



# Effects of different phosphorus fertilizers on cadmium absorption and accumulation in rice under low-phosphorus and rich-cadmium soil

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## Abstract

Rice is the main food crops with the higher capacity for cadmium (Cd) uptake, necessitating the urgent need for remediation measures to address Cd in paddy soil. Reasonable agronomic methods are convenient and favorable for fixing the issue. In this study, a pot experiment was employed to evaluate the effects of two foliar ( $\text{NaH}_2\text{PO}_4$ , SDP;  $\text{KH}_2\text{PO}_4$ , PDP) and two solid phosphate fertilizers (double-superphosphate, DSP; calcium-magnesium phosphate, CMP) on uptake and remobilization of Cd in rice plants under the low-P and rich-Cd soil. The results revealed that these four phosphorus fertilizer significantly down-regulated the relative expression of *OsNRAMP5* involved in Cd absorption, while up-regulated *OsPCS1* expression and increased distribution of Cd into the cell wall in roots. Furthermore, phosphorus fertilizer resulted in a significant decrease in the relative expression of *OsLCT1* in stems and *OsLCD* in leaves, decreased the transfer factor of Cd from shoots to grains, and ulterior reduced the Cd accumulation in three protein components of globulin, albumin, and glutelin, making the average Cd concentration of brown rice decreased by 82.96%. These results comprehensively indicate that in situations with similar soil backgrounds, the recommended application of solid CMP and foliar PDP can alleviate the toxicity of Cd by reducing its absorption and remobilization.

**Keywords** Rice · Cadmium stress · Phosphorus · Accumulation · Protein composition

## Introduction

The overuse of pesticides and fertilizers, coupled with the indiscriminate discharge of industrial and mining sewage, has resulted in the increasingly grave issue of heavy metal

pollution in farmland (Peera et al. 2023). Cadmium (Cd), one of these inorganic pollutants, has been a cause for serious concern due to its highly water-soluble and fat-soluble nature, which can lead to its accumulation in vegetation and potentially impact the quality and safety of agricultural produce, subsequently posing a risk to public health (Chen et al. 2020). Rice is a staple cereal crop with the highest Cd uptake capacity. Previous studies have shown that the average Cd concentration in rice grains is significantly markedly greater than that in wheat and other cereals (Zeng et al. 2021; Zhao et al. 2021). Elevated soil Cd concentrations may affect the amino acid metabolism of rice grains, leading to increased prolamin levels and decreased albumin and globulin concentrations (Zeng et al. 2021). The outer layer of brown rice is the main storage site of albumin and globulin. Therefore, the protein components in milled rice are mainly composed of nutrient-rich glutelin and taste-reducing prolamin (Teixeira et al. 2021). Moreover, high soil Cd concentration leads to a decrease in the breakdown value of rice-starch spectrum. However, it significantly increases the amylose content as well as its final

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viscosity and setback value (Huang et al. 2021). Cooking and eating quality of rice depend largely on the amount of amylose. Better flavor quality rice generally has relatively larger breakdown value and smaller setback value (Amagliani et al. 2016). Thus, stressing soil with Cd will have an adverse effect on the nutritious and gastronomic properties of paddy grains.

Based on the physiological and metabolic characteristics of Cd, experiments were conducted to determine how Cd levels can be reduced in rice plants through improved breeding, soil remediation, and agronomic regulation. The most cost-effective, efficient, and successful method among these is agronomic regulation. This process leverages cation competition and antagonistic effects to both control Cd uptake and minimize Cd accumulation in edible parts of the plant (Xiao et al. 2021). Some researchers have shown that foliar spraying of phosphorus (P), zinc (Zn), and silicon (Si) nutrition effectively dropped the amount of Cd in brown rice while ensuring food safety (Farooq et al. 2022; Barman and Kundu 2023).

Phosphorus promotes carbohydrate and plays a crucial role in the material and energy metabolism of plants (Dissanayaka et al. 2018). The utilization of phosphate fertilizers enhances the soil's physical and chemical properties, amplifies heavy metal dispersal in root cell walls, competes with heavy metal transporters, and consequentially restrains heavy metal absorption by roots (Jiang et al. 2014; Zhao et al. 2020). Furthermore, phosphorus can facilitate the creation of immovable phosphate compounds and protein connective substances within cells, impeding the passage of heavy metals (Fu et al. 2011; Siebers et al. 2013). Phosphate fertilizers commonly used in paddy production include water-soluble fertilizers of potassium dihydrogen phosphate (PDP), sodium dihydrogen phosphate (SDP), and solid fertilizers of double superphosphate (DSP) and calcium magnesium phosphate (CMP) (Gong et al. 2022). However, the effects of various phosphate fertilizers on Cd accumulation in rice plants and the changes in grain chemical composition remain uncertain under Cd stress. To address this, we examined Cd concentration, subcellular distribution, antioxidant enzyme activity, gene expression, and their response to phosphate fertilizer in different rice sections under Cd stress. Furthermore, a study was conducted to investigate the impact of phosphorus on the accumulation of starches, proteins, and other elements in rice grains. The ultimate goal was to formulate an agronomic strategy that would enhance the nutritional and edible quality of rice while simultaneously combating heavy metal stress.

## Materials and methods

### Plant material and experiment design

A pot experiment using Nanjing 9108 japonica rice as the material was conducted at the Crop Production Experimental Center of Huaiyin Institute of Technology, Jiangsu Province, China. Three weeks after sowing the rice seeds, seedlings with uniform growth were transplanted into experimental pots, with five seedlings per pot. Before this experiment, we also conducted a previous pot experiment to observe the effects of water management on wheat growth under Cd stress with the conventional fertilization conditions (before wheat sowing, the soil was added with  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$  solution as Cd source; the soil was obtained from the 0–20 cm surface layer in the rice cultivation region of Huaiyin District, Huai'an City, Jiangsu Province, China, with a latitude of  $33.64^\circ\text{N}$  and a longitude of  $118.90^\circ\text{E}$ ). Therefore, in order to analyze the mechanism of Cd impact on rice-wheat rotation system, the soil used in this experiment was the soil retained after wheat harvest. Prior to loading the soil into the test pots, it was centrally stacked, dried, and screened to remove wheat roots and small stones. In each experimental pot, 15 kg of sieved, dry soil, along with 0.8 g of urea and 1.2 g of  $\text{K}_2\text{O}$  basal fertilizers (with no phosphate fertilizer), was applied. The soil's physical and chemical properties utilized in this research are displayed in Table 1. As per the Soil Environmental Quality Risk Control Standard (GB 15618–2018,  $5.5 < \text{pH} \leq 6.5$ ,  $0.4 \text{ mg kg}^{-1}$ ), it suggests the potential for soil contamination with Cd.

The experiment comprised five treatments with three replicates: CK, no phosphate fertilizer was applied, only foliar deionized water spray; SDP, 100 mL  $\text{pot}^{-1}$  of  $\text{NaH}_2\text{PO}_4$  ( $50 \text{ mmol L}^{-1}$ ) was sprayed at the booting stage and early filling stage (September 2, 2021) of rice, respectively; PDP, 100 mL  $\text{pot}^{-1}$  of  $\text{KH}_2\text{PO}_4$  ( $50 \text{ mmol L}^{-1}$ ) was sprayed in the same manner as SDP; DSP, double superphosphate ( $0.1 \text{ g kg}^{-1}$ ,  $\text{P}_2\text{O}_5/\text{soil}$ , w/w) was evenly added into the each pot soil before rice transplanting; CMP, calcium magnesium phosphate ( $0.1 \text{ g kg}^{-1}$ ,  $\text{P}_2\text{O}_5/\text{soil}$ , w/w) was evenly added as the same way of DSP. Meanwhile, an appropriate amount of 0.02% (v/v) Tween 20 (surfactant) was added to the SDP and PDP solutions to enhance the absorption and utilization of phosphate fertilizer by the rice plants. Additionally, an equal volume of a mixture of Tween 20 and deionized water was sprayed on the CK, DSP, and CMP treatments.

**Table 1** The physical and chemical properties of the soil ( $\text{mg kg}^{-1}$ )

pH	Organic matter	Total N	Available P	Available K	Cr	Cu	Zn	As	Hg	Pb	Cd
6.43	16,880	1120	13.13	119.21	19.88	26.19	58.88	15.81	0.25	66.89	0.89

At the mature stage of rice growth (November 3, 2021), dry plant samples with similar characteristics were chosen for analysis. The roots, stems, leaf sheaths, leaves, glumes, and brown rice of the plant samples were separated, and the brown rice (100 g) was ground into two components: milled rice and aleurone layer with a stainless steel grinder (model JLGJ3, Zhejiang, China). The samples underwent a thorough washing with deionized water followed by heating in an oven at 105 °C for an hour. After that, the samples were dried at 70 °C until reaching a constant weight. Subsequently, the samples were crushed and screened to analyze their chemical elements and grain storage components. Additionally, this experiment collected fresh samples from the same parts of paddy plants during the early flowering stage (September 7). After separation, the fresh sample is immediately frozen and stored in liquid nitrogen tank for further investigation of the expression levels of Cd-related genes, Cd subcellular components, superoxide dismutase (SOD) activity, as well as the contents of malondialdehyde (MDA), phytochelatin (PCs), and glutathione (GSH).

### Heavy metal and essential element concentrations in plant or soil samples

Paddy plant samples were digested with 10 mL of a mixed acid solution composed of HNO<sub>3</sub> and HClO<sub>4</sub> in a 3:1 ratio, while soil samples were digested with a solution of HNO<sub>3</sub>–HClO<sub>4</sub>–HF–HCl. The available Cd content in soil was extracted using DTPA. According to our early research work, inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the concentrations of Cd, Zn, and other elements in plants and soil samples (Wang et al. 2023). Total As of rice grains was measured using the hydrogen-generation atomic-fluorescence spectrometer (WHG-690A, Beijing Hanshi Instruments Co., China) as described by Shi et al. (2015).

The translocation factor (Tf) of Cd between various organs in rice plants was calculated according to Huang et al. (2021).

$$\begin{aligned} \text{Tf}_{\text{soil-roots}} &= \text{Cd}_{\text{roots}} / \text{Cd}_{\text{soil}} \\ \text{Tf}_{\text{roots-stems, leaves, glumes}} &= \text{Cd}_{\text{stems, leaves, glumes}} / \text{Cd}_{\text{roots}} \\ \text{Tf}_{\text{stems, leaves, glumes-grains}} &= \text{Cd}_{\text{grains}} / \text{Cd}_{\text{stems, leaves, glumes}} \end{aligned}$$

Symbols such as Cd<sub>soil</sub> and Cd<sub>roots</sub> indicate the concentration of Cd in paddy field soil and different parts of rice plants (mg kg<sup>-1</sup>, DW).

### Calculation of Cd related intake and risk factors

Daily intake of heavy metals (DIM, mg kg<sup>-1</sup> day<sup>-1</sup>) = (C × IR)/BW (US EPA 2004).

Health risk index (HRI) = DIM/RFD (Cui et al. 2005).

C is the concentration of Cd in milled rice; IR is the ingestion rate of rice (mg daily<sup>-1</sup>, calculated as 300 g per day for adults) (Majumdar et al. 2020); BW is the body weight (kg, calculated based on an average weight of 60 kg); RFD is the oral reference dose (calculated as 1.00E–03 of Cd) (WHO/FAO 2013). RFD represents the degree of harm to human health from consuming food contaminated with Cd. If the HRI < 1, people can safely consume it (Cui et al. 2005).

### Starch and protein component contents in paddy grains and the Cd concentrations in four protein components

The total starch content of rice grains was measured by polarimetry. The amylose content was determined by iodine colorimetry of Zhu et al. (2021). The difference between the total starch content and amylose content corresponds to the amylopectin content. The each protein component content in rice grains was analyzed with reference to the extraction method of Dos Reis et al. (2020). Refer to the study method of Wei et al. (2017) to separate the protein components. The sifted paddy flour was soaked in n-hexane and dried to obtain defatted rice flour. Defatted rice flour (15 g) was extracted with ultrapure water, sodium chloride (NaCl, 5%), and sodium hydroxide solution (NaOH, 0.1 mol L<sup>-1</sup>), respectively. After centrifugation (10,000 r/min, 15 min), the supernatant was added with acetone (4 °C) and freeze drying to collect three protein components (albumin, globulin, and glutelin). Another equivalent amount of defatted rice flour was soaked and extracted using 70% ethanol. The supernatant was then concentrated in a nitrogen evaporator to collect the prolamin, following the same centrifugation conditions as used in the previous protein components. The concentration of Cd in the four protein components was determined with the same method used for the plant samples above.

### Subcellular fractions of Cd, content of PCs, GSH, MDA, and SOD activity determination in shoots and roots of rice plants

According to our previous research method (Wang et al. 2023), fresh rice shoots (including leaves, stems-sheaths) and roots samples were extracted by adding precooling extraction buffer solution of Tris–HCl (50 mM, pH 7.5), sucrose (250 mM), and DL-dithioerythritol (1 mM). Subsequently, the samples underwent two rounds of centrifugal separation, leading to the isolation of F1 (cell wall), F2 (cell organelle), and F3 (soluble) fractions. Fresh rice shoots and roots (0.2 g) were accurately weighed, pulverized using liquid nitrogen, and extracted by adding 2 mL of a liquid mixture consisting of the extraction buffer (0.1% trifluoroacetic

acid (TFA) and  $6.3 \text{ mmol L}^{-1}$  DTPA). The contents of GSH and NPT (total non-protein thiol) in supernatant after centrifugation ( $12,000 \times g$ ,  $4 \text{ }^\circ\text{C}$ , 10 min) were determined by the kit (JianCheng, Nanjing) according to the instructions. The PC content was calculated based on the difference between NPT and GSH values (Huang et al. 2021). The SOD activity and MDA content were determined by spectrophotometry according to the kit of Nanjing JianCheng Institute of Biological Engineering (Nanjing, China). Fresh shoot and root samples were pulverized in liquid nitrogen, and normalized with 10 mL of 0.1 M phosphate buffer (pH 7.8). The centrifuged supernatant was utilized to determine SOD and MDA (Zhen et al. 2021).

### Gene expression analysis

Total RNA was isolated from the stems, top three leaves, and roots of paddy plants at the early flowering period (September 7, 2021) using the FastPure Universal Plant Total RNA Lsolation Kit. The cDNA was synthesized with the Oligo dT Primer or dNTP mixture. The primer sequences used are listed in Table S1, and  $\beta$ -actin was used as the internal control. The qRT-PCR was performed using a FQD-48A (A4) machine (parameters: 30 s at  $95 \text{ }^\circ\text{C}$ , followed by 40 cycles of 5 s at  $95 \text{ }^\circ\text{C}$ , 20 s at  $60 \text{ }^\circ\text{C}$ , and 15 s at  $72 \text{ }^\circ\text{C}$ ). All the reactions were carried out in triplicate (technical replicates) for each biological replicate (three for each treatment). The  $2^{-\Delta\Delta\text{CT}}$  method was calculated to quantify gene expression (Chakrabarti et al. 2002).

### Statistical analysis

The experimental data were statistically analyzed using Excel 2010 (Microsoft, Redmond, WA) and SPSS 17.0 (Statistical Product and Service Solutions, IBM). A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ( $P < 0.05$ ) was carried out to test the significance of treatments. Additionally, multivariate analysis was conducted with principal component analysis (PCA) with the Graphpad Prism software. And spearman's correlation analysis was used to uncover the relationships among the different indicators.

## Results

### Plant height, dry weight, yield parameters of rice, and soil bioavailable Cd concentrations under different treatments

The use of four different types of phosphate fertilizers substantially increased the height of rice plants. Specifically, PDP proved to be the most effective (72.53 cm, Fig. 1b).

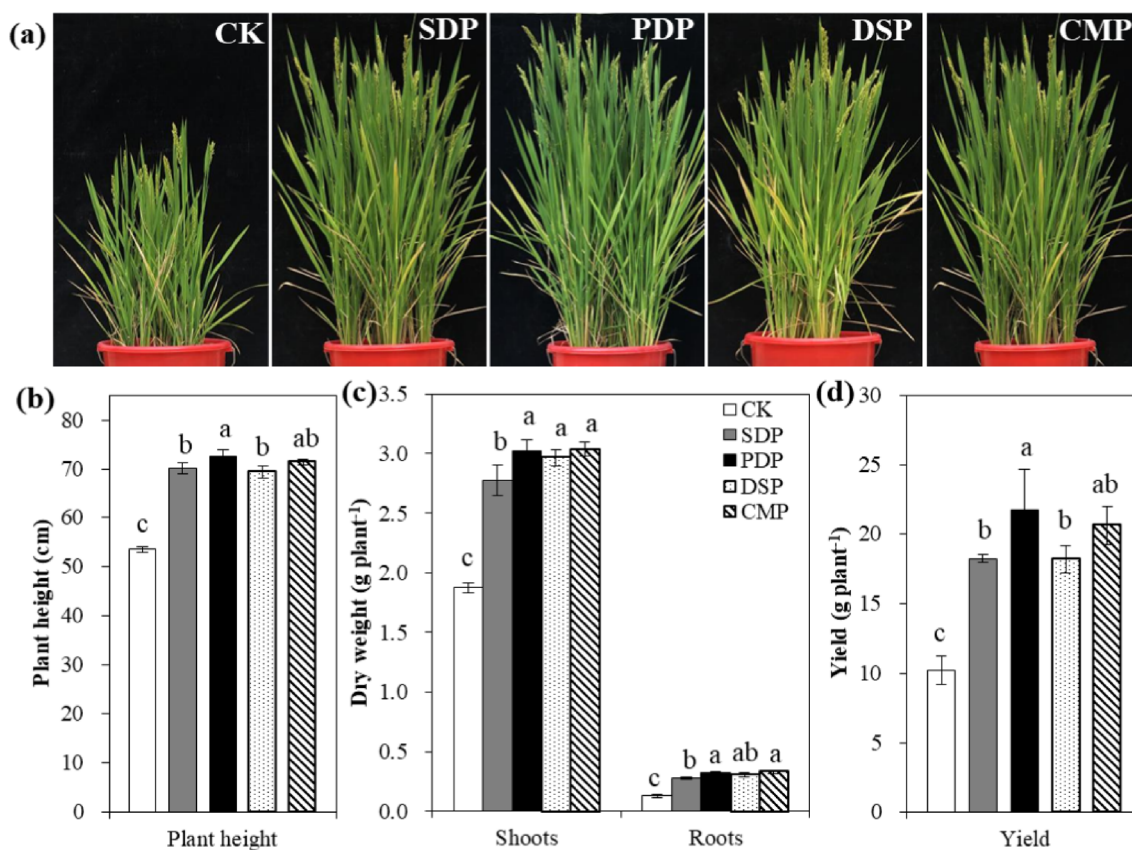
Furthermore, the dry weight of the shoots and roots significantly increased under the treatment of both PDP and CMP. Notably, the application of these four phosphate fertilizers led to a significant increase in dry matter accumulation and overall plant growth, ultimately resulting in a higher yield of grain. The maximum yield was achieved under PDP and CMP treatments, specifically 21.79 g per plant and 20.64 g per plant, respectively (Fig. 1a–d). In addition, after rice harvest, compared with CK, soil available Cd concentrations under each phosphate fertilizer treatment showed a decreasing trend, and CMP treatment reached a significant level (Table S2).

### Cd concentration in in different parts of rice plant

The Cd concentration in rice plants is the highest in roots, followed by leaf sheaths, stems, and leaves, and lowest in four grain parts (Fig. 2). Overall, different phosphate fertilizer treatments efficiently lower the Cd concentration in vegetative organs, and the largest reduction was observed in CMP treatment. It is worth noting that the aleurone layer of grains exhibited the highest Cd concentration, while the milled rice had the lowest. All phosphate fertilizer treatments, whether applied to the soil or foliage, significantly reduced the concentration of Cd in both brown and milled rice, and the lowest reduction was noted under CMP treatment. Additionally, SDP and CMP treatments had a substantial effect on reducing the concentration of Cd in the rice aleurone layer, with the lowest values being  $0.638 \text{ mg kg}^{-1}$  and  $0.671 \text{ mg kg}^{-1}$ , respectively (Fig. 2). Furthermore, the four phosphate fertilizer treatments were observed to significantly decrease daily metal intake (DIM) and health risk index (HRI) when compared to CK. Each of the HRI indicators has decreased below 1, with CMP exhibiting the lowest HRI of 0.214, indicating an 80.77% decrease in comparison to CK (Table S4).

### Translocation factor of Cd between different organs

The translocation factor (Tf) values of Cd between various plant organs are presented Table S3. Among them, the values of  $\text{Tf}_{\text{soil} \rightarrow \text{roots}}$  was the highest, followed by the  $\text{Tf}_{\text{roots-stems, -sheaths, -leaves, -glumes}}$  and the lowest was the  $\text{Tf}_{\text{stems, sheaths, leaves, glumes-grains}}$ . Phosphate fertilizer treatments significantly affected  $\text{Tf}_{\text{soil} \rightarrow \text{roots}}$ , with CMP treatment causing a decline of 60.38%. However, PDP, DSP, and CMP significantly increased the values of  $\text{Tf}_{\text{roots-stems, -sheaths, -leaves}}$ . Furthermore, all treatments markedly reduced the Cd remobilization and translocation in the four values of  $\text{Tf}_{\text{aboveground-grains}}$  and  $\text{Tf}_{\text{roots-glumes}}$  (Table S3).



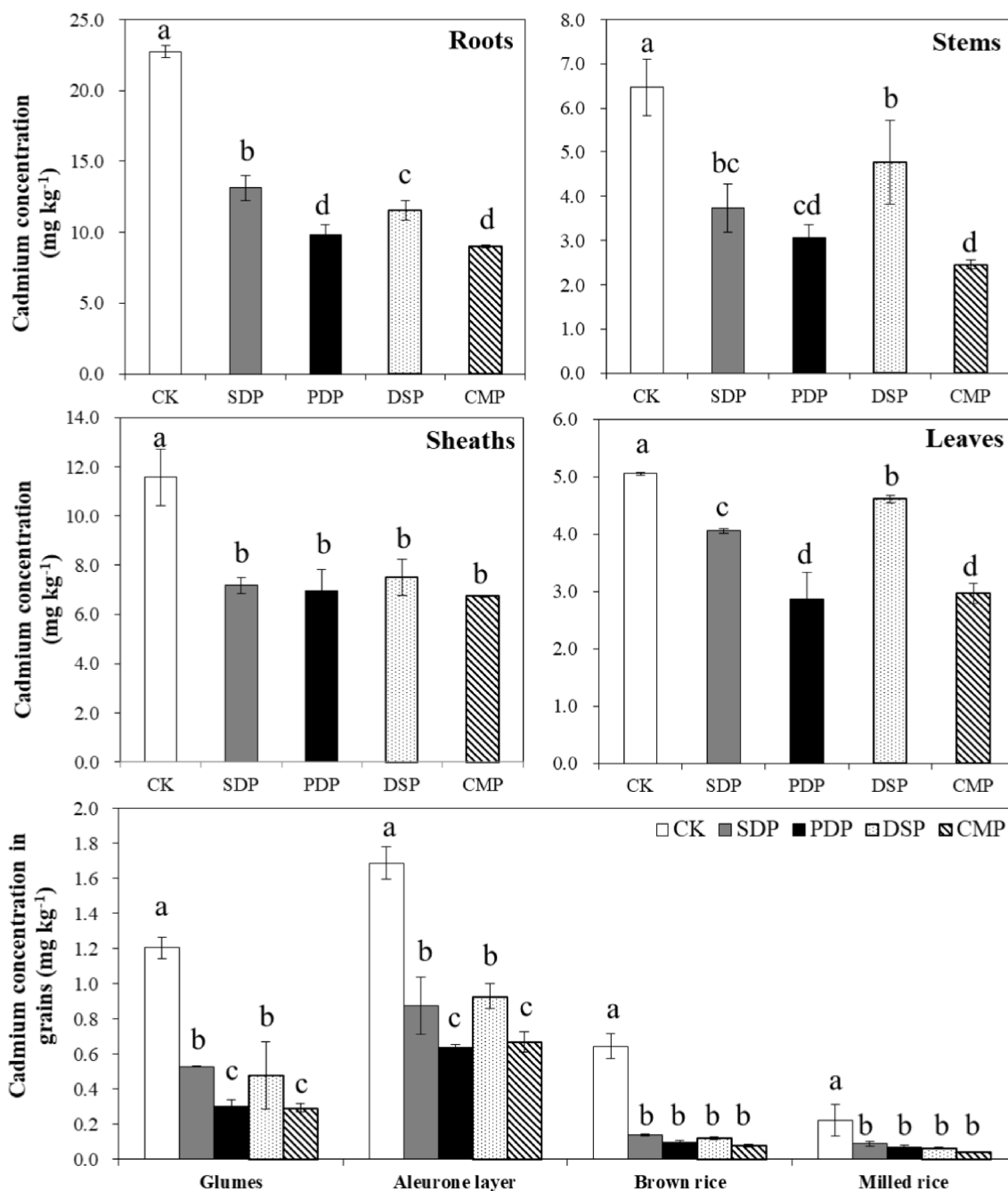
**Fig. 1** Effects of phosphate fertilizer application on plant growth (a), plant height (b), plant dry weight (c), and yield (d) of rice plants. CK, no phosphate fertilizer was applied, only foliar deionized water spray; SDP, sprayed  $\text{NaH}_2\text{PO}_4$  ( $50 \text{ mmol L}^{-1}$ ); PDP, sprayed  $\text{KH}_2\text{PO}_4$  ( $50 \text{ mmol L}^{-1}$ ); DSP, added double superphosphate ( $0.1 \text{ g kg}^{-1}$ ,

$\text{P}_2\text{O}_5/\text{soil w/w}$ ) into soil; CMP, added calcium magnesium phosphate ( $0.1 \text{ g kg}^{-1}$ ,  $\text{P}_2\text{O}_5/\text{soil w/w}$ ) into soil. Data are expressed as the average  $\pm$  SD ( $n=3$ ). Different lowercase letters above each bar indicate significant differences among different treatments (Duncan's multiple range test,  $P < 0.05$ )

### Subcellular distribution of Cd, PCs, and GSH content, MDA, and SOD accumulation in shoots and roots

Figure 3 shows the subcellular distribution of Cd in rice shoots and roots. The order of subcellular distribution of Cd was highest in the cell wall, followed by soluble fraction, and lowest in cell organelles (Fig. 3a). As compared with the CK, the four phosphate fertilizer treatments significantly increased the distribution of Cd in the cell wall of rice shoots and roots, while decreased the accumulation of Cd in soluble fraction. For instance, the treatment with CMP led to a maximum Cd proportion in the cell wall of roots, recorded as 74.89%, while minimizing its soluble fraction to 15.48%. Furthermore, rice shoot cell organelles experienced a significant increase in Cd proportion under PDP and CMP treatments, reaching 18.51% and 17.80%, respectively. However, the distribution of Cd in root cell organelles demonstrates a significant downward trend under the various treatments, particularly in the case of CMP treatment, where it dropped to 9.63% (Fig. 3a).

In current study, the content of GSH was higher than that of PCs in shoots, but markedly lower than that of PCs in roots. The accumulation of both PCs and GSH in paddy shoots reached its peak following the PDP treatment. Moreover, the four phosphate fertilizers considerably increased the synthesis of PCs and GSH in roots, with CMP displaying a stronger influence. Furthermore, CMP markedly increased PCs content by 32.96% and GSH by 120.47% in roots. Furthermore, there was a higher increase in accumulation of these two substances in rice shoots when treated by two foliar fertilizers as compared to two solid fertilizers. However, the trend was opposite in the roots (Fig. 3b). The content of MDA in shoots and roots decreased significantly with each treatment. Conversely, SOD activity exhibited a reduction in shoots and a considerable increase in roots, and was notably higher in roots than in shoots (Fig. 3c).



**Fig. 2** The Cd concentration in different organs of rice plants at maturity under four phosphate fertilizer application. Data are expressed as the average ±SD (n=3). Different lowercase letters above each bar

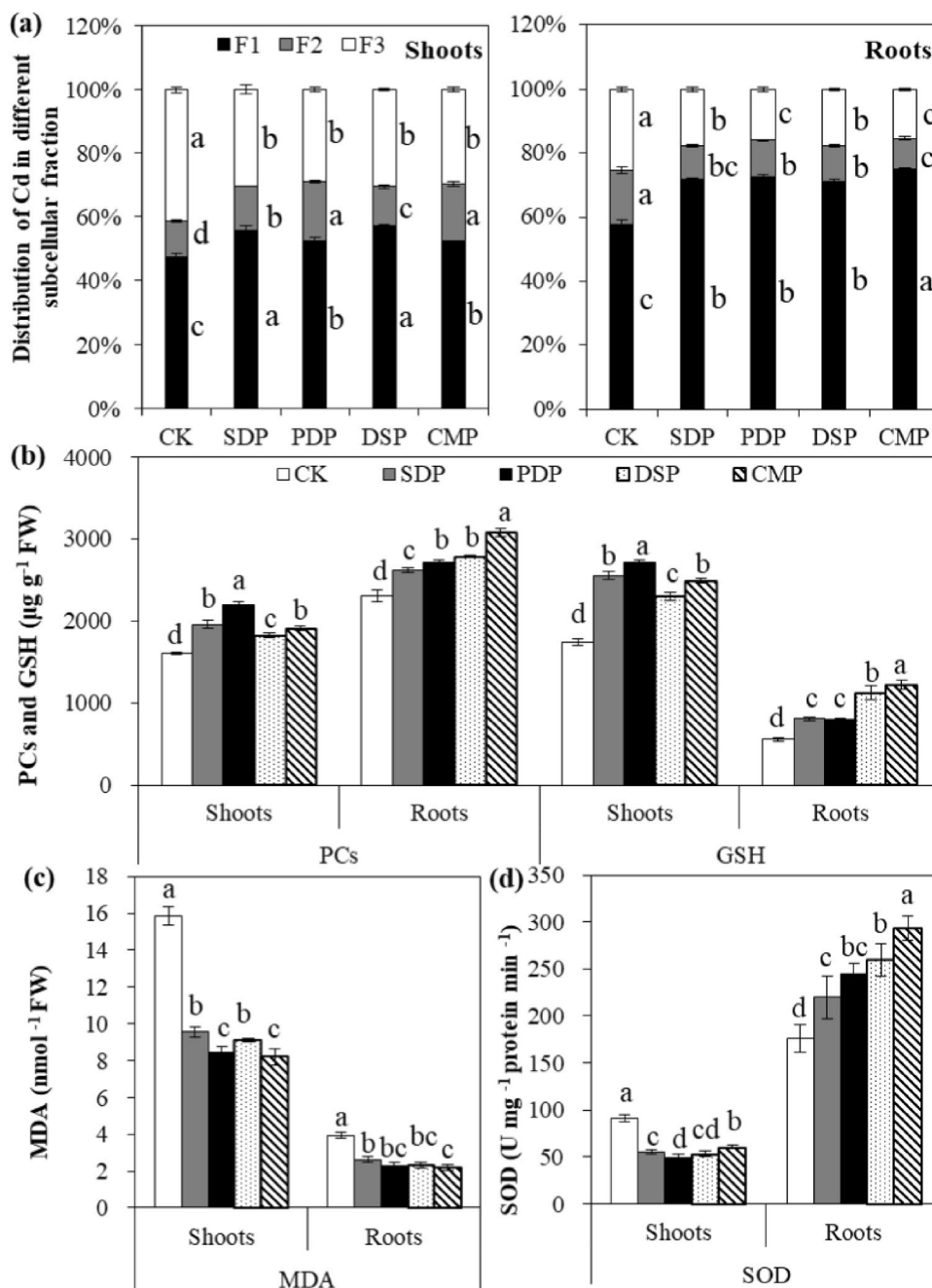
indicate significant differences among different treatments (Duncan’s multiple range test, P < 0.05)

**Gene expression quantification**

The relative expressions of *OsLCT1*, *OsPCSI*, *OsLCD*, and *OsNRAMP5* involved in Cd metabolism were analyzed to comprehend the molecular and physiological mechanisms of P in alleviating Cd stress in paddy plants (Fig. 4). Compared with the control, the four phosphate fertilizers significantly down-regulated the *OsLCT1* expression level

in stems and *OsLCD* expression in leaves, but markedly up-regulated the *OsPCSI* expression level in the three organs (Fig. 4a–d, f). Furthermore, under Cd stress, the presence of phosphate fertilizer resulted in a significant reduction of the expression of *OsNRAMP5* in the roots of rice. Of these, the greatest effects were observed with DSP and CMP, which resulted in a decrease of 45.41% and 34.86%, respectively (Fig. 4e).

**Fig. 3** The subcellular distribution of Cd (a), PCs and GSH content (b), and MDA and SOD accumulation (c) in shoots and roots at early filling stage under four phosphate fertilizer application. Cell wall (F1), organelle (F2), and soluble (F3) fractions. Data are expressed as the average  $\pm$  SD ( $n=3$ ). Different lowercase letters above each bar indicate significant differences among different treatments (Duncan's multiple range test,  $P < 0.05$ )

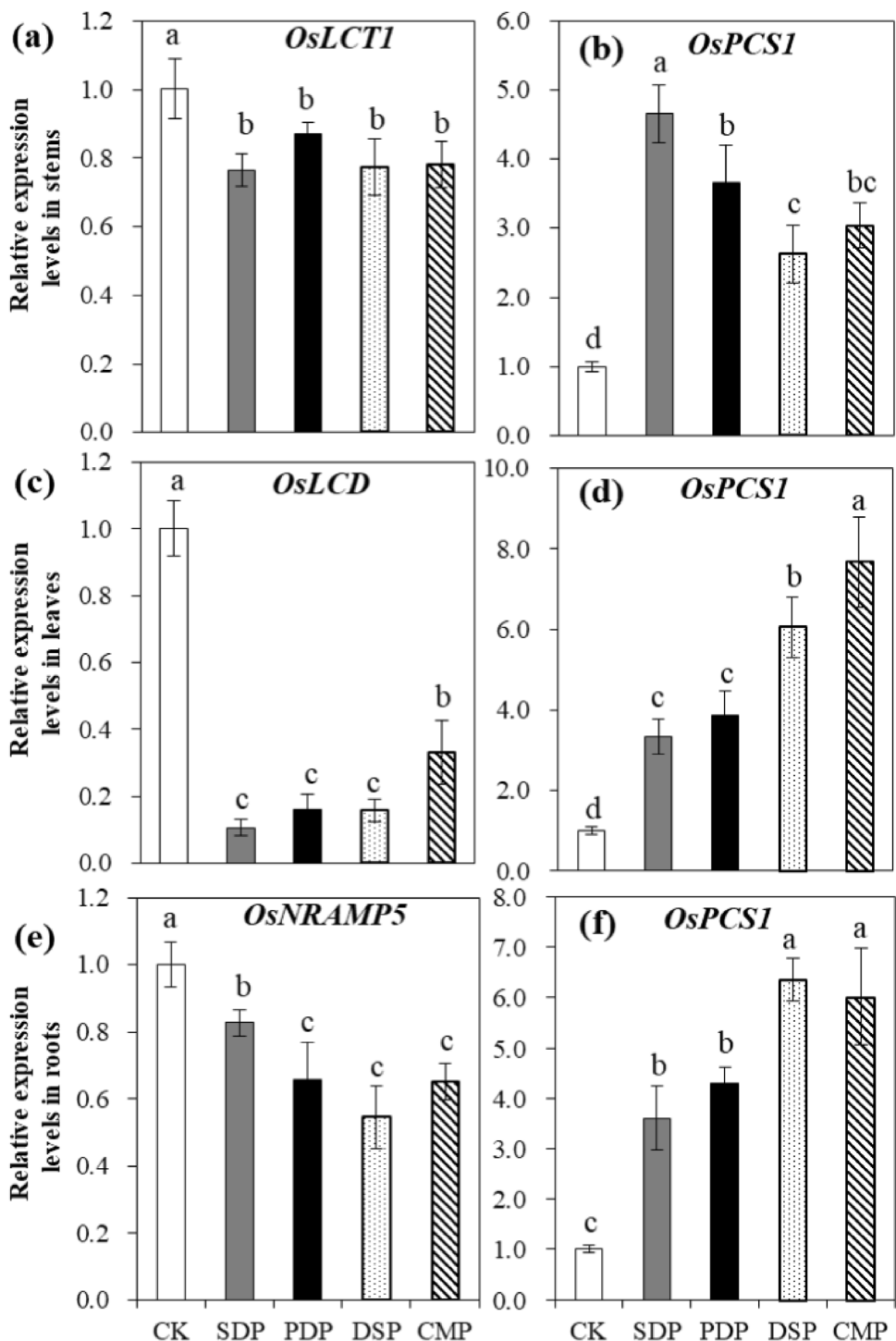


### Starch and protein component content in paddy grains and the Cd concentrations in four protein components

Starch and protein are two important storage components in rice grains. Different treatments did not have a significant impact on the total starch and amylose levels in the grains. However, the use of phosphate treatments significantly heightened the amylopectin levels in contrast to CK (Fig. 5a). The grain albumin content increased significantly under SDP treatment, but decreased markedly under CMP

treatment. The four phosphate fertilizer treatments seemed to have little influence on the globulin and prolamin content. Furthermore, these treatments significantly increased glutelin content, leading to a significant raise in the content of total protein, and reaching a higher level under DSP and CMP treatments (Fig. 5b). Furthermore, the concentrations of Cd in the four rice protein components are ranked from highest to lowest as globulin, albumin, glutelin, and gliadin. This suggests that Cd potentially binds with the globulin and albumin components to a greater extent. Notably, the use of phosphate fertilizers significantly reduced the amount

**Fig. 4** The gene relative expression of *OsLCT1* (a), *OsPCS1* (b, d, f), *OsLCD* (c), and *OsNRAMP5* (e) of paddy plants at early flowering period (September 7, 2021) under four phosphate fertilizer applications. Data are expressed as the average  $\pm$  SD ( $n=3$ ). Different lowercase letters above each bar indicate significant differences among different treatments (Duncan’s multiple range test,  $P<0.05$ )



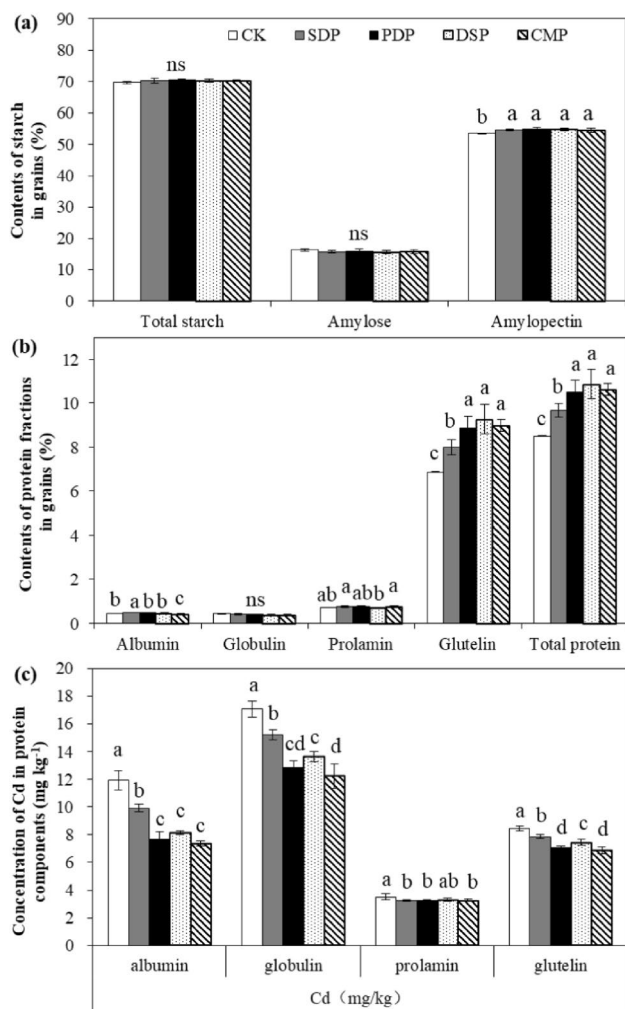
of Cd in all four protein fractions, particularly in globulin, albumin, and glutelin (Fig. 5c).

**Concentration of heavy metals and essential elements in grains**

Compared with CK, four phosphate fertilizers enhanced the Cr accumulation in grains, but none of them reached a

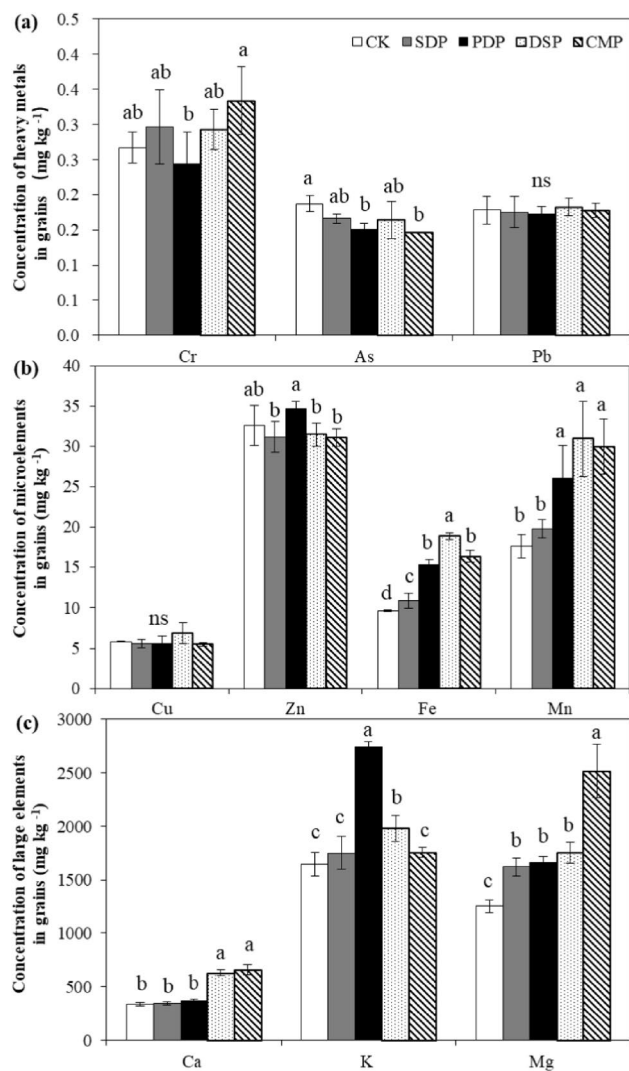
significant level (Fig. 6). And these treatments decreased the concentration of As in grain, in which PDP and CMP reached a significant level. Moreover, the Pb concentration in rice grain was not apparently affected by these treatments (Fig. 6a). In terms of microelements, different treatments mainly had significant effects on Zn, Fe, and Mn. Compared with the other three phosphate fertilizer





**Fig. 5** The starch (a) and protein components (b) contents and the Cd concentrations in four protein components (c) of grains under four phosphate fertilizer applications. Data are expressed as the average  $\pm$  SD ( $n=3$ ). Different lowercase letters above each bar indicate significant differences among different treatments (Duncan's multiple range test,  $P < 0.05$ )

treatments, PDP significantly increased the grain Zn concentration, but there no remarkable difference between PDP and CK. Both Fe and Mn exhibited the same behavior, demonstrating a significant upward trend under various phosphate fertilization procedures, with the increase in solid phosphate fertilizer being greater than that of foliar spraying (Fig. 6b). When it comes to the macro-elements, Ca was significantly improved mainly by the two solid phosphate fertilizer treatments (DSP and CMP). Under the PDP treatment, the grain K concentration was significantly increased to the highest, reaching 2738 mg kg<sup>-1</sup>. And these four phosphate fertilizer treatments markedly promoted the increase of grain Mg concentration, and the CMP treatment had the greatest effect (Fig. 6c).



**Fig. 6** The concentration of heavy metals (a), micro (b), and macro essential (c) elements in rice grains under four phosphate fertilizer applications. Data are expressed as the average  $\pm$  SD ( $n=3$ ). Different lowercase letters above each bar indicate significant differences among different treatments (Duncan's multiple range test,  $P < 0.05$ )

### Correlation matrix and principal component analysis (PCA)

The principal component analysis (PCA) results showed that the first two principal components explained 91% of the data variation. According to the projection of  $\cos^2$  on variables, PC1 is explained by the shoot *OsPCS1* expression, shoot PCs and GSH content, and subcellular distribution of Cd in shoots cell wall (F1) and organelles (F2). PC2 is mainly explained by indicators such as prolamins-Cd content, leaves *OsLCD* expression, and roots SOD activity. The GSH and PC content, root *OsPCS1* expression, SOD activity, and the shoot *OsPCS1* expression have a promoting effect on the formation of PC3 (Fig. S1).

Correlation analysis shows that the concentration of Cd in brown rice and milled rice is mainly positively correlated with the subcellular distribution of Cd in soluble components, MDA content, SOD activity, albumin-Cd, prolamin-Cd, and the expression of *OsLCD* in leaves. There was a significant negative correlation with the subcellular distribution of Cd in the cell wall components of root, GSH content, SOD activity in the stem, and the expression of *OSPCSI* in the roots and leaves (Fig. S2).

## Discussion

### Effects of phosphate fertilizer application on plant height, dry weight, and yield parameters of rice

The application of chemical fertilizers is crucial for enhancing and stabilizing rice yield. NPK, which comprises three essential nutritive elements, is particularly significant in the growth and development of rice (Phares and Akaba 2022). This study showed that four kinds of phosphate fertilizer treatments all promoted the increase of rice plant height and dry matter under Cd stress, thus improving rice yield (Fig. 1). Plant performance and yield are better under PDP and CMP compared to SDP and DSP, indicating that K, Ca, and Mg elements in these fertilizers may provide superior nutrition for rice plants under Cd stress. In addition, in current study, the available phosphorus content (Olsen-P) in text soil is 13.13 mg kg<sup>-1</sup>, which belongs to the middle low level. Under this soil condition, the application of phosphate fertilizer can help to raise the yield of rice and other crops (Gong et al. 2022). Therefore, when managing varied Cd contaminated soils, adding suitable macronutrients or micronutrients according to soil characteristics can produce dual benefits of decreasing Cd stress and enhancing yield.

### Effects of phosphate fertilizer application on remobilization and concentration of Cd in rice vegetative organs

Root uptake and redistribution of vegetative organs post-anthesis is essential for the accumulation of Cd in grain (Huang et al. 2017). Firstly, in current study, Cd is mainly stored in the cell wall fraction in shoots and roots (Fig. 3a), which has been consistent with numerous existing research findings (Zhong et al. 2022). In contrast to CK, the other treatments promoted the proportion of Cd in cell wall of shoots and roots, and decreased the distribution of Cd in soluble fraction, indicating that these applications promoted Cd binding to the cell wall under Cd stress (Fig. 3a). Polysaccharides and proteins play a role in Cd fixation, either by uptake or precipitation through their active groups, thus decreasing Cd transportation to the grain (Wei et al.

2023). In addition, *OsLCD* and *OsLCT1* were specifically expressed in vascular tissues of rice leaves and stem nodes, and were involved in Cd accumulation in rice (Shimo et al. 2011; Tian et al. 2019). The results of this study showed that the application of phosphate fertilizer significantly down-regulated the expressions of these two genes in leaves and stems, leading to a reduction of Cd redistribution to paddy grains (Fig. 4).

Secondly, synthesizing sulfhydryl compounds is of vital importance for plants to cope with heavy metal stress. PCs are a type of metal sulfhydryl protein with a unique structure that is synthesized based on GSH. PCs aid in the sequestration of heavy metals in cellular vacuoles, thereby reducing toxicity (Huang et al. 2021). In these physiological processes, *OsPCs* (including *OsPCSI* and *OsPCS2*) have essential regulatory roles (Das et al. 2017; Huang et al. 2021). Our experiment showed that application of phosphate fertilizer up-regulated the *OsPCSI* genes in rice plants and promoted the PCs and GSH synthesis and accumulation in shoots and roots (Fig. 3b), which is similar to the research results of Pál et al. (2017). Furthermore, this research showed that GSH content was higher than PCs in shoots and lower in roots (Fig. 3b), suggesting that these two substances may exhibit tissue specificity in heavy metal detoxification (Wang et al. 2021b).

Finally, the subcellular distribution and physiological characteristics of Cd in vegetative organs of rice plants affect the accumulation of Cd in these organs. In this study, it was found that the concentration of Cd in roots was significantly higher than that in shoots at maturity, which was in agreement with the results of previous studies (Huang et al. 2021). In addition, the phosphate fertilizer treatments significantly decreased the concentration,  $Tf_{\text{soil-roots}}$ , and  $Tf_{\text{aboveground-grains}}$  of Cd in different vegetative organs, among which CMP treatment decreased the most (Fig. 2; Table S3). It is widely recognized that Cd competes with Ca, Zn, and other transporters in rice, resulting in an antagonistic effect with these cations (Zhen et al. 2021). In this study, relative gene expression analysis showed that the gene *OsNRAMP5* involved in Cd uptake in roots was significantly down-regulated under each treatment, especially in CMP treatment. These findings suggest that the use of phosphate fertilizer primarily decreases Cd absorption in rice roots, thereby affecting transportation and remobilization to grains.

### Effects of phosphate fertilizer application on the concentration of Cd and other elements in rice grains

This study showed that the Cd concentration in aleurone layer, brown rice, and milled rice decreased significantly to a safe level under various phosphate fertilizer treatments, which may be the common result of the above absorption and remobilization. Phosphorus fertilizer treatment resulted in a significant reduction

of the daily intake of metal (DIM) and health risk index (HRI) in milled rice. The application of these treatments resulted in a reduction in HRI indicators below 1, effectively mitigating the toxic hazards to human health caused by Cd (Table S4).

For other heavy metal elements, this study found that except PDP, the other three phosphate fertilizers increased the Cr concentration in grain, but the difference was not significant and far below the safe value ( $1 \text{ mg kg}^{-1}$ ). In addition, each phosphate fertilizer treatment reduced the concentration of As, but had no significant effect on Pb (Fig. 6a). Note that P and As belong to group VA in the Periodic Table. Foliar phosphate fertilizer can hinder arsenic uptake in rice through competition among transporters, leading to diminished root-stem transport and stem-grain remobilization, ultimately reducing arsenic levels in rice grains (Jiang et al. 2014). Xie et al. (2018) showed that the application of Pb, Cd, Cr, and Cu significantly affected the accumulation of Cd, Cr, and Cu in grains, indicating that there was a synergistic or antagonistic mechanism among heavy metal elements. Therefore, it is necessary to deeply study the absorption, transportation, and remobilization of heavy metals in rice plants.

For macronutrient elements, in this study, two kinds of solid phosphate fertilizers (DSP and CMP) significantly increased grain Ca concentration. And the PDP and CPM treatments markedly enhanced the concentration of K and Mg in grains (Fig. 6c). Prior research has indicated that CMP fertilizers promoted the absorption of Ca and Mg in root systems, resulting in an increase of their contents in paddy soil. The summarized results indicate that applying phosphate fertilizer might enhance the absorption of other vital elements due to their varied abundance (Wang et al. 2021a). In addition, for micronutrient elements, different phosphate fertilizer treatments had no effect on Cu, but significantly increased the concentrations of Fe and Mn in grains. Compared to the CK control group, the PDP treatment resulted in an increase in grain Zn concentration, while the other three phosphate treatments did not significantly contribute to grain Zn accumulation (Fig. 6b). At present, reports on the effects of phosphate fertilizer on microelements concentrations in crops are not consistent, particularly with regard to Zn in grains. Most studies have shown that exogenous phosphorus supply reduced Zn uptake by roots and inhibited Zn transport into paddy grains (Zhang et al. 2017; Su et al. 2018). However, some researchers found that phosphorus had a slight synergistic effect on Zn concentration in grains (Naeem et al. 2018). The above differences may be caused by changes in the texture or physical and chemical properties of text soil (Hagh et al. 2016; Zhang et al. 2021). In addition, phosphorus exists mainly in the form of phytic acid in rice grains, and phytic acid can chelate with cations such as Zn, affecting human nutrition absorption (Yamaji et al. 2017). Thence, the effect of P on Zn was shown in soil availability,

root absorption, and grain chelation. Consequently, further research is necessary to understand the P–Zn interaction mechanism in rice, taking into account the determination of different soil phosphorus levels.

### **Effects of phosphate fertilizer application on the starch and protein component contents and the Cd concentrations in four protein components**

Starch makes up around 90% of rice's dry weight and is a vital biochemical component for determining rice quality (Amagliani et al. 2016). Consumers tend to prefer medium- to low-amylose rice, which is softer and bonds more easily due to its lower amylose content. Amylopectin found in rice can increase its stickiness and sweetness, thus improving its palatability (Amagliani et al. 2016; Zhang et al. 2018). Previous research has indicated a close correlation between mineral elements and starch quality, particularly in the context of managing nitrogen, phosphorus, and potassium fertilizers (Zhang et al. 2018). The results of this study showed that four kinds of phosphate fertilizer treatments decreased the amylose content of grains, but significantly enhanced the amylopectin content (Fig. 5a). Like starch, the storage protein in rice endosperm is additionally a vital indicator to evaluate the nutrition and taste quality of rice (Baxter et al. 2004). Research shows that most Cd binds to protein in grain with sulfamino acids playing a key role in this process (Wei et al. 2017). Recent studies revealed a significant increase in glutelin content under various treatments, resulting in a higher level of overall protein (Fig. 5b). Glutelin is rich in amino acids, which have a vital catalytic role in improving the nutritional quality of rice (Teixeira et al. 2021). Coordinating the accumulation of amylopectin and glutelin in paddy grains, the application of P fertilizer could potentially mitigate the decline in rice quality under Cd stress.

Moreover, in this research, the Cd concentrations in protein components of globulin, albumin, and glutelin are higher than in prolamin (Fig. 5c). However, consistent with the aleurone layer, albumin and globulin were largely stored in the outer layer of unpolished rice and were mostly grinded off in the milling process (Dos Reis et al. 2020). Accordingly, this indicates that Cd is likely to be closely associated with these two protein fractions in the aleurone layer (Wei et al. 2017). The study demonstrated that the use of phosphate fertilizer significantly reduced Cd concentration in four protein fractions, particularly in globulin, albumin, and glutelin. Similarly to grains, Cd primarily binds with proteins and thiol compounds in the phloem of crop plant leaves and stems' vegetative organs (Gu et al. 2023). Hence, further research is required to investigate the physiological process of Cd binding and remobilization characteristics in vegetative organs' impact on grain Cd accumulation.

## Effect mechanism of foliar and soil phosphate fertilizer on Cd accumulation in rice plants

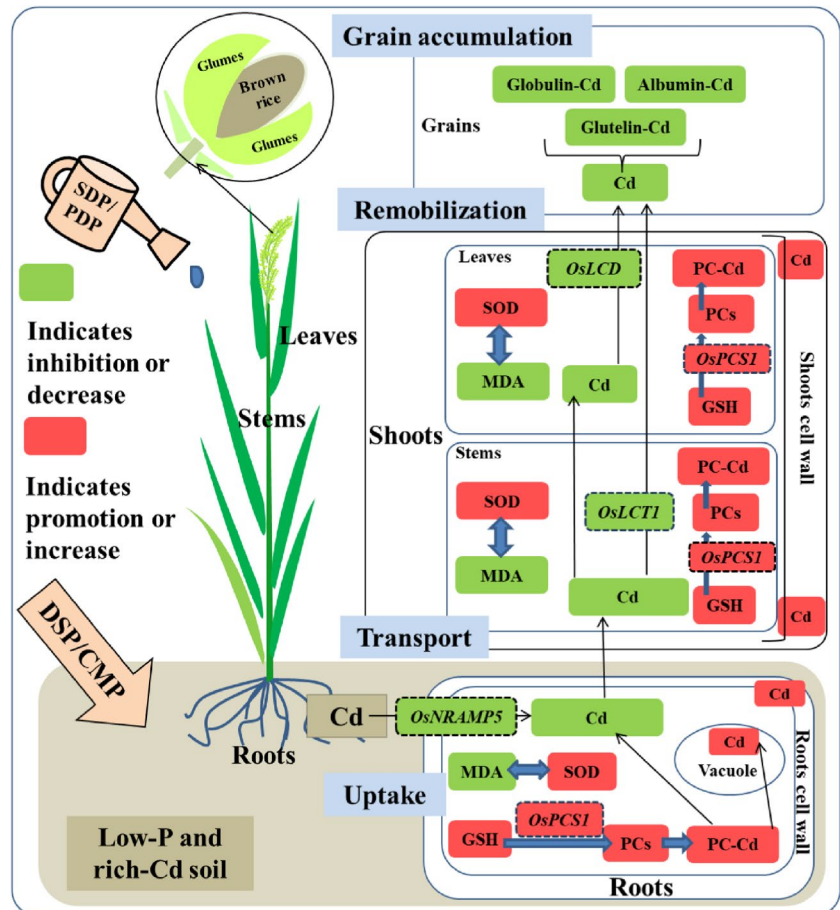
In this study, four types of foliar and solid phosphate fertilizers were used to study the effect of phosphate fertilizer on Cd accumulation in rice plants under the background of low-P and rich-Cd soil. The effects of these two phosphate fertilizers on the Cd accumulation model in rice may exhibit dissimilar mechanisms. Foliar phosphate fertilizer significantly augmented the accumulation of PCs and GSH in rice shoots, in addition to substantially enhancing the expression of *OsPCSI* in shoots when compared to solid phosphate fertilizer (Figs. 3 and 4). These results suggest that foliar phosphate fertilizer may alleviate the effect of Cd stress by inhibiting root uptake and reducing retransfer from shoots to grains. For the solid phosphate fertilizer treatment, there was a substantial increase in root PC and GSH contents, along with a significant reduction in *OsNRAMP5* expression in roots (Figs. 3 and 4). It is suggested that solid phosphate fertilizer may prevent the transport of Cd to aboveground mainly by inhibiting absorption and increasing root fixation (Figs. 7, S1, and S2). In addition, the amount of phosphate fertilizer used in this study is converted according to the conventional amount of local farmers ( $P_2O_5$ , 150 kg  $hm^{-2}$ ).

Therefore, solid phosphate fertilizer is applied to the soil as a base fertilizer at one time. In addition, the yield of PDP and CMP was better among the four kinds of phosphate fertilizers. Hence, in cases where the soil background is similar, CMP can be used preferentially as a base fertilizer, while PDP can be used as a quick and convenient remedy.

## Conclusions

This study clarified the response to different kinds of phosphate fertilizer (foliar fertilizers of SDP and PDP, solid application of DSP and CMP) on the root absorption, vegetative organ redistribution, and grain storage of Cd in paddy plants under low-P and rich-Cd soil, and revealed the effect of P on the chemical components of starch and protein in rice grains. First of all, phosphate fertilizer treatment decreased the absorption of Cd by down-regulating the expression level of *OsNRAMP5*, and promoted the synthesis of chelate PC-Cd and the regionalization of Cd to vacuole and cell wall in roots. Second, phosphate fertilizer treatments increased the distribution Cd in the cell wall of shoots, thus reducing the remobilization of Cd from source organs to grains. Finally, the application of the four

**Fig. 7** Schematic presentations of the effects of different phosphate fertilizers on the absorption, transport, redistribution, and grain storage of Cd in paddy plants



phosphate fertilizer resulted in a significant reduction of Cd accumulation in the globulin, albumin, and glutelin protein components, thus contributing greatly to the decrease of Cd concentration in the grain to a safe level. The study demonstrates that foliar or soil application of phosphorus effectively regulates the metabolic processes of Cd absorption, remobilization, and grain accumulation in rice, which plays a vital role in mitigating heavy metal stress and enhancing nutritional quality (Figs. 7, S1, and S2). Furthermore, under the background of low-P and rich-Cd soil, CMP can be used preferentially as a base fertilizer, while PDP can be used as a quick and convenient remedy. Thence, appropriate micronutrients or micronutrients can be added according to different soil backgrounds to achieve the dual effects of alleviating Cd stress and increasing yield.

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**Data availability** All data generated or analyzed during this study are included in this published article.

## Declarations

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