

Cadmium accumulation in plants: Insights from physiological/molecular mechanisms to evolutionary biology

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Abstract

Cadmium (Cd) contamination in the environment has becoming a hot issue worldwide, as it has posed a great risk to human health through food chain. Cd accumulation in the edible parts of crops are involved in four processes: uptake, translocation, sequestration and (re)distribution, which are all controlled by membrane transporters. In this review, the advance in studies on physiological and molecular mechanisms of Cd accumulation in plants was summarized, and then the functional evolution was discussed based on oneKP database. Cd accumulation in plants is a derived and polyphyletic trait that has evolved convergently by several times. During their evolution, the membrane transporter families, such as NRAMPs, HMAs, ABCs, ZIPs, CDFs, CAXs and OPTs, have undergone the lineage specific expansion due to gene duplication. The orthologues of OsHMA2 in higher plants are stepwisely evolved from monophyletic evolutionary lineage with one common ancestor; whereas the orthologues of OsNRAMP5 from a polyphyletic evolutionary lineage with several ancestors. In addition, phylogenetic clusters of the orthologues of OsNRAMP5 have occurred rampant intermixing, suggesting horizontal gene transfer. It may be concluded that evolution of Cd accumulation in plants could provide an adaptive advantage for colonization of plants to the new habitats like metalliferous soil.

Cadmium accumulation in plants: Insights from physiological/molecular mechanisms to evolutionary biology

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Running title: Advances in Cd accumulation by plants

ABSTRACT

Cadmium (Cd) contamination in the environment has becoming a hot issue in the world, as it has posed a great risk to human health through the food chain. Cd accumulation in the edible parts of plant crops are

involved in four processes: uptake, translocation, sequestration and (re)distribution, which are all controlled by membrane transporters. In this review, the advance in studies on physiological and molecular mechanisms of Cd accumulation in plants was summarized, and then the functional evolution of Cd accumulation in plants was discussed based on oneKP database. Plant Cd accumulation is a derived and polyphyletic trait that has evolved convergently by several times. In the course of evolution, the membrane transporter families, such as NRAMPs, HMAs, ABCCs, ZIPs, CDFs, CAXs and OPTs, have undergone the lineage specific expansion due to gene duplication. The orthologues of OsHMA2 in higher plants are stepwisely evolved from monophyletic evolutionary lineage with one common ancestor; whereas the orthologues of OsNRAMP5 from a polyphyletic evolutionary lineage with several different ancestors. In addition, phylogenetic clusters of the orthologues of OsNRAMP5 have occurred rampant intermixing, suggestive of horizontal gene transfer. It may be concluded that such an evolution of Cd accumulation in plants could provide an adaptive advantage for colonization of plants to the new habitats like metalliferous soil.

KEYWORDS: Cadmium, Monophyletic evolution, Polyphyletic evolution, Tissue accumulation, Transporters, Vacuole sequestration

1 | INTRODUCTION

Cadmium (Cd) contamination in soil has become one of the environmental issues receiving more and more attention in the world (Rai et al., 2019). Cd contamination can be caused by several human activities, such as the application of municipal sewage sludge, atmospheric deposition of Cd, mining, application of chemical fertilizers and pesticides containing Cd (Clemens, 2019). It is estimated that about 30,000 tons of cadmium pollutants are discharged into the environment annually, and 13,000 tons of them are produced from human activity (Gallego et al., 2012). Cd is easily taken up and accumulated in plant tissues, resulting in reduced crop yield and also threatening human health via food chain. (Ismael et al., 2019; Rai et al., 2019).

Cd accumulation in plants may occur through soil-plant, water-plant, and air-plant interfaces, with soil-plant interface being dominant (Shahid et al., 2017). It is well documented that there is a close association between Cd level in soil and food crops (Khan et al. 2015). In soil-plant systems, Cd in rhizosphere enters are plant roots in ionic forms, and then translocated into above-ground tissues, including grains of cereal crops (Clemens and Ma, 2016). As a non-essential and toxic metal element, the excessive Cd accumulation in plants will causes various detrimental impacts on plants, such as reducing plant growth and development, inhibiting photosynthesis and respiration, interfering with biochemical and physiologically related signaling pathways, disturbing nutrients uptake and water relations, damaging cell membrane permeability and disrupting the cellular redox homeostasis etc., as a consequence resulting in yield reduction and even plant death (Shahid et al., 2017; Ismael et al., 2019). In turn, by consumption of the Cd-contaminated foods, Cd intake and accumulation occur inevitably in human body, thus arising serious health risks, such as kidney cancer, breast cancer, anemia, heart failure, hypertension, cerebral infarction, proteinuria, eye cataract formation, osteoporosis, emphysema, and renal insufficiency (Clemens, 2019). It has been well known that crop foods are the main sources of Cd exposure in population, which account for 90% of the total dietary intake (Khan et al., 2015). Therefore, controlling Cd accumulation in plants, in particular the edible parts is crucial for reducing its threat to human health. Accordingly, a much better understanding of the mechanisms underlying Cd uptake, translocation, detoxification, and accumulation is imperative.

In the last decades, the rapid advance has been made in revealing the physiological and molecular mechanisms of Cd uptake and accumulation (reviewed by Clemens, 2019; Clemens and Ma, 2016; Manara et al., 2018). A series of genes associated with Cd transport in plants have been identified and functionally verified (reviewed by Clemens and Ma, 2016; Shahid et al., 2017; Ismael et al., 2019; Shi et al., 2019). Meanwhile, the specific plant genotypes or lines with extremely low or high Cd accumulation have been developed by genetic manipulation, such as transformation, gene editing and molecular breeding (reviewed by Fasani et al., 2018). However, there is no review available which systematically reports the role of heavy metal transporters in Cd absorption and accumulation, and their evolution. In this review, the advance in studies on the physiological and molecular mechanisms of Cd accumulation in plants is first summarized and the functional evolution

of Cd accumulation in plants was further discussed in terms of the most important membrane transporter families mediating Cd accumulation.

2 | CADMIUM UPTAKE AND TRANSPORT BY PLANTS

The mechanisms for Cd accumulation in plants have been extensively investigated, (reviewed by Clemens, 2019; Clemens and Ma, 2016; Shahid et al., 2017; Ismael et al., 2019; Shi et al., 2019). In general, the whole process of Cd accumulation from the growth medium (soil) to above-ground plant parts can be divided into 4 steps, including (1) root Cd uptake from soil, (2) root-to-shoot translocation via xylem, (3) Cd sequestration and (4) Cd accumulation in above-ground tissues or edible parts through phloem.

2.1 | Cd uptake by roots

Plants can take up Cd from soil by their roots, and the rate and amount of Cd absorption in roots are dependent on Cd bio-availability or concentration in soil (Clemens, 2006). Meanwhile root Cd uptake is controlled by genetic factors of plants and greatly affected by environmental conditions. The uptake of Cd^{2+} by plant roots consist of two phases: apoplastic binding and symplastic uptake (Zhao et al. 2002). In the first phase, the positively-charged Cd^{2+} arises an electrostatic interaction with the negatively-charged carboxylate groups on cell walls, resulting in Cd^{2+} accumulation of in the root apoplast (Meychik and Yermakov, 2001). It is rapid and spontaneous, suggesting the phase of apoplastic binding require no energy (passive system). Compared to apoplastic binding, the second phase, symplastic uptake is a slow active process, requiring a huge of energy and depending on the metabolic activity (Sloof et al., 1995). Cd transport across the root cell plasma membrane is the initial step for its symplastic uptake (Yin et al., 2015). It is a concentration-dependent process, exhibiting the saturable kinetics in the relationship of Cd concentration in the medium and uptake velocity, indicating that Cd is taken up via a carrier-mediated system (Verbruggen et al., 2009). Since it is a non-essential element for plants and interferes with the uptake of other ions, it may be assumed that Cd^{2+} is likely to enter root cells through the transporters for essential elements such as Fe^{2+} , Zn^{2+} , and Mn^{2+} , due to the similarity of Cd^{2+} to these ions in chemical and physical properties. In Arabidopsis and rice, for instance, many transporters from the families of Natural Resistance-Associated Macrophage Proteins (NRAMPs, such as OsNRAMP1, OsNRAMP5, and AtNRAMP6) and Zinc/Iron-regulated transporter-like Protein (ZIP, such as AtIRT1) transporters have been demonstrated to transport Cd^{2+} in root cells (Schaaf et al., 2004; Lux et al., 2011; Sasaki et al., 2012). In addition, Cd^{2+} may also enter root cells through the transport pathway for Ca^{2+} (White and Broadley, 2003; Li et al., 2012; Chen et al., 2018), because of their similarities in charge and ionic radius. Adding La^{3+} and Gd^{3+} (potent Ca channel inhibitors) or increasing the concentration of Ca (ca. 5mM) appeared to suppress the metabolically-dependent Cd uptake substantially in the Zn hyperaccumulator *Thlaspi caerulescens* (Zhao et al. 2002), the halophyte *Suaeda salsa* (Li et al., 2012) and rice (Chen et al., 2018). Recently, one member of the major facilitator superfamily (MFS), OsCd1, was also detected to be associated with root Cd uptake in rice (Yan et al., 2019). However, although a great number of membrane proteins have been demonstrated to be involved in the uptake of Cd in plants, little is still known about transport of Cd^{2+} across root plasma membranes at molecular level.

2.2 | Root-to-shoot Cd translocation

After its uptake at the root epidermis or exodermis, Cd is radially transported across the cortex, endodermis, and pericycle cells, and then loaded into root xylem for the subsequent translocation from roots to shoots via xylem (Lu et al., 2009; Ueno et al., 2008; Uraguchi et al., 2009b). Loading Cd into root xylem is a crucial step for Cd translocation to the aerial plant parts (Uraguchi et al., 2009b; Ismael et al., 2019). The radial movement of Cd toward the root xylem occurs via the symplastic and/or apoplastic transport of free Cd^{2+} or Cd-complexes with various chelates (Shahid et al., 2017). Symplastic transport is considered as an energy-consuming (positive) pathway involving both influx and efflux transporters (Clemens and Ma, 2016). Apoplastic transport is known to be a passive pathway, usually driven by transpiration (Qiu et al., 2012). It was suggested that the symplastic transport play the dominant role in the radial transport of Cd to root

xylem in plants (Lu et al., 2009; Ueno et al., 2008). However, a recent study on *Sedum alfredii* found that the apoplastic transport contributed up to 37% of the transported Cd when exposed to high Cd level (Tao et al., 2017). After crossing the barriers from root epidermis to root cortex, either free Cd ions or Cd-chelates may enter symplasm and are then loaded into root xylem (Gallego et al., 2012). The xylem loading of Cd in roots a energy-consuming process because it occurs against membrane potential (Clemens and Ma, 2016), and is mediated by heavy metal P1B-ATPases (HMAs), such as orthologues of AtHMA2 and AtHMA4, and possibly also by YSL proteins (Lux et al., 2011; Ismael et al., 2019).

2.3 | Cd sequestration

Cd sequestration has a significant contribution to preventing Cd translocation, thereby controlling Cd accumulation in the plant tissues. The main sites for Cd sequestration in plant cells are cell walls and vacuoles. Cell wall is the “first line of defense” against toxic metals from the external environment. When captured by root cells, metal ions are largely bound by the functional components of cell walls, such as cellulose, hemicellulose, lignin, and pectin (Shi et al., 2019). Many studies found that there was the highest Cd concentration in the apoplast of roots, particularly in the cell walls of the rhizodermis and cortical cells, while root cells had much lower Cd concentrations (Lux et al., 2011). Such binding of Cd to cell walls can efficiently prevent Cd from being transported across the plasma membrane into the protoplasts, thereby reducing the translocation of Cd in plant cells and tissues. Once inside the root cells, Cd is mainly concentrated in vacuole, which is generally considered to be the main storage site for metals in plant cells (Salt and Rauser, 1995). Some studies on the cell fractionation have shown that Cd-sensitive plants have even higher Cd concentrations in vacuole than in cell wall (Uraguchi et al., 2009a). Wu et al. (2005) found that 51% of the Cd in barley root is present in the soluble fraction of vacuole, and only 36% is present in cell wall. After its translocation from roots to the aerial plant parts, Cd is mainly sequestered in vacuoles of parenchyma cells in the leaf mesophyll, stem pith, and cortex in plant shoots (Qiu et al. 2011; Tian et al., 2017). Obviously Cd sequestration into vacuoles plays a vital role in reducing Cd concentrations in the cytoplasm and alleviating Cd toxicity to enzymes involved in cytosolic biochemical reactions (Shi et al., 2019). Several families of transporters have been identified to be responsible for the process of vacuole sequestration, such as HMAs, Ca^{2+} exchangers (CAXs), NRAMPs, MTPs and ATP-binding cassette subfamily C proteins (ABCCs) (Korenkov et al., 2007; Park et al., 2012; Sharma et al., 2016). It is generally assumed that Cd ions in the cytosol first form the low molecular weight (LMW) complexes by binding with metal ligands, such as glutathione (GSH), phytochelatins (PCs), metallothioneins (MTs), nicotianamine, organic acids and amino acids, and subsequently are transported into the vacuole, where more Cd^{2+} and thiol-containing chelators like GSH and PCs are incorporated to produce the high molecular weight (HMW) complexes (Zhang et al., 2018). In addition, the formation of Cd-malate complex in the vacuoles is also found to reduce the subsequent Cd efflux from vacuole to cytoplasm (Ueno et al., 2005).

2.4 | Cd accumulation in shoots and grains

Following the process of root uptake and root-to-shoot translocation, the accumulation of Cd in shoots and grains (the edible part in cereals) is the final destination of Cd, which consists of three processes, including xylem unloading, phloem translocation and inter-vascular transfer (Clemens and Ma, 2016). Corresponding to the xylem loading of Cd in root, Cd xylem unloading in shoot also occurs via both apoplastic and symplastic transport (Clemens et al., 2002). Phloem translocation represents the main pathway of Cd accumulation in plant shoots and grains. It was reported that Cd in the phloem sap is mainly complexed with nicotianamine, glutathione (GSH), and phytochelatins (PCs) (Mendoza-Cózatl et al., 2008). However, how these Cd-chelates are loaded into the phloem is poorly understood up to date, and none responsible transporter has been identified (Khan et al., 2014). Inter-vascular transfer is closely associated with Cd accumulation in grains (Clemens and Ma, 2016). In cereals, nodes are the complex but well-organized vascular systems, consisting of two major vascular bundles: enlarged vascular bundles (EVBs) and diffuse vascular bundles (DVBs) (Yamaji and Ma, 2014; Clemens and Ma, 2016). EVBs come from the lower nodes and are connected to leaves, whereas DVBs surrounding the EVBs start at the node and are connected to the

upper nodes or panicles (Clemens and Ma, 2016). Fujimaki et al. (2010) performed a noninvasive detection of Cd in rice plant, and found that Cd was more concentrated in the nodes than in the internodes. In rice, increasing evidences suggests that phloem loading by inter-vascular transfer from the EVBs to the DVBs in node I is a major pathway for Cd movement toward grains (Yamaji and Ma, 2014). OsHMA2 and low-affinity cation transporter 1 (OsLCT1) are involved in the inter-vascular transfer, with OsHMA2 loading Cd into the phloem of EVBs and DVBs and OsLCT1 exporting Cd from phloem parenchyma cells into the sieve tubes (Uraguchi et al., 2011; Yamaji et al., 2013). However, the molecular mechanisms underlying Cd distribution in dicots shoots is still unclear.

3 | CADMIUM TRANSPORTERS

As a non-essential element, Cd normally enters into plant roots through the same pathways as essential or beneficial elements, such as Zn^{2+} , Fe^{2+} and Mn^{2+} (Clemens, 2006). In recent decades, a large number of genes conferring Cd transportation across membrane in plants have been identified and functionally verified (Clemens and Ma, 2016; Shahid et al., 2017; Ismael et al., 2019; Shi et al., 2019). These transporters belong to Natural Resistance-Associated Macrophage Proteins (NRAMPs), Heavy Metal ATPases (HMAs), Zinc/Iron-regulated transporter-like Proteins (ZRT1/IRT1-Like Protein, ZIPs), Cation Diffusion Facilitators (CDFs), oligopeptide transporter family (OPTs), ATP-binding cassette subfamily C proteins (ABCCs) and Cation/ H^+ Exchangers (CAXs). All these transporters are involved in uptake, translocation, sequestration, and distribution of Cd in plants, and their tissue-specific localization and substrate specificity are summarized in Table 1.

3.1 | NRAMPs

NRAMPs represent a family of metal transporters that are located at the membrane of root cells and evolutionarily conserved in a wide range of organisms, including bacteria, fungi, animals, and plants (Uiih et al., 2018). In plants, NRAMP genes are participated in the uptake of the divalent cations, such as Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , and Cd^{2+} as well. In Arabidopsis, six NRAMP family members have been identified, with AtNRAMP3 and AtNRAMP4 being localized at tonoplast and responsible for Cd^{2+} efflux from the vacuole to the cytosol (Lanquar et al., 2005). In rice, OsNRAMP1 and OsNRAMP5 are plasma membrane (PM)-localized transporters and participated in taking up Cd from the external solution to root cells (Takahashi et al., 2011; Sasaki et al., 2012). The knockdown or CRISPR/Cas9-mediated editing of OsNRAMP5 caused a dramatic reduction of Cd and Mn concentrations in both rice roots and shoots (Sasaki et al., 2012; Tang et al., 2017). However, OsNRAMP3, OsNRAMP4 and OsNRAMP6 appeared having no ability of Cd transportation (Yamaji et al., 2013; Peris-Peris et al., 2017; Xia et al., 2010). In barley, HvNRAMP5, which shares 84% identity with OsNRAMP5, can also mediate the uptake of Cd (Wu et al., 2016). In the hyperaccumulator *Noccaea caerulescens*, NcNRAMP1 is one of the main transporters involved in the influx of Cd across the endodermal PM, and thus plays a key role in Cd influx into the stele and contributes to Cd root-to-shoot transport (Milner et al., 2014).

3.2 | HMAs

HMAs, also known as P_{1B} -ATPase, have a great contribution to transporting cations across the membrane by consuming energy from ATP hydrolysis (Williams and Mills 2005). There are eight HMA members identified in Arabidopsis, with AtHMA1-AtHMA4 showing the ability of transporting divalent cations like Cd^{2+} , Zn^{2+} and Pb^{2+} (Williams and Mills, 2005). Among them, AtHMA2 and AtHMA4 are predominately expressed in the tissues surrounding the vascular vessels of roots and other tissues such as stems and leaves, and they mediate efflux of Cd from xylem parenchyma cells to xylem vessels and are necessary for the root-to-shoot Cd translocation (Mills et al. 2003). In the hyperaccumulator *T. caerulescens*, *S. plumbizincicola* and *A. halleri*, HMA4 shows much higher gene copy numbers and transcript levels than that in *A. thaliana* (Craciun et al., 2012; Hanikenne et al., 2008; Liu et al., 2017), and is considered to be a candidate in determining the evolution of Cd hyperaccumulator phenotype (Hanikenne et al., 2008). In rice, OsHMA2 is localized

to PM mainly in root cells and has been proven to play the crucial role in Cd xylem loading and root-to-shoot translocation, and the loss of its function significantly decreases Cd accumulation in leaves and grains (Sato-Nagasawa et al., 2012; Takahashi et al., 2012). OsHMA3 is a tonoplast-localized transporter and involved in restricting Cd translocation by mediating Cd sequestration into the vacuoles (Miyadate et al., 2011). The loss-of-function of OsHMA3 could cause high Cd accumulation in rice shoots and grains (Yan et al., 2016), whereas its overexpression shows a great opportunity to produce Cd-free rice by reducing Cd concentration in brown rice by 94-98% (Lu et al., 2019). In addition, some recent studies suggested that the natural variation in the promoter or coding region of HMA3 contributes to the genotypic difference in Cd accumulation in rice and *Brassica rapa* (Liu et al., 2019; Zhang et al., 2019).

3.3 | ZIPs

ZIPs family members are generally involved in Cd uptake and translocation in plants (Colangelo and Gueriot, 2006). IRT1 is the first identified member of the ZIP family in Arabidopsis, and participated in taking up Fe^{2+} , Zn^{2+} , Cu^{2+} , Ni^{2+} , and Cd^{2+} from soil (Vert et al., 2002). In rice, 17 ZIPs transporters have been identified. OsIRT1, which is highly homologous to AtIRT1, is predominantly expressed in roots and up-regulated by Fe deficiency and Cd exposure (Bughio et al., 2002; Chen et al., 2018). A recent comparative study of the genome-wide expression profiles revealed a distinct difference between Arabidopsis and rice in the expression profile of ZIPs genes in response to Cd stress (Zheng et al., 2018). In addition, other ZIP transporters like OsZIP1 and OsZIP3 have been also proved to be involved in Cd uptake in rice (Zheng et al., 2018). It is noteworthy that the involvement of ZIP genes in Cd uptake in hyperaccumulators has also been suggested. In *N. caerulescens*, NcZNT1, a homolog of AtZIP4, mediates low-affinity Cd uptake when expressed in *Saccharomyces cerevisiae zhy3* cells (Pence et al. 2000). Recently, it has been demonstrated that NcZNT1 is a PM-localized $\text{Zn}^{2+}/\text{Cd}^{2+}$ transporter and its promoter is mainly active in cells of the cortex, endodermis, and pericycle of roots in *N. caerulescens* (Lin et al., 2016).

3.4 | CDFs

CDF proteins, also known as Metal Tolerance Proteins (MTPs), are a family of heavy metal transporters involved in the transport of Zn^{2+} , Cd^{2+} , and Co^{2+} , and has been identified in the diverse organisms, including bacteria, fungi, animals, and plants. (Williams et al., 2000). The CDFs found in plant cells, generally named as MTPs (Fu et al., 2017), are known to mediate heavy metals efflux from the cytoplasm either to the extracellular space or into the vacuoles and organelles (Peiter et al., 2007). MTPs consists of seven phylogenetic groups, with Zn-CDFs in groups 1 (MTP1–MTP4), 5 (MTP5) and 12 (MTP12); the Fe/Zn-CDFs in groups 6 (MTP6) and 7 (MTP7); and Mn-CDFs in groups 8 (MTP8) and 9 (MTP9–MTP11) (Gustin et al., 2011). In rice, OsMTP1 has been demonstrated to be a PM-localized transporter involved in translocation of Cd and other heavy metals in both roots and shoots (Yuan et al., 2012). Other MTPs, such as TgMTP1 from *Thlaspi goesingense* (Kim et al., 2004), CsMTP1 and CsMTP4 from *Cucumis sativus* (Migocka et al., 2015), and CitMTP1 from *Citrus sinensis* (Fu et al., 2017), have also been proved to be involved in Cd sequestration into vacuoles or Cd efflux from root cells.

3.5 | OPTs

OPT family, which contains Yellow Stripe-Like (YSL) transporters, is involved in transporting metal-nicotianamine (NA) complexes through the plant cell membrane. Thus, when Cd is chelated, it can be taken up through OPT or YSL proteins (Zheng et al., 2018). To enhance the availability of metal ions in rhizosphere, plant roots secrete LMW organic acids, such as mugineic acids (Mas) and phytosiderophore (PS), to form the metal-ligand complexes, which are then transported by YSL transporters (Negishi et al., 2002). This strategy is very efficient for Gramineae plants to take up Fe from Fe-deficient soils (Morrissey and Gueriot, 2009). In addition, YSLs also play an important role in Cd transport. In *Zea mays*, ZmYS1 has been suggested to transport the complexes of Cd-PS and Cd-NA at a low rate (Schaaf et al., 2004). Two orthologues of ZmYS1 isolated from rice and Cd hyperaccumulator *Solanum nigrum*, OsYSL2 and SnYSL3,

have been also reported to transport NA-complexes containing Cd when heterologously expressed in yeast (Koike et al., 2004; Ishimaru et al., 2010; Feng et al., 2017).

3.6 | ABCCs

ABC is one of the largest protein families in living organisms (Higgins, 1992), which have various substrates, including carbohydrates, lipids, xenobiotics, antibiotics, drugs, and heavy metals (Martinoia et al., 2002). Unlike NRAMP3/4, HMA3 and CAX2/4 transporting the free Cd^{2+} ions, ABCCs are identified to transport the conjugates of Cd-PCs (Zhang et al., 2018). In Arabidopsis, AtABCC1 and AtABCC2 are responsible for the transport of Cd-PCs into the vacuoles (Park et al., 2012). Likewise, AtABCC3 has been also suggested to mediate transport of Cd-PC complex (Bovet et al. 2003; Brunetti et al., 2015). In addition to ABCCs, some members from the other subfamilies of ABC transporters have been found to confer Cd tolerance as well. AtABCG36/AtPDR8, a member of the pleiotropic drug resistance (PDR) subfamily of ABC transporters in Arabidopsis, is proposed to have a role in resistance to Cd by pumping Cd^{2+} or Cd conjugates out of the plasma membrane of root epidermal cells (Kim et al., 2007). AtATM3, which belongs to the mitochondria subfamily of Arabidopsis ABC proteins, contributes to Cd resistance by mediating the transport of glutamine synthetase-conjugated Cd across the mitochondrial membrane (Kim et al., 2006). In rice, OsABCG36/OsPDR9 has been recently demonstrated to be involved in Cd tolerance by exporting Cd^{2+} or Cd conjugates from the root cells (Fu et al., 2019).

3.7 | CAXs

The CAXs are tonoplast-localized transporters that export cations out of the cytosol to maintain ion homeostasis across biological membranes (Pittman et al. 2002). Most of CAXs are calcium (Ca^{2+}) specific. However, two CXAs identified in Arabidopsis, AtCAX1 and AtCAX2, have been demonstrated to be capable of pumping not only Ca^{2+} but also other cations like Cd^{2+} , Zn^{2+} and Mn^{2+} into the vacuoles (Korenkov et al., 2007; Korenkov et al., 2009). A later report has shown that the ectopic expression of *AtCAX1* in *Petunia* can significantly increase Cd tolerance and accumulation (Wu et al., 2010). In the Cd hyperaccumulator *A. halleri*, Cd tolerance was shown to be highly associated with the expression of *AhCAX1* (Baliardini et al., 2015), suggesting an involvement of *AhCAX1* in conferring Cd tolerance in this plant. A CAX2-like protein in the hyperaccumulator *S. alfredii*, *SaCAX2*, endows Cd tolerance and accumulation when heterologously expressed in yeast and tobacco (Zhang et al. 2016).

3.8 | Other transporters

In addition to the above transporter families, some other transporters are also involved in Cd transport. OsLCT1, a rice homolog of wheat low affinity cation transporter 1, is localized to the plasma membrane of the cells surrounding the EVBs and DVBs of node 1 and the phloem parenchyma cells of DVBs (Uraguchi et al., 2011). It mediates the efflux of various cations including Ca^{2+} , Cd^{2+} , K^+ , Mg^{2+} , and Mn^{2+} , with a high affinity for Cd^{2+} (Uraguchi et al., 2011). The knockdown of *OsLCT1* can lead to a reduction up to 50% of grain Cd in rice plants grown in Cd contaminated soil, indicating that OsLCT1 is involved in xylem-to-phloem distribution of Cd (Clemens and Ma, 2016). Recently, a gene belonging to the major facilitator superfamily, *OsCd1*, was proved to be involved in root uptake and grain accumulation of Cd in rice (Yan et al., 2019). Furthermore, a natural variation in *OsCd1* caused by a missense mutation was found to be associated with the divergence of grain Cd accumulation between *Indica* and *Japonicarice*. Luo et al. (2018) have also identified a QTL in rice, namely CAL1, which encodes a defensin-like protein. CAL1 is preferentially expressed in root exodermis and xylem parenchyma cells, and acts by chelating Cd in the cytosol and facilitating Cd secretion into apoplast, thus lowering cytosolic Cd concentration (Luo et al., 2018).

Because of sharing a similarity with Ca^{2+} in charge and ionic radius, Cd^{2+} can enter the plant symplast by passive transport through channel proteins transporting Ca^{2+} (Perfus-Barbeoch et al., 2002; Li et al., 2012; Chen et al., 2018). Indeed, several kinds of calcium-permeable channels, such as depolarization-activated calcium channels (DACCs), hyperpolarization-activated calcium channels (HACCs), and voltage-insensitive

cation channels (VICCs), are capable of transporting Cd^{2+} , as evidenced by the significant suppression of Cd^{2+} influxes by the application of putative calcium channel inhibitors (Perfus-Barbeoch et al., 2002; L.Z. Li et al., 2012; Chen et al., 2018). However, the function of these channels in facilitating Cd transport is poorly understood. In a recent study on rice, the expression of the genes belonging to the two Ca channel families, annexins and glutamate receptors (GLRs), was shown to co-segregate with Cd influx and uptake by root cells (Chen et al., 2018), suggesting the possibility to identify the candidate channels responsible for Cd transport from these two families.

4 | EVOLUTION OF CADMIUM ACCUMULATION IN PLANTS

4.1 | Phylogenetic variation in Cd accumulation in plants

Phylogenetic variation in Cd accumulation has been found not only among species, but also among genotypes within a species (Cappa and Pilon-Smits, 2014). Algae constitute an important component of our environment and ecosystem as a primary producer, contributing to nearly 40% of the global productivity of biomass (Issa et al., 2016). Although there is no evidence showing Cd is necessary for growth of algae, they have the ability to concentrate Cd from the environments, with a manner of algae species dependent (Klimmek et al., 2001). In fresh water green algae, such as *Scenedesmus spp.* and *Spirogyra spp.*, the maximum concentration of Cd ranges between 3.0 to 11.4 mg/g dry biomass at 1.0-40.0 mg/L Cd in wastewater. (Brahmbhatt et al., 2012). However, the marine brown algae, such as *Ecklonia radiata*, *Macrocystis pyrifera*, *Laminaria digitata* and *Sargassum spp.* have been shown to be the strong biosorbents of Cd^{2+} , with the highest Cd concentration ranging from 76.4 to 1634 mg/g dry biomass (Davis et al., 2004; Park and Lee, 2002). Such high capacity of Cd^{2+} adsorption for these marine brown algae would be explained by the high abundance of alginate in cell wall matrix, which is a biopolymer segment and has high affinity for divalent metals (Davis et al., 2004; Park and Lee, 2002). On the other hand, the ability of algae to absorb Cd^{2+} is strongly influenced by water pH. In the freshwater green algae *Pseudokirchneriella subcapitata*, the intracellular flux of Cd is at least 20 times higher at pH 7.0 than at pH 5.0 (Vigneault and Campbell, 2005). Similar result has been observed in *Scenedesmus obliquus*, with the highest value of Cd absorption occurring at pH 7.0 (Monteiro et al., 2009). Nevertheless, for the marine brown algae *Ecklonia radiata* there was appreciable Cd uptake at the pH range of 2.0-6.0 and maximum at pH 4.0 (Park and Lee, 2002).

Bryophytes are the first green plants to colonize the terrestrial environment (Nickrent et al. 2000), and they are usually divided into three large phyla: the liverworts (Marchantiophyta), mosses (Bryophyta), and hornworts (Anthoceroophyta) (Shaw et al. 2011). It has been demonstrated that both mosses and liverworts possess the ability to absorb heavy metal ions over the entire plant surface, due to the lack of the cuticle layer, pronounced ion-exchange properties and a large surface-to-weight ratio (reviewed by Stanković et al., 2018). Thus, both mosses and liverworts have been widely used as the biomonitor or bioindicator of heavy metal pollution in both terrestrial and aquatic environments (Mahapatra et al., 2019; Stanković et al., 2018). Nevertheless, this ability is highly species-specific and strongly dependent on the environmental conditions. Vukojević et al. (2005) reported that two moss species, *Bryum capillare* and *Ceratodon purpureus*, could accumulate up to 0.1% DW of Cd (appx.1125-1250 mg/kg) in their shoots. In a later research on the other two moss species *Fabrionia ciliaris* and *Leskea angustata*, however, their Cd concentrations only varies from <0.1 to 7.3 mg/kg, with an average value of 1.3 mg/kg in the rainy season (Macedo-Miranda et al., 2016). The authors also found that Cd is enriched much higher in the rainy season than in dry-cold season. Furthermore, Wells and Brown (1990) found that in the moss *Rhytidiadelphus squarrosus* lowering of pH not only reduced the extracellular binding of Cd, but also inhibited its intracellular uptake.

Ferns and lycophytes are the largest groups in the vascular plants beside the seed plants, consisting of about 10,578 and 1,338 species, respectively (Pteridophyte Phylogeny Group I, 2016). They play the important role in early land plant evolution (Pryer et al., 2001), and are remarkably adapted to a wide range of environments, including both tropical and cold temperate climates, alpine and lowland regions, as well as aquatic and xeric conditions (Mehltreter, 2008). In general, both ferns and lycophytes can accumulate a large amount of Aluminum (Al) and Arsenic (As), but they have much less Cd accumulation (Meharg, 2002;

Schmitt et al., 2017). However, Arora et al. (2006) measured Cd concentrations in three *Azolla* (a small aquatic fern) species, and found that *Azolla* would accumulate Cd as high as 2759 $\mu\text{g/g}$ in shoot when exposed to 10 mg/L Cd, suggesting a potential of *Azolla* in Cd hyperaccumulation.

Angiosperms, appeared during the Early Cretaceous period and within 10-30 Myr, are the largest and most diverse group of vascular plants, consisting of about 400-500 families and perhaps as many as 400,000 species (Taylor et al., 2009). Angiosperms represent approximately 80% of all known living Viridiplantae, including five major groups: Eudicots, Monocots, Magnoliids, Chloranthales, and Ceratophyllales (Yang et al., 2020). Angiosperms inhabit all seven continents, as well as the oceans. They are able to occupy any environment on the earth, such as high mountaintops, deep oceans, freezing tundras, and warm, wet rainforests as well (Field and Arens, 2005). It has been well documented that the angiosperm ecological incursion is highly driven by the environmental circumstances and biotic factors (Field and Arens, 2005). Broadley et al. (2001) summarized the early records of shoot Cd content in angiosperms, and found a significant variation in shoot Cd content among the tested 108 angiosperm species. All available results indicate that there is a large difference in absorbing Cd from the environment among the angiosperms. In fact, about 20 angiosperm species (~10 families) have been already reported as Cd hyperaccumulators (summarized in Cappa and Pilon-Smits, 2014; Reeves et al., 2017; Qiu et al., 2012; Xu et al., 2020). These Cd accumulators are not randomly distributed over the families of angiosperms, but mainly belong to the families of Brassicaceae and Asteraceae. Two Brassicaceae species, *A. halleri* and *T. caerulescens*, which are the most intensively studied species of Cd hyperaccumulating plants, also display an extraordinary ecotypic variation in Cd accumulation (Reeves et al., 2018; Verbruggen et al., 2013). Likewise, the Cd hyperaccumulator *S. alfredii* also exhibit the fixed ecotypic differences in Cd sequestration and translocation (Cappa and Pilon-Smits, 2014; Reeves et al., 2018), indicative of local adaptation of these species to the natural habitat (Reeves et al., 2018), or probably to the appearance of anthropogenic metal pollution (Sobczyk et al., 2017).

Taken together, plants, especially angiosperms, display a wide adaptation over the plant phylogeny to the presence of Cd in the environment. Such adaptation has occurred throughout their evolution (Broadley et al., 2001). The phylogenetic distribution of Cd accumulation across eight to ten orders indicates that Cd accumulation is a derived, polyphyletic trait that evolved convergently within eight to ten angiosperm clades (Broadley et al., 2001; Cappa and Pilon-Smits, 2014). Similar convergent patterns of evaluation in angiosperms have also been observed for accumulation of other metals like selenium (Se) and nickel (Ni) etc. (Cappa and Pilon-Smits, 2014; Reynolds et al., 2017). Hence, here arises a question as to which selection pressures favor the evolution of Cd accumulation? It is well known that the emergence of Cd hyperaccumulation trait in some plant species like *A. halleri*, *T. caerulescens* and *S. alfredii* was coincident with the appearance of anthropogenic metal-polluted sites in the mining regions (Reeves et al., 2017; Qiu et al., 2012; Xu et al., 2020). So it is generally believed that the colonization of anthropogenically heavy metal contaminated soils is likely to represent a recent event in the evolutionary history of plant species (Hanikenne and Nouet, 2011). However, a comparison study between *A. halleri* and *A. lyrata* suggests that ecological differentiation may have occurred at the onset of speciation in this species pair (5 Mya, Koch et al., 2000) and *A. halleri* has well fostered the spread of Cd-polluted areas long before the expansion of anthropogenic environments (Roux et al., 2011). Therefore, Cd accumulation might thus have evolved either on the calamine outcrops before metal pollution by mining activities, or on nonmetalliferous soils followed by a later colonization of metalliferous soils and an increase in metal tolerance (Hanikenne and Nouet, 2011; Reeves et al., 2018). The other hypotheses proposed on the selective factors of Cd accumulation by plants include: protection against herbivores or pathogens, allelopathy and positive physiology effect (reviewed by Cappa and Pilon-Smits, 2014).

4.2 | Evolution of membrane transporters in plants

Evolution of the membrane transporters may play an important role in adapting to metalliferous environments in plants. As described above, there are many membrane transporters involved in Cd accumulation in plants, mainly including NRAMPs, HMAs, ABCCs, ZIPs, CDFs, CAXs and OPTs. Thus several intriguing evolutionary questions arise. Are these membrane transporters conserved in all plant lineages? Is there a

stepwise evolution of membrane transporters in plants? If yes, do they occur from monophyletic or polyphyletic evolutionary events? The ancestors of modern land plants colonized the terrestrial habitat about 500 to 470 Mya. Since then, the dramatic changes have taken place for the living environments of land plant, with a large fluctuation of water availability, illumination, light intensity, temperature and the concentration ratio of carbon dioxide and oxygen, as compared to the aquatic environments for seagrasses grown in the ocean (Chen et al., 2017). As a consequence, an adaptive and stepwise evolution would happen persistently for the membrane transporters in plants. A comparative genomics analysis of seven Cd transporter gene families was performed using 41 plant species ranging from Rhodophyte to Eudicots to reveal the difference of these gene families accompanied by species evolution (Figure 1; Table 2). By applying the strict selection criteria (E-value < 10^{-10} and query coverage > 50%), the OPTs protein family is no longer detected in all algae species with the exception of *Klebsormidium flaccidum*, which has only one OPT homolog (Figure 1; Table 2). Hanikenne et al. (2005) attempted to search for YS1-like proteins in the genome sequences of green alga *Chlamydomonas reinhardtii* and red alga *Cyanidioschyzon merolae*, but could not identify any homolog. Likewise, a previous phylogenetic analysis of 325 OPTs family members from prokaryotes and eukaryotes revealed that OPT family members in eukaryotes were found only in fungi and land plants (Gomolplitinant and Saier Jr., 2011). Thus it may be suggested that the OPTs family have evolved after the emergence of land plants. By contrast, the families of NRAMPs, HMAs, ABCCs, ZIPs, CAXs and CDFs are identified across all the plant species, although there is lack of CAX (in *Cyanidioschyzon merolae* and *Mesotaelium endlicherianum*) and ZIP (in *Porphyra yezoensis* and *Volvox carteri*) homologs in several algae species (Table 2). As been recognized as the ancient gene families that pre-date the origin of eukaryotes (Emery et al., 2012; Gustin et al., 2011; Hanikenne and Baurain, 2014; Hanikenne et al., 2005; Montanini, et al., 2007; Williams and Mills, 2005; Ullah et al., 2018), these Cd transporter families seem to be conserved throughout the evolutionary lineage of entire Viridiplantae species, indicating an evolutionarily conserved function of them in metal homeostasis. Furthermore, it is notable that the vascular plants, including lycophytes, ferns, gymnosperms and angiosperms, contain much more family members of NRAMPs, HMAs, ABCCs, ZIPs, CAXs, CDFs and OPTs in their genomes than do algae and bryophytes (liverworts and mosses) (Table 2), suggesting that these transporter families underwent lineage specific expansion, which might be contributed by gene duplication resulting from segmental duplication [whole genome duplication (WGD) or duplications of large chromosomal regions] and/or tandem duplication (arised through unequal crossing over) (Cannon et al., 2004; Liu et al., 2012; Ullah et al., 2018). During the course of evolution, the expansion of membrane transporter families in the vascular plants may provide an adaptive advantage for colonizing new habitats like metalliferous soil before significant vascular development occurred in early land plants.

OsNRAMP5 and OsHMA2, which mediate Cd uptake and xylem loading respectively, are recognized as the most important transporters for Cd accumulation in rice (Clemens and Ma, 2016). In order to understand the origin of these two Cd transporters, a phylogenetic analysis was performed across the entire plant kingdom using oneKP database (One Thousand Plant Transcriptomes Initiative, 2019). In the phylogenetic tree with 396 species, the orthologues of OsHMA2 from algae, mosses, ferns, lycophytes, gymnosperms and angiosperms form a distinct cluster, but they are all basal to Rhodophyta species *Rhodochaete parvula* (Figure 2A), indicating that this transporter has an early evolution in Viridiplantae. While the angiosperms cluster can be grouped into two subclusters of monocots and eudicots, and all of them are basal to *Amborella trichopoda*, which is the only living species on the sister lineage to all other flowering plants (Amborella Genome Project, 2013). In addition, eudicots show a closer orthologous relationship with *Myristica fragrans* (Figure 2A). These results indicate that monocots and eudicots share a common origination of HMA2 transporter from *A. trichopoda*, which is dating back to about 130 Mya; however, they have evolved separately thereafter, forming a monophyletic evolutionary lineage. In phylogenetic analysis the orthologues of OsNRAMP5 proteins from 592 Viridiplantae species are grouped into two clusters (Figure 2B). Cluster 1 includes green algae, Glaucophyta, Rhodophyta and mosses; and Cluster 2 contains green algae, hornworts, liverworts, lycophytes, ferns, gymnosperms and angiosperms. Each cluster can be further divided into three subclusters; the subclusters in cluster 2 are deep-branching, while those in cluster 1 are not. In the three subclusters of cluster 2, green algae form a distinct sub-subcluster; lycophytes, ferns and gymnosperms are included in the other two sub-subclusters; whereas hornworts, liverworts, and angiosperms can only be found

in the last sub-subcluster. In addition, the higher plants are clearly divided into two groups, including both monocots and eudicots, occupying their specific ancestral gymnosperms and basal angiosperms (Figure 2B). It may be suggested that the orthologues of OsNRAMP5 in higher plants underwent a polyphyletic evolutionary lineage originated from different ancestors. Furthermore, the divergent presence of green algae, lycophytes, ferns and gymnosperms in the phylogenetic tree indicates a rampant occurrence of horizontal gene transfer during the evolution of the orthologues of OsNRAMP5 (Figure 2B), which has been previously evidenced in the studies on the evolution of NRAMPs in bacteria (Richer et al., 2003) and OPTs in plants (Gomolplitinant and Saier Jr., 2011). However, further studies are necessary to make insight into the molecular mechanisms and adaptive roles of horizontal gene transfer events in the evolution of Cd transporters in plants.

5 | CONCLUSIONS AND PERSPECTIVES

Cadmium is one of the most hazardous toxic heavy metals in the environments, posing great risks to human health. In soil-plant system, Cd accumulation in plants is controlled by both genetic and environmental factors via affecting the whole process, including uptake from soil, root-to-shoot translocation, sequestration and (re)distribution in shoots. In essence, all these processes are governed by membrane metal transporters, including NRAMPs, HMAs, ZIPs, CDFs, OPTs, ABCCs, CAXs and some other transporters or channels like OsLCT1, OsCd1 and CAL1. Across the entire Viridiplantae kingdom, Cd accumulation displays a wide phylogenetic variation among species and genotypes within a species. By applying oneKP database, the functional evolution of Cd accumulation in plants was carefully examined, indicating that Cd accumulation in plants is a derived and polyphyletic trait that has evolved convergently by several times. During the course of evolution, the membrane transporter families, such as NRAMPs, HMAs, ABCCs, ZIPs, CDFs and CAXs, are conserved throughout the evolutionary lineage of entire Viridiplantae species, indicating that their functions are evolutionarily conserved for metal homeostasis. However, the OPTs protein family is missed in algae species, suggesting that the OPTs family may evolve after the emergence of land plants. Moreover, the vascular plants contain much more family members of the above transporters in their genomes than do algae and bryophytes, suggesting that these transporter families underwent lineage specific expansion, which might be conferred by gene duplication due to segmental duplication and/or tandem duplication. Furthermore, the orthologues of OsHMA2 in higher plants are stepwisely evolved from monophyletic evolutionary lineage with one common ancestor; whereas the orthologues of OsNRAMP5 came from a polyphyletic evolutionary lineage with the different ancestors. In addition, the phylogenetic clusters showed rampant intermixing have occurred for the orthologues of OsNRAMP5, indicating the horizontal gene transfer involved its evolution. All these evolutionary patterns may provide an adaptive advantage for colonization of plants to new habitat like metalliferous soil. Because of lacking the information on the known Cd hyperaccumulators in oneKP database, we still have no clear answer as to whether and what extent such evolutionary patterns of membrane transporters contributes to Cd (hyper)accumulation in plants. Ideally, the non-accumulator, accumulator and hyperaccumulator plants should be comprehensively compared, in terms of genome sequencing, ecological distribution patterns and the ability of Cd uptake and transport, to fully elucidate the evolutionary mechanisms associated with the accumulation and adaptive response of Cd in plants.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

F. R. Z. and G. P. Z. planned and designed the outline of this review.

G. C. and X. H. C. prepared the figures and tables.

F. R. Z., Q. L., X. J. W. and G. P. Z. wrote the manuscript.

SUPPLEMENTARY MATERIALS

Table S1 Information of NRAMPs, HAMs, ABCCs, ZIPs, CDFs, CAXs and OPTs families for evolutionary bioinformatics analysis.

Supplemental file 1 List of references for Table 1.

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FIGURE LEGENDS

Figure 1 Similarity heat map of key membrane Cd transporters in different species. Genesis software was used to estimate the similarity among protein sequences based on Tables 1 and S1. Candidate protein sequences were selected by BLASTP searches which satisfied E value $<10^{-10}$ and query coverage $>50\%$. Colored squares indicate protein sequence similarity from zero (blue) to 100% (red). White squares indicate that no homologous genes were found.

Figure 2 Phylogenetic analysis of OsHMA2 (A) and OsNRAMP5 (B). The mRNA sequences of OsHMA2 and OsNRAMP5 were queried from the One Thousand Plant Transcriptome (1KP) database (<https://sites.google.com/a/ualberta.ca/onekp/>). Amino acid sequence of *Oryza Sativa* OsHMA2 and OsNRAMP5 were employed as the query sequences to access the transcriptome data with the criterion of E-value $< 10^{-10}$ and coverage $> 50\%$ using BLASTP. The sequences were aligned with MAFFT and the phylogenies constructed with the online toolkit RAxML (Stamatakis, A., 2014) of CIPRES (Miller, M.A. et al., 2010). Genes sampled from Chromia algae were used as the outgroup (in the shade of light grayish magenta) and the root of the tree, and the Interactive Tree of Life resource (<http://www.itol.embl.de>) was used to annotate gene trees. Bootstraps (1-100) were displayed as the width of branches (1-10 px).

Table 1 The identified transporters mediating Cd uptake, translocation, sequestration and distribution in plants sequestration and distribution in plants

| Family | Gene symbol | Expression organ and localization | Plant species | Possible properties | References |
|--------|-------------------|------------------------------------|-----------------------------|---------------------|---|
| NRAMPs | <i>AtNRAMP3/4</i> | root, leave (tonoplast) | <i>Arabidopsis thaliana</i> | Cd, Fe, Mn | Thomine et al., 2000; Lanquar et al., 2005 |
| | <i>NcNRAMP1</i> | root, shoot (PM, tonoplast) | <i>Noccaea caerulea</i> | Cd | Milner et al., 2014 |
| | <i>TcNRAMP3/4</i> | root, shoot (tonoplast) | <i>Thlaspi caerulea</i> | Cd, Fe, Mn | Oomen et al., 2009 |
| | <i>OsNRAMP1</i> | root, shoot (PM) | <i>Oryza sativa</i> | Cd | Takahashi et al., 2011 |
| | <i>OsNRAMP5</i> | root (PM) | <i>Oryza sativa</i> | Cd, Fe, Mn | Ishimaru et al., 2012; Yang et al., 2014 |
| HMAs | <i>HvNRAMP5</i> | root (PM) | <i>Hordeum vulgare</i> | Cd, Mn | Wu et al., 2016 |
| | <i>AtHMA1</i> | root, shoot (chloroplast envelope) | <i>Arabidopsis thaliana</i> | Cd, Zn, Cu | Moreno et al., 2008; Kim et al., 2009 |
| | <i>AtHMA3</i> | root, collar, leaf (tonoplast) | <i>Arabidopsis thaliana</i> | Cd, Zn, Pb, Co | Gravot et al., 2004; Morel et al., 2009; Chao et al., 2012 |
| | <i>AtHMA2/4</i> | root, stem, leaf (PM) | <i>Arabidopsis thaliana</i> | Cd, Zn | Hussain et al., 2004; Lekeux et al., 2019; Verret et al., 2004; Wong et al., 2009 |

| Family | Gene symbol | Expression organ and localization | Plant species | Possible properties | References | |
|-----------------|------------------|---|-----------------------------|----------------------------|---|--|
| ABCCs | <i>OsHMA2</i> | root (PM) | <i>Oryza sativa</i> | Cd, Zn | Takahashi et al., 2012; Yamaji et al., 2013 | |
| | <i>OsHMA3</i> | root (tonoplast) | <i>Oryza sativa</i> | Cd, Zn | Ueno et al., 2010; Miyadate et al., 2011; Sasaki et al., 2014 | |
| | <i>OsHMA9</i> | vascular bundle and anther (PM) | <i>Oryza sativa</i> | Cd, Cu, Zn, Pb | Lee et al., 2007 | |
| | <i>TaHMA2</i> | root, shoot (PM) | <i>Triticum aestivum</i> | Cd, Zn | Tan et al., 2013 | |
| | <i>GmHMA3</i> | root (ER) | <i>Glycine max</i> | Cd, Zn | Wang et al., 2012 | |
| | <i>TcHMA3</i> | root, shoot (tonoplast) | <i>Thlaspi caerulescens</i> | Cd | Ueno et al., 2011 | |
| | <i>SaHMA3</i> | root, shoot (tonoplast) | <i>Sedum alfredii</i> | Cd | Zhang et al., 2016 | |
| | <i>SpHMA3</i> | root, shoot (tonoplast) | <i>S. plumbizincicola</i> | Cd, Zn | Liu et al., 2017 | |
| | <i>AtABCC1/2</i> | root, shoot (tonoplast) | <i>Arabidopsis thaliana</i> | Cd-PC; Hg-PC; As(III)-PC | Park et al., 2012 | |
| | <i>AtABCC3</i> | root, shoot (tonoplast) | <i>Arabidopsis thaliana</i> | Cd-PC | Song et al., 2010; Brunetti et al., 2015 | |
| | <i>AtPDR8</i> | root, shoot (PM) | <i>Arabidopsis thaliana</i> | Cd | Kim et al., 2007 | |
| | <i>OsABCG36</i> | root, shoot (PM) | <i>Arabidopsis thaliana</i> | Cd | Fu et al., 2019 | |
| | CDFs | <i>OsMTP1</i> | root, leaf (tonoplast) | <i>Oryza sativa</i> | Cd, Ni, Fe | Yuan et al., 2012 |
| | | <i>TgMTP1</i> | root, leaf (tonoplast) | <i>Thlaspi goesingense</i> | Cd, Zn, Co, Ni | Kim et al., 2004; Persans et al., 2001 |
| <i>CitMTP1</i> | | root, leaf (tonoplast) | <i>Citrus sinensis</i> | Cd, Zn, Mn, Cu | Fu et al., 2017 | |
| <i>CsMTP1/4</i> | | root, hypocotyl, cotyledon, petiole, leaf (tonoplast) | <i>Cucumis sativus</i> | Cd, Mn, Zn | Migocka et al., 2015 | |
| OPTs | <i>ZmYS1</i> | leaf blade and sheath, crown, seminal root (PM) | <i>Zea mays</i> | Cu, Ni, Cd, Fe, Zn, Mn | Schaaf et al., 2004 | |
| | <i>OsYSL2</i> | shoot phloem (PM) | <i>Oryza sativa</i> | Fe(II)-NA, Mn-NA, Cd-NA | Koike et al., 2004; Ishimaru et al., 2010 | |

| Family | Gene symbol | Expression organ and localization | Plant species | Possible properties | References |
|---------------|-----------------|------------------------------------|-----------------------------|-------------------------|--------------------------------------|
| ZIPs | <i>AtOPT3</i> | root, shoot (PM) | <i>Arabidopsis thaliana</i> | Cd, Zn, Fe | Mendoza-Cozatl et al., 2014 |
| | <i>SnYSL3</i> | root, shoot (PM) | <i>Sedum nigrum</i> | Fe(II)-NA, Mn-NA, Cd-NA | Feng et al., 2017 |
| | <i>OsZIP1</i> | root, shoot (ER, PM) | <i>Oryza sativa</i> | Cd, Zn, Cu | Liu et al., 2019 |
| | <i>TcIRT1</i> | root (PM) | <i>Thlaspi caerulescens</i> | Cd, Zn, Fe(II, III); Mn | Lombi et al., 2002 |
| | <i>TcZNT1</i> | root, shoot (PM) | <i>Thlaspi caerulescens</i> | Cd, Zn | Pence et al., 2000; Lin et al., 2016 |
| CAXs | <i>AtCAX2/4</i> | root (tonoplast) | <i>Arabidopsis thaliana</i> | Cd, Zn, Mn | Korenkov et al., 2007; 2009 |
| | <i>AhCAX1</i> | root, shoot (tonoplast) | <i>Arabidopsis halleri</i> | Cd | Baliardini et al., 2015 |
| | <i>SaCAX2</i> | root, shoot (tonoplast) | <i>Sedum alfredii</i> | Cd | Zhang et al., 2016 |
| Others | <i>OsLCT1</i> | leaf, node, phloem parenchyma (PM) | <i>Oryza sativa</i> | Cd | Uraguchi et al., 2011;2014; |
| | <i>OsLCD</i> | root, shoot (cytoplasm, nucleus) | <i>Oryza sativa</i> | Cd | Shimo et al., 2011 |
| | <i>OsCd1</i> | root (PM) | <i>Oryza sativa</i> | Cd | Yan et al., 2019 |
| | <i>CAL1</i> | root, leaf sheath, internode (CW) | <i>Oryza sativa</i> | Cd | Luo et al., 2018 |

Note: The references were listed as Supplemental materials. PS: Phytosiderophore; PC: Phytochelatin; NA: Nicotianamine. PM: Plasma membrane; ER: Endoplasmic reticulum; CW: Cell wall.

Table 2 Number of predicted and published Cd transporter families in 41 plant and algal species

| Clade | Plant species | Plant species | NRAMPs | HMAAs | ZIPs | CDFs | ABCCs |
|-----------------|-----------------------------|---------------|--------|-------|------|------|-------|
| Eudicots | <i>Arabidopsis thaliana</i> | | 6 | 8 | 15 | 12 | 15 |
| | <i>Brassica rapa</i> | | 9 | 15 | 28 | 18 | 17 |
| | <i>Gossypium raimondii</i> | | 10 | 9 | 21 | 15 | 26 |
| | <i>Theobroma cacao</i> | | 6 | 7 | 12 | 13 | 15 |
| | <i>Eucalyptus grandis</i> | | 9 | 9 | 20 | 18 | 33 |
| | <i>Malus domestica</i> | | 10 | 17 | 16 | 26 | 24 |
| | <i>Medicago truncatula</i> | | 7 | 10 | 13 | 13 | 38 |
| | <i>Glycine max</i> | | 13 | 18 | 19 | 23 | 37 |

| Clade | Plant species | Plant species | NRAMPs | HMAAs | ZIPs | CDFs | ABCCs |
|--------------------------|----------------------------|---------------|--------|-------|------|------|-------|
| Monocots | Populus trichocarpa | | 8 | 13 | 16 | 22 | 24 |
| | Vitis vinifera | | 6 | 5 | 13 | 11 | 23 |
| | Solanum lycopersicum | | 4 | 8 | 11 | 12 | 14 |
| | Chenopodium quinoa | | 10 | 18 | 18 | 15 | 45 |
| | Spirodela polyrhiza | | 3 | 8 | 9 | 11 | 12 |
| | Zostera marina | | 5 | 7 | 13 | 9 | 10 |
| | Phoenix dactylifera | | 10 | 21 | 14 | 19 | 32 |
| | Triticum aestivum | | 18 | 26 | 25 | 19 | 53 |
| | Hordeum vulgare | | 7 | 11 | 12 | 8 | 16 |
| | Brachypodium distachyon | | 7 | 9 | 11 | 10 | 20 |
| | Phyllostachys heterocycla | | 7 | 9 | 11 | 10 | 11 |
| | Zea mays | | 8 | 12 | 10 | 11 | 12 |
| | Sorghum bicolor | | 8 | 11 | 12 | 8 | 16 |
| | Oryza sativa | | 7 | 8 | 11 | 9 | 16 |
| Basal angiosperms | Amborella trichopoda | | 3 | 7 | 8 | 8 | 14 |
| Gymnosperms | Pinus taeda | | 13 | 8 | 11 | 8 | 11 |
| | Pinus lambertiana | | 9 | 11 | 13 | 10 | 10 |
| | Picea abies | | 5 | 5 | 10 | 2 | 6 |
| Ferns | Azolla filiculoides | | 9 | 18 | 11 | 16 | 22 |
| | Salvinia cucullata | | 2 | 9 | 3 | 7 | 16 |
| Lycophytes | Selaginella moellendorffii | | 6 | 12 | 5 | 8 | 23 |
| Mosses | Physcomitrella patens | | 6 | 18 | 7 | 12 | 15 |
| | Sphagnum fallax | | 6 | 8 | 5 | 9 | 16 |
| Liverworts | Marchantia polymorpha | | 5 | 6 | 5 | 5 | 15 |
| Streptophyte | Mesotaenium endlicherianum | | 3 | 7 | 1 | 3 | 8 |
| | Spirogloea muscicola | | 9 | 19 | 3 | 17 | 11 |
| | Chara braunii | | 1 | 3 | 2 | 2 | 2 |
| | Klebsormidium flaccidum | | 3 | 6 | 2 | 5 | 3 |
| | Chlamydomonas reinhardtii | | 2 | 4 | 2 | 4 | 4 |
| Chlorophyta | Volvox carteri | | 2 | 4 | 0 | 1 | 4 |
| | Ostreococcus sp. | | 1 | 5 | 1 | 3 | 2 |
| | Cyanidioschyzon merolae | | 2 | 1 | 1 | 1 | 1 |
| Rhodophyta | Porphyra yezoensis | | 1 | 3 | 0 | 2 | 2 |

Note: Numbers are based on both literature search and bioinformatic analysis. Query Cd transporter genes are listed in Table S1. Genome sequence data were downloaded from oneKP database. Genesis software was used to estimate the similarity between protein sequences. Candidate protein sequences were selected by BLASTP searches which satisfied E value $<10^{-10}$ and query coverage $>50\%$. The abbreviations are: NRAMPs, Natural Resistance-Associated Macrophage Proteins; HMAAs; Heavy Metal ATPases; ZIPs, Zinc/Iron-regulated transporter-like Proteins (ZRT1/IRT1-Like Protein); CDFs, Cation Diffusion Facilitators; OPTs, Oligopeptide transporter family; ABCCs, ATP-binding cassette subfamily C proteins; CAXs, Cation/H⁺ Exchangers.

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