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Membrane Transporter Families of Metal Microelements make Plants Grow Better and Healthier

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ABSTRACT

Appropriate supplies of metal microelements, such as Fe, Mn, Cu, Zn, Mo, are essential for plant normal growth and development. Plants can also uptake toxic metals, such as Cd, Pb, Hg, when they are exposed to contaminated soils. In response to external fluctuations of these metal element supplies, plants have developed an active and complex network of membrane transport system for optimizing the nutrient uptake, translocation and compartmentalization to maintain their cellular homeostasis. The application of powerful genetic and molecular techniques has contributed a lot to the identification and characterization of gene families that are involved in membrane transport of metal elements. In this review, we summarized recent advances in the functional analysis of transporters involved in the essential metal microelements as well as the toxic metals, including ZIPs, MTPs, HMAs, NRAMPs and YSLs, in both dicot Arabidopsis and monocot rice together with other plants especially the metal hyper-accumulating plants. Well understanding of this complicated but effective membrane transport system provides us a foundation for the development of novel strategies to increase the acquisition and distribution of beneficial elements for crop production as well as the efflux and sequestration of toxic elements for human health.

Keywords: Microelement, Transporter, Gene family, Arabidopsis, Rice

INTRODUCTION

In order to keep the normal growth and preferential development, plants need a range of microelements, such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni) and chloride (Cl) in addition of the macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S). Fe and Cu are essential trace elements involving in a range of electron transport reactions in both photosynthesis and photorespiration processes, while Zn and Mn activate a number of enzymes during the biological and metabolic processes [1]. Therefore, when these nutrients are not available to the roots, plants develop specific deficiency symptoms. However, these nutrients can also become extremely toxic to plants causing symptoms, such as leaf chlorosis and necrosis, stunting and root growth inhibition because of the overproduction of reactive oxygen species and oxidative injury when they present in excess levels [1-3]. Thus, the abilities of plants uptaking micronutrients by the roots, transport and distribution among the tissues and organs, and regulation of the cytosolic concentrations to keep ion homeostasis are particularly important to healthy plant growth and preferential development.

Plant genomes encode large families of nutrient transporters that vary in their substrate specificities, cellular localization and expression patterns, to ensure that all tissues and organs receive an adequate level of the nutrients required for vital cellular processes but prevent them from accumulating to toxic levels [4,5]. Until recently, a wide range of gene families have been identified in plants that are likely to be involved in metal microelements transport. These include ZIP (ZRT-, IRT-like proteins) family [6], CDF (cation diffusion facilitator) family [7], HMA (heavy metal ATPase) family [8-10], NRAMP (natural resistance-associated macrophage protein) family [11] and YSL (yellow stripe-like) proteins [12-14]. The mineral micro nutrition of crops is of fundamental importance to both yield production and human health. However, there are still many physiological and molecular mechanisms remaining to be investigated, particularly in

relation to the distribution and accumulation of either essential micronutrients or non-essential and toxic heavy metal elements in edible parts of crops. This paper will summarize the general properties of metal micronutrient transporter families, including substrate specificities, cellular and subcellular locations, biological functions in both Arabidopsis and rice, as well as some of other crops and metal hyper-accumulating plants. It aims to provide an overview of the potential transport systems that thought to be involved in the acquisition, distribution and homeostasis of metal microelements in plants. Figure 1 summarized the organ/tissue expressions and responses to element sufficient or deficient conditions of *ZIP*, *MTP*, *HMA*, *NRAMP* and *YSL* genes in Arabidopsis and rice, Figure 2 summarized the subcellular expressions and transport substrates of ZIPs, MTPs, HMAs, NRAMPs and YSLs in Arabidopsis and rice.

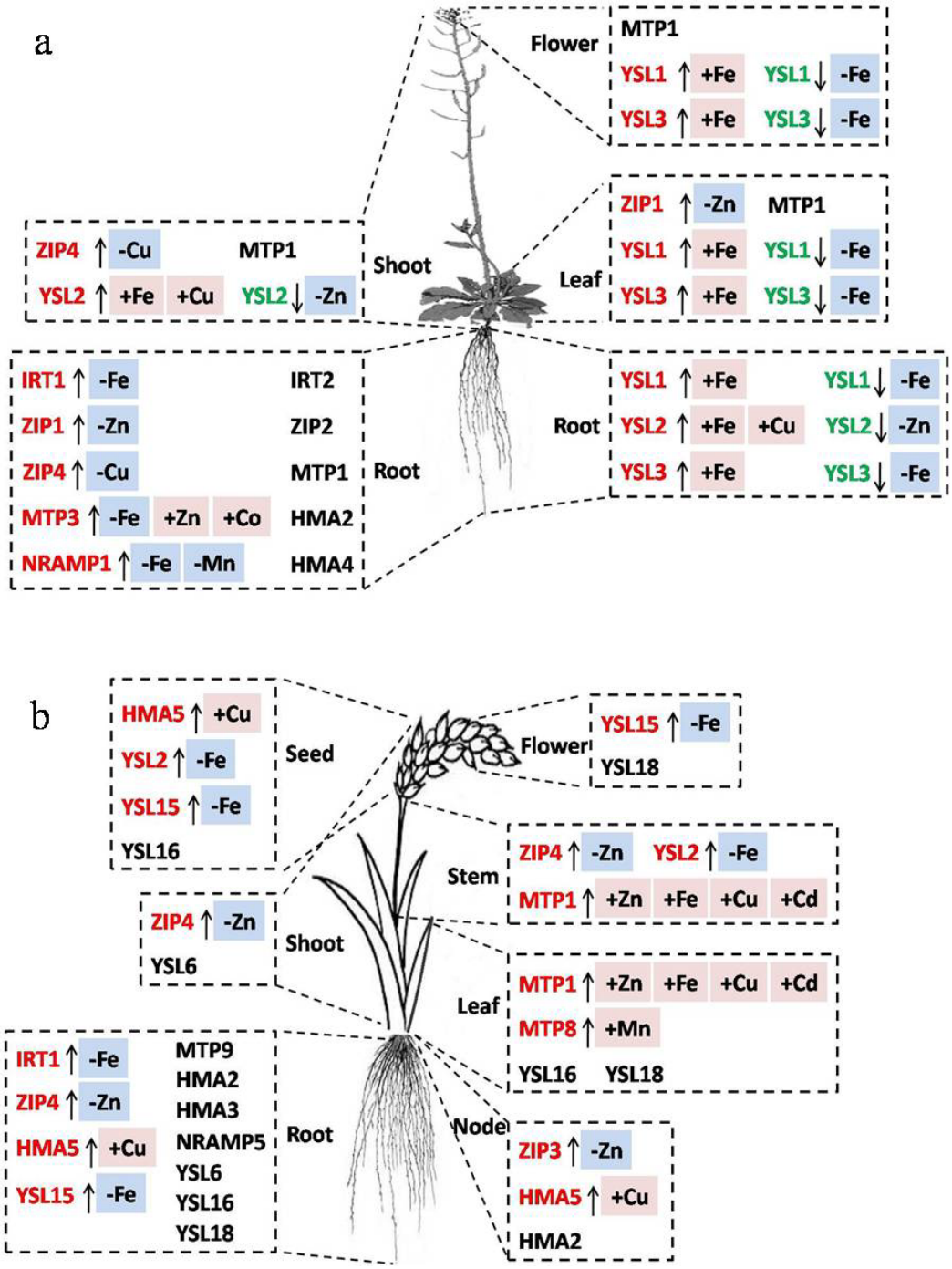

Figure 1: The diagram of organ/tissue expressions and responses to element sufficient or deficient conditions of *ZIP*, *MTP*, *HMA*, *NRAMP* and *YSL* genes in Arabidopsis (a) and rice (b). ↑ and ↓indicates the gene expression level is up- and down-regulated by element sufficiency (+) or deficiency (-), respectively

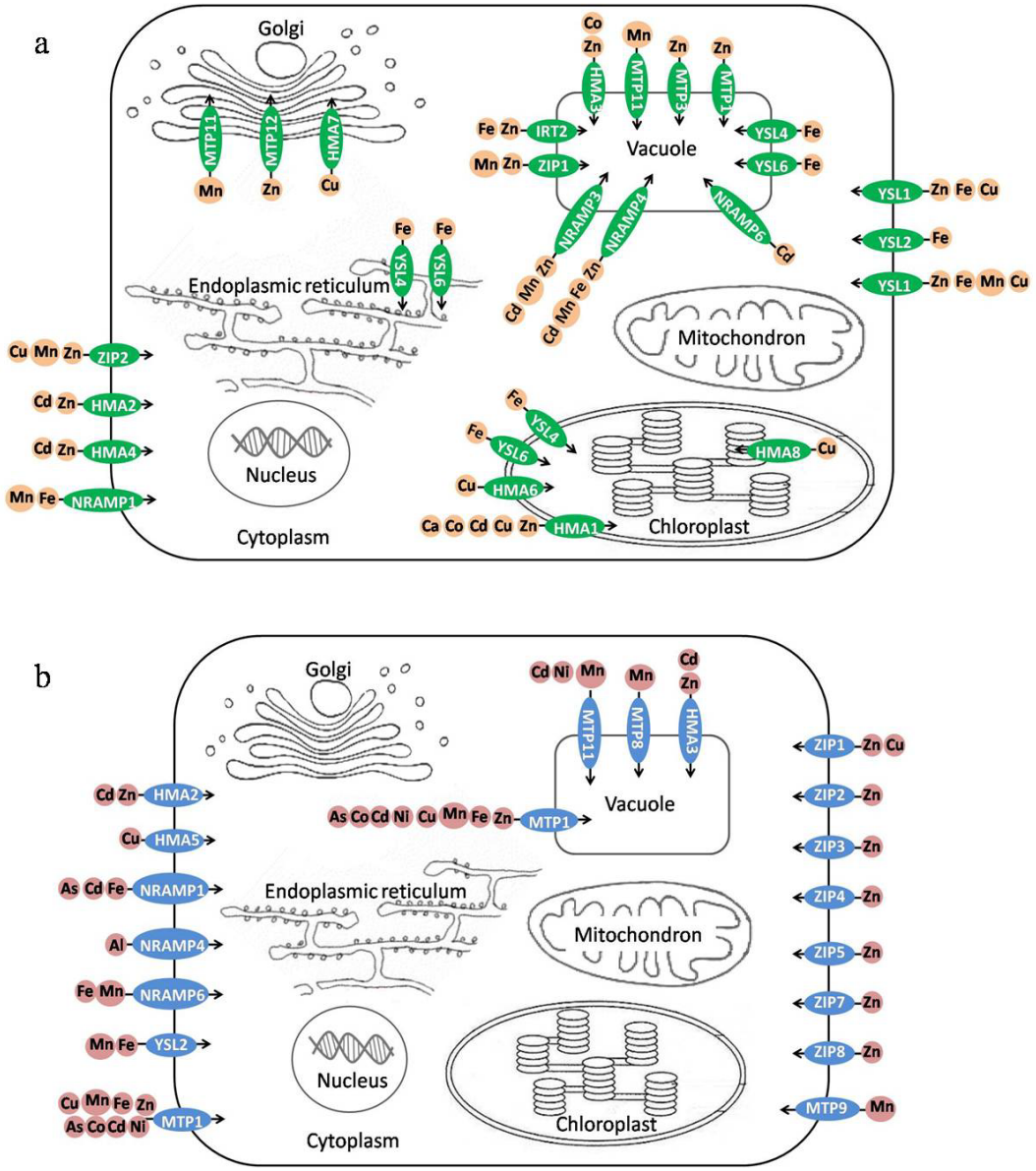

Figure 2: The diagram of subcellular expressions and transport substrates of ZIPs, MTPs, HMAs, NRAMPs and YSLs in Arabidopsis (a) and rice (b). → indicates the transport direction of elements

ZIP GENE FAMILY

General function of *ZIP* genes

ZIP (ZRT-, IRT-like proteins) family are named for their sequence similarity to the ZRT1 (Zn regulated transporter 1) from yeast and IRT1 (iron regulated transporter 1) from Arabidopsis. It is a divalent metal transporter family and considered to be the primary group of transporters controlling plant Zn influx at the plasma membrane [6,15-18]. ZIP family is well conserved among bacteria, fungi, archaea, plants and mammals [17,19]. To date, many *ZIP* genes have been isolated and characterized in plants, and they are involved in transport of varies metal ions in addition to Zn, such as Fe, Mn, Cu, Ni, Co (cobalt) and Cd (cadmium) [20]. ZIP transporters have been implicated in uptake of Zn from soil, transport of Zn to leaves and translocation of Zn to seeds [17,21-23]. ZIP proteins consist of 309-476 amino acid residues and are predicted to have a similar membrane topology with 7-8 TM (transmembrane) domains. The N-terminal and C-terminal regions of ZIP proteins exposed to the outside surface of plasma membrane have been demonstrated to transport divalent heavy metal ions into the cytoplasm [6,18].

There is a variable intracellular loop between TM III and TM IV containing a conserved potential metal binding domain rich in histidine residues [6,24]. Mutations in the HRD (histidine rich domain) of human ZIP1 and ZIP4

resulted in a reduction of cellular Zn uptake and the HRD of human ZIP4 is essential for Zn-stimulated ubiquitination and degradation of the protein [25,26]. However, replacement of all histidine residues with glutamine in the HRD of the yeast ZRT1 had no apparent effect on transporter function, but altered the subcellular localization and protein turnover of ZRT1 [27]. Although, most ZIP transporters appear to be localized to the plasma membrane, several ZIP proteins that have amino terminal signal peptides are predicted to localize to other membranes [17,28,29], which suggests diverse functions of ZIP proteins existed in different biological processes.

ZIP genes in Arabidopsis

In *Arabidopsis thaliana* genome, fifteen *ZIP* genes have been identified [20]. *AtIRT1*, the first identified plant *ZIP* gene, was cloned from *Arabidopsis* by functional complementary assay of a Fe uptake deficient yeast mutant, *fet3/fet*4 [15]. Although AtIRT1 is a divalent cation transporter that transports a wide range of trace elements, including Fe, Zn, Mn, Ni and Cd, it is considered to be the major Fe transporter at the root surface in Arabidopsis under Fe deficient conditions [30-32]. Rogers et al. also reported that the extracellular loop between TM II and TM III was extremely important for the Fe selectivity of AtIRT1 [33]. *AtIRT1* is predominantly expressed in the external cell layers of the root and up-regulated in Fe deficiency conditions but was rarely detected after plants were returned to Fe sufficient conditions [15,31,34].

Knock out of *AtIRT1* gene in Arabidopsis results in typical Fe deficient symptoms that causing severe leaf chlorosis and plant lethality with a drastic reduction of chloroplast thylakoid and lack of palisade parenchyma differentiation in leaves, reduced number of vascular bundles in stems, and irregular patterns of enlarged endodermal and cortex cells in roots. Furthermore, the mutation in *AtIRT1* gene also reduces Fe and Zn accumulation through altering the expression levels of certain Fe and Zn transporter genes, including the activation of *AtZIP1* in shoots and *AtIRT2* in roots, as well as the reduction of *AtZIP2* in roots [35]. Compare to the wild type, the mutant also showed a significant reduction in root Mn and Ni concentrations when grown under Fe deficient conditions, which indicates that *AtIRT1* may mediate Mn and Ni translocation in Arabidopsis [32,36]. Additionally, transgenic plants over-expressing *AtIRT1* accumulate more Zn and Cd than wild type plants under Fe starvation, which indicates that *AtIRT1* can also transport Zn and Cd [34].

Similar to *AtIRT1*, *AtIRT2* is also a divalent cation transporter and specifically induced in the external cell layers of the root subapical zone under Fe deficient conditions. Unlike *AtIRT1*, *AtIRT2* can transport only Fe and Zn in yeast but not Mn, Ni and Cd. However, the insertional mutant of *AtIRT2* gene did not show any Fe deficient symptom [31]. Although it is reported that the expression level of *AtIRT2* was enhanced in the root of *AtIRT1* defective mutant, over-expression of *AtIRT2* in *AtIRT1* defective mutant failed to restore the wild type phenotype, which indicates that *AtIRT2* does not play a significant role in Fe uptake from the soil. Together with the localization of *AtIRT2* to intracellular vesicles, it may function in Fe trafficking and compartmentalization into internal storage vesicles to avoid metal toxicity [35-38]. AtIRT3 is another member of ZIP family in Arabidopsis similar to AtIRT1. Different to *AtIRT2*, the mRNA level of *AtIRT3* is increased in response to Zn deficiency, and over-expression of *AtIRT3* in *AtIRT1* defective mutant could rescue the plant growth phenotype under Fe deficiency. Moreover, transgenic *Arabidopsis* plants over-expressing *AtIRT3* accumulated more Zn in shoots and more Fe in roots [39-41]. These results indicate that AtIRT3 is involved in both Zn and Fe translocation.

AtZIP1 is mainly expressed in the root stele and the leaf vasculature and up-regulated in the root under Zn deficient conditions. Subcellular localization analysis showed that*AtZIP1* is a vacuolar transporter. In yeast, *AtZIP1* can complement the Zn and Mn uptake mutant *zrt1/zrt2*, suggesting it may transport both Zn and Mn. However, the functional studies with *Arabidopsis AtZIP1* knockout lines suggest it plays a role in Mn remobilizing from the vacuole to cytoplasm in root stellar cells, and contributes to Mn radial movement to the xylem parenchyma [17,42]. Moreover, *AtZIP1* can rescue yeast mutants deficient in Cu transport suggesting that it may also transport Cu [43]. Similar to *AtZIP1*, *AtZIP2* can complement the yeast Zn and Mn uptake mutant *zrt1/zrt2*.

AtZIP2 is localized to the plasma membrane of the root stele cell and functional studies with Arabidopsis *AtZIP2* knockout lines suggest that it may mediate the uptake of Mn/Zn into root stellar cells, and contribute to the movement of Mn/Zn to the xylem parenchyma for subsequent xylem loading and transport to the shoot [42]. AtZIP3 is also proposed to play a role in Zn transport from soil to the *Arabidopsis* root, as it can complement Zn uptake in the yeast mutant, *zrt1/zrt2* and its expression is up-regulated in the root in response to Zn deficiency [17]. *AtZIP4* is expressed either in roots and shoots, showing a delicate regulation by Zn to control the Zn homeostasis in plant [6,20]. In addition, AtZIP4 is up-regulated in Cu-deficient roots and can rescue a Cu transport-deficient yeast mutant, which suggests that it may also transport Cu [43].

***ZIP* genes in rice**

In rice genome, seventeen *ZIP* genes have been identified [44]. O*sIRT1*, highly homologous to *AtIRT1*, is predominantly expressed in rice roots and up-regulated by Fe deficiency [45,46]. Expression of *OsIRT1* in yeast can reverse the growth defect of *Dftr1/Dfet4/Dfre1* mutant on Fe depleted medium, while in rice, over-expression of *OsIRT1* leads to increased Fe and Zn accumulations in roots, shoots and mature seeds, suggesting it is a functional metal transporter not only involved in Fe and Zn uptake from rhizosphere to root, but also involved in Fe and Zn distribution to leaves and seeds [46]. *OsZIP1, OsZIP3, OsZIP4, OsZIP5* and *OsZIP8* are plasma membrane Zn transporters and their expression levels can be induced by Zn deficiency in rice [47-51]. *OsZIP1, OsZIP2* and *OsZIP3* were identified from rice genome by searching GenBank database with amino acid sequences of *AtZIP1*. *OsZIP1* is up-regulated under either Zn deficient or Cu deficient conditions, while *OsZIP2* and *OsZIP3* are up-regulated only by Zn deficiency. Both *OsZIP1* and *OsZIP3* can complement the growth defective phenotype of yeast mutant *ZHY3* on low Zn medium, suggesting they may be involved in Zn transport [47,48,52].

However, Sasaki et al. reported that neither *OsZIP3* gene expression nor encoded protein was affected by either deficiency or toxic levels of Zn [53]. *OsZIP3* is highly expressed in the nodes of rice and localized at the xylem intervening parenchyma cells and xylem transfer cells of the enlarged vascular bundle in both basal and upper nodes. Knockdown of *OsZIP3* results in significantly reduced Zn levels in the shoot basal region but increased Zn levels in the transpiration flow. More Zn is distributed to the lower leaves, but less to the shoot elongating zone and nodes in the knockdown lines compared with wild type, indicating the important role of *OsZIP3* responsible for unloading Zn from the xylem of enlarged vascular bundles and distribution of Zn to the developing tissues in rice [53]. *OsZIP4, OsZIP5, OsZIP6* and *OsZIP7* are homologous with *OsIRT1*. Among them, *OsZIP4* is up-regulated in roots and shoots by Zn deficiency, while *OsZIP5* and *OsZIP7* are up-regulated only in shoots by Zn deficiency. *OsZIP4* is expressed in the phloem cells of stem, and vascular bundles of leaves and roots and can complement a Zn uptake deficient yeast mutant, suggesting it may be responsible for the root to shoot translocation of Zn in rice [47,48].

However, over-expressing *OsZIP4* results in increased Zn contents in roots, while Zn levels in seeds was significant lower than in wild type plants [54]. O*sZIP6* has been found to be transcriptionally activated in shoot and root in response to Fe, Zn or Mn deficiency. However, when expressed in Oocytes, *OsZIP6* has the pH dependent transport activity of Fe, Co and Cd, but not Zn, Mn and Ni, and the enhanced transport is observed at acidic pH [55]. *OsZIP8* is also strongly up-regulated in roots and shoots under Zn deficiency and can complement the growth defect of Zn uptake yeast mutant. The *OsZIP8* over-expressing plants are shorter than the wild type and show lower levels of Zn in shoots and mature seeds, but an increase of Zn in roots, which demonstrates that *OsZIP8* is a transporter that functions in Zn uptake and distribution [49,50].

***ZIP* genes in other plants**

Studies of metal hyper-accumulating plant species, such as *Noccaea caerulescens* (previously known as *Thlaspi caerulescens*), *Arabidopsis halleri* and *Thlaspi japonicum*, show that high expression of *ZIP* genes contributes to high Zn accumulation [40,44,56]. NcZNT1, a ZIP family member isolated from *Noccaea caerulescens*, is the first metal transporter identified in the metal hyper-accumulating plant species. It is suggested to be mediated in high affinity Zn uptake and low affinity Cd uptake in yeast [57]. *NcZNT1* is not only expressed in the root epidermis but also highly expressed in the root and shoot vasculature. But different to the yeast, *NcZNT1* was reported to be a plasma membrane transporter that mediated the long distance transport of Zn but not Cd, Fe, Mn or Cu from the root to the shoot *via* the xylem in *Noccaea caerulescens* [44].

The predominant expression of *AhIRT3*, *AhZIP3*, *AhZIP6* and *AhZIP12* in roots and shoots and *AhZIP9* in roots is suggested to be the main reason for the Zn/Cd uptake and hyper-accumulation in *Arabidopsis halleri* [58]. Among them, AhIRT3 is a plasma membrane protein that can functionally complement the yeast Zn uptake defective single mutant *Spzrt1* and double mutant *zrt1/zrt2*, as well as the Fe uptake defective mutant *fet3/fet4*, which suggests that AhIRT3 confreres both Zn and Fe uptake activity [40]. Furthermore, over-expressing *AhIRT3* in *Arabidopsis thaliana* leads to increased accumulation of Zn in the shoot and Fe in the root of transgenic lines, suggesting AhIRT3 functions as a Zn and Fe uptake transporter in *Arabidopsis halleri* [40]. In *Thlaspi japonicum*, *TjZNT1* and *TjZNT2* share high sequence similarity (78% identity) and have been identified as excess Ni resistance genes in this Ni hyper-accumulating plant [59,60]. *TjZNT1* has Zn-, Mn- and Cd-transporting abilities while *TjZNT2* only has Zn- and Mn-transporting abilities [59]. Unlike many ZIP members that have one *HRD, TjZNT1* and *TjZNT2* have two long HRDs in the putative cytoplasmic domain between TM III and TM IV, and the sequences of the HRDs are apparently different between *TjZNT1* and *TjZNT2*, which may be involved in differential ion selectivity [59]. Nishida et al. reported that the deletion of HRD did not affect the localization of *TjZNT1* but increased the specificity for Zn [61]. Furthermore, Nishida et al. demonstrated that the first 25 amino acid region of the N-terminus was important for the Zn transport of *TjZNT2* [36].

In *Medicago truncatula*, six *MtZIP* members showed different metal specificities have been identified. *MtZIP1, MtZIP5* and *MtZIP6* can restore yeast growth on Zn limited medium, *MtZIP3, MtZIP5* and *MtZIP6* can restore yeast growth on Fe limited medium, *MtZIP4* and *MtZIP7* can restore yeast growth on Mn limited medium [28]. The diverse expression patterns of *MtZIPs* also have been observed. *MtZIP1* transcripts are only detected in Zn deficient roots and leaves, *MtZIP3* and *MtZIP4* expression levels are down-regulated in leaves under Fe and Mn deficient conditions and appeared to be up-regulated in both roots and leaves under Zn deficient conditions, *MtZIP5* is up-regulated in leaves under Zn and Mn deficiency, while the expression levels of *MtZIP6* and *MtZIP7* are not affected by the metal supply, at least in root and leaf tissues [28].

HvIRT1, the first identified ZIP protein in barley, is reported to be a plasma membrane localized Mn transporter [62]. According to the yeast uptake assay, in addition to Mn, it also can transport Zn, Fe and Cd. Both Fe and Mn deficiency can induce an up-regulation of *HvIRT1* and the higher expression of *HvIRT1* correlates with an increased Mn uptake rate in barley [62]. Pedas et al. also isolated and identified another three *HvZIPs, HvZIP3*, *HvZIP5* and *HvZIP8*, as specific Zn transporters [63]. When expressing in Zn uptake defective yeast mutant, *Dzrt1/Dzrt2*, *HvZIP3, HvZIP5* and *HvZIP8* can restore the yeast growth, while expressing in Fe and Mn uptake defective yeast mutant, they are unable to restore the yeast growth [63]. Additionally, HvZIP7 is a low-affinity Zn transporter in barley. *HvZIP7* is strongly induced by Zn deficiency, primarily in vascular tissues of roots and leaves, and its protein is localized in the plasma membrane [64]. Over-expression of *HvZIP7* in barley plants increases Zn uptake when supplying moderately high concentrations of Zn, and there is a specific enhancement of shoot Zn accumulation but no measurable increase in Fe, Mn, Cu or Cd [64].

Nine ZIP members were identified in maize genome and they share a conserved transmembrane domain and a variable region between *TM III* and *TM IV*. *ZmZIPs* have been shown to be localized to the plasma membrane and endoplasmic reticulum and dramatically induced in response to Zn and Fe deficiency [65]. When over-expressing *ZmIRT1* in Arabidopsis plants, the Zn and Fe concentration increases in roots and seeds while the Fe content decreases in shoots. When over-expressing *ZmZIP3* in Arabidopsis plants, the Zn accumulation is enhanced in roots while that is repressed in shoots. And all of the transgenic plants showed altered tolerance to various Zn and Fe conditions compared with wild type plants [65]. The various expression patterns of *ZmZIP* genes have been also observed in different stages of embryo and endosperm development. *ZmZIP4* is up-regulated during the early development of embryo, and the *ZmZIP5* is dramatically induced in the middle stage development of embryo and endosperm, while the transcripts of *ZmIRT1* and *ZmZIP6* are increased in the late developmental stages of embryo, which indicate that they may be essential for ion translocation and storage during differential stages of embryo and endosperm development [65].

MTP GENE FAMILY

General function of *MTP* genes

Members of CDF (cation diffusion facilitator, also known as cation efflux) family, first identified by Nies and Silver, ubiquitously presents in numerous organisms, including bacteria, fungi, animals and plants [66,67]. They have been shown to be important for maintenance of cation homeostasis and efflux the divalent cations, such as Zn, Fe, Mn, Co and Cd, from the cytoplasm through efflux from the cell or through sequestration into internal compartments [68,69]. In plants, the CDF members are usually called MTPs (metal tolerance proteins). To date, *MTP* genes have been cloned from a number of plant species and they are identified to be involved in compartmentalization or efflux of metal ions to reduce the toxicity, thereby maintaining the cellular homeostasis during metal stress [70-78]. Based on the comprehensive phylogenetic analysis of putative CDF proteins representative for all six kingdoms of life (Archaea, Bacteria, Protista, Fungi, Plantae and Animalia) and the available data on sequences with experimentally confirmed specificities, the CDF transporters can be classified into three major clusters: Zn-CDFs, Mn-CDFs and Fe/Zn-CDFs according to their respective major metal substrate [69,74]. Among them, the Zn-CDF cluster is well characterized.

According to the recent phylogenetic analysis, the MTP proteins from diverse plant species can be divided into seven groups: G1, G5, G6, G7, G8, G9 and G12, and each of them is designated based on the annotation of *Arabidopsis thaliana* MTPs [69]. Following this classification, the Zn-CDF cluster comprises groups 1, 5 and 12, the Mn-CDF cluster comprises groups 8 and 9, and the last two groups 6 and 7 form the Fe/Zn-CDF cluster [69]. Most of the CDF proteins possess six putative TMs with cytoplasmic N- and C-terminus [79]. A histidine-rich region, either between TM IV and TM V or at the N- and/or C-terminus, is thought to be involved in metal recognition and function as a potential metal binding domain [79-81]. Thus such region is vital for transporter specificity and might act as a chaperone to determine the identity of metal ions to be transported [76,77]. A signature sequence between TM I

and TM II, proposed by Paulsen and Saier and modified by Montanini et al. are present in all members and enables predictions regarding uncharacterized CDF family members [74,79]. However, the cluster of Mn-CDFs in plants shows a predicted 4-5 TM homology and lacks the histidine-rich domain [67,82]. Moreover, some human and yeast CDFs are predicted to have 12-15 TMs [83-85].

***MTP* genes in Arabidopsis**

In the genome of *Arabidopsis thaliana*, twelve genes encoding putative MTP transporters have been identified [69,86,87]. Among them, *AtMTP1* to *AtMTP4* form a phylogenic subgroup and have a long histidine-rich region between *TM IV* and *TM V* which is predicted to act as a Zn-binding pocket and a sensor of Zn levels in the cytosol [74,76,88]. *AtMTP8* to *AtMTP11* are predicted to form another subgroup in the phylogenetic tree [74]. *ZAT* (zinc transporter of *Arabidopsis*) is the first *MTP* gene identified in plants [89]. Because of its involvement in heavy metal tolerance of *Arabidopsis*, ZAT was later renamed as *AtMTP1* (metal tolerance protein 1) [67,82]. AtMTP1 is a vacuolar membrane protein. Its expression is detected in all organs and is not affected by the Zn concentration in the medium [72].

But heterologous expression of AtMTP1 protein can complement the yeast Zn-sensitive mutant and mediates the influx of Zn in Xenopus oocytes [72,90,91]. Furthermore, Bloß et al. expressed *AtMTP1* in *Escherichia coli* and studied the purified protein in reconstituted proteoliposomes, which demonstrated that the AtMTP1protein could transport Zn into proteoliposomes based on the Zn gradient across the membrane but not on a proton gradient [90]. The *AtMTP1* knock-out mutant or RNAi lines display enhanced sensitivity to high Zn concentrations with reduced Zn content in stems and leaves [72,91]. While the *AtMTP1* ectopically over-expressing plants exhibit enhanced Zn tolerance with increased Zn concentration in roots, which suggests an improved ability of sequestration of excess Zn in the cytoplasm into vacuoles to maintain Zn homeostasis in transgenic plants [72,89]. Similar to AtMTP1, AtMTP3 is also localized to the vacuolar membrane and mediates vacuolar sequestration of Zn in Arabidopsis roots [67,92]. Unlike to *AtMTP1*, it is mainly expressed in roots, and is strongly induced in epidermal and cortex cells in the root hair zone by high but non-toxic concentrations of Zn or Co, or Fe deficiency. Heterologous expression of *AtMTP3* in yeast double mutant *zrc1D/cot1D* can restore Zn and Co tolerance. Knock-out *AtMTP3* mutants or RNAi lines also show Zn hyper-sensitivity with increased Zn content in above-ground organs, while the *AtMTP3* ectopic over-expression increases Zn accumulation in Arabidopsis leaves and enhances Zn tolerance [92].

Sequence analysis shows that *AtMTP11* belongs to Mn-CDF cluster [74]. It is a Mn transporter localized to the pre-vacuolar compartment or the golgi network and involved in both Mn tolerance and homeostasis in Arabidopsis [73,75]. Expression of *AtMTP11* in a Mn-hypersensitive yeast mutant can restore Mn tolerance to wild type level with the enhanced Mn transport activity in the microsomes of the mutant. In Arabidopsis, *Atmtp11* mutant displays increased sensitivity to Mn but not to Zn or Cu. When at a sufficient but non-toxic supply level of Mn, the mutants accumulate higher levels of Mn in shoots and roots than the wild type plants, but not show any obvious deleterious effects on plant growth. However, when Mn supply is high to toxic, the mutants accumulate Mn concentrations in shoots similar to those in wild type plants, and showing Mn toxic symptoms [73]. AtMTP12, localized to the Golgi apparatus, has unique structural characteristics which appear to have approximately twice the length of amino acid sequence as the other *AtMTPs*. It is predicted to consist of 798 amino acids and form 14 TM segments [93]. Heterologous expression of *AtMTP12* in a yeast mutant lacking *Msc2p* (encoding an endoplasmic reticulum Zn importer in yeast) can complement the growth phenotype and cause *MTP5t1* gene co-expressed. Moreover, *AtMTP12* and *AtMTP5t1* were determined to interact in the Golgi of Arabidopsis using a bimolecular fluorescence complementation assay, which suggests that *AtMTP12* and *AtMTP5t1* form a functional complex to transport Zn into the Golgi. However, the expression of *AtMTP12* in suspension-cultured cells was not affected by Zn deficiency or excess [93].

***MTP* genes in rice**

Ten *MTP* genes have been identified in rice genome [69]. *OsMTP1*, highly similar to *AtMTP1*, is a bivalent cation transporter which is necessary for efficient translocation of Zn, Cd and other heavy metals to maintain ion homeostasis in rice [94-96]. *OsMTP1* is localized in rice plasma membrane and highly expressed in mature leaves and stem and significantly induced by exposure to Zn as well as Fe, Cu and Cd [94,95,97]. *OsMTP1* RNAi rice seedlings showed heavy metal sensitivity and changed heavy metal accumulation in different organs of mature rice under low-concentration heavy metal stress [95]. However, the subcellular localization of *OsMTP1* is altered when it is heterologously expressed in yeast or Arabidopsis. OsMTP1 is found to be localized to the vacuole and increases the tolerance of Zn, Fe, Ni, Co, Cd in yeast and transport ability of Zn in Arabidopsis [95,96]. While heterologous expression of *OsMTP1* in tobacco results in the reduction of Cd toxic effects, including growth inhibition, lipid peroxidation and cell death. The transgenic tobacco plants also shows moderate tolerance and accumulation of As upon exogenous As stress, which significantly broad substrate specificity of *OsMTP1* [97].

Furthermore, site-directed mutagenesis studies revealed two substitutions in *OsMTP1* can alter the transport function of this protein. A substitution of Leu (82) to a Phe in *OsMTP1* leads to a decreased Zn transport activity, but an enhanced affinity for Fe and Co and a gain of function for Mn transport, whereas a substitution of His (90) with an Asp completely abolishes Zn transport activity but improves Fe transport, which suggest that these amino acid residues are important in determining substrate specificity of *OsMTP1* [96]. *OsMTP8*, a tonoplast localized Mn-specific transporter, is important for Mn detoxification by the sequestration of Mn into vacuoles in rice shoot cells. *OsMTP8* is mainly expressed in leaf blades and induced by Mn supply.

Heterologous expression of *OsMTP8.1* in yeast enhances the Mn accumulation and tolerance. While disruption of *OsMTP8.1* in rice results in growth inhibition under the high Mn levels, decreased chlorophyll levels in leaves and decreased accumulation of Mn in roots and shoots, but no significant difference in the accumulation of other metals, including Zn, Fe, Cu, Ca, Mg and K [98]. OsMTP9 is also a Mn transporter which is reported to be polarly localized to the plasma membrane of both exodermis and endodermis cells in rice roots. Knockout of *OsMTP9* in rice results in growth inhibition at both the vegetative and reproductive stages with significantly decreased Mn uptake in roots and translocation to shoots [99]. Recently, Zhang and Liu reported that *OsMTP11* can complement the hypersensitivity of yeast mutant strains to Mn as well as Co and Ni. *OsMTP11* is expressed constitutively and universally in different tissues in rice plant and is substantially enhanced under Mn, Zn, Ni, and Cd treatments. High signals of *OsMTP11* fused GFP have been observed in the vacuolar membrane of onion epidermal cells may indicate its function in cation sequestration [100].

***MTP* genes in other plants**

MTP genes have also been cloned from other plants, especially from some heavy metal hyper-accumulating plants such as *Arabidopsis halleri*, *Thlaspi caerulescens*, *Thlaspi goesingense* and *Stylosanthes hamata* L. [71,82,101]. All these MTPs have been found to respond to various metal ions including Zn and Cd. AhMTP1, an AtMTP1 homolog identified from the Zn hyper-accumulator *Arabidopsis halleri*, localizes to the vacuolar membrane. The expression of *AhMTP1* is substantially higher than *AtMTP1* in roots and leaves, and up-regulated under high Zn concentrations in the roots, which is thought to be the main reason for Zn tolerance in *Arabidopsis halleri* [101]. TcMTP1 is also a tonoplast protein and has been proposed to be a major contributor of Zn accumulation in the vacuole of leaf cells in *Thlaspi caerulescens* and higher expression level of *TcMTP1* has been observed in the highly Zn-tolerant accessions [102,103]. TgMTP1, identified from the Ni hyper-accumulator *Thlaspi goesingense*, confers Zn resistance in yeast *zrc1cot1* mutants deficient in *ZNTs*.

Yeast cells expressing *TgMTP1* exhibit enhanced efflux of Zn at the plasma membrane and show more resistant to high concentrations of Ni, Co and Cd [71,104]. Interestingly, *TgMTP1* localizes either to the plasma membrane of Arabidopsis cells or to the plasma membrane and vacuolar membrane of yeast, which may suggest that TgMTP1 contribute to both metal efflux across the plasma membrane and metal sequestration within vacuoles [71,104,105]. *ShMTP1*, isolated from the Mn hyper-accumulator *Stylosanthes hamata* and renamed to *ShMTP8* following the nomenclature for Mn-MTP proteins, shows a predicted 4-5 TMs topology and does not contain the histidine-rich domain [67,82]. *ShMTP1* protein localizes to the tonoplast of Arabidopsis cells but appears to localize to the endoplasmic reticulum of yeast. Furthermore, *ShMTP1* was found to confer Mn tolerance of yeast by internal sequestration into vacuole rather than by efflux of Mn. Expression of *ShMTP1* in a range of yeast mutants suggests that it functions as a proton/Mn anti-porter on the internal organelle membrane. Similarly, when expressed in Arabidopsis, *ShMTP1* confers Mn tolerance through internal sequestration and thus confers plant resistance to excess environmental Mn [82].

Additionally, in non-hyper accumulating plants, MTP transporters are probably assumed to contribute to the adjustment of heavy metal homeostasis in the cytoplasm within narrow concentration ranges [4,106]. In *Brassica juncea*, the MTP proteins are considered as essential contributors to increase tolerance to heavy metals. The yeast complementation assay shows that three *BjMTP* proteins (*BjCET2, BjCET3* and *BjCET4*) are involved in the efflux of Zn, Ni, Co and Cd from plant cells and thus play a substantial role in plant resistance to heavy metal stress [107,108]. *MtMTP1*, a Zn transporter in the legume model plant *Medicago truncatula*, has been detected in all vegetative organs with the highest expression level in leaves. Similar to other MTPs, heterologous expression of *MtMTP1* can complement the Zn-susceptible *zrc1cot1* yeast double mutant [109]. In barley, two HvMTP8 (HvMTP8.1 and HvMTP8.2) proteins are considered to be involved in Mn loading to the Golgi apparatus and play an important role in Mn homeostasis by delivering Mn to Mn-dependent enzymes and/or by facilitating Mn efflux *via* secretory vesicles [110].

In roots, *HvMTP8.1* transcripts increased with external Mn supply ranging from deficiency to toxicity, while *HvMTP8.2* transcripts decreased under the same conditions, while in leaves, the expression of both two *HvMTP8* genes declined in

response to toxic Mn additions [110]. In cucumber, CsMTP8, a Mn transporter localized in the vacuolar membrane, is considered to participate in the maintenance of Mn homeostasis in root cells. *CsMTP8* is expressed almost exclusively in roots, and markedly up-regulated or reduced under elevated Mn or Mn deficiency, respectively [111]. Expression of *CsMTP8* in yeast leads to increased Mn accumulation in yeast cells and fully restores the growth of mutant's hyper-sensitive to excessive Mn conditions. Similarly, the over-expression of *CsMTP8* in *Arabidopsis thaliana* enhances plant tolerance to high Mn in medium as well as the accumulation of Mn in plant tissues [111]. In addition, CsMTP9 is reported to be a plasma membrane H^+-coupled Mn and Cd anti-porter involved in the efflux of Mn and Cd from cucumber root cells by the transport of both metals from endodermis into vascular cylinder. The relative abundance of *CsMTP9* transcript and protein in roots is significantly increased under Mn excess and Cd [112]. Since CsMTP9 transports Mn and Cd *via* a proton-anti-port mechanism, expression of *CsMTP9* in yeast rescues the Mn- and Cd-hypersensitive phenotypes through the enhanced efflux of Mn and Cd from yeast cells. Similarly, the over-expression of *CsMTP9* in *Arabidopsis thaliana* confers increased resistance of plants to Mn excess and Cd but not to other heavy metals and leads to the enhanced translocation of Mn and Cd from roots to shoots [112].

HMA GENE FAMILY

General function of *HMA* genes

The HMAs (heavy metal ATPases), also known as the P1B-type ATPase, is a subfamily of P-type ATPases and has also been described as heavy metal-transporting P-type ATPases and CPx-ATPases [8-10]. The typical HMA proteins consist of approximately 6-8 TMs, both N-terminal and C-terminal regions comprise metal binding domains that interact with and bind specific metal ions (such as Cd and Pb (plumbum)) [113,114]. In addition, a phosphorylation domain, a soluble nucleotide binding domain and a soluble actuator domain are located in HMA proteins, and the interactions of these three domains play an important regulatory role in the heavy metal transport [115]. The main function of HMA proteins is involved in the transport of heavy metal cations across biological membranes *via* an ATP dependent process. Unlike other P-type ATPase subfamilies, HMA proteins can transport multiple heavy metals, such as Zn, Cu, Co, Cd and Pb in a wide range of eukaryotic organisms, and can be clustered into two major phylogenetic subclasses: Cu/Ag (argentum) monovalent (Cu-ATPases) and Zn/Cd/Co/Pb divalent heavy metal cation transporters (Zn-ATPases), based on their metal substrate specificity [113,116].

HMA genes in Arabidopsis

So far, all eight members of HMAs (*AtHMA1* to *AtHMA8*) in *Arabidopsis thaliana* have been functionally characterized. Based on the phylogenetic analysis, *AtHMA5, AtHMA6, AtHMA7* and *AtHMA8* belong to the P1B1 subclass, which are specific to transport monovalent heavy metal cations, such as Cu and Ag [114], whereas *AtHMA2, AtHMA3* and *AtHMA4* fit in a *P1B2* subgroup, which are specific to transport divalent heavy metal cations, such as Zn and Cd [10,117,118]. In contrast to the above mentioned *AtHMAs, AtHMA1* is clustered in *P1B4* subgroup and occupies an intermediate position between the monovalent and divalent heavy metal cation-transporting HMAs on the phylogenetic tree, and likely to have the transport activity of both monovalent and divalent heavy metal cations, including Cu, Zn, Cd, Co as well as Ca [113,117,119]. AtHMA1 is located on the chloroplast envelope and is involved both in delivering Cu into the chloroplastic stroma and in exporting Zn from the chloroplast for Zn detoxification [120]. The *Athma1* mutant is sensitive to high concentrations of Zn in Arabidopsis [119]. AtHMA1 has also been reported to be a Ca or a heavy metal transporter in the intracellular organelle [119-121].

AtHMA2 and *AtHMA4*, located on the plasma lemma of stellar cells in *Arabidopsis thaliana*, have been recognized as the major transporters involved in the root to shoot translocation of Zn as well as Cd [10,118,122,123]. When expressed in yeast membrane vesicles, the ATPase activity of AtHMA2 can be activated by both Zn and Cd [124], while only Cd tolerance increases by *AtHMA4* expression [8,125,126]. In the transgenic plants over-expressing *AtHMA4*, increased Cd tolerance and higher Cd and Zn concentrations in the shoot have been also observed [122,127]. While *AtHMA4* knock out mutant is more sensitive to excess Zn or Cd, and has lower shoot Zn content but higher root Zn content than wild type plants [122,125]. In *Athma2* or *Athma4* single mutants, no visible growth phenotype has been observed when grown in soil [10]. Interestingly, when the *Athma2* mutation is present in an *Athma4* mutant background, it amplifies the phenotypic change that shows dwarf, sterile and Zn deficient in the upper parts. And these symptoms can be resolved by feeding *Athma2/Athma4* double mutants with a high Zn concentration in the nutrient solution [123,125].

Additionally, in *Athma2/Athma4* double mutants and *Athma4* single mutants, but not *Athma2* single mutants, the root to shoot translocation of Cd was decreased to about 2% and 60%, respectively, of that in the wild type. And the Cd

sensitivity increased approximately two fold in the *Athma2/Athma4* double mutants [123]. These results indicates that *AtHMA2* is partially functional redundant with *AtHMA4*. *AtHMA2* and *AtHMA4* have a unique feature of an extended C-terminal region which may be have important roles in metal transport [126,128,129]. The C-terminal regions of *AtHMA2* and *AtHMA4* consist of 244 and 470 amino acid residues, respectively, which contain numerous cysteine pairs and histidine residues and have been shown a fundamental function to bind to Zn and Cd ions [129,130]. Although AtHMA2 shares partially redundant functionality with *AtHMA4* for xylem loading of Zn and Cd, C-terminal truncated AtHMA4 has been shown to efflux Cd in yeast and fails to rescue the phenotype of the *Athma2/Athma4* double mutant of *Arabidopsis thaliana*, whereas the C-terminal truncated *AtHMA2* complements the phenotype of the *Athma2/Athma4* double mutant [118,131].

AtHMA3 has been characterized through a heterologous expression in yeast mutants and found to be localized in the tonoplast and function in the vacuolar sequestration of excess Zn and Co as well as Cd and Pb. And it plays a critical role in the heavy metal homeostasis, detoxification and tolerance in plants [132,133]. Over-expression lines of *AtHMA3* accumulate higher levels of Cd as well as Zn in plants showing a 2.5- and 2-fold higher Cd concentration in the root and shoot, respectively [132]. A further study of diverse population of 349 *Arabidopsis thaliana* accessions demonstrated that *AtHMA3* is the major locus responsible for the variation in leaf Cd accumulation [133]. However, the remaining AtHMA5 to AtHMA8 proteins were shown to engage in Cu transport. AtHMA5 interacts with Cu chaperones and promotes Cu translocation from roots to shoots, which indicates it contributes to the compartmentalization and detoxification of excessive Cu in roots [134,135]. AtHMA6 is localized at the chloroplast periphery and has been proposed to transport Cu over the chloroplast envelope, whereas AtHMA8 is localized at the thylakoid membranes and most likely transports Cu into the thylakoid lumen to provide Cu to Cu-dependent Cu/Zn-superoxide dismutase and plastocyanin [136,137]. AtHMA7 is localized in the post-golgi compartment and responsible for transporting Cu into the post-golgi lumen to supply Cu to the Cu-dependent ETR1 (ethylene receptor apoprotein) [138-140].

HMA genes in rice

There are nine members of HMA in rice and only four members (*OsHMA2*, *3, 5* and *9*) have been functionally characterized. *OsHMA2*, localized at the plasma membrane of root pericycle cells, functions in root to shoot translocation of Zn and Cd [141-145]. Yamaji et al. found that *OsHMA2* is also localized at the phloem region of the nodes at both the vegetative and reproductive growth stages, which suggests *OsHMA2* is involved in the inter-vascular transfer of Zn and Cd and responsible for preferential distribution of Zn to the developing tissues, including young leaves and panicles [145]. Knockout of *OsHMA2* in rice not only results in Cd reduction in the shoots and grain, but also causes the growth and yield reduction [143-145]. Similar to AtHMA4 in Arabidopsis, C-terminal truncated *OsHMA2* is also shown to functionally efflux Cd from yeast cells, and the root to shoot translocation of Cd and Zn is remarkably low in rice mutants with C-terminal truncated *OsHMA2* proteins [143].

OsHMA3 has been identified as a key gene controlling Cd translocation from the root to the shoot in rice [142,143, 146]. *OsHMA3* is located on the tonoplast of root cells and functions in sequestration of Cd into vacuoles, and therefore limits root to shoot Cd translocation to approximately 20% in rice plants [141-145]. Over-expression of *OsHMA3* only reduces Cd accumulation in the grain, but not affects the concentration of Zn and Fe [141]. Further study showed that over-expression of *OsHMA3* enhances the vacuolar sequestration of Cd in the roots and the plant tolerance to toxic Cd. When compared with the wild type rice and vector control line, the over-expressed line shows higher Cd concentration in the roots but lower Cd concentration in the shoots, and displays an alleviated Cd-inhibited growth. However, the Zn concentration in the roots of the *OsHMA3*-overexpressed line is also constantly higher than that of vector control, but the Zn concentration in the shoots is similar between the over-expressed line and vector control [147].

This result suggests *OsHMA3* is also involved in the Zn sequestration into vacuoles in rice roots. The loss of function of *OsHMA3* results in greater Cd translocation from root to shoots and Cd over-accumulation in rice shoots [142,146]. Ueno et al. also reported that the allelic variation in *OsHMA3* can account for a major QTL for Cd accumulation in rice shoots and mutation of *OsHMA3* through single amino acid substitution can result in high Cd accumulation in the grain [103,141,142,148]. Similar to *AtHMA2* and *AtHMA4, OsHMA3* also has a long 273 amino acids C-terminal region, including nine cysteine pairs but no histidine residue [114,142]. Recently, Kumagai et al. concluded that the C-terminal region, particularly the region containing the first 105 amino acids, has an important role in *OsHMA3* activity [149].

The plasma membrane protein *OsHMA5*, localized at the root pericycle cells and xylem region of diffuse vascular bundles in node I, vascular tissues of peduncle, rachis and husk, is involved in Cu loading to the xylem in roots and other organs [150]. The expression of *OsHMA5* can be elevated by excess Cu but not by the deficient Cu at the vegetative stage. *Oshma5* knockout mutant shows a lower Cu concentration of xylem sap and a decreased Cu concentration in

the shoots but an increased Cu concentration in the roots at the vegetative stage, while at the reproductive stage, the concentration of Cu in the brown rice was significantly lower in the mutants than in the wild type rice [150]. However, the *Oshma9* knockout lines accumulants more Zn, Cu, Cd and Pb in the vascular cells, which indicates that OsHMA9 is implicated in the efflux of these metals from vascular tissues [151].

HMA genes in other plants

Some members of HMA have also been identified in other plant species, including metal hyper-accumulators *Arabidopsis halleri* and *Noccaea caerulescens*, barley, wheat, soybean and so on. In both *Arabidopsis halleri* and *Noccaea caerulescens*, Zn and Cd hyper-accumulators, highly expressed *HMA3* and *HMA4* genes are thought to be a part of Zn and Cd detoxification systems comprising enhanced vacuolar sequestration or root to shoot translocation [56,87,103,152-156]. In *Arabidopsis halleri*, AhHMA4 is co-localized with a major QTL controlling Zn and Cd tolerance as well as the Zn and Cd accumulation [157-159]. RNAi mediated lines of *AhHMA4* shows a decreased Zn and Cd accumulation in shoots as well as a decreased Zn and Cd tolerance [56]. In *Noccaea caerulescens*, four copies of AtHMA4 orthologs have been recently identified, and their expression levels determine the capacity of Cd tolerance and accumulation in different ecotypes [156]. Recently, Liu et al. have isolated a homolog of *HMA3* (*SpHMA3*) from the Cd hyper accumulator *Sedum plumbizincicola*, a Crassulaceae species native to the Cd/Zn mining areas in southeast China. *SpHMA3* is a tonoplast-localized transporter specific to Cd and highly expressed in the shoots. Overexpressing *SpHMA3* in the non-hyper accumulating ecotype of *S. alfredii* greatly increases its tolerance to and accumulation of Cd, but not Zn, while the SpHMA3-RNAi lines are hypersensitive to Cd but not to Zn, with the inhibited growth of shoots and young leaves by Cd [160].

In barley, only HvHMA1 has been characterized as an effluxer of not only Zn and Cd, but also Mn, Cu, Co and Ca, from chloroplasts and involved in the Zn and Cu mobilization from the aleurone layer during seed germination [161]. In wheat, *TaHMA2* is localized to the plasma membrane and functions in root to shoot translocation of Zn and Cd. *TaHMA2*-transformed yeast shows Zn and Cd resistance, and the over-expression of TaHMA2 improves the root to shoot translocation of Zn and Cd [162]. Recently, twenty HMA family members in the soybean genome have been identified and divided into six clusters by a phylogenetic analysis. Six *GmHMAs* (*GmHMA5, 19, 13, 16, 14,* and *18*) have been classified as Zn-ATPases, while the other HMA members in soybean have been clustered as Cu-ATPases [163]. Moreover, studies showed that GmHMA13 is involved in Cd response and *GmHMA8* is able to transport Cu [164-166]. In cucumber, eight genes encoding putative HMAs have been identified [167].

According to the study reported by Migocka et al., *CsHMA3* and *CsHMA4* play an important role in Zn homeostasis and the detoxification of Cd and Pb in cucumber cells. Immuno-staining analysis reveals the tonoplast localization of *CsHMA3* and plasma membrane localization of *CsHMA4* in root cells, and the gene of *CsHMA3* is predominantly expressed in roots and up-regulated by excess Zn, Cd or Pb, whereas the *CsHMA4* transcript is most abundant in roots and flowers of cucumber plants and elevated under excess Zn or Pb. Expression of *CsHMA3* in yeast enhances yeast tolerance to Cd and Pb, whereas *CsHMA4* confers increased resistance of yeast cells to Zn and Cd [168]. In Camelina, *CsHMA3* gene is expressed in all organs and is induced in roots and leaves especially after Pb treatment. The transgenic lines over-expressing *CsHMA3* display better root growth than wild type plant under Zn, Cd or Pb stress and show enhanced Zn and Pb tolerance and translocation from roots to shoots [169].

NRAMP GENE FAMILY

General function of *NRAMP* genes

The first member of the NRAMP family is named natural resistance-associated macrophage protein 1 (*NRAMP1*), because mutations in this macrophage-specific protein confer increased sensitivity to intracellular bacterial pathogens [170]. *NRAMPs* are now recognized as proton-coupled metal ion transporters, which have nearly 12 highly hydrophobic transmembrane domains and constitute a large evolutionarily conserved family existed widely in bacteria, yeast, algae, plants and animals [7,171-177]. *NRAMP* transporters, also known as DCT1 (divalent cation transporter 1) or DMT1 (divalent metal transporter 1), have a broad range of metal cation substrates, including Zn, Fe, Mn, Cu, Al, Ni, Cd, Co and Pb and have divergent functions in different species [174,178-180]. In plants, the NRAMP family transporters have been identified in various species and many of them function as Fe or Mn transporters [59,180-189].

NRAMP genes in Arabidopsis

In Arabidopsis, six members of the NRAMP family have been identified and partially characterized. Most of them can complement yeast mutants deficient for Fe, Mn or Cd uptake, revealing their conserved function as metal transporters

among both the plant and animal kingdoms [182,183]. *AtNRAMP1* is localized to the plasma membrane of root cells and has Fe and Mn transport activities in yeast [182,183,190-192]. The expression level of *AtNRAMP1* gene can be induced by Fe deficiency in Arabidopsis roots but not in leaves and over-expression of *AtNRAMP1* in Arabidopsis increases the resistance to toxic Fe [182]. In addition, *AtNRAMP1* is reported to be a high affinity Mn influx protein in root plasma membrane and essential for uptake of Mn from the soil in low Mn conditions. The expression of *AtNRAMP1* is restricted to the root and stimulated by Mn deficiency, and knockout of this gene results in a significant growth reduction failing to take up Mn at low conditions [192]. *AtNRAMP3* and *AtNRAMP4* are close homologs and function redundantly to release metals from the vacuolar to the developing tissues [191,193]. They are localized to the vacuolar membrane and can complement Zn, Fe, Mn and Cd deficiency in yeast mutants. The *AtNRAMP3* and *AtNRAMP4* transcripts accumulate in response to Fe deficiency in both roots and aerial parts of Arabidopsis plants [183,190,191,194]. Over-expression of *AtNRAMP3* leads to Fe over-accumulation in *Arabidopsis thaliana* [183]. *Atnramp3/Atnramp4* double mutants display a strong chlorotic phenotype when seeds are germinated in the absence of Fe in the medium due to their inability to remobilize Fe out of vacuolar seed stores [191,195].

Meanwhile, both genes also play important roles in Mn homeostasis in photosynthetic tissues of adult plants as they release Mn from mesophyll vacuoles to supply the Mn required in the chloroplasts [192,193]. Although leaf *AtNRAMP3* and *AtNRAMP4* protein levels are unaffected by Mn deficiency, the vacuolar Mn accumulation in mesophyll cells of rosette leaves is dramatically increased associated with reduced growth and less functional photosystem II in the adult *nramp3/nramp4* double mutants when compared with the wild type [193]. AtNRAMP3 and AtNRAMP4 are also involved in Cd transport [183,187,195,196]. Over-expression of AtNRAMP3 results in Cd hypersensitivity and disruption of *AtNRAMP3* gene leads to slightly enhanced Cd resistance of Arabidopsis root growth [183]. *Atnramp3/Atnramp4* double mutants show hypersensitivity to Cd stress [191,195]. In addition, *AtNRAMP4* can transport Zn [194]. *AtNRAMP6* is targeted to a vesicular-shaped endomembrane compartment, which is distinct from the vacuole or mitochondria. However, AtNRAMP6 only functions as an intracellular metal transporter to affect distribution/availability of Cd but not Fe within the cell. When expressed in yeast, *AtNRAMP6* increases sensitivity to Cd without affecting Cd content in the cell. Likewise, Arabidopsis transgenic plants over-expressing *AtNRAMP6* are hypersensitive to Cd, although plant Cd content remained unchanged while a null allele of *AtNRAMP6*, named *atnramp6-1*, is more tolerant to Cd toxicity [196].

***NRAMP* genes in rice**

In rice, there are seven members of *NRAMP* family and only four of them have been well characterized. *OsNRAMP1*, localized to the plasma membrane and highly up-regulated by Fe deficiency, can rescue the growth of a Fe defective yeast mutant (*fet3fet4*) and enhance Cd and As accumulation [197]. The expression of *OsNRAMP1* in roots is increased in the presence of Cd and its over-expression plants show a slight increase in Cd in the leaves. *OsNRAMP1* expression is higher in the roots of high-Cd-accumulating indica cultivars such as *Anjana Dhan, Jarjan*, *Habataki* and *Cho-ko-koku* than in the roots of low-Cd-accumulating *japonica* cultivars such as *Nipponbare, Sasanishiki* and *Tsukinohikari* [198-201]. Chakrabarty et al. also suggested that *OsNRAMP1* expression is up-regulated during As exposure in rice [202].

Over-expression of *OsNRAMP1* in *Arabidopsis* improves plant tolerance to As with enhanced As accumulation in root and shoot. Cellular localization reveals that *OsNRAMP1* resides on plasma membrane of endodermis and pericycle cells, which suggests its function in As xylem loading for root to shoot mobilization [197]. *OsNRAMP3*, a vascular bundle-localized Mn-influx transporter, is highly selective for Mn and involves in Mn distribution and contributes to remobilization of Mn from old to young leaves [203,204]. The *OsNRAMP3* knockout lines show serious necrosis on young leaves and root tips under low Mn conditions, and high Mn supplies could rescue this phenotype [204]. *OsNRAMP4* shares relatively low similarity with other NRAMP members. In contrast with other *NRAMP* members, it has no transport activity for divalent cations, including Zn, Fe and Mn but it is identified as the first transporter for the trivalent Al and shown to be highly selective for Al. Knockout of *OsNRAMP4* results in a greater reduction in Al tolerance compared with wild type rice [180,205]. Recently, Li et al. investigated natural variation in the rice *NRAT1* (*OsNRAMP4*) gene and found that sequence variation in both coding and regulatory regions is closely associated with changes in *OsNRAT1* expression, Al transport properties, Al tolerance and the Al content of root cell wall and cell sap.

These results indicate that *OsNRAT1* plays an important role in rice Al tolerance by reducing the level of toxic Al in the root cell wall and transporting Al into the root cell, where it is ultimately sequestered in the vacuole [206]. *OsNRAMP5*, a plasma-membrane-localized transporter, is constitutively expressed in the roots through the whole growth period and is polarly localized at the distal side of the exodermis and endodermis [201]. Several studies have demonstrated that OsNRAMP5 is a major transporter of Mn and Cd and is responsible for the transport of Mn and Cd

from the external solution to root cells [189,200,201]. Knockout of *NRAMP5* causes a significant reduction in growth and grain yield, especially when grown at low Mn concentrations, which could be partially rescued by supplying high concentrations of Mn. Moreover, the knockout lines lost the ability to take up Mn and Cd, and show lower concentration of Mn and Cd in both the roots and shoots than wild type rice [201]. Recently, Peris-Peris et al. have reported that *OsNRAMP6* has two isoforms and both of them are plasma membrane-localized proteins that function as Fe and Mn transporters [207].

NRAMP genes in other plants

NRAMP genes have also been cloned and characterized from other plant species, such as *Noccaea caerulescens*, *Thlaspi japonicum*, tomato, soybean and peanut [59,181,184,185]. NcNRAMP1, one of the main transporters involved in Cd hyper-accumulation in *Noccaea caerulescens*, is involved in the influx of Cd across the endodermal plasma membrane and thus plays a key role in Cd influx into the stele and contributes Cd root to shoot transport [208]. *NcNRAMP3*, identified as a tonoplast protein, is predominantly expressed in roots of *Noccaea caerulescens* and is able to transport Cd, as well as Fe and Ni [188,209]. The expression of *TcNRAMP3* can be induced by Fe starvation and also by Ni and Cd exposure. When expressed in yeast, *TcNRAMP3* is able to rescue the growth of a Fe uptake mutant and increase Cd sensitivity and Cd content, while it enhances Ni resistance but reduces Ni accumulation in yeast cells, indicating that TcNRAMP3 could accumulate Fe and Cd and exclude Ni in yeast. Furthermore, over-expression of *TcNRAMP3* in tobacco results in slight Cd sensitivity of root growth but not influence Ni resistance [188].

TcNRAMP4, also a tonoplast protein, is able to transport Zn, Fe, Mn and Cd in yeast [187]. *TjNRAMP4*, identified from *Thlaspi japonicum*, an Ni hyper-accumulating plant, specifically shows transport activity for Ni but not for Zn, Mn or Cd in yeast and might therefore contribute to Ni hyper-accumulation in this plant [59]. In tomato, *LeNRAMP1* localizes to the vascular parenchyma of the root hair zone as well as the root epidermis and the cortex behind the root tip, and is thought to play a role in Fe distribution in the vascular parenchyma upon Fe deficiency as well as the involvement of Mn transport [184]. *GmDMT1*, a soybean *MRAMP* homologue located on the peribacteroid membrane of root nodules, is a symbiotic divalent metal transporter of Fe, Mn and possibly Zn and Cu [185]. *AhNRAMP1*, a peanut *NRAMP* gene expressed in roots and leaves, is up-regulated by Fe deficiency and is a functional Fe transporter that might be responsible for Fe acquisition and distribution in peanut plants [210].

YSL GENE FAMILY

General function of *YSL* genes

YSL (yellow stripe-like) proteins, suggested to transport metal complexes, belong to the OPT (oligopeptide transporter) family but have low similarity to other plant OPT members [12-14]. *ZmYS1* is the first member of *YSL* gene in plant. It is isolated from maize (*Zea mays* L.) *yellow stripe 1* mutant, which exhibits a severe yellow leaf phenotype and is deficient in Fe(III)-PS (phytosiderophores) uptake [12]. Afterwards, ZmYS1 is characterized as a high-affinity proton-coupled symporter not only for Fe(III)-PS but also for various metal complexes including Fe(III)-, Zn(II)-, Cu(II)- and Ni(II)-MA (mugineic acids) complexes and Fe(II)-, Fe(III)- and Ni(II)-NA (nicotianamine) complexes by heterologous expression in yeast and *Xenopus oocytes* [12,211,212]. To date, several members of the YSL family have been functionally identified in both monocots and dicots, suggesting that YSL-mediated metal uptake and mobilization may be a conserved transport mechanism across the plant kingdom [13].

YSL genes in Arabidopsis

To date, eight YSL members were identified in Arabidopsis by sequence similarity to *ZmYS1*. Arabidopsis *YSL1*, *YSL2* and *YSL3* are located in the plasma membrane and expressed in the vascular bundle parenchyma. They are proposed to function in the remobilization of Zn, Fe, Mn and Cu in the form of metal-NA chelates from senescent leaves and the loading of these metals into inflorescences and seeds [213-217]. *AtYSL2* is expressed in many cell types in both roots and shoots, and the fluorescence of *AtYSL2*: GFP fusion protein is observed on the lateral sides of the xylem parenchyma plasma membrane, which suggests it may be involved in the lateral movement of metals in the vasculature for maintaining Zn and Fe homeostasis. Interestingly, the expression of *AtYSL2* is strongly down-regulated by Zn or Fe deficiency, while it is up-regulated by the presence of Fe and Cu [213,215]. In yeast, DiDonato et al. reported that the expression of *AtYSL2* is able to restore the growth of *fet3/fet4*, a Fe uptake-defective yeast mutant, specifically when Fe is provided as Fe(II)-NA but not as Fe(III)-NA. *AtYSL2* is also able to mediate the transport of Cu-NA as well as Zn-PS complex, and its expression depends on the Zn status [213]. However, another study reported that they have obtained contradictory results that they did not observe any Fe(II)-NA dependent complementation in *fet3/fet4* mutant

or Fe(II)-NA inducible currents in *Xenopuslaevis oocytes* by *AtYSL2* and furthermore demonstrated that DiDonato et al.'s growth restoration was independent of Fe or NA supply [215].

AtYSL1 and *AtYSL3* are responsible for the mobilization of micronutrients such as Zn, Fe, Mn and Cu in the form of metal-NA chelates from senescent leaves and the loading of these metals into developing parts, such as inflorescences and seeds [214,216,217]. *AtYSL1* and *AtYSL3* mRNAs are mainly expressed in roots, leaves and flowers; the level is highest in senescing rosette leaves and cauline leaves. In addition, both *AtYSL1* and *AtYSL3* mRNAs are down-regulated by Fe deficiency but up-regulated by Fe excess [214,216]. The seeds of *Atysl1* knockout mutant germinate slowly and have less Fe and NA than wild type seeds, but this defect can be rescued by an exogenous Fe supply. However, the leaves of *Atysl1* have excess NA, while Fe levels are normal [214]. Unlike *Atysl1* or *Atysl3* single mutant, the *Atysl1/Atysl3* double mutants exhibited Fe deficiency symptoms, such as interveinal chlorosis, and greatly reduced fertility due to defective anther and embryo development, which can be alleviated by application of Fe-ethylene diamine-N,N'-bis (2-hydroxyphenylacetic acid) solution to the soil [216]. In addition, the concentrations of several metals are specifically altered in this double mutant.

In leaves, the concentration of Fe is decreased, whereas concentrations of Zn, Mn and especially Cu are elevated. In seeds, the concentrations of Zn, Fe and Cu are lower than wild type [216]. These results demonstrated that double mutants failed to mobilize Zn and Cu from leaves. Chu et al. also have reported that AtYSL1 and *AtYSL3* are able to transport Fe(II)-NA. *AtYSL3* but not *AtYSL1* also transports Fe(III)-DMA and Fe(III)-PS in yeast, although *Arabidopsis* does not synthesize PS [217]. Interestingly, Chen et al. hypothesized that the *AtYSL1* and *AtYSL3* could be regulated by SA and probably involved in the pathogen defense. The expression of *AtYSL1* and *AtYSL3* are dramatically higher in the *siz1* mutant, which shows high levels of salicylic acid (SA) and SA glucoside, induced expression of pathogenesis-related (PR) genes and increased resistance of the bacterial pathogen *Pseudomonas syringae* pv. tomato (Pst) DC3000 [218].

Jaquinod et al. identified AtYSL4 and AtYSL6 as members of the tonoplast proteins, whereas Conte et al. reported that *AtYSL4* and *AtYSL6* are localized to both vacuole membranes and internal membranes resembling endoplasmic reticulum [219,220]. Meanwhile, Divol et al. concluded that *AtYSL4* and *AtYSL6* proteins are located in plastids/chloroplast envelope and demonstrated a fundamental role for *AtYSL4* and *AtYSL6* in managing chloroplastic Fe [221]. Knock out both *AtYSL4* and *AtYSL6* greatly reduces the plant's ability to cope with excess Fe in the chloroplasts and ubiquitous expression of *AtYSL4* or *AtYSL6* dramatically reduces plant tolerance to Fe deficiency and decreases chloroplastic Fe content [221]. Interestingly, Hofstetter et al. reported that *AtYSL7* and *AtYSL8* are major *SylA* uptake transporters in Arabidopsis. AtYSL7 and AtYSL8 can render yeast cells sensitive to growth inhibition by *SylA* and the greatest *SylA* sensitivity is conferred by *AtYSL7* and *AtYSL8* expression levels. An *Atysl7* mutant exhibits strongly reduced *SylA* sensitivity in a root growth inhibition assay. In leaves of *Atysl7* and *Atysl8* mutants, *SylA* mediated quenching of salicylic-acid-triggered *PATHOGENESIS-RELATED GENE1* transcript accumulation is greatly reduced compared with the wild type [222].

YSL genes in rice

To date, eighteen *YSL* genes have been identified in rice genome that exhibit 36-76% sequence similarity to the maize *YS1* gene. *OsYSL2* is suggested to be involved in the phloem transport of Fe and Mn, including the translocation of Fe and Mn into the rice grains. It is a plasma membrane protein mainly expressed in the phloem cells of the vascular bundles and developing seeds [223]. The transcripts of *OsYSL2* cannot be detected in the roots of either Fe-sufficient or Fe-deficient plants, but the dramatic expression is induced in the leaves, particularly in the phloem of leaf blade and leaf sheaths, by Fe deficiency [223-225]. Electrophysiological measurements using *Xenopuslaevis oocytes* have shown that OsYSL2 transports Fe(II)-NA and Mn(II)-NA but not Fe(III)-DMA [223]. An RNAi line of *OsYSL2* shows decreased Fe translocation to seeds, lower Fe concentrations in shoots and seeds, and greater accumulation of Fe in the roots [226]. OsYSL15 is the dominant Fe(III)-DMA but not Fe(III)-NA, Fe(II)-NA or Mn(II)-NA transporter responsible for Fe uptake from the rhizosphere and is also responsible for phloem transport and distribution of Fe in rice [224,225].

The expression of *OsYSL15* is strongly induced by Fe-deficiency and is dominant in the epidermis/exodermis and phloem cells in roots under conditions of Fe deficiency but detected only in phloem under Fe sufficiency [212,224,225,227]. *OsYSL15* is also expressed in flowers, developing seeds, and in the embryonic scutellar epithelial cells during seed germination. OsYSL15 functionally complements a yeast mutant defective in Fe uptake when supplied with Fe(III)-DMA and transports Fe(III)-DMA in *Xenopuslaevis oocytes*. *Osysl15* mutants exhibit Fe deficiency chlorotic phenotypes and have reduced Fe concentrations in their roots, shoots and seeds, while over-expression of *OsYSL15* increases the Fe concentration in leaves and seeds [224,225]. The mutant seedlings are severely arrested in their

germination and early growth but are rescued by a high Fe supply, which revealed that *OsYSL15* is also crucial in Fe homeostasis during early stages of growth [224].

OsYSL16, 85% similarity to both *OsYSL2* and *OsYSL15*, plays a role in the allocation of Fe *via* the vascular bundles and facilitates Fe distribution within a plant [228,229]. *OsYSL16* is highly expressed in the root epidermis, vascular bundles of root, leaf and spikelet, and leaf mesophyll cells. It has been detected in the xylem of old leaves, the phloem of new leaves and the vascular bundles of un-elongated nodes [228,229]. OsYSL16 functionally complements a yeast mutant defective in Fe uptake when grown on medium containing Fe(III)-DMA, but not when grown on medium containing Fe(II)-NA [228]. *OsYSL16* knockdown seedlings are smaller than wild type when only $FeCl_3$ is supplied as a Fe source. The *OsYSL16* knockdown plants show more severe chlorosis than wild type, whereas the Fe concentration in shoots was similar to that of the wild type under Fe deficient conditions. Furthermore, *OsYSL16* knockdown plants accumulated more Fe in the vascular bundles of the leaves [228]. In the *OsYSL16* activation lines, the Fe concentration in shoots is higher than in the wild type and the rate of Fe utilization from the seeds is also higher than in the wild type seeds during germination [229].

Additionally, *OsYSL6* is constitutively expressed in all cells of both the shoots and roots and its expression level increases with leaf age but is not affected by either deficiency or toxicity of various metals [230]. *OsYSL6* is a Mn(II)-NA but not Mn-MA transporter that is required for the detoxification of excess Mn in rice. Knockout of *OsYSL6* results in decreased growth of both roots and shoots only in the high Mn condition. There is no difference in the concentration of total Mn and other essential metals between the wild type rice and the knockout line, but the knockout line shows a higher Mn concentration in the leaf apoplastic solution and a lower Mn concentration in the symplastic solution than wild type rice [230]. *OsYSL18*, an Fe-PS transporter involved in the translocation of Fe in reproductive organs and phloem in joints, transports Fe(III)-DMA, but not Fe(II)-NA, Zn(II)-DMA or Zn(II)-NA. In vegetative organs, *OsYSL18* is specifically expressed in lamina joints, the inner cortex of crown roots and phloem parenchyma and companion cells at the basal part of every leaf sheath. However, more *OsYSL18* transcripts have been observed in flowers than in shoots or roots, and it is expressed independently of Fe conditions [231].

YSL genes in other plants

Three YSLs have been isolated from a metal hyper-accumulator, *Thlaspi caerulescens*, which is a model plant for the study of Zn, Cd and Ni hyper-accumulation [57,152,232,233]. The three *YSL* genes are expressed at high rates compared with their *Arabidopsis thaliana* homologs but with distinct patterns. The expression of *TcYSL3* is equivalent in all the organs, while *TcYSL5* is highly expressed in the shoots and *TcYSL7* is more highly expressed in the flowers. Interestingly, the high and constitutive expression of the *TcYSL* gene is not affected by the exposure to heavy metals [234]. Through yeast complementation and uptake assays, *TcYSL3* has been identified as a Fe/Ni-NA influx transporter, which is suggested to be involved in the entry of Ni-NA into symplastic transport in the roots for delivery to the xylem and unloading of the Ni-NA complexes from the xylem in the leaves [234].

BjYSL7 from the hyper-accumulator *Brassica juncea*, over 90% identical to *TcYSL7* and *AtYSL7*, is a Fe(II)-NA influx transporter, which might be involved in the transport of Fe, Ni and Cd to the shoot and improving heavy metal resistance in plants. *BjYSL7* is mainly expressed in the stem under normal condition, whereas its expression level is up-regulated in roots and shoots under Fe, Ni and Cd stresses. The *BjYSL7* over-expressing transgenic tobacco plants exhibits longer root lengths, lower relative inhibition rate of length and superior root hair development compared to that of wild type plants in the presence of Cd and Ni [235]. Furthermore, the concentrations of Cd and Ni in shoots of *BjYSL7* over-expressing plants are significantly higher than that of wild type plants, while the Fe concentrations are higher in the shoots and seeds but lower in the roots compared to the wild type plants [235].

In barley, *HvYS1* is limited to the root epidermis under Fe deficient conditions and highly specific for Fe(III)-MAs while demonstrating a low transport activity for MAs chelated to Zn(II), Cu(II), Ni(II) or Co(II), which partially explains the important role played by *HvYS1* in the uptake of Fe under Fe deficient conditions [12,212,227]. *HvYSL2*, exhibits 67.3% identity to *HvYS1*, is localized to the endodermis of shoot and root and is induced under Fe deficient conditions. It constitutes a broader range of substrate preference than *HvYS1* and transports PS complexes with Fe(III), Zn(II), Mn(II), Cu(II), Ni(II) or Co(II) [236]. *HvYSL5*, shares 50% identity with *HvYS1*, is localized either to vesicles or the tonoplast and expressed in the roots and the expression is greatly induced by Fe deficiency, but not by deficiency of other metals including Zn, Mn and Cu. Furthermore, the expression of *HvYSL5* shows a diurnal rhythm, being the highest in the morning, but with no expression in the afternoon [236]. Although knock down of *HvYSL5* did not result in any detectable phenotype changes, Zheng et al. suggested that *HvYSL5* is involved in the transient storage of Fe or phytosiderophores [236,237].

CONCLUSION AND FUTURE PERSPECTIVES

Based on the incensement of the knowledge and functions of these well characterized transporter genes in rice and other crops, most of them could be applied in the molecular breeding of crops to promote the uptake and distribution of beneficial elements to improve the yield production and micronutrient concentrations in grains, especially Zn and Fe, which are inadequate in our daily diet. For example, the plasma membrane localized ZIP transporters have been suggested to be involved in both Fe and Zn uptake from rhizosphere to roots and distribution form roots to leaves and seeds in rice as well as in barley and maize [46,49,50,53,63-65]. *OsYSL2*, *OsYSL15* and *OsYSL18* genes also have been reported to play important roles in Fe uptake and preferential distribution into new leaves and seeds in rice [223-225,231]. Most importantly, several genes could be used to reduce the root acquisition of toxic elements and/or the translocation to shoots and seeds by efflux and sequestration the toxic heavy metals into vacuoles, which can avoid the original entry of toxic heavy metals into our diet and is beneficial for human health. For example, *OsHMA2, OsNRAMP1, OsNRAMP5* have been identified to function in root uptake and root-to-shoot mobilization of Cd and As. Thereafter, the knockout lines lost the ability to take up Cd and As and show lower concentration of Cd and As in both the roots and shoots than wild type rice [143-145,197,200-202]. *OsHMA3* is a tonoplast localized protein involving in valvuolar sequestration of Cd. Rice plants with functional allele of *OsHMA3* and overexpression transgenic plants exhibit significantly decreased concentrations of Cd in shoots and seeds [141-143,146]. *OsNRAMP4* is also a tonoplast localized transporter which contributes to Al tolerance in rice [180,205].

Furthermore, from the plant genomic sequencing and bioinformatics analysis, we can find that large transporter gene families exist in the genomes of Arabidopsis and rice. Although a range of plant transporter genes involved in metal microelement uptake and translocation have been cloned and characterized in last decades, undoubtedly, further study is still needed. (1) As described above, there is amount of transporter genes await for identification and functional analysis, and some of the transporter genes have a wide range of substrates while others have specific substrate. Thereafter, the most important work in the future will focus on the cellular and subcellular expression and function of these transporter genes to reveal their specific roles and substrates distinct to other genes within the same family. (2) In addition, transcriptional and post-transcriptional regulation of transporter genes plays important roles for plants to cope with the variety of metal supplies. Therefore, studies aimed to isolate and characterize the regulator genes, including transcription factors, protein kinases and hormone-related genes, controlling the transport activity of metal microelements in response to environmental changes are particularly meaningful. (3) An appropriate structure of transport proteins is essential for the effective metal recognition, binding and transition from the outside to the inside of the membrane. Hence, more studies should be contributed to the structure analysis of metal microelement transporters to reveal the basic mechanism of the substrates selection and transport activity.

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REFERENCES

[1] Marschner H. Mineral nutrition of higher plants, 2nd edn. *UK Academic Press*, London, **1995**.

[2] Van Assche F, Clijsters H. Effects of metals on enzyme activity in plants. *Plant Cell Environ*, **1990**, 13: 195-206.

[3] Schutzendubel A, Polle A. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. *J Exp Bot*, **2002**, 53: 1351-1365.

[4] Clemens S. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, **2001**, 212: 475-486.

[5] Hall JL. Cellular mechanisms for heavy metal detoxification and tolerance. *J Exp Bot*, **2002**, 53: 1-11.

[6] Guerinot ML. The ZIP family of metal transporters. *Biochimica et Biophysica Acta*, **2000**, 1465: 190-198.

[7] Williams LE, Pittman JK, Hall JL. Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta*, **2000**, 1465: 104-126.

[8] Mills RF, Krijger GC, Baccarini PJ, Hall JL, Williams LE. Functional expression of AtHMA4, a P1B-type ATPase in the Zn/Co/Cd/Pb subclass. *Plant J*, **2003**, 35: 164-175.

[9] Gravot A, Lieutaud A, Verret F, Auroy P, Vavasseur A, et al. AtHMA3, a plant P1B-ATPase, functions as a Cd/Pb transporter in yeast. *FEBS Lett*, **2004**, 561:22-28.

[10] Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, et al. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. *Plant Cell*, **2004**, 16:1327-1339.

[11] Vidal SM, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of a candidate for BCG. *Cell*, **1993**, 73: 469-485.

[12] Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, et al. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature*, **2001**, 409: 346-349.

[13] Curie C, Cassin G, Couch D, Divol F, Higuchi K, et al. Metal movement within the plant: Contribution of nicotianamine and yellow stripe 1-like transporters. *Ann Bot*, **2009**, 103: 1-11.

[14] Yen MR, Tseng YH, Saier MH Jr. Maize Yellow Stripe 1, an iron phytosiderophore uptake transporter, is a member of the oligopeptide transporter (OPT) family. *Microbiol*, **2001**, 147: 2881-2883.

[15] Eide DM, Broderius JF, Guerinot ML. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc Natl Acad Sci USA*, **1996**, 93: 5624-5628.

[16] Zhao H, Eide D. The yeast *ZRT1* gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proc Natl Acad Sci U S A*, **1996**, 93: 2454-2458.

[17] Grotz N, Fox T, Connolly E, Park W, Guerinot ML Eide D. Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. *Proc Natl Acad Sci U S A*, **1998**, 95: 7220-7224.

[18] Eide DJ. Zinc transporters and the cellular trafficking of zinc. *Biochimica Et Biophysica Acta-Molecular Cell Research*, **2006**, 1763: 711-722.

[19] ChenWR, FengY, ChaoYE. Genomic analysis and expression pattern of *OsZIP1, OsZIP3* and *OsZIP4* in two rice (*Oryza sativa* L.) genotypes with different zinc efficiency. *Russian J Plant Physiol*, **2008**, 55: 400-409.

[20] Grotz N, Guerinot ML. Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochimica Et Biophysica Acta Mol Cell Res*, **2006**, 1763: 595-608.

[21] Waters BM, Sankaran RP. Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective. *Plant Sci*, **2011**, 180: 562-574.

[22] White PJ, Broadley MR. Physiological limits to zinc biofortification of edible crops. *Front Plant Sci*, **2011**, 2: 80.

[23] Olsen LI, Palmgren MG. Many rivers to cross: The journey of zinc from soil to seed. *Front Plant Sci*, **2014**, 5: 30.

[24] Eng BH, Guerinot ML, Eide D, Saier MH. Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. *J Memb Biol*, **1998**, 166: 1-7.

[25] Milon B, Wu Q, Zou J, Costello LC, Franklin RB. Histidine residues in the region between transmembrane domains III and IV of hZip1 are required for zinc transport across the plasma membrane in PC-3 cells. *Biochim Biophys Acta*, **2006**, 1758: 1696-1701.

[26] Mao X, Kim BE, Wang F, Eide DJ, Petris MJ. A histidine-rich cluster mediates the ubiquitination and degradation of the human zinc transporter, hZIP4, and protects against zinc cytotoxicity. *J Biol Chem*, **2007**, 282: 6992-7000.

[27] Gitan RS, Shababi M, Kramer M, Eide DJ. A cytosolic domain of the yeast Zrt1 zinc transporter is required for its post-translational inactivation in response to zinc and cadmium. *J Biol Chem*, **2003**, 278: 39558-39564.

[28] López-Millán AF, Ellis DR, Grusak MA. Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. *Plant Mol Biol*, **2004**, 54: 583-596.

[29] Moreau S, Thomson RM, Kaiser BN, Trevaskis B, Guerinot ML, et al. GmZIP1 Encodes a symbiosis-specific zinc transporter in soybean. *J Biol Chem*, **2002**, 277: 4738-4746.

[30] Korshunova YO, Eide D, Clark WG, Guerinot ML, Pakrasi HB. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Mol Biol*, **1999**, 40: 37-44.

[31] Vert G, Briat JF, Curie C. Arabidopsis *IRT2* gene encodes a root–periphery iron transporter. *Plant J*, **2001**, 26: 181-189.

[32] Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, et al. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell*, **2002**, 14: 1223-1233.

[33] Rogers EE, Eide DJ, Guerinot ML. Altered selectivity in an Arabidopsis metal transporter. *Proc Natl Acad Sci U S A*, **2000**, 97: 12356-12360.

[34] Connolly EL, Fett JP, Guerinot ML. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell*, **2002**, 14: 1347-1357.

[35] Henriques R, Jasik J, Klein M, Feller U, Schell J, et al. Knock-out of Arabidopsis metal transporter gene *IRT1* results in iron deficiency accompanied by cell differentiation defects. *Plant Mol Biol*, **2002**, 50: 587-597.

[36] Nishida S, Tsuzuki C, Kato A, Aisu A, Yoshida J, et al. AtIRT1, the primary iron uptake transporter in the root, mediates excess nickel accumulation in *Arabidopsis thaliana*. *Plant Cell Physiol*, **2011**, 52: 1433-1442.

[37] Varotto C, Maiwald D, Pesaresi P, Jahns P, Salamini F, et al. The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*. *Plant J,* **2002**, 31: 589-599.

[38] Vert G, Barberon M, Zelazny E, Séguéla M, Briat JF, et al. *Arabidopsis* IRT2 cooperates with the high-affinity iron uptake system to maintain iron homeostasis in root epidermal cells. *Planta*, **2009**, 229: 1171-1179.

[39] Talke IN, Hanikenne M, Kramer U. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyper- accumulator *Arabidopsis halleri*. *Plant Physiol*, **2006**, 142: 148-167.

[40] Lin YF, Liang HM, Yang SY, Boch A, Clemens S, et al. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. *New Phytol*, **2009**, 182: 392-404.

[41] Shanmugam V, Lo JC, Wu CL, Wang SL, Lai CC, et al. Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana* - the role in zinc tolerance. *New Phytol*, **2011**, 190: 125-137.

[42] Milner MJ, Seamon J, Craft E, Kochian LV. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *J Exp Bot*, **2013**, 64: 369-381.

[43] Wintz H, Fox T, Wu YY, Feng V, Chen W, et al. Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. *J Biol Chem*, **2003**, 278: 47644-47653.

[44] Milner MJ, Craft E, Yamaji N, Koyama E, Ma JF, et al. Characterization of the high affinity Zn transporter from *Noccaea caerulescens*, *NcZNT1* and dissection of its promoter for its role in Zn uptake and hyperaccumulation. *New Phytol*, **2012**, 195: 113-123.

[45] Bughio N, Yamaguchi H, Nishizawa NK, Nakanishi H, Mori S. Cloning an iron-regulated metal transporter from rice. *J Exp Bot*, **2002**, 53: 1677-1682.

[46] Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M, et al. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. *Plant J*, **2006**, 45: 335-346.

[47] Ramesh SA, Shin R, Eide DJ, Schachtman DP. Differential metal selectivity and gene expression of two zinc transporters from rice. *Plant Physiol*, **2003**, 133: 126-134.

[48] Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, et al. OsZIP4, a novel zinc-regulated zinc transporter in rice. *J Exp Bot*, **2005**, 56: 3207-3214.

[49] Yang X, Huang J, Jiang Y, Zhang HS. Cloning and functional identification of two members of the *ZIP* (Zrt, Irt-like protein) gene family in rice (*Oryza sativa* L.). *Mol Biol Rep*, **2009**, 36: 281-287.

[50] Lee S, Kim S, Lee J, Guerinot M, An G. Zinc deficiency-inducible OsZIP8 encodes a plasma membrane-localized zinc transporter in rice. *Mol Cells*, **2010**, 29: 551-558.

[51] Suzuki M, Bashir K, Inoue H, Takahashi M, Nakanishi H, et al. Accumulation of starch in Zn-deficient rice. *Rice*, **2012**, 5: 1-8.

[52] Bashir K, Ishimaru Y, Nishizawa NK. Molecular mechanisms of zinc uptake and translocation in rice. *Plant Soil*, **2012**, 361: 189-201.

[53] Sasaki A, Yamaji N, Mitani-Ueno N, Kashino M, Ma JF. A node-localized transporter OsZIP3 is responsible for the preferential distribution of Zn to developing tissues in rice. *Plant J*, **2015**, 84: 374-384.

[54] Ishimaru Y, Masuda H, Suzuki M, Bashir K, Takahashi M, et al. Overexpression of the OsZIP4 zinc transporter confers disarrangement of zinc distribution in rice plants. *J Exp Bot*, **2007**, 58: 2909-2915.

[55] Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, et al. Evolution of metal hyper accumulation required cis-regulatory changes and triplication of HMA4. *Nature*, **2008**, 453: 391-395.

[56] Pence NS, Larsen PB, Ebbs SD, Letham DLD, et al. The molecular physiology of heavy metal transport in the Zn/Cd hyper accumulator *Thlaspi caerulescens*. *Proc Natl Acad Sci U S A*, **2000**, 97: 4956-4960.

[57] Weber M, Harada E, Vess C, Roepenack-Lahaye EV, Clemens S. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyper accumulation factors. *Plant J*, **2004**, 37: 269-281.

[58] Mizuno T, Usui K, Horie K, Nosaka S, Mizuno N, et al. Cloning of three *ZIP*/*Nramp* transporter genes from a Ni hyperaccumulator plant *Thlaspi japonicum* and their Ni^{2+}-transport abilities. *Plant Physiol Biochem*, **2005**, 43: 793-801.

[59] Mizuno T, Usui K, Nishida S, Unno T, Obata H. Investigation of the basis for Ni tolerance conferred by the expression of TjZnt1 and TjZnt2 in yeast strains. *Plant Physiol Biochem*, **2007**, 45: 371-378.

[60] Nishida S, Mizuno T, Obata H. Involvement of histidine-rich domain of ZIP family transporter TjZNT1 in metal ion specificity. *Plant Physiol Biochem*, **2008**, 46: 601-606.

[61] Pedas P, Ytting CK, Fuglsang AT, Jahn TP, Schjoerring JK, et al. Manganese efficiency in barley: Identification and characterization of the metalion transporter HvIRT1. *Plant Physiol*, **2008**, 148: 455-466.

[62] Pedas P, Schjoerring JK, Husted S. Identification and characterization of zinc-starvation-induced ZIP transporters from barley roots. *Plant Physiol Biochem*, **2009**, 47: 377-383.

[63] Tiong J, McDonald G, Genc Y, Shirley N, Langridge P, et al. Increased expression of six ZIP family genes by zinc (Zn) deficiency is associated with enhanced uptake and root-to-shoot translocation of Zn in barley (*Hordeum vulgare*). *New Phytol*, **2015**, 207: 1097-1109.

[64] Li S, Zhou X, Huang Y, Zhu L, Zhang S, et al. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. *BMC Plant Biol*, **2013**, 13: 144.

[65] Kavitha PG, Kuruvilla S, Mathew MK. Functional characterization of a transition metal ion transporter, OsZIP6 from rice (*Oryza sativa* L.). *Plant Physiol and Biochem*, **2015**, 97: 165-174.

[66] Nies DH, Silver S. Ion efflux systems involved in bacterial metal resistances. *J Ind Microbiol Biotechnol*, **1995**, 14: 186-199.

[67] Maser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, et al. Phylogenetic relationships within cation transporter families of Arabidopsis. *Pl*ant *Physiol*, **2001**, 126: 1646-1667.

[68] Haney CJ, Grass G, Franke S, Rensing C. New developments in the understanding of the cation diffusion facilitator family. *J Ind Microbiol Biotechnol*, **2005**, 32: 215-226.

[69] Gustin JL, Zanis MJ, Salt DE. Structure and evolution of the plant cation diffusion facilitator family of ion transporters. *BMC Evol Biol*, **2011**, 11: 76.

[70] Blaudez D, Kohler A, Martin F, Sanders D, Chalot M. Poplar metal tolerance protein 1 confers zinc tolerance and is an oligomeric vacuolar zinc transporter with an essential leucine zipper motif. *Plant Cell*, **2003**, 15: 2911-2928.

[71] Kim D, Gustin JL, Lahner B, Persans MW, Baek D, et al. 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*. *Plant J*, **2004**, 39(2): 237-251.

[72] Kobae Y, Uemura T, Sato MH, Ohnishi M, Mimura T, et al. Zinc transporter of *Arabidopsis thaliana* AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. *Plant* Cell *Physiol*, **2004**, 45: 1749-1758.

[73] Delhaize E, Gruber BD, Pittman JK, White RG, Leung H, et al, Richardson AE. A role for the *AtMTP11* gene of Arabidopsis in manganese transport and tolerance. *Plant J*, **2007**, 51: 198-210.

[74] Montanini B, Blaudez D, Jeandroz S, Sanders D, Chalot M. Phylogenetic and functional analysis of the cation diffusion facilitator (CDF) family: Improved signature and prediction of substrate specificity. *BMC Genomics*, **2007**, 8: 107.

[75] Peiter E, Montanini B, Gobert A, Pedas P, Husted S, et al. A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *Proc Natl Acad Sci U S A*, **2007**, 104: 8532-8537.

[76] Kawachi M, Kobae Y, Mimura T, Maeshima M. Deletion of a histidine-rich loop of AtMTP1, a vacuolar Zn(2+)/H(+) anti-porter of *Arabidopsis thaliana*, stimulates the transport activity. *J Biol Chem*, **2008**, 283: 8374-8383.

[77] Podar D, Scherer J, Noordally Z, Herzyk P, Nies D, et al. Metal selectivity determinants in a family of transition metal transporters. *J Biol Chem*, **2012**, 87: 3185-3196.

[78] Sharma SS, Dietz KJ, Mimura T. Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant Cell Environ*, **2016**, 39: 1112-1126.

[79] Paulsen IT, Saier MH Jr. A novel family of ubiquitous heavy metal ion transport proteins. *J Membr Biol*, **1997**, 156: 99-103.

[80] Anton A, Grosse C, Reissmann J, Pribyl T, Nies DH. CzcD is a heavy metal ion transporter involved in regulation of heavy metal resistance in *Ralstonia* sp. strain CH34. *J Bacteriol*, **1999**, 181: 6876-6881.

[81] Wei Y, Fu D. Binding and transport of metal ions at the dimer interface of the *Escherichia coli* metal transporter YiiP. *J Biol Chem*, **2006**, 281: 23492-23502.

[82] Delhaize E, Kataoka T, Hebb DM, White RG, Ryan PR. Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *Plant Cell*, **2003**, 15: 1131-1142.

[83] Li L, Kaplan J. The yeast gene *MSC2*, a member of the cation diffusion facilitator family, affects the cellular distribution of zinc. *J Biol Chem*, **2001**, 276: 5036-5043.

[84] Cragg RA, Christie GR, Phillips SR, Russi RM, Kury S, et al. A novel zinc-regulated human zinc transporter, hZTL1, is localized to the enterocyte apical membrane. *J Biol Chem*, **2002**, 277: 22789-22797.

[85] Kambe T, Narita H, Yamaguchi-Iwai Y, Hirose J, Amano T, et al. Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic b cells. *J Biol Chem*, **2002**, 277: 19049-19055.

[86] Cho M, Chardonnens AN, Dietz KJ. Differential heavy metal tolerance of *Arabidopsis halleri* and *Arabidopsis thaliana*: A leaf slice test. *New Phytol*, **2003**, 158, 287-293.

[87] Becher M, Talke IN, Krall L, Kramer U. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyper accumulator *Arabidopsis halleri*. *Plant J*, **2004**, 37: 251-268.

[88] Tanaka N, Kawachi M, Fujiwara T, Maeshima M. Zinc-binding and structural properties of the histidine-rich loop of *Arabidopsis thaliana* vacuolar membrane zinc transporter MTP1. *FEBS Open Bio*, **2013**, 3: 218-224.

[89] Van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC, Hooykaas PJJ. Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiol*, **1999**, 119: 1047-1055.

[90] Bloß T, Clemens S, Nies DH. Characterization of the ZAT1p zinc transporter from *Arabidopsis thaliana* in microbial model organisms and reconstituted proteoliposomes. *Planta*, **2002**, 214: 783-791.

[91] Desbrosses-Fonrouge AG, Voigt K, Schroder A, Arrivault S, Thomine S, et al. *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Lett*, **2005**, 579: 4165-4174.

[92] Arrivault S, Senger T, Kramer U. The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *Plant J*, **2006**, 46: 861-879.

[93] Fujiwara T, Kawachi M, Sato Y, Mori H, Kutsuna N, et al. A high molecular mass zinc transporter MTP12 forms a functional heteromeric complex with MTP5 in the *Golgi* in *Arabidopsis thaliana*. *FEBS J*, **2015**, 282: 1965-1979.

[94] Lan HX, Wang ZF, Wang QH, Wang MM, Bao YM, et al. Characterization of a vacuolar zinc transporter OZT1 in rice (*Oryza sativa* L.). *Mol Biol Rep*, **2012**, 40: 1201-1210.

[95] Yuan L, Yang S, Liu B, Zhang M, Wu K. Molecular characterization of a rice metal tolerance protein, OsMTP1. *Plant Cell Rep*, **2012**, 31: 67-79.

[96] Menguer PK, Farthing E, Peaston KA, Ricachenevsky FK, Fett JP, et al. Functional analysis of the rice vacuolar zinc transporter OsMTP1. *J Exp Bot*, **2013**, 64: 2871-2883.

[97] Das N, Bhattacharya S, Maiti MK. Enhanced cadmium accumulation and tolerance in transgenic tobacco overexpressing rice metal tolerance protein gene *OsMTP1* is promising for phytoremediation. *Plant Physiol Biochem*, **2016**, 105: 297-309.

[98] Chen Z, Fujii Y, Yamaji N, Masuda S, Takemoto Y, et al. Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. *J Exp Bot*, **2013**, 64: 4375-4387.

[99] Drager DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, et al. Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. *Plant J*, **2004**, 39: 425-439.

[100] Assunção AGL, Martins PD, DeFolter S, Vooijs R, Schat H, et al. Elevated expression of metal transporter genes in three accessions of the metal hyper accumulator *Thlaspi caerulescens*. *Plant Cell Environ*, **2001**, 24: 217-226.

[101] Ueno D, Milner MJ, Yamaji N, Yokosho K, Koyama E, et al. Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *Plant J*, **2011**, 66: 852-862.

[102] Persans MW, Nieman K, Salt DE. Functional activity and role of cation-efflux family members in Ni hyper accumulation in *Thlaspi goesingense*. *Proc Natl Acad Sci USA*, **2001**, 98: 9995-10000.

[103] MacDiarmid CW, Milanick MA, Eide DJ. Biochemical properties of vacuolar zinc transport systems of *Saccharomyces cerevisiae*. *J Biol Chem*, **2002**, 277: 39187-39194.

[104] Clemens S, Palmgren MG, Kramer U. A long way ahead: understanding and engineering plant metal accumulation. *Trends Plant Sci*, **2002**, 7: 309-315.

[105] Xu J, Chai T, Zhang Y, Lang M, Han L. The cation-efflux transporter BjCET2 mediates zinc and cadmium accumulation in *Brassica juncea* L. leaves. *Plant Cell Rep*, **2009**, 28: 1235-1242.

[106] Lang M, Hao M, Fan Q, Wang W, Mo S, et al. Functional characterization of BjCET3 and BjCET4, two new cation-efflux transporters from *Brassica juncea* L. *J Exp Bot*, **2011**, 62: 4467-4480.

[107] Chen M, Shen X, Li D, Ma L, Dong J, et al. Identification and characterization of MtMTP1, a Zn transporter of CDF family, in the *Medicago truncatula*. *Plant Physiol Biochem*, **2009**, 47: 1089-1094.

[108] Pedas P, Schiller Stokholm M, Hegelund JN, Ladegard AH, Schjoerring JK, et al. Golgi localized barley MTP8 proteins facilitate Mn transport. *PLoS ONE*, **2014**, 9: e113759.

[109] Migocka M, Papierniak A, Maciaszczyk-Dziubinska E, Pozdzik P, Posyniak E, et al. Cucumber metal transport protein MTP8 confers increased tolerance to manganese when expressed in yeast and *Arabidopsis thaliana*. *J Exp Bot*, **2014**, 65: 5367-5384.

[110] Migocka M, Papierniak A, Kosieradzka A, Posyniak E, Maciaszczyk-Dziubinska E, et al. Cucumber metal tolerance protein CsMTP9 is a plasma membrane H^+-coupled antiporter involved in the Mn^{2+} and Cd^{2+} efflux from root cells. *Plant J*, **2015**, 84: 1045-1058.

[111] Ueno D, Sasaki A, Yamaji N, Miyaji T, Fujii Y, et al. A polarly localized transporter for efficient manganese uptake in rice. *Nat Plants*, **2015**, 9: 15170.

[112] Zhang M, Liu B. Identification of a rice metal tolerance protein OsMTP11 as a manganese transporter. *PLoS ONE*, **2017**, 12: e0174987.

[113] Williams LE, Mills RF. P1B-ATPases–an ancient family of transition metal pumps with diverse functions in plants. *Trends Plant Sci*, **2005**, 10: 491-502.

[114] Argüello JM, Eren E, Gonzalez-Guerrero M. The structure and function of heavy metal transport P1B-ATPases. *Biometals*, **2007**, 20: 233-248.

[115] Smith AT, Smith KP, Rosenzweig AC. Diversity of the metal-transporting P1B-type ATPases. *J Biol Inorg Chem*, **2014**, 19: 947-960.

[116] Axelsen KB, Palmgren MG. Evolution of substrate specificities in the P-type ATPase superfamily. *J Mol Evol*, **1998**, 46: 84-101.

[117] Argüello JM. Identification of ion-selectivity determinants in heavy-metal transport P1B-type ATPases. *J Membr Biol*, **2003**, 195: 93-108.

[118] Wong CK, Jarvis RS, Sherson SM, Cobbett CS. Functional analysis of the heavy metal binding domains of the Zn/Cd-transporting ATPase, HMA2, in Ar*a*bidopsis *thaliana*. *New Phytol*, **2009**, 181: 79-88.

[119] Seigneurin-Berny D, Gravot A, Auroy P, Mazard C, Kraut A, et al. HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. *J Biol Chem*, **2006**, 281: 2882-2892.

[120] Moreno I, Norambuena L, Maturana D, Toro M, Vergara C, et al. AtHMA1 is a thapsigargine-sensitive Ca^{2+}/ heavy metal pump. *J Biol Chem*, **2008**, 283: 9633-9641.

[121] Kim YY, Choi H, Segami S, Cho HT, Martinoia E, et al. AtHMA1 contributes to the detoxification of excess Zn(II) in *Arabidopsis*. *Plant J*, **2009**, 58: 737-753.

[122] Verret F, Gravot A, Auroy P, Leonhardt N, David P, et al. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Lett*, **2004**, 576: 306-312.

[123] Wong CK, Cobbett CS. HMA P-type ATPases are the major mechanism for root-to-shoot Cd translocation in *Arabidopsis thaliana*. *New Phytol*, **2009**, 181: 71-78.

[124] Eren E, Argüello JM. *Arabidopsis* HMA2, a divalent heavy metal transporting P(1B)-type ATPase, is involved in cytoplasmic Zn^{2+} homeostasis. *Plant Physiol*, **2004**, 136: 3712-3723.

[125] Mills RF, Francini A, Ferreira da Rocha PS, Baccarini PJ, Aylett M, et al. The plant P1B-type ATPase AtHMA4 transports Zn and Cd and plays a role in detoxification of transition metals supplied at elevated levels. *FEBS Lett*, **2005**, 579: 783-791.

[126] Verret F, Gravot A, Auroy P, Preveral S, Forestier C, et al. Heavy metal transport by AtHMA4 involves the N-terminal degenerated metal binding domain and the C-terminal His 11 stretch. *FEBS Lett*. **2005**, 579: 1515-22.

[127] Cun P, Sarrobert C, Richaud P, Chevalier A, Soreau P, et al. Modulation of Zn/Cd P1B2-ATPase activities in *Arabidopsis* impacts differently on Zn and Cd contents in shoots and seeds. *Metallomics*, **2014**, 6: 2109.

[128] Cobbett CS, Hussain D, Haydon MJ. Structural and functional relationships between type 1B heavy meta-transporting P-type ATPases in Arabidopsis. *New Phytol*, **2003**, 159: 315-321.

[129] Eren E, Kennedy DC, Maroney MJ, Argüello JM. A novel regulatory metal binding domain is present in the C terminus of Arabidopsis Zn^{2+}-ATPase HMA2. *J Biol Chem*, **2006**, 281: 33881-33891.

[130] Baekgaard L, Mikkelsen MD, Sorensen DM, Hegelund JN, Persson DP, et al. A combined zinc/cadmium sensor and zinc/cadmium export regulator in a heavy metal pump. *J Biol Chem*, **2010**, 285: 31243-31252.

[131] Mills RF, Valdes B, Duke M, Peaston KA, Lahner B, et al. Functional significance of AtHMA4 C-terminal domain in planta. *PLoS ONE*, **2010**, 5: e13388.

[132] Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, et al. AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. *Plant Physiol*, **2009**, 149: 894-904.

[133] Chao DY, Silva A, Baxter I, Huang YS, Nordborg M, et al. Genome-wide association studies identify heavy metal ATPase3 as the primary determinant of natural variation in leaf cadmium in *Arabidopsis thaliana*. *PLoS Genet*, **2012**, 8: e1002923.

[134] Andres-Colas N, Sancenon V, Rodriguez-Navarro S, Mayo S, Thiele DJ, et al. The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. *Plant J*, **2006**, 45: 225-236.

[135] Kobayashi Y, Kuroda K, Kimura K, Southron-Francis JL, Furuzawa A, et al. Amino acid polymorphisms in strictly conserved domains of a P-type ATPase HMA5 are involved in the mechanism of copper tolerance variation in Arabidopsis. *Plant Physiol*, **2008**, 148: 969-980.

[136] Shikanai T, Muller-Moule P, Munekage Y, Niyogi KK, Pilon M. PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. *Plant Cell*, **2003**, 15: 1333-1346.

[137] Abdel-Ghany SE, Muller-Moule P, Niyogi KK, Pilon M, Shikanai T. Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. *Plant Cell*, **2005**, 17: 1233-1251.

[138] Hirayama T, Kieber JJ, Hirayama N, KoganM, Guzman P, et al. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis. *Cell*, **1999**, 97: 383-393.

[139] Woeste KE, Kieber JJ. A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype. *Plant Cell*, **2000**, 12: 443-455.

[140] Binder BM, Rodriguez FI, Bleecker AB. The copper transporter RAN1 is essential for biogenesis of ethylene receptors in *Arabidopsis*. *J Biol Chem*, **2010**, 285: 37263-37270.

[141] Ueno D, Yamaji N, Kono I, Huang CF, Ando T, et al. Gene limiting cadmium accumulation in rice. *Proc Natl Acad Sci U S A*, **2010**, 107:16500-16505.

[142] Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, et al. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol*, **2011**, 189: 190-199.

[143] Satoh-Nagasawa N, Mori M, Nakazawa N, Kawamoto T, Nagato Y, et al. Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. *Plant Cell Physiol*, **2012**, 53: 213-224.

[144] Takahashi R, Bashir K, Ishimaru Y, Nishizawa NK, Nakanishi H. The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. *Plant Signal Behav*, **2012**, 7:1605-1607.

[145] Yamaji N, Xia J, Mitani-Ueno N, Yokosho K, Ma JF. Preferential delivery of zinc to developing tissues in rice is mediated by P-type heavy metal ATPase OsHMA2. *Plant Physiol*, **2013**, 162: 927-939.

[146] Tezuka K, Miyadate H, Kato K, Kodama I, Matsumoto S, et al. A single recessive gene controls cadmium translocation in the cadmium hyperaccumulating rice cultivar Cho-Ko-Koku. *Theor Appl Genet*, **2010**, 120: 1175-1182.

[147] Sasaki A, Yamaji N, Ma JF. Overexpression of OsHMA3 enhances Cd tolerance and expression of Zn transporter genes in rice. *J Exp Bot*, **2014**, 65: 6013-6021.

[148] Ueno D, Koyama E, Kono I, Ando T, Yano M, et al. Identification of a novel major quantitative trait locus controlling distribution of Cd between roots and shoots in rice. *Plant Cell Physiol*, **2009**, 50: 2223-2233.

[149] Kumagai S, Suzuki T, Tezuka K, Satoh-Nagasawa N, Takahashi H, et al. Functional analysis of the C-terminal region of the vacuolar cadmium-transporting rice OsHMA3. *FEBS Lett*, **2014**, 588: 789-794.

[150] Deng F, Yamaji N, Xia J, Ma JF. A member of the heavy metal P-type ATPase OsHMA5 is involved in xylem loading of copper in rice. *Plant Physiol*, **2013**, 163: 1353-1362.

[151] Lee S, Kim YY, Lee Y, An G. Rice P1B-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. *Plant Physiol*, **2007**, 145: 831-842.

[152] Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N. A novel CPx-ATPase from the cadmium hyper accumulator *Thlaspi caerulescens*. *FEBS Lett*, **2004**, 569: 140-148.

[153] Papoyan A, Kochian LV. Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyper accumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. *Plant Physiol*, **2004**, 136: 3814-3823.

[154] Hanikenne M, Kramer U, Demoulin V, Baurain D. A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae*. *Plant Physiol*, **2005**, 137: 428-446.

[155] Chiang HC, Lo JC, Yeh KC. Genes associated with heavy metal tolerance and accumulation in Zn/Cd hyper accumulator *Arabidopsis halleri*: A genomic survey with cDNA microarray. *Environ Sci Tech*, **2006**, 40: 6792-6798.

[156] Craciun AR, Meyer CL, Chen J, Roosens N, De Groodt R, et al. Variation in *HMA4* gene copy number and expression among *Noccaea caerulescens* populations presenting different levels of Cd tolerance and accumulation. J *Exp Bot*, **2012**, 63: 4179-4189.

[157] Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, et al. A major quantitative trait locus for cadmium tolerance in *Arabidopsis halleri* colocalizes with HMA4, a gene encoding a Heavy Metal ATPase. *Plant Physiol*, **2007**, 144: 1052-1065.

[158] Willems G, Drager DB, Courbot M, Gode C, Verbruggen N, et al. The genetic basis of zinc tolerance in the metallophyte *Arabidopsis halleri* ssp. *halleri* (Brassicaceae): An analysis of quantitative trait loci. *Genetics*, **2007**, 176: 659-674.

[159] Willems G, Frerot H, Gennen J, Salis P, Saumitou-Laprade P, et al. Quantitative trait loci analysis of mineral element concentrations in an *Arabidopsis halleri* - *Arabidopsis lyratapetraea* F2 progeny grown on cadmium-contaminated soil. *New Phytol*, **2010**, 187: 368-379.

[160] Liu H, Zhao H, Wu L, Liu A, Zhao FJ, et al. Heavy metal ATPase 3 (HMA3) confers cadium hypertolerance on the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. *New Phytol*, **2017**, 215: 687-698.

[161] Mikkelsen MD, Pedas P, Schiller M, Vincze E, Mills RF, et al. Barley HvHMA1 is a heavy metal pump involved in mobilizing organellar Zn and Cu and plays a role in metal loading into grains. *PLoS ONE*, **2012**, 7: e49027.

[162] Tan J, Wang J, Chai T, Zhang Y, Feng S, et al. Functional analyses of TaHMA2, a P1B-type ATPase in wheat. *Plant Biotech J,* **2013**, 11: 420-431.

[163] Fang X, Wang L, Deng X, Wang P, Ma Q, et al. Genome-wide characterization of soybean P1B-ATPases gene family provides functional implications in cadmium responses. *BMC Genomics*, **2016**, 17: 376.

[164] Bernal M, Testillano PS, Alfonso M, del Carmen RM, Picorel R, et al. Identification and subcellular localization of the soybean copper P1B-ATPase GmHMA8 transporter. *J Struct Biol*, **2007**, 158: 46-58.

[165] Benitez ER, Hajika M, Takahashi R. Single-base substitution in P1B-ATPase gene is associated with a major QTL for seed cadmium concentration in soybean. *J Hered*, **2012**, 103: 278-286.

[166] Wang Y, Yu KF, Poysa V, Shi C, Zhou YH. A single point mutation in GmHMA3 affects cadmium (Cd) translocation and accumulation in soybean seeds. *Mol Plant*, **2012**, 5: 1154-1156.

[167] Huang S, Li R, Zhang Z, Li L, Gu X, et al. The genome of the cucumber, *Cucumis sativus* L. *Nat Genet*, **2009**, 41: 1275-1281.

[168] Migocka M, Papierniak A, Maciaszczyk-Dziubinska E, Posyniak E, Kosieradzka A. Molecular and biochemical properties of two P1B2-ATPases, CsHMA3 and CsHMA4, from cucumber. *Plant Cell Environ*, **2015**, 38: 1127-1141.

[169] Park W, Feng Y, Ahn SJ. Alteration of leaf shape, improved metal tolerance, and productivity of seed by over expression of CsHMA3 in *Camelina sativa*. *Biotech Biofuels*, **2014**, 7: 96.

[171] Gruenheid S, Cellier M, Vidal S, Gros P. Identification and characterization of a second mouse Nramp gene. *Genomics*, **1995**, 25: 514-525.

[172] Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, et al. Nramp defines a family of membrane proteins. *Proc Natl Acad Sci U S A*, **1995**, 92:10089-10093.

[173] Cellier MF, Bergevin I, Boyer E, Richer E. Polyphyletic origins of bacterial Nramp transporters. *Trends Genet*, **2001**, 17: 365-370.

[174] Nevo Y, Nelson N. The NRAMP family of metal-ion transporters. *Biochim Biophys Acta*, **2006**, 1763: 609-620.

[175] He XM, Fang MX, Zhang ZT, Hu YS, Jia XZ, et al. Characterization of chicken natural resistance-associated macrophage protein encoding genes (*Nramp1* and *Nramp2*) and association with salmonellosis resistance. *Genet Mol Res*, **2013**, 12: 618-630.

[176] Kwong RW, Hamilton CD, Niyogi S. Effects of elevated dietary iron on the gastrointestinal expression of *Nramp* genes and iron homeostasis in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol Biochem*, **2013**, 39: 363-372.

[177] Peracino B, Buracco S, Bozzaro S. The Nramp (Slc11) proteins regulate development, resistance to pathogenic bacteria and iron homeostasis in *Dictyostelium discoideum*. *J Cell Sci*, **2013**, 126: 301-311.

[178] Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*, **1997**, 388: 482-488.

[179] Colangelo EP, Guerinot ML. Put the metal to the petal: metal uptake and transport throughout plants. *Curr Opin Plant Biol*, **2006**, 9: 322-330.

[180] Xia J, Yamaji N, Kasai T, Ma JF. Plasma membrane-localized transporter for aluminum in rice. *Proc Natl Acad Sci USA*, **2010**, 107: 18381-18385.

[181] Belouchi A, Kwan T, Gros P. Cloning and characterization of the OsNramp family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. *Plant Mol Bi*ol, **1997**, 33: 1085-1092.

[182] Curie C, Alonso J, Le Jean M, Ecker J, Briat J. Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochem J*, **2000**, 347: 749-755.

[183] Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI. Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to *Nramp* genes. *Proc Natl Acad Sci U S A*, **2000**, 97: 4991-4996.

[184] Bereczky Z, Wang HY, Schubert V, Ganal M, Bauer P. Differential regulation of NRAMP and IRT metal transporter genes in wild type and iron uptake mutants of tomato. *J Biol Chem*, **2003**, 278: 24697-24704.

[185] Kaiser BN, Moreau S, Castelli J, Thomson R, Lambert A, et al. The soybean NRAMP homologue, GmDMT1, is a symbiotic divalent metal transporter capable of ferrous iron transport. *Plant J*, **2003**, 35: 295-304.

[186] Xiao H, Yin L, Xu X, Li T, Han Z. The iron-regulated transporter, MbNRAMP1, isolated from *Malus baccata* is involved in Fe, Mn and Cd trafficking. *Ann Bot*, **2008**, 102: 881-889.

[187] Oomen RJ, Wu J, Lelievre F, Blanchet S, Richaud P, et al. Functional characterization of NRAMP3 and NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytol*, **2009**, 181: 637-650.

[188] Wei W, Chai T, Zhang Y, Han L, Xu J, et al. The *Thlaspi caerulescens* NRAMP homologue TcNRAMP3 is capable of divalent cation transport. *Mol Biotech*, **2009**, 41: 15-21.

[189] Ishimaru Y, Takahashi R, Bashir K, Shimo H, Senoura T, et al. Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Sci Rep*, **2012**, 2: 286.

[190] Thomine S, Lelievre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H. AtNRAMP3, a multi specific vacuolar metal transporter involved in plant responses to iron deficiency. *Plant J*, **2003**, 34: 685-695.

[191] Lanquar V, Lelievre F, Bolte S, Hamès C, Alcon C, et al. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO J*, **2005**, 24: 4041-4051.

[192] Cailliatte R, Schikora A, Briat JF, Mari S, Curie C. High affinity manganese uptake by the metal transporter NRAMP1 is essential for Arabidopsis growth in low manganese conditions. *Plant Cell*, **2010**, 22: 904-917.

[193] Lanquar V, Ramos MS, Lelievre F, Barbier-Brygoo H, Krieger-Liszkay A, et al. Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is required for optimal photosynthesis and growth under manganese deficiency. *Plant Physiol*, **2010**, 152: 1986-1999.

[194] Lanquar V, Leli_evre F, Barbier-Brygoo H, Thomine S. Regulation and function of AtNRAMP4 metal transporter protein. *Soil Sci Plant Nutr*, **2004**, 50: 477-484.

[195] Molins H, Michelet L, Lanquar V, Agorio A, Giraudat J, et al. Mutants impaired in vacuolar metal mobilization identify chloroplasts as a target for cadmium hypersensitivity in *Arabidopsis thaliana*. *Plant Cell Environ*, **2013**, 36: 804-817.

[196] Cailliatte R, Lapeyre B, Briat JF, Mari S, Curie C. The NRAMP6 metal transporter contributes to cadmium toxicity. *Biochem J*, **2009**, 422: 217-228.

[197] Tiwari M, Sharma D, Dwivedi S, Singh M, Tripathi RD, et al. Expression in *Arabidopsis* and cellular localization reveal involvement of rice NRAMP, OsNRAMP1, in arsenic transport and tolerance. *Plant Cell Environ*, **2014**, 37: 140-152.

[198] Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa NK. Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. *Plant Signal Behav*, **2011**, 6: 1813-1816.

[199] Takahashi R, Ishimaru Y, Senoura T, Shimo H, Ishikawa S, et al. The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. *J Exp Bot*, **2011b**, 62: 4843-4850.

[200] Ishikawa S, Ishimaru Y, Igura M, Kuramata M, Abe T, et al. Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proc Natl Acad Sci U S A*, **2012**, 109: 19166-19171.

[201] Sasaki A, Yamaji N, Yokosho K, Ma JF. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell*, **2012**, 24: 2155-2167.

[202] Chakrabarty D, Trivedi PK, Misra P, Tiwari M, Shri M, et al. Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings. *Chemosphere*, **2009**, 74: 688-702.

[203] Yamaji N, Sasaki A, Xia JX, Yokosho K, Ma JF. A node based switch for preferential distribution of manganese in rice. *Nat Commun*, **2013**, 4: 2442.

[204] Yang M, Zhang W, Dong H, Zhang Y, Lv K, et al. OsNRAMP3 Is a vascular bundles-specific manganese transporter that is responsible for manganese distribution in Rice. *PLoS ONE*, **2013**, 8: e83990.

[205] Xia J, Yamaji N, Ma JF. Further characterization of an aluminum influx transporter in rice. *Plant Signal Behav*, **2011**, 6: 160-163.

[206] Li JY, Liu J, Dong D, Jia X, McCouch SR, et al. Natural variation underlies alterations in Nramp aluminum transporter (NRAT1) expression and function that play a key role in rice aluminum tolerance. *Proc Natl Acad Sci U S A*, **2014**, 111: 6503-6508.

[207] Milner MJ, Mitani-Ueno N, Yamaji N, Yokosho K, Craft E, et al. Root and shoot transcriptome analysis of two ecotypes of *Noccaea caerulescens* uncovers the role of NcNramp1 in Cd hyper accumulation. *Plant J*, **2014**, 78: 398-410.

[208] van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, et al. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyper accumulator *Thlaspi caerulescens*. *Plant Physiol*, **2006**, 142: 1127-1147.

[209] Xiong H, Kobayashi T, Kakei Y, Senoura T, Nakazono M, et al. AhNRAMP1 iron transporter is involved in iron acquisition in peanut. *J Exp Bot*, **2012**, 63: 4437-4446.

[210] Peris-Peris C, Serra-Cardona A, Sanchez-Sanuy F, Campo S, Arino J, et al. Two NRAMP6 isoforms function as iron and manganese transporters and contribute to disease resistance in rice. *MPMI*, **2017**, 30: 385-398.

[211] Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, et al. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *J Biol Chem*, **2004**, 279: 9091-9096.

[212] Murata Y, Ma JF, Yamaji N, Ueno D, Nomoto K, et al. A specific transporter for iron(III)-phytosiderophore in barley roots. *Plant J*, **2006**, 46: 563-572.

[213] DiDonato RJ Jr, Roberts LA, Sanderson T, Eisley RB, Walker EL. Arabidopsis Yellow Stripe-Like2 (*YSL2*): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant J*, **2004**, 39: 403-414.

[214] Le Jean M, Schikora A, Mari S, Briat JF, Curie C. A loss-of-function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. *Plant J*, **2005**, 44: 769-782.

[215] Schaaf G, Schikora A, Haberle J, Vert G, Ludewig U, et al. A putative function for the Arabidopsis Fe-Phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. *Plant Cell Physiol*, **2005**, 46: 762-774.

[216] Waters BM, Chu HH, DiDonato RJ, Roberts LA, Eisley RB, et al. Mutations in Arabidopsis Yellow Stripe-Like1 and Yellow Stripe-Like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiol*, **2006**, 141: 1446-1458.

[217] Chu HH, Chiecko J, Punshon T, Lanzirotti A, Lahner B, et al. Successful reproduction requires the function of *Arabidopsis* YELLOW STRIPE-LIKE1 and YELLOW STRIPE-LIKE3 metal-nicotianamine transporters in both vegetative and reproductive structures. *Plant Physiol*, **2010**, 154: 197-210.

[218] Chen CC, Chien WF, Lin NC, Yeh KC. Alternative functions of Arabidopsis Yellow Stripe-Like3: From metal translocation to pathogen defense. *PLoS ONE*, **2014**, 9: e98008.

[219] Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, et al. A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. *Mol Cell Proteomics*, **2007**, 6: 394-412.

[220] Conte SS, Chu HH, Rodriguez DC, Punshon T, Vasques KA, et al. *Arabidopsis thaliana* YELLOW STRIPE1-LIKE4 and YELLOW STRIPE1-LIKE6 localize to internal cellular membranes and are involved in metal ion homeostasis. *Front Plant Sci*, **2013**, 4: 283.

[221] Divol F, Couch D, Conejero G, Roschzttardtz H, Mari S, et al. The Arabidopsis YELLOW STRIPE LIKE4 and 6 transporters control iron release from the chloroplast. *Plant Cell*, **2013**, 25: 1040-1055.

[222] Hofstetter SS, Dudnik A, Widmer H, Dudler R. Arabidopsis YELLOW STRIPE-LIKE7 (YSL7) and YSL8 transporters mediate uptake of Pseudomonas virulence factor Syringolin A into plant cells. *MPMI*, **2013**, 26: 1302-1311.

[223] Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, et al. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J*, **2004**, 39: 415-424.

[224] Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, et al. Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. *J Biol Chem*, **2009**, 284: 3470-3479.

[225] Lee S, Chiecko JC, Kim SA, Walker EL, Lee Y, et al. Disruption of OsYSL15 leads to iron inefficiency in rice plants. *Plant Physiol*, **2009**, 150: 786-800.

[226] Ishimaru Y, Masuda H, Bashir K, Inoue H, Tsukamoto T, et al. Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *Plant J*, **2010**, 62: 379-390.

[227] Sasaki A, Yamaji N, Xia J, Ma JF. OsYSL6 is involved in the detoxification of excess manganese in rice. *Plant Physiol*, **2011**, 157: 1832-1840.

[228] Ueno D, Yamaji N, Ma JF. Further characterization of ferric phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley. *J Exp Bot*, **2009**, 60: 3513-3520.

[229] Kakei Y, Ishimaru Y, Kobayashi T, Yamakawa T, Nakanishi H, et al. OsYSL16 plays a role in the allocation of iron. *Plant Mol Biol*, **2012**, 79: 583-594.

[230] Lee S, Ryoo N, Jeon JS, Guerinot ML, An G. Activation of rice Yellow Stripe1-Like 16 (OsYSL16) enhances iron efficiency. *Mol Cells*, **2012**, 33: 117-126.

[231] Aoyama T, Kobayashi T, Takahashi M, Nagasaka S, Usuda K, et al. OsYSL18 is a rice iron(III)-deoxymugineic acid transporter specifically expressed in reproductive organs and phloem of lamina joints. *Plant Mol Biol*, **2009**, 70: 681-692.

[232] Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV. Molecular physiology of zinc transport in the Zn hyper accumulator *Thlaspi caerulescens*. *J Exp Bot*, **2000**, 51: 71-79.

[233] Milner MJ, Kochian LV. Investigating heavy-metal hyper accumulation using *Thlaspi caerulescens* as a model system. *Ann Bot*, **2008**, 102: 3-13.

[234] Gendre D, Czernic P, Conejero G, Pianelli K, Briat JF, et al. TcYSL3, a member of the *YSL* gene family from the hyper-accumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. *Plant J*, **2007**, 49: 1-15.

[235] Wang JW, Li Y, Zhang YX, Chai TY. Molecular cloning and characterization of a *Brassica juncea* yellow stripe-like gene, BjYSL7, whose overexpression increases heavy metal tolerance of tobacco. *Plant Cell Rep*, **2013**, 32: 651-662.

[236] Araki R, Murata J, Murata Y. A novel barley yellow stripe 1-like transporter (HvYSL2) localized to the root endodermis transports metal-phytosiderophore complexes. *Plant Cell Physiol*, **2011**, 52: 1931-1940.

[237] Zheng L, Fujii M, Yamaji N, Sasaki A, Yamane M, et al. Isolation and characterization of a barley yellow stripe-like gene, HvYSL5. *Plant Cell Physiol*, **2011**, 52: 765-774.