiangzhan2*, SOD activity did not remarkably differ between CK and sodium selenite treatments in Guangzhou, but in Zengcheng, SOD activity under Se2 treatment was significantly higher than in CK, Se1 and Se3, which were 278.32, 229.13, 235.68 and 244.83 $U\ g^{-1}\ min^{-1}\ FW$, respectively. Compared with CK, CAT activities under Se1 treatment in Guangzhou and Zengcheng were 1.24 and 1.08 fold higher for *Xiangyaxiangzhan*. For *Meixiangzhan2*, the CAT activity was improved by both Se1 and Se2, but was maximized in Se2 treatment in two locations. The effects of sodium selenite on soluble protein accumulation differed among treatments and between cultivars. For *Xiangyaxiangzhan*, the trend of soluble protein contents was $Se3 < CK < Se2 < Se1$ in Guangzhou, but did not significantly differ among treatments in Zengcheng. For *Meixiangzhan2*, the highest protein concentration in Guangzhou was recorded in Se1; the contents in Se1 and Se2 were significantly higher than CK and Se3 in Zengcheng.

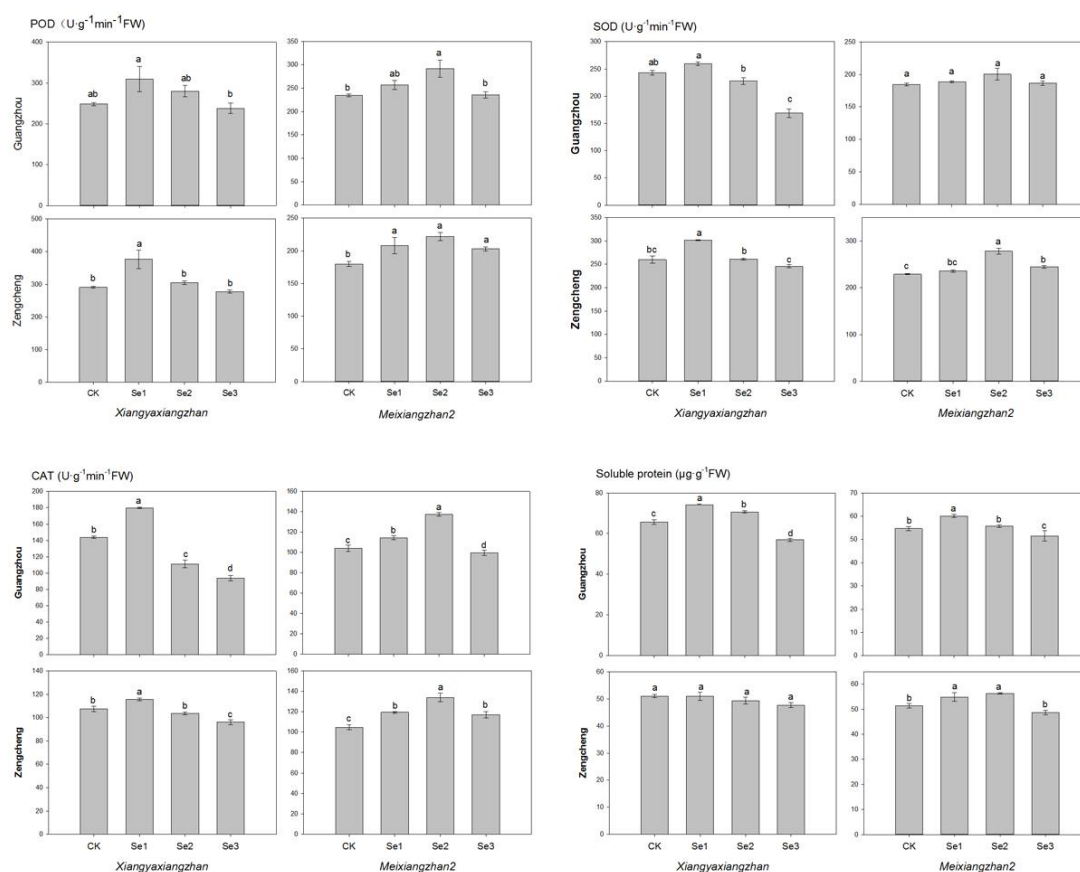


Figure 2. Effects of sodium selenite application on anti-oxidant responses

Chlorophyll

Chlorophyll contents were significantly affected by sodium selenite applications (Figure 3). For *Xiangyaxiangzhan*, the total Chl content maximized in Se1 and minimized in Se3 for both Guangzhou and Zengcheng, and a similar trend was found in Chl a contents. For *Meixiangzhan2* in Guangzhou, total Chl contents under Se1, Se2 and Se3 were 1.10, 1.18 and 1.06 fold higher than CK, respectively, and the trends of

both Chl a and Chl b were recorded as: CK < Se1 < Se3 < Se2. In Zengcheng, however, total Chl contents were not remarkably different between Se1 and Se2, but were both higher than CK and Se3, and similar trends were also found in both Chl a and Chl b.

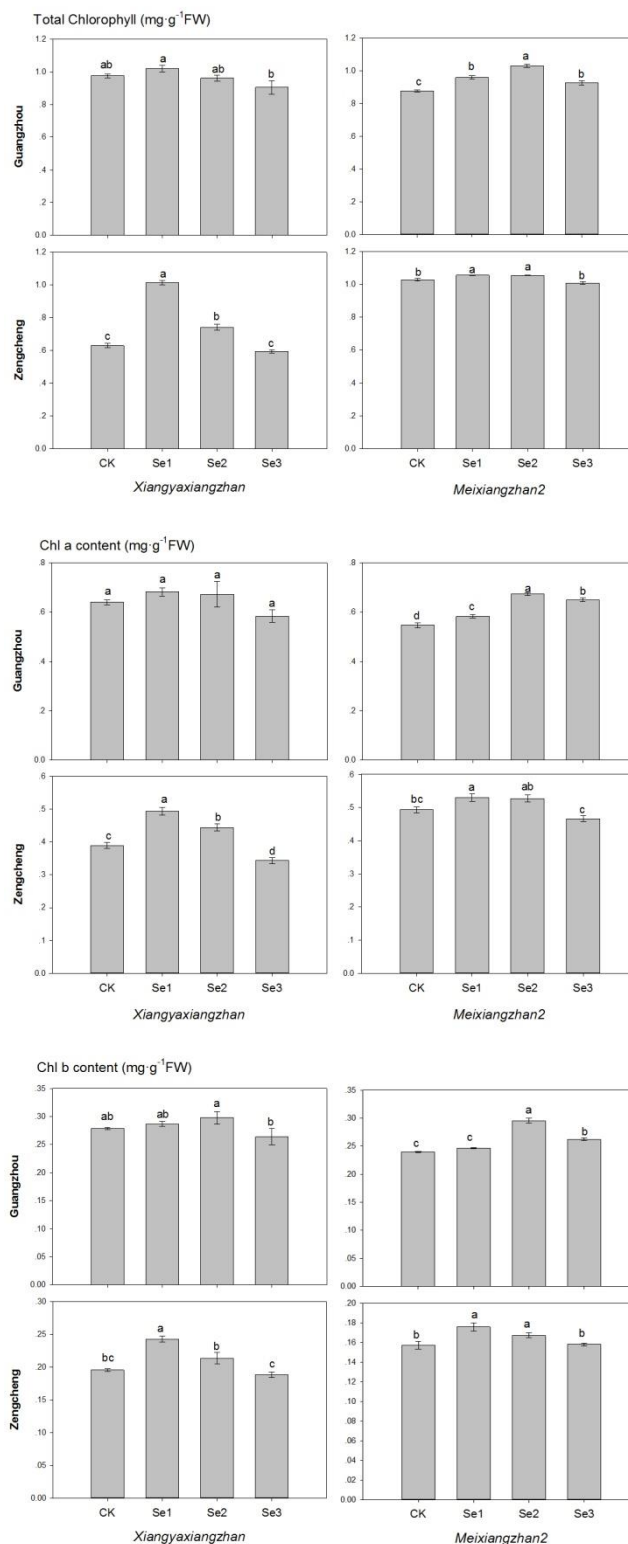


Figure 3. Effects of sodium selenite application on chlorophyll contents

Yield and yield-related traits

No significant difference among CK, Se1, Se2 and Se3 was found in panicle number per m² or grain number per panicle irrespective of locations or cultivars (*Table 1*). For *Xiangyaxiangzhan*, however, seed-setting rate of Se2 was higher than CK, Se1 and Se3 in Zengcheng, which were 78.85%, 70.81%, 66.53% and 71.43%, respectively. In Guangzhou, 1000-grain weights of Se1 and Se2 remained higher than CK and Se3. Furthermore, Se1 significantly improved grain yield compared with CK. For *Meixiangzhan2*, the sodium selenite treatments had no remarkable effect on seed-setting rate, grain weight or yield.

Discussion

As one of the important life elements, Se has many similar chemical properties as sulfur (S). The most important biofunction of Se in animals and humans is as a component of the glutathione peroxidase system (GSH-PX), which is involved in the REDOX reaction in the body, scavenging free radicals, and reducing the body peroxidation damage caused by biofilms (Qiang et al., 2011). Se could significantly enhance the antioxidant capacity of higher plants, such as wheat, corn, soybean and rape, and maintains the normal growth of plants (Wang et al., 1996; Nawaz et al., 2014). The mechanism is that Se can significantly improve the transcriptional levels of SOD and guaiacol peroxidase in plants, thus activating the antioxidant protection ability. The present study proves that sodium selenite treatments improved the activities of antioxidant enzymes, including POD, SOD and CAT. Furthermore, the sodium selenite applications also reduced oxidative damage by decreasing lipid per-oxidation (MDA concentration), while inducing dynamics in soluble proteins. Normally, the MDA production might be related to oxidation of polyunsaturated fatty acids (e.g., linoleic and linolenic acid) that play an important role in the arrangement of photosystem reaction centers. MDA could even react with free amino acids and cause ethylene over-production in cellular membranes (Rakwal et al., 2003). Thus, MDA content could be an important indicator of oxidative stress. We observed the treatments Se1 and Se2 reduced MDA concentration and enhanced the activities of POD, SOD and CAT. This finding indicates that low-rate sodium selenite applications at the rupturing stage could improve the antioxidant performance and strength of filling-stage rice under external environment stress. This study also agreed with (Ríos et al., 2009) that low-rate selenite application could enhance the activities of H₂O₂-detoxifying enzymes, especially glutathione (GSH), POD and SOD. Moreover, selenite treatment could regulate the accumulation of soluble proteins that help in maintaining cellular structures and functions when the antioxidant system quenches reactive oxygen species.

In our study, sodium selenite applications significantly affected the chlorophyll content, and the total Chl contents first increased and then decreased with the increase of sodium selenite concentration. This finding agrees with (Wu et al., 2000) that the electron transfer of chloroplast is accelerated at low Se concentration.

Interestingly, the sensitivity to sodium selenite was quite different between *Xiangyaxiangzhan* and *Meixiangzhan2*. As reported, different genotype cultivars responded differently to selenium fertilizers and differed in terms of selenium uptake and distribution. For *Xiangyaxiangzhan*, low concentration selenite applications such as Se1 significantly improved the activities of antioxidant enzymes, up-regulated the chlorophyll contents and increased the grain yields.

Table 1. Effects of sodium selenite application on yield and yield-related traits

Location	Cultivar	Treatment	Panicle number per m ²	Grains number per panicle	Seed-setting rate (%)	1000-grain weight (g)	Yield (t hm ⁻²)
Guangzhou	Xiangyaxiangzhan	CK	271.33±13.49a	105.51±11.56a	84.49±1.73a	18.26±0.36b	4.90±0.21b
		Se1	253.02±16.33a	87.47±11.05a	86.45±2.57a	19.49±0.06a	6.65±0.19a
		Se2	246.85±33.99a	111.59±8.15a	85.33±0.24a	19.78±0.13a	5.59±0.43ab
		Se3	286.17±29.17a	93.63±1.13a	86.58±1.57a	18.48±0.23b	5.64±0.66ab
	Meixiangzhan2	CK	227.40±28.23a	178.37±1.04a	79.26±1.95a	18.84±0.21a	5.33±0.72a
		Se1	219.66±14.76a	176.69±3.67a	80.52±0.35a	18.91±0.15a	5.18±0.29a
		Se2	250.6±9.66a	176.68±12.19a	81.75±2.59a	18.93±0.18a	7.51±0.11a
		Se3	202.64±13.75a	175.59±13.42a	78.58±4.19a	19.07±0.11a	5.64±1.06a
Zengcheng	Xiangyaxiangzhan	CK	292.1±11.45a	110.23±9.02a	70.81±3.36ab	20.27±0.35a	4.23±0.61a
		Se1	292.1±33.11a	104.62±2.95a	66.53±0.27b	20.55±0.12a	4.95±0.28a
		Se2	283.87±25.69a	117.19±7.37a	78.85±4.84a	20.36±0.11a	4.88±0.53a
		Se3	306.5±20.88a	106.12±6.34a	71.43±3.43ab	20.67±0.13a	3.72±0.22a
	Meixiangzhan2	CK	266.07±32.19a	138.56±2.25a	85.35±0.24a	18.07±0.39a	3.65±0.15a
		Se1	262.2±14.11a	116.14±3.17a	85.13±0.40a	18.18±0.14a	3.54±0.26a
		Se2	230.49±12.67a	120.63±7.73a	81.51±3.76a	18.41±0.07a	3.85±0.20a
		Se3	259.11±14.94a	124.54±6.62a	78.36±2.28a	18.00±0.02a	3.47±0.14a

However, high-concentration selenite application such as Se3 remarkably restricted the antioxidant system while inducing the over-production of MDA. For *Meixiangzhan2*, the best concentration of sodium selenite seemed to be Se2 with regard to the lowest MDA content and highest activities of POD, SOD and CAT, while antioxidant responses or chlorophyll contents were not significantly different among CK, Se1 and Se2. The result of our study indicated different rice cultivars had different sensibility to exogenous selenite and thus more study should be done in rice sensibility for Se.

Conclusions

Sodium selenite applications at low concentration could enhance the antioxidant system in term of POD, SOD and CAT, lower the MDA production and increase the chlorophyll contents. For *Meixiangzhan 2*, we considered that $30 \mu\text{mol}\cdot\text{L}^{-1}$ was the most suitable concentration for application. For *Xiangyaxiangzhan*, we considered that $10 \mu\text{mol}\cdot\text{L}^{-1}$ was the most suitable concentration for application. In the future, sodium selenite application could be used in the rice production to improve the rice oxidation resistance and prevent the yield loss caused by environmental stress.

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REFERENCES

- [1] Anjum, S. A. (2016): Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes and yield formation in maize. – *Pakistan Journal of Agricultural Sciences* 53: 751-757.
- [2] El-Shintinawy, F. (2000): Structural and Functional Damage Caused by Boron Deficiency in Sunflower Leaves. – *Photosynthetica* 36: 565-573.
- [3] Gladyshev, V. N., Martín-Romero, F. J., Xu, X. M., Kumaraswamy, E., Carlson, B. A., Hatfield, D. L., Lee, B. J. (1999): Molecular biology of selenium and its role in cancer, AIDS and other human diseases. – *Recent Research Developments in Biochemistry*.
- [4] Gong, R., Ai, C., Zhang, B., Cheng, X. (2018): Effect of selenite on organic selenium speciation and selenium bioaccessibility in rice grains of two Se-enriched rice cultivars. – *Food Chemistry* 264: 443-448.
- [5] Gotsis, O. (1982): Combined effects of selenium/mercury and selenium/copper on the cell population of the alga *Dunaliella minuta*. – *Marine Biology* 71: 217-222.
- [6] Hu, Y., Norton, G. J., Duan, G., Huang, Y., Liu, Y. (2014): Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. – *Plant & Soil* 384: 131-140.
- [7] Kong, L., Ashraf, U., Cheng, S., Rao, G., Mo, Z., Tian, H., Pan, S., Tang, X. (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.
- [8] Liu, Q., Wang, D. J., Jiang, X. J., Cao, Z. H. (2004): Effects of the Interactions Between Selenium and Phosphorus on the Growth and Selenium Accumulation in Rice (*Oryza Sativa*). – *Environmental Geochemistry & Health* 26: 325-330.

- [9] Nawaz, F., Ashraf, M. Y., Ahmad, R., Waraich, E. A., Shabbir, R. N. (2014): Selenium (Se) Regulates Seedling Growth in Wheat under Drought Stress. – *Advances in Chemistry* (2014-7-22): 670-675.
- [10] Qiang, X., Shu, Y., Hai-Lei, A. Z. (2011): Effects of Exogenous Nitric Oxide Donor SNP on Lipid Peroxidation Caused by Selenium in Rice Seedlings. – *Acta Agronomica Sinica* 37: 177-181.
- [11] Rakwal, R., Agrawal, G. K., Kubo, A., Yonekura, M., Tamogami, S., Saji, H., Iwahashi, H. (2003): Defense/stress responses elicited in rice seedlings exposed to the gaseous air pollutant sulfur dioxide. – *Environmental & Experimental Botany* 49: 223-235.
- [12] Ríos, J. J., Blasco, B., Cervilla, L. M., Rosales, M. A., Sanchezrodriguez, E., Romero, L., Ruiz, J. M. (2009): Production and detoxification of H₂O₂ in lettuce plants exposed to selenium. – *Annals of Applied Biology* 154: 107-116.
- [13] Sairam, R. K., Srivastava, G. C., Saxena, D. C. (2000): Increased Antioxidant Activity under Elevated Temperatures: A Mechanism of Heat Stress Tolerance in Wheat Genotypes. – *Biologia Plantarum* 43: 245-251.
- [14] Srivastava, M., Ma, L. Q., Rathinasabapathi, B., Srivastava, P. (2009): Effects of selenium on arsenic uptake in arsenic hyperaccumulator *Pteris vittata* L. – *Bioresource Technology* 100:1115.
- [15] Terry, N., de Souza, M. P., Am, T. A. Z. (2000): Selenium in higher plants. – *Annual Review of Plant Physiology & Plant Molecular Biology* 51: 401-432.
- [16] Wang, Y. D., Wang, X., Wong, Y. S. (2012): Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. – *Journal of Proteomics* 75: 1849-1866.
- [17] Wang, Z., Xie, S., Peng, A. (1996): Distribution of Se in soybean samples with different Se concentration. – *Journal of Agricultural & Food Chemistry* 44: 2754-2759.
- [18] Wu, Y. Y., Lu, X. Y., Peng, Z. K., Luo, Z. M. (2000): Effect of Se on physiological and biochemical characters of paddy rice. – *Scientia Agricultura Sinica* 33(1): 100-103.