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Running Title: COMBINED MEASURES ON RICE CD REDUCTION

Effects of soil amendments, foliar silicon and selenium, and their combination applications on the reduction of cadmium accumulation in rice (*Oryza sativa* L.)

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Abstract: Agricultural soils contaminated by cadmium (Cd)is becoming one of the most serious environmental issues and public concerns. Factorial experiments were performed to explore the effects of two soil amendments (SSBA: sodium sulfide and biofuel ash; and lime) (0.1%) and three foliar applications (silicon (Si: 2.5 mM), selenium (Se: 40 mg L^{-1}), and Si+Se (SS: 2.5 mM Si and 40 mg L^{-1} Se) on Cd reduction in rice in pot experiment and then verified in the field experiment, respectively. The results show that SSBA and lime can significantly reduce $CaCl_2$-extractable Cd and brown rice Cd concentrations by 63--44% and 57--72% in the pot and field experiments, respectively. Foliar Si, Se, and SS applications significantly reduce Cd accumulations in brown rice by 64--62%, 83--72%, and 73--39%, respectively, but showed different trends in pot and field experiments. Combination of SSBA and lime with Si, Se, and SS had non-significant synergistic effect on Cd reductions in brown rice compared to only foliar sprayings or soil applications in both pot and field experiments, although SSBA+Se and SSBA+Si can reduce Cd concentrations by 16%--34% and 14%--24% in brown rice compared to only foliar Si and Se and soil SSBA applications, respectively. Soil lime application and foliar Si spraying were the most cost-effective strategies to reduce Cd accumulation in the brown rice in field and pot experiments, respectively. The major implication of this study is that pot experiments should be verified by field experiments and the combination of soil amendments and foliar treatment failed to exhibit a synergistic effect on the Cd concentrations in brown rice.

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INTRODUCTION

Cadmium (Cd) is highly mobile in the soil-plant system (Dutta *et al.*, 2020; Feng *et al.*, 2021), and is easily transferred from soil to the food chain (Nie *et al.*, 2018). Rice is a primary staple crop and its consumption is regarded as an important exposure route to humans (Rizwan *et al.*, 2016). Agricultural soils contaminated by Cd are becoming one of the most serious environmental issues and public concerns because rice production in Cd-contaminated soils challenges the food security (Jallad and Karim, 2015) and critically threatens human health (Xiaofang and Dongmei, 2019; Du *et al.*, 2020). A severe health risk can potentially happened with chronic Cd exposure at a relatively low level. Therefore, minimization of Cd accumulation in crops, especially for staple foods, is a reasonable approach to reduce risk to human health.

Many measures have been performed on the Cd reduction in rice to guarantee food security in Cd-contaminated soils, which include foliar sprayings(Shahbaz *et al.*, 2019), soil amendment applications (Shafaqat *et al.*, 2019), plant growth regulators (Xie *et al.*, 2019), low Cd-accumulating rice varieties (Qi *et al.*, 2020), water management (Islam *et al.*, 2021), crop rotation (Yang *et al.*, 2020), planting patterns (Ge *et al.*, 2019), and microbial remediation (Liu *et al.*, 2020). Soil amendments can substantially minimize the Cd accumulations in brown rice, such as lime and sulfur fertilizer (Rajendran *et al.*, 2019). Lime increased soil Ca^{2+} and consequently promotes the soil Cd-holding capacity and the competition with Cd^{2+} on root surfaces (Zhu *et al.*, 2016). Sulfur applications reduce Cd absorption, because thiol pool is synthesized in plant, such as glutathione (GSH), phytochelatins (PCs), and non-protein thiol (NPT) (Zhang *et al.*, 2013). Reduction of rice Cd uptake and Cd toxicity alleviation by sulfur is mainly by formations of CdS precipitates or by complexations with thiols, which are subsequently move into vacuolar compartment (Rajendran *et al.*, 2019).

Crop Cd reduction in situ immobilization may be enhanced by foliar spraying of appropriate microelement applications, as foliar application can also provide a potential to alleviate Cd toxicity in plants (Shiyu *et al.*, 2020). Numerous researches have shown that selenium (Se) and silicon (Si) can significantly decrease the Cd concentrations in plants in pot, field, and hydroponic experiments (Hussain *et al.*, 2020; Huang *et al.*, 2021). The mechanisms of Cd reduction by Se and Si applications are possible: (1) increasing the oxidization of trace elements by capability of the plant rhizosphere (Rizwan *et al.*, 2019); (2) co-precipitation reactions between Si/Se and trace elements to form precipitates (Lima *et al.*, 2018); (3) enhancing the soil pH of rhizosphere soils (Li *et al.*, 2020); (4) enhancing antioxidant system(Rizwan *et al.*, 2019); and (5) regulating Cd, Si, and Se uptake (Zhou *et al.*, 2021).

Performance of the amendments largely depends on their application amounts and the soil physical and chemical properties. Furthermore, reduction in Cd concentrations by individual applications of soil amendments are not always to the level of standards set for Cd-safe foods (Sohail *et al.*, 2020). However, it still remains unclear whether the combined treatments of amendments and foliar sprayings have the synergistic effects on the Cd reduction in rice. Additionally, the dosages of most amendments should be controlled as the adverse effects of excess amendments on rice growth are also showed (Ma *et al.*, 2020). Furthermore, the Cd concentrations reduced by individual application cannot always measure up to food standard. Thus, it is feasible to operate foliar sprayings in combination with soil amendments thereby reduction of soil amendment dosages to decrease Cd accumulation in rice grains. In this study, we hypothesized that the combined applications of soil amendments and foliar sprayings have synergistic effects on the reduction of rice Cd accumulation, as individual measure could significantly decrease the Cd concentrations in rice. The factorial experiments of two soil amendments (SSBA: sodium sulfide and biofuel ash; and lime) and three foliar sprayings (Se, Si, and Se+Si (SS)) were applied to investigate either in individual or in combination on the soil Cd bioavailability and rice Cd accumulation by pot experiment and further verification by field experiment.

MATERIALS AND METHODS

Pot experiment design

The pot soil was sampled from the paddy field of Guixi, Jiangxi Province, China, which was contaminated by a large copper smelter about 6 km away. The collected soil was air-dried and crushed to screen a 10-mesh sieve for the pot experiment. The basic soil properties are presented in Table S1 (see Supplementary Material for Table S1). Then 10 kg soil was potted in each pot (diameter: 35 cm, height: 25 cm). The Cd concentrations in the soil is 0.82 mg kg^{-1} and bioavailable Cd ($CaCl_2$-extractable Cd) is 0.37 mg kg^{-1}. Basal fertilizers (334 mg N kg^{-1}, 667 mg P kg^{-1}, and 267 mg K kg^{-1}) were applied to the tested soil and mixed thoroughly.

Two amendments (SSBA: sodium sulfide and biofuel ash; and lime) were applied to the test soil. SSBA is a combined amendment composited by sodium sulfide and biofuel ash with ratio of 3:7 that was obtained through our soil culture experiments in the laboratory, and lime is CaO. The biofuel ash was residue from the combustion of rice husk. SSBA and lime were applied to soil samples at the ratio of 0.1% (w/w). The basic elements of lime, biofuel ash and sodium sulfide are shown in Table S2. Three foliar reagents were sprayed to the rice leaf: Si, Se and SS with the concentrations of 2.5 mM Si, 40 mg/L Se, and 2.5 mM Si and 40 mg L^{-1} Se, respectively. Si and Se were prepared by dissolving tetraethylorthosilicate ($C_8H_{20}O_4Si$) and sodium selenite (Na_2SeO_3), respectively, and the reagents preparation are showed in Text S3. 0.1% Tween 80 was applied as surfactant to enhance the adhesive capacity on foliar surface. Soil applications of SSBA and lime (two treatments), foliar applications of Se, Si and SS (three treatments), their factorial applications of SSBA/lime in soil and Se/Si/SS on leaf (six treatments) and a control group consisted 12 treatments (Table 1). Each treatment was conducted in triplicate and 36 pots were performed. For foliar application, rice plant in each pot was sprayed with 100 mL of solution by a hand-operated knapsack sprayer at both of the tillering and heading stages.

An early rice cultivar of Jingzao 47 widely planted in the Jiangxi province was selected and one plant was cultivated in each plot. Before germination, rice seed was sterilized by sodium hypochlorite solution for 10 minutes and then washed with ultrapure water. Seedlings were cultivated on April 11 and collected on July 19, 2016. The pots were kept in a net house at 29 °C/21 °C day/night temperatures under about 13 h daylight and 33–81% humidity. Water management followed local rice conventional operating practices. The paddy was naturally dried for 7 days at tillering stage and 15 days at grain-filling to maturity stage, and 3--4 cm of water above soil surface was maintained under flooded conditions at other stages.

Field experiment design

In order to verify the effects of the amendments, foliar sprayings, and their combinations on rice Cd reduction in practical application, a field experiment was conducted in the paddy field of Guixi where from the soil was collected for pot experiment.. The size of experimental plots was 4 m × 4 m (16 m^2), including 0.5 m ridge between plots to avoid cross impact. Soil fertilization and water irrigation accorded to local conventional management, which were same as the pot experiment.

This field experiment also consisted of 12 treatments that were same as the pot experiment (Table 1), each treatment was treated with 3 replications, resulting in total 36 plots. The amendments were mixed evenly with a 15 cm depth of the surface soil in each plot before 15 days of rice transplanting, and 1.8 kg of amendments (0.1%) were used in each plot. Fertilizer and soil were fully mixed and equilibrated for a week. The method and period of foliar application followed pot experiment operating practices, with a dosage of 1.6 L $plot^{-1}$ at both of the tillering and heading stages. Each treatment was in triplicate and in a fully-randomized distribution.

Sampling and measurement

Surface morphologies of biofuel ash were determined by scanning electron microscope (SEM) (XL-30 ESEM, Philips). UV2550 (Shimadzu Co., Ltd., Japan) were used to analyze the fourier transform infrared spectroscopy (FTIR) and biofuel ash over the range of 500--4000 cm^{-1} to measure the surface functional groups. One week after foliar spray of the field experiment at the tillering stage, antioxidant enzymes catalase (CAT) and peroxidase (POD) activities of rice leaves were measured in each plot. Antioxidant enzymes activities were determined spectrophotometrically, which was detailed in the Text S4. Briefly, Fresh leaves were ground by using ice with potassium phosphate as a buffer and polyvinylpyrrolidone as a homogenizing solution, and then centrifuged. The spectrophotometer are used to measure the enzyme activity after preparing the assay mixtures.

At maturity, totally, 72 whole rice plant samples and corresponding soil samples were collected. Grain yield were recorded and rice plant was divided into root, stem, leaf, and brown rice. After separation, the rice plants were rinsed with tap water, ultrapure water, and oven-dried at 70 °C to 48 hours. All the rice plants were ground to flours to prepare for the acid digestion. Mixed acid of HNO_3 and $HClO_4$ (5:2) was used to digest the plant samples (Zhou et al., 2021).

After rice harvest, topsoil (0--15 cm) were sampled, air-dried, ground, screened through a 0.15 mm diameter sieve, homogenized, and preserved. Soil pH was measured by a pH meter at a liquid-solid ratio of 2.5:1. The bioavailable Cd in soils were extracted by the methods of $CaCl_2$ (Hamid et al., 2020). Soil (2 g) was extracted with 20 mL of 0.01 M $CaCl_2$ and shaken for 2 h at 25 ℃, and then supernatant was obtained. Cd concentrations in the solutions of all the digested samples were measured by Inductively Coupled Plasma Mass Spectrometry (Perkin Elmer® Optima). Duplicates, method blanks, matrix spikes, and certified reference materials (CRM: spinach GBW10015(GSB-6) and soil GBW07427(GSS-13)) were included for quality control and quality assurance. Limit of determination was 0.05 μg kg^{-1} for Cd according to the three times of the SD of blank measurements. All the Cd in blanks were under 10% of the samples, and were deducted in the concentration calculations. All the measured Cd concentrations were > 20 times higher than the detection limits. An average Cd concentration of 0.15 ± 0.008 mg kg^{-1} (n = 5) was determined from CRM (0.15 ± 0.005 mg kg^{-1}). The variation coefficient of precisions, measured from eight duplicated determinations of the CRM, were 3.1–5.2% for all the elements.

Calculations and data analysis

The plant Cd bioconcentration factor (BCF) were calculated as follows:

$$BCF=Cd_i/Cd_{soil} \qquad (1)$$

Where Cd_i is the brown rice, stem, leaf and root Cd concentration; Cd_{soil} is soil Cd concentration.

The Cd translocation factor (TF) was calculated using the concentration ratio of upper part to lower part of the rice plant and accorded to:

$$TF_{m\text{–}n} = Cd_m/Cd_n \qquad (2)$$

where m and n represent the Cd concentrations in the upper part and lower part, respectively.

Statistical analysis of the mean and standard deviation (SD) of all the tissue Cd concentration, plant parameters of rice plants were analyze by SPSS Statistics 19.0 (IBM, USA). The Cd concentrations in rice tissues were compared among all the treatments using two-way analyses of variance (ANOVAs). All differences in means were significant at the $p = 0.05$ level (two-tailed).

RESULT AND DISCUSSION

Foliar Se and Si applications on plant growth and antioxidant enzyme activities

All treatments can increase 4%--10% ($p > 0.05$) of grain yield compared with CK-control (Table S5) and significantly increase the Se concentrations in the Se applications ($p < 0.05$, Fig. S4). CAT and POD in the rice leaves after foliar spraying were determined and shown in Fig. 1. Compared with control, both Se and SS significantly enhanced the leaves CAT and POD activities by 54--24% and 19--38% ($p <0.05$), respectively. Meanwhile, Si also increased the CAT and POD activities for the rice by 10--3%, but not significantly ($p >0.05$). Besides higher Cd accumulation in rice plant, redox imbalance also has an important adverse effect on rice (Naeem *et al.*, 2018), as antioxidative system plays acritical role response to Cd toxicity (Zhou *et al.*, 2021). In this study, the increased activities of CAT and POD suggested that some superoxide anion free radicals were produced in rice leaf when foliar Se and Si were sprayed. Our results are consistent with previous study that confirmed foliar Se spraying can significantly facilitate the increased of POD and CAT activities (Wu *et al.*, 2018). For example, Se alleviated the oxidative stress as reflected by reduction of $O_2^{\cdot-}$, H_2O_2, and MDA induced by Cd in rice tissues (Huang *et al.*, 2020) and regulated ROS metabolism (Huang *et al.*, 2018).

Effect of amendments on soil and rice Cd

Significant increase of soil pH and reduction of soil Cd bioavailability were observed by applications of soil amendments compared to CK (Fig. S1, $p < 0.05$). SSBA and lime increased pH by 0.17--0.20 and 0.18--0.25 unite compared to CK in both pot and field experiments, respectively. Soil pH of SSBA and lime additions showed inconsistent variations in the pot and field experiments. Soil pH values of SSBA and lime were showed no significant difference in the pot experiment ($p > 0.05$), but in the field, lime showed significantly higher soil pH compared to

SSBA ($p < 0.05$). These would be due to that sodium sulfide was oxidized and soil was acidified during the rice ripening stage in field plot, where the water was drained in this period (Huang *et al.*, 2019; Morgan and Graham, 2019). Compared to CK, $CaCl_2$-extractable Cd significantly decreased by 30%--39% and 31%--40% in the pot and field soils by addition of SSBA and lime ($p < 0.05$), respectively, but not significantly different between the two amendments ($p > 0.05$).

Applications of alkaline soil amendments can reduce soil Cd bioavailability by changing soil pH values (Hussain Lahori *et al.*, 2017). The increase soil pH by combined application of biofuel ash and sodium sulfide was related to the fact that biofuel ash and sodium sulfide were alkaline, with pH of 10.35 and 12.10, respectively (Table S2). The SEM image revealed that many well-developed pores were clearly displayed on the surface of biofuel ash (Fig. S2). FTIR characterization indicated the presence of different functional groups mainly hydroxyl groups (Fig. S3). Biofuel ash can reduce the available cadmium in the soil due to its large specific surface area and contains alkaline -OH related to Cd adsorption, such as the broad peaks at 3621 and 3698 cm^{-1} presents the -OH bonding. Presence of hydroxyl groups may be linked to the sorption of Cd on the surface of lime (Hamid *et al.*, 2020). These groups can combine with free heavy metal ions and effectively reduce their activity in soils (Pizarro *et al.*, 2015). Furthermore, sodium sulfide in soil may induce NaOH production via the process of dissolution ($Na_2S + H_2O \rightarrow NaHS + NaOH$) that resulted in a tendency to elevate the soil pH (Mahar *et al.*, 2016). Soil Eh, DOC, Fe(II), and S(-II) concentrations were increased because the sulfur application reduced the soil pH and promoted root oxygen secretion, resulting in reduction of soil Cd activity (Zhao et al., 2021). Reactions of S^{2-} with water or with heavy metals can generate stable hydroxides to reduce the bioavailability of soil heavy metals (Chen *et al.*, 2019). For example, an increased S application can enhance the formation of CdS precipitate in flooded soil, as sulfur can be readily transformed into S^{2-} in anaerobic environment (Zheng *et al.*, 2019). Once upon drainage, CdS precipitate can be oxidized and released as soluble Cd into soil solutions (Fulda *et al.*, 2013). Studies have reported that lime has two probable possibilities for Cd immobilization: (1) higher H^+ concentration can suppress Cd adsorptive capacity and then facilitate Cd desorption from the soil and the solubility of carbonated Cd, thereby increasing Cd mobility (Wang *et al.*, 2006), and lime neutralizes H^+ and reduces Cd bioavailability (Ardestani and van Gestel, 2013); (2) reduction of bioavailable Cd concentrations would be due to the pH-induced increases in surface negative charges and soil Ca^{2+} (Hamid *et al.*, 2020). Lime increased pH by 0.18 and 0.25 compared to CK in pot and field experiments, respectively. However, soil pH remained quite acidic after lime, perhaps Cd concentrations in rice grain could be further reduced by a heavier lime application, as pH 6--7 is generally considered optimum for rice production.

Fig. 2 shows applications of SSBA and lime can significantly ($p < 0.05$) reduce the Cd uptake in rice in both pot and field experiments. For the two amendments, both SSBA and lime additions significantly reduced Cd accumulations in brown rice compared to the CK ($p < 0.05$), with the reduction by 63--44% and 53--72% in the pot and field experiments, respectively. SSBA showed more effectively reduce Cd in brown rice in pot experiment, but less effectively in the field experiment, although Cd concentrations were not significantly different between the two amendments in pot and field experiments ($p > 0.05$). Compared to CK, treatments of SSBA and lime decreased Cd concentrations by 37--29% in roots and 47--38% in stems in the pot and field experiments, respectively. SSBA application significantly decreased Cd concentration by 19% in rice leaf ($p < 0.05$), but lime application showed no significant differences with CK ($p > 0.05$).

The applications of SSBA and lime resulted in significant reductions of $TF_{\text{leaf-brown rice}}$ and $TF_{\text{stem-brown rice}}$ ($p < 0.05$) (Table 2). Meanwhile, SSBA and lime caused a 63% and 53% decrease in $BCF_{\text{brown rice}}$, 37% and 29% decrease in BCF_{root}, and 47% and 38% decrease in BCF_{stem}. SSBA caused a 19% significantly decrease in BCF_{leaf}. BCF under the SSBA was lower compared to the lime, suggesting that SSBA was more effectively reduce brown rice Cd concentrations mainly by inhibiting root Cd uptake from soil. Meanwhile, SSBA and lime decreased 31% and 23% of $TF_{\text{stem-brown rice}}$ compared with CK, but $TF_{\text{root-stem}}$ under SSBA and lime showed no significant differences ($p > 0.05$), suggesting that the amendments decrease root Cd uptake and transfer capacity from belowground to aerial parts.

Applications of SSBA and lime increased 4.8%--5.1% ($p > 0.05$) of grain yield compared with CK, although yield were not significantly different between the applications of two amendments (Table S3). Reduction of Cd accumulation in plant is induced by the reduction of soil Cd bioavailability (Lin *et al.*, 2017). SSBA and lime incrementally raised the pH and decrease the $CaCl_2$-extractable Cd, which caused the reduction of rice Cd accumulation as we stated above. Within rice plant, sulfur plays a critical role in rice growth (Zhang *et al.*, 2013), which could alleviate Cd toxicity because of S-induced increase of GSH for synthesis PCs correlated to Cd tolerance (Fan *et al.*, 2010). Sulfur-induced Cd accumulation and translocation in brown rice may be attributed to the increase root Fe plaque formation, Cd chelation production and Cd vacuolar sequestration (Cao et al., 2018). As Ca and Cd compete for absorption by rice roots, the higher Ca concentrations in lime crucially reduces the absorption of Cd by crops (Liu *et al.*, 2020). The reduction Cd accumulated in rice plant by the lime application would be attributed to the increase of iron plaque formation that decreased Cd uptake (Zeng *et al.*, 2020).

Effects of foliar Se and Si applications on Cd in rice

In both pot and field experiments (Fig. 2), Cd accumulation in all the tissues significantly decreased with foliar spraying compared to control ($p < 0.05$). In pot and field experiments, Cd concentrations were significantly decreased by 26--27%, 59--46%, and 45--25% in roots, and by 49--51%, 74--60%, and 60--25% in stems, and by 32--29%, 49--37%, and 9--23% in leaves, by 64--62%, 83--72%, and 73--39% in brown rice following Se, Si, and SS sprayings relative to control ($p < 0.05$), respectively. Rice Cd concentration after foliar Se, Si, and SS applications showed inconsistent variations in the pot and field experiments. Cd concentration of roots and leaves with foliar spraying with Si was not significantly different with that of Se and SS ($p > 0.05$) in the field experiment, but showed significantly lower Cd concentration compared Se and SS in the pot experiment ($p < 0.05$).

BCF was ordered roots>stems>leaves>brown rice in all treatments (Table 3). In pot experiment, foliar spraying with Si had the most significant effect, which had the lowest BCF for Cd in root, stem, leaf and brown rice ($p < 0.05$) compared with the other three treatments. However, in field experiment, the effects of Se and Si were much similar in BCF. For TF, control treatment had the highest Cd translocation, except $TF_{stem-leaf}$. A significant increase in $TF_{stem-leaf}$ for foliar Se, Si and SS applications ($p < 0.05$). In both pot and filed experiments, foliar sprayings with Se and Si have much similar in $TF_{root-stem}$ and $TF_{stem-brown\ rice}$ ($p > 0.05$). In the field experiment, foliar spraying with Se and Si significantly increased $TF_{stem-leaf}$ ($p < 0.05$), but foliar application with Si impacted Cd uptake and translocation by decreasing $TF_{root-stem}$, $TF_{leaf-brown\ rice}$, and $TF_{stem-brown\ rice}$. Meanwhile, foliar Se and Si applications treatments caused a 3.65%--4.14% ($p > 0.05$) increase grain yield compared with control (Table S4).

Although Si is not regarded as an essential element for plant, but can alleviate the phytotoxicity induced by non-essential heavy metals and excess essential elements (Vaculik *et al.*, 2020), which reduced the Cd concentrations and its proportions in cell walls of roots (Wu *et al.*, 2016). Si application can reduce Cd uptake and translocation through enhancing the binding of Cd to the cell wall as well as the Cd compartmentation into the vacuoles (Adrees et al., 2015, Wu et al., 2019). The Cd in rice tissues had significant lower concentrations in foliar spraying with Si than those of other treatments ($p < 0.05$). Si can ameliorate Cd translocation in rice via various mechanisms occurring within rice plant. For instance, Si application could be tightly bound with the plant cell walls, compositing organosilicon (Si-O-C linkage) and compartmentation into the vacuoles, which could co-localize Cd and form insoluble Cd-Si complexes in the cell wall (Neumann and zur Nieden, 2001; Fan *et al.*, 2016; Wu *et al.*, 2018). Exogenous Si can also reduce Cd stress via defense metabolites, involving regulation of antioxidative enzymes activities (Hussain *et al.*, 2019), improvement of the stability of the plasma membrane (Chen *et al.*, 2019), immobilization by synthesis of various complex agents (Naeem *et al.*, 2018), promotion of various photosynthetic parameters (Gao *et al.*, 2018), and regulation the mineral nutrients (Singh and Prasad, 2018). Furthermore, Si applications could regulate Cd transporters and phytochelatin gene transcription to decrease Cd uptake and translocation at the molecular level (Aprile *et al.*, 2018; Zhou *et al.*, 2021). Si regulated the expressions of Cd transporter genes (e.g. *OsLCT1* and *OsNramp5*) (Zhou *et al.*, 2021), and simultaneously, Si positively regulated phytochelatin genes involved in metal detoxification (e.g. *Lsi1*)in order to reduce the Cd stress (Wang *et al.*, 2020).

Our findings showed that Se application can significantly increase Se accumulation in rice brown rice (Fig. S4), and significantly decreased Cd concentrations in rice tissues, which indicated Se antagonism for Cd bioaccumulation in rice (Lin *et al.*, 2012). Se alleviates Cd toxicity possibly by regulating the antioxidant enzyme activities (Cartes *et al.*, 2010) and repairing the damaged cells (Zhang *et al.*, 2014; Huang *et al.*, 2021). Foliar Se spraying positively effects on physiological and biochemical process. Unlike Si, Se application can enhance the production of phytochelatins and activate phytochelatin-Cd chelation to detoxify in rice, which is related to the synthesis of selenoenzyme (e.g. glutathione) (Rizwan *et al.*, 2020; Zhang *et al.*, 2020). Se restricted Cd from root to shoot translocation due to the formation and retention of Cd-Se complex in roots (Huang *et al.*, 2018).

Foliar application of Si and Se composite sols significantly decreased the transcription of gene, Cd transporter-related genes (e.g. OsLCT1, OsCCX2, TaCNR2 and OsPCR1) were downregulated to decrease Cd translocation from leaf or peduncle to brown rice (Wang et al., 2020). SS application had less effect on the reduction of Cd concertation in brown rice compared to Si or Se applications individually. The reason would be that the mixed Se and Si solutions increased the colloid aggregates and formed relatively large colloidal particles in the SS treatment (Gao *et al.*, 2018). Plant cell walls are a structure which is composed of cellulose which permits the entry of small particles and restricting the larger one, therefore smaller colloidal particles can go through this layer in a comparatively easy way in respect to larger colloidal particles (An et al., 2017). One study has showed that the colloidal particle size of SS was much larger (161 nm), which corresponded to the lowest effect on reducing the Cd concentration in brown rice. The colloidal particle size of Si was the largest (59 nm), which corresponded to the strongest effect on decreasing the Cd concentration in brown rice (Gao *et al.*, 2018). One study has found that compared to the cells cultured with silica nanoparticles, the Cd^{2+} influx in the treatments of silica nanoparticles (19, 48 and 202 nm) reduced by 15.7-, 11.1- and 4.6-folds, respectively (Cui *et al.*, 2017), which indicated that small particles have more effects on the decrease of rice Cd uptake.

Effect of soil amendment combined with foliar application on rice Cd concentration

All treatments can significantly reduce roots and stems Cd concentration compared to CK+control in the pot experiment and have 4%--10% ($p > 0.05$) increase of grain yield compared with CK-control (Table S5) ($p < 0.05$, Fig. S4). SSBA+Se can significantly reduce Cd concentrations in roots and stem compared with soil additions of SSBA and foliar spraying of Se alone ($p < 0.05$). SSBA+Si reduced the Cd concentration in roots, stems and leaves compared with soil additions of SSBA and foliar spraying of Si alone, but showed no significant differences (p >0.05). Fig. 2 shows that applications of soil amendment combined with foliar application can significantly decrease the Cd uptake in brown rice in both pot and field experiments (p < 0.05). For the pot and field experiments, Cd concentrations were significantly decreased by 74%--63%, 86%--67% and 69%--70% in brown rice following SSBA+Se, SSBA+Si and SSBA+SS, and by 62%--70%, 66%--69% and 68%--62% following lime+Se, lime+Si and lime+SS compared with CK-control, respectively. In pot experiment, SSBA+Se and SSBA+Si can reduce Cd concentrations by 29%--20% compared to foliar spraying, and by 29%--62% compared to soil SSBA application in brown rice, respectively, but showed no significant differences (p >0.05). In field experiment, SSBA+Se and SSBA+Si can also reduce Cd concentrations by 16%--34% compared to foliar spraying, and 14%--24% compared to soil SSBA application in brown rice (p >0.05), respectively. In addition, SSBA+SS reduce Cd concentrations by 43% compared to foliar spraying of SS and by 31% compared to soil SSBA application in brown rice (p >0.05), respectively. On the contrary, the combination of lime with Se, Si, and SS can elevate the Cd concentrations in brown rice compared to lime applications alone in the field experiment and foliar applications alone in the pot experiment. These results suggests that there were no synergistic effects on the combination of SSBA and foliar applications in both pot and field experiments (p >0.05), which is partly in conflict with our hypothesis.

Sulfur, Si and Se are essential elements to maintain plants growth, which can promote the transfer of Cd to vacuoles, and can inhibit the toxicity of Cd to plants (Jacob et al., 2003; Mendoza-Cozatl et al., 2005). Studies have reported that application of Si, Se and sulfur fertilizer can increase the formation of iron plaque on the surface of rice root, which can hamper Cd uptake by rice (Wang et al. 2016; Hu et al2007), which can also promote the transfer of Cd to vacuoles and reduce the retention of Cd by cell wall. Although sodium sulfide, biofuel ash and foliar spraying of Si and Se could be utilized in minimizing metal toxicity in rice, pot and field experiments are scarce regarding soil amendments combined with foliar applications aiming to decrease Cd toxicity in rice. The physiological mechanisms still remain unknown on how combined applications of soil amendments and foliar benefit elements affect the Cd reduction in rice, which need further investigation.

Our results showed that Cd concentrations showed higher reduction rates in brown rice in the pot than in field experiments. The results from the pot experiment were not predictive of the field conditions; therefore, field experiments are needed to verify the results from pot experiment (Tlustos *et al.*, 2016). Pot experiment incorporates fewer environmental disturbances, while experiment in the field is more difficult to control (Kim *et al.*, 2010). In the field, contact time between foliar fertilizer and leaf would be shorter, because foliar fertilizer sprayed on rice leaves were easily blown by natural wind and falls into the soil, affecting the absorption of nutrients in foliar fertilizer by leaf, meanwhile, rice is completely exposed to the environment filled with fresh pollutants, which usually have high bioavailability, e.g. atmospheric metal deposition (Liu *et al.*, 2019). Additionally, other factors also significantly impact on the Cd accumulation in rice plants, such as even spraying, sunlight, soil water condition, and soil microorganism (Xu *et al.*, 2021). Furthermore, heterogeneity in field conditions at a smaller scale can result in different metal levels even in the same plot, which led to the different heavy metal absorption in rice, affecting the foliar effects (Hou *et al.*, 2020). There are many uncontrollable factors in the field experiment, which are limited by natural conditions and complex ecosystems, resulting in some differences between the results of field and pot experiments. To verify the Cd concentration in brown rice of soil amendment combination with foliar spraying in the field, more researches are needed.

Our results suggested that individual applications of Si in the pot experiment and lime in the field experiment can significantly reduce the Cd accumulation in the brown rice. Therefore, to decrease the financial costs during the safe use of contaminated soil, foliar Si and soil lime applications are recommend base on our experiments. Based on the dose in field trial and the current price of the materials in China, the estimated direct financial costs are about 3600 CNY ha^{-1} for the foliar Si application and 1600 CNY ha^{-1} for the soil lime application, which are relatively economical compare to the combination applications.

CONCLUSION

Foliar Se and Si applications increased 4%--10% of grain yield, improved the antioxidant enzyme activities, and significantly reduced the Cd accumulation in the brown rice by 39--83%. Amendments of SSBA and lime applications significantly decreased $CaCl_2$-extractable Cd concentrations, and resulted in significant reduction of root Cd bioaccumulation and transfer capacity from root to aerial tissues. Combined soil applications of SSBA+Si, SSBA+Se, and SSBA+SS can reduce Cd accumulation in rice plants compared to foliar applications and soil SSBA application, but showed no significantly synergistic effects on the reduction of Cd accumulation in brown rice. Individual application of Si and combination application of SSAB+Si in the pot experiment and individual application of lime in the field experiment were the most effective in Cd reduction in brown rice. Combination of soil amendments and foliar applications had no synergistic effects on reduction of Cd accumulation in rice. The major implication of this study is that pot experiments should be verified by field experiments and the combination of soil amendments.

CONTRIBUTION OF AUTHORS

Demin LI and Hongyan LIU contributed equally to this work.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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Table 1. Experiment design for the soil amendment and foliar element applications.

Abbreviation	Soil and foliar application of composition	Addition amount in % (w/w)	Foliar application mount (100 mL pot^{-1} or m^{-3})
Control	Deionized water	0%	100 mL per time
CK-Si	2.5 mM Si	0%	100 mL per time
CK-Se	40 mg/L Se	0%	100 mL per time
CK-SS	Equal volumes of 2.5 mM Si and 40 mg L^{-1} Se	0%	100 mL per time
SSBA-Control	SSBA + Deionised water	0.1%	100 mL per time
SSBA-Si	SSBA+ 2.5 mM Si	0.1%	100 mL per time
SSBA-Se	SSBA+40 mg/L Se	0.1%	100 mL per time
SSBA-SS	SSBA + Equal volumes of 2.5 mM Si and 40 mg L^{-1} Se	0.1%	100 mL per time
LIME-Control	LIME + Deionized water	0.1%	100 mL per time
LIME-Si	LIME + 2.5 mM Si	0.1%	100 mL per time
LIME-Se	LIME+40 mg/L Se	0.1%	100 mL per time
LIME-SS	LIME + Equal volumes of 2.5 mM Si and 40 mg L^{-1} Se	0.1%	100 mL per time

Table 2. Effects of amendment treatments on bioconcentration factor (BCF) and translocation factor (TF) for Cd in different tissues of rice in pot and field experiment.

Treatment	BCF					TF	
	Brown rice (pot)	Brown rice (field)	Root (pot)	Stem (pot)	Leaf (pot)	Stem/Root (pot)	Brown rice/Leaf (pot)
Control	1.5±0.24a	2.5±0.12a	14±0.99a	11±0.77a	2.9±0.35a	0.53±0.06a	0.14±0.01a
SSBA	0.56±0.06b	1.1±0.39b	8.7±0.78b	5.8±0.21c	2.4±0.15b	0.24±0.03b	0.097±0.01b
LIME	0.73±0.07b	0.72±0.2b	9.7±0.96b	6.8±0.44b	3.1±0.24a	0.24±0.03b	0.11±0.02b

Different letters represent significant difference between treatments at the $p < 0.05$ level.

Table 3. Effects of foliar spraying treatments on bioconcentration factor (BCF) and translocation factor (TF) for Cd in different tissues of rice in pot and field experiment.

Experiment	Treatments	BCF				TF			
		Brown rice	Root	Stem	Leaf	Stem/Root	Leaf/Stem	Brown Rice/Leaf	Brown Rice/Stem
Pot	Control	1.5±0.24a	14±0.99a	11±0.77a	2.9±0.35a	0.8±0.02a	0.26±0.02c	0.53±0.06a	0.14±0.01a
	Se	0.55±0.09b	10±0.74b	5.6±0.4b	2±0.2b	0.55±0.04b	0.36±0.04b	0.28±0.05b	0.098±0.02b
	Si	0.26±0.06c	5.6±0.78d	2.8±0.47d	1.5±0.17c	0.51±0.05b	0.53±0.09a	0.18±0.05c	0.098±0.03b
	SS	0.41±0.09bc	7.6±0.75c	4.3±0.48c	2.6±0.31a	0.57±0.05b	0.61±0.05a	0.16±0.05c	0.095±0.03b
Field	Control	1.9±0.4a	15±2.99a	12±0.74a	3.2±0.38a	0.82±0.17a	0.27±0.03b	0.16±0.03a	0.58±0.06a
	Se	0.72±0.16bc	11±0.66b	5.8±0.84c	2.3±0.25b	0.53±0.09b	0.4±0.04a	0.12±0.01a	0.31±0.04c
	Si	0.54±0.13c	8.1±0.81b	4.8±0.33c	2±0.15b	0.6±0.08b	0.42±0.02a	0.11±0.03a	0.27±0.09c
	SS	1.2±0.23b	11±1.02b	8.9±1.08b	2.5±0.3b	0.67±0.13a	0.28±0.04b	0.15±0.04a	0.25±0.2b

Different letters represent difference significant between treatments at the $p < 0.05$ level.

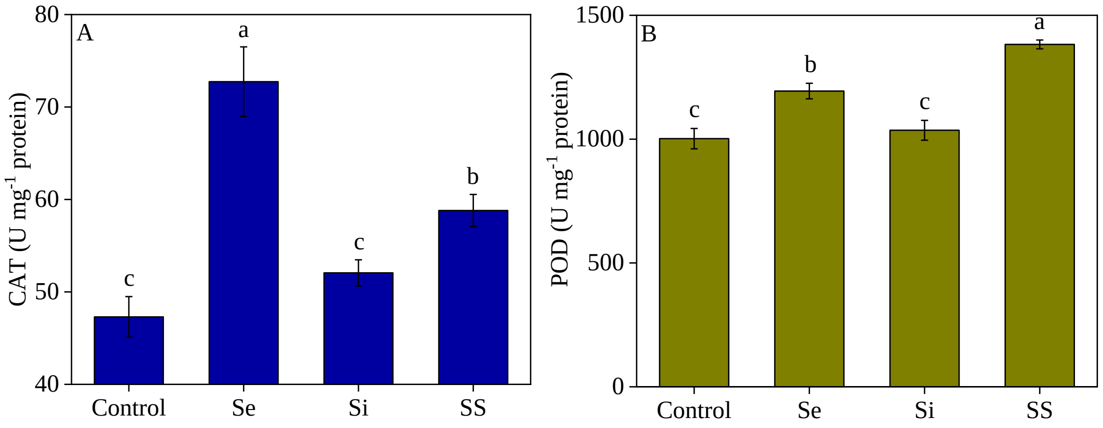

Fig. 1. Antioxidant enzyme activities in the leaves of the rice following foliar spray treatments in field experiment (Control, Si, Se, and SS). Panels A-B = CAT and POD, respectively. Different letters represent difference significant between treatments at the $p < 0.05$ level.

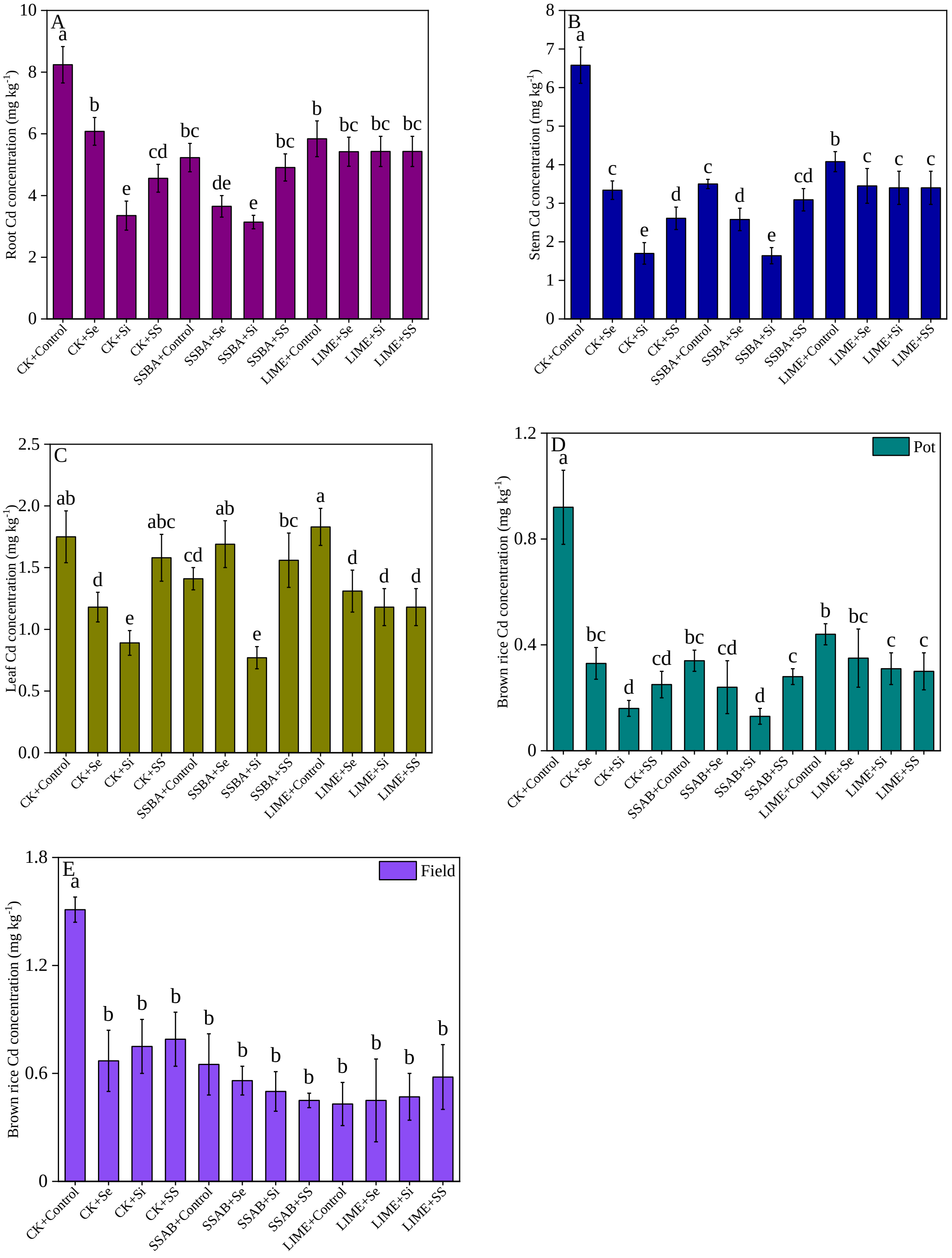

Fig. 2. Effect of soil amendments combined with foliar applications on rice Cd concentration. Panels A-E=Cd concentration in roots (A), stems (B), leaves (C), brown rice (D) in pot experiment, and brown rice (E) in field experiment, respectively. Different letters represent difference significant between treatments at the $p<0.05$ level.