# Cadmium Adsorption in Leaf Cell Walls Prevents Redistribution to Silique in Arabidopsis Thaliana Ecotypes Jm-1 and Kyo-0

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# Abstract

**Background:** Along with the rapid progress of industrialization and urbanization in the world, soil Cd pollution has become an increasingly serious problem. Phytoremediation has been widely used to mitigate heavy metal pollution in soils; however, it is difficult to reduce the Cd content in the grains of food crops using Cd pollution remediation techniques.

**Results:** Here, we found that the Cd concentrations in the leaves, stems, and siliques of *Arabidopsis thaliana* (*A. thaliana*) ecotype Jm-1 were higher than in the ecotype Kyo-0. The Cd concentrations in the cell walls (CW) of the leaves were lower in Jm-1 than in Kyo-0, while the concentrations in the CW of the stem and silique were significantly higher in Jm-1 than in Kyo-0. The Cyclohexane Diamine Tetraacetic Acid (CDTA)-pectin and hemicellulose in Kyo-0 had higher Cd concentrations than those of Jm-1. The pectin methylesterase (PME) activity was higher in Kyo-0 than in Jm-1, and the expression levels of *PME1*, *PME2*, *PME12*, and *PME25* were upregulated in Kyo-0 after Cd treatment. In addition, no significant differences in the Cd concentrations were found in the xylem of the two ecotypes, while the Cd concentration in the phloem was significantly higher in Jm-1 than in Kyo-0. The expression of iron transport-related genes showed that only *YSL3* and *ZIP11* had significant differences between the two ecotypes after Cd treatment, and the expression of the vacuolar Cd compartment-related genes that are responsible for transferring Cd from the cytoplasm to the vacuole showed that only *CAX1* expression was significantly higher in Kyo-0 than in Jm-1.

**Conclusions:** Kyo-0 accumulated less Cd than Jm-1 in the silique, which may be because (1) the activity of PME that is mainly regulated by *PME1*, *PME2*, *PME12*, and *PME25* was higher in Kyo-0 leaves, leading to more Cd chelation in the pectin of the CWs, and (2) the expression of *YSL3* was induced to regulate the transport of Cd in the phloem, thus reducing the transport of Cd to the silique. This study would benefit future research and agricultural practices.


# Background

Cadmium (Cd) is a toxic heavy metal in the environment, which has adverse effects on plants and animals[1]. Cadmium accumulation may induce toxic effects in crops, such as by inhibiting normal cell division, reducing photosynthesis efficiency of the blade, increasing membrane lipid peroxidation, and inhibiting the activity of antioxidant enzymes[2,3]. These effects inhibit normal plant growth and reduce yields. In addition, cadmium can enter the body through the food chain and pose a health risk[4,5], potentially leading to prostate cancer, lung cancer, bone deformities, and other diseases[6,7]. Therefore, reducing the toxicity of Cd, and its transportation and accumulation in plant seeds, is essential for normal crop growth and human safety.

The cell wall (CW) is one of the main storage sites for heavy metals in the cell[8], and by accumulating an excess of Cd it acts as a barrier[9]. The CW matrix is mainly composed of polysaccharides, such as hemicellulose, cellulose, and pectin[10]. Pectin in the CW is one of the main binding sites for cations[11],

and there is increasing evidence that CW pectin polysaccharides play a role in the resistance of plants to heavy metals. The CW pectin is a polysaccharide that is rich in galacturonic acid, which accounts for approximately 70% of the CW[12]. During synthesis, some galacturonic acid carboxyl groups of pectin are catalyzed by methyltransferase to form methyl groups, or methylesterified. When the highly methylated pectin is secreted into the CW, the ester group is absorbed by the pectin. Esterase performs different degrees of demethylation via pectin methylesterase (PME)[13], which exposes the charge on the carboxyl group. Demethylation creates a negative charge on pectin polysaccharides, which plays an important role in growth, adsorbing cations, and binding to proteins and homogalacturonic acid (HG) cross-linking complexes ($Ca^{2+}$-pectate cross-linked complexes, the so called “egg-boxes”) in the CW[14,15]. The degree of methyl esterification of pectin in different parts of the CW varies, and it directly affects the structure and properties of pectin[16]. Homogalacturonan (HG) demethylesterification appears to be a key element controlling the chemistry and the rheology of the CW. Hocq[17] postulate that precise and dynamic modulation of extracellular pH plays a central role in the control of HG-modifying enzyme activities and in particular those of pectin methylesterases and polygalacturonases.

Cd accumulation in plants depends on the uptake of Cd by the roots, and its redistribution and transport from the roots to the shoots[18]. Cd is typically absorbed and accumulated in three stages: 1) after absorption in the roots, Cd is transmitted from the roots to the shoots by the xylem; 2) Cd is distributed directionally in the phloem of the stems and nodes to the aboveground plant parts; and 3) the Cd in the leaves is redistributed through the phloem. Cd translocation via xylem loading is a key process in accumulation in the shoots[19][20]. Furthermore, xylem-mediated Cd transport from the root to the shoot is key for its accumulation in rice stalks and grains, rather than the absorption capacity for Cd by the roots[20]. As Cd moves from the root to the shoot, it is transported via the phloem to the grains, and the capacity of the phloem to transport Cd determines the level of Cd accumulation in the grain. This suggests that the transport of the Cd from the xylem to the phloem is necessary before it is transported to the grain[21,22] (Kato et al., 2010). Phloem- and xylem-mediated Cd transport are the keys to Cd accumulations in plant grains. Studies have shown that 91%–100% of Cd in rice grains is deposited from the phloem[21]. However, the transport of Cd in the phloem has not previously been widely investigated.

In this study, we identified differences of Cd accumulation in silique between the two *Arabidopsis ecotypes*. We verified that Cd redistribution to silique was hindered by adsorption of leaf CWs. *YSL3*, gene expressed in the phloem, which was differentially expressed, might be involved in Cd redistribution in phloem. The aim of this study was to provide theoretical research for Cd redistribution in grains by studying the *Arabidopsis thaliana* (*A. thaliana*) ecotypes with significant differences in the accumulation of Cd in silique.

## Materials And Methods

# Plant materials and growth conditions

Jm-1 and Kyo-0 *Arabidopsis thaliana* ecotypes were provided by Dr. Chao Daiying at the Shanghai Institute of Physiology and Ecology, Institute of Botany. *CAX1* overexpressed material, *CAX1*-OE, and the *T-DNA* insertion mutants, Colo.0 and *cax1* were used. The growth conditions were as previously described by Xiao[55]. The nutrient solution, which contained 1.25 mM $KNO_3$, 0.625 mM $KH_2PO_4$, 0.5 mM $MgSO_4$, 0.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.025 mM Fe-EDTA, and 0.25 mL·$L^{-1}$ micronutrients (70 mM $H_3BO_3$, 14 mM $MnCl_2$, 1 mM $ZnSO_4$, 0.5 mM $CuSO_4$, and 0.2 mM $NaMoO_4$) [56], was renewed every 4 days. After 20, 25, and 33 days of hydroponic growth, the plants were treated with 10 μM $CdCl_2$ for 4 days and were used for the determination of Cd concentration in different organs. The 25-day-old plants treated with 10 μM $CdCl_2$ for 4 days were used for the experiment, and those in the control group were not treated with Cd. The treatment with 10 μM $CdCl_2$ for 4 days was the same for all investigations. Each measurement contained at least four biological replicates.

# Determination of Cd and metal concentrations and dry weight

After 25 days of hydroponic growth, and treatment with $CdCl_2$ for the experimental group, the siliques, stems, leaves, and roots of the plants were collected. Each group was first cleaned with $CaCl_2$ for 1 min and then washed with deionized water four times as described previously by Jian[57]. They were then dried in an oven to a constant weight, and the dry weight value was recorded. The dried samples were digested with 2 mL of nitric acid, and the Cd and metal concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS) on a NexION 350X instrument (PerkinElmer, Massachusetts, USA) after dilution by 10 times[57].

# Determination of the Cd and metal concentrations in the xylem sap and phloem

For xylem sap collection, plants were cultured for 25 days before $CdCl_2$ treatment. The stems were cut 2–3 cm from the base with a razor blade, kept in the dark for 15 min, and then the xylem sap was collected[58]. For each replicate of the experiment, six samples were collected from each plant as biological repeats, and there were four replicates in total. The Cd concentrations were determined by ICP-MS after being diluted 10 times. For the phloem sap collection, 25-day-old *Arabidopsis* plants were treated with or without $CdCl_2$, then the leaves and stems were cut, and then cut again under deionized water. The cut leaves were extracted in 20 mM EDTA (pH 7.0) and kept in the dark for 4 h at 23 °C[20,59]. The concentrations of Cd and other metals were determined by ICP-MS after the extracting solution was digested by nitric acid.

# Extraction of subcellular components and the determination of Cd concentrations

Twenty-five-day-old *Arabidopsis* plants were treated with or without $CdCl_2$. The silique, stem, leaf, and roots of the plants were then collected. The subcellular components were extracted by differential centrifugation[60]. Briefly, fresh samples (0.5 g) were mixed with 8 mL of extracting agent (250 mmol/L sucrose, 50 mmol/L Tris-HCl pH 7.5, 1 mmol/L dithiothreitol) and ground on ice to form homogenates. Centrifugation was carried out at 300 × *g* and 2000 × *g* to obtain precipitates of the CW and organelles, respectively, and the remaining liquid was the soluble fraction. The extracted CWs and organelles were dried in an oven, and after drying, the CWs, cell organelles and the soluble fraction were digested in a 1:4 mixture (vol/vol) of $HNO_3$ and $HClO_4$, respectively, before the Cd concentration was determined by ICP-MS.

# Determination of Cd in the pectin, cellulose, hemicellulose, and pectin methylesterase (PME) activity

Twenty-five-day-old *Arabidopsis* plants were treated with or without $CdCl_2$. The silique, stem, leaf, and roots of the plant materials were then collected. The extraction of the CW was in accordance with the method previously described by Brummell[61]. Briefly, the plant samples were homogenized in 80% ethanol and incubated for 20 min at 90 °C. After cooling, the samples were centrifuged at 6000 × *g* for 10 min, and the residual precipitates were washed once with 1.5 mL of 80% ethanol and 1.5 mL of acetone in turn. After soaking in dimethyl sulfoxide for 15 h to remove the starch, they were centrifuged for 10 min at 6000 × *g*, the supernatant was discarded, and the CW precipitate was dried for further use.

Pectin, cellulose, and hemicellulose were extracted according to the method previously described by Wu[62]. Briefly, the CW fraction was mixed with 50 mM sodium acetate buffer (containing 50 mM CDTA, pH 6.5) then shaken for 12 h and centrifuged at 10000 × *g*, and the CDTA-soluble pectin was extracted from the supernate. Then, the residue was soaked in a 50 mM sodium carbonate solution and shaken for 12 h. The $Na_2CO_3$-soluble pectin was obtained from the supernate after centrifugation at 5000 × *g*. Next, the residue was shaken with 5 mL of 4 M KOH (containing 1% $NaBH_4$) for 3 h and centrifuged at 5000 × *g* for 20 min. The cellulose sediment fraction and hemicellulose solution were then separated by centrifugation at 5000 × *g* after being soaked in KOH and oscillated for 3 h. Then the previously extracted hemicellulose, CDTA-soluble pectin, $Na_2CO_3$-soluble pectin, and cellulose precipitate were dried and digested with nitric acid. Cd concentrations were determined by ICP-MS after dilution. PME activity was determined by titration with a pectinesterase assay kit (Suzhou Comin Biotechnology, Co., Ltd).

# RNA extraction and determination of gene expression

Twenty-five-day-old *A. thaliana* plants were treated with or without 10 μM $CdCl_2$ for 4 days. The leaves of the plant materials were collected and placed in liquid nitrogen. Total RNA was extracted with TRIzol (Ambion, Inc., Austin, USA). Complementary DNA templates were then synthesized using the Evo M-MLVRT Premix Kit (Accurate Biotechnology Co., Ltd., Hunan, China). Relative gene expression levels were determined using quantitative reverse-transcription PCR (qRT-PCR) with the SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biotechnology Co., Ltd., Hunan, China). The primer sequences used in the assays are shown in Supplementary Table S1, and the primers for the qRT-PCR analysis were designed using Primer Premier 6.0 software (http://www.premierbiosoft.com/primerdesign/). The PCR conditions were: 95 °C for 30 sec, followed by 40 cycles at 95 °C for 5 sec, 60 °C for 30 sec, and 95 °C for 10 sec. Melting curve analysis was performed to confirm PCR specificity with a heat dissociation protocol from 65 °C to 95 °C following the final cycle of PCR.

# Statistical analysis

The data were analyzed using minimum differential multiple-range comparisons with SPSS software, and each experiment was carried out with at least four biological replicates. $P < 0.05$ was considered to indicate a significant difference, and $P < 0.01$ was considered to indicate a highly significant difference[54]. Charts were prepared with GraphPad Prism 8.

## Results

# The silique of ecotype Jm-1 accumulated more Cd than ecotype Kyo-0

The $CdCl_2$ treatment was applied after 20 days of normal culture and the Cd concentrations in the roots of the two ecotypes showed no differences, while the Cd concentrations in the leaves, stems, and siliques of Jm-1 were significantly higher, as they were 0.19, 0.20, and 0.72 times higher than those of Kyo-0, respectively (Figure 1A). After 25 days of normal culture, the two *Arabidopsis* ecotypes were treated with the same concentration of $CdCl_2$, and similarly, the Cd concentrations in the leaves, stems, and siliques of Jm-1 were significantly higher than those of Kyo-0, as the levels in Jm-1 were 1.01, 0.21, and 1.56 times greater, respectively, but no difference was found in the roots (Figure 1B). Furthermore, for the 33-day-old ecotypes receiving the $CdCl_2$ treatment, differences in the Cd concentrations were found only in the leaves, stems, and siliques of the two ecotypes, as the levels in Jm-1 were 0.24, 0.17, and 1.22 times higher, respectively.

# No differences in biomass and xylem sap Cd between Jm-1 and Kyo-0

After 25 days of normal culture, there were no significant differences in the dry weights between the different organs of the two *Arabidopsis* ecotypes without treatment (Figure 2A), and even after the $CdCl_2$ treatments there were no marked differences in their growth performances (Figure 2B). With the $CdCl_2$ treatment, there was no significant difference in the Cd concentrations of the xylem sap (Figure 2C). This indicates that the Cd long-distance transport from the root to the shoot had no impact on the silique Cd accumulations between Jm-1 and Kyo-0.

# Cd subcellular distribution between Jm-1 and Kyo-0

To further investigate the differences in Cd concentration between the two ecotypes, the subcellular components were then extracted from the different organs, and their Cd concentrations were determined. We found that the Cd concentrations in the CW of the leaves of Jm-1 were significantly lower than those of Kyo-0, and were higher in the stems and siliques of Jm-1, but there was no difference in the Cd concentrations between the roots of the two ecotypes (Figure 3A). In the cell organs that were gradient centrifuged and extracted, the Cd concentrations of the leaves, stems, and siliques did not differ between the two ecotypes, while the Cd concentration in the roots of Jm-1 was higher than in those of Kyo-0 (Figure 3B). In Jm-1, the concentration of Cd in the soluble fractions of the siliques was significantly higher than that in Kyo-0, while differences in Cd concentration were not found in the leaves, stems, or roots between Jm-1 and Kyo-0 (Figure 3C).

# Cd content in CW fractions and PME activity of Jm-1 and Kyo-0

Due to the difference of Cd concentration in the CWs of the two ecotypes, the CWs were then gradient centrifuged and their Cd concentrations measured. For EDTA-pectin, it was found that the Cd concentrations in the leaves of Kyo-0 were significantly higher than that of Jm-1, but these differences were reversed in the stem and silique, and there was no significant difference in the Cd concentrations of the roots of the two ecotypes (Figure 4A). We did not find differences in the $Na_2CO_3$ pectin and cellulose of the two ecotypes for the different organs (leaves, stems, or roots), while the concentrations of Cd in the siliques of Jm-1 were significantly higher than those in Kyo-0 (Figure 4B, D). Except for the roots, the Cd concentrations in the hemicellulose of the other organs (leaves, stems, or roots) were higher than in those of Jm-1 (Figure 4C). The PME activity was higher in Kyo-0 than in Jm-1 with the Cd treatments (Figure 4E).

# Metal concentrations in different organs and phloem sap

In Cd-free/control conditions (Figure 5A), the Ca concentrations in the leaves and stems were strikingly higher in Jm-1 than in Kyo-0, while this difference was not found in the siliques or roots of the two

ecotypes. The concentrations of Fe, Cu, and Zn were higher in the leaves, stems, and roots of Jm-1, but no difference was found in the siliques. The Mg and Mn concentrations in the Jm-1 leaves, stems, and siliques were significantly higher than in Kyo-0, but this difference was not found in the roots.

After the $CdCl_2$ treatment (Figure 5B), the concentrations of Ca, Fe, and Mg in the different organs showed the same trend between the two ecotypes, that is, concentrations in the stems and siliques of Jm-1 were higher than those in Kyo-0, but differences in the concentrations in the leaves and roots were not found. Furthermore, the other three metal concentrations (Cu, Mn, and Zn) showed the same trend in different organs between the two ecotypes, i.e., concentrations in the stems were significantly higher in Jm-1, but we did not detect any clear differences between their leaves, siliques, or roots.

We then examined the metal concentrations in the phloem sap, and found that the Ca, Mg, and Mn concentrations were remarkable higher in Jm-1 than in Kyo-0 in the Cd-free/control conditions, and no differences were detected in the Fe, Cu, or Zn concentrations (Figure 6A). After the $CdCl_2$ treatment, concentrations of Cd, Fe, Mg, and Mn were found to be high in Jm-1, among which the Fe and Mg concentrations were significantly high (Figure 6B). However, differences were not found in the Ca, Cu, and Zn concentrations for these two ecotypes (Figure 6B).

# Associated transporter gene expression

We measured the expression levels of related genes, including those for PMEs, yellow strip-like proteins (YSLs), natural resistance-associated macrophage proteins (NRAMPs), zinc regulated transporter/iron-regulated transporter (ZRT/IRT1)-related proteins (ZIPs), and the vacuolar Cd compartment-related genes that are responsible for transferring Cd from the cytoplasm to the vacuole. The expression of *PME1*, *PME2*, *PME3*, *PME12*, *PME18*, *PME31*, and *PME35* was high in Kyo-0 under normal conditions, and after the Cd treatment the expression of *PME1*, *PME2*, *PME12*, *PME25*, *PME18*, *PME31*, and *PME41* was high in Kyo-0. Furthermore, the expression levels for most *PMEs* were increased in Kyo-0 after Cd treatment (Figure 7A). In the Cd-free/control conditions, the expression of *YSL6* was obviously higher in Jm-1, while there was no significant difference in the expression of the other *YSLs* (Figure 7B). After the Cd treatment, only the expression of *YSL3* showed differences between the two ecotypes, and its expression was much higher in Kyo-0 compared with that in Jm-1 (Figure 7B). There was no significant difference in the *MTP8* expression between the two ecotypes with or without treatment (Figure 7C). Among the NRAMPs, only the expression of *NRAMP3*, *NRAMP4,* and *NRAMP6* showed differences between the two ecotypes, and their expression was higher in Jm-1. However, with the Cd treatment, only the expression of *NRAMP6* was higher in Jm-1, while the other NRAMPs showed no significant differences (Figure 7C). Similarly, in the ZIPs, only the expressions of *ZIP4* and *ZIP11* were different without the Cd treatment, as their expression levels in Jm-1 were higher. However, after the Cd treatment, there were no significant differences in the other ZIPs, and the expression of *ZIP11* in Kyo-0 was significantly higher than that in Jm-1 (Figure 7D). For the relative genes responsible for transferring cadmium from cytoplasm to vacuoles, the expression levels for *CAX2*, *CAX3,* and *ABCC2* were not significantly different in either the control or Cd treatment

groups (Figure 7E). However, *CAX1* expression was significantly upregulated after the Cd treatment, but the expression levels in Kyo-0 were not significantly higher than those in Jm-1, and there was no remarkable difference compared with those under the Cd-free/control conditions (Figure 7E).

Then, *cax1* and *CAX1*-OE material was treated with $CdCl_2$ after 25 days of hydroponic growth, and Colo.0 was used as the control. The Cd concentrations were also measured in the leaves, stems, siliques, and roots. In the leaves and stems, the Cd concentrations in *CAX1*-OE were lower than in Colo.0, while there was no difference between Colo.0 and *cax1* (Supplementary Figure 1). Cd concentrations in the silique of *cax1* were much higher than those in Colo.0, but there was no difference between Colo.0 and *CAX1*-OE (Supplementary Figure 1). Furthermore, there were no significant differences in the Cd concentrations of the roots (Supplementary Figure 1).

# Discussion

The mechanisms of Cd accumulation have been the focus of several previous investigations, but it is still not well understood. In the present study, we found that the Cd concentrations in the Kyo-0 ecotype were more than one-fold lower those in Jm-1 when treated with 10 μM $CdCl_2$, especially when grown for 25 days, as Kyo-0 was 1.56 times lower (Figure 1).

Previous studies have found that preventing the entry of aqueous Cd into root cells plays a key role in enhancing the resistance to Cd[23,24]. Thus, the CW is the first effective barrier protecting cells from Cd[23]. Previous studies have shown that the CW polysaccharides of *Sedum alfredii* were involved in the super accumulation of Cd. Since the higher concentration of CW polysaccharide and PME activity in the non-hyperaccumulating ecotype (NHE) resulted in more free pectic acid residues in the NHE, the NHE CW could bind more Cd than it does in the hyperaccumulating ecotype[25]. Zhu[26] also found that 80% of Cd was fixed in the root CW in hemp, whereas in rice, it was 70% to 90%[27]. The subcellular distribution of the Cd showed that most of it is adsorbed by the CW, and more Cd was absorbed by CWs in the leaves of Kyo-0, and less was absorbed by the CWs of the stems and siliques (Figure 4A). This led us to speculate that the ability of the CWs in the Kyo-0 leaves to absorb more Cd may help to explain why the Kyo-0 ecotype allocates less Cd to other organs than Jm-1 does. Plant CW components (cellulose, hemicellulose, and pectin) can also chelate Cd in the CWs with their negatively charged groups[24]. The pectin in CWs is one of the main cationic binding sites[28]. We measured the Cd concentrations of different CW components by graded extraction and found that the Cd concentrations in the CDTA-pectin were the highest (Figure 4), and only the Cd concentrations in the CDTA-pectin and hemicellulose between the two ecotypes showed the same trend for Cd concentrations in the CWs (Figure 4A, C). This suggests that CDTA-pectin and the hemicellulose in the Kyo-0 leaves are responsible for the accumulation of more Cd in the CW of the leaves. However, pectin is one of the main components of the CW and one of the main sites for Cd adsorption[29]. PMEs can promote the demethylation of pectin, resulting in free carboxyl groups and other negatively charged groups, which can bind more Cd[14,24]. Our results showed that the PME activity in the Kyo-0 leaves was significantly higher than that in the Jm-1 leaves after receiving the

Cd treatment (Figure 4E). However, while the expression of the PME genes in the Kyo-0 leaves was higher than that in JM-1 under normal treatments, most of the PME gene expression in the Kyo-0 leaves was upregulated after the Cd treatment (Figure 7A). This indicated that with the Cd treatment, these PME genes were involved in the demethylation of pectin in the Kyo-0 leaves, and this promoted the CWs to bind more Cd.

The initial distribution by the xylem, the redistribution by the phloem, and the transfer from the xylem to the phloem must be regarded as important elements in the process of distribution throughout the plant. Transport in the xylem is from the root to the shoot, while transport in the phloem is from sources to sinks, with higher selectivity[30]. Xylem-to-phloem transfer may occur on the entire path from the root to shoot, with the stem playing an important role in this case[31-33], probably by transfer cells[34]. The process of accumulation of Cd in rice grains may be controlled by the following factors: absorption by the roots, movement by xylem from the root to the shoot, migration from the xylem to the phloem, and transportation from the source to the sink through the phloem[35]. The primary determinant of Cd concentrations in the bud tissues is the ability to transfer Cd from the root to the bud through the xylem rather than the plant's ability to absorb Cd[36]. Studies have shown that 91%–100% of the Cd in rice grains is deposited from the phloem[21]. However, the mechanisms of Cd transport in the phloem are still unclear. It was of interest that there was no difference in the Cd between the xylem in the two ecotypes, and that less Cd was transported in Kyo-0 phloem (Figure 2C, Figure 6B).

Because Cd is toxic and non-essential for plant growth, it was suggested that Cd transport in plants is mediated by a transporter for an essential element, such as Zn, Ca, or Fe. In fact, antagonism between Cd and Zn has been observed in many plant species[37,38]. Research has shown that mineral transporters, such as those for Zn and Fe, are partly responsible for Cd transport in plants[19]. After the Cd treatment, only the Fe and Ca concentrations in the Kyo-0 ecotype increased in the leaves and significantly decreased in the silique compared with those in the control (Figure 5). In addition, for metals in the phloem, only the trends for the Fe concentrations were consistent with those of Cd (Figure 6A, B), while under normal culture, the Ca concentrations in Kyo-0 were significantly lower than those in Jm-1 (Figure 6A). However, after the Cd treatment, the Ca concentrations of the two ecotypes showed no difference (Figure 6B). Thus, we hypothesized that the genes that mediate the transport of Fe in the phloem may be involved in the transport of Cd. Because there are few studies on Fe transporter genes in the phloem, we could only detect the expression of Fe transporter gene families expressed in the leaves. YSLs are heavy metal absorption and transport proteins widely found in plants, involved in the transport of metal ions such as Fe[39]. The functional analysis of ZIPs indicates that the gene family plays an important role in the transport of Fe, Zn, Cd, and other metal elements from outside to inside the cell[40]. NRAMPs participate in the absorption and transport of a variety of divalent metal ions including Mn, Fe, and Cd[41]. *AtMTP8* regulates the distribution of Mn and Fe in seeds, and the mutants with *mtp8* dysfunction are sensitive to high levels of Mn, while the tolerance of the *MTP8*-overexpressed strain to high Mn increases[42]. The experimental results showed that under the treatment with Cd, only *YSL3* and *ZIP11* of

the Fe transport genes showed differences in expression between the two ecotypes, and all of them had higher expression in Kyo-0 (Figure 7B–D).

There are a few previous studies on the transporters of heavy metals in the phloem. *OsLCT1* is a membrane-bound Cd transporter that participates in phloem Cd transport[20]. Our results showed that the difference in Cd concentrations between the phloem of the two ecotypes may be one reason that Kyo-0 accumulates less Cd in the silique (Figure 1, Figure 2C, Figure 6), because the expression of *YSL3* in the leaves of Jm-1 was significantly higher than that of Kyo-0 (Figure 7B). *AtYSL3* may act as a transporter of the metal-NA complex in vascular cells[43]. GUS staining was performed on a cross section of a vein of *Arabidopsis* rosette leaves, revealing the expression of YSL3p::GUS in the phloem and parts of the xylem parenchyma[44]. We suggested that *YSL3* may be involved in the Cd transport in the phloem, but this mechanism requires further investigation. Some studies have also shown that *AtZIP5*, *AtZIP9*, *AtZIP12,* and *AtIRT3* may all have the function of transporting Zn and Cd, which could enhance the absorption of Cd by plants[45,46], while research proved that *ZIP11* acts as a transcriptional activator and could be regulated by abiotic stresses such as salt and cytokinin[47,48]. The present study showed that the expression of *ZIP11* in the ecotype Kyo-0 was upregulated with the Cd treatment (Figure 7D), but the specific function of *ZIP11* requires further investigation.

Vacuolar transport is an important mechanism for metal storage in vacuoles. Therefore, the regulation of vacuolar transport activity may be an important strategy to improve Cd tolerance and accumulation[49]. In the present study, the expression of *CAX1* in the leaves of Kyo-0 was higher than in Jm-1 (Figure 7E), which suggested that *CAX1* had a role in vacuole storage. There was no significant difference in the Cd concentrations in the soluble parts of the leaves of the two ecotypes (Figure 3C). However, the concentrations of Ca in the Kyo-0 leaves increased after the Cd treatment (Figure 5A, B), suggesting that with the Cd treatment, the upregulated expression of *CAX1* in the Kyo-0 leaves could transport more Ca. The $Ca^{2+}/H^{+}$ antiporter *CAX1* is positioned in the vacuole membrane and participated in Cd transportation into the vacuole[49,50], indicating that *CAX1* has the potential to store Cd in the vacuoles. Thus, we used the existing *cax1* mutant and *CAX1*-overexpression (OE) in the laboratory, with the Cd treatment used in this investigation, and found that the concentrations of Cd in the siliques of *cax1* increased, while those in *CAX1*-OE decreased (Figure S1), suggesting that *CAX1* could reduce the accumulation of the Cd in the seeds. This could be applied in future phytoremediation research strategies.

Transpiration also plays an important role in the absorption of inorganic ions. A large number of studies have shown that transpiration affects the absorption and transport of cadmium in plants[51,52]. However, this has been less studied in *Arabidopsis thaliana*. The transpiration rate of vegetable crops varies with species. Cucumber has a higher transpiration rate due to having larger leaves and poor protective tissue. Lu[53] found that cadmium transport from roots to aboveground tissues is driven by transpiration and is transported upward through the xylem. Lai[54] found that in *Impatiens*, Cd accumulation was significantly positively correlated with leaf area and transpiration rate. In our study, the Cd concentrations in the roots

of the two ecotypes showed no differences with $CdCl_2$ treatment (Figure 1A). This indicates that the absorption of Cd is not different in roots. Furthermore, there were no significant differences between the dry weights of different organs in the two *Arabidopsis* ecotypes without treatment (Figure 2A), and even after the $CdCl_2$ treatments there were no marked differences in their growth performances (Figure 2B). With the $CdCl_2$ treatment, there was no significant difference in the Cd concentrations of the xylem sap (Figure 2C). This indicates that the Cd long-distance transport from the root to the shoot had no impact between Jm-1 and Kyo-0. We presume that transpiration does not affect the absorption, transport, and distribution of cadmium in the two ecotypes.

## Conclusion

In this study, we found that with the Cd treatment, there was less Cd accumulation in the siliques of Kyo-0 than those of Jm-1. The different mechanisms for this may be as follows: (1) the activity of the PME, mainly regulated by *PME1*, *PME2*, *PME12*, and *PME25*, was higher in the Kyo-0 leaves, leading to more Cd chelation in the pectin of the CWs, which prevented its redistribution to the silique; and (2) regulating the expression of *YSL3* could regulate the transport of Cd in the phloem, thus reducing the transport of Cd to the silique. Research on the directed distribution of phloem Cd to grains is currently lacking, but of vital importance. Our research on Cd accumulation in the silique of *Arabidopsis thaliana* provides some theoretical guidance and direction to help elucidate these mechanisms in the future.

## Declarations

# Ethics approval and consent to participate

Not applicable

# Consent for publication

Not applicable

# Availability of data and materials

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

# Competing interests

The authors declare that there are no conflicts of interest

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# Author Contributions

YX, DL, ZZ, and JL designed the experiments and analyzed the data; YX, DL, and LL performed most of the experiments; and YX, DL, ZZ, and JL wrote the manuscript. All authors contributed to the initial design of the project and read and approved the manuscript.

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# Figures

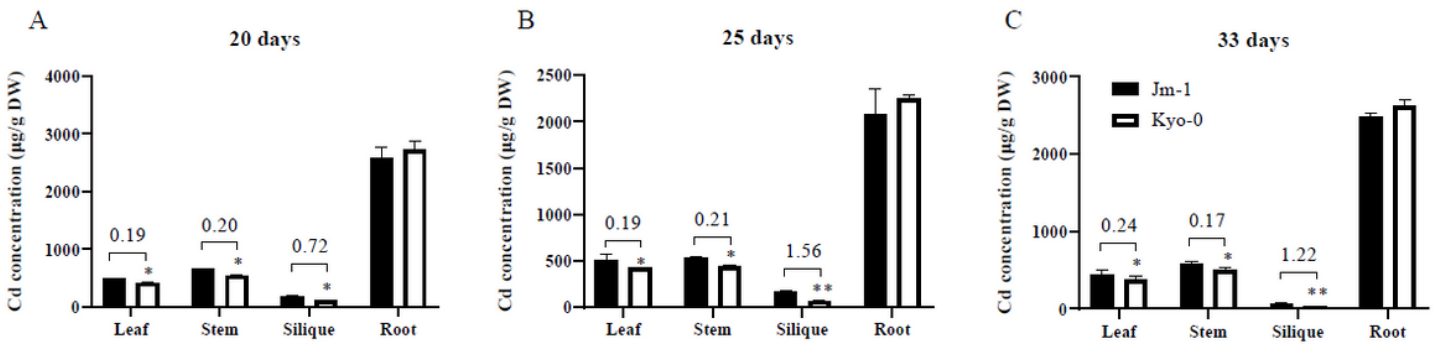
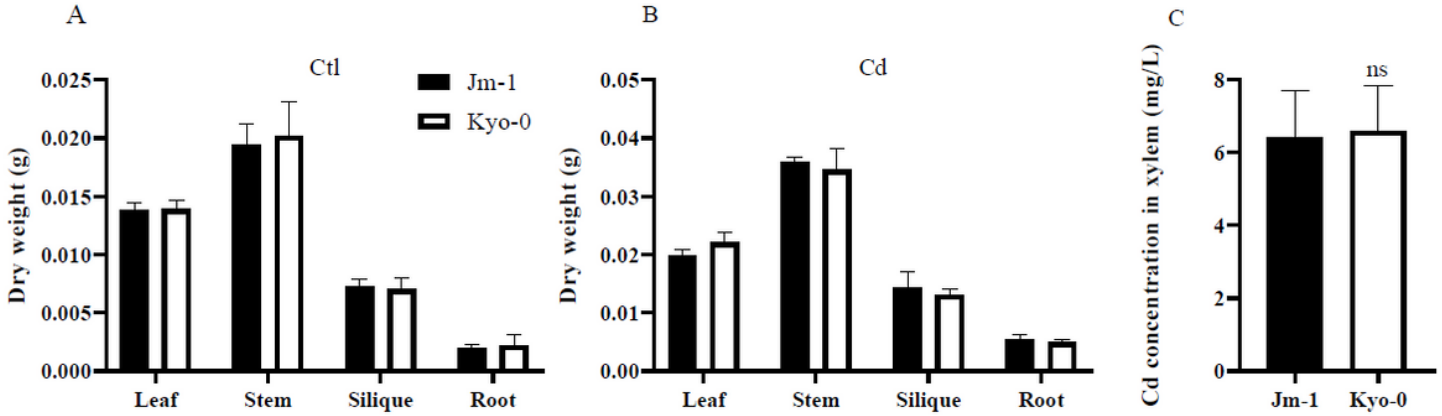


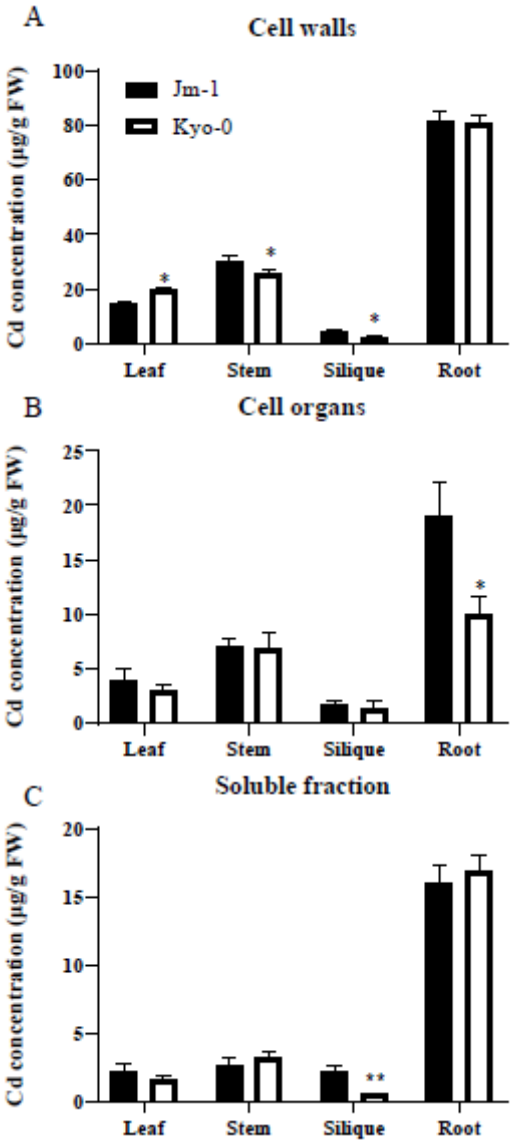

**Figure 1**

Cd concentrations in different organs of two Arabidopsis thaliana ecotypes after 4 days of 10 μM CdCl2 treatment at different culture times. After normal culture for (A) 20 days, (B) 25 days, and (C) 30 days, Cd concentrations in leaves, stems, silique, and roots were treated with 10μM CdCl2 for 4 days. Date presented means of 4 independent biological replicates (n = 4) and vertical bars represent the SD., * and ** indicate significant differences from the control at P < 0.05, and 0.01, respectively.



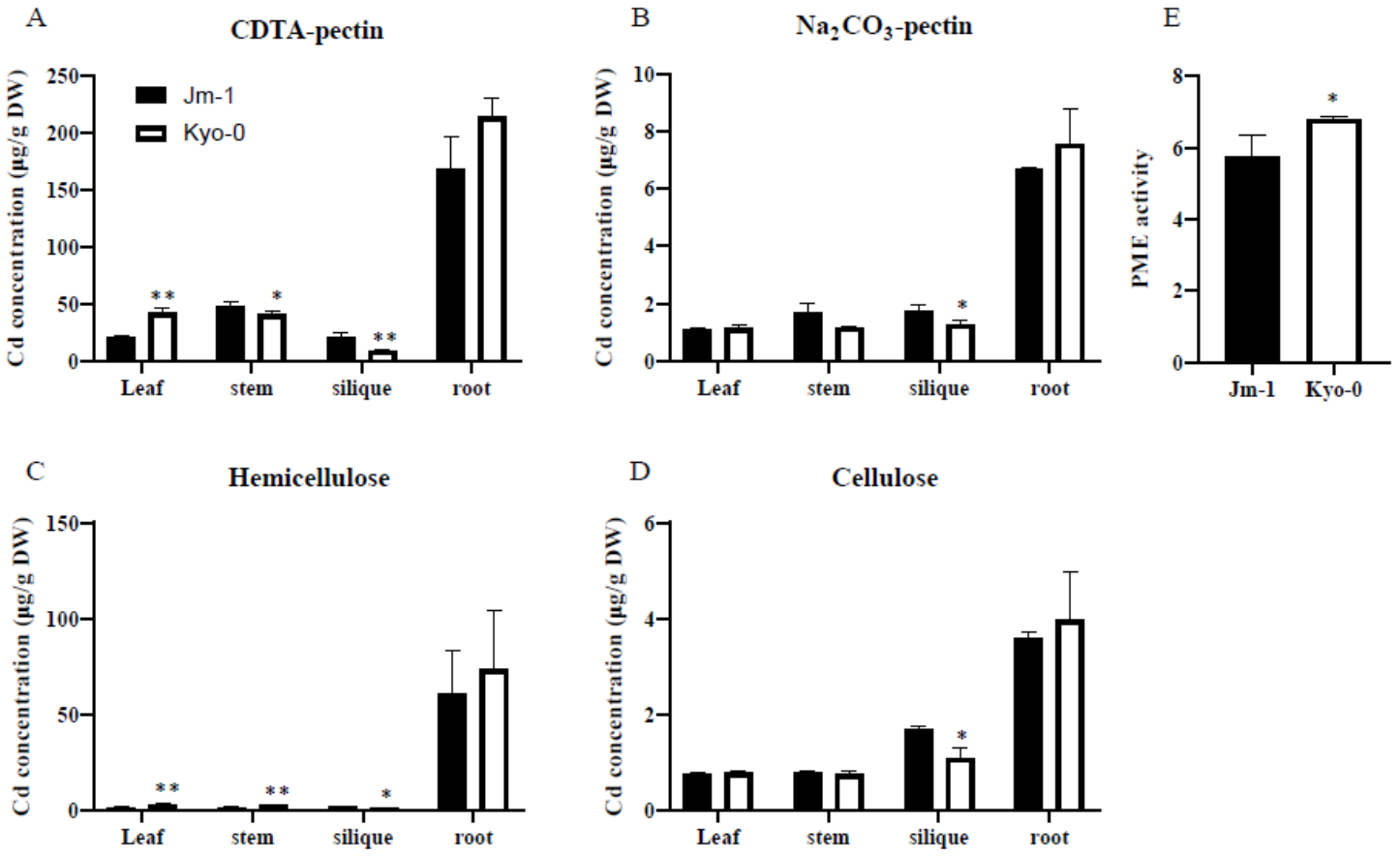

## Figure 2

Dry mass and Cd concentrations. The dry mass (DM) of Leaves, stems, silique and roots treated without (A) or with (B) 10μM CdCl2 for 4 days. C. the Cd concentration of xylem sap after 25-day-old Arabidopsis treated with 10μM CdCl2 for 4 days. Ctl means control treatment, with normal culture conditions; Cd, treatment with CdCl2. Date are means of 4 independent biological replicates (n = 4) and the vertical bars indicate the SD, ns = differences are not significant.



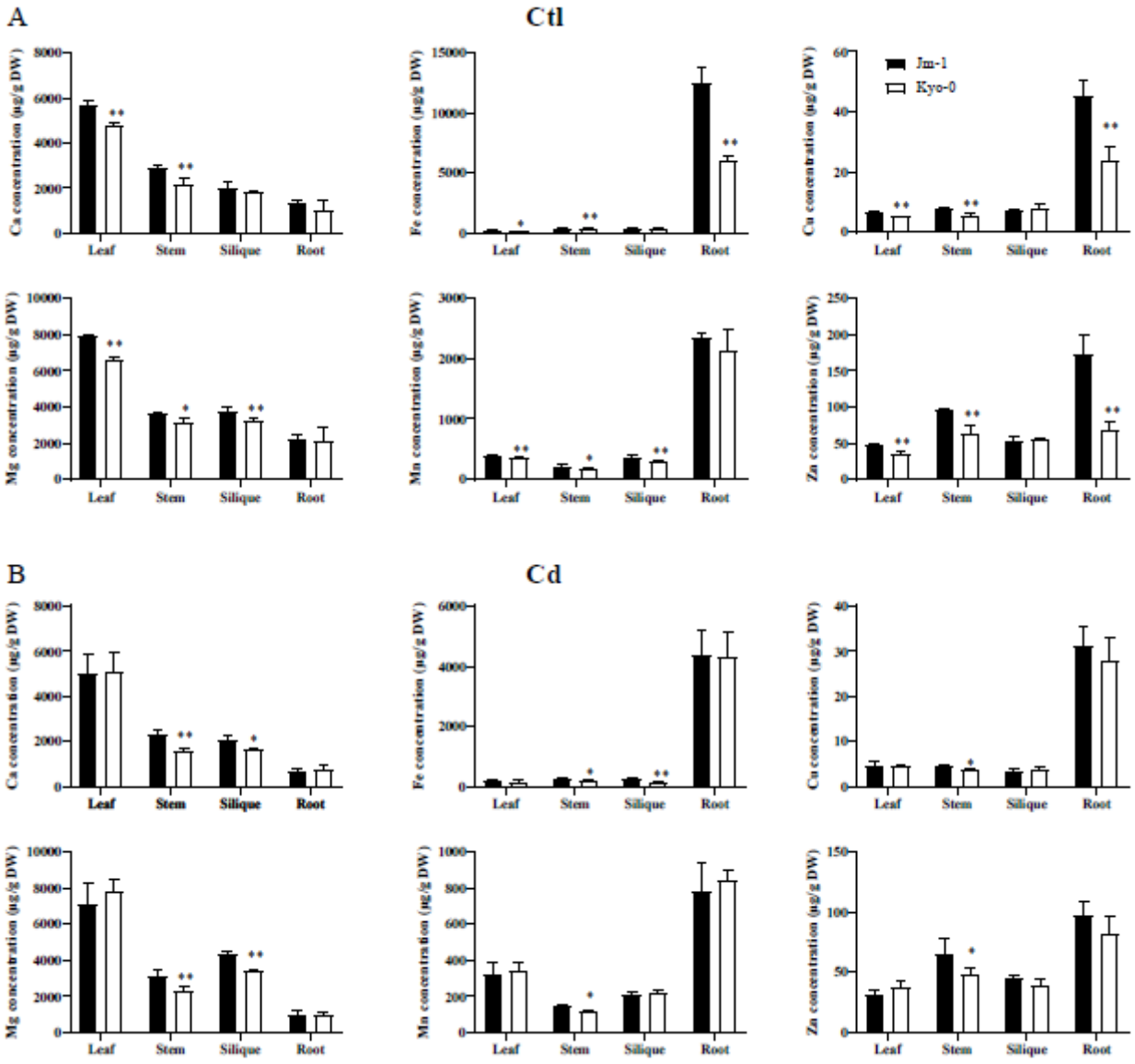

## Figure 3

Subcellular distribution of Cd in plant different organs. Cd concentration in (A) cell walls, (B) organelles, and (C) soluble parts of plant different organs after 25-day-old Arabidopsis treated with 10μM CdCl2 for 4 days. Date presented means of 4 independent biological replicates (n = 4) and vertical bars represent the SD., * and ** indicate significant differences from the control at P < 0.05, and 0.01, respectively.



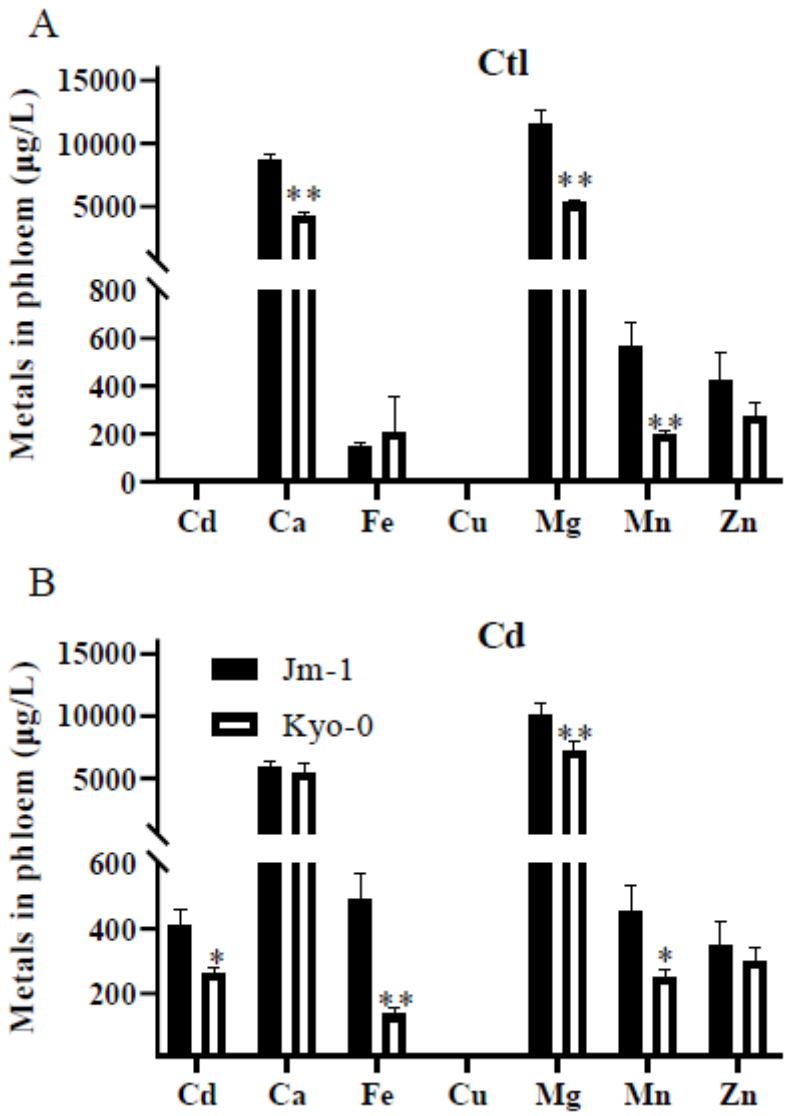

## Figure 4

Concentration of Cd of cell wall components in different organs and PME activity. Cd concentrations in different organs of (A) CDTA-pectin, (B) Na2CO3-pectin, (C) hemicellulose and (D) cellulose after 25-day-old Arabidopsis treated with 10μM CdCl2 for 4 days. (E) PME activity in leaves after 25-day-old Arabidopsis treated with 10μM CdCl2 for 4 days. Date presented means of 5 independent biological replicates (n = 5) and vertical bars represent the SD., * and ** indicate significant differences from the control at P < 0.05, and 0.01, respectively.




## Figure 5

Metal ion concentrations at different plant organs. Ca, Fe, Cu, Mg, Mn and Zn concentrations in leaves, stems, silique and roots after 25-day-old Arabidopsis treated without (A) or with (B) 10μM CdCl2 for 4 days. Ctl means control treatment, with normal culture conditions; Cd, treatment with CdCl2. Date presented means of 4 independent biological replicates (n = 4) and vertical bars represent the SD., * and ** indicate significant differences from the control at $P < 0.05$, and 0.01, respectively.




## Figure 6

Metal concentrations in phloem sap. Concentrations of Cd, Ca, Fe, Cu, Mg, Mn and Zn in 25-day-old Arabidopsis with (A) Cd-free treatment and (B) 10μM CdCl2 treatment for 4 days. Ctl means control treatment, with normal culture conditions; Cd, treatment with CdCl2. Date presented means of 5 independent biological replicates (n = 5) and vertical bars represent the SD., * indicate significant differences from the control at $P < 0.05$, and 0.01, respectively.

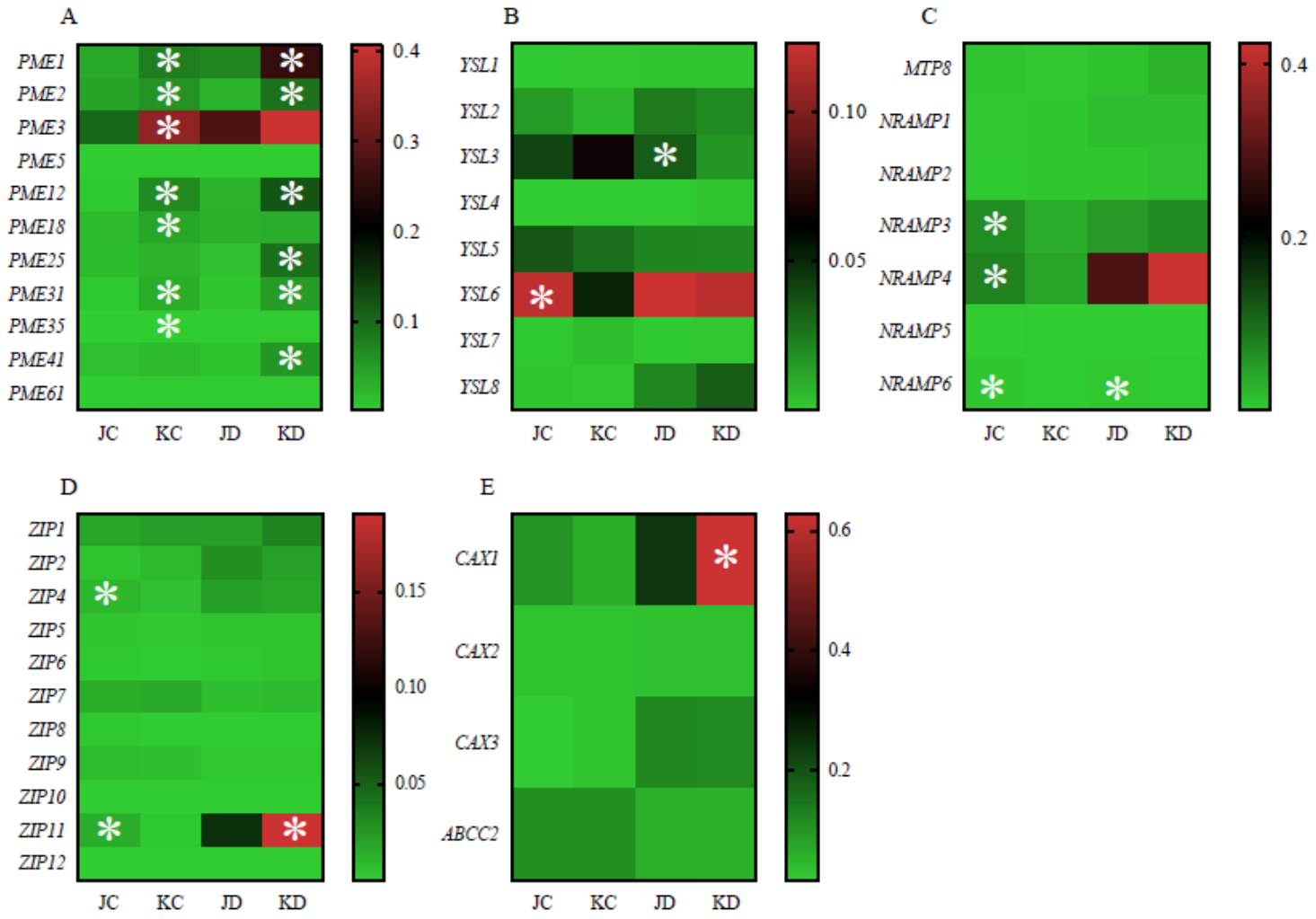



**Figure 7**

Expression of PMEs, YSLs, ZIPs, MTP8 and NRAMPs relative genes. (A) PMEs expression, (B) YSLs expression, (C) MTP8 and NRAMPs expression, (D) ZIPs expression, (E) relative gene expression of vacuole cadmium compartment. JC and KC meant Jm-1 and Kyo-0 with Cd-free treatment, respectively; JD and KD meant Jm-1 and Kyo-0 under 10μM CdCl2 treatment for 4 days, respectively. Date presented means of 4 independent biological replicates (n = 4), * and ** indicate significant differences from the control at P < 0.05, and 0.01, respectively.

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