Nano-selenium controlled cadmium accumulation and improved photosynthesis in indica rice cultivated in lead and cadmium combined paddy soils

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Abstract

Selenium nanoparticles (Se NPs) are less toxic and more biocompatible than selenite or selenate. However, studies involving spraying with Se NPs for reducing accumulation of cadmium (Cd) and lead (Pb) in rice grains have been rarely reported as yet. Herein, indica rice seedlings cultivated in Cd-Pb-spiked paddy soils (denoted as positive control) were sprayed with Se NPs soils for four times from tillering to booting stage. Compared to positive control, 50–100 μmol/L Se NPs downregulated Cd transporters-related genes such as OsLCT1, OsHMA2 and OsCCX2 in leaves and OsLCT1, OsPCR1 and OsCCX2 genes in node I at filling stage. Meanwhile, Se-binding protein I was distinctly elevated, involving the repression of Cd and Pb transportation to rice grains. Se NPs also differentially improved RuBP carboxylase and chlorophylls especially some key genes and proteins involving photosynthetic system. Besides, 25–50 μmol/L Se NPs diminished reactive oxygen species overproduction from NADPH oxidases whereas boosted glutathione peroxidase, reducing protein carbonylation in rice seedlings. However, the antioxidant isozymes and oxidatively modified proteins were slightly rebounded at 100 μmol/L. Se contents were noticeably elevated and confirmed to exist as selenomethionine in the rice grains following all the treatments by Se NPs. Thus, the optimal dosage of Se NPs for foliar application is 50 μmol/L, which significantly decreased Cd accumulation, improved photosynthesis and Se enrichment whereas caused no distinct reduction of Pb in the grains. Thus, an appropriate dosage of Se NPs can be conducted to decrease Cd accumulation, improve photosynthesis, and organic Se contents in rice grains.

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Introduction

Paddy rice (Oryza sativa L.) is the staple food for more than half of the population in the world. Cadmium (Cd) can be readily absorbed by rice seedlings and transferred to grains (Rizwan et al., 2016). Food chain contamination by Cd through rice consumption will bring about healthy risk to people (Wu et al., 2020). Particularly, many soils are contaminated by more than one heavy metal or metalloid (Liao et al., 2016; Murtaza et al., 2019). Moreover, lead (Pb) can also enter food chain via rice or other crops and pose potential health harm in the mining and smelting regions contaminated by Pb (Hu et al., 2014).

Currently, most studies have been concentrated on decreasing single heavy metal contamination (e.g., Cd, Pb, arsenic (As), etc.) in crops by soil regulation methods (Wu et al., 2020). However, information concerning simultaneously reduction of more than one heavy metal in crops remains limited (Russin et al., 2020; Murtaza et al., 2019), especially through foliar pathway. Thus, exploring measures to repress the upward translocation of Cd and Pb to rice grains seems to be very interesting and challenging.

Heavy metals are taken up through root cortical tissues, and adopt symplastic and/or apoplastic pathway to reach xylem, then transport to shoots (Qi et al., 2020). At nodes, ions are transferred from xylem to phloem, followed by upward translocation via phloem to rice grains (Fujimaki et al., 2010). In these processes, the phloem-mediated Cd remobilization to rice grains is crucial for Cd deposition in rice grains (Hao et al., 2018). Generally, Cd previously accumulated in leaves will be translocated via phloem to rice grains following redirection at nodes at filling stage, leading to almost half of Cd contents in the grains (Uruguchi and Fujiwara, 2013). So far, a few transporters such as OsLCT1, OsHMA2, etc. have been specified in phloem, which selectively transport metal ions such as Cd$^{2+}$ to rice grains (Hao et al., 2018; Uruguchi et al., 2011, 2014). These studies suggest that downregulation of these transporters-related genes or proteins at filling stage have the potential to diminish Cd accumulation in rice grains.

Soil pollution by toxic heavy metals can diminish seed germination, inhibit seedling growth, and reduce mineral nutrients as well as decrease biomass, photosynthesis and grain yields in many plants (Ji et al., 2017; Konate et al., 2017; Rizwan et al., 2016). Both Cd and Pb usually cause reactive oxygen species (ROS) overproduction through indirect mechanisms such as activating NADPH oxidase, resulting in oxidative damage in plants (Sharma and Dietz, 2009).

Selenium (Se) is an essential microelement for humans and animals. It is also beneficial for plants at proper concentrations (Yin et al., 2019). To some extent, treatments by selenium or selenate could reduce the uptake, transport and accumulation of Cd, As, Pb or mercury in rice tissues or grains, improve resistance to these toxic metals or metalloid, and thus alleviate their oxidative damage to plants (Cui et al., 2018; Liu et al., 2018; Lv et al., 2020; Ulhassan et al., 2019; Wu et al., 2017). The potential mechanisms involved inhibition of the Cd transporters-related genes (Shao et al., 2017) and improvement of the defense systems or/and secondary metabolites in plants under Se treatments (Handa et al., 2019; Malik et al., 2012; Shahid et al., 2019). Besides, Se treatments could elevate photosynthesis-related proteins, enzymes or chlorophyll contents, and thus promoted the photosynthesis and productivity in crops (Hu et al., 2014). Se was also able to improve Se enrichment and nutritional quality in crops (Singh et al., 2018; Wan et al., 2016; Zhang et al., 2014).

As evidenced by some studies that Se could markedly decrease Cd concentrations, but caused no distinct reduction of Pb in rice grains (Hu et al., 2014). Thus, Se might exert different effects on the accumulation of Cd and Pb in rice grains. In addition, the protective effects of Se against Cd or other toxic metals were closely related to the dosages of Se and Cd (Ding et al., 2014), involved with the aerobic or anoxic conditions in the culture medium (Affolder et al., 2019; Huang et al., 2019), and were also relevant to the growth stages of rice seedlings (Huang et al., 2018).

Selenium nanoparticles (Se NPs) have peculiar characteristics of nano materials, underlying their lower toxicity, higher bioavailability and biological activities versus other species of Se (Wadhwani et al., 2016; Wang et al., 2007). Thus, Se NPs have the potential to be used as nano fertilizer in agriculture for crop protection and nutrition enhancement (El-Ramady et al., 2016; Skalickova et al., 2017). However, studies involving foliar application of Se NPs for controlling Cd or/and Pb translocation to rice grains remains limited (Hussain et al., 2020).

Silicon (Si) and Se composite soils were confirmed to be able to diminish Cd and Pb accumulation in rice grains through foliar application (Wang et al., 2020). Due to the difference in particle sizes and component, Se NPs have different physicochemical and physiobiochemical properties compared with the Si and Se composite soils.

Thus, pot experiments were carried out to: (1) investigate the potential effects and mechanisms of foliar application of Se NPs soils for repressing Cd and Pb translocation to rice grains and also for improving photosynthesis and productivity at filling stage of rice seedlings; (2) explore the optimum concentration of Se NPs based on ecological risk assessment in rice plants cultivated in Cd+/Pb-spiked paddy soils. These achievements will provide new insight for the prevention of Cd or/Pb accumulation while improving grain yields and Se contents in rice grains by foliar spraying with Se NPs.

1. Materials and methods

1.1. Preparation and characterization of Se NPs soils

Se NPs soils were prepared according to method described by Wang et al. (2020) with a little modification. 200 mL of 0.4324% (M/V) selenite solution was completely mixed with 400 mL of 0.5% (M/V) bovine serum albumin, and then was magnetically stirred for 30 min (40°C, 350 r/min). 0.25% (M/V) reduced glutathione (GSH) solution was slowly added into the mixture until constant orange-red color appeared, then was normalized to 1000 mL with deionized water, constituting stock solution of Se NPs soils (5000 μmol/L Se). The soils were characterized by transmission electron microscope (TEM) coupled with energy dispersive spectroscopy (EDS). The TEM image and
1.2. Treatments of soils and rice seedlings

The native soil was collected from a field in Huainan district, Anhui Province, China, and the physicochemical properties and native contents of Cd and Pb in the soil were included in Appendix A Table S1 of Wang et al. (2020). 35 kg of powdered soil was weighed and transferred to a plastic container (70 cm × 50 cm × 60 cm). The soils were spiked with deionized water or mixture of Cd(NO$_3$)$_2$ and Pb(NO$_3$)$_2$, respectively. Un-polluted soils accompanied by spraying with deionized water were labeled as negative controls. 3.0 mg/kg Cd+300 mg/kg Pb-added soils (abbreviated as Cd+Pb) accompanied by spraying with deionized water were labeled as positive controls. The other treatments were as follows: Cd+Pb+foliary spraying of 25 μmol/L Se NPs; Cd+Pb+foliary spraying of 50 μmol/L Se NPs; Cd+Pb+foliary spraying of 100 μmol/L Se NPs. The tested soils were incubated in these containers under the same water-flooded condition at room temperature for nearly 60 days, respectively. Each treatment was equipped with four parallels.

Rice seeds (Oryza sativa L. subsp. Indica) are the same as adopted in Wang et al. (2020). After sterilization with 5% sodium hypochlorite, the seeds were sown in trays filled with moist fine gravel. The culture conditions for subsequent germination, translocation and transplantation were conducted according to protocols of Wang et al. (2020). From tillering to booting stage, the seedlings were sprayed with 300 mL of deionized water or concentrations of Se NPs soils as designed above for 4 times at an interval of 2 weeks in each container. During the cultivation, each of the containers maintained the same flooded condition, and was regularly added with the same volume of nutrient solution (Yoshida et al., 1976).

At filling period, flag leaves were collected to analyze some biochemical and molecular parameters in the seedlings. Up to maturing stage, grains were harvested for determination of Cd, Pb or Se contents in brown rice. 1000 grains were randomly selected and measured for assessment of grain yields in present experiment.

1.3. Determination of mRNA abundance concerning Cd transporters or photosynthesis in rice plants by real-time PCR

Leaves and node I (the uppermost node in culms) were excised from rice plants at the filling stage. The extraction, quantification and purity identification of total RNA were carried out with reference to protocols of Wang et al. (2020). 1.8 μg of total RNA was reversely transcribed to cDNA for real-time PCR (qPCR) using the revertAd Reverse transcriptase (Thermo Scientific) with reference to the instructions. The qPCR of Cd transporters- or photosynthesis-related genes were carried out in StepOnePlus™ Real-Time PCR System (ABI, USA) according to the protocol of Hao et al. (2018). The qPCR program was as follows: 4 min at 95 °C, 40 cycles (30 sec at 95 °C, 45 sec at 55 ºC, 1 min at 72 ºC), and extended at 72 ºC for 10 min. The primer sequences are exhibited in Appendix A Table S2. Relative abundances of mRNA products were calculated with the method of 2$^{-\Delta\Delta CT}$.

1.4. Immunoblotting of photosynthesis-related proteins, SBP products and oxidatively modified proteins in rice tissues

Crude protein extract in rice leaves and node I were prepared according to method of Wang et al. (2014). Soluble proteins in the extract were quantified using method of Bradford (1976), and were used for determination of some key isozyme’s patterns and protein’s immunoblotting.

Several crucial photosynthesis-related proteins (Psba, Rbc and Lhcβ1), selenium binding protein 1 (SBP1), and carbonylated proteins were visualized using immunoblotting protocols of Wang et al. (2014, 2020), respectively. Simultaneously, β-Actin protein was immunoblotted as the loading control.

1.5. Measurement of RuBP carboxylase activities and chlorophyll contents in the leaves

RuBP carboxylase (Rubisco) activities were determined by the method of Marco and Tricoli (1983). The reaction system was the same as described in the previous report (Wang et al., 2020). 0.44 mmol/L RuBP was added to initiate the reaction, and absorbance at 340 nm was consecutively monitored for 90 sec at 25 ºC.

Acetone was used to extract chlorophylls in rice leaves, and the absorbances were detected at 663 nm and 645 nm, respectively. Contents of chlorophyll a, b and total chlorophyll were calculated by the protocols of Lichtenthaler and Wellburn (1983), respectively.

1.6. Measurement of photosynthetic indexes in the leaves

At 10:00 AM to 11:30 AM, a portable photosynthesis system (TARGAS-1, USA) was used to measure four photosynthetic indexes such as net photosynthetic rate, transpiration rate, and stomatal conductance as well as intercellular CO$_2$ concentrations in rice seedlings. The detection conditions were as follows: external CO$_2$ concentration was about 300 μL/L, leaf temperature was about 27 ºC, illumination intensity was about 600 μmol/m²/sec.

1.7. Resolving crucial isozyme’s patterns in the leaves

Native polyacrylamide gel electrophoresis (PAGE) was performed with Mini-PROTEIN 3 electrophoresis system (Bio-Rad, USA) to separate these isozymes. The performance of native PAGE was referred to protocols of Wang et al. (2020). The running buffer consisted of 25 mmol/L Tris and 192 mmol/L glycine (pH 8.3). Isozymes of NADPH oxidases were visualized by the methods of Sagi and Fluhr (2001). Isozymes of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POD) were visualized with reference to protocols of Garcia-Limones et al. (2002). Isozymes of glutathione peroxidase (GPx) were determined according to the protocol of Lin et al. (2002).

1.8. Measurement of Cd and Pb contents in rice seedlings and brown rice

Leaves and node I were rinsed successively with 0.1 mol/L hydrochloric acid (HCl) and deionized water. These tissues and
brown rice were completely dried at 60 °C, then were ground into fine powders, respectively. Contents of Cd and Pb were measured with an atomic absorption spectrometer (Jena) with reference to the methods of Wang et al. (2014). Certified reference materials for Cd and Pb were obtained from Guobiao (Beijing) Testing and Certification Co., Ltd. The standard’s recoveries ranged from 91% to 102% for Cd, and 89% to 99% for Pb, respectively.

1.9. Detection of Se speciation, total Se contents and yields in the grains

Se speciation was detected by HPLC-AFS (Persee, China) with reference to method described by Wang et al. (2020). Certified reference materials of Se species were obtained from the National Research Center for Reference Materials in China. Contents of total Se in rice grains were analyzed using AFS with reference to protocol of Kumar et al. (2016). Se standard solution (from the National Institute of Metrology, China) was adopted in present experiment.

1.10. Statistical analysis

SPSS 19.0 for windows was used to conduct all the statistical analyses. One-way ANOVA test was carried out to obtain mean ± standard deviations. Duncan’s t-test was adopted to examine the difference (p < 0.05) between each treatment in present experiments.

2. Results

2.1. Transcript levels of Cd transporters-related genes in rice leaves and node 1

In the leaves, as exhibited in Fig. 1, mRNA transcripts of OsLCT1 and OsHMA2 genes in the positive control were significantly elevated more than that of the negative control. Following by spraying with 25–100 μmol/L Se NPs, the mRNA transcripts were decreased with the increase of Se NPs and were significantly less than the positive control. Meanwhile, the mRNA abundances of OsCCX2 gene were slightly increased under foliar application of 25 μmol/L Se NPs, then tended to decline with the increase of Se NPs, but no significance was observed under all the treatments by Se NPs in comparison to the positive control. For OsPCR1 gene, its mRNA abundance in the positive control was significantly elevated above the negative control. The mRNA abundances were reduced to less than the positive control following the treatments by Se NPs from 25
Results of Rubisco activities, chlorophyll contents and photosynthetic parameters in rice leaves

Results exhibited that activities of Rubisco and contents of chlorophyll a, b or total chlorophyll in the positive control were slightly increased in comparison to control plants, respectively. After foliar spraying with 25–100 μmol/L Se NPs, the Rubisco activities were slightly increased higher than the positive control. Meanwhile, the levels of chlorophyll a, b or total chlorophylls were also slightly elevated higher than the positive control in spite of their decreasing with the increase of Se NPs in the leaves (Table 1). Generally, the Pb+i-Cd-spiked soils caused a slight reduction of net photosynthetic rate, transpiration rate, and stomatal conductance except for intercellular...
CO₂ concentration in the seedlings. Following the treatments by Se NPs, the three former parameters were slightly elevated, and the latter were declined with the increase of Se NPs in the leaves. However, no significance was observed in these parameters compared with the positive control (Appendix A, Fig. S2).

### 2.4. Accumulation of Cd or Pb in rice tissues and grains

Compared with the negative control, contents of Cd or Pb in the leaves and node I were significantly elevated in the positive control (p < 0.05). Cd contents in the leaves or Pb accumulation in the node I were slightly enhanced higher than the positive control at 25 μmol/L Se NPs, then tended to decline and were less than the positive control at 50–100 μmol/L Se NPs, respectively. Nevertheless, Pb contents in the leaves or Cd accumulation in the node I were lower than that of the positive control at 25 μmol/L Se NPs, then enhanced above the positive control and increased with the increase of Se NPs (Appendix A, Table S1).

Similarly, Cd contents in brown rice were slightly elevated at 25 μmol/L Se NPs, then showed a tendency to decrease along the treatments by Se NPs and were distinctly decreased versus the positive control from 50 to 100 μmol/L Se NPs. Meanwhile, Pb contents in brown rice were also slightly elevated at 25–50 μmol/L Se NPs, then were slightly reduced at 100 μmol/L versus the positive control (Table 2).

### 2.5. Alterations in contents and speciation of Se in brown rice and changes in grain yields

As shown in Table 2, Se NPs caused a significant enrichment of Se in the brown rice versus the negative control and positive control, respectively. Furthermore, Se speciation was detected by HPLC-AFS and proved to exist as form of Se-Met in brown rice (Appendix A, Fig. S3). Also, the average weights of 1000 grains were increased higher than the positive control following the treatments by 25–50 μmol/L Se NPs, then were decreased at 100 μmol/L (Table 2).

### 2.6. Alterations in isozymes of NADPH oxidases and some antioxidative enzymes in rice leaves

The integrated intensity of bands in an isozyme denotes the total activity of an enzyme. Results indicated that, apparently,
Table 2 – Alterations in contents of Cd, Pb and Se elements in brown rice as well as in grain yields*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cd contents in brown rice (μg/kg)</th>
<th>Pb contents in brown rice (μg/kg)</th>
<th>Se contents in brown rice (μg/kg)</th>
<th>Average weight of 1000 grains (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>unpolluted soil + spraying deionized water (negative control)</td>
<td>80.9 ± 14.5c</td>
<td>64.9 ± 11.0b</td>
<td>37.4 ± 5.4d</td>
<td>28.65±1.46a</td>
</tr>
<tr>
<td>Cd+Pb+ spraying deionized water (positive control)</td>
<td>778.2 ± 145.6a</td>
<td>180.6 ± 37.0a</td>
<td>42.0 ± 3.2d</td>
<td>26.78±2.45a</td>
</tr>
<tr>
<td>Cd+Pb+ spraying 25 μmol/L Se NPs</td>
<td>827.6 ± 129.1a</td>
<td>213.0 ± 35.2a</td>
<td>218.9 ± 36.3c</td>
<td>27.26±3.06a</td>
</tr>
<tr>
<td>Cd+Pb+ spraying 50 μmol/L Se NPs</td>
<td>608.4 ± 118.2b</td>
<td>191.3 ± 32.5a</td>
<td>551.4 ± 42.1b</td>
<td>28.23±4.98a</td>
</tr>
<tr>
<td>Cd+Pb+ spraying 100 μmol/L Se NPs</td>
<td>534.2 ± 104.4b</td>
<td>160.9 ± 31.2a</td>
<td>1096.6 ± 140.0a</td>
<td>26.08±2.72a</td>
</tr>
</tbody>
</table>

n = 4, superscript letters with the same letter are indicative of no significant difference between each other at p<0.05.

![Image](image-url)

**Fig. 4 – Isozymes of NADPH oxidase, SOD, CAT, APX, GPx, and POD enzymes in rice leaves.**

the total activities in NADPH oxidases, SOD, or CAT as well as in APX and POD isozymes were differentially decreased below the positive control at 25–50 μmol/L Se NPs and were slightly enhanced thereafter. Conversely, GPx activities were obviously elevated compared to the positive control following the application of Se NPs (Fig. 4).

2.7. Production of SBP1 protein and carbonylated proteins in rice plants

As revealed by immunoblotting, SBP1 products were enhanced higher than the positive control in the leaves and node I after foliar spraying with the increasing concentrations of Se NPs (Fig. 5a, b). Meanwhile, carbonylated protein products were distinctly diminished and were less than the positive control in the leaves and node I at 25–50 μmol/L Se NPs, then were slightly enhanced at 100 μmol/L (Fig. 5c, d). Additionally, β-actin was employed to normalize the sample loading in each treatment (Fig. 5e).

The results indicated that foliar spraying with Se NPs significantly diminished Cd and Pb deposition in rice grains through the foliar pathway seems to be more challenging and significant than the reduction of individual heavy metal in rice grains.

Se element was evidenced to be able to reduce Cd accumulation and alleviate Cd oxidative stress to plants (Gao et al., 2018; Wu et al., 2016). The present results exhibited that the foliar spraying with Se NPs sols induced changes of Cd or Pb accumulation in the leaves, node I and grains (Table 2, Appendix A Table S1). Compared to the positive control, treatments by 50–100 μmol/L Se NPs resulted in a significant reduction of Cd contents in the leaves or brown rice. However, no distinct decrease of Pb contents was detected in the leaves, node I and grains. Therefore, the foliar spraying with Se NPs had the potential to diminish Cd accumulation, but exerted no distinct...
effect on Pb deposition in the rice plants and grains. The potential mechanisms are not clear, which needs to be clarified in the future.

At filling stage, Cd ions are remobilized and translocated upwards from leaves and culms to node I, where they are redirected from xylem to phloem and resumed to be transferred to rice grains (Hao et al., 2018; Uraguchi et al., 2011; Wu et al., 2015). In phloem, Cd transport is usually performed by specified transporters such as OsLCT1 (Uraguchi et al., 2014), OsHMA2 (Takahashi et al., 2012; Yamaji et al., 2013), OsPCR1 (Wang et al., 2019), and OsCCX2 (Hao et al., 2018), which function in translocation of Cd from leaves to node I then from node I to rice grains (Uraguchi and Fujiwara, 2013). Therefore, downregulation of these transporter genes at the filling stage may be possible to repress Cd remobilization to rice grains. The present results revealed that the mRNA abundances of OsLCT1, OsHMA2 or OsCCX2 in the leaves as well as OsLCT1, OsPCR1 or OsCCX2 gene in the node I at the filling stage of rice seedlings were differentially decreased below the positive control at 50–100 μmol/L Se NPs. These downregulated transporter genes might diminish Cd translocation from the leaves to node I then from the node I to rice grains, underlying the distinct reduction of Cd in the brown rice (Table 2). Particularly, 25 μmol/L Se NPs slightly improved the transcript of OsLCT1 gene in node I (Fig. 2), which might improve Cd translocation from the node I to rice grains through this transporter, resulting in Cd accumulation more than the positive control in the brown rice at 25 μmol/L Se NPs (Table 2).

The present results also confirmed that 50–100 μmol/L Se NPs promoted Pb accumulation in the leaves, thus diminishing Pb remobilization from the leaves to node I and resulting in Pb reduction in the node I versus the positive control at the filling stage (Appendix A Table S1). In terms of OsLCT1, OsHMA2, and OsCCX2 genes in the leaves, their mRNA transcripts tended to decline with the increase of Se NPs (Fig. 1), which might be responsible for Pb retention in the leaves (Appendix A Table S1). It suggests that these transporter genes might also involve in Pb translocation from the leaves to node I.

In contrast to OsLCT1, OsPCR1 and OsCCX2 genes in node I, OsHMA2 mRNA levels were increased higher than that of the positive control under the treatments by Se NPs in the node I at the filling stage (Fig. 2). Combined with the declined tendency of Pb accumulation in the node I (Appendix A Table S1) and brown rice (Table 2) along the treatments, it may suggest that OsLCT1, OsPCR1 and OsCCX2 genes might play roles in the transportation of Pb from the node I to rice grains at the filling stage. However, data concerning Pb transporter genes have been rarely reported as yet. As a result, these assumed transporter genes of Pb ions need to be further validated in rice plants.

Dutilleul et al. (2008) reported that SBP1 protein functioned as a potential new player, and could directly bind Cd, forming SBP-Cd complexes and acting in parallel with phytochelatins and reduced GSH in Cd detoxification in Arabidopsis (Arabidopsis thaliana). In present study, SBP1 products were distinctly enhanced under the foliar application of 25–100 μmol/L Se NPs versus the positive control in the leaves and node I (Fig. 3a, b). It suggests that the treatments by Se NPs improved the chelation and detoxification of Cd and Pb through the elevated SBP1 synthesis in the rice plants.

Toxic heavy metals adversely affect the growth, development and photosynthesis of plants, threatening crop productivity and environmental safety (An et al., 2019; Cao et al., 2015). Photosystem is consist of photosystem II (PS II) and PS I. The subunits of D1 and D2 proteins in PS II transfer electron from water to plastoquinone pool (Mulo et al., 2012). The D1 protein is also named as psbA, since it is encoded by psbA gene. The mRNA abundance of psbA gene was confirmed to be closely correlated with chlorophyll a/b contents or PS II activities (Duan et al., 2006; Yuan et al., 2005). Besides, Lhcb1, Lhcb2 and Lhcb3 are three function closely proteins, which constitute a major light-harvesting complex in PS II. The Rubisco catalyzes the fixation of CO2 in Calvin cycle. It is composed of eight large subunits and eight small subunits, in which the former are coded by RbcL gene and the latter are coded by RbcS genes. The enhancements of RbcL or RbcS gene were evidenced to be able to decrease intercellular CO2 concentrations and improve photosynthesis in rice plants (Suzuki and Makino, 2012).

In general, the mRNA levels of psbA, Lhcb1 or RbcL gene at the filling stage tended to increase more than the positive control in the leaves along the treatments by Se NPs (Fig. 3a). Meanwhile, the increasing concentrations of Se NPs not only promoted the expression of psbA, Lhcb1 or RbcL protein (Fig. 3b), but also elevated the Rubisco activities and contents of chlorophyll a, b or total chlorophyll compared to the positive control (Table 1). These photosynthesis-related genes, proteins, Rubisco and chlorophylls would play important roles in the photosynthesis (Duan et al., 2006; Suzuki and Makino, 2012; Yuan et al., 2005). Thus, the foliar treatments by appropriate concentrations of Se NPs would be beneficial for the photosynthesis and productivity in rice plants, which were evidenced by the elevated photosynthetic parameters.
(Appendix A Fig. S2) and increased grain yields in the present experiments (Table 2).

In order to enhance resistance against heavy metal(s), a series of defense systems including antioxidant enzymes (e.g. SOD, CAT, APX, GPx, and POD, etc.) and antioxidants (e.g. ascorbate, GSH, carotenoids, and tocopherol) have been developed in plants. These antioxidant enzymes have distinct isozymes, which usually cooperate to control ROS overproduction in plants (Mittler, 2002).

NADPH oxidase is a source of ROS products in plants (Sagi et al., 2001). Excess ROS can attack side chain groups of protein molecules and generate oxidatively modified proteins such as carbonylated proteins, indicating oxidative damage in organisms (Møller and Kristensen, 2004). As shown in Fig. 4, the total activities in NADPH oxidases, SOD, or CAT, as well as in APX or POD isozymes in the leaves were decreased at 25–50 μmol/L Se NPs versus the positive control. These results indicate that foliar treatments by 25–50 μmol/L Se NPs restricted ROS production from the NADPH oxidases, thus diminishing the production of the oxidatively damaged proteins in the seedlings (Fig. 5). ROS also act as signals for defense responses in plants subjected to abiotic stress (Sagi et al., 2001).

The declined accumulation of ROS products might play roles in downregulation of the expressions of SOD, CAT, APX, or POD isozymes, resulting in the decrease of these isozyme’s activities in the leaves following the treatments by Se NPs (Fig. 4).

However, the activity of NADPH oxidase isozymes was apparently boosted at 100 μmol/L Se NPs, indicative of ROS burst and thus aggravating protein’s oxidation in the plants (Fig. 5c, d). Moreover, the rebounded NADPH oxidases also upregulated the isozymes of SOD, CAT, APX or POD in the seedlings. Therefore, the foliar application of 25–50 μmol/L Se NPs might mitigate the oxidative damage through control of ROS production from the NADPH oxidases in the rice plants exposed to the combined stress of Pb and Cd in the paddy soils.

Se element serves as a cofactor in GPx and thioredoxin reductase in organisms. It can activate protective mechanisms or act as an antioxidant in plants, thus reducing the oxidative stress due to Cd pollution (Kumar et al., 2012; Lin et al., 2002). The increasing Se NPs evidently elevated the activities of GPx, improving the antioxidant potential at the filling stage of rice plants under the joint stress of Pb and Cd in the soils (Fig. 4).

Organic Se in food is beneficial for human health. Our results also evidenced that the foliar application of 25–100 μmol/L Se NPs significantly promoted Se enrichment in the brown rice versus the positive control (p<0.05, Table 2). Se element mainly exists as organic Se in rice grains (Farooq et al., 2019). As illustrated in Fig. 53, Se speciation was confirmed to be Se-Met in the brown rice.

4. Conclusions

As evidenced by the results, foliar application of 50–100 μmol/L Se NPs at filling stage of rice seedlings diminished Cd translocation to rice grains through downregulation of the expression of genes concerning Cd transporters and upregulation of the production of SBP1, and also promoted the photosynthesis in rice seedlings and Se enrichment in rice grains.

Meanwhile, the contents of Cd in grains were slightly elevated at 25 μmol/L Se NPs, then were notably reduced below the positive control with the increase of Se NPs. This result indicates that Cd accumulation in rice grains can be improved by spraying with lower concentrations of Se NPs (<25 μmol/L), whereas repressed by Se NPs at no less than 50 μmol/L. Combined with the changes of the antioxidant isozymes, oxidatively modified proteins and grains yields, it is concluded that the optimum concentration of Se NPs may be preliminarily defined as 50 μmol/L in the present condition. Contrastly, the foliar spraying of Se NPs induced no distinct reduction of Pb in the grains. The related mechanisms need to be further elucidated in the future.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.11.005.

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