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A novel family of PLAC8 motif-containing/PCR genes mediates Cd tolerance and Cd accumulation in rice

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Abstract

Background: Cd is one of the highly toxic heavy metals to most organisms, including humans and plants, and Cd-contaminated rice from China has become a global food safety issue. The early prediction of OsPCR (the plant cadmium resistance protein) which contained a PLAC8 domain was related with the accumulation of Cd in rice. To further understand the biological function of the *OsPCR* genes on the Cd tolerance and Cd accumulation in rice, we used a low grain-Cd-accumulating rice (xiushui 11) and a high grain-Cd-accumulating rice (xiushui 110) varieties to analyze the relationship between the expression levels of the two most abundant expression genes (*OsPCR1* and *OsPCR3*) and the Cd concentrations in different tissues at different growth periods during Cd stress, and transgenic experiments of *OsPCR1* and *OsPCR3* were carried out.

Results: *OsPCR1* and *OsPCR3* were closely related with Cd accumulation. Overexpression of *OsPCR1* and *OsPCR3* could not only increase the Cd tolerance, but also decrease the Cd accumulation obviously in different parts of the transgenic rice plants (especially in the rice grains), while the RNAi expression plants showed the opposite results.

Conclusions: These results indicate that *OsPCR1* and *OsPCR3* play critical roles in Cd accumulation in rice, which provides a theoretical basis for the safe production of rice.

Keywords: Rice, *OsPCR1*, *OsPCR3*, Transgenic rice plants, Cd accumulation

Background

Heavy metals have a serious impact if released into the environment even in trace quantities which can enter into the food chain from aquatic and agricultural ecosystems and threaten human health indirectly [1]. Cd is one of the most toxic heavy metals in the soil; it has strong chemical activity and can be absorbed by plants easily [2, 3]. Rice is one of the most important food crops in the world. The problem of Cd pollution has been highly valued by the government departments of all countries. Therefore, how to reduce the Cd content in rice grain and clarify its accumulation rule to realize the production of low FAO/WHO and the national standard of rice grain in the heavy metal contaminated soil is of great significance.

Many studies have been carried out on the molecular mechanisms of Cd accumulation in rice, and several genes involved in Cd translocation and accumulation have been identified [4, 5]; for example, phytochelatin synthase genes (*OsPCS1* and *OsPCS2*) [6], the natural resistance-associated macrophage protein (Nramp) family genes (*OsNRAMP1* and *OsNRAMP5*) [7, 8], heavy metal ATPase gene (*OsHMA2)* [9], and low-affinity ion transporter gene (*OsLCT1*) [10], the Fe transporters (*OsIRT1* and *OsIRT2*) [11]. *OsLCD* gene was expressed in the phloem of vascular bundle and leaf in rice root which was involved in the Cd accumulation of rice [12]; *OsLCT1* protein was a membrane protein, involved in the transport process of Cd from the cell to the outside world [3]; Ueno reported that heavy metal ATP enzyme (*OsHMA3*)

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in rice could decrease Cd transport to the shoot [10, 13]; and OsCCX2 was reported as a node-expressed transporter participated in Cd accumulation in rice grain of rice [14]. But the uptake, translocation and accumulation of Cd in rice seedlings were still not clear, and the discovery of Cd-accumulation-related genes was still very poor.

The plant cadmium resistance protein (PCR) which belonged to a membrane protein family should contain CCXXXXCPC or CL/FXXXXCPC conserved amino acid sequences to be effective, and a PLAC8 domain was reported as associate with cadmium resistance in plants [15–18]. Many functions have been reported for PLAC8 domain-containing proteins of plants; *Arabidopsis thaliana* plant cadmium resistance 2 (AtPCR2) acts as a Zn efflux transporter and related with Zn resistance [19]. The similar proteins such as *Solanum lycopersium* (tomato) fruit weight 2.2 (fw2.2), *Zea mays* (maize) cell number regulator (ZmCNR1) and an animal protein (onzin) were also contain the PLAC8 domain, but they all played important roles in the control of cell growth [20–22], and only onzin was involved in the pathogen defense and autothagy [23, 24]. *Brassica juncea* plant cadmium resistance 1 protein (BjPCR1) facilitated the radial transport of calcium in the root and so on [25]. Most studies suggested that the CCXXXXCPC motif was likely to take part in the binding of divalent cations, then complete the transport of the divalent cations. In our previous research work, we predicted that *OsPCR1* (Loc_Os02g0578900) was important in Cd accumulation in rice [26]. And similar results were shown by Song et al. about the function of *OsPCR* genes in rice. Their recent reports suggested that *OsPCR* (Loc_Os10g02300) could influence Zn accumulation and grain weight in rice, and the *OsPCR*-knockdown rice seedlings showed lower Cd concentrations than the control rice grain which indicated that *OsPCR* played an important role in Cd accumulation in rice grain [27]. Recently, Xiong et al. [28] reported a *FW2.2*-like family gene in rice (*OsFWL4, Os03g614440*) which contained a PLAC8 that could not only regulate grain size and plant height, but also involved in Cd translocation form roots to shoots in rice.

Therefore, it was urgent to understand the information of the functional *OsPCRs* genes in rice, and clarify the expression patterns of *OsPCR* genes in different rice cultivars. This study selected the two most abundantly expressed genes (*OsPCR1* and *OsPCR3*(LOC_Os02g52550.1)) predicted by bioinformatics as the target genes. The relationship between the expression levels and the Cd concentrations in different tissues at different growth periods during Cd stress in xiushui110 (high-Cd in grains) and xiushui11 (low-Cd in grains) (*Oryza sativa* L.) was analyzed, and the transgenic rice plants of *OsPCR1* and *OsPCR3* genes response to Cd stress were studied, respectively. The results of this study will provide a reference to guide future experiments that focus on the function and mechanism of *OsPCRs* genes in the growth and development of rice, and provide a theoretical basis for the study on the molecular mechanisms of Cd tolerance and accumulation in rice.

Materials and methods

Plant materials and treatments

The pre-screened low grain-Cd-accumulating rice (xiushui 11) and high grain-Cd-accumulating rice (xiushui 110) seeds were used in these experiments. The rice seed germination and seedling cultivation with nutrient solutions were according to Wang et al. [29]. The uniform 5th-leaf stage rice seedlings were treated with 2 μM $CdCl_2$ (determination of Cd accumulation) or 10 μM $CdCl_2$ (determination of physiological indicators); then different rice samples (leaf, stem, root, flag leaf, and panicle) of the two rice cultivars were collected at different growth development for the further experiments.

Expression patterns of *OsPCR1* and *OsPCR3* in response to Cd stress

Different rice samples (leaf, stem, root, flag leaf, and panicle) at different growth development of rice seedlings were collected after Cd treatments. After RNA was extracted from different rice samples, cDNA was synthesized from total RNA using PrimerscriptT^{M} RT Reagent Kit (TaKaRa, Japan), then qRT-PCR was performed according to Wang et al. in Fluorescence quantitative PCR instrument (Roor-Gene Q, QIAGEN, Germany) with *OsPCR1*-specific primer sets (Table 1) [30]. qRT-PCR conditions were 45 cycles of 95 °C for 60 °s, 95 °C for 10 s, 55 °C for 15 s, 72 °C for 15 s. The expression level was normalized by that of actin.

Construction of transgenic rice plants

The fragment amplifications of *OsPCR* genes and their interference fragments used the specific primers in

Table 1 Primer sequences of *OsPCR1* for qRT-PCR

Primer name	Gene	Forward sequence (5′ → 3′)	Reverse sequence (5′ → 3′)
OsPCR1	Os02g0578900	GCCAAGATGCGGTCCCAGTA	GCGTGCCATCCGAGGTTCAT
OsPCR3	Os02g0763000	TTCCTCGTCCATTTCTGCTGC	CGCCTCTGCCTGTCCACATT

Table 2 PCR amplification of *OsPCR* gene and its interference fragment sequence primer information

Primer	Primer sequence	Tm	Sequence length (bp)
OsPCR1-F	5′-GGTACCATGTACTCGAAACCGGAG G-3′	55	489
OsPCR1-R	5′-GTCGACGCGTGTCATCCCGGGGA-3′		
OsPCR3-F	5′-GGTACCATGTATCCCTCCGCCCCT-3′	58	546
OsPCR3-R	5′-GTCGACCCTCATCATGCCGACCGG -3′		
OsPCR1RNAi-F	5′- CACCATGTACTCGAAACCGGA GGA-3′	59	140
OsPCR1RNAi-R	5′-CACAGGCACGTCACGCAACAGTT-3′		
OsPCR3RNAi-F	5′-CACCATGTATCCCTCCGCCCCTCC-3′	64	170
OsPCR3RNAi-R	5′-CGTCCATGCAGTGGAAAAGGCCGG TG-3′		

Table 2, and then they were cloned into overexpression vector (35S-1300-EGFP) and interference expression vector [pH7GWIWG2(I),O], respectively. Cultivars of xiushi 11 were used for this transformation, and the transformation and transgenic rice seedlings screening were followed by the method of Ding et al. [31]. Transgenic rice seedlings (Overexpression transgenic plants OE:OsPCR1 and OE:OsPCR3; Interference expression transgenic plants Ri:OsPCR1 and Ri:OsPCR3) were selected using 50 mg/L hygromycin and PCR analysis with genomic DNA from their leaves. All the putative T3 transgenic plants were used for the experiments.

Estimation of hydrogen peroxide and lipid peroxidation

The difference of peroxidation level induced by Cd stress between wild-type (WT) xiushi 11 and transgenic rice seedlings was estimated by the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). H_2O_2 and MDA determinations were carried out by the methods of Wang et al. [30] and Wang et al. [29], respectively.

Extraction and determination of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity

SOD activity, CAT activity and POD activity were assayed according to Ma et al. [32] and Wang et al. [33]. 0.3 g fresh rice leaves samples were ground with liquid nitrogen, and then added with 3 ml cold buffer (50 mM PBS pH 7.0, 3 mM DTT, 1 mM EDTA-Na_2, 5% PVP). The homogenate was centrifuged at 15,000*g* for 20 min, and the supernatant was used for analyzing the enzyme activities. SOD activity was measured by the inhibition of the photochemical reduction of β-nitroblue-tretrazolium chloride (BNT), CAT activity was analyzed with the rate of decrease in H_2O_2 absorbance at 240 nm, POG activity was assayed by the reaction of oxidation of guaiacol at 470 nm. All operations were performed at 4 °C.

Cd determination in rice samples

0.2 g rice samples were used in the digestion, and the specific steps refereed to Liu et al. [34]. Except brown rice was digested in a HNO_3/HF mixture (3:1) in a microwave digester (CEM-MARS: Boston, MA, USA); other rice samples were all digested with 6 ml HNO_3. Cd concentrations in different samples were detected by AA-7000 (SHIMADZU: Kyoto, Japan).

Statistical analysis

The Excel 2003 and SPSS (Product and Service Solutions Statistical, 18.0) were used to analyze the data, and the experiments in this manuscript were performed at least three repetitions and all the data reported in this paper were means of three replicates.

Results and analysis

Expression patterns of *OsPCR1* and *OsPCR3* in different Cd accumulating rice

Different distribution of Cd in xiushui 11 and xiushui 110

Low grain-Cd-accumulating rice (xiushui 11) and a high grain-Cd-accumulating rice (xiushui 110) seeds were used to analyze the Cd accumulation in different tissues at different growth periods of the two rice cultivars during Cd stress (Fig. 1). Here, we found that the Cd contents of the different tissues in xiushui 11 were all lower than that of xiushui 110, and the pattern of Cd accumulation both in xiushui 11 and xiushui 110 was root > stem > leaf (or flag leaf) > spike; while root and stem tissues showed the largest Cd accumulations. The accumulation of Cd in root in xiushui 11 and xiushui 110 was increased remarkably in tillering stage, and then tends to be stable. In the stem, the Cd accumulation was increased from seedling stage to tillering stage both in xiushui 11 and xiushui 110, then decreased till to the blooming stage in xiushui 11 and to the heading stage in xiushui 110, respectively, and finally both increased. In the leaf tissues, the tendency of Cd accumulation increased first and then decreased at the heading stage in the two rice cultivars, then increased at the later stages continuously, and the tillering stage was the key period of Cd accumulation. The Cd accumulation patterns in flag leaf and spike tissues were uniform in different stages. In general, the Cd was mainly accumulated in root, and the Cd contents in different parts of xiushui 11 were all lower than that of xiushui 110.

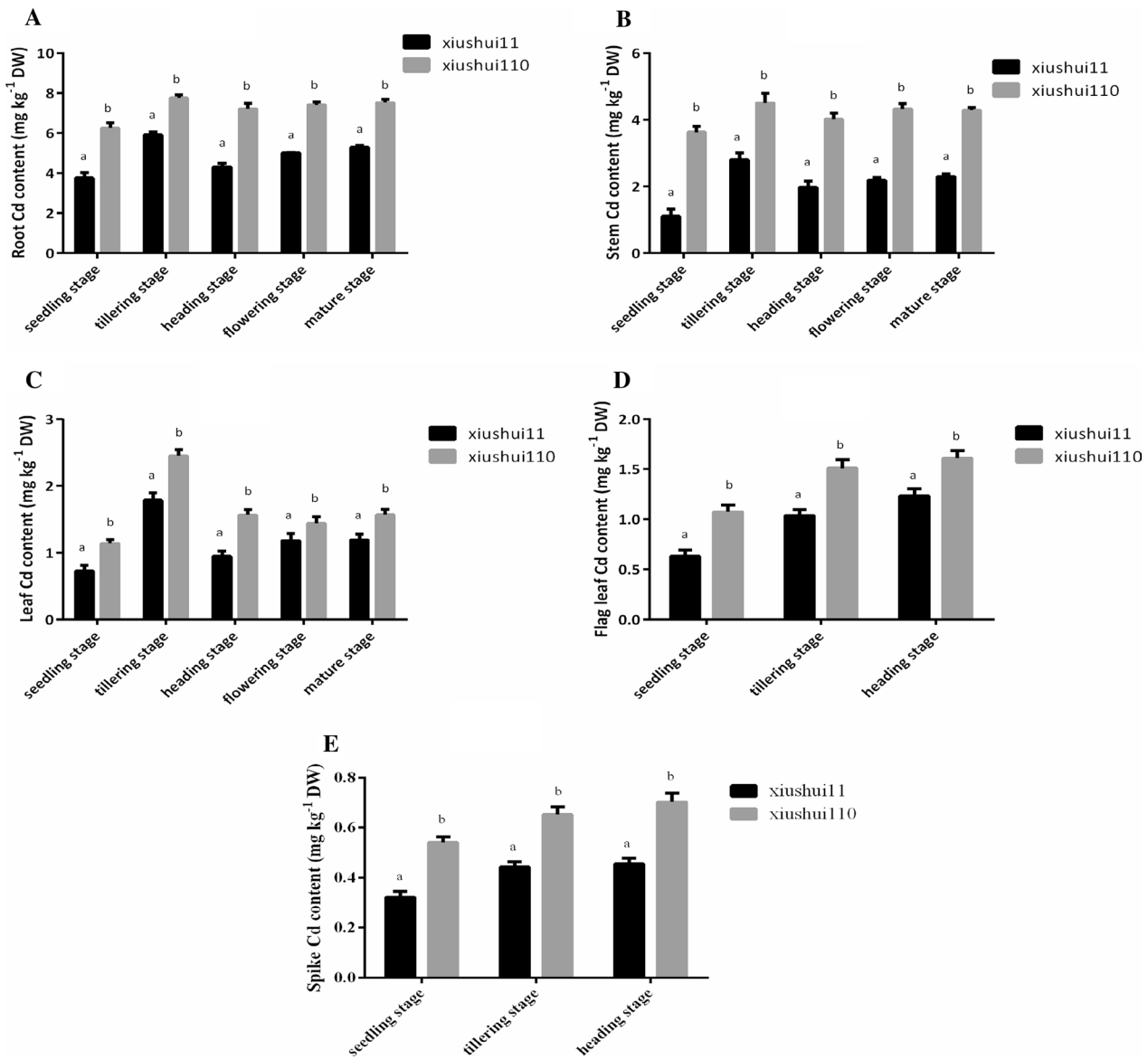

Fig. 1 Changes of Cd contents in different parts of the two rice varieties under Cd stress. **A** Root; **B** stem; **C** leaf; **D** flag leaf; **E** spike

Expression analysis of OsPCR1 and OsPCR3 in different tissues at different growth stages of rice with qRT-PCR

As indicated in Figs. 2 and 3, as well as *OsPCR1*, *OsPCR3* was inhibited in xiushui 11, while it was induced in xiushui 110 during Cd stress. The expression levels of the two genes in different tissues were almost higher in xiushui 11 than that of xiushui 110 in the contrast, but were notable lower in xiushui 11 than xiushui 110 during the Cd treatment in different parts at different growth stages. Furthermore, the expression levels of the two genes were lower in different parts at different growth stage in the presence of Cd than in the absence of Cd in xiushui 11, but were opposite in xiushui 110. Moreover, the expression levels of the two genes in xiushui 11 were all lower than that of xiushui 110 in different parts at different growth stages under Cd stress.

Effect of different expression levels of *OsPCR1* and *OsPCR3* on H_2O_2 accumulation, lipid peroxidation and antioxidant enzymes' activities

To explore possible mechanisms of the *OsPCR1* and *OsPCR3* in Cd tolerance in rice, we compared the responses among WT plants, *OsPCR1* and *OsPCR3* overexpression transgenic plants (OE:OsPCR1 and

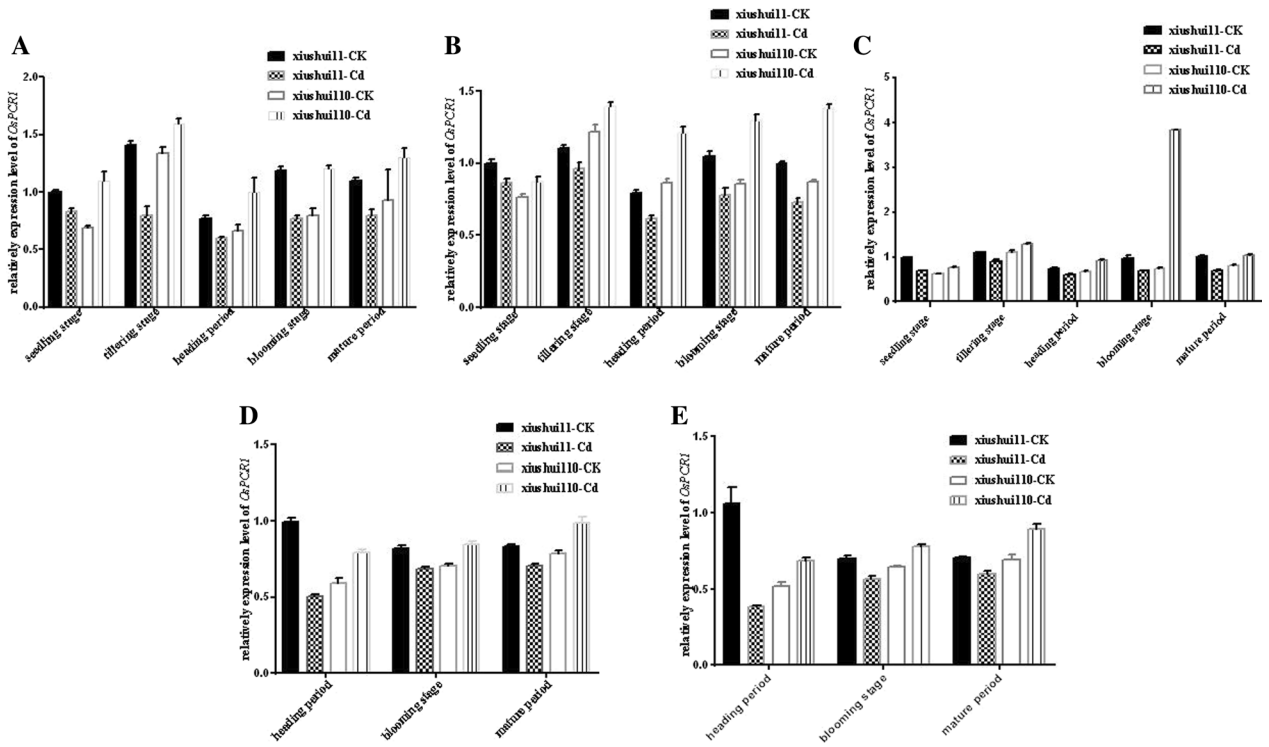

Fig. 2 Expression changes of *OsPCR1* in the two rice varieties at different growth stages. **A** Root; **B** stem; **C** leaf; **D** flag leaf; **E** spike

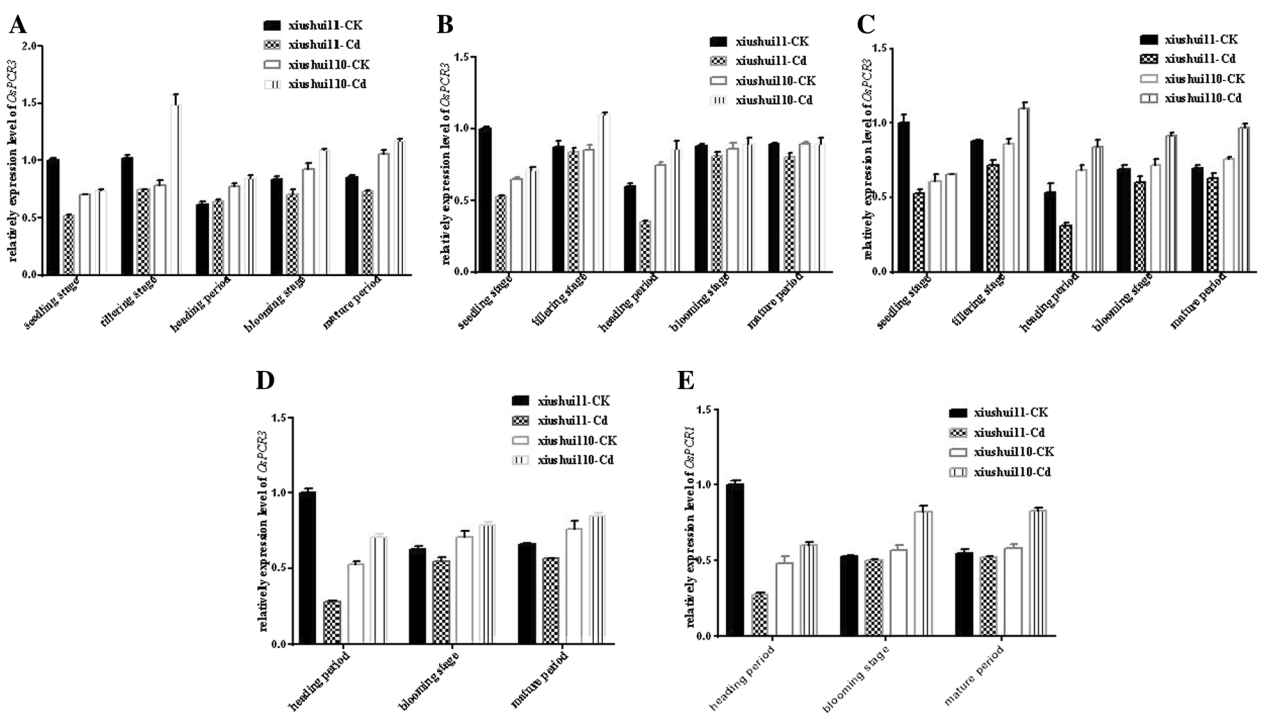

Fig. 3 Expression changes of *OsPCR3* in the two rice varieties at different growth stages. **A** Root; **B** stem; **C** leaf; **D** flag leaf; **E** spike

OE:OsPCR3) and the interference expression transgenic plants (Ri:OsPCR1 and Ri:OsPCR3) to Cd stress. The 5th-leaf stage rice seedlings were treated with 10 μM for 14 days, and then used to determinate the levels of oxidative stress in transgenic plants and WT plants. Results showed that OE:OsPCR1 and OE:OsPCR3 transgenic plants showed enhanced Cd tolerance, while Ri:OsPCR1 and Ri:OsPCR3 transgenic plants showed enhanced Cd sensitivity (Fig. 4).

From Fig. 5, the results of the indicator of oxidized membrane lipid (MDA) showed that although the MDA levels in the overexpression transgenic plants (OE:OsPCR1 and OE:OsPCR3) were higher than that of WT, Cd could induce higher MDA levels in WT than that of OE:OsPCR1 or OE:OsPCR3. Meanwhile, the MDA levels in the interference expression transgenic plants (Ri:OsPCR1 and Ri:OsPCR3) were significant higher than that of WT, and Cd stress induced higher MDA levels in the interference expression transgenic plants (Ri:OsPCR1 and Ri:OsPCR3) than that of WT, while there was almost no change in WT during without Cd or with Cd treatment. On the other hand, from Fig. 4, the difference of H_2O_2 accumulation between WT and OE:OsPCR1 plants was not notable, while it was significantly higher in OE:OsPCR3 than that of WT both during without Cd or with Cd stress. Furthermore, the H_2O_2 accumulation of Ri:OsPCR1 and Ri:OsPCR3 was similar; they were both significantly

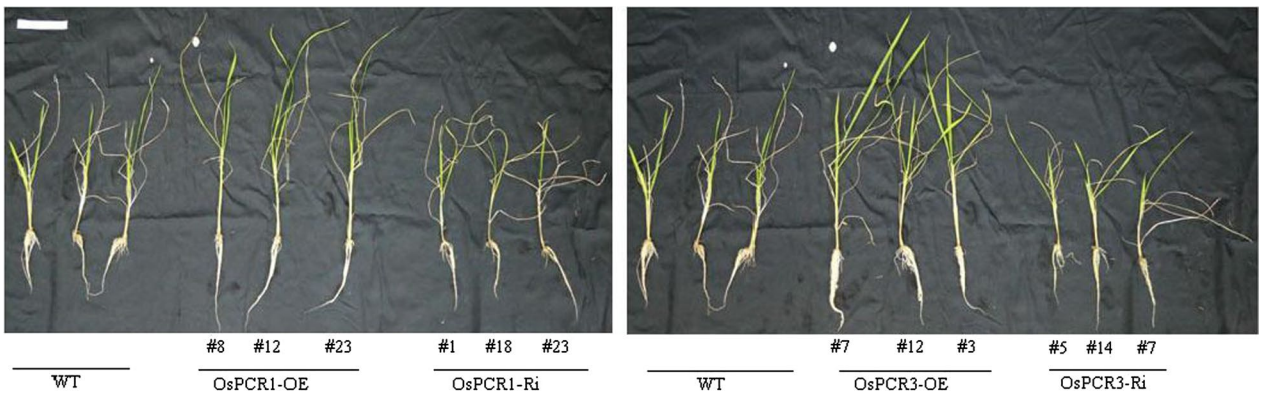

Fig. 4 Phenotypic appearance of different rice plants under Cd stress

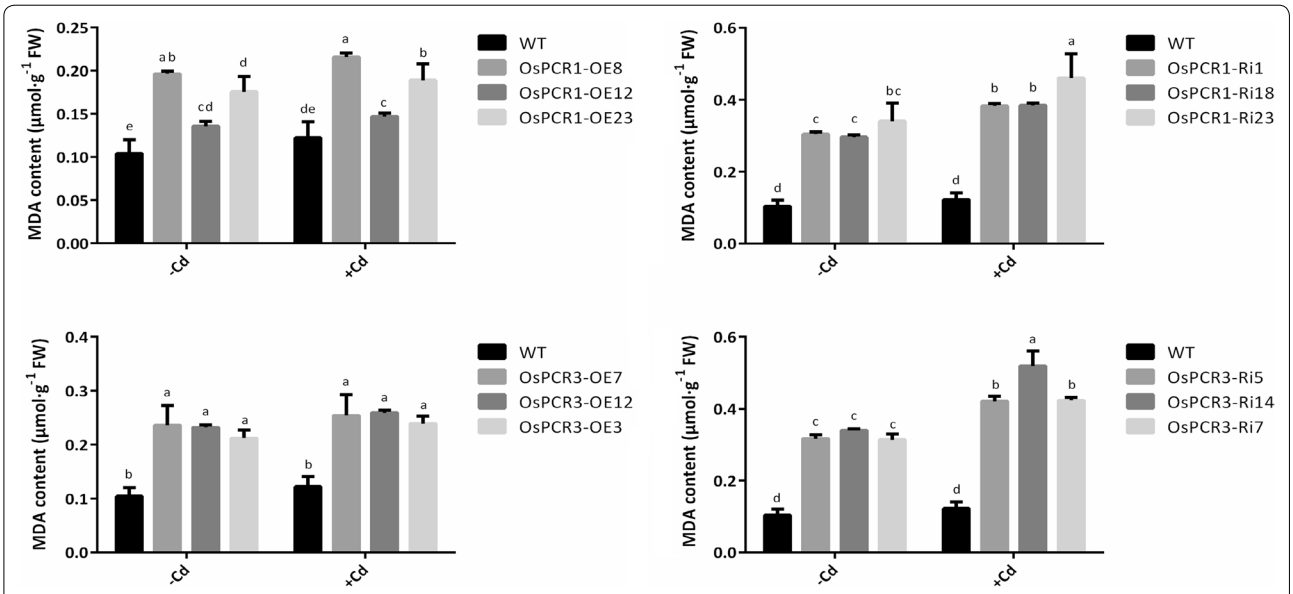

Fig. 5 Effect of Cd stress on MDA levels in different rice plants. Different lowercase letters above the different columns indicated significant difference at $P < 0.05$

lower in interference expression transgenic plants (Ri:OsPCR1 or Ri:OsPCR3) than that of WT.

From Fig. 6, the H_2O_2 accumulation in OE:OsPCR1 was considerably lower than that of WT during Cd stress, while it was opposite between OE:OsPCR3 and WT. However, the H_2O_2 accumulation both in Ri:OsPCR1 and Ri:OsPCR3 were all obviously lower than that of WT.

Cd treatment also could moderate the antioxidant activities in rice seedlings. From Fig. 7, it showed that Cd stress induced the higher CAT activities in OE:OsPCR1 and OE:OsPCR3 than that of WT, and Ri:OsPCR1 showed lower CAT activities than that of WT, while the Ri:OsPCR3 was similar to WT. Moreover, the activities of POD in OE:OsPCR1 and OE:OsPCR3 were similar to WT during Cd stress, as well as the pattern of SOD

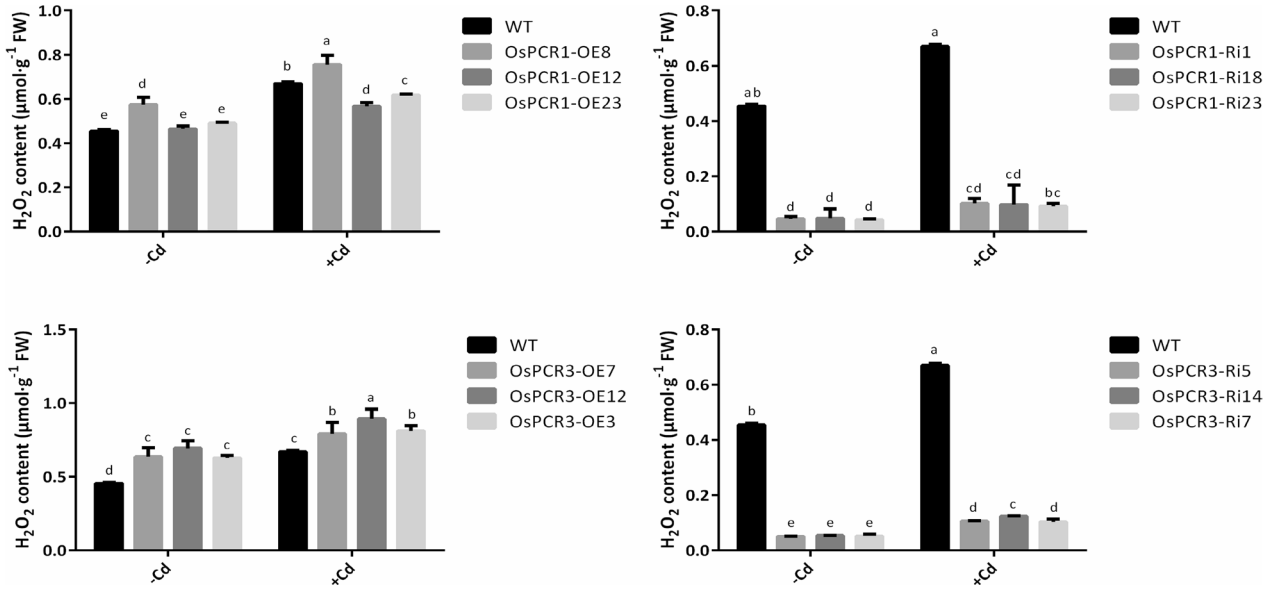

Fig. 6 Effect of Cd stress on H_2O_2 accumulation in different rice plants. Different lowercase letters above the different columns indicated significant difference at $P < 0.05$

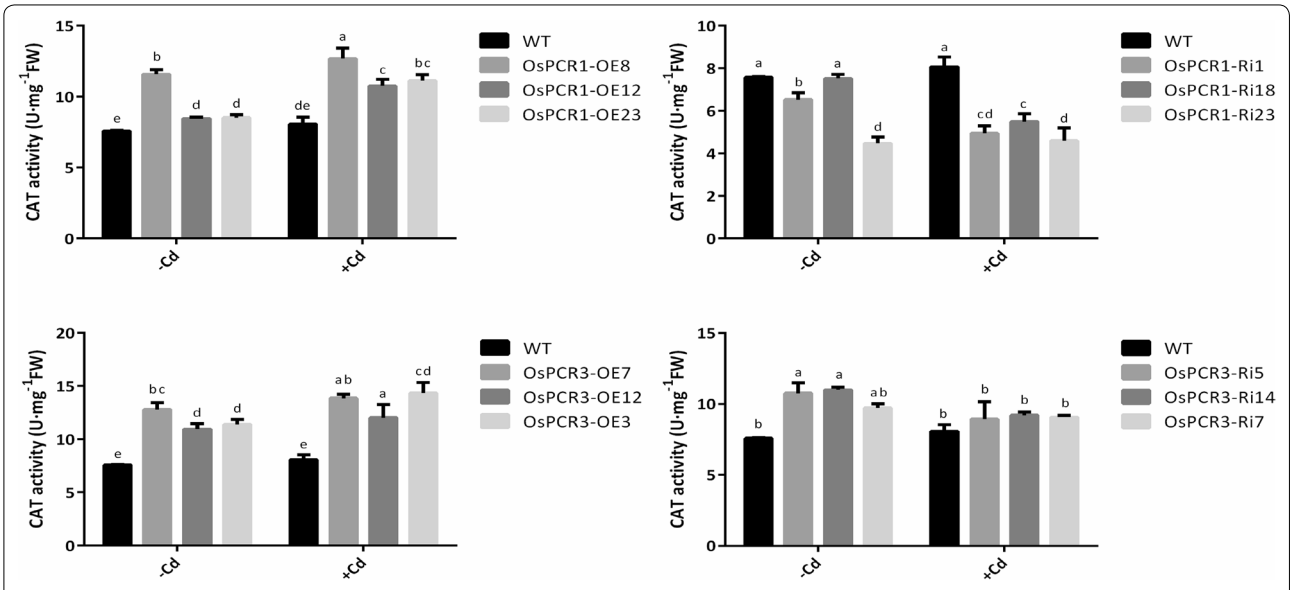

Fig. 7 Effect of Cd stress on CAT activities in different rice plants. Different lowercase letters above the different columns indicated significant difference at $P < 0.05$

activities between OE:OsPCR3 and WT, except that the SOD activities in OE:OsPCR1 were higher than that of WT under Cd stress. Under Cd treatment, the activities of POD in Ri:OsPCR1 or Ri:OsPCR3 were both lower than that of WT. The activities of SOD in Ri:OsPCR3 was lower than that of WT under Cd treatment, except that the activities of SOD in Ri:OsPCR1 were similar to WT (Figs. 8 and 9).

Thus, the results indicated that the interference expression of *OsPCR1* or *OsPCR3* could cause higher membrane oxidation and higher Cd sensibility under Cd stress, while the overexpression of *OsPCR1* or *OsPCR3*

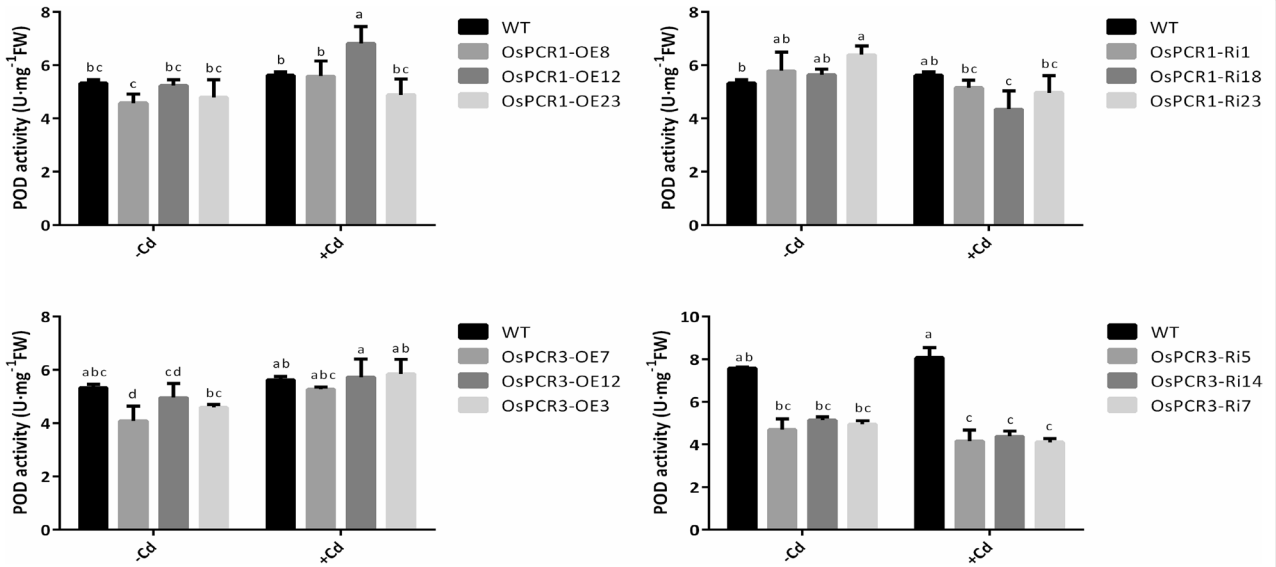

Fig. 8 Effect of Cd stress on POD activities in different rice plants. Different lowercase letters above the different columns indicated significant difference at $P < 0.05$

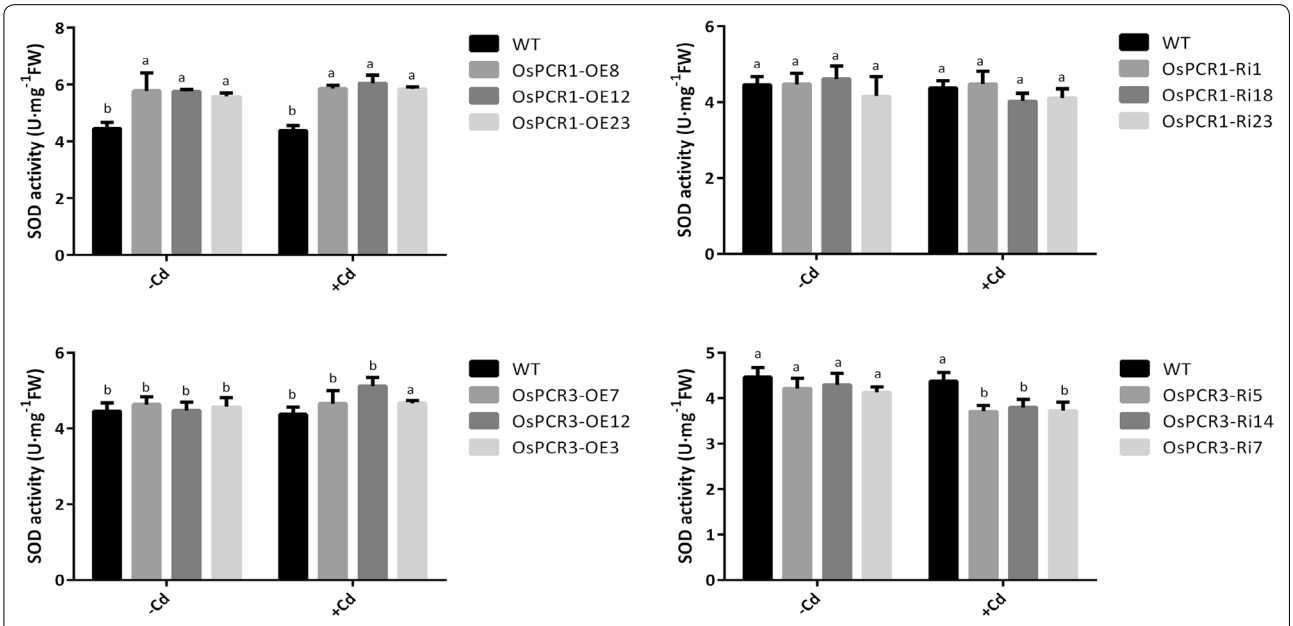

Fig. 9 Effect of Cd stress on SOD activities in different rice plants. Different lowercase letters above the different columns indicated significant difference at $P < 0.05$

could induce higher antioxidant activities to arise the higher Cd tolerance.

Overexpression of *OsPCR1* and *OsPCR3* decreased Cd accumulations in rice

To further explore the functions of *OsPCR1* and *OsPCR3* in the Cd accumulation of rice, we measured Cd levels in the different parts of rice plants at seedling stage (Cd stress for 14 days) and maturity stage that have grown hydroponically in the presence of 2 μM $CdCl_2$, respectively. From Figs. 10 and 11, we found that the Cd contents in the roots of OE:OsPCR1 and OE:OsPCR3 at the seedling stage were significantly lower than WT, while the WT and the interference expression transgenic plants (Ri:OsPCR1 and Ri:OsPCR3) were nearly the same. Moreover, although the Cd contents in the stems and leaves of OE:OsPCR1 and OE:OsPCR3 both showed no obvious difference compared with the WT, the Cd contents in OE:OsPCR1 and OE:OsPCR3 were significantly lower than that of Ri:OsPCR1 and Ri:OsPCR3 separately.

On the other hand, compared to the seedling stage, the Cd accumulations in different parts of rice were changed in stems and leaves at the maturity stage except the roots. As shown in Figs. 12 and 13, the pattern of Cd accumulation in different parts of rice plants (OE:OsPCR1,Ri:OsPCR1 and WT) was Ri:OsPCR1 > WT > OE:OsPCR1. The Cd accumulation trends of transgenic rice lines of *OsPCR3* were similar to the transgenic rice lines of *OsPCR1*. Moreover, the Cd contents in the brown rice of OE:OsPCR1 and OE:OsPCR3 transgenic rice lines were obviously lower than that of WT, as well as the Cd contents in the brown rice of Ri:OsPCR1 and Ri:OsPCR3 transgenic rice lines were significantly higher than WT.

Discussions

Cd is a highly toxic heavy metal to all forms of life including plants and humans. Cd is absorbed by plant roots and accumulated in plant tissues, when grown slightly or moderately Cd-polluted soil, plant growth and development may not be substantially affected but the accumulated Cd can enter the food chain and cause harmful effects to human health. In recent years, many experiments have shown genotypic differences in Cd accumulation among rice varieties [8, 35]. The Cd accumulations in indica subspecies were more and easier than the

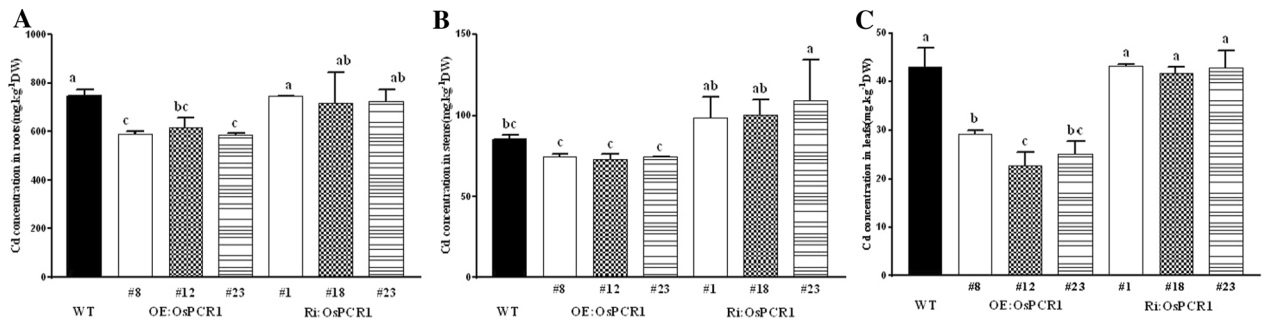

Fig. 10 Cd contents in different transgenic rice plants (OE:OsPCR1 and Ri:OsPCR1) after exposure to 2 μM $CdCl_2$ for 14 days. Different panels indicated different rice plants and different lowercase letters above the different columns indicated significant difference at $P < 0.05$

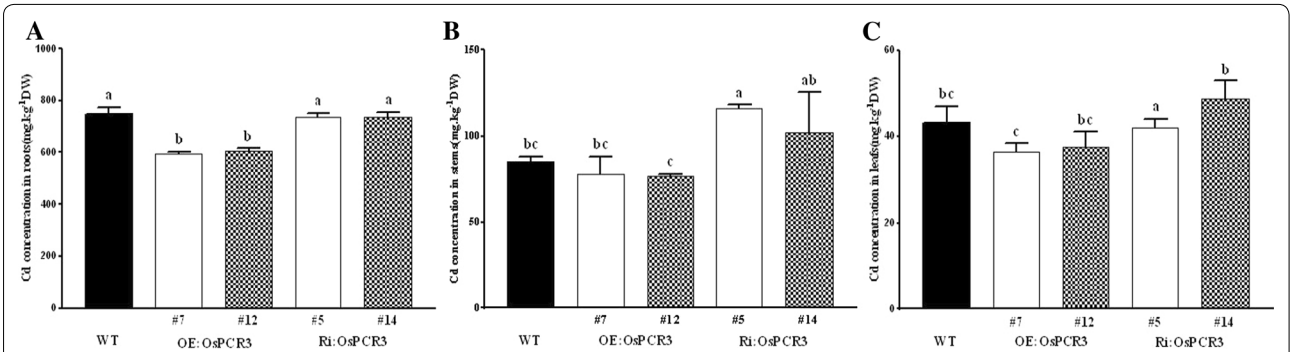

Fig. 11 Cd contents in different transgenic rice plants (OE:OsPCR3 and Ri:OsPCR3) after exposure to 2 μM $CdCl_2$ for 14 days. Different panels indicated different rice plants and different lowercase letters above the different columns indicated significant difference at $P < 0.05$

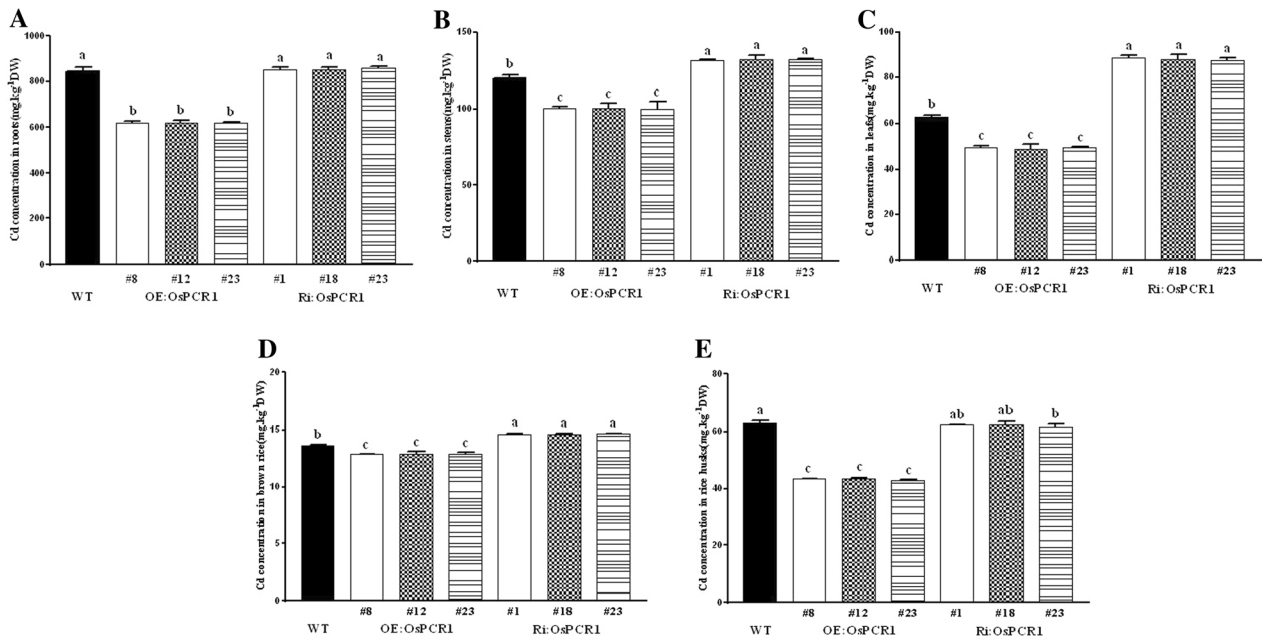

Fig. 12 Cd contents in different rice plants after exposure to 2 μM $CdCl_2$ to maturity. Different panels indicated different rice plants and different lowercase letters above the different columns indicated significant difference at $P < 0.05$

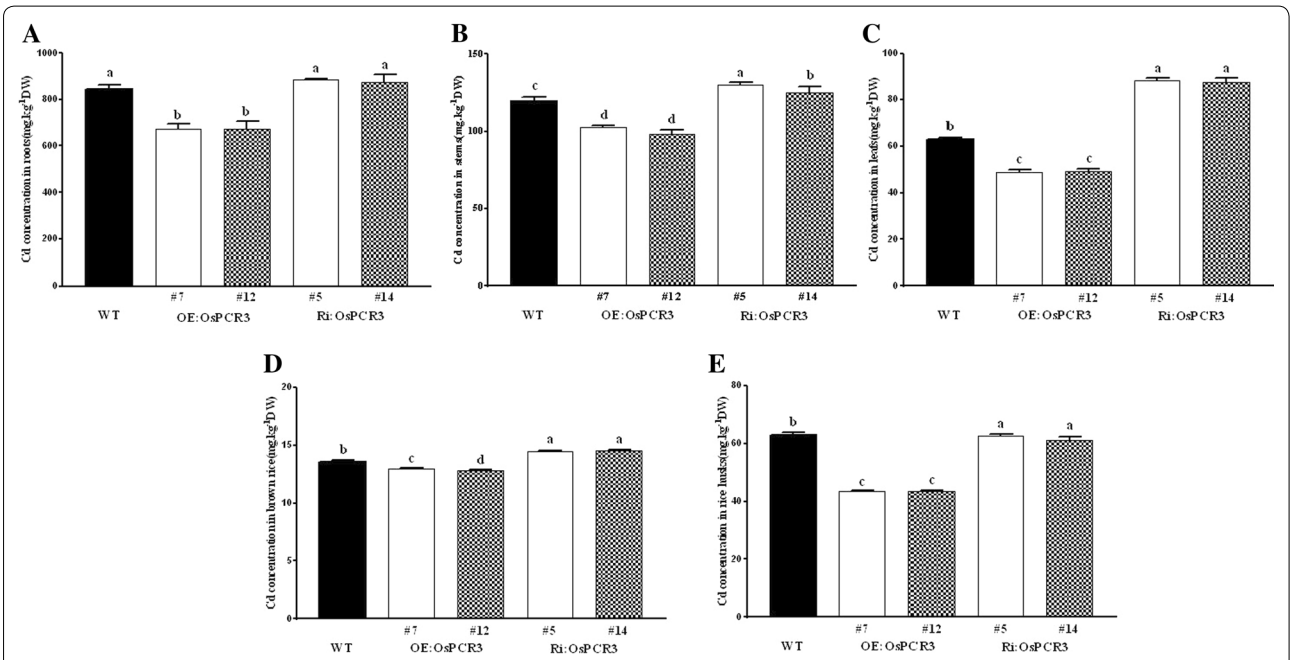

Fig. 13 Cd contents in different rice plants after exposure to 2 μM $CdCl_2$ to maturity. Different panels indicated different rice plants and different lowercase letters above the different columns indicated significant difference at $P < 0.05$

japonica subspecies. In this study, low grain-Cd-accumulating rice (xiushui 11) and a high grain-Cd-accumulating rice (xiushui 110) varieties were used as experimental materials. Owing to the genotypic difference, the accumulation of Cd in xiushui110 was obviously higher than that of xiushui11. Furthermore, the Cd accumulation in

rice grain was correlated with the uptake of Cd by roots, and the root-to-shoot or shoot-to-grain translocation abilities.

Many functions have been reported for PLAC8 domain-containing proteins of plants (such as *Arabidopsis thaliana, Solanum lycopersium, Zea mays, Brassica juncea*) which showed that these proteins including the CCXXXXCPC or CLXXXXCPC motif were reported as associate with Cd resistance in plants [15–17]. Two members of this family, *AtPCR1* and *AtPCR2,* played an important role in the Cd tolerance and transport of Zn, the CPC motif had a much more important role in the function of the AtPCR proteins than CC motif in Cd stress [17]. BjPCR1 protein also had a hydrophobic domain composed of CC-CPC, which was a Ca^{2+} efflux transporter in mustard [16]. In this study, we demonstrated the functions of *OsPCR1* and *OsPCR3* which were homologous to *AtPCR2,* and found that the expression levels of *OsPCR1* and *OsPCR3* were closely related with the Cd contents in rice grains (Figs. 2 and 3). Moreover, *OsPCR3* overexpression transgenic plants (OE:OsPCR1 and OE:OsPCR3) enhanced Cd tolerance, while the interference expression transgenic plants (Ri:OsPCR1 and Ri:OsPCR3) suffered higher Cd injure (Fig. 4). The results were similar to the reports on the heterologous expression of the OsFWL4 which also belonged to PLAC8 domain-containing proteins in yeast cells [28].

One mechanism for regulating metal ion uptake and transport is through alteration of gene expression. As Cd is a non-essential ion, there are no specific Cd transporters. However, many transporters for divalent transition metals (Mn, Fe, and Zn) can absorb Cd. For example, the expression of the root ZIP transporter IRT1, which plays a critical role in the uptake of Fe and non-essential heavy metals including Cd, is highly regulated at the transcriptional level [36, 37]. *OsNramp5* was found contribute to Mn, Cd and Fe transport [7, 38, 39]. *OsHMA2* was revealed to play a pivotal role in Zn and Cd accumulation in rice [9, 40]. As we predicted, *OsPCR* genes take effect as a transporter which may play an important role in the transport of Cd from roots to shoots in rice. In our experiments, we found overexpression of *OsPCR1* and *OsPCR3,* both, could decrease the Cd contents in brown rice, as well as roots, stems, leaves and husks. However, the interference expression of the two genes would increase the Cd contents in brown rice, as well as roots, stems and leaves (Figs. 11 and 12). Due to the grain-ripening stage is a critical period for grain Cd accumulation [30, 41], the lower Cd accumulation in rice grain of the *OsPCR1* or *OsPCR3* overexpression transgenic rice plants may be due to the reduction of Cd uptake and transportation in rice.

Moreover, because of the transmembrane structure, we conjectured that *OsPCR1* and *OsPCR3* did not induce Cd accumulation by acting as an intracellular chelator, but acted as a Cd transporter. This prediction was in agreement with that of Song et al. [17] and Xiong et al. [28]. They found that the expression level of *AtPCR1* and *OsFWL4* was closely related with Cd contents in plants and yeast, respectively. Qiao et al. also found that overexpression of wheat cell number regulator 2 (TaCNR2) which contained the CC/LXXXXCPC conserved motif could increase the Cd tolerance and change the Cd translocation from roots to shoots in rice [42]. In addition, our early bioinformatics prediction showed that OsPCR1 and OsPCR3 were mainly located in Cytoplasm or periplasm and contained the CC/LXXXXCPC conserved motif. We suggest that OsPCR1 and OsPCR3 may take part in inducing transcriptional changes in rice seedlings when exposed to Cd stress, then reduced DNA damage to increase Cd tolerance and influenced the Cd accumulation in rice [18]. A working model for *OsPCR* was suggested in Fig. 14. Taken as a whole, this study is the preliminary exploration of *OsPCR1* and *OsPCR3*; the results of this study will be beneficial to the further research on the function of *OsPCR* genes and its

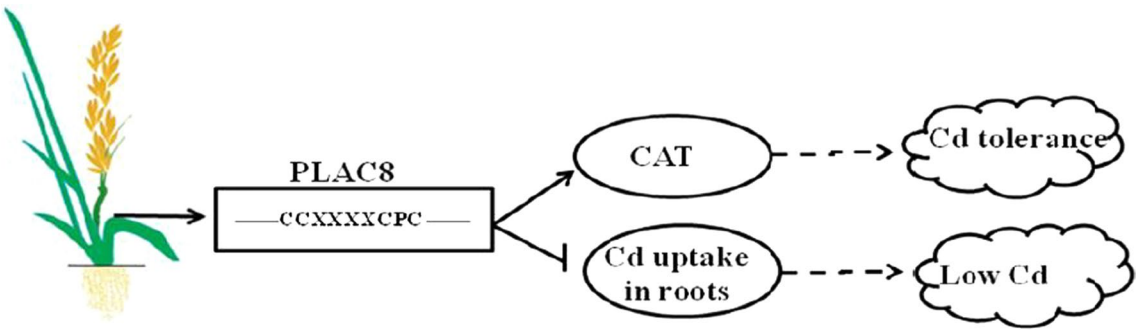

Fig. 14 A working model for *OsPCR*

encoding proteins. However, we need further cloning and functional analysis of the genes' functional motifs to study the molecular mechanisms of Cd tolerance and accumulation of *OsPCR* genes in rice.

Conclusions

Many studies have been carried out on the molecular mechanisms of Cd accumulation in rice. Several genes involved in Cd translocation and accumulation have been identified, but the uptake, translocation and accumulation of Cd in rice seedlings were still not clear, and the discovery of Cd-accumulation-related genes was still very poor. These results elaborated that there was a negative correlation between the expression levels of *OsPCR1* or *OsPCR3* and Cd accumulation in rice. Lower Cd accumulation in the overexpression transgenic rice plants may due to the decrease of Cd uptake and transport in rice; the overexpression of *OsPCR1* or *OsPCR3* in rice could increase Cd tolerance by enhancing antioxidant levels in vivo. These results indicated that *OsPCR1* and *OsPCR3* play critical roles in Cd tolerance and accumulation in rice, which provides a theoretical basis for the safe production of rice. However, many questions remain to be answered. Are *OsPCR1* and *OsPCR3* involved in the regulation of gene expression in response to Cd? Do *OsPCR1* and *OsPCR3* contain any other recognizable domains which contribute to Cd tolerance and what are the mechanisms by which *OsPCR1* and *OsPCR3* confer Cd tolerance? How do *OsPCR1* and *OsPCR3* interact with Cd transporters and regulatory approaches? Therefore, more mechanisms of *OsPCR1*- and *OsPCR3*-mediated Cd tolerance and low-Cd-accumulation in rice should be studied in the future research.

Abbreviations

PCR: the plant cadmium resistance; PCS: phytochelatin synthase; Nramp: the natural resistance-associated macrophage protein; HMA: heavy metal ATPase; LCT1: low-affinity ion transporter; IRT: Fe transporters; fw2.2: fruit weight 2.2; CNR: cell number regulator; FWL: *FW2.2*-like family; OE: OsPCR: *OsPCR* overexpression transgenic plants; Ri: OsPCR1: *OsPCR1* interference expression transgenic plants; H_2O_2: hydrogen peroxide; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; BNT: β-nitroblue-tetrazolium chloride; TaCNR2: wheat cell number regulator 2; WT: wild-type.

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Authors' contributions

This study was designed by CZ, ZC and FW. Construction of transgenic rice plants was conducted by XH and YD. The other experiments and the data analyze were performed by HT, JH, YZ and FW. FW wrote this manuscript, CZ and ZC polished the manuscript. All authors contributed equally to this work. All authors read and approved the final manuscript.

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Availability of data and materials

All essential data are part of the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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