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RESEARCH ARTICLE

Differences of cadmium uptake and accumulation in roots of two maize varieties (*Zea mays* L.)

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Abstract

Different maize varieties respond differentially to cadmium (Cd) stress. As the first organ in contact with the soil, the response of the root is particularly important. However, the physiological mechanisms that determine the response are not well defined. Here, we compared the differences in Cd-induced related gene expression, ionic homeostasis, and ultrastructural changes in roots of Cd-tolerant maize variety (XR57) and Cd-sensitive maize variety (LY296), and assessed their effects on Cd uptake and accumulation. Our findings indicate that XR57 absorbed a significantly lower Cd than LY296 did, and that the expression levels of genes related to Cd uptake (*Zm*NRAMP5 and *Zm*ZIP4) and efflux (*Zm*ABCG4) in the root were consistent with the Cd absorption at the physiological levels. Compared with LY296, the lower Cd concentration in the roots of XR57 caused less interference with the ion balance. Transmission electron microscope images revealed that the roots from XR57 exposed to Cd had developed thicker cell walls than LY296. In addition, the large increase *Zm*ABCC1 and *Zm*ABCC2 expression levels in XR57 mediated the appearance of numerous electron-dense granules in the vacuoles present in the roots. As a result, the high Cd tolerance of XR57 is the result of a multi-level response that involves increased resistance to Cd uptake, a stronger capacity for vacuolar regionalization, and the formation of thicker cell walls. These findings may provide a theoretical basis for maize cultivation in Cd-contaminated areas.

Keywords Cadmium stress · Roots · Ionic homeostasis · Ultrastructure · Gene expression · Non-invasive micro-test technology

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Highlights

1. Roots of Cd-sensitive maize varieties accumulate more Cd than those of Cd-tolerant varieties.
2. Cd tolerance is associated with *Zm*NRAMP5 and *Zm*ZIP4 suppression, as well as *Zm*ABCG4 elevation.
3. Cd-stress-induced upregulation of *Zm*ABCC1 and *Zm*ABCC2 expression boosts vacuolar regionalization.
4. Roots of Cd-tolerant maize varieties respond to Cd stress by developing thicker cell walls and larger vacuoles.

Novelty statement We investigated the difference in performance of two maize varieties subjected to Cd stress by combining measurements of the expression levels of genes associated with Cd tolerance with observations of changes in the root ultrastructure. Our study provided us with an in-depth understanding of the mechanisms governing Cd tolerance in Cd-tolerant maize varieties.

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Introduction

Heavy metal contamination of agricultural soil poses a serious threat to global crop production, and for this reason, it has attracted widespread public attention (Zhou et al. 2019; Zhang et al. 2020). Cadmium (Cd) is a toxic heavy metal for which no known physiological functions have ever been detected in plants. Nevertheless, Cd seriously impedes crop growth and yield formation, and poses a threat to human health through the food chain (Li et al. 2020). Cd is extremely soluble in water, has high mobility in the soil–plant system, is easily absorbed by plant roots, and is readily accepted by the xylem for transportation to the shoot (Wu et al. 2021), and consequently, the high Cd absorption and accumulation potential of maize (Xu et al. 2014), which represents the most important cereal crop produced by the agricultural sector in China, has made it one of the main sources of human Cd intake today. For this reason, guaranteeing food safety in the future urgently requires the development of strategies aiming at reducing the Cd accumulation in maize grain.

It has been reported that the accumulation of Cd is facilitated by several major transport processes that are essential for supporting normal plant functions, including (1) uptake by roots; (2) xylem loading, followed by transportation from the root system to the shoot; (3) redistribution by Cd nodes via inter-vascular transfer; and (4) redistribution from leaves to grains via the phloem (Wu et al. 2015; Ismael et al. 2019). Among these processes, Cd uptake by roots is the primary determinant of the level of Cd accumulation in grains (Rodda et al. 2011; Xiao et al. 2021). One of the proteins involved in the Cd, Mn, and Fe uptake by the roots system of maize is NRAMP5 (Sasaki et al. 2012), which serves as cell membrane metal transporters and are located on the outside of the plasma membrane. It has been reported that responses involving downregulation of NRAMP5 expression significantly reduced Cd uptake by roots, which ultimately resulted in a reduced Cd content in stems and grains (Ishimaru et al. 2012; Ishikawa et al. 2012). Another important factor associated with the Cd uptake by roots is the mediation of cation uptake systems by zinc-regulated transporter-like proteins (ZIP) and iron-regulated transporters (IRTs) (Khan et al. 2019).

Irrespective of the channel involved, the uptake of Cd has a number of detrimental effects upon a plant's physiology: it limits the uptake of essential nutrients, interferes with essential physiological functions, and inhibits the activity of key metabolic enzymes (Lavres et al. 2019). So far, strategies aimed at counteracting Cd toxicity have explored various approaches including metal efflux enhancement, cell wall retention, cytoplasmic sequestration, and vacuole compartmentalization (Zuzana et al. 2018; Tao et al. 2020; Huang et al. 2021b). The response mechanisms involved are all manifested in the ultrastructure of the cell; typically, they induce thickening of the cell wall, precipitation in the cytoplasm, and changes in the number and volume of vacuoles. With respect to metal efflux enhancement, it has been suggested that since the *Zm*ABCG1, *Zm*ABCG3, *Zm*ABCG4, and *Zm*ABCG8 genes are in the same evolutionary branch as the class II Arabidopsis PDR known to be involved in the Cd efflux mechanisms in maize, developing maize varieties exhibiting responses that upregulate those genes might strengthen their Cd tolerance (Kim et al. 2007; Pang et al. 2013). Previous research has also indicated that a plant's Cd tolerance is closely related to its ability to accumulate Cd in vacuoles (Huang et al. 2021b), which is regulated by HMA3, one of the P_{1B}-ATPase genes encoding a transporter protein on the vacuole membrane. HMA3 is mainly expressed in the root and has the function of transporting Cd^{2+} from the root cytoplasm to vacuoles, which effectively isolates and sequesters it instead of being transported to the shoot (Ueno et al. 2011; Cui et al. 2018). Free Cd^{2+} ions in cells can also bind to glutathione and phytochelatin, enabling proteins encoded by the *At*ABCC1 and *At*ABCC2 genes to mediate the vacuolar regionalization of the resulting chelates, thereby protecting the protoplasm (Park et al. 2012). It should also be noted that not all studies in this area are in agreement about the relative importance of factors influencing Cd accumulation in crops: a study by Wu et al. (2015) highlighted that xylem transport played a decisive role in the aboveground Cd accumulation by two oilseed rape varieties, while root uptake contributed little. Additionally, Zhang et al. (2020) pointed out that the level of Cd accumulation in different wheat varieties was limited by nodes, especially those connected to the spike.

Most previous studies investigating the mechanism governing Cd translocation to explain differences in Cd accumulation between varieties have been limited to observing changes in either ultrastructure or the gene expression levels in isolation, especially with respect to maize. In our study, however, we focused on root response in combination with non-invasive micro-test (NMT), transmission electron microscopy observations, and expression of relevant genes. Our study had the following objectives: (1) investigate the root causes explaining the differences between the Cd uptake levels exhibited by the roots of the two maize varieties included in our experiment and (2) clarify the mechanisms endowing Cd-tolerant maize varieties with a high degree of Cd tolerance. The results of our study might be used to facilitate future evaluations of Cd-tolerant maize varieties and help to develop approaches aimed at the production of safe maize crops in Cd-contaminated areas.

Materials and methods

Experimental subjects and treatments

After screening 50 maize varieties, we selected the Cd-tolerant variety Xinrui 57 (XR57) and the Cd-sensitive variety Liyuan 296 (LY296) as experimental subjects (Table S1, S2).

We prepared a number of randomly selected maize seeds for hydroponic cultivation by sterilizing them in a 10% H_2O_2 solution for 30 min, rinsing them thoroughly with deionized water, submerging them in a 0.5 mmol L^{-1} $CaSO_4$ solution and leaving them in the dark for 3 days at 30 °C to germinate. After germination, the seedlings were transferred to a greenhouse that was kept at 30 ℃ during the day and at 25 ℃ during the night, at a relative humidity of 70%. After 7 days, randomly selected seedlings with similar height were transplanted to black plastic hydroponic culture boxes containing 25 L of nutrient solution. In order to allow the seedlings to adapt to hydroponic cultivation, we started off with a half-strength solution, switching to a full-strength solution after the trefoil stage. The nutrient solution contained the following nutrients (at the parenthesized concentrations in μmol L^{-1}): $Ca(NO_3)_2{\cdot}4H_2O$ (4000); KNO_3 (6000); $MgSO_4{\cdot}7\ H_2O$ (2000); $NH_4H_2PO_4$

(2); H_3BO_3 (46); NaCl (53); $MnSO_4{\cdot}4H_2O$ (5); $ZnSO_4{\cdot}7H_2O$ (0.9); $CuSO_4{\cdot}5H_2O$ (0.4); $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.02); and EDTA-Fe (150). The culture medium was aerated using an air pump, and the nutrient solution was replaced every 3 days. We subjected the experimental subjects to the following two treatments, each including five replicates: (1) a CK treatment (control treatment), which deployed the nutrient solution without adding any Cd, and (2) a Cd treatment following the method suggested by Fioroto et al. (2020), which consisted of infusing the nutrient solution with Cd^{2+} through the addition of 10 mg L^{-1} of $CdCl_2{\cdot}2.5H_2O$ to the nutrient solution. During both treatments, the pH of the nutrition solution was adjusted to a value between 5.5 and 6 by adding 1.0 mol L^{-1} HCl or NaOH solutions as required. The Cd treatment started at the trefoil stage. The net Cd^{2+} flux in the subjects' roots was measured 24 h after commencing the treatment, while the other indexes were measured at the jointing stage.

Measurement of the total root length and analysis of the root activity

We removed a number of randomly selected seedlings from the cultures and measured the root length and shoot height. Next, we removed the root and divided it into primary, seminal, and crown roots. Each part was scanned with an Epson scanner (Epson, perfection v700 photo), and the resulting images were analyzed using the root analysis software WinRhizo Pro 2016 (Regent instruments, Quebec, Canada) to determine the total root length.

The root activity was measured using the modified triphenyl tetrazolium chloride (TTC) method proposed by Qi et al. (2012).

Measurement of the concentration of cadmium and nutrients

We finely powdered the roots and shoots, and separately digested each part in an electric digester at 200 ℃ using an acid mixture comprised of HNO_3 and $HClO_4$ at a ratio of 7:1 (v/v). The concentration of Cd, Mg, Ca, Fe, K, and Na in the sample solutions was measured using an atomic absorption spectrophotometer (NovAA400, Jena, Germany). The measured Cd concentration in each plant part was combined with the dry weight to calculate the Cd accumulation in that part.

Measurement of the Cd^{2+} influx and efflux associated with the root

To measure the Cd^{2+} influx and efflux associated with the roots of our test subjects, we used an NMT system (NMT100-IMXY, Younger, USA). Details about the method we used to determine the Cd^{2+} flux are presented in Supporting information (S1.1).

Observation of roots transmission electron microscopy

We prepared randomly selected root fragment samples by fixing them for 8 h at 4 ℃ in a vacuum tube with a 2.5% (v/v) glutaraldehyde solution (pH = 7.4), and subsequently post-fixing them for 4 h with a 1% (w/v) aqueous osmium tetroxide solution. The resulting preparations were dehydrated through an ascending series of ethanol gradients (comprising 45, 55, 75, 85, 95, 100, and finally 100% solutions) and then saturated for 12 h using an embedding agent (spur 812). The embedded samples were sectioned into ultrathin sections with an ultra-microtome (Leica EM UC 7, Germany) using a diamond knife, and the resulting slices were placed on copper grids. Finally, the sections were imaged using a TEM (JEOL JEM1400PLUS JPN) at 120 kV, and ImageJ was used to measure the cell wall thickness by inspection.

Measurement of gene expression levels

Root tips were subjected to total RNA extraction, and the extracted RNA was subsequently used to synthesize cDNA. The resulting cDNA was subjected to an RT-PCR analysis according to the method proposed by Cui et al. (2018). The primers of gene related to Cd uptake can be found in Table S3.

Statistical analysis

All data analyses were performed using SPSS 21.0, SigmaPlot 14.0 and Origin95 software. We also performed a one-way ANOVA followed by Duncan's test to analyze the significant differences between the treatments at a significance level of $P \leq 0.05$ and $P \leq 0.01$.

Results and discussion

Effect of Cd stress on the growth of maize

The root is the first plant organ to come into direct contact with Cd present in the soil it grows in, and it is safe to assume that morphological responses enabling the root to compete for resources more effectively will facilitate the plant's survival under adverse conditions (Lux et al. 2011; Huang et al. 2021a; Wei et al. 2021). Our study tried to shed a light on the mechanisms involved in responses to Cd contamination through a quantitative analysis of the morphological changes in the roots of plants exposed to Cd stress. Our results show that the root morphology of LY296 subjected to the Cd treatment was more strongly affected than that of XR57, and the total length of the primary, seminal, and crown roots of LY296 exposed to Cd stress was

11.36%, 38.02%, and 61.42% shorter than that of the same root sections from the control group, whereas the shortening of the corresponding root sections from XR57 was 17.80%, 28.90%, and 51.37% (Fig. 1F). Our findings are in line with the results obtained by Qin et al. (2018) from their studies on wheat. The significantly shorter total root length is probably due to Cd inhibiting lateral root growth (Kollárováa et al. 2018; Wang et al. 2021): Cd contamination of the soil creates an environment that exposes root cells to oxidative stress, which makes them prone to cell membrane damage, and, in cases of severe contamination, cell death, both of which typically result in stunted root growth (Riaz et al. 2021). Our study indicated that exposure to Cd significantly affected the shoot height of both varieties included in our experiment: the shoot height of XR57 subjected to Cd was on average 17.57% smaller than that of CK, while the shoot height of LY296 was 29.03% smaller (Fig. 1B). With respect to the root length, we did not observe a significant differences between the various treatments for either variety (Fig. 1D). The dry weight of shoots from XR57 subjected to the Cd treatment was 33.88% lower than that of shoots from the CK treatment, and for LY296, the corresponding reduction was 42.70% (Fig. 1C). With respect to the dry weight of roots, the corresponding reduction amounted to

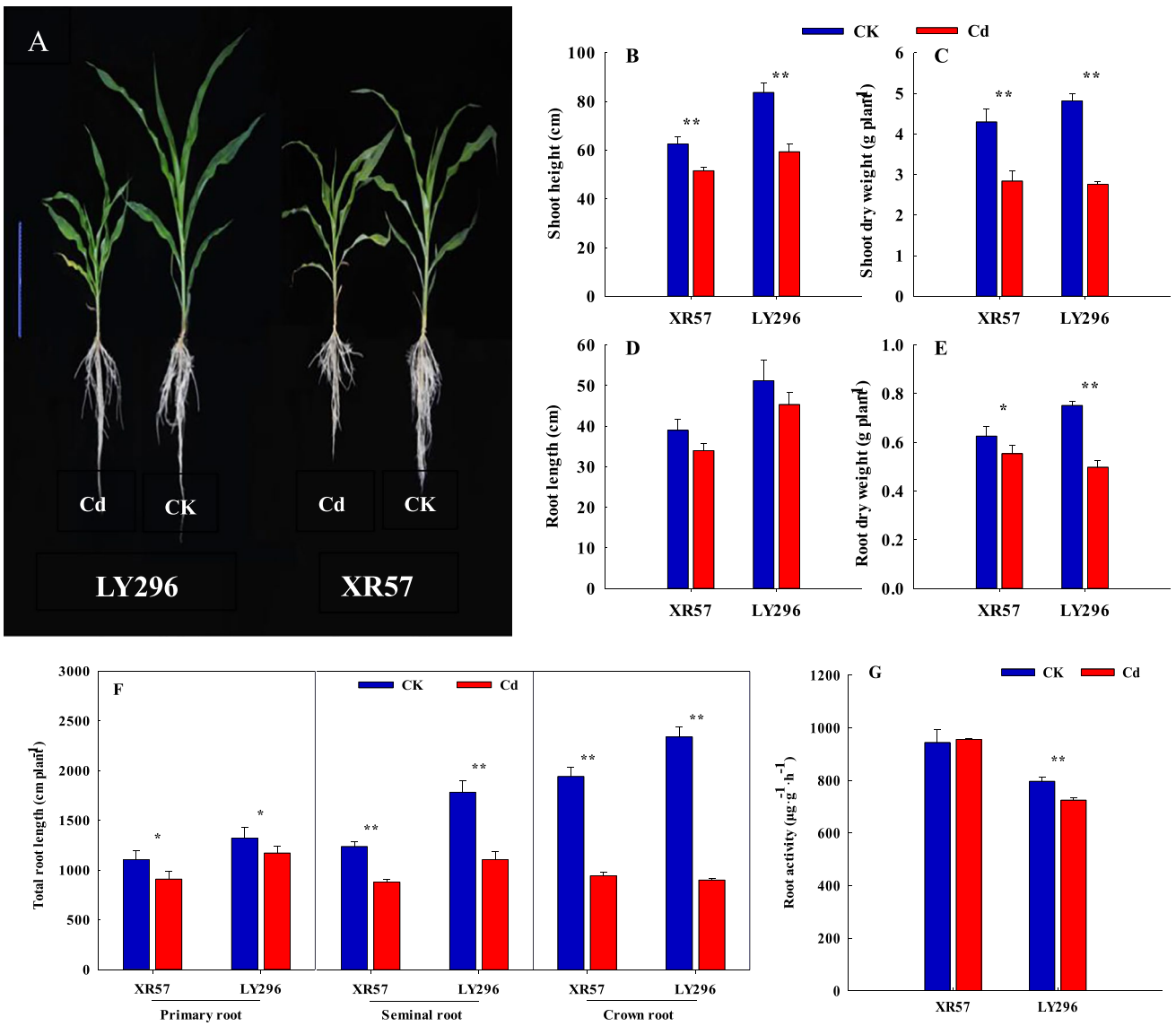


Fig. 1 Effect of Cd stress on the growth of the two maize varieties, broken down by treatment and variety. **A** Images of representative plants of both varieties after having been subjected to the treatments. **B** Shoot height, where the length of the blue bar shown is 30 cm. **C** Shoot dry weight. **D** Root length. **E** Root dry weight. **F** Total root length. **G** Root activity. Error bars are shown as mean ± SD. Bars being annotated with * and ** indicate they are significantly different between treatments of the same variety at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to one-way ANOVA followed by Duncan's test

Table 1 Nutrient concentration in roots and shoots from experimental plants, broken down by variety and treatment. Data shown as means of three replicates ± SD. Values being annotated with different lowercase letters for the same variety indicate they are significantly different between treatments at $P \leq 0.05$ according to one-way ANOVA followed by Duncan's test

Nutrient	Locus	Concentration			
		XR57		LY296	
		CK	Cd	CK	Cd
Na (mg g^{-1})	Root	1073.53 ± 23.4 b	1227.75 ± 47.1 a	1068.57 ± 51.6 a	1093.49 ± 41.9 a
	Shoot	524.12 ± 26.0 a	405.20 ± 8.3 b	523.05 ± 14.4 a	422.84 ± 13.8 b
K (mg g^{-1})	Root	9.86 ± 0.2 b	14.91 ± 0.3 a	12.81 ± 0.6 b	16.83 ± 0.5 a
	Shoot	28.36 ± 0.8 a	27.98 ± 0.3 a	25.18 ± 0.5 a	26.02 ± 0.7 a
Ca (μg g^{-1})	Root	321.67 ± 26.9 a	234.46 ± 27.8 b	360.84 ± 10.1 a	153.28 ± 10.2 b
	Shoot	135.30 ± 11.1 a	74.46 ± 2.2 b	222.93 ± 2.9 a	65.15 ± 2.4 b
Mg (μg g^{-1})	Root	150.69 ± 1.3 b	177.08 ± 3.1 a	152.64 ± 8.6 a	161.01 ± 8.0 a
	Shoot	172.71 ± 4.3 b	215.08 ± 2.7 a	189.86 ± 3.4 b	209.31 ± 5.5 a
Fe (μg g^{-1})	Root	2717.10 ± 30.1 a	2791.58 ± 38.9 a	2557.31 ± 86.9 b	2972.16 ± 25.0 a
	Shoot	107.90 ± 3.4 a	87.41 ± 1.1 b	86.59 ± 3.7 b	118.88 ± 5.6 a

12.70% for XR57 and 33.33% for LY296 (Fig. 1E). Evidently, XR57 are more resistant to Cd stress in terms of biomass formation and plant height than LY296. With respect to the plant root activity, which comprises the plant's capacity for absorbing, synthesizing, oxidizing, and reducing nutrients during metabolic activity (Liu et al. 2014). Our results indicated that LY296 subjected to the Cd treatment exhibited a significantly lower level of activity than the CK treatment (Fig. 1G), suggesting that exposure to Cd impairs the root function, which in turn inhibits plant growth.

Effect of Cd stress on the nutrient uptake of maize

The reduction of biomass is largely attributed to the interference of Cd with other ions, such as ion uptake and translocation, stronger affinity for Cd, and substitution of binding sites within proteins (Lavres et al. 2019; Wang and Björn 2014).

Potassium (K^+) is a highly mobile ion that plays an important role in the maintenance of cell turgor, as well as in processes that control stomatal movement and gas exchange (Gupta and Seth 2022). The results of our experiment indicate that the K concentration in the roots of both varieties subjected to the Cd treatments was significantly higher than the control groups, especially with respect to XR57 (Table 1). Previous researchers have reported a reduced Cd uptake by roots after the exogenous application of K channel inhibitors (TEA +) (Chen et al. 2018), which can be explained from the fact that if the K concentration in roots is elevated, K^+ is able to compete with Cd^{2+} during the uptake process, thereby enhancing the Cd tolerance of the crop (Javed et al. 2017). On the other hand, it has also been suggested that the presence of Cd^{2+} inhibits K uptake, which may be influenced by genetic factors of the variety as well as Cd concentration (Sun et al. 2021). Additionally, the presence of Cd in the roots of XR57 was also associated with an elevated Na concentration (Table 1). This finding is in agreement with the results reported by Han et al. (2023), who suggested that the presence of Na^+ inhibits Cd uptake and transport.

The presence of Cd significantly increased the Mg concentration in the roots and shoots of XR57, while with respect to LY296, only the shoots were affected (Table 1). Previous studies have indicated that Mg^{2+} are able to compete with Cd^{2+} in terms of uptake through ion channels or membrane surface ion ligands, and that they can also

Table 2 Cd intake indicators in various parts of XR57 and LY296 after having been subjected to the Cd treatment. Data shown as means of three replicates ± SD. Values being annotated with different lowercase letters for the same variety indicate they are significantly different between treatments at $P \leq 0.05$ according to one-way ANOVA followed by Duncan's test

Indicator	XR57	LY296
Shoot Cd concentration (μg g^{-1} of dry weight)	200.99 ± 6.83 b	231.49 ± 10.06 a
Root Cd concentration (μg g^{-1} of dry weight)	1895.58 ± 29.59 b	2448.35 ± 39.96 a
Cd transport coefficient (shoot Cd concentration/root Cd concentration)	0.11 ± 0.002 a	0.09 ± 0.004 b
Shoot Cd accumulation (μg $plant^{-1}$)	570.82 ± 19.39 b	634.29 ± 27.55 a
Root Cd accumulation (μg $plant^{-1}$)	1049.96 ± 16.39 b	1219.77 ± 19.91 a
Total Cd accumulation (μg $plant^{-1}$)	1626.19 ± 27.23 b	1854.06 ± 29.25 a
Relative Cd accumulation (shoot Cd accumulation/root Cd accumulation)	0.55 ± 0.01 a	0.52 ± 0.03 a

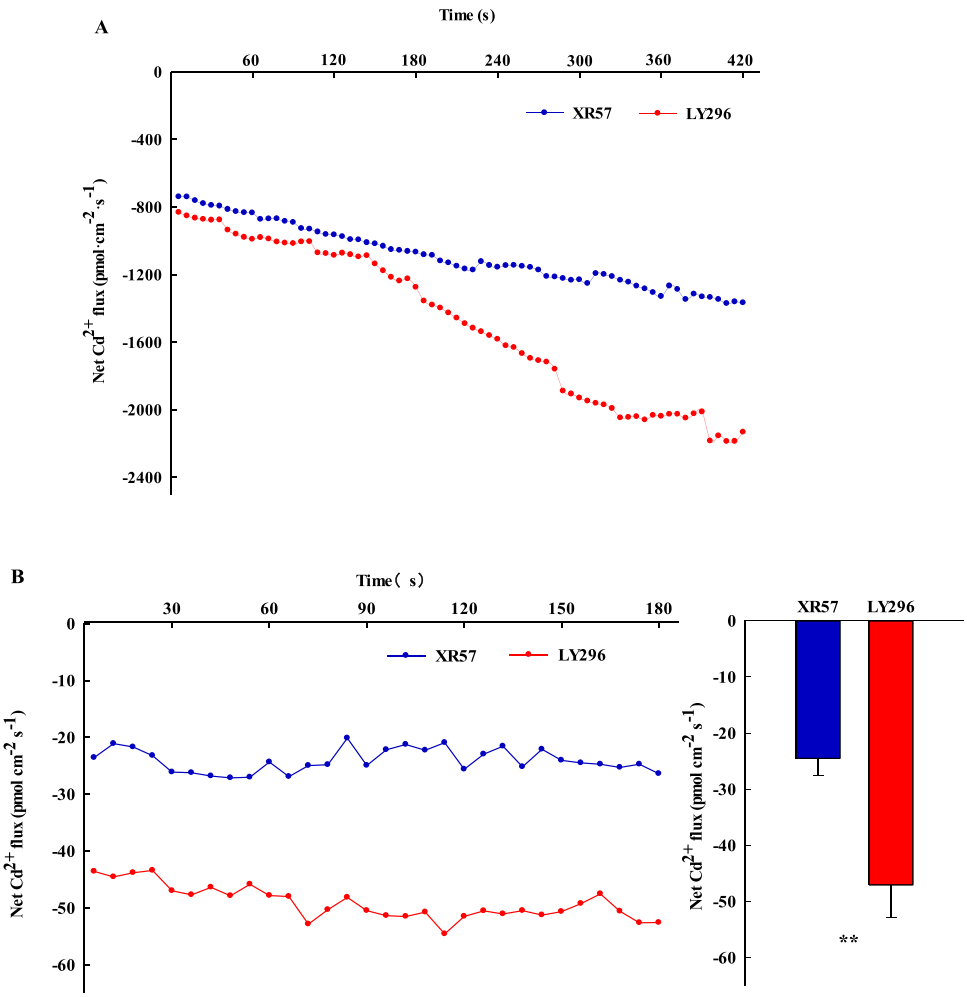


Fig. 2 Cd^{2+} flux in the roots of plants subjected to Cd stress, broken down by variety. **A** Net flux measurements at 6 s intervals. **B** Average net flux over the measurement period. Error bars are shown as mean ± SD ($n = 5$). Bars being annotated with * and ** indicate they are significantly different between varieties at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to one-way ANOVA followed by Duncan's test

enhance the synthesis of cell walls (Tang and Luan 2017). Hence, the Mg concentration in XR57 subjected to Cd stress was significantly elevated, which may have impeded Cd uptake by the roots. By contrast, the Ca concentration in both the roots and shoots of XR57 and LY296 subjected to the Cd treatment was significantly lower than the control groups, where it should be noted that the reduction was more pronounced in LY296 (Table 1). This finding suggests that exposure to Cd inhibits Ca uptake, which ultimately results in plants malnutrition. Han et al. (2023) showed that Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} in roots all inhibit the uptake and transport of Cd. Among those ions, Ca^{2+} is the strongest inhibitor, which can be explained from the fact that Cd^{2+} and Ca^{2+} ions have a similar radius. Hence, an excessive Cd concentration in the root inhibits the intracellular flow of Ca and competes for the uptake sites responsible for transporting nutrients to the shoot (Andosch et al. 2012; Javed et al. 2017). Of course, the reverse is also true, and the exogenous application of Ca can reduce Cd concentration in plants and help to sustain the physiological processes in the growth and development of plants, for example photosynthesis and respiration (Zeng et al. 2017). We also found elevated Fe concentrations in plants exposed to Cd stress. In LY296, these may have been caused by an upregulation of *Zm*ZIP4 expression (Table 1). Muneer et al. (2014) have pointed out that in cases of Cd stress, the addition of appropriate amounts of Fe can increase the expression of phytochelatin-related genes and alleviate the ionic disorders.

Cd uptake and Cd accumulation of maize exposed to Cd stress

As shown in Table 2, the Cd concentration in shoots from LY296 subjected to the Cd treatment was 15.17% higher than XR57, and in the roots, it was 29.16% higher. Similarly, the Cd accumulation in shoots and roots from LY296 subjected to the Cd treatment was 11.12% and 16.17% higher than XR57, respectively, and the total Cd accumulation was 14.01% higher. These findings suggest that LY296 are more prone to Cd accumulation than XR57. Typically, plant roots readily absorb heavy metals, before passing them on to xylem conduits for transportation to shoot. Our results

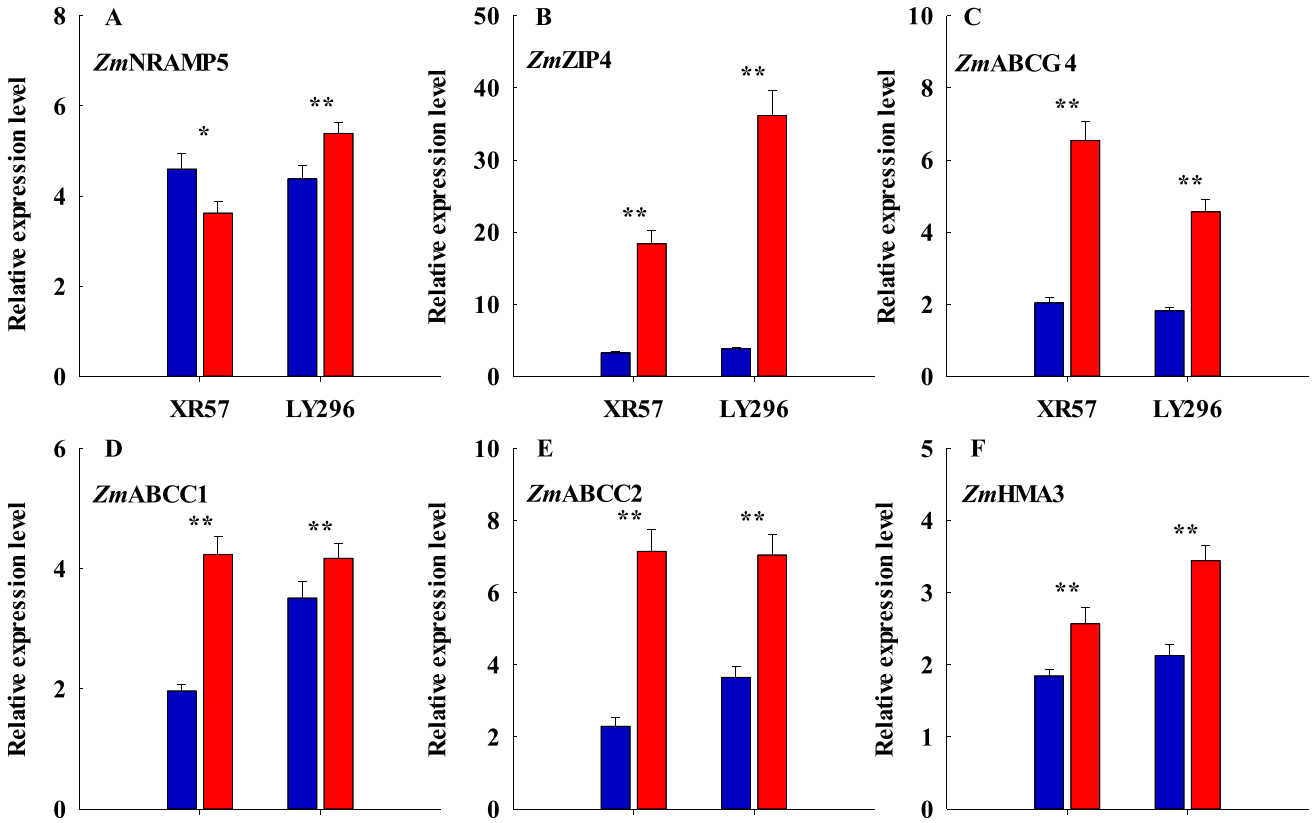
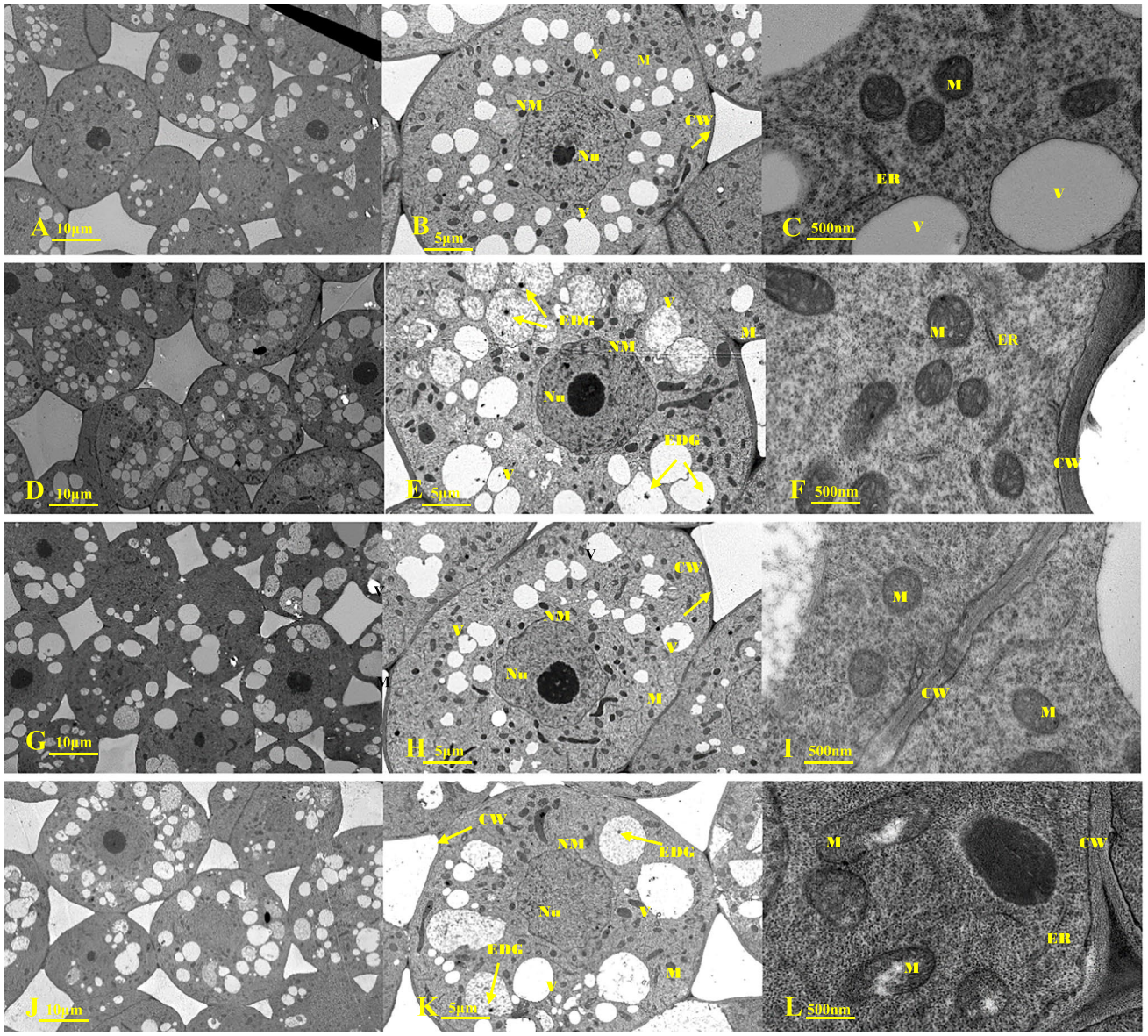


Fig. 3 Effect of Cd stress on Cd-related gene expression levels, broken down by variety and treatment. **A** Relative expression level of *Zm*NRAMP5. **B** Relative expression level of *Zm*ZIP4. **C** Relative expression level of *Zm*ABCG4. **D** Relative expression level of *Zm*ABCC1. **E** Relative expression level of *Zm*ABCC2. **F** Relative expression level of *Zm*HMA3. Error bars are shown as mean ± SD ($n = 5$). Bars being annotated with * and ** indicate they are significantly different between treatments of the same variety at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to one-way ANOVA followed by Duncan's test

show that the amount of heavy metals stored in the roots is higher than shoot, which is consistent with previous findings by Rizvi and Khan (2018). This might be due to the fact that although roots contain a large amount of metal transporter proteins that enhance Cd^{2+} extraction, the transport process of Cd to shoot is affected by factors like the Cd concentration in the culture solution, which causes the amount of Cd transported to the shoot to be much lower than that taken up by the roots (Wang et al. 2023).

Although the Cd transport coefficient of XR57 was significantly higher than LY296, the higher shoot biomass of the former diluted the Cd concentration, resulting in no significant difference in the relative Cd accumulation between the two varieties (Table 2). It has been reported that the Cd content of shoots is primarily determined by the process by which Cd is transported from the root to the shoot (Wu et al. 2015). In contrast to previous studies, however, we found little difference between the translocation capacity of the two varieties included in our study, which suggests that the key process perturbing the normal growth of plants under Cd stress is the root uptake of Cd. LY296 exhibited a higher level of Cd uptake than XR57 (Fig. 2), resulting in the Cd accumulation in the shoots of the former being significantly higher than that in the latter, and its growth being more severely limited (Table 2; Fig. 1). It is also notable that the differences in the Cd uptake and accumulation rates of XR57 and LY296 were directly proportional to their Cd tolerance. In addition, the lower net Cd^{2+} influx and Cd concentration we measured in the roots of XR57 were consistent with the relatively high biomass and root activity (Figs. 1 and 2). Zhang et al. (2020) have shown that the Cd concentration in wheat grain depends on the Cd concentration in the roots and shoots of the wheat. Therefore, we speculate that the higher Cd uptake rate of the roots of LY296 might boost the Cd accumulation in grains, thereby increasing the potential risk of Cd entry into the food chain. To increase our confidence level, however, this claim requires further verification.

Many literatures have confirmed that NRAMP5 is the main transporter for Cd to enter root cells (Sasaki et al. 2012; Yue et al. 2016; McLaughlin et al. 2020; Ma et al. 2021). In addition, Cui et al. (2018) have noted that the increase in Cd uptake induced by elevated NRAMP5 expression levels was


A 10μm
B 5μm
C 500nm
D 10μm
E 5μm
F 500nm
G 10μm
H 5μm
I 500nm
J 10μm
K 5μm
L 500nm
CW
M
NM
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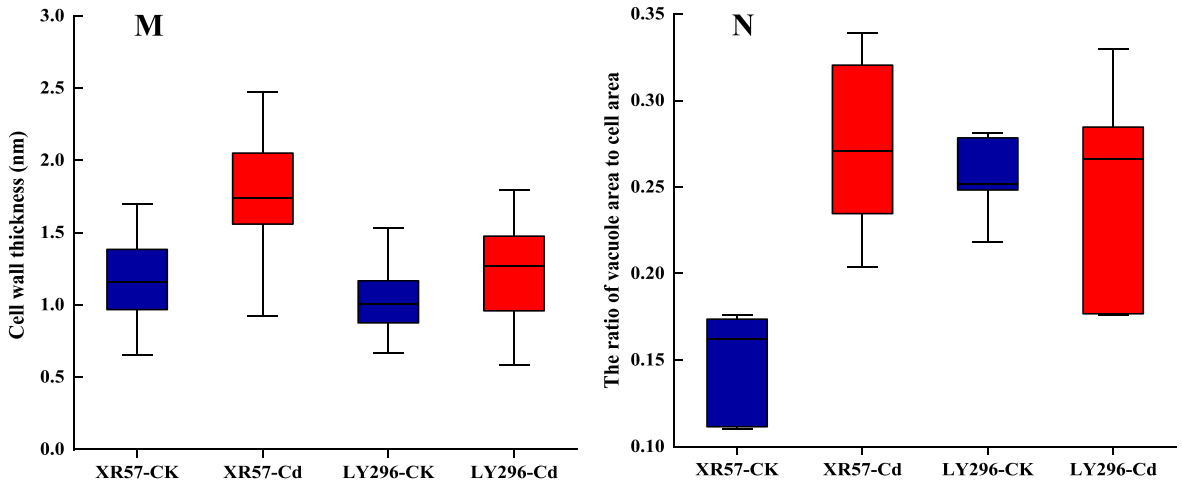
M
Cell wall thickness (nm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
XR57-CK
XR57-Cd
LY296-CK
LY296-Cd
N
The ratio of vacuole area to cell area
0.35
0.30
0.25
0.20
0.15
0.10
XR57-CK
XR57-Cd
LY296-CK
LY296-Cd

◂**Fig. 4** Effect of Cd stress on the ultrastructure of the root meristem. **A–C** Roots from XR57 subjected to the CK treatment; **D–F** roots from XR57 subjected to the Cd treatment; **G–I** roots from LY296 subjected to the CK treatment; **J–L** roots from LY296 subjected to the Cd treatment; **M** thickness of root cell walls; **N** ratio of the vacuole area to the cell area. Abbreviations: CW, cell wall; ER, endoplasmic reticulum; EDG, electron-dense granules; M, mitochondria; Nu, nucleus; NM, nuclear membrane; V, vacuole

higher in rice than in wheat or maize. In our study, we found that exposure to Cd stress induced a significant upregulation of the expression level of *Zm*NRAMP5 in LY296, whereas in XR57, it induced a downregulation (Fig. 3). This effect might go a long way towards explaining the differences in Cd uptake exhibited by the roots of the two varieties we studied. However, after prolonged exposure to Cd, the NRAMP5 expression level exhibited a decreasing trend; we suspect that the decrease might be directly proportional to the duration of the exposure. It should be noted that the elevated NRAMP5 expression level we observed could be due in part to a mechanism to compensate for mineral nutrient deficiencies caused by competition with Cd for root uptake transport proteins (Romè et al. 2016). ZIP4 is another gene with an important regulatory effect upon the Cd uptake by the root (Zeng et al. 2017). Our results show that exposure to Cd induced a significant upregulation of *Zm*ZIP4 expression, which is consistent with the high Cd concentration we found in LY296 subjected to the Cd treatment (Fig. 3). Since *Zm*ABCG4 belongs to the same evolutionary branch as *At*PDR8, an ABC transporter protein localized on the root plasma membrane that mediates Cd efflux, it is safe to assume that the upregulation of its expression level enhances the Cd tolerance of maize (Pang et al. 2013). Compared to LY296, the expression of *ZmABCG4* in XR57 roots increased more than that in the control (Fig. 3), which is conducive to reduce Cd concentration.

Effect of Cd stress on the ultrastructure of root cells

It has been well documented that excessive Cd accumulation in plants inhibits growth, disrupts the cellular ultrastructure, and alters the primary and secondary metabolic processes in plants (Zuzana et al. 2018; Huang et al. 2021a). In our study, we found that the cells making up the root tips in the CK groups exhibited a typical ultrastructure. The plasma membrane was spread out and uniform in shape at all loci, as shown in Fig. 4A, B, G, and H. We also found a healthy number of endoplasmic reticula, mitochondria, and vacuoles in the cytoplasm, as shown in Fig. 4C and I. In contrast, the cells making up the root tips of both varieties subjected to the Cd treatment exhibited obvious symptoms of Cd poisoning, such as mitochondrial vacuolation, indistinct nuclear membrane, as shown in Fig. 4D–F and J–L.

As the first barriers to metal ions coming into contact with a plant, the cell walls of root cells play a crucial role in protecting the protoplasm against toxic metals (Ovečka and Takáč 2014). In our study, we found that the cell walls of the roots from both varieties exposed to Cd stress were thicker than those of the roots from the control groups (Fig. 4M). The thickening effect, which was especially notable in the roots from XR57, enhances Cd tolerance by facilitating the fixation of free Cd^{2+}. This is in agreement with findings by Yang et al. (2015) and Ramos et al. (2002), who reported that Cd immobilization in the thickened cell walls of Salix matsudana Koidz and lettuce exposed to Cd contributed to the Cd tolerance of those crops. Liu et al. (2004) suggested that the obstruction to metal ions posed by thickened cell walls is associated with increased peroxidase activity and the effect of cell wall lignification. In our study, we found that the mitochondrial structure of LY296 roots exposed to Cd stress was severely damaged: it exhibited swelling of the cristae and even vacuolization, as evident from Fig. 4L, similar to the Cd-induced mitochondrial swelling observed in cucumber by Khan et al. (2013). It should be noted that because mitochondria are one of the main sites of reactive oxygen species production, disruption of their structure may induce oxidative stress in plant cells (Zhao et al. 2018).

Regionalization of Cd in the vacuoles in root cells is an effective way to reduce Cd translocation to the shoot and to reduce the exposure of other organelles to Cd toxicity (Huang et al. 2021b). Because vacuoles occupy most of the cytoplasmic volume of root cells, they are capable of sequestering heavy metals through ion-chelator interactions with transport proteins. In our study, we found that exposure to Cd stressed induced an increase in the volume of vacuoles in the root cells of XR57, and that they contained clearly visible electron-dense granules, as evident from Fig. 4N. A similar response was observed in cotton by Daud et al. (2009). Electron-dense granules found in vacuoles are deposits of Cd chelates that can be sequestered into the vacuoles by ABC transporter proteins (Ge et al. 2012; Park et al. 2012). The elevated number of electron-dense granules we found in the vacuoles of roots from XR57 exposed to Cd stress (Fig. 3) might be the result of the high level of *Zm*ABCC1 and *Zm*ABCC2 expression, but another explanation could be that its roots are capable of responding by synthesizing more phytochelators that can bind free Cd^{2+} (Huang et al. 2021a). It has been demonstrated that heavy metals can induce the synthesis of phytochelatin peptides, which in turn triggers synthesis of its precursor glutathione and isolates its protein complexes into vacuoles (Kumar et al. 2023). Our findings imply that the response mechanism endowing XR57 with Cd tolerance is more active than that observed in LY296. The HMA3 transporter protein, which is located on the vacuolar membrane, is responsible for the transport of Cd^{2+} to

the vacuoles, and plays an important role in regulating a plant's Cd tolerance, as demonstrated for rice by Cui et al. (2018), and for wheat by Zhou et al. (2020). HMA3 is also involved in the transport of Cd to shoot (Qin et al. 2022), which explains why shoots from LY296 exposed to Cd had a higher Cd concentration than those from XR57 (Table 2). In our study, we found that exposure to Cd stress induced a significant upregulation of *Zm*HMA3 (Fig. 3), which facilitated the regionalization of Cd^{2+} into vacuoles. It was interesting to note that although the *Zm*HMA3 elevation in LY296 exposed to Cd was higher than that in XR57, its root cells were nevertheless severely damaged, probably because the Cd concentration in LY296 roots was too high to compensate for the damage it caused.

Conclusion

Our study explores the reasons why the two maize varieties exhibited different levels of Cd accumulation after exposure to Cd stress. After considering several factors including the ultrastructure, nutrient ion uptake, and the expression levels of several genes associated with Cd uptake, we identified the following response mechanisms that enhance the Cd tolerance of maize: (1) suppression of *Zm*NRAMP5 and *Zm*ZIP4 expression levels and elevation of *Zm*ABCG4 expression level combined with a thickening of the walls of root cells limits the uptake of Cd by the root, which in turn reduces the Cd accumulation in grains, and (2) the combined effect of vacuolar regionalization and regulation of ion homeostasis in the root reduces cell damage from exposure to Cd stress, allowing the plant to maintain a high level of physiological activity and sustain its biomass formation.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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