Cadmium pollution leads to selectivity loss of glutamate receptor channels for permeation of $Ca^{2+}/Mn^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+} in rice plant

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HIGHLIGHTS

- Cd ions inhibit expression of *OsGLR3.1–3.4* and synthesis of Glu in rice cells.
- Cd toxicity destroys the selective permeation of GLRs for Ca^{2+}/Mn^{2+} over Cd^{2+}.
- Low-Cd-accumulation genotype preferentially transports $Ca^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+}.

GRAPHICAL ABSTRACT
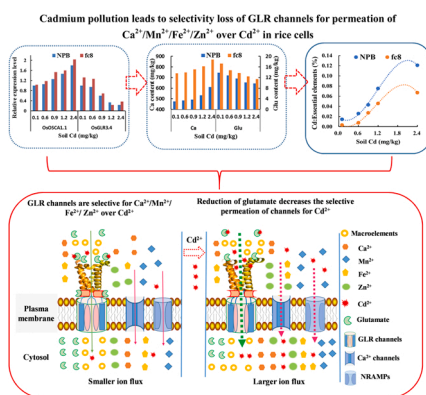
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ABSTRACT

The selective permeation of glutamate receptor channels (GLRs) for essential and toxic elements in plant cells is poorly understood. The present study found that the ratios between cadmium (Cd) and 7 essential elements (i.e., K, Mg, Ca, Mn, Fe, Zn and Cu) in grains and vegetative organs increased significantly with the increase of soil Cd levels. Accumulation of Cd resulted in the significant increase of Ca, Mn, Fe and Zn content and the expression levels of Ca channel genes (*OsCNGC1,2* and *OsOSCA1.1,2.4*), while remarkable reduction of glutamate content and expression levels of *GLR3.1–3.4* in rice. When planted in the same Cd-polluted soil, mutant fc8 displayed significantly higher content of Ca, Fe, Zn and expression levels of *GLR3.1–3.4* than its wild type NPB. On the contrary, the ratios between Cd and essential elements in fc8 were significantly lower than that in NPB. These results indicate that Cd pollution may damage the structural integrity of GLRs by inhibiting glutamate synthesis and expression levels of *GLR3.1–3.4*, which leads to the increase of ion influx but the decrease of preferential selectivity for $Ca^{2+}/Mn^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+} through GLRs in rice cells.

1. Introduction

Cadmium (Cd) is one of the most widely distributed toxic heavy metals in the farm lands around the world, and its toxicity to plant metabolism increases with Cd levels in the top soil [27,48]. As a non-essential element, Cd ions are enriched in rice roots and transported to the shoots mainly through pathways for essential elements and water [34,35,6]. Thus, the ion species and concentrations in rice rhizosphere, water management conditions and transpiration have significant effects on Cd uptake and transport [37,43,7]. Meanwhile, Cd content in soil also significantly affects the absorption of essential elements by rice plant. High Cd pollution generally inhibits the uptake and accumulation of K, Ca, Fe, etc., but promotes the uptake of Mn in rice [11,24,27]. Therefore, there are not only antagonistic, but also promoting and synergistic actions between Cd and cations. However, the unfavorable interference of Cd on physiological processes and redox state of cells in rice is much greater than essential elements [3,5,46] (Yuan et al., 2018). Intake of Cd-contaminated grains even increases the risk of bone, liver and cardiovascular disease in human body [42].

Water and nutrients are transported in plants mainly by apoplastic and symplastic pathways. The transport efficiency of mineral elements by apoplast is mainly regulated by the Casparian band, cork layer and lignin [2,29,34]. Compared with apoplast, the plasma membrane in symplast displays a selective permeation to ions. Transporters and channels are responsible for active or passive transport of ions across the plasma membrane [12,33]. Selectivity filter (SF) in ion channels is a key structure for determining flow rates of different ions [16]. Nonselective cation channels (NSCCs) are the most common ion channels in the cell membrane, which allow the passage of cations in different valence at the same time, and are more selective to cations than anions. NSCCs are involved in many physiological processes, such as nutrient absorption, turgor regulation and signal transduction. Some NSCCs are permeable to a wide range of nutrient cations (i.e., K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, etc.), and even some toxic ions including Pb^{2+}, Hg^+ and Cd^{2+} [10,20,33]. NSCCs play a more important role when cells are under environmental stress or are in urgent need of nutrients to maintain normal cellular metabolic function [4,41]. However, the regulatory mechanism for NSCCs to preferentially transport essential elements remains unclear.

Activities of ion channels are modulated by many cellular factors including membrane voltage and agonists. NSCCs gated by amino acids, specifically by glutamate and glycine, are called ionotropic glutamate receptor channels (GLRs). Recent studies have demonstrated that GLRs are ubiquitous ligand-gated channels, and are involved in ion transport, defense response to wound and disease, signal transduction, and developmental processes [13,18,21,44]. After binding to the ligand-binding domain (LBD) in the GLRs, ligands such as glutamate are able to change the angle of the peptide chains in the transmembrane domain (TMD), thereby regulating the selectivity of channels for different cations [1,18]. The rapid responses of GLRs to environmental stress are related with the release of glutamate in the apoplast and Ca^{2+} influx into the cytosol of plant cells [32,36,40]. More than 20 GLR homologous proteins have been identified in *Arabidopsis thaliana* [18,21]. Low concentration of glutamate and other amino acid can induce a fast increase of free cytosolic Ca^{2+} in *Arabidopsis* root cells [1,38]. However, it is unclear if GLRs in rice cells have been involved in the selectively conducting essential ions over toxic ions like Cd^{2+}.

The concentration of metal ions inside and outside the cells also has a significant effect on the selectivity and conductance of ion channels. Some micronutrients (i.e., Zn, Mn and Cu) have significant inhibitory effects on ion channels when their concentrations exceed a certain value. Toxic heavy metals (i.e., Cd, La, Gd and Ba) can greatly influence permeation and selectivity of ion channels at very low concentrations (Bouron, 2015) [20,19,49]. For example, Cd^{2+} at 20–100 μM strongly blocks the current of BK-type K^+ channel [55]. However, the effects of Cd concentration on the selective permeation of GLRs in rice cells have not been reported. As Cd accumulation in rice was always companied by

increase of some essential elements and reduction of glutamate in rice, we assumed that the selective permeability of GLRs in rice cells may be destroyed by Cd ions. Therefore, in this study, the effects of soil Cd content on the uptake of essential elements, gene expression levels of GLRs in rice plants were examined. The mechanism of Cd toxicity in destroying selective permeation of GLRs for $Ca^{2+}/Mn^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+} in rice cells was demonstrated. The molecular regulatory mechanism of rice grains and vegetative organs alleviating the physiological toxicity of Cd was analyzed at cellular and gene levels, which provided theoretical basis for producing Cd-free rice in Cd-polluted fields.

2. Materials and methods

2.1. Plant materials and growth conditions

Pot experiments were carried out in a greenhouse in Tianjin (39°10′N, 117°16′E). Soil samples with 0.1, 0.6, 0.9, 1.2, and 2.4 mg kg^{-1} Cd were prepared as previous description [47]. These 5 kinds of Cd-polluted soils were ground and mixed completely every year before planting rice. Then, 5.0 kg of soil was put into 8.0 L pots and incubated with 3 L water for 45 days. A wild *japonica* rice (*Oryza sativa* L.) Nipponbare (NPB) and its fragile-culm mutant fc8 were used in this experiment. The seeds of these two genotypes were provided by Biomass and Bioenergy Research Centre, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China. The homozygous mutant fc8 was developed from a T-DNA mutagenesis pool of Nipponbare (NPB) in 2008 as described in previous report [45]. After growth for 5 weeks in seed bed, 12 uniform seedlings were evenly placed in four holes of each pot. 2.0 g of NH_4NO_3 (0.7 g N) was added in each pot at tillering stage and elongation stage, respectively. The pot soil was flooded (4 cm deep) during the whole growth period of rice. The gene expression levels of the developing grains were determined on the seventh day after flowering. At mature stage, all plants were divided into grains, rachises, the upmost internodes (internode1) and nodes (node1), flag leaves, second internodes (internode2) and nodes (node2), second leaves, stem bases, roots and other parts. All samples were dried to constant weight at 75 °C. Then, these samples were ground and filtered by the sieve with 20 meshes (<0.83 mm).

2.2. Determination of elements and amino acids

The samples were digested with a digestion instrument DigiBloc ED54 (LabTech, Beijing, China) by concentrated HNO_3 and 30% H_2O_2 with a volume ratio of 7:1. The digestion solution was fixed to 25 mL with deionized water [27]. The content of elements including K, Mg, Ca, Fe, Mn, Zn, Cu and Cd was determined by ICP-MS (iCAP Q, Thermo Fisher Scientific, Waltham, MA, USA). The content of free amino acids in samples was measured as described by Xue et al. [48] by using an Agilent 1200 HPLC systems (Agilent Technologies, Palo Alto, CA). C18 column (4.6 mm × 150 mm, 5 μm Agilent Technologies, CA) was used in the experiment. Column temperature was set to 40 °C. The parameters of mobile phase: A, $CH_3CN/CH_3OH/H_2O$: 45/45/10; B, 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7 \cdot 10\ H_2O$, pH 8.2. Gradient elution parameters: 0–0.35 min, 2% A and 98% B; 0.35–13.4 min, 2~57% A and 98~43% B; 13.4–15.7 min 100% A; 15.7–18 min 100~2% A and 0~98% B. Flow rate was 1.0 mL min^{-1}.

2.3. Gene expression analysis

Total RNA of fresh panicles was extracted with Trizol reagent with 4 replications. The complementary DNA (cDNA) was synthesized from total RNA with HiScript II Q RT SuperMix for qPCR (Vazyme R223). Relative quantification of gene expression by real-time PCR was performed on a BioRad CFX Manager System (BioRad, USA) with ChamQ Universal SYBR qPCR Master Mix (Vazyme Q711). Primer pairs for Actin gene were designed using Primer-BLAST software (http://www.ncbi.

nlm.nih.gov/tools/primerblast/) based on the corresponding sequences available in the database (http://www.ncbi.nlm.nih.gov/), and used as an internal reference. The primer pairs for GLRs, Ca and Mn channels were designed based on references (Table S1) [25,30,31,47]. All gene expression analysis was performed with at least four independent biological replicates. Relative expression levels of genes were calculated according to the $2^{-\Delta\Delta Ct}$ method.

2.4. Statistical analysis

The data in the experiment were presented as the mean$\pm$ SD (n = 4). The sum of K, Mg, Ca, Fe, Mn, Zn and Cu was used to represent essential elements (EE). In order to reflect the selective permeation of channels for essential elements over Cd, the fault-tolerant rate (FTR) of channels for Cd was calculated by the equation: FTR(%) = 100 × [Cd] / [N], where [Cd] represented the content of Cd in each organ, and [N] represented content of any essential element or content of EE in corresponding organ. ΔEE = [C] - [EE], where [C] and [EE] represented the content of essential elements in each organ when soil Cd content was in the range of 0.6–2.4 mg kg^{-1} and soil Cd content was 0.1 mg kg^{-1}, respectively. ΔCd was the difference of Cd content in each organ between every treatment and CK with 0.1 mg kg^{-1} soil Cd.

The significant differences among treatments were analyzed by one-way analysis of variance (ANOVA) with multiple comparisons by Duncan's test (p < 0.05), a mixed model ANOVA (p < 0.05) utilizing SPSS 20.0. Tables and figures were generated utilizing Microsoft Excel 2013 and Cytoscape. Principal component analysis was performed using Origin 2021.

3. Results

3.1. Effects of soil Cd content and genotypes on the mineral elements in grains

Levels of soil Cd and rice genotype had significant influence on the content of Cd and other mineral elements in grains. With the increase of soil Cd in the range of 0.6–2.4 mg kg^{-1}, the content of Cd and Mn in grains of two rice genotypes increased significantly. Mutant fc8 displayed significantly lower Cd and Mn than its wild type NPB when soil Cd was at the same level (Fig. 1A). When soil Cd increased from 0.6 to 2.4 mg kg^{-1}, rice Cd increased from 0.97 to 4.78 mg kg^{-1} in NPB, and increased from 0.34 to 3.40 mg kg^{-1} in fc8. Rice Cd in fc8 was 25.7–65.3% lower than that in NPB, while rice Mn in fc8 was only 5.4–12.2% lower than that in NPB.

With the increase of soil Cd, the increase amount of Fe and Zn content in grains changed with genotypes. Grains of fc8 displayed significantly lower Fe than those of NPB in all treatments, but only significantly higher Zn than those of NPB when soil Cd was in the range of 0.9–2.4 mg kg^{-1}. When soil Cd increased from 0.9 to 2.4 mg kg^{-1}, rice Zn in fc8 increased from 32.5 to 37.4 mg kg^{-1}, while rice Zn in NPB remained on the similar level (29.4 $\pm$ 0.04 mg kg^{-1}) (Fig. 1B).

Mutant fc8 had much higher rice Ca than its wild type NPB. When Cd content in soil increased from 0.1to 0.9 mg kg^{-1}, rice Ca increased from 741.2 to 778.3 mg kg^{-1} in fc8 and from 476.8 to 492.1 mg kg^{-1} in NPB (Fig. 1C). Heavy Cd pollution in soil stimulated significant increase of Ca in rice. When soil Cd was raised to 1.2–2.4 mg kg^{-1}, rice Ca increased to 806.4–870.2 mg kg^{-1} in fc8 and 533.1~ 611.6 mg kg^{-1} in NPB. In
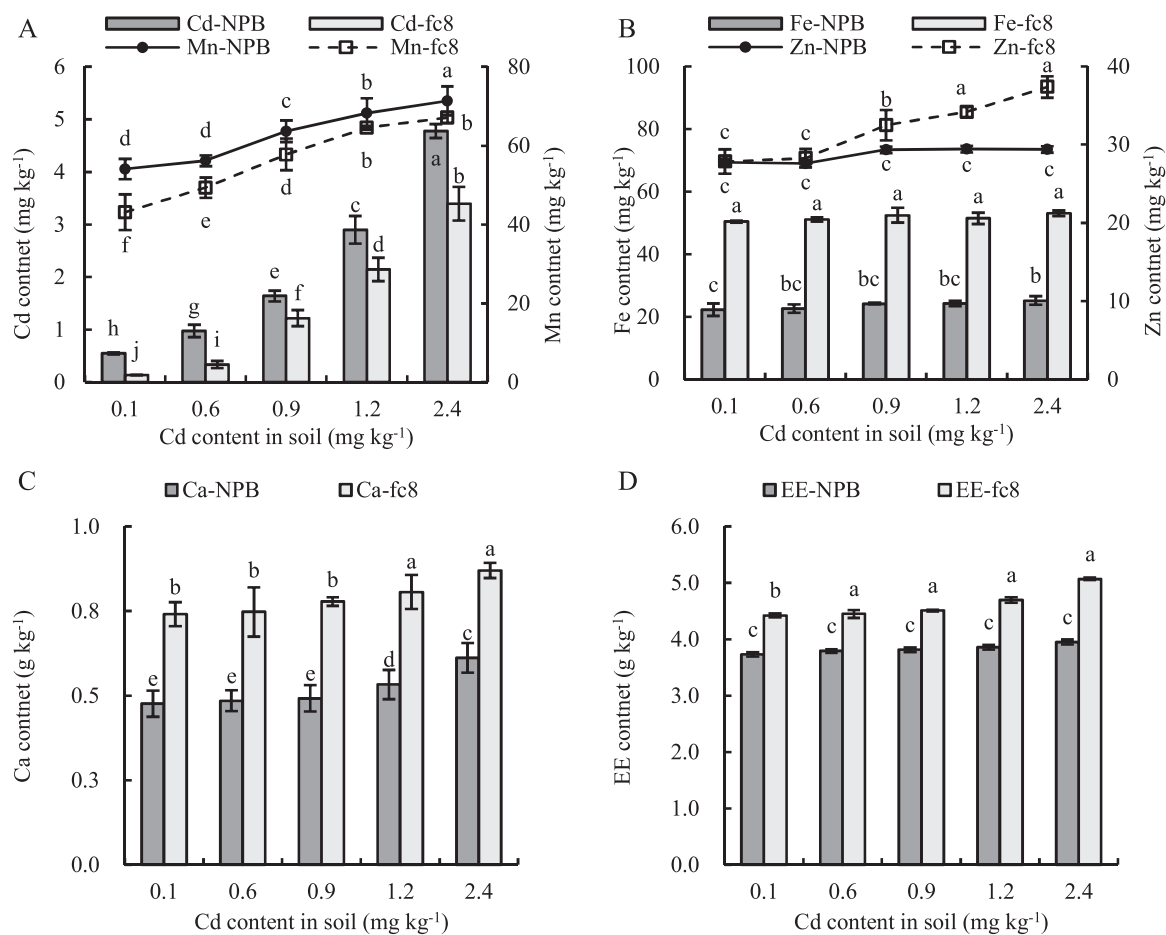
Fig. 1. Effects of soil Cd on the content of Cd and Mn (A), Fe and Zn (B), Ca (C) and essential elements (D) in grains. NPB represents rice cultivar Nipponbare and fc8 represents its fragile-culm mutant, respectively. Error bars represent SD of four replications. Different letters indicate significant difference (p < 0.05) among treatments and genotypes for each element or sum of essential elements (EE).

comparison with the treatment of 0.1 mg kg^{-1} soil Cd, 2.4 mg kg^{-1} soil Cd made the rice Ca increased by 17.4% in fc8 and by 28.3% in NPB.

The sum of 7 essential elements (EE) in grains was increased with the Cd content in soil, but the differences among treatments were not significant at $p < 0.5$. However, the difference of EE between genotypes was significant in all treatments (Fig. 1D). Content of EE in grains of fc8 was significantly higher than that of NPB. In spite of soil Cd levels changed in the range of 0.1–1.2 mg kg^{-1}, the content of EE increased slightly in grains of fc8 (4.4–4.7 g kg^{-1}) and NPB (3.7–3.9 g kg^{-1}). When soil Cd was raised to 2.4 mg kg^{-1}, content of EE in grains of fc8 showed a significant increase. Relatively, content of EE in grains of NPB was not sensitive to Cd stress and maintained stable (3.7–3.9 g kg^{-1}) when soil Cd changed in the range of 0.1–2.4 mg kg^{-1}.

3.2. Effects of soil Cd content and genotypes on the mineral elements in vegetative organs

Among vegetative organs, nodes and stem bases displayed much higher Cd than internodes. Mutant fc8 had significantly lower Cd in internodes and nodes than its wild type NPB (Table 1). Most micronutrients (i.e., Ca, Mn, Fe, and Zn) were also concentrated in nodes, and their content in vegetative organs generally increased with soil Cd, especially when soil Cd increased to 1.2–2.4 mg kg^{-1} (Table S2). However, K and Mg were primarily concentrated in the upmost internode. Therefore, internode1 and node1 accumulated more essential elements than internode2 and node2 as well as stem base in all treatments. When planted in the same Cd-polluted soil, mutant fc8 concentrated more essential elements than its wild type NPB in all vegetative organs.

3.3. Effects of soil Cd content and genotypes on the selective permeation of channels in grains

The fault-tolerant rate (FTR) reflects the selective permeability of ion channels for essential elements over harmful elements to some extent. Low TFR indicates the low permission of channels for toxic ions. Rice genotypes and Cd levels in soil had great influence on the FTR of channels for Cd. When Cd content in soil increased from 0.6 to 2.4 mg kg^{-1}, the net increase of EE (ΔEE) in grains was only about 0.22 g kg^{-1} in NPB, while ΔEE was as high as 0.65 g kg^{-1} in fc8. In contrary

to ΔEE, the net increase of Cd content (ΔCd) in grains of NPB (4.23 mg kg^{-1}) was significantly higher than that of fc8 (3.26 mg kg^{-1}) (Fig. 2A). Therefore, mutant fc8 showed significantly lower TFR than its wild type NPB in grains when they were planted in the same Cd-polluted soil. However, with the increase of Cd content in soil, the FTR of channels in both genotypes increased significantly. The correlationship between the FTR for EE over Cd and content of soil Cd was well fitted by S-curve equation (Fig. 2B). When soil Cd was less than 0.6 mg kg^{-1}, the FTR of channels was about 0.01% in fc8 and 0.03% in NPB. When soil Cd was raised to 1.2–2.4 mg kg^{-1}, the FTR of channels in fc8 and NPB increased to 0.05–0.07% and 0.08–0.12%, respectively.

Among different ion channels, Fe-Zn channels had the highest FTR and K-Mg channels had the lowest FTR. Both genotypes and Cd levels in soil had significant influence on the FTR of Fe-Zn channels. When soil Cd increased from 0.6 to 2.4 mg kg^{-1}, the FTR of Fe-Zn channels for Cd increased from about 0.91–7.60% in fc8 and from about 4.12–17.94% in NPB, respectively (Fig. 2C). Different from Fe-Zn channels, K-Mg channels permitted very limited Cd through them. Rice genotypes and Cd levels in soil had slight influence on the FTR of K-Mg channels for Cd. When soil Cd increased from 0.6 to 2.4 mg kg^{-1}, the FTR of K-Mg channels increased from 0.01% to 0.17% in fc8, and from 0.03% to 0.30% in NPB (Fig. 2D). These results indicated that K-Mg channels in grains were able to recognize and intercept Cd effectively.

The correlation between the FTR of Mn channels and content of soil Cd was well fitted by S-curve equation (Fig. 2E). When soil Cd was raised to 1.2–2.4 mg kg^{-1}, the FTR of Mn channels in fc8 and NPB grains increased to 3.32–5.06% and 4.44–6.69%, respectively. In comparison with Mn channels, Ca channels displayed much lower FTR for Cd in grains. When Cd content in soil increased from 0.6–2.4 mg kg^{-1}, the FTR of Ca channels in fc8 grains increased from 0.04% to 0.30%, and that in NPB grains increased from 0.20% to 0.78% (Fig. 2F). Both Mn and Ca channels in fc8 displayed much lower FTR than those in NPB in all treatments, especially when soil Cd was in the range of 0.9~2.4 mg kg^{-1}. Apparently, Ca channels were more specific and had much lower permission for Cd than Mn channels in grains.

Table 1

Effects of soil Cd content and genotypes on the mineral elements in vegetative organs.

Genotypes	Organs	Soil Cd (mg kg^{-1})				
		0.1	0.6	0.9	1.2	2.4
		Cd (mg kg^{-1})				
NPB	Internode1	2.06 ± 0.03e	3.85 ± 0.06d	5.95 ± 0.18c	11.87 ± 0.41b	22.66 ± 1.82a
	Internode2	1.87 ± 0.03e	3.27 ± 0.06d	5.86 ± 0.06c	9.95 ± 0.29b	17.43 ± 0.27a
	Node1	18.69 ± 0.77e	29.56 ± 1.78d	36.98 ± 2.51c	71.09 ± 5.06b	98.79 ± 3.46a
	Node2	11.00 ± 0.58e	16.19 ± 1.01d	22.14 ± 1.08c	34.03 ± 1.13b	73.02 ± 1.87a
	Stem base	7.98 ± 0.41d	12.85 ± 0.43c	17.39 ± 0.78c	38.10 ± 1.62b	41.07 ± 0.43a
fc8	Internode1	0.54 ± 0.01e	1.29 ± 0.06d	4.28 ± 0.07c	6.22 ± 0.12b	12.77 ± 0.29a
	Internode2	0.28 ± 0.01e	0.82 ± 0.02d	3.16 ± 0.05c	4.64 ± 0.21b	9.33 ± 0.49a
	Node1	2.11 ± 0.12e	9.30 ± 0.20d	30.93 ± 1.56c	65.07 ± 3.62b	72.35 ± 6.81a
	Node2	1.07 ± 0.08e	4.18 ± 0.33d	11.38 ± 0.41c	22.84 ± 1.51b	51.95 ± 2.80a
	Stem base	1.43 ± 0.06d	4.07 ± 0.27d	11.04 ± 0.35c	17.34 ± 0.48b	21.86 ± 1.72a
		^{a}Essential elements (g kg^{-1})				
NPB	Internode1	24.68 ± 0.83a	25.36 ± 0.26a	25.21 ± 0.17a	25.19 ± 0.21a	25.67 ± 0.81a
	Internode2	8.38 ± 0.18b	8.71 ± 0.27b	9.12 ± 0.20ab	9.43 ± 0.25ab	9.80 ± 0.11a
	Node1	18.89 ± 0.29c	19.18 ± 0.30bc	19.67 ± 0.23b	21.63 ± 0.64ab	22.84 ± 0.54a
	Node2	14.15 ± 0.14a	14.19 ± 0.10a	14.21 ± 0.10a	14.92 ± 0.28a	16.37 ± 0.18a
	Stem base	9.81 ± 0.16a	9.92 ± 0.10a	10.12 ± 0.29a	10.28 ± 0.13a	10.49 ± 0.11a
fc8	Internode1	28.87 ± 0.92a	29.39 ± 1.13a	29.88 ± 0.21a	30.01 ± 1.44a	30.46 ± 0.21a
	Internode2	12.52 ± 0.21b	12.66 ± 0.33a	12.81 ± 0.35a	12.94 ± 0.17a	13.30 ± 0.40a
	Node1	22.37 ± 0.40c	23.77 ± 0.31c	25.77 ± 0.40c	26.18 ± 0.47b	27.15 ± 0.38a
	Node2	16.07 ± 0.28b	16.30 ± 0.21b	16.96 ± 0.27b	16.99 ± 0.30a	17.51 ± 0.36a
	Stem base	11.56 ± 0.19a	11.65 ± 0.26a	11.65 ± 0.23a	11.95 ± 0.28a	12.10 ± 0.22a

^{a} Essential elements represent the sum of K, Mg, Ca, Fe, Mn, Zn and Cu content. Data show means ± standard deviation (SD) (n = 4). Different letters indicate significant difference ($p < 0.05$) among treatments in same organ according to the Duncan's test.
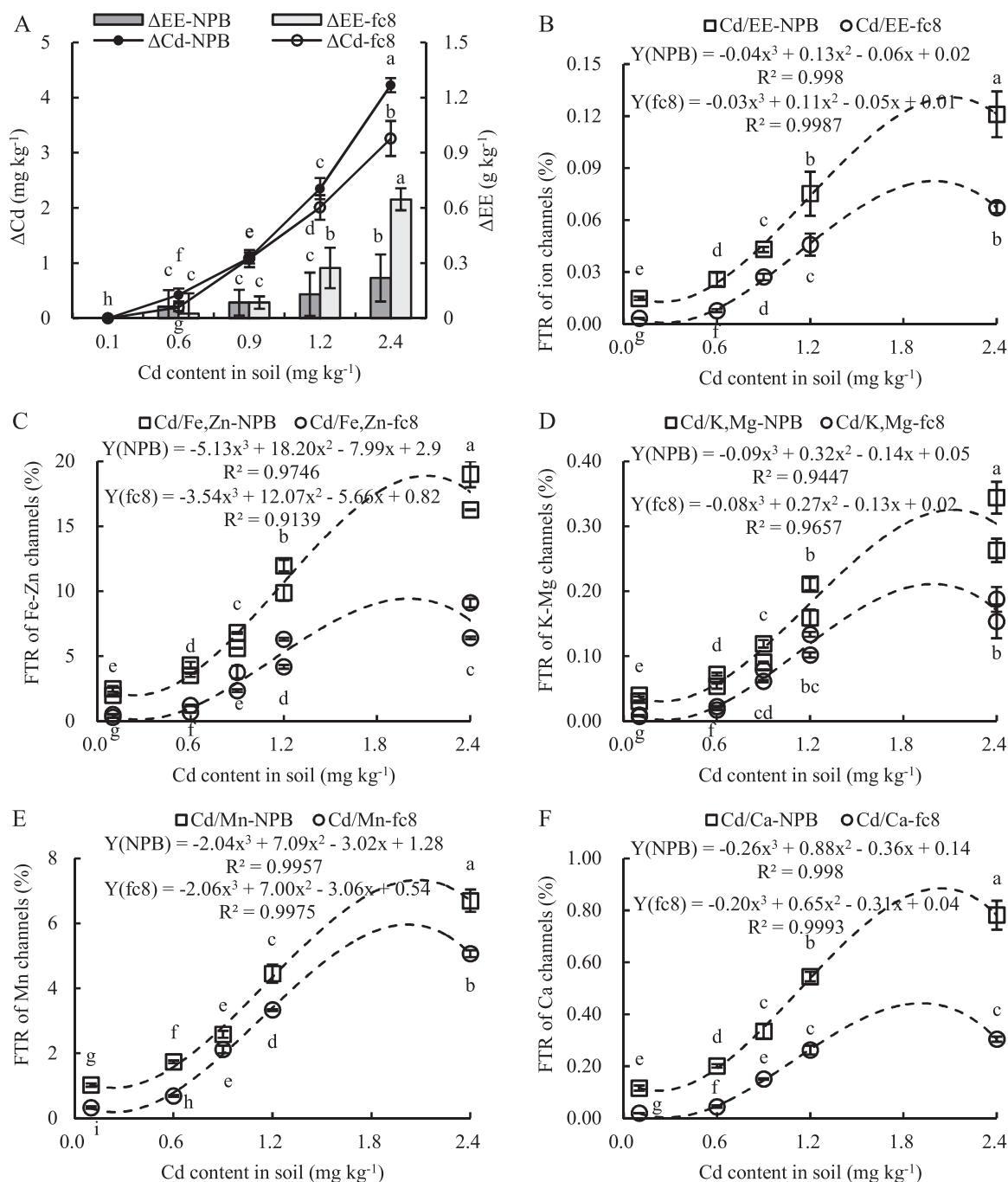
Fig. 2. Effects of soil Cd on the net increase of Cd and essential elements (A), and the fault-tolerant rate (FTR) of essential element (EE) channels for Cd (B), the FTR of Fe-Zn channels (C), the FTR of K-Mg channels (D), the FTR of Mn channels (E), and the FTR of Ca channels for Cd (F) in grains. Error bars represent SD of four replications. Different letters indicate significant difference (p < 0.05) among treatments and genotypes according to the Duncan's test.

3.4. Effects of soil Cd content and genotypes on the selective permeation of channels in vegetative organs

Among essential elements in vegetative organs, Ca and Mn were more sensitive to Cd stress than the others. The FTR of Ca channels for Cd in the internode1 and internode2 was linearly correlated with the Cd content in soil, and NPB had more than 2 times higher FTR than its mutant fc8 (Fig. 3A). The FTR of Mn channels for Cd in the internode1 and internode2 was also linearly correlated with the Cd content in soil (Fig. 3B). In comparison, Mn channels in internodes displayed much higher FTR for Cd than Ca channels. When soil Cd increased from 0.6 m to 2.4 mg kg^{-1}, the FTR of Ca channels in internodes of fc8 and NPB was

about 0.03–0.26% and 0.14–0.71%, while the FTR of Mn channels in them was about 0.86–3.88% and 1.84–5.76%, respectively.

Ca or Mn channels in nodes and stem bases generally had higher FTR for Cd than that in internodes. Mutant fc8 showed significantly higher FTR of Ca channels in node1, node2 and stem base than its wild type NPB in all kinds of Cd-polluted soils (Fig. 3C). However, the FTR of Mn channels for Cd in rice nodes was not significantly different, especially when soil Cd was in the range of 0.9–2.4 mg kg^{-1} (Fig. 3D). These results indicated that Ca and channels in internodes had much lower permission to Cd than those in nodes and stem bases. Vegetative organs of fc8 were able to intercept Cd more efficiently than those of NPB.
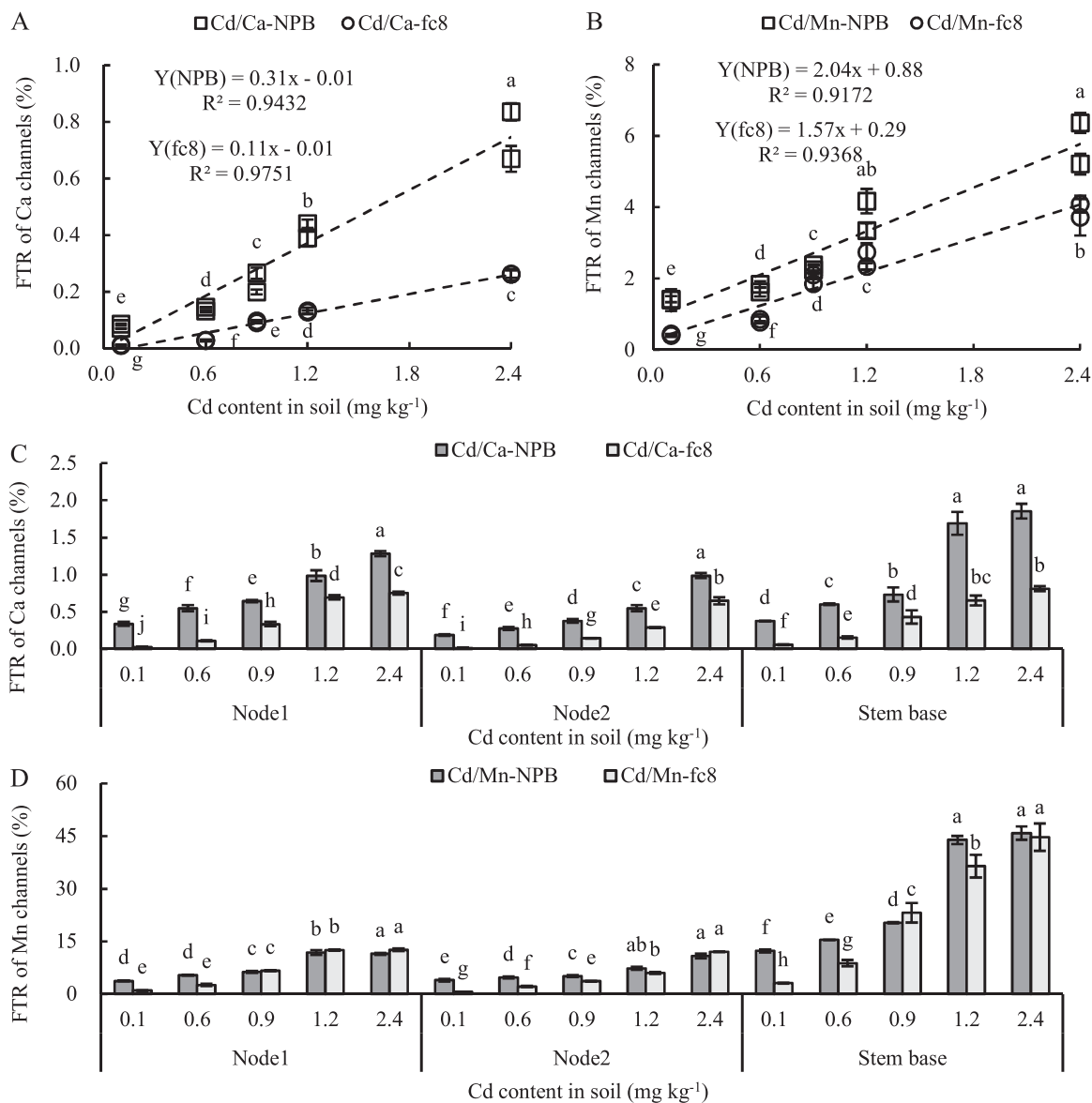
Fig. 3. Effects of soil Cd on the FTR of Ca channels (A) and Mn channels (B) for Cd in internodes, and the FTR of Ca channels (C) and Mn channels (D) for Cd in nodes and stem bases. Error bars represent SD of four replications. Different letters indicate significant difference (p < 0.05) among treatments and genotypes in same organ according to the Duncan's test.

3.5. The correlations between Cd and other elements in rice organs
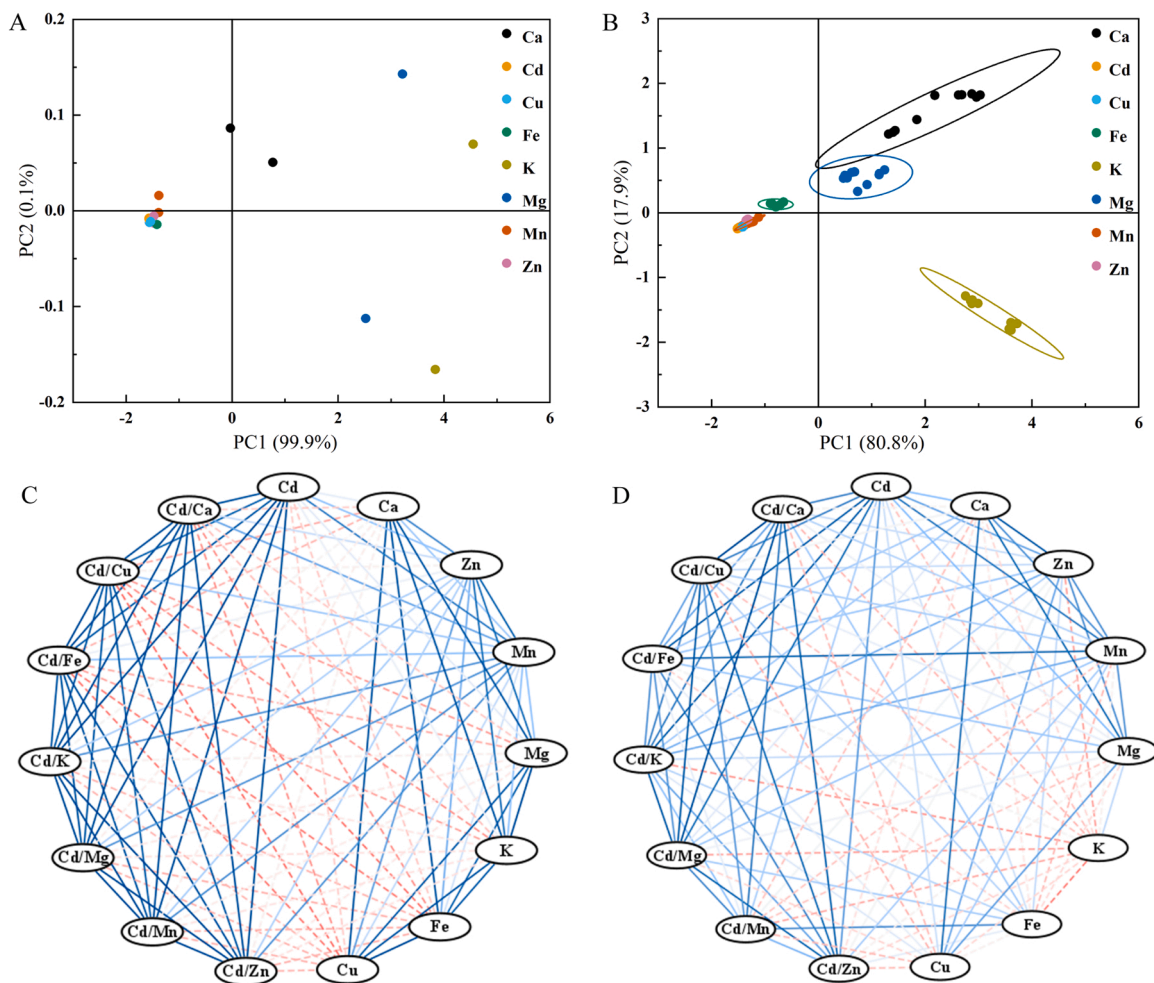
Principal component analysis (PCA) was employed to investigate the relationship between the content of seven essential elements and Cd in grains among treatments with different soil Cd levels. The variance contribution ratio of PC1 reached 99.9%. The load coefficients of K and Mg on PC1 were positive, while those of Cd, Mn, Fe, Zn and Cu on PC1 were negative (Fig. 4A). The scores of Cd, Mn, Fe, Zn and Cu on PC1 were all about − 1.5, which indicated that Cd had the greatest similarity with Mn, Fe, Zn and Cu on PC1, and was closely related to these four essential elements in grains.

The PCA results in vegetative organs among the 5 treatments with different soil Cd levels showed that the variance contribution rates of PC1 and PC2 were 80.8% and 17.9%, respectively. The cumulative contribution rates of PC1 and PC2 reached 98.7%. Among elements, the biggest contribution to PC1 was dominated by K and Ca, followed by Cd, Mn, Cu, Zn and Fe (Fig. 4B). The scores of Cd and Mn on PC1 were both about − 1.45, indicating that Cd content in vegetative organs was closely related to Mn content.

Pearson's correlation analysis showed that Cd content was positively correlated with only Mn content, as well as with the ratios between content of Cd and essential elements (i.e., Ca, Fe, Zn, Cu, K and Mg) in grains (Fig. 4C). However, Cd content in nodes and internodes was positively correlated with the content of Mn, Ca, Zn and Fe (Fig. 4D). Again, there were significant and positive correlations between Cd content and essential elements in nodes and internodes. These results confirmed that the selective permeation of ion channels in plasma memberane for essential elements over Cd made a great contribution to Cd content in grains and vegetative organs.

3.6. Effects of soil Cd and genotypes on the content of amino acids and gene expression

Content of soil Cd significantly influenced the synthesis of amino acids in grains. The content of glutamate (Glu) in grains was remarkably higher than that of other amino acids, and was dramatically decreased with the increase of Cd soil. Mutant fc8 had much higher Glu content than its wild type NPB (Fig. 5A). Meanwhile, the content of other non-

Fig. 4. Principal component analysis of seven mineral elements and Cd content in grains (A) and vegetative organs (B). Pearson's correlation analysis of 8 elements and ratios between Cd and each essential element in grains (C) and vegetative organs (D). Only significant correlations are displayed in the correlations wheels ($P < 0.05$). Positive correlations are denoted by blue lines, negative correlations are denoted by red lines. Darker color of lines means higher R values ($R > 0.51$).

essential amino acids (NEAAs) including Asp and Arg was also significantly declined with Cd content in soil. Relatively, the sensitivity of essential amino acids (EAAs) to Cd stress was less than that of NEAAs. The reduced amount of EAAs with the increase of soil Cd was apparent smaller than that of NEAAs (Fig. 5B). When Cd content in soil increased from 0.1 to 2.4 mg kg^{-1}, the content of EAAs in grains of NPB was

decreased by 25.4%, while NEAAs was decreased only by 8.9%.

Inhibition of soil Cd on the synthesis of NEAAs and EAAs in grains varied with rice genotypes. Mutan fc8 displayed significantly higher NEAAs and EAAs than its wild type NPB, especially when planted in polluted soil with high Cd content (0.9–2.4 mg kg^{-1}). When Cd content in soil increased from 0.1 to 2.4 mg kg^{-1}, the content of NEAAs in grains
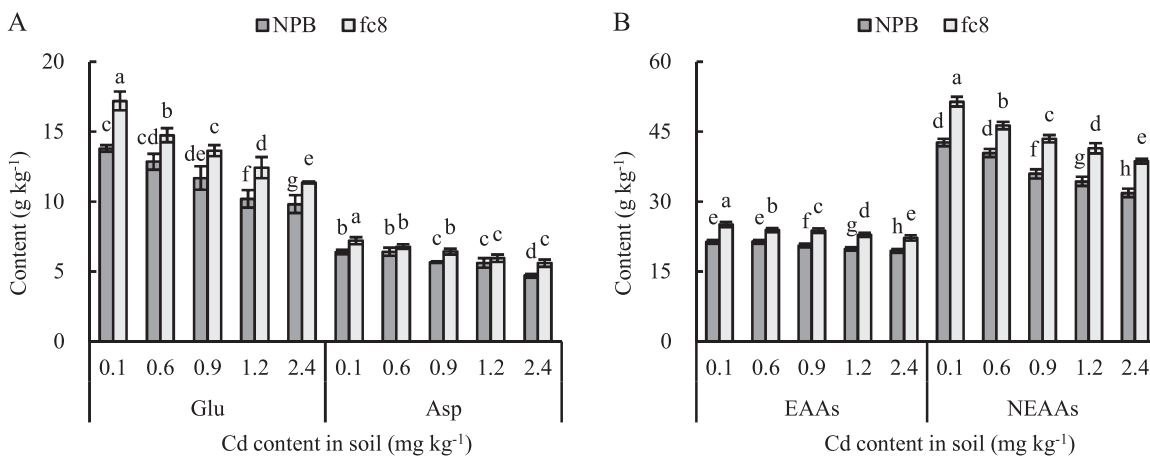
Fig. 5. Effects of Cd content in soil on the content of Glu and Asp (A), and essential amino acids (EAAs) and nonessential amino acids (NEAAs) in grains (B). Error bars represent SD of four replications. Different letters indicate significant difference ($p < 0.05$) among treatments and genotypes according to the Duncan's test.

of fc8 decreased from 51.4 to 38.6 g kg^{-1}, and that of NPB decreased from 42.6 to 31.8 g kg^{-1}.

Several genes encoding Ca channels have been identified in rice, such as *OsGLR*, *OsCNGC*, *OsOSCA*, etc. These genes play vital roles in regulating flux of Ca^{2+} into cells. The expression levels of these genes were determined in the developing grains on the 7th day after anthesis. With the increase of soil Cd content, expression levels of *OsGLR3.1–3.4* in panicles of rice plants were down-regulated. Mutant fc8 displayed higher expression levels than its wild type NPB in *OsGLR3.1–3.3*, especially when soil Cd was in the range of 1.2–2.4 mg kg^{-1} (Fig. 6A,B). However, expression levels of *OsCNGC1,2*, *OsOSCA1.1* and *OsOSCA2.4* were significantly up-regulated with the increase of soil Cd content (Fig. 6C,D). Mutant fc8 displayed significantly higher expression levels of *OsCNGC2*, *OsOSCA1.1* and *OsOSCA2.4*) than its wild type NPB, especially when planted in soil with 0.9–2.4 mg·kg^{-1} Cd.

OsNRAMPs are major transport proteins for Mn and Cd in rice cells. Expression levels of *OsNRAMP1–3* and *OsNRAMP5* in panicles of rice plants were up-regulated by Cd stress. Their expression levels in fc8 were significantly lower than those in NPB when soil Cd was at the same level.

4. Discussion

Rice cells have evolved complicated mechanisms to deal with various stresses. Toxic heavy metals and water deficiency generally stimulate rice cells to actively accumulate inorganic ions, such as K$^+$, Mg^{2+}, and Ca^{2+}, and organic compounds to regulate cell osmotic potential and ensure the normal physiological and biochemical processes [15,20,27]. In Cd-polluted environments, cell walls and vacuoles in the vegetative organs of rice plants become the main sites for storing essential elements and compartmenting Cd ions [28,54,8]. During grain filling period, only about one tenth of the Cd in vegetative organs is remobilized and transferred to the rice grains [48,50,51]. In this study, it was found that the content of essential elements in the internode1 was significantly higher than that in other vegetative organs below it. However, both the Cd content and fault-tolerant rates of Ca and Mn channels for Cd in nodes and stem bases were significantly higher than those in internode1. With the increase of soil Cd levels, the fault-tolerant rate of ion channels for Cd in vegetative organs significantly increased. These results indicate that Cd pollution has a destructive effect on the selective permeability of cell membrane and reduces the functions of filter and interception of vegetative organs to toxic elements like Cd in rice plant. As nodes and stem bases are pivotal organs to recognize and intercept Cd, exploring
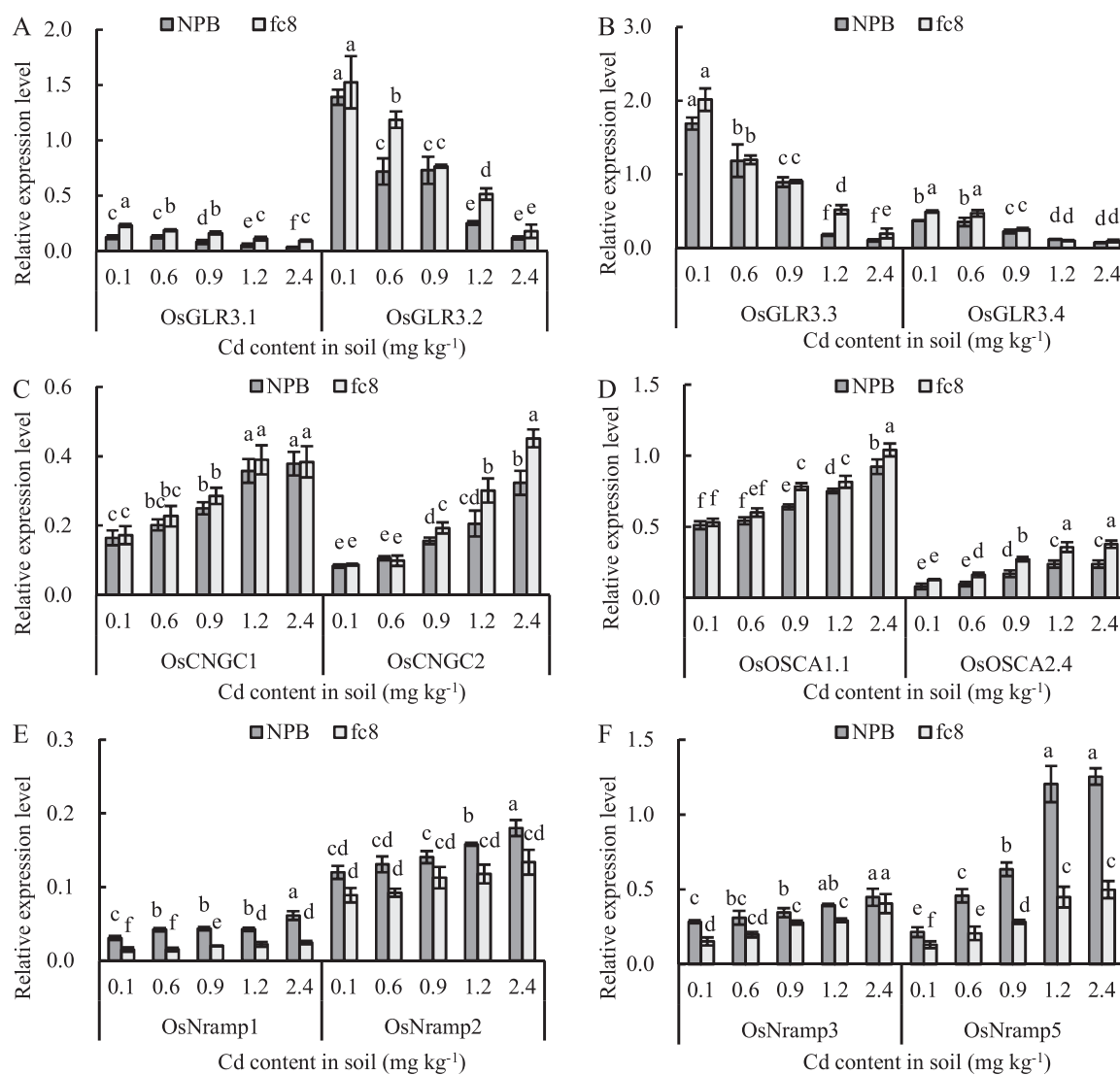
Fig. 6. Relative expression levels of *OsGLR3.1–3.2* (A), *OsGLR3.3–3.4* (B), *OsCNGC1–2* (C), *OsOSCA1.1* and *OsOSCA2.4* (D), *OsNRAMP1,2* (E) and *OsNRAMP3,5* (F) in panicles of rice plants on the 7th day after anthesis. Error bars represent SD of four replications. Different letters indicate significant difference (p < 0.05) among treatments and genotypes for the same gene according to the Duncan's test.

their potential of Cd compartmentalization is an effective means to reduce Cd content in rice grains.

In order to exchange message and energy quickly, many channels are formed on the cell membrane which can selectively absorb and excrete ions. Among them, non-selective cation channels (NSCCs) play a very important role in maintaining the normal metabolic function of cells [4] (Tunc-ozdemir et al., 2013). GLR CHANNELs, CYCLIC NUCLEOTIDE-GATE CHANNELs (CNGCs) and HYPEROSMOLALITY-GATED CALCIUM-PERMEABLE CHANNELs (OSCAs) make a great contribution to Ca transport [9,25,30,52]. Some natural resistance-associated macrophage proteins (NRAMPs) are also involved in the transmembrane transport of Mn, Cd and other ions [22]. Experimental results in this study showed that the expression levels of genes related with OsNRAMP, OsCNGCs and OsOSCAs were significantly up-regulated in the developing grains with the increase of Cd content in them. Meanwhile, the content of Ca, Mn, Fe, Zn and Cd in grains and vegetative organs was also significantly increased with the increase of Cd levels in soil. These results indicate that Cd stress promotes the permeation of these ion channels for Ca^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+} etc., and rice cells alleviate the toxicity of Cd by taking in more micro-nutrients.

Rice vegetative organs are able to convert more than 85% of Cd into insoluble forms, and their amino acids are consumed by chelating Cd ions to eliminate Cd toxicity [23,48]. Glutamate is the most abundant amino acid in plant, and plays a central role in the metabolism of amino acids and proteins. The two carboxyl groups (- coo-) of Glu can bind with Cd to form a stable chelate [48,53]. The results of this study showed that the content of total amino acids, especially Glu and Asp, in rice decreased significantly with the increase of Cd levels in soil. These amino acids may be consumed by cells to form chelates with Cd when rice plants are grown in Cd-contaminated soil.

GLRs are ubiquitous on the plasma membranes of plants, animals, and microorganisms and perform multiple physiological functions [18, 26,36]. In prokaryotes, GluR0 is a dimer consisting of a ligand-binding domain (LBD) and a transmembrane domain (TMD). In eukaryotes, the GLRs have evolved into a tetramer structure (ATD-LBD-TMD-CTD), where an amino-terminal domain (ATD) is extended to the extracellular direction and a carbon-terminal domain (CTD) is added to the intracellular direction (Fig. 7A). The development of ATD and CTD make GLRs more sensitive to environmental signals inside and outside the cell than GluR0. With the perfection of the recognition system to ions and ligands, the flow rate of ions across GLRs is slowed down [17,39,44]. The kinds and arrangement of amino acids in the ATD form different spatial configurations (Fig. 7B) and nanogaps between filter pairs (Fig. 7C). Therefore, ATD acts as a selective filter to regulate the diffusion rates of different ions through this region. The ligand-binding domain is particularly sensitive to small molecules. Some agonists like glutamate and glycine can bind with LBD, and regulate flow rates of cations through channels by altering the nanopore size of TMD [1,14, 26]. Changing the composition of amino acid residues in selective filters and regulating the concentration of extracellular ligands have significant effects on the ion selectivity of channels [1,16,55]. Results in this study showed that the increased Cd pollution in soil led to significant reductions in glutamate content and expression levels of *GLR3.1–3.4*, while significant increase of essential elements such as K, Mg, Ca, Mn, Fe, and Zn. Meanwhile, the ratios between Cd and essential nutrients including Ca, Mn, Fe and Zn decreased significantly in rice and vegetative organs. It seems that Cd pollution promotes the influx of cations through GLRs but down-regulates the selectivity of these channels for essential elements over Cd, which makes it easy for Cd to slip through channels (Fig. 7D). The selective permeation of GLRs for different cations may be destroyed by Cd through two pathways: (1) Cd pollution increases nano-pores in the ATD of GLRs by inhibiting the synthesis of Glu, which allows fast passage of Cd and other cations through this region; (2) Down-regulation of *GLR3.1–3.4* damages the structural integrity of GLRs and decreases the sensitivity of ATD to Cd and other toxic
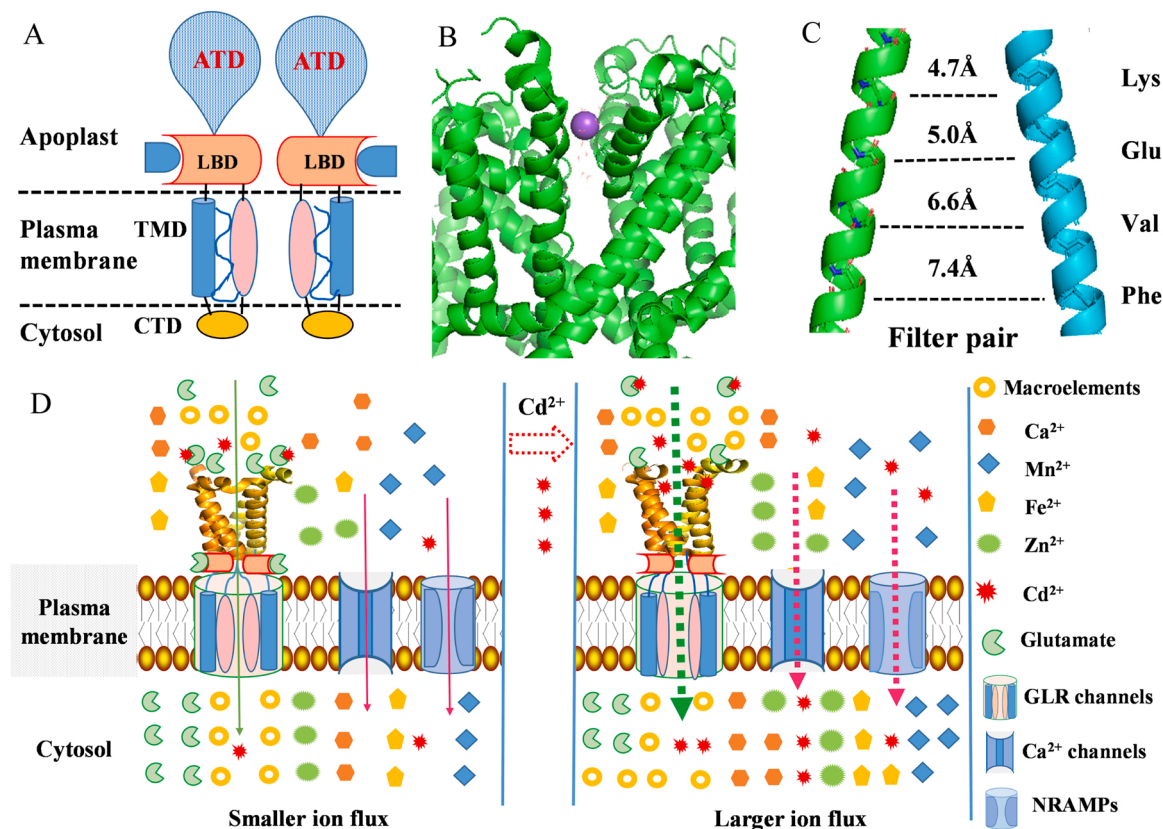

Fig. 7. Schematic of GLR channel structure (A), vertical section (B), distances of nano-pores in the cross section of filter pairs in amino-terminal domain (C), and effects of Cd ions on the selective permeation of channels for essential and toxic elements in plasma memberane of rice cell.

ions. In summary, damage of Cd ions to the integrity of amino-terminal domain in GLRs may directly lead to the selectivity loss of GLRs for Ca^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, etc. over Cd^{2+} in rice plants.

In spite of the destructive effects of Cd on the selective permeation of GLRs, there were significant differences in the fault-tolerant rate of channels for Cd and glutamate content between genotypes tested in this study. Mutant fc8 accumulated significantly less Cd in grains and displayed much lower fault-tolerant rates of channels for Cd in all organs than its wild type NPB. Meanwhile, fc8 had much higher Glu, Ca, Fe and Zn content and higher expression levels of genes related with GLRs and Ca-permeable channels (i.e., OsCNGCs, OsOSCAs) in panicles than NPB. These results indicate that low-Cd-accumulating genotype like fc8 preferentially transports $Ca^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+} and inhibits Cd accumulation by promoting synthesis of glutamate. It is possible to screen rice lines with low-Cd-accumulating characteristics based on selective permeation of GLRs to toxic elements.

5. Conclusions

Results in this study proved that Cd pollution significantly down-regulated the expression of *GLR3.1–3.4*, but up-regulated the expression of genes related with Ca-permeable channels (OsCNGCs and OsOSCAs) and Mn-transport protein OsNRAMP. With the increase of Cd levels in soil, the fault-tolerant rates of ion channels for Cd increased in an S-shaped curve. Both Cd content in soil and rice genotype displayed significant influence on the ratios between Cd and essential elements. When soil Cd increased from 0.1 to 2.4 mg kg^{-1}, mutant fc8 accumulated much less Cd and higher Glu, displayed significantly higher expression levels of *GLR3.1–3.4* and lower fault-tolerant rates of ion channels for Cd than its wild type NPB in grains and vegetative organs. These results indicate that Cd ions destroy the integrity of GLRs by inhibiting amino acid synthesis and down-regulating *GLR3.1–3.4*, which reduces the preferential selectivity of GLRs for Ca^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+} over Cd^{2+} in rice plants.

Environmental Implication

As one of the most toxic heavy metals, cadmium (Cd) accumulation in rice results in a serious of damage to quality and production of rice. Here we first found that heavy Cd pollution in farmland destroyed the structural integrity of glutamate receptor channels (GLRs) in cell membrane by inhibiting amino acid synthesis and down-regulating the expression levels of *GLR3.1–3.4*, which leads to the increase of ion influx but the decrease of preferential selectivity for $Ca^{2+}/ Mn^{2+}/ Fe^{2+}/ Zn^{2+}$ over Cd^{2+} through GLRs in rice cells. Considering GLRs are ubiquitous on the plasma membranes of plants, animals, and microorganisms, the damage of Cd ions to GLRs may take place in other plants. Therefore, the change of selective permeation of GLRs in plant cells can be used for monitoring Cd pollution in environment.

CRediT authorship contribution statement

Xin Zhang: Investigation, Methodology, Resources, Writing – original draft. **Weijie Xue**: Investigation, Methodology, Resources, Writing – review & editing. **Changbo Zhang**: Methodology, Software, Resource. **Changrong Wang**: Conceptualization, Formal analysis. **Yongchun Huang**: Resources, Visualization, Formal analysis. **Yanting Wang**: Investigation, Resources, Visualization. **Liangcai Peng**: Resource, Methodology, Supervision, Project administration, Funding acquisition. **Zhongqi Liu**: Conceptualization, Validation, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131342.

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