

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 become a hot issue worldwide, as it has posed a great risk to human health via food chain. Cd accumulation in the edible parts of crops is involved in four processes: uptake, translocation, sequestration and redistribution, which are all controlled by membrane transporters. In this review, advances in the studies on the physiological and molecular mechanisms of Cd accumulation in plants are summarized, and then the functional evolution of Cd accumulation in plants

is discussed based on oneKP database. Plant Cd accumulation is a derived and polyphyletic trait that has evolved convergently several times. In the course of evolution, 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 and OsGLR3.4 in higher plants are stepwisely evolved from a common ancestor; whereas the orthologues of OsNRAMP5 evolved from a polyphyletic evolutionary lineage with several different ancestors. In addition, abundant genetic intermixing have occurred in the phylogenetic clusters of the orthologues of OsNRAMP5, indicating horizontal gene transfer from one species to another one. It may be concluded that such an evolution of Cd accumulation in plants could provide an adaptive advantage for colonization of plants to the novel habitats like metalliferous soil.

KEYWORDS: Cadmium, Evolution, Horizontal gene transfer, Xylem loading, Vacuole sequestration, Transporters

1 | INTRODUCTION

Cadmium (Cd) contamination in soil has become one of the most important environmental issues (Rai et al., 2019). It can be caused by many artificial activities, such as application of municipal sewage sludge, chemical fertilizers and pesticides containing Cd and mining (Clemens, 2019). It is estimated that about 30,000 tons of Cd 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 the reduced crop yield and harm to 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 the most prominent (Shahid et al., 2017). There is a close association between soil Cd level and plant Cd content (Khan et al. 2015). In soil-plant system, Cd in the rhizosphere enters plant roots mainly in ionic forms, and is then translocated to above-ground tissues, including grains (Clemens and Ma, 2016). As a non-essential and toxic metal element, the excessive Cd accumulation in plants will cause various detrimental impacts on plants, such as reducing growth, inhibiting photosynthesis and respiration, interfering with biochemically- 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). On the other hand, excessive intake of Cd by humans through consumption of agricultural products containing high Cd content will induce a variety of serious diseases, including 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 food is the main source of Cd exposure for human, accounting for 90% of the total dietary intake (Khan et al., 2015). Therefore, minimizing Cd accumulation in plants, in particular the edible parts is crucial for alleviating its harm to human health. So a comprehensive understanding of the mechanisms underlying the accumulation in plants is imperative for developing the crop cultivars with less Cd accumulation.

In the last decade, a great deal of research has been done in revealing the physiological and molecular mechanisms of Cd uptake and accumulation (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 (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 transformation and gene editing (Fasani et al., 2018). Moreover, there is increasing evidences indicating that Cd accumulation and tolerance in some plant species might have evolved under the selection pressure for their colonization in metalliferous soils or protecting them against herbivores or pathogens (Cappa and Pilon-Smits, 2014). However, there is no report on the relationship between physiological/molecular mechanisms of Cd accumulation and evolutionary adaptation. In this review, the advances in the studies on the physiological and molecular mechanisms of Cd accumulation in plants is summarized and the functional evolution of Cd accumulation in plants is also discussed in terms of the most important membrane transporter families mediating Cd accumulation.

2 | CADMIUM UPTAKE AND TRANSPORT BY PLANTS

The mechanisms of Cd accumulation in plants have been extensively investigated (Clemens, 2019; Clemens and Ma, 2016; Shahid et al., 2017; Ismael et al., 2019; Shi et al., 2019). In general, Cd accumulation in plants can be divided into 4 processes, 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 take up Cd from soil mainly by their roots, and the rate and amount of Cd uptake in roots are dependent on Cd bio-availability or concentration in soil (Clemens, 2006), and also controlled by genetic factors of plants. Cd uptake 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 accumulation in the root apoplast (Meychik and Yermakov, 2001). It is rapid and spontaneous, suggesting no energy requirement in this phase (a passive system). In the second phase, Cd is symplastically taken up in a slow active process, which requires a great deal of energy and is highly dependent 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, reflected by a saturation kinetics in the relationship of Cd uptake velocity and concentration in the medium, indicating that Cd is taken up *via* a carrier (transporter)-mediated system (Verbruggen et al., 2009). 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 are responsible for Cd transport in root cells (Schaaf et al., 2004; Lux et al., 2011; Sasaki et al., 2012). In addition, Cd may also enter root cells through the transport pathway for Ca (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+} (the potent Ca channel inhibitors) or increasing Ca concentration in the culture solution suppressed 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 Cd uptake, little is known about transport of Cd across plant root plasma membranes at the 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 root to shoot *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* symplastic and/or apoplastic transport in the form 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 in both influx and efflux transporters (Clemens and Ma, 2016). Apoplastic transport is a passive pathway, usually driven by transpiration (Qiu et al., 2012). It was suggested that symplastic transport play the dominant role in the radial transport of Cd (Lu et al., 2009; Ueno et al., 2008). However, a recent study on *Sedum alfredii* found that apoplastic pathway contributed up to 37% of Cd transported to the xylem (Tao et al., 2017). After crossing the barriers from root epidermis to root cortex, either free Cd^{2+} or Cd-chelates may enter symplasm and are then loaded into root xylem (Gallego et al., 2012). The xylem loading of Cd in roots is an energy-consuming process because it occurs against membrane potential (Clemens and Ma, 2016), and is mediated by heavy metal P1B-ATPases (HMAs) 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 reducing Cd accumulation in cereal grains. The main sites for Cd sequestration in plants 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 some components of cell walls, such as cellulose, hemicellulose, lignin, and pectin (Shi et al., 2019). As a result, the highest Cd concentration in root tissues occurs in apoplasm, particularly in the cell wall of rhizodermis and cortical cells (Lux et al., 2011). Such binding of Cd to cell walls can efficiently prevent Cd from being transported across the plasma membrane into protoplasts, thereby reducing Cd translocation between cells and tissues.

Within the root cells, Cd is mainly located in vacuoles, which are generally considered to be the main storage site for metals (Salt and Rauser, 1995). Wu et al. (2005) found that 51% of Cd in barley roots was present in the soluble fraction of vacuole, and 36% was present in cell wall. After entering aerial plant parts, Cd is also mainly sequestered in the vacuoles of parenchyma cells in the leaf mesophyll, stem pith, and cortex (Qiu et al. 2011; Tian et al., 2017). Obviously Cd sequestration into vacuoles plays a vital role in reducing Cd concentrations in cytoplasm and alleviating Cd injury (Shi et al., 2019). Several families of transporters have been identified to be responsible for vacuolar sequestration of Cd, 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^{2+} in cytosol firstly 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 the complexes are transported into vacuoles, 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 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

Cd accumulation in shoots and grains (the edible parts in cereals) is the final destination of Cd transportation, which consists of three processes, including xylem unloading, phloem translocation and inter-vascular transfer (Clemens and Ma, 2016). Like 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 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 and no responsible transporter has been identified up to date (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 suggest that phloem loading by inter-vascular transfer from the EVBs to the DVBs in the 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 eudicots shoots is still unclear.

3 | CADMIUM TRANSPORTERS

Since Ca is a non-essential element for plants and interferes with the uptake of other ions, it is likely to enter plant cells through the transporters for essential elements such as Fe^{2+} , Zn^{2+} , and Mn^{2+} , due to the similarity of Cd^{2+} and these ions in chemical and physical properties. In recent decades, a large number of genes conferring Cd transportation across membranes in plants has 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). The functions of these transporters, including uptake, translocation, sequestration, and distribution of Cd in plants, and their tissue-specific localization and substrate specificity are summarized in Table 1 and Table S1.

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, plants, and animals (Uiiah et al., 2018). In plants, *NRAMP* genes are participated in 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 vacuole to the cytosol (Lanquar et al., 2005). In rice, *OsNRAMP1* and *OsNRAMP5* are plasma membrane (PM)-localized transporters involved 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* have 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 Cd uptake (Wu et al., 2016). In the hyperaccumulator *Noccaea caerulescens*, *NcNRAMP1* is one of the main transporters involved in the influx of Cd^{2+} across the endodermal PM, and thus plays a key role in Cd^{2+} influx into the stele and contributes to Cd root-to-shoot transport (Milner et al., 2014).

3.2 | HMAs

HMAs, also known as $\text{P}_{1\text{B}}$ -ATPase, are involved in transporting cations across the membrane by consuming energy from ATP hydrolysis (Williams and Mills 2005). Eight HMA members have been 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). Of them, *AtHMA2* and *AtHMA4* are predominately expressed in the tissues surrounding the vascular vessels of roots, and they mediate Cd^{2+} efflux from xylem parenchyma cells to xylem vessels, which is necessary for the root-to-shoot Cd translocation (Mills et al. 2003). In the hyperaccumulator *Thlaspi caerulescens*, *Sedum plumbizincicola* and *Arabidopsis halleri*, *HMA4* have much more gene copy numbers and transcript levels than that in *Arabidopsis thaliana* (Craciun et al., 2012; Hanikenne et al., 2008; Liu et al., 2017), and it is a candidate in determining the evolution of Cd hyperaccumulator phenotype (Hanikenne et al., 2008). In rice, *OsHMA2* is localized at 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 (Satoh-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 through reducing Cd concentration in brown rice by 94-98% (Lu et al., 2019). In addition, several 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 Guerinot, 2006). IRT1 is the first identified member of the ZIP family in *Arabidopsis*, and participates 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). Recently, Zheng et al. 2018 showed a distinct difference between *Arabidopsis* and rice in the expression profiles of ZIPs genes in responses to Cd stress. 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 was also found in hyper-accumulator plants. In *Noccaea caerulescens*, *NcZNT1*, a homolog of *AtZIP4*, mediates low-affinity Cd uptake when expressed in *Saccharomyces cerevisiae zhy3* cells (Pence et al. 2000). Recently, it was reported 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 *Noccaea 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+} (Williams et al., 2000). They have been identified in the diverse organisms, including bacteria, fungi, animals, and plants. 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 to 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 been proved to be involved in Cd sequestration into vacuoles or 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^{2+} is chelated, it can be taken up through the 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 some species of Poaceae to take up Fe from Fe-deficient soils (Morrissey and Guerinot, 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 found to transport Cd-NA complex 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 organisms (Higgins, 1992) and it has various substrates, including carbohydrates, lipids, xenobiotics, antibiotics, drugs, and heavy metals (Martinoia et al., 2002). Unlike NRAMP3/4, HMA3 and CAX2/4 transporting free Cd^{2+} ions, ABCCs are found to transport the Cd-PCs complex (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). Besides ABCCs, some other

members of ABC transporters also confer Cd tolerance. AtABCG36/AtPDR8, a member of the pleiotropic drug resistance (PDR) subfamily of ABC transporters in *Arabidopsis*, is proposed to have a role in Cd tolerance by pumping Cd²⁺ or Cd complexes 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 tolerance 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 Cd²⁺ 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 CAX members are calcium (Ca²⁺) specific. However, two CXAs identified in *Arabidopsis*, AtCAX1 and AtCAX2, have been found to be capable of pumping Ca²⁺ and other cations like Cd²⁺, Zn²⁺ and Mn²⁺ into vacuoles (Korenkov et al., 2007; Korenkov et al., 2009). Wu et al. (2011) reported that the ectopic expression of *AtCAX1* in *Petunia* significantly increased Cd tolerance and accumulation (Wu et al., 2011). In the Cd hyperaccumulator *Arabidopsis halleri*, Cd tolerance is 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 *Sedum alfredii*, SaCAX2, confers 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 at PM 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 Ca²⁺, Cd²⁺, K⁺, Mg²⁺, and Mn²⁺, with a high affinity for Cd²⁺ (Uraguchi et al., 2011). Knockdown of *OsLCT1* leads 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 *Japonica* rice. Luo et al. (2018) 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 Ca²⁺ in charge and ionic radius, Cd²⁺ can enter the plant symplast by passive transport through channel proteins transporting Ca²⁺ (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 Cd²⁺. Increasing evidences has been obtained using channel blockers and flux measurement to supporting the effects of Ca on Cd uptake and accumulation in plants (Perfus-Barbeoch et al., 2002; L.Z. Li et al., 2012; Chen et al., 2018). However, the functions 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 Cd²⁺ influx and uptake by root cells (Chen et al., 2018), suggesting the possibility to identify the candidate channels responsible for Cd transport.

4 | EVOLUTION OF CADMIUM ACCUMULATION IN PLANTS

4.1 | Phylogenetic variation in Cd accumulation in plants

The great 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 no evidence proves Cd is necessary for growth of algae, they have the ability to concentrate Cd from the environment, with a manner of algae species dependent (Klimmek et al., 2001). In fresh water green algae (Chlorophyta), such as *Scenedesmus* spp. (Scenedesmaceae) and *Spirogyra* spp. (Zygnemataceae), 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 (Ochrophyta), such as *Ecklonia radiata* (Lessoniaceae), *Macrocystis pyrifera* (Laminariaceae), *Laminaria digitata* (Lessoniaceae) and *Sargassum* spp. (Sargassaceae) have been shown to be the strong biosorbents of Cd²⁺, with the highest Cd concentration ranging from 76.4 to 1634 mg/g dry biomass (Davis et al., 2004; Park and Lee, 2002). Such a high capacity of Cd²⁺ adsorption for these marine brown algae could be attributed to high alginate content in cell wall matrix, as alginate is a biopolymer with high affinity for divalent metals (Davis et al., 2004; Park and Lee, 2002). On the other hand, the ability of algae to absorb Cd²⁺ is strongly influenced by water pH. In the freshwater green alga *Pseudokirchneriella subcapitata* (Selenastraceae), the intracellular flux of Cd²⁺ is at least 20 times higher at pH 7.0 than at pH 5.0 (Vigneault and Campbell, 2005). The similar result has been observed in *Scenedesmus obliquus* (Coelastraceae), with the highest value of Cd absorption occurring at pH 7.0 (Monteiro et al., 2009). Nevertheless, for the marine brown alga *Ecklonia radiata* (Lessoniaceae) considerable Cd uptake occurs at the pH ranging 2.0-6.0 and the 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 clades: the liverworts (Marchantiophyta), mosses (Bryophyta), and hornworts (Anthoceroophyta) (Shaw et al. 2011). Both mosses and liverworts possess the ability to absorb heavy metal ions over their entire surface, due to the lack of cuticle layer, pronounced ion-exchange property 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 also 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% Cd DW (appx. 1125-1250 mg/kg) in their shoots. However, in other two moss species *Fabrya ciliaris* and *Leskea angustata*, Cd concentrations only varies from 0.1 to 7.3 mg/kg, with an average value of 1.3 mg/kg (Macedo-Miranda et al., 2016). Furthermore, Wells and Brown (1990) found that in the moss *Rhytidiadelphus squarrosus* low 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 µg/g in shoot when exposed to 10 mg/L Cd, suggesting a potential of *Azolla* in Cd hyperaccumulation.

Gymnosperms are an ancient and widespread plant clade, which represents four of the five main lineages of seed plants, including cycads, ginkgos, gnetophytes, and conifers (De La Torre et al., 2017). Gymnosperm lineages separated from each other during the Late Carboniferous to the Late Triassic (311–212 Mya), earlier than the occurrence of the earliest extant angiosperms (around 300 Mya) (Magallón et al., 2013). Living

gymnosperms comprise only a little more than 1000 species, with conifers (pines, cypresses and relatives) being the largest group (Wang and Ran, 2014). The available research on Cd accumulation in gymnosperms is mostly focused on conifers. Kim et al. (2003) reported that *Pinus sylvestris* would accumulate Cd up to 33.2 mg/kg in shoots under the treatment of 10 mg/kg Cd for 11 weeks. In a later study, however, another *Pinus* species, *Pinus pinaster*, was found to accumulate only 11.9 mg/kg Cd in shoots when exposed to 15 mg/kg Cd for 6 months (Sousa et al., 2014). Hashemi and Farajpour (2016) found that *Picea abies*, grown in the metal contaminated soils (Cd 6.8 mg/kg), could accumulate 1.5 mg/kg Cd in saplings, whereas Österås and Greger (2006) found the plant could accumulate Cd up to 72.4 mg/kg in barks and 26.1 mg/kg in wood after exposure to 0.5 μ M Cd for 3 months. Guo et al. (2016) showed that *Platycladus orientalis* exhibited an exceptional ability of Cd tolerance, accumulating about 69.3 mg/kg Cd in stems and 406 mg/kg in roots after exposure to 100 mg/kg of soil for 220 days. Recently, Zeng et al. (2018) reported that the growth of *Platycladus orientalis* was significantly inhibited under the soil containing 9.6 mg/kg Cd, and accumulated over 2 mg/kg Cd in stems and about 41.5 mg/kg in roots. Although the results of Cd accumulation were not consistent, both authors have suggested that *Platycladus orientalis* is a promising plant for phytostabilization of Cd-contaminated soil. In addition, a hybrid larch shows an extraordinary potential for Cd phytoextraction, as it can tolerate a 4-week exposure to 0.25 mM Cd (or 1-week exposure to 1.5 mM Ca) and accumulate as high as 208-220 mg/kg Cd in shoots (Bonet et al., 2016; Moudouma et al., 2013).

Angiosperms, appeared during the Early Cretaceous period (around 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 plants, including five major groups: Eudicots, Monocots, Magnoliids, Chloranthales, and Ceratophyllales (APG IV, 2016). Angiosperms 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 is 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, founding 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 identified as Cd hyperaccumulators (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, and mainly belong to the families of Brassicaceae and Asteraceae. Two Brassicaceae species, *Arabidopsis halleri* and *Thlaspi caerulescens*, which are the most intensively studied species of Cd hyperaccumulating plants, also show an extraordinary ecotypic variation in Cd accumulation (Reeves et al., 2018; Verbruggen et al., 2013). Likewise, the Cd hyperaccumulator *Sedum alfredii* exhibits the fixed ecotypic difference in Cd sequestration and translocation (Cappa and Pilon-Smits, 2014; Reeves et al., 2018), indicating the local adaptation of these species to the natural habitats (Reeves et al., 2018), or probably to the anthropogenic metal pollution (Sobczyk et al., 2017).

Overall, plants, especially angiosperms, display a wide adaptation to the presence of Cd in the environments. 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). The similar convergent patterns of evolution 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 hyper-accumulation trait in some plant species like *Arabidopsis halleri*, *Thlaspi caerulescens* and *Sedum 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 plants in the 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 *Arabidopsis halleri* and *Arabidopsis lyrata* suggests

that ecological differentiation may have occurred at the onset of speciation in this species pair (5 Mya, Koch et al., 2000) and *Arabidopsis 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).

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 plants, 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.

We made a comparative genomics analysis of seven Cd transporter gene families 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 find the YS1-like proteins in the genome sequences of green alga *Chlamydomonas reinhardtii* and red alga *Cyanidioschyzon merolae*, but failed to 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 CAX (in *Cyanidioschyzon merolae* and *Mesotaenium endlicherianum*) and ZIP (in *Porphyra yezoensis* and *Volvox carteri*) homologs were not detected in several algae species (Table 2). 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 plantae, 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 (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 experienced an early evolution in plants. While the angiosperms cluster can be grouped into two sub-clusters 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 *Amborella trichopoda*, which can be dated 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 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 sub-clusters; the sub-clusters in cluster 2 are deeply-branching, while those in cluster 1 are not. In the three sub-clusters 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, 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.

Our previous study revealed that the expression of OsGLR3.4 was co-segregated with Cd²⁺ influx and uptake into rice roots (Chen et al., 2018). Recently, the contribution of GLR channels to Cd²⁺ influx was verified using mammalian glutamate receptor antagonists AP-5, and our results showed that net Cd²⁺ influx in rice root epidermis was significantly inhibited by AP-5 (Figure 3A). Moreover, relative expression of *OsGLR3.4* was up-regulated when rice plants were exposed to 5 and 50 μ M Cd stress (Figure 3B). All these results support the assumption that GLR channel is involved in mediating Cd uptake in rice roots (Lux et al., 2011; Clemens, 2019). Here we present the origin of OsGLR3.4 across the entire plant kingdom (Figure 3C). Accordingly, the phylogenetic analysis of OsGLR3.4 was conducted using 850 species-based oneKP database. In the phylogenetic tree, the orthologues of OsGLR3.4 from Streptophyta to Eudicots formed 5 distinct clusters. Interestingly, each phylogenetic cluster includes very specific clades of species (Figure 3C). Among the major clusters, GLR3.4 can be traced to Streptophyta species, and the significant presence are observed in hornworts, liverworts, mosses, lycophytes, ferns, gymnosperms and angiosperms (Figure 3C), in accordance with the phylogenetic analysis of OsHMAs (Figure 2A), indicating an ancient origin of OsGLR3.4 orthologues in plants (Bortoli et al., 2016). Moreover, angiosperms are grouped into 2 sub-clusters, with each sub-cluster containing the specific basal angiosperms, monocots and Eudicots, suggesting that GLR3.4 could have recently diverged into two evolutionary branches, possibly giving rise to the distinct functions between them, adapting to Cd-contaminated environments.

5 | CONCLUSIONS AND PERSPECTIVES

Cd is one of the most hazardous toxic heavy metals in the environments, posing great risks to human health. 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 plantae 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 plantae 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 and OsGLR3.4 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 rampant intermixing have occurred for the orthologues of OsNRAMP5 in the phylogenetic clusters, indicating the horizontal gene transfer in its evolution. All these evolutionary patterns may provide an adaptive advantage for colonization of plants to new habitat like metalliferous soil. Due to lack of 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, so as 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.

REFERENCES

- Amborella Genome Project. (2013). The Amborella Genome and the Evolution of Flowering Plants. *Science* , 342 , 1241089-1241089.
- Arora, A., Saxena, S., & Sharma, D.K. (2006). Tolerance and phytoaccumulation of chromium by three *Azolla* species. *World Journal of Microbiology and Biotechnology* , 22 (2), 97-100.

- Baliardini, C., Meyer, C. L., Salis, P., Saumitou-Laprade, P., & Verbruggen, N. (2015). CATION EXCHANGER1 cosegregates with cadmium tolerance in the metal hyperaccumulator *Arabidopsis halleri* and plays a role in limiting oxidative stress in *Arabidopsis* Spp. *Plant Physiology*, 169 (1), 549-559.
- Bonet, A., Lelu-Walter, M.A., Céline F., Gloaguen, V., & Saladin, G. (2016). Physiological responses of the hybrid larch (*larix xeurolepis henry*) to cadmium exposure and distribution of cadmium in plantlets. *Environmental Science & Pollution Research*, 23 (9), 8617-8626.
- Bortili, S.D., Teardo, E., Szabò, I., Morosinotto, T. & Alboresi, A. (2016). Evolutionary insight into the ionotropic glutamate receptor superfamily of photosynthetic organisms. *Biophysical Chemistry*, 218,14-26.
- Bovet, L., Eggmann, T., Meylan-Bettex, M., Polier, J., Kammer, P., Marin, E., Feller, U., & Martinoia, E. (2003). Transcript levels of *AtMRPs* after cadmium treatment: induction of *AtMRP3*. *Plant, Cell & Environment*, 26 (3), 371-381.
- Brahmbhatt, N.H., Patel, V.R., & Jasrai, R.T. (2012). Bioremediation Potential of *Spirogyra Sps* and *Oscillatoria Sps* for Cadmium. *Asian Journal of Biochemical and Pharmaceutical Research*, 2 (2), 102-107.
- Brunetti, P., Zanella, L., De Paolis, A., Di Litta, D., Cecchetti, V., Falasca, G., Barbieri, M., Altamura, M. M., Costantino, P., & Cardarelli, M. (2015). Cadmium-inducible expression of the ABC-type transporter *AtABCC3* increases phytochelatin-mediated cadmium tolerance in *Arabidopsis*. *Journal of Experimental Botany*, 66 (13), 3815-3829.
- Bughio, N., Yamaguchi, H., Nishizawa, N. K., Nakanishi, H., & Mori, S. (2002). Cloning an iron-regulated metal transporter from rice. *Journal of Experimental Botany*, 53 (374), 1677-1682.
- Cannon, S.B., Mitra, A., Baumgarten, A., Young, N.D. & May, G. (2004). The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Evolutionary Biology*, 4, 10.
- Cappa, J.J., & Pilon-Smits, E.A.H. (2014). Evolutionary aspects of elemental hyperaccumulation. *Planta*, 239 (2), 267-275.
- Chen, X., Ouyang, Y., Fan, Y., Qiu, B., Zhang, G., & Zeng, F. (2018). The pathway of transmembrane cadmium influx via calcium-permeable channels and its spatial characteristics along rice root. *Journal of Experimental Botany*, 69 (21), 5279-5291.
- Chen, Z.H., Chen, G., Dai, F., Wang, Y., Hills, A., & Ruan, Y.L., et al. (2016). Molecular evolution of grass stomata. *Trends in Plant Science*, 22 (2), 124-139.
- Clemens, S. (2006). Evolution and function of phytochelatin synthases. *Journal of Plant Physiology*, 163 (3), 319-332.
- Clemens, S. (2019). Safer food through plant science: reducing toxic element accumulation in crops. *Journal of Experimental Botany*, 70 (20), 5537-5557.
- Clemens, S., & Ma, J. F. (2016). Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annual Review of Plant Biology*, 67, 489-512.
- Clemens, S., Palmgren, M. G., & Kramer, U. (2002). A long way ahead: Understanding and engineering plant metal accumulation. *Trends in Plant Science*, 7 (7), 309-315.
- Colangelo, E. P., & Guerinot, M. L. (2006). Put the metal to the petal: metal uptake and transport throughout plants. *Current Opinion in Plant Biology*, 9 (3), 322-330.
- Craciun, A. R., Meyer, C.L., Chen, J., Roosens, N., De Groodt, R., Hilson, P., & Verbruggen, N. (2012). Variation in *HMA4* gene copy number and expression among *Noccaea caerulea* populations presenting different levels of Cd tolerance and accumulation. *Journal of Experimental Botany*, 63 (11), 4179-4189.

- De La Torre A.R., Li Z., de Peer, Y.V. & Ingvarsson P.K. (2017). Contrasting rates of molecular evolution and patterns of selection among gymnosperms and flowering plants. *Molecular Biology & Evolution*, *34* (6), 1363-1377.
- Emery, L., Whelan, S., Hirschi, K., & Pittman, K. (2012). Protein phylogenetic analysis of Ca^{2+} /cation antiporters and insights into their evolution in plants. *Frontiers in Plant Science* , *3* , 1.
- Fasani, E., Manara, A., Martini, F., Furini, A., & DalCorso, G. (2018). The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. *Plant, Cell & Environment*, *41* (5), 1201-1232.
- Feng, S., Tan, J., Zhang, Y., Liang, S., Xiang, S., Wang, H., & Chai, T. (2017). Isolation and characterization of a novel cadmium-regulated Yellow Stripe-Like transporter (SnYSL3) in *Solanum nigrum* .*Plant Cell Reports*, *36* (2), 281-296.
- Fu, S., Lu, Y., Zhang, X., Yang, G., Chao, D., Wang, Z., Shi, M., Chen, J., Chao, D.-Y., Li, R., Ma, J. F., & Xia, J. (2019). The ABC transporter ABCG36 is required for cadmium tolerance in rice. *Journal of Experimental Botany*, *70* (20), 5909-5918.
- Fu, X.Z., Tong, Y.H., Zhou, X., Ling, L.L., Chun, C.P., Cao, L., Zeng, M., & Peng, L.Z. (2017). Genome-wide identification of sweet orange (*Citrus sinensis*) metal tolerance proteins and analysis of their expression patterns under zinc, manganese, copper, and cadmium toxicity. *Gene*, *629* , 1-8.
- Fujimaki, S., Suzui, N., Ishioka, N.S., Kawachi, N., Ito, S., & Nakamura, C.S.I. (2010). Tracing cadmium from culture to spikelet: noninvasive imaging and quantitative characterization of absorption, transport, and accumulation of cadmium in an intact rice plant. *Plant Physiology* , *152* (4), 1796-1806.
- Gallego, S. M., Pena, L. B., Barcia, R. A., Azpilicueta, C. E., Iannone, M. F., Rosales, E. P., Zawoznik, M. S., Groppa, M. D., & Benavides, M. P. (2012). Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. *Environmental and Experimental Botany*, *83* , 33-46.
- Gomolplitinant, K.M., & Saier, M.H. (2011). Evolution of the oligopeptide transporter family. *Journal of Membrane Biology*, *240* (2), 89-110.
- Guo, B., Liu, C., Ding, N., Fu, Q., Lin, Y., Li, H. & Li, N. (2016). Silicon alleviates cadmium toxicity in two cypress varieties by strengthening the exodermis tissues and stimulating phenolic exudation of roots. *Journal of Plant Growth and Regulation* , *35*, 420-429.
- Gustin, J. L., Zanis, M. J., & Salt, D. E. (2011). Structure and evolution of the plant cation diffusion facilitator family of ion transporters. *BMC Evolutionary Biology*, *11* (1), 76.
- Hanikenne, M. & Baurain, D. (2014). Origin and evolution of metal p-type ATPases in plantae (archaeplastida). *Frontiers in Plant Science* , *4* , 544.
- Hanikenne, M., & Cecile, N. (2011). Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. *Current opinion in plant biology*, *14* (3), 252-259.
- Hanikenne, M., Krame,r U., Demoulin, V., & Baurain, D. (2005). A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae* . *Plant Physiology*, *137* (2):428-446.
- Hanikenne, M., Talke, I. N., Haydon, M. J., Lanz, C., Nolte, A., Motte, P., Kroymann, J., Weigel, D., & Kramer, U. (2008). Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. *Nature*, *453* (7193), 391-395.
- Hashemi, S.A. & Farajpour, G. (2016). Investigation of cadmium pollution in the spruce saplings near the metal production factory. *Toxicology and Industrial Health*, *32*(2), 323-327.

- Higgins, C. F. (1992). ABC Transporters: From Microorganisms to Man. *Annual Review of Cell Biology*, 8 (1), 67-113.
- Ishimaru, Y., Masuda, H., Bashir, K., Inoue, H., Tsukamoto, T., Takahashi, M., Nakanishi, H., Aoki, N., Hirose, T., Ohsugi, R., & Nishizawa, N. K. (2010). Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *The Plant Journal*, 62 (3), 379-390.
- Ismael, M. A., Elyamine, A. M., Moussa, M. G., Cai, M. M., Zhao, X. H., & Hu, C. X. (2019). Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics*, 11 (2), 255-277.
- Jiao, Y., & Guo, H. (2014). Prehistory of the Angiosperms: characterization of the ancient genomes. *Advances in Botanical Research*, 69, 223-245.
- Khan, A. R., Ullah, I., Khan, A. L., Park, G.S., Waqas, M., Hong, S.J., Jung, B. K., Kwak, Y., Lee, I.J., & Shin, J.H. (2015). Improvement in phytoremediation potential of *Solanum nigrum* under cadmium contamination through endophytic-assisted *Serratia* sp. RSC-14 inoculation. *Environmental Science and Pollution Research*, 22 (18), 14032-14042.
- Khan, M. A., Castro-Guerrero, N., & Mendoza-Cozatl, D. G. (2014). Moving toward a precise nutrition: preferential loading of seeds with essential nutrients over non-essential toxic elements. *Frontiers in plant science*, 5, 51-51.
- Kim, C.G., Bell, J.N.B., & Power S.A. (2003). Effects of soil cadmium on *Pinus sylvestris* L. seedlings. *Plant and Soil*, 257: 443-449.
- Kim, D., Gustin, J. L., Lahner, B., Persans, M. W., Baek, D., Yun, D.J., & Salt, D. E. (2004). The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. *The Plant Journal*, 39 (2), 237-251.
- Kim, D.Y., Bovet, L., Kushnir, S., Noh, E. W., Martinoia, E., & Lee, Y. (2006). AtATM3 Is Involved in Heavy Metal Resistance in *Arabidopsis*. *Plant Physiology*, 140 (3), 922-932.
- Kim, D.Y., Bovet, L., Maeshima, M., Martinoia, E., & Lee, Y. (2007). The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. *The Plant Journal*, 50 (2), 207-218.
- Klimmek, S., Stan, H. J., Wilke, A., Bunke, G., & Buchholz, R. (2001). Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. *Environmental Science & Technology*, 35 (21), 4283-4288.
- Koch, M.A., Haubold, B., & Mitchell-Olds, T. (2000). Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabidopsis* and related genera. *Molecular Biology and Evolution*, 17 (10), 1483-98.
- Korenkov, V., Hirschi, K., Crutchfield, J. D., & Wagner, G. J. (2007). Enhancing tonoplast Cd/H antiport activity increases Cd, Zn, and Mn tolerance, and impacts root/shoot Cd partitioning in *Nicotiana tabacum* L. *Planta*, 226 (6), 1379-1387.
- Korenkov, V., King, B., Hirschi, K., & Wagner, G. J. (2009). Root-selective expression of *AtCAX4* and *AtCAX2* results in reduced lamina cadmium in field-grown *Nicotiana tabacum* L. *Plant Biotechnology Journal*, 7 (3), 219-226.
- Lanquar, V., Lelievre, F., Bolte, S., Hames, C., Alcon, C., Neumann, D., Vansuyt, G., Curie, C., Schroder, A., Kramer, U., Barbier-Brygoo, H., & Thomine, S. (2005). Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *The EMBO Journal*, 24 (23), 4041-4051.
- Li, L., Liu, X., Peijnenburg, W. J. G. M., Zhao, J., Chen, X., Yu, J., & Wu, H. (2012). Pathways of cadmium fluxes in the root of the halophyte *Suaeda salsa*. *Ecotoxicology and Environmental Safety*, 75, 1-7.

- Lin, Y.F., Hassan, Z., Talukdar, S., Schat, H., & Aarts, M. G. M. (2016). Expression of the ZNT1 zinc transporter from the metal hyperaccumulator *noccaea caerulescens* confers enhanced zinc and cadmium tolerance and accumulation to *Arabidopsis thaliana*. *PLoS One*, 11 (3), e0149750-e0149750.
- Liu, H., Zhao, H., Wu, L., Liu, A., Zhao, F.J., & Xu, W. (2017). Heavy metal ATPase 3 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. *New Phytologist*, 215 (2), 687-698.
- Liu, T., Zeng, J., Xia, K., Fan, T., Ly, T., Wang, Y., Xu, X. & Zhang, M. (2012). Evolutionary expansion and functional diversification of oligopeptide transporter gene family in rice. *Rice*, 5, 12.
- Lu, L.I., Tian, S.k., Yang, X.e., Li, T.q., & He, Z.I. (2009). Cadmium uptake and xylem loading are active processes in the hyperaccumulator *Sedum alfredii*. *Journal of Plant Physiology*, 166 (6), 579-587.
- Lu, C., Zhang, L., Tang, Z., Huang, X.Y., Ma, J.F. & Zhao, F.J. (2019) Producing cadmium-free *Indica* rice by overexpressing OsHMA3. *Environ International*, 126 (1), 619-626.
- Luo, J.S., Huang, J., Zeng, D.L., Peng, J.S., Zhang, G.B., Ma, H.L., Guan, Y., Yi, H.Y., Fu, Y.L., & Han, B. (2018). A defensin-like protein drives cadmium efflux and allocation in rice. *Nature Communications*, 9 (1), 1-9.
- Lux, A., Martinka, M., Vaculik, M., & White, P. J. (2011). Root responses to cadmium in the rhizosphere: a review. *Journal of Experimental Botany*, 62 (1), 21-37.
- Macedo-Miranda, G., Avila-Perez, P., Gil-Vargas, P., Zarazua, G., Sanchez-Meza, J.C., Zepeda-Gomez, C., & Tejeda, S. (2016). Accumulation of heavy metals in mosses: a biomonitoring study. *Springerplus*, 5 (1), 715-728.
- Magallon, S., Hilu, K.W. & Quandt, D. (2013). Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *American Journal of Botany*, 100(3): 556–573.
- Martinoia, E., Klein, M., Geisler, M., Bovet, L., Forestier, C., Kolukisaoglu, U., Muller-Rober, B., & Schulz, B. (2002). Multifunctionality of plant ABC transporters – more than just detoxifiers. *Planta*, 214 (3), 345-355.
- Meharg, A.A. (2003). Variation in arsenic accumulation – hyperaccumulation in ferns and their allies: rapid report. *New Phytologist*, 157 (1), 25-31.
- Mehrtreter, K. (2008). Phenology and habitat specificity of tropical ferns. In Ranker, T.A., & Haufler, C.H. [eds.], *Biology and evolution of ferns and lycophytes*. Cambridge University Press, Cambridge, pp. 201-221.
- Mendoza-Cozatl, D. G., Butko, E., Springer, F., Torpey, J. W., Komives, E. A., Kehr, J., & Schroeder, J. I. (2008). Identification of high levels of phytochelators, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. *The Plant Journal*, 54 (2), 249-259.
- Meychik, N., & Yermakov, I. (2001). Ion exchange properties of plant root cell walls. *Plant and Soil*, 234 (2), 181-193.
- Migocka, M., Kosieradzka, A., Papierniak, A., Maciaszczyk-Dziubinska, E., Posyniak, E., Garbiec, A., & Filleur, S. (2015). Two metal-tolerance proteins, MTP1 and MTP4, are involved in Zn homeostasis and Cd sequestration in cucumber cells. *Journal of Experimental Botany*, 66 (3), 1001-1015.
- Mills, R. F., Krijger, G. C., Baccarini, P. J., Hall, J. L., & Williams, L. E. (2003). Functional expression of AtHMA4, a P1B-type ATPase of the Zn/Co/Cd/Pb subclass. *The Plant Journal*, 35 (2), 164-176.
- Milner, M. J., Mitani-Ueno, N., Yamaji, N., Yokosho, K., Craft, E., Fei, Z., Ebbs, S., Clemencia Zambrano, M., Ma, J. F., & Kochian, L. V. (2014). Root and shoot transcriptome analysis of two ecotypes of *Noccaea*

- caerulescens* uncovers the role of *NcNramp1* in Cd hyperaccumulation. *The Plant Journal*, 78 (3), 398-410.
- Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., Watanabe, A., Fujimura, T., & Akagi, H. (2011). OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytologist*, 189 (1), 190-199.
- Montanini, B., Blaudez, D., Jeandroz, S., Sanders, D & Chalot M. (2007). Phylogenetic and functional analysis of the Cation Diffusion Facilitator (CDF) family: improved signature and prediction of substrate specificity. *BMC Genomics* , 8 ,107
- Morrissey, J., & Guerinot, M. L. (2009). Iron uptake and transport in plants: the good, the bad, and the ionome. *Chemical Reviews*, 109 (10), 4553-4567.
- Moudouma, C.F.M., Riou, C., Gloaguen, V. & Saladin, G. (2013). Hybrid larch (*larix x eurolepis* henry): a good candidate for cadmium phytoremediation? *Environmental Science & Pollution Research*, 20 (3), 1889-1894.
- Negishi, T., Nakanishi, H., Yazaki, J., Kishimoto, N., Fujii, F., Shimbo, K., Yamamoto, K., Sakata, K., Sasaki, T., Kikuchi, S., Mori, S., & Nishizawa, N. K. (2002). cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. *The Plant Journal*, 30 (1), 83-94.
- Nickrent, D.L., Parkinson, C.L., Palmer, J.D., & Duff, R.J. (2000). Multigene phylogeny of land plants with special reference to bryophytes and the earliest land plants. *Molecular Biology and Evolution* ,17 (12), 1885-1895.
- One Thousand Plant Transcriptomes Initiative. (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* ,574 , 679-685.
- Park, E., & Lee, S. (2002). Cadmium uptake by non-viable biomass from a marine brown alga *Ecklonia radiata* turn. *Biotechnol Bioproc Engineering*, 7 (4), 221-224.
- Park, J., Song, W.Y., Ko, D., Eom, Y., Hansen, T. H., Schiller, M., Lee, T. G., Martinoia, E., & Lee, Y. (2012). The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *The Plant Journal*, 69 (2), 278-288.
- Peiter, E., Montanini, B., Gobert, A., Pedas, P., Husted, S., Maathuis, F. J. M., Blaudez, D., Chalot, M., & Sanders, D. (2007). A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *Proceedings of the National Academy of Sciences*, 104 (20), 8532.
- Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., & Forestier, C. (2002). Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant Journal*, 32 (4), 539-548.
- Peris-Peris, C., Serra-Cardona, A., Sanchez-Sanuy, F., Campo, S., Arino, J., & San Segundo, B. (2017). Two NRAMP6 isoforms function as iron and manganese transporters and contribute to disease resistance in rice. *Molecular Plant-Microbe Interactions*, 30 (5), 385-398.
- Pittman, J. K., Sreevidya, C. S., Shigaki, T., Ueoka-Nakanishi, H., & Hirschi, K. D. (2002). Distinct N-terminal regulatory domains of $\text{Ca}^{2+}/\text{H}^{+}$ antiporters. *Plant Physiology*, 130 (2), 1054-1062.
- Pteridophyte Phylogeny Group I. (2016). A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution*, 54 (6), 563-603.
- Qiu, R. L., Tang, Y. T., Zeng, X. W., Thangavel, P., Tang, L., Gan, Y. Y., Ying, R. R., & Wang, S. Z. (2012). Mechanisms of Cd hyperaccumulation and detoxification in heavy metal hyperaccumulators: how plants cope with Cd. *Progress in Botany* , 73 , 127-159.

- Qiu, R.L., Thangavel, P., Hu, P.J., Senthilkumar, P., Ying, R.R., & Tang, Y.T. (2011). Interaction of cadmium and zinc on accumulation and sub-cellular distribution in leaves of hyperaccumulator *Potentilla griffithii*. *Journal of Hazardous Materials*, 186 (2-3), 1425-1430.
- Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K.H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International*, 125, 365-385.
- Reeves, R.D., Baker, A.J.M., Jaffre, T., Erskine, P.D., Echevarria, G., & Ent, A.V.D. (2017). A global database for plants that hyperaccumulate metal and metalloid trace elements. *New Phytologist*, 218 (2), 407-411.
- Reeves, R.D., Ent, A.V.D., & Baker, A.J.M. (2018). Global Distribution and Ecology of Hyperaccumulator Plants. *Agromining: Farming for Metals*, pp. 75-92.
- Reynolds, R.J.B., Cappa, J.J., & Pilon-Smits, E.A.H. (2017). Evolutionary aspects of plant selenium accumulation. In E.A.H. Pilon-Smits et al. (eds.), "*Selenium in plants*". Springer International Publishing, pp. 189-205.
- Richer, E., Courville, P., Bergevin, I., & Cellier, M.F.M. (2003). Horizontal gene transfer of "prototype" Nramp in Bacteria. *Journal of Molecular Evolution*, 57 (4), 363-376.
- Roux, C., Castric, V., Pauwels, M., Wright, S.I., Saumitou-Laprade, P., & Vekemans, X. (2011). Does speciation between *Arabidopsis halleri* and *Arabidopsis lyrata* coincide with major changes in a molecular target of adaptation? *PLoS ONE*, 6 (11), e26872.
- Salt, D. E., & Rauser, W. E. (1995). MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiology*, 107 (4), 1293-1301.
- Sasaki, A., Yamaji, N., Yokosho, K., & Ma, J. F. (2012). Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell*, 24 (5), 2155-2167.
- Satoh-Nagasawa, N., Mori, M., Nakazawa, N., Kawamoto, T., Nagato, Y., Sakurai, K., . . . Akagi, H. (2012). Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (*OsHMA2*) restrict the translocation of zinc and cadmium. *Plant and Cell Physiology*, 53 (1), 213-224.
- Schaaf, G., Ludewig, U., Erenoglu, B. E., Mori, S., & Wiren, N. V. (2004). ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *Journal of Biological Chemistry*, 279 (10), 9091-9096.
- Schmitt, M., Mehltreter, K., Sundue, M., Testo, W., & Jansen, S. (2017). The evolution of aluminum accumulation in ferns and lycophytes. *American Journal of Botany*, 104 (4), 573-583.
- Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., & Niazi, N. K. (2017). Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of Hazardous Materials*, 325, 36-58.
- Sharma, S. S., Dietz, K. J., & Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant, Cell & Environment*, 39 (5), 1112-1126.
- Shaw, A.J., Szovenyi, P., & Shaw, B. (2011). Bryophyte diversity and evolution: windows into the early evolution of land plants. *American Journal of Botany*, 98 (3), 352-369.
- Shi, G. L., Li, D. J., Wang, Y. F., Liu, C. H., Hu, Z. B., Lou, L. Q., Rengel, Z., & Cai, Q. S. (2019). Accumulation and distribution of arsenic and cadmium in winter wheat (*Triticum aestivum* L.) at different developmental stages. *Science of The Total Environment*, 667, 532-539.
- Sloof, J. E., Viragh, A., & Van Der Veer, B. (1995). Kinetics of cadmium uptake by green algae. *Water, Air, and Soil Pollution*, 83 (1), 105-122.

- Sobczyk, M.K., Smith, J.A.C., Pollard, A.J., & Filatov, D.A. (2016). Evolution of nickel hyperaccumulation and serpentine adaptation in the *Alyssum serpyllifolium* species complex. *Heredity*, 118 (1), 31-41.
- Sousa, N.R., Ramos, M.A., Marques, A.P.C.C. & Castro P.M.L. (2014). A genotype dependent-response to cadmium contamination in soil is displayed by *Pinus pinaster* in symbiosis with different mycorrhizal fungi. *Applied Soil Ecology* , 76, 7-13.
- Takahashi, R., Ishimaru, Y., Nakanishi, H., & Nishizawa, N. K. (2011). Role of the iron transporter Os-NRAMP1 in cadmium uptake and accumulation in rice. *Plant Signaling & Behavior*, 6 (11), 1813-1816.
- Takahashi, R., Ishimaru, Y., Shimo, H., Ogo, Y., Senoura, T., Nishizawa, N. K., & Nakanishi, H. (2012). The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant, Cell & Environment*, 35 (11), 1948-1957.
- Tang, Z., Cai, H., Li, J., Lv, Y., Zhang, W., & Zhao, F.J. (2017). Allelic variation of NtNramp5 associated with cultivar variation in cadmium accumulation in tobacco. *Plant and Cell Physiology*, 58 (9), 1583-1593.
- Tao, Q., Jupa, R., Luo, J., Lux, A., Kovač, J., Wen, Y., Zhou, Y., Jan, J., Liang, Y., & Li, T. (2017). The apoplasmic pathway via the root apex and lateral roots contributes to Cd hyperaccumulation in the hyperaccumulator *Sedum alfredii* . *Journal of Experimental Botany*, 68 (3), 739-751.
- The Angiosperm Phylogeny Group. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* , 181, 1-20.
- Tian, S., Xie, R., Wang, H., Hu, Y., Hou, D., Liao, X., Brown, P. H., Yang, H., Lin, X., Labavitch, J. M., & Lu, L. (2017). Uptake, sequestration and tolerance of cadmium at cellular levels in the hyperaccumulator plant species *Sedum alfredii* . *Journal of Experimental Botany*, 68 (9), 2387-2398.
- Ueno, D., Iwashita, T., Zhao, F.J., & Ma, J. F. (2008). Characterization of Cd translocation and identification of the cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri* . *Plant and Cell Physiology*, 49 (4), 540-548.
- Ueno, D., Ma, J. F., Iwashita, T., Zhao, F.J., & McGrath, S. P. (2005). Identification of the form of Cd in the leaves of a superior Cd-accumulating ecotype of *Thlaspi caerulescens* using ¹¹³Cd-NMR. *Planta*, 221 (6), 928-936.
- Ullah, I., Wang, Y., Eide, D. J., & Dunwell, J. M. (2018). Evolution, and functional analysis of Natural Resistance-Associated Macrophage Proteins (NRAMPs) from *Theobroma cacao* and their role in cadmium accumulation. *Scientific Reports*, 8 (1), 14412.
- Uraguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida, A., Kyojuka, J., Ishikawa, S., & Fujiwara, T. (2011). Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. *Proceedings of the National Academy of Sciences*, 108 (52), 20959-20964.
- Uraguchi, S., Kiyono, M., Sakamoto, T., Watanabe, I., & Kuno, K. (2009a). Contributions of apoplasmic cadmium accumulation, antioxidative enzymes and induction of phytochelatins in cadmium tolerance of the cadmium-accumulating cultivar of black oat (*Avena strigosa* Schreb.). *Planta*, 230 (2), 267-276.
- Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T., & Ishikawa, S. (2009b). Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *Journal of Experimental Botany*, 60 (9), 2677-2688.
- Verbruggen, N., Hermans, C., & Schat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist*, 181 (4), 759-776.
- Verbruggen, N., Juraniec, M., Baliardini, C., & Meyer, C.L. (2013). Tolerance to cadmium in plants: the special case of hyperaccumulators. *BioMetals*, 26 (4), 633-638.

- Vert, G., Grotz, N., Dédaldéchamp, F., Gaymard, F., Guerinot, M. L., Briat, J.F., & Curie, C. (2002). IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell*, 14 (6), 1223-1233.
- Vigneault, B., & Campbell, P.G.C. (2005). Uptake of cadmium by freshwater green algae: effects of pH and aquatic humic substances. *Journal of Phycology*, 41 (1), 55-61.
- Vukojević, V., Sabovljević, M., & Jovanović, S. (2005). Mosses accumulate heavy metals from the substrata of coal ash. *Archives of Biological Sciences*, 57 (2), 101-106.
- Wells, J.M., & Brown, D.H. (1990). Ionic control of intracellular and extracellular Cd uptake by the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. *New Phytologist*, 116 (3), 541-553.
- White, P. J., & Broadley, M. R. (2003). Calcium in plants. *Annals of Botany*, 92 (4), 487-511.
- Williams, L. E., & Mills, R. F. (2005). P1B-ATPases – an ancient family of transition metal pumps with diverse functions in plants. *Trends in Plant Science*, 10 (10), 491-502.
- Williams, L. E., Pittman, J. K., & Hall, J. L. (2000). Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1465 (1), 104-126.
- Wu, D., Yamaji, N., Yamane, M., Kashino-Fujii, M., Sato, K., & Ma, J. F. (2016). The HvNramp5 transporter mediates uptake of cadmium and manganese, but not iron. *Plant Physiology*, 172 (3), 1899-1910.
- Wu, Q., Shigaki, T., Williams, K. A., Han, J. S., Kim, C. K., Hirschi, K. D. & Park, S. (2011). Expression of an *Arabidopsis* $\text{Ca}^{2+}/\text{H}^{+}$ antiporter CAX1 variant in petunia enhances cadmium tolerance and accumulation. *Journal of Plant Physiology*, 168(2), 167-173.
- Wu, F.B., Dong, J., Qian, Q. Q., & Zhang, G.P. (2005). Subcellular distribution and chemical form of Cd and Cd-Zn interaction in different barley genotypes. *Chemosphere*, 60 (10), 1437-1446.
- Xia, J., Yamaji, N., Kasai, T., & Ma, J. F. (2010). Plasma membrane-localized transporter for aluminum in rice. *Proceedings of the National Academy of Sciences*, 107 (43), 18381-18385.
- Xu, W.M., Xiang, P., Liu, X., & Ma, L.Q. (2020). Closely-related species of hyperaccumulating plants and their ability in accumulation of As, Cd, Cu, Mn, Ni, Pb and Zn. *Chemosphere*, 251, 126334.
- Yamaji, N., & Ma, J. F. (2014). The node, a hub for mineral nutrient distribution in graminaceous plants. *Trends in Plant Science*, 19 (9), 556-563.
- Yamaji, N., Sasaki, A., Xia, J. X., Yokosho, K., & Ma, J. F. (2013). A node-based switch for preferential distribution of manganese in rice. *Nature Communications*, 4 (1), 1-11.
- Yan, H., Xu, W., Xie, J., Gao, Y., Wu, L., Sun, L., Feng, L., Chen, X., Zhang, T., Dai, C., Li, T., Lin, X., Zhang, Z., Wang, X., Li, F., Zhu, X., Li, J., Li, Z., Chen, C., Ma, M., Zhang, H., & He, Z. (2019). Variation of a major facilitator superfamily gene contributes to differential cadmium accumulation between rice subspecies. *Nature Communications*, 10 (1), 2562.
- Yan, J., Wang, P., Wang, P., Yang, M., Lian, X., Tang, Z., Huang, C.F., Salt, D. E., & Zhao, F. J. (2016). A loss-of-function allele of *OsHMA3* associated with high cadmium accumulation in shoots and grain of *Japonica* rice cultivars. *Plant, Cell & Environment*, 39 (9), 1941-1954.
- Yang, M., Zhang, Y., Zhang, L., Hu, J., Zhang, X., Lu, K., Dong, H., Wang, D., Zhao, F.J., Huang, C.F., & Lian, X. (2014). OsNRAMP5 contributes to manganese translocation and distribution in rice shoots. *Journal of Experimental Botany*, 65 (17), 4849-4861.
- Yuan, L., Yang, S., Liu, B., Zhang, M., & Wu, K. (2012). Molecular characterization of a rice metal tolerance protein, OsMTP1. *Plant Cell Reports*, 31 (1), 67-79.

Zeng, P., Guo, Z., Xiao, X., Cao, X. & Peng, C. (2018). Response to cadmium and phytostabilization potential of *Platycladus orientalis* in contaminated soil. *International Journal of Phytoremediation*, 20(13), 1337-1345.

Zhang, L., Wu, J., Tang, Z., Huang, X.Y., Wang, X., Salt, D. E., & Zhao, F.J. (2019). Variation in the *BrHMA3* coding region controls natural variation in cadmium accumulation in *Brassica rapa* vegetables. *Journal of Experimental Botany*, 70 (20), 5865-5878.

Zhang, M., Zhang, J., Lu, L. L., Zhu, Z. Q., & Yang, X. E. (2016). Functional analysis of CAX2-like transporters isolated from two ecotypes of *Sedum alfredii*. *Biologia Plantarum*, 60 (1), 37-47.

Zhang, X., Rui, H., Zhang, F., Hu, Z., Xia, Y., & Shen, Z. (2018). Overexpression of a functional *Vicia sativa* PCS1 homolog increases cadmium tolerance and phytochelatin synthesis in *Arabidopsis*. *Frontiers in plant science*, 9, 107.

Zhao, F. J., Hamon, R. E., Enzo, L. J., M. M., & P., M. S. (2002). Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*, 53 (368), 535-43.

Zheng, X., Chen, L., & Li, X. (2018). *Arabidopsis* and rice showed a distinct pattern in ZIPs genes expression profile in response to Cd stress. *Botanical Studies*, 59 (1), 22.

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).

Figure 3 Kinetics of Cd²⁺ fluxes in response to glutamate receptor antagonists AP-5 (A), gene expression of OsGLR3.4 in response to external Cd (B) and phylogenetic analysis of OsGLR3.4 (C). Transient Cd²⁺ fluxes were measured from mature zone in rice root in response to 50 μM Cd pretreated with or without 100 μM AP-5, Data $\pm\text{SE}(n=8)$. Gene expression of *OsGLR3.4* was quantified by qRT-PCR under 5 or 50 μM Cd at the exposure time indicated in the figure, , Data $\pm\text{SE}(n=3)$. Phylogenetic analysis of OsGLR3.4 was conducted similarly as described in Figure 2.

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 caerulescens</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
	<i>HvNRAMP5</i>	root (PM)	<i>Hordeum vulgare</i>	Cd, Mn	Wu et al., 2016
HMA s	<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
	<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

Family	Gene symbol	Expression organ and localization	Plant species	Possible properties	References
ABCCs	<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
	<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
ZIPs	<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

Family	Gene symbol	Expression organ and localization	Plant species	Possible properties	References
Others	<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
	<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
	<i>Populus trichocarpa</i>		8	13	16	22	24
	<i>Vitis vinifera</i>		6	5	13	11	23
	<i>Solanum lycopersicum</i>		4	8	11	12	14
	<i>Chenopodium quinoa</i>		10	18	18	15	45
Monocots	<i>Spirodela polyrhiza</i>		3	8	9	11	12
	<i>Zostera marina</i>		5	7	13	9	10
	<i>Phoenix dactylifera</i>		10	21	14	19	32
	<i>Triticum aestivum</i>		18	26	25	19	53
	<i>Hordeum vulgare</i>		7	11	12	8	16
	<i>Brachypodium distachyon</i>		7	9	11	10	20
	<i>Phyllostachys heterocycla</i>		7	9	11	10	11
	<i>Zea mays</i>		8	12	10	11	12
	<i>Sorghum bicolor</i>		8	11	12	8	16
	<i>Oryza sativa</i>		7	8	11	9	16
Basal angiosperms	<i>Amborella trichopoda</i>		3	7	8	8	14

Clade	Plant species	Plant species	NRAMPs	HMA	ZIPs	CDFs	ABCCs
Gymnosperms	<i>Pinus taeda</i>		13	8	11	8	11
	<i>Pinus lambertiana</i>		9	11	13	10	10
	<i>Picea abies</i>		5	5	10	2	6
Ferns	<i>Azolla filiculoides</i>		9	18	11	16	22
	<i>Salvinia cucullata</i>		2	9	3	7	16
Lycophytes	<i>Selaginella moellendorffii</i>		6	12	5	8	23
Mosses	<i>Physcomitrella patens</i>		6	18	7	12	15
	<i>Sphagnum fallax</i>		6	8	5	9	16
Liverworts	<i>Marchantia polymorpha</i>		5	6	5	5	15
Streptophyte	<i>Mesotaenium endlicherianum</i>		3	7	1	3	8
	<i>Spirogloea muscicola</i>		9	19	3	17	11
	<i>Chara braunii</i>		1	3	2	2	2
	<i>Klebsormidium flaccidum</i>		3	6	2	5	3
	<i>Chlamydomonas reinhardtii</i>		2	4	2	4	4
Chlorophyta	<i>Volvox carteri</i>		2	4	0	1	4
	<i>Ostreococcus sp.</i>		1	5	1	3	2
	<i>Cyanidioschyzon merolae</i>		2	1	1	1	1
Rhodophyta	<i>Porphyra yezoensis</i>		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; HMAs; 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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