

# SELENIUM REGULATION OF THE GRAIN YIELD, DRY MATTER, ANTIOXIDANT ATTRIBUTES, AND METAL CONTENTS OF FRAGRANT RICE UNDER STRESS OF CADMIUM AND LEAD

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**Abstract.** The pot experiment was carried out with two fragrant rice cultivars (Yuxiangyouzhan and Xiangyaxiangzhan) grown under six experimental treatments (CK: no application of selenium, Pb(NO<sub>3</sub>)<sub>2</sub>, and CdCl<sub>2</sub>; Se: application of 80 mg selenium fertilizer per pot; Pb: application of 80 mg Pb(NO<sub>3</sub>)<sub>2</sub> per pot; Cd: application of 10 mg CdCl<sub>2</sub> per pot; SePb: application of 80 mg of Se fertilizer and 80 mg Pb(NO<sub>3</sub>)<sub>2</sub> per pot; SeCd: application of 80 mg of Se fertilizer and 10 mg CdCl<sub>2</sub> per pot). The results showed that selenium application improved the yield of fragrant rice under lead and cadmium stress. The application of Se fertilizer treatment increased the grain yield of Yuxiangyouzhan under the Pb and Cd stress by 84.55% and 22.44%, respectively, and improved the grain yield of Xiangyaxiangzhan by 43.07% and 46.55%, respectively, as compared to no Se fertilizer treatment. The application of selenium fertilizer treatment significantly increased the total dry weight of fragrant rice as compared to no selenium fertilizer treatment under the Pb and Cd stress. The application of Se fertilizer regulated the activities of SOD, POD, and CAT and the content of MDA. Under the influence of selenium fertilizer, the variations in Cd and Pb concentration were cultivar-dependent. Our results suggested that the application of selenium fertilizer regulated the yield formation, due to the improvement in dry matter accumulation and the change in antioxidant response in fragrant rice.

**Keywords:** *fragrant rice, selenium, lead and cadmium, heavy metal stress, yield*

## Introduction

Rice is one of the most important staple food crops which feeds more than half of the world's population (Dawe, 2002). Fragrant rice is known for its flavor compounds, and the demand for fragrant rice has gradually risen (Chen et al., 2008; Shen et al., 2015; Zhao et al., 2020). Therefore, improvement of the grain yield and quality of fragrant rice has become a priority for rice researchers.

Heavy metal pollution has been reported seriously threaten the agricultural production (Chen et al., 2021; Xiang et al., 2021). Excessive heavy metal deposition in agricultural fields affected the ecological environment, plant development, crop yield, and human health (Zhang

et al., 2007; Luo et al., 2017; Etesami, 2018). Among the heavy metals, lead (Pb) and cadmium (Cd) are the two most common pollutants in the soil which influenced the metabolism of fragrant rice and cause grain yield and quality losses (Cheng, 2003; Duan et al., 2013; Ashraf et al., 2017; Kanu et al., 2019; Li et al., 2021). Previous research has reported that heavy metals in the soil affected the photosynthesis and respiration in leaves and the antioxidant enzyme activity of rice plants (Ashraf et al., 2017; Li et al., 2021). Therefore, it is of great significance to investigate how to alleviate the adverse effects of Pb and Cd stress on the grain yield and quality of fragrant rice.

Selenium (Se) is an important essential trace element (Zhou et al., 2018). Se is important in antioxidants in repairing DNA damage, boosting immunity, and lowering the risk of cancer (Wallace et al., 2009). Se fertilizer could improve crop yield, and the improvement of the Se content in crop grain by applying selenium fertilizer is beneficial to human health (Farooq et al., 2018; Xu et al., 2019; Cao et al., 2020). Many studies have shown that Se could mitigate the toxicity of Cd to a variety of plants (Farooq et al., 2019; Zou et al., 2020). However, there are few studies on the mitigated effect of Se fertilizer on the heavy metal stress in fragrant rice.

Therefore, a pot experiment was conducted with two popular fragrant rice cultivars (Yuxiangyouzhan and Xiangyaxiangzhan) to evaluate the changes in grain yield, dry matter weight, superoxide dismutase (SOD) activity, catalase (CAT) activity, peroxidase (POD) activity, malondialdehyde (MDA) content, Cd, and Pb content under different Se, Cd, and Pd treatments. With this study, we hope to establish a theoretical framework for further research into the effects of selenium fertilizer on heavy metal stress in fragrant rice.

## Material and methods

### *Experimental site and materials*

A pot experiment was carried out on the teaching experimental farm of South China Agricultural University from July to November 2018. This region has an annual average temperature of 20.3°C. Two fragrant rice varieties, Yuxiangyouzhan and Xiangyaxiangzhan, were used in this experiment, which are popular fragrant rice cultivars in South China. The seeds of the two fragrant rice cultivars were obtained from the College of Agriculture, South China Agricultural University. The specifications of the pot are 30 cm in diameter and 25 cm in height, containing 10 kg of dry paddy soil. The soil was sandy loam, consisting of 15.06 g kg<sup>-1</sup> organic matter, 0.83 g kg<sup>-1</sup> total nitrogen (N), 1.10 g kg<sup>-1</sup> total phosphorus (P), 20.10 g kg<sup>-1</sup> total potassium (K), with a pH value of 6.56. The selenium (Se) fertilizer used in this experiment was a Se ore powder, which is a powder of shale containing 80 mg kg<sup>-1</sup> Se and was exploited from Se deposits in Enshi, a well-known selenium-rich region (Zhu et al., 2008), in Hubei Province, China.

### *Experimental design and crop management*

This experiment used a randomized block design with 6 treatments. The six experimental treatments were CK: no application of any chemical; Se: application of 80 mg selenium fertilizer per pot; Pb: 80 mg Pb(NO<sub>3</sub>)<sub>2</sub> was applied to per pot; Cd: 10 mg CdCl<sub>2</sub> was applied to per pot; SePb: each pot was applied with 80 mg of Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: 80 mg selenium fertilizer and 10 mg CdCl<sub>2</sub> were applied to each pot. The seeds were disinfected with 3% NaClO solution for 15 minutes and immersed for 12 h. Seeds are seeded in a substrate by direct seeding and then transplanted into the pot at the two-leaf stage. Five hills of rice seedlings were transplanted in each pot, with five rice seedlings per hill. There were 8

pots in total for each treatment. Irrigation and pest management were carried out in accordance with conventional management methods. Maintain a water layer of 2-4 cm from transplanting until 7 days before harvest, and then allow the soil to dry naturally.

### ***Sampling and measurements***

#### *Determination of yield and yield-related traits*

The determination of yield and yield-related traits was performed according to a previously reported method (Li et al., 2020). At the maturity stage (MS), three hills of rice plants were randomly selected from each pot. After manual threshing, the grain yield of each pot was measured, and the effective panicle number, grain number per panicle, seed-setting rate, and 1000-grain weight were investigated.

#### *Determine the weight of dry matter in different parts*

At the heading stage (HS) and MS, representative rice plants were randomly selected from each pot. The plant is divided into leaf, stem, and panicle, then dried at a constant temperature of 80 °C and weighed for measurement of dry weight.

#### *Determination of MDA content and antioxidant activities in leaves*

The flag leaves from the represent rice plants were sampled under HS and MS, and stored at -80 °C pending biochemical analysis.

The activity of antioxidant enzymes and the content of MDA were measured following the previously reported method (Li et al., 2020). The fresh leaf sample (0.3 g) was homogenized with 3 mL of 100 mM phosphate buffered saline (PBS) solution and centrifuged at 14000 g and 4 °C for 15 min. The supernatant was used for measuring the activity of SOD, CAT, POD, and the content of MDA.

For SOD activity, the nitro-blue tetrazolium (NBT) method was employed. The absorbance was measured by using a spectrophotometer at 560 nm. The SOD activity was defined in the unit  $U\ g^{-1}\ min^{-1}\ FW$ . For POD activity, a spectrophotometer was used to measure the absorbance at 470 nm with five replicates at an interval of 30 s, and the POD activity was expressed as  $U\ g^{-1}\ min^{-1}\ FW$ . For CAT activity, a spectrophotometer was used to measure the absorbance at 470 nm, and the absorbance was recorded every 30 s, with four replicates. The CAT activity was defined as  $U\ g^{-1}\ min^{-1}\ FW$ . For MDA content, the absorbance was recorded by a spectrophotometer at 450, 600, and 532 nm. The MDA content was defined as  $\mu mol\ g^{-1}\ FW$ .

#### *Determination of Pb and Cd contents in different parts*

The Cd and Pb contents in plant tissues were determined according to Liu (2008). Approximately 0.5 g of the dry sample was soaked in 4 mL of nitric acid and 3 mL of perchloric acid for 12 h and heated in a graphite furnace (HYP-340, Shanghai, China). The heating process consisted of four stages: 90 °C for 30 min, 140 °C for 40 min, 195 °C for 120 min, and 220 °C for 240 min. The filtered digesting solution was then placed in a 25-mL volumetric flask with a constant volume of 25 mL. Absorbent values were then determined by an atomic absorption spectrophotometer (GFA-EX7i), and the Cd and Pb contents were expressed as  $mg\ kg^{-1}$ .

## Data analysis

Analysis of variance (ANOVA) and correlation analysis were performed using Statistics version 8 (Analytical, Tallahassee, Florida, USA). The differences amongst means were separated by using the least significant difference (LSD) test at a 5% significance level.

## Results

### Grain yield and yield components

The application of Se significantly affected the grain yield. Compared to the CK, the Pb and Cd treatment significantly reduced the grain yield in Yuxiangyouzhan (37.55% reduction by Pb) and Xiangyaxiangzhan (23.97% reduction by Pb; 23.09% reduction by Cd), respectively. Compared with Pb and Cd treatments, the yields of Yuxiangyouzhan and Xiangyaxiangzhan were increased by 84.55% and 43.07% under SePb treatment, respectively, and increased by 22.44% and 46.55% under SeCd treatment, respectively.

Moreover, compared with CK, for Yuxiangyouzhan, the panicle per pot, grain number per panicle, seed-setting rate, and 1000-grain weight were reduced under Pb treatment, and the seed-setting rate, and 1000-grain weight were reduced under Cd treatment; for Xiangyaxiangzhan, both Pb and Cd treatments reduced the panicle per pot. The SePb treatment significantly increased the panicle per pot, grain number per panicle, seed-setting rate in Yuxiangyouzhan, and the seed-setting rate and 1000-grain weight in Xiangyaxiangzhan as compared to the Pb treatment. The SeCd treatment significantly increased the panicle number per pot and 1000-grain weight in both cultivars and the seed-setting rate, in Xiangyaxiangzhan as compared to the Cd treatment. The improvement in the yield components after the Se application under Pb and Cd contributed to the grain yield improvement (Table 1).

**Table 1.** Effects of selenium on yield and yield components of fragrant rice under Pb and Cd conditions

Cultivars	Treatments	Panicle number per pot	Grain number per panicle	Seed-setting rate (%)	1000-grain weight (g)	Grain yield (g pot <sup>-1</sup> )
Yuxiangyouzhan	CK	22.25±1.03c	90.99±4.08c	71.80±1.97a	22.61±0.43ab	27.22±2.84ab
	Se	24.50±0.65ab	103.98±3.21ab	71.20±0.78a	23.24±0.22a	31.89±1.64a
	Pb	18.00±0.41d	71.88±2.40d	58.13±0.83c	21.94±0.24c	17.02±0.25c
	Cd	22.50±0.65bc	90.33±2.81c	61.22±2.00bc	21.80±0.18c	22.95±1.32b
	SePb	24.50±0.87ab	106.15±1.15a	72.18±1.56a	22.36±0.28bc	31.41±1.34a
	SeCd	25.25±0.63a	96.56±3.50bc	62.84±1.08b	22.92±0.38ab	28.10±1.69ab
Xiangyaxiangzhan	CK	29.75±0.95a	85.09±3.36a	61.15±2.29bc	18.22±0.10c	26.20±1.40a
	Se	29.75±1.11a	87.25±1.58a	67.93±2.13ab	18.62±0.13bc	28.83±2.80a
	Pb	25.00±0.71cd	88.55±1.64a	53.56±3.75c	18.22±0.13c	19.92±0.87b
	Cd	22.75±0.85d	84.23±2.55a	59.20±2.73bc	18.96±0.39b	20.15±0.74b
	SePb	27.00±0.41bc	87.02±1.55a	64.41±4.92ab	20.20±0.18a	28.50±2.01a
	SeCd	28.75±0.48ab	87.89±1.52a	73.43±1.78a	20.60±0.27a	29.53±1.47a

The different low case letters following the mean value of each variety in the same column was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

### Dry weight accumulation

Compared with CK treatment, Pb and Cd treatment significantly decreased the total dry weight of Yuxiangyouzhan and Xiangyaxiangzhan at HS and MS. The Se treatment significantly increased the total dry weight in Yuxiangyouzhan at MS which was attributed to the increment in panicle dry weight at MS. Significant decrement in leaf dry weight at HS under Cd treatment and at MS under Pb, Cd, SePb, and SeCd treatment for Yuxiangyouzhan and at HS under Se, Pb, Cd, SePb, and SeCd treatment and at MS under Cd and SeCd treatment for Xiangyaxiangzhan were detected as compared with CK. The Cd treatment significantly decreased the stem and sheath dry weight at HS and the Pb and Cd treatment significantly decreased the stem and sheath dry weight at MS for Yuxiangyouzhan when compared to CK. The Pb and Cd treatment significantly decreased the panicle dry weight at MS for Yuxiangyouzhan and the Pb and Cd treatment significantly decreased the panicle dry weight at MS for Xiangyaxiangzhan as compared with CK. The SePb (compared to Pb) and SeCd treatment (compared to Cd) significantly increased the panicle dry weight at MS in Yuxiangyouzhan and Xiangyaxiangzhan, respectively (Table 2).

**Table 2.** Effects of selenium on dry weight (g pot<sup>-1</sup>) of different parts of fragrant rice under Pb and Cd conditions

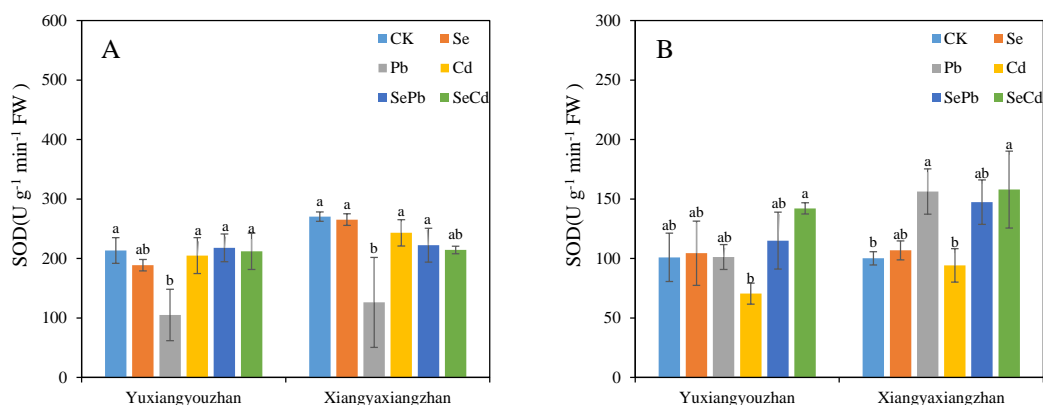
Cultivar	Treatment	Leaf dry weight	Stem and sheath dry weight	Panicle dry weight	Total dry weight
<b>HS</b>					
Yuxiangyouzhan	CK	11.01±0.56ab	42.96±2.74ab	7.17±0.60ab	61.13±2.81ab
	Se	11.24±1.02a	49.36±4.08a	6.19±0.62ab	66.79±4.47a
	Pb	8.13±0.54bc	32.04±0.61bc	7.71±0.46a	47.88±0.55cd
	Cd	7.18±0.29c	32.15±1.59c	5.37±0.61b	44.70±0.76d
	SePb	11.03±1.58ab	36.65±1.88bc	7.15±1.05ab	54.84±2.94bc
	SeCd	9.19±1.24abc	36.92±2.77bc	6.47±0.53ab	52.58±2.49cd
Xiangyaxiangzhan	CK	11.95±0.74a	38.06±1.65abc	5.00±0.02ab	55.01±1.70a
	Se	11.07±0.84c	43.61±1.14a	4.72±0.28ab	59.4±1.88a
	Pb	7.96±0.86c	33.55±3.34c	4.37±0.88ab	45.87±4.35b
	Cd	7.39±0.48bc	34.18±1.30bc	3.32±0.60b	44.9±0.74b
	SePb	9.19±0.45bc	38.47±1.33abc	5.39±0.33a	53.06±1.58a
	SeCd	9.24±0.45c	39.66±1.80ab	4.58±1.15ab	53.48±2.30a
<b>MS</b>					
Yuxiangyouzhan	CK	8.42±0.57a	34.75±0.58ab	28.18±0.69b	71.34±1.56bc
	Se	8.25±0.59a	39.14±2.53a	38.98±2.24a	86.36±2.50a
	Pb	6.36±0.35b	27.95±2.07c	17.81±1.84c	52.12±2.24e
	Cd	4.23±0.46c	27.73±1.24c	28.18±1.01b	60.14±1.22d
	SePb	6.50±0.57b	31.16±2.17bc	38.26±2.03a	75.92±3.69b
	SeCd	5.37±0.49bc	29.95±2.95bc	32.71±1.73b	68.03±2.09c
Xiangyaxiangzhan	CK	8.30±0.87a	32.47±4.58ab	27.85±1.06b	68.62±5.21a
	Se	8.64±0.87a	34.54±1.31a	28.64±0.92ab	71.82±1.60a
	Pb	6.92±0.56abc	25.99±2.41b	22.64±1.13c	55.55±1.82b
	Cd	5.03±0.38c	27.82±2.03ab	22.35±0.88c	55.2±2.27b
	SePb	7.73±0.63ab	30.71±2.81ab	28.21±1.07ab	66.66±3.01a
	SeCd	6.27±0.33bc	31.19±1.03ab	31.18±1.48a	68.63±2.34a

The different low case letters following the mean value of each variety in the same column was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

Besides, compared with the Pb treatment, the SePb treatment significantly increased the total dry weight at MS in Yuxiangyouzhan and Xiangyaxiangzhan by 45.66% and 20.00%, respectively. Compared with the Cd treatment, the SeCd treatment significantly increased the total dry weight at MS in Yuxiangyouzhan and Xiangyaxiangzhan by 13.12% and 24.33%, respectively. The total dry weight at HS in Xiangyaxiangzhan was improved after Se application under Cd and Pb when compared to that without Se application under Cd and Pb. Compared with Cd, the SeCd treatment significantly increased the panicle dry weight at MS for Xiangyaxiangzhan. The SePb significantly increased the panicle dry weight at MS for both Yuxiangyouzhan and Xiangyaxiangzhan (Table 2).

### Antioxidant response

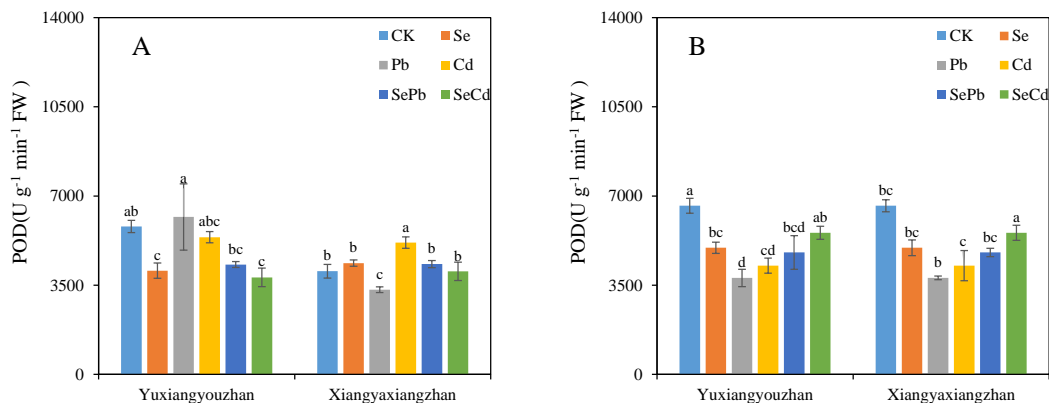
Compared with CK treatment, the Pb treatment significantly decreased the SOD activity in leaves of Yuxiangyouzhan and Xiangyaxiangzhan at HS by 50.80% and 53.35%, respectively (Figure 1A), and significantly increased the SOD activity in leaves of Xiangyaxiangzhan at MS by 56.08% (Figure 1B). Compared with the Pb treatment, the SePb treatment significantly increased the SOD activity in leaves of Yuxiangyouzhan and Xiangyaxiangzhan at HS by 107.41% and 76.99%, respectively (Figure 1A). Compared with the Cd treatment, the SeCd treatment significantly increased the activity of SOD in leaves of Yuxiangyouzhan and Xiangyaxiangzhan at MS by 101.65% and 67.66%, respectively (Figure 1B).



**Figure 1.** Effects of selenium on the activities of SOD in leaves at HS (A) and MS (B) under Pb and Cd conditions. The different low case letters following the mean value of each variety was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied  $Pb(NO_3)_2$ ; Cd: applied  $CdCl_2$ ; SePb: applied Se fertilizer and  $Pb(NO_3)_2$ ; SeCd: applied Se fertilizer and  $CdCl_2$ .

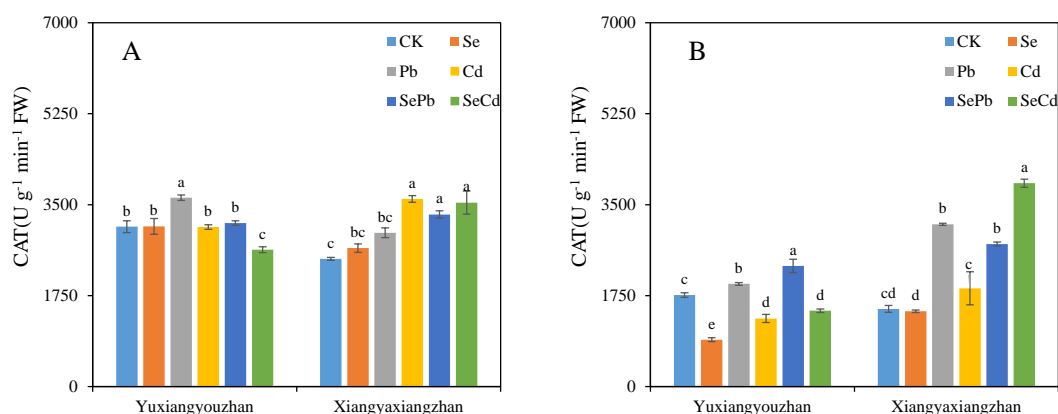
A significantly decreased in the POD activity in leaves at HS for Yuxiangyouzhan under Se and SeCd treatment and for Xiangyaxiangzhan under Pb treatment was detected as compared to CK (Figure 2A). The Cd treatment significantly decreased the POD activities in leaves of Xiangyaxiangzhan at HS as compared to CK. Compared with CK treatment, the POD activity in leaves at MS for Yuxiangyouzhan under Se, Pb, Cd and SePb treatment significantly decreased, whilst the SeCd treatment significantly increased the POD activity in leaves at MS for Xiangyaxiangzhan (Figure 2B). Compared with the Pb treatment, the SePb treatment significantly decreased POD activity in leaves of

Yuxiangyouzhan at HS by 30.22%, but increased POD activity in leaves of Xiangyaxiangzhan at HS by 23.28%. Compared with Cd treatment, the POD activities in leaves of Yuxiangyouzhan and Xiangyaxiangzhan were increased at MS but decreased at HS (Figure 2A).



**Figure 2.** Effects of selenium on the activities of POD in leaves at HS (A) and MS (B) under Pb and Cd conditions. The different low case letters following the mean value of each variety was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

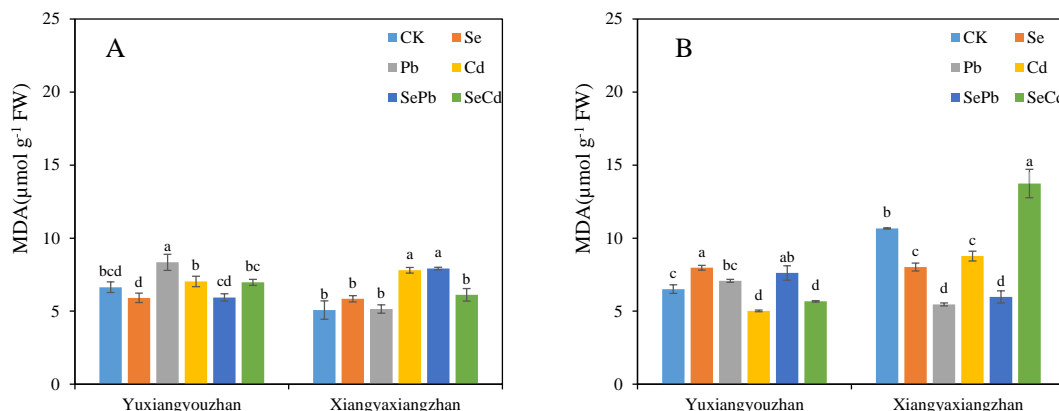
Compared with CK treatment, the Se treatment resulted in a decrease in CAT activity in leaves of Yuxiangyouzhan at MS; the Pb treatment significantly increased the CAT activity in leaves of Yuxiangyouzhan and Xiangyaxiangzhan at HS and MS; the Cd treatment significantly increased the CAT activity in leaves of Xiangyaxiangzhan at HS but decreased the CAT activity in leaves of Yuxiangyouzhan at MS; the SeCd treatment decreased the CAT activity in leaves of Yuxiangyouzhan at HS; the SeCd and Se Pb treatment significantly increased the CAT activity in leaves of Xiangyaxiangzhan at HS and MS (Figure 3A, B).



**Figure 3.** Effects of selenium on the activities of CAT in leaves at HS (A) and MS (B) under Pb and Cd conditions. The different low case letters following the mean value of each variety was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

Compared with the Pb treatment, the SePb treatment significantly decreased the CAT activity in leaves of Yuxiangyouzhan but increased the CAT activity in leaves of Xiangyaxiangzhan at HS and increased the CAT activity in leaves of Yuxiangyouzhan at MS. Compared with Cd treatment, the SeCd treatment significantly decreased the CAT activity in leaves of Yuxiangyouzhan at HS but significantly increased the CAT activity in leaves of Xiangyaxiangzhan at MS (Figure 3A, B).

The Se treatment resulted in a decrease in the MDA content in leaves of Xiangyaxiangzhan but an increase in the MDA content in leaves of Yuxiangyouzhan at MS as compared to CK. Compared with CK, the Pb treatment significantly increased the MDA content in leaves of Yuxiangyouzhan at HS by 25.60%, and significantly decreased the MDA content in leaves of Xiangyaxiangzhan at MS by 48.78%. Compared with CK, the Cd treatment significantly reduced the MDA content in leaves of Yuxiangyouzhan and Xiangyaxiangzhan at MS by 22.89% and 23.29%, respectively. The content of MDA in leaves of Xiangyaxiangzhan at HS under Cd and SePb treatments were increased as compared with CK. The SePb treatment significantly increased the MDA in leaves of Yuxiangyouzhan but decreased the MDA in leaves of Xiangyaxiangzhan at MS, whilst the SeCd treatment significantly decreased the MDA in leaves of Yuxiangyouzhan but increased the MDA in leaves of Xiangyaxiangzhan at MS. Compared with Pb treatment, the SePb treatment significantly reduced the MDA content in leaves of Yuxiangyouzhan at HS by 28.78%, significantly increased the MDA content of in leaves of Xiangyaxiangzhan at HS. Compared with Cd, the MDA content in leaves of Xiangyaxiangzhan at HS was decreased but the MDA content in leaves of Xiangyaxiangzhan at MS was increased (Figure 4).



**Figure 4.** Effects of selenium on the MDA content in leaves at HS (A) and MS (B) under Pb and Cd conditions. The different low case letters following the mean value of each variety was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

### Pb and Cd content

Compared with the Cd treatment, the content of Cd in the brown rice, rice husk, and grain of Yuxiangyouzhan was significantly increased under the SeCd treatment, and the content of Cd in the brown rice, husk, and grain of Xiangyaxiangzhan was significantly decreased under SeCd treatment. Compared with the Pb treatment, the SePb treatment significantly increased the Pb content in brown rice, rice husk, and grain of



Yuxiangyouzhan and in the husk and grain of Xiangyaxiangzhan but decreased the Pb content in the brown rice and straw in Xiangyaxiangzhan (Table 3).

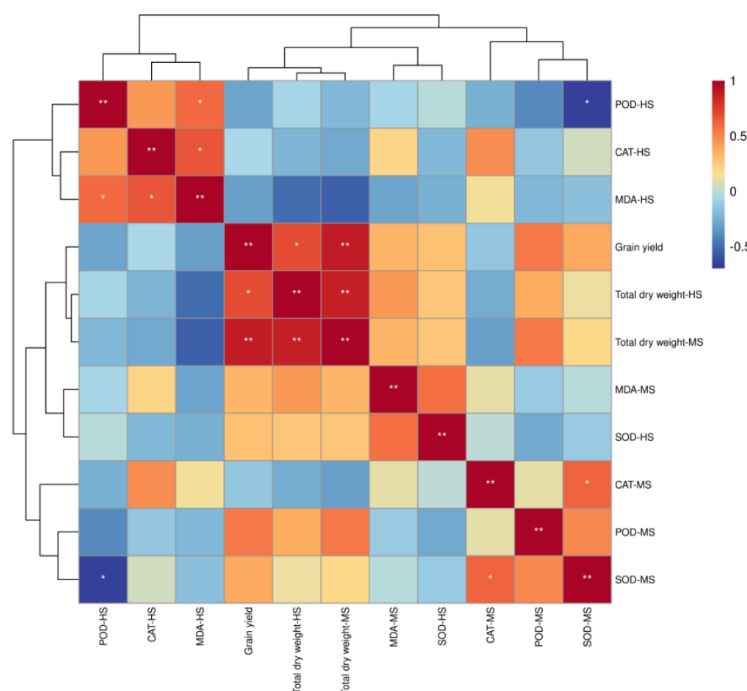
**Table 3.** Effects of selenium on Cd and Pb content in fragrant rice under Pb and Cd conditions

Cultivar	Treatment	Brown rice	Husk	Grain	Straw
<b>Cd content (mg kg<sup>-1</sup>)</b>					
Yuxiangyouzhan	SeCd	0.143±0.003a	0.196±0.008a	0.338±0.011a	0.829±0.024a
	Cd	0.134±0.002b	0.175±0.004b	0.309±0.005b	0.785±0.023a
Xiangyaxiangzhan	SeCd	0.162±0.001b	0.217±0.019b	0.379±0.02b	0.989±0.028a
	Cd	0.172±0.00a	0.278±0.008a	0.45±0.008a	1.041±0.009a
<b>Pb content (mg kg<sup>-1</sup>)</b>					
Yuxiangyouzhan	SePb	0.094±0.004a	0.061±0.002a	0.156±0.003a	1.72±0.026b
	Pb	0.022±0.001b	0.052±0.002b	0.073±0.002b	2.609±0.148a
Xiangyaxiangzhan	SePb	0.035±0.002b	0.322±0.008a	0.357±0.01a	3.133±0.114b
	Pb	0.076±0.000a	0.159±0.004b	0.235±0.004b	3.442±0.019a

The different low case letters following the mean value of each variety in the same column was significant according to LSD test ( $p < 0.05$ ). CK: control; Se: applied Se fertilizer; Pb: applied Pb(NO<sub>3</sub>)<sub>2</sub>; Cd: applied CdCl<sub>2</sub>; SePb: applied Se fertilizer and Pb(NO<sub>3</sub>)<sub>2</sub>; SeCd: applied Se fertilizer and CdCl<sub>2</sub>.

### Correlation analysis

The correlation analysis suggested that the grain yield was significantly correlated with the total dry weight at HS and MS (Figure 5).



**Figure 5.** Correlation analyses of the investigated parameters. \* and \*\* represents significance at 0.05 and 0.01 level. Total dry weight-HS: total dry weight at heading stage, Total dry weight-MS: total dry weight at maturity stage, SOD-HS: SOD activity at heading stage, SOD-MS: SOD activity at maturity stage, POD-HS: POD activity at heading stage, POD-MS: POD activity at maturity stage, CAT-HS: CAT activity at heading stage, CAT-MS: CATD activity at maturity stage, MDA-HS: MDA content at heading stage, MDA-MS: MDA content at maturity stage

## Discussion

Compared to the Pb treatment, the SePb treatment improved the effective panicles, grains per panicle, and seed setting rate of fragrant rice, influenced the yield of Yuxiangyouzhan. Compared with the Pb treatment, the SePb treatment improved the effective panicle number, seed setting rate, and 1000-grain weight, affected the grain yield of Xiangyaxiangzhan. Compared with the Cd treatment, the SeCd treatment affected the effective panicles, grains per panicle, seed setting rate, and the grain yield of the two fragrant rice cultivars. The Se treatment increased the grain yield of fragrant rice. It can be seen that selenium can mitigate the inhibition of lead and cadmium on the grain yield formation of fragrant rice.

Study have shown that rice yield is highly related to dry weight (Fageria et al., 2011). As shown in *Table 2*, Pb and Cd stress reduced the total dry weight of the two fragrant rice cultivars, while the application of Se fertilizer increased the total dry weight of fragrant rice under heavy metal stress. Further analysis suggested that the grain yield was significantly correlated with the total dry weight at HS and MS. Those results indicated that selenium application could significantly alleviate the stress of Pb and Cd on aboveground dry matter accumulation of fragrant rice which finally improves the grain yield of fragrant rice.

Studies have reported that the accumulation and transportation of the heavy metals in different plant tissues (Hu et al., 2014; Wan et al., 2016; Zhang et al., 2020). Compared with the Cd treatment, the contents of Pb in stems and leaves of two fragrant rice varieties were not significantly affected by Se fertilizer. The Se fertilizer significantly reduced the Cd content in the grain of Yuxiangyouzhan but increased the Cd content in the grain of Xiangyaxiangzhan. Therefore, the Se fertilizer effects on the Cd content in grain of fragrant rice was cultivar-dependent. The selenium fertilizer reduced the Pb content in straw but increased the Pb content in the grain of the two fragrant rice cultivars. The selenium fertilizer may promote the transport of lead from the stems and leaves to the grains. It suggested that selenium application effects on the content of Cd and Pb in grains of fragrant rice were different, so the appropriate application rate of selenium fertilizer is important when the field soil contains different heavy metals.

Oxidative damage is generally found when the plant is exposed to environmental stress (Zandi and Schnug, 2022). Studies have shown that the antioxidant response, like the protective enzyme system (SOD, POD, and CAT) is important for plant growth (Singh et al., 2015; Ahmad et al., 2022). Because the antioxidant enzymes such as SOD, POD and CAT in the plant can remove excessive active oxygen in the plant (Sattar et al., 2022). The increase of malondialdehyde (MDA) content is an important marker of plant cell membrane permeability under stress (Mittler, 2002; Du et al., 2019). The results showed that the antioxidant response was affected by cultivars, Se application, and heavy metal types. The activities of SOD, POD, CAT, and the content of MDA were regulated by the selenium fertilizer application which finally affected the growth and grain yield.

## Conclusion

The pot experiment showed that the application of selenium fertilizer could significantly alleviate the inhibition effect of lead and cadmium on fragrant rice. Compared with lead and cadmium treatment, the application of selenium fertilizer affected the physiological and biochemical attributes of fragrant rice and significantly improved the grain yield and dry weight of fragrant rice. Therefore, an appropriate amount of selenium fertilizer can be applied in rice fields polluted by lead and cadmium to reduce the harm caused by heavy metal pollution.

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## REFERENCES

- [1] Ahmad, S., Bani Mfarrej, M. F., El-Esawi, M. A., Waseem, M., Alatawi, A., Nafees, M., Saleem, M. H., Rizwan, M., Yasmeen, T., Anayat, A., Ali, S. (2022): Chromium-resistant *Staphylococcus aureus* alleviates chromium toxicity by developing synergistic relationships with zinc oxide nanoparticles in wheat. – *Ecotoxicology and Environmental Safety* 230: 113142.
- [2] Ashraf, U., Kanu, A. S., Deng, Q. Q., Mo, Z. W., Pan, S. G., Tian, H., Tang, X. R. (2017): Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice. – *Frontiers in Plant Science* 8: 259.
- [3] Cao, J. J., Zhang, Z. Q., Yang, F. L., Kong, F. L., Yu, H. H., Li, H., Li, G., Sun, C. B. (2020): The effect of selenium fertilizer on spring wheat yield and selenium enrichment in grain of Jilin province by spraying selenium fertilizer on leaves. – *Journal of Northeast Agricultural Science* 45(5): 6-8+46. (In Chinese).
- [4] Chen, S. H., Yang, Y., Shi, W. W., Ji, Q., He, F., Zhang, Z. D., Cheng, Z. K., Liu, X. N., Xu, M. L. (2008): *Badh2*, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. – *Plant Cell* 20(7): 1850-1861.
- [5] Chen, R., Wang, Q., Lv, J. G., Wang, Z. S., Gao, T. (2021): Multivariate correlation analysis of bio-accumulation with soil properties and potential health risks of cadmium and lead in rice seeds and cabbage in pollution zones, China. – *Environmental Geochemistry and Health* 43(9): 3485-3503.
- [6] Cheng, S. P. (2003): Heavy metal pollution in China: origin, pattern, and control. – *Environmental Science and Pollution Research international* 10(3): 192-198.
- [7] Dawe, D. (2002): The changing structure of the world rice market, 1950-2000. – *Food Policy* 27(4): 355-370.
- [8] Du, B., Luo, H. W., He, L. X., Zhang, L. H., Liu, Y. F., Mo, Z. W., Pan, S. G., Tian, H., Duan, M. Y., Tang, X. R. (2019): Rice seed priming with sodium selenate: effects on germination, seedling growth, and biochemical attributes. – *Scientific Reports* 9: 4311.
- [9] Duan, G. L., Liu, W. J., Chen, X. P., Hu, Y., Zhu, Y. G. (2013): Association of arsenic with nutrient elements in rice plants (Review). – *Metallomics* 5(7): 784-792.
- [10] Etesami, H. (2018): Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: mechanisms and future prospects. – *Ecotoxicology and Environmental Safety* 147: 175-191.
- [11] Fageria, N. K., Moreira, A., Coelho, A. M. (2011): Yield and yield components of upland rice as influenced by nitrogen sources. – *Journal of Plant Nutrition* 34(3): 361-370.
- [12] Farooq, M. U., Tang, Z. C., Zeng, R., Liang, Y. K., Zhang, Y. J., Zheng, T. D., Ei, H. H., Ye, X. Y., Jia, X. M., Zhu, J. Q. (2018): Accumulation, mobilization and transformation of selenium in rice grain provided with foliar sodium selenite. – *Journal of the Science of Food and Agriculture* 99(6): 2892-2900.
- [13] Farooq, M. U., Tang, Z. C., Zheng, T. D., Asghar, M. A., Zeng, R., Yang, S., Ei, H. H., Liang, Y. K., Zhang, Y. J., Ye, X. Y., Jia, X. M., Zhu, J. Q. (2019): Cross-talk between cadmium and selenium at elevated cadmium stress determines the fate of selenium uptake in rice. – *Biomolecules* 9(6): 247.
- [14] Hu, Y., Norton, G. J., Duan, G. L., Huang, Y. C., Liu, Y. X. (2014): Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. – *Plant and Soil* 384(1-2): 131-140.

- [15] Kanu, A. S., Ashraf, U., Mo, Z. W., Sabir, S. U. R., Baggie, I., Charley, C. S., Tang, X. R. (2019): Calcium amendment improved the performance of fragrant rice and reduced metal uptake under cadmium toxicity. – *Environmental Science and Pollution Research* 26(24): 24748-24757.
- [16] Li, Y. Z., Liang, L. X., Fu, X. M., Gao, Z. F., Liu, H. C., Tan, J. T., Potcho, M. P., Pan, S. G., Tian, H., Guan, M. Y., Tang, X. R., Mo, Z. W. (2020): Light and water treatment during the early grain filling stage regulates yield and aroma formation in aromatic rice. – *Scientific Reports* 10(1): 14830.
- [17] Li, Y., Liang, L., Li, W., Ashraf, U., Ma, L., Tang, X., Pan, S., Tian, H., Mo, Z. (2021): ZnO nanoparticle-based seed priming modulates early growth and enhances physio-biochemical and metabolic profiles of fragrant rice against cadmium toxicity. – *Journal of Nanobiotechnology* 19: 75.
- [18] Liu, Q. L., Nong, X. M. (2008): Determination of heavy metal cadmium content in Mingmu Dihuang Jiaonang by graphite furnace atomic absorption spectrometry. – *Strait Pharmaceutical Journal* 4: 44-45. (In Chinese).
- [19] Luo, H. F., Zhang, J. Y., Jia, W. J., Ji, F. M., Yan, Q., Xu, Q., Ke, S., Ke, J. S. (2017): Analyzing the role of soil and rice cadmium pollution on human renal dysfunction by correlation and path analysis. – *Environmental Science and Pollution Research* 24(2): 2047-2054.
- [20] Mittler, R. (2002): Oxidative stress, antioxidants and stress tolerance. – *Trends in Plant Science* 7(9): 405-410.
- [21] Sattar, A., Wang, X. K., Ul-Allah, S., Sher, A., Ijaz, M., Irfan, M., Abbas, T., Hussain, S., Nawaz, F., Al-Hashimi, A., Al-Munqedhi, B. M., Skalicky, M. (2022): Foliar application of zinc improves morpho-physiological and antioxidant defense mechanisms, and agronomic grain biofortification of wheat (*Triticum aestivum* L.) under water stress. – *Saudi Journal of Biological Sciences* 29(3): 1699-1706.
- [22] Shen, J. K., Xie, Z. Y., He, Z. Z., Yin, M., Yi, J. L. (2015): Research and development prospect of fragrant rice. – *Chinese Journal of Tropical Agricultural* 35(8): 46-51. (In Chinese).
- [23] Singh, R., Hemantaranjan, A., Patel, P. K. (2015): Salicylic acid improves salinity tolerance in field pea (*Pisum sativum* L.) by intensifying antioxidant defense system and preventing salt-induced nitrate reductase (NR) activity loss. – *Legume Research: An International Journal* 38(2): 202-208.
- [24] Wallace, K., Kelsey, K. T., Schned, A., Morris, J. S., Andrew, A. S., Karagas, M. R. (2009): Selenium and risk of bladder cancer: a population-based case-control study. – *Cancer Prevention Research* 2(1): 70-73.
- [25] Wan, Y. N., Yu, Y., Wang, Q., Qiao, Y. H., Li, H. F. (2016): Cadmium uptake dynamics and translocation in rice seedling: Influence of different forms of selenium. – *Ecotoxicology and Environmental Safety* 133: 127-134.
- [26] Xiang, M. T., Li, Y., Yang, J. Y., Lei, K. G., Li, Y., Li, F., Zheng, D. F., Fang, X. Q., Cao, Y. (2021): Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. – *Environmental Pollution* 278: 116911.
- [27] Xu, Q., Wang, M., Xie, Y. M., Chen, T. T., Zhou, L., Tan, Y. H., Shi, L., Xing, D. Y. (2019): Effects of selenium application on rice appearance quality and grain selenium, cadmium, and arsenic contents. – *Agricultural Science and Technology* 21(5): 135-140. (In Chinese).
- [28] Zandi, P., Schnug, E. (2022): Reactive oxygen species, antioxidant responses and implications from a microbial modulation perspective. – *Biology* 11(2): 155.
- [29] Zhang, Y., Zhang, H. W., Su, Z. C., Zhang, C. G. (2007): Effects of long-term heavy metal stress on farmland soil microbial population, biomass, and activity. – *The Journal of Applied Ecology* 18(7): 1491-1497. (In Chinese).

- [30] Zhang, Z. Y., Ji, X. H., Xie, Y. H., Liu, S. H., Tian, F. X., Yi, H. W. (2020): Study on the absorption and transportation of cadmium and arsenic in rice. – *Hybrid Rice* 35(6): 68-74. (In Chinese).
- [31] Zhao, Q. Y., Yousaf, L., Xue, Y., Shen, Q. (2020): Changes in flavor of fragrant rice during storage under different conditions. – *Journal of the Science of Food and Agriculture* 100(8): 3435-3444.
- [32] Zhou, X. B., Li, Y. Y., Lai, F. (2018): Effects of different water management on absorption and accumulation of selenium in rice. – *Saudi Journal of Biological Sciences* 25(6): 1178-1182.
- [33] Zhu, J. M., Wang, N., Li, S. H., Li, L., Su, H. C., Liu, C. X. (2008): Distribution and transport of selenium in Yutangba, China: impact of human activities. – *Sci. Total Environ.* 392: 252-261.
- [34] Zou, C. L., Zhai, R. N., Huang, K. J., Tan, H., Zheng, D. B., Huang, A. H., Wei, X. X., Mo, R. X., Xiong, F. Q., Wei, H., Wei, S. Z. (2020): Effect of foliar application of selenium fertilizer on yield, selenium content and heavy metal contents of waxy maize. – *Asian Agricultural Research* 12(8): 40-44,48. DOI:10.19601/j.enki.issn1943-9903.2020.08.010