Natural Resistance-Associated Macrophage Proteins (NRAMPs): Functional Significance of Metal Transport in Plants

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Abstract

In plants, there are several families of metal transporters that play an important role in metal uptake and sequestration, thus protecting the plant from stress due to heavy metals. Crop plants grown on heavy metal-contaminated agricultural land present an indirect hazard to the health of their consumers. Hence, it is very important to study the transporters associated with metal transport in plants. Natural resistance-associated macrophage protein (NRAMP) transporters are present in a wide range of organisms. This family of NRAMP transporters has been identified and functionally characterized in several plant species like *Arabidopsis*, rice, soya bean, etc. that is, in both monocot and dicot plants. In plants, several members of the NRAMP gene family have been identified and functionally characterized. They are involved in the transport of several divalent metal ions in the plant based on their localization. The presence of NRAMP transporter genes has also been computationally predicted in the genomes of all the plants studied so far.

This chapter discusses the general properties, structure, function, and expression of NRAMP transporters in several plants based on existing literature. It mainly focuses on the functional significance of the NRAMP family of transporters in both monocot and dicot plants.

Keywords

Metal · Plant · NRAMP · Structure · Function · Expression

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Abbreviations

NRAMP	Natural resistance-associated macrophage protein
CDF	Cation diffusion facilitator
CTR	Copper transporters
YSL	Yellow stripe-like
ZIP	ZRT1-IRT1-like protein
ABC	ATP binding cassette
CAX	Cation proton exchanger

5.1 Introduction

Fourteen different minerals are required by the plants for their overall growth and development (Marschner 2012). These mineral elements present in the soil are absorbed by the roots, transported to the shoots, and then distributed to various tissues and parts of the plant based on their requirement (Marschner 2012). The quality of food, to a large extent, is determined by the uptake and sequestration of essential, as well as nonessential heavy metals, by the plants. In some types of soil, the bioavailability and abundance of essential metals can be limiting, and thus plants have developed several methods for efficient absorption.

In plants, there exist several large families of metal transporter genes. These transporters are involved in the uptake and efflux of metal ions. The families of metal transporters include ZRT1-IRT1-like protein (ZIP), ATPases, cation diffusion facilitator (CDF), copper transporters (CTR), and natural resistance-associated macrophage protein (NRAMP) homologs (Guerinot 2000). Apart from them, members of the ABC transporter family and vacuolar cation proton exchanger (CAX) are involved in plant metal homeostasis (Kushnir et al. 2001).

After the uptake of these metals, plants transport them to the cellular compartments and ultimately reach the growing organs where they are required. Metals play many important roles in photosynthesis but can cause severe oxidative damage when in excess, thus necessitating a highly regulated influx/efflux mechanism in the photosynthetic tissues (Hall and Williams 2003). Plants being sessile also have to combat toxicity of nonessential heavy metals such as lead, cadmium, and mercury or essential metals when present in excessive concentrations. The transporters function to either exclude metals at the root or sequester metals in certain cell compartments like the vacuole and thus minimize damages caused due to them.

Members of the NRAMP family of transporters are found in a wide variety of living beings like animals, plants, fungi, and bacteria (Nevo and Nelson 2006). The NRAMP family of transporters are highly conserved membrane proteins that are involved in metal ion transport. The NRAMP gene was first identified in phagosomes inside infected murine macrophages. It was believed to determine

sensitivity to bacterial infection by regulating the concentrations of essential divalent metal ions. Homologs of *NRAMP1* were later characterized in plants as well (Hall and Williams 2003).

In plants, the NRAMP gene family has been identified to function on several divalent metal ions, regulating their acquisition, transportation, and homeostasis. NRAMP transporters primarily transport metal ions like Fe^{+2}, Mn^{+2}, Co^{+2}, and Zn^{+2} (Nevo and Nelson 2006). The rice NRAMPs were one of the first NRAMP families to be identified in plants with the ESTs of OsNRAMP1-3 from rice being identified and cloned (Belouchi et al. 1995). Subsequently genes from this family of transporters were identified in several higher plants. A total of six NRAMP transporters have been identified in *Arabidopsis* (Mäser et al. 2001). The NRAMP transporters in plants are divided into two subfamilies, with AtNRAMP1 and AtNRAMP6 belonging to the first subfamily and NRAMP2–5 to the second subfamily (Mäser et al. 2001). The rice OsNRAMP1 and OsNRAMP3 are grouped in the first subfamily while OsNramp 2 has been grouped into the second subfamily.

A variable number of NRAMP proteins have been identified in the genomes of several plant species. A basal angiosperm, *A. trichopoda*, has three copies of NRAMP; these copies underwent a lineage-specific expansion to 10 copies found in *Panicum virgatum*, a monocot species, and 13 in *Glycine max*, a eudicot species (Ullah et al. 2018). NRAMP genes are present in all plant families in both the grass and non-graminaceous species. These two groups of plants use different approaches to transport iron from the soil (Marschner and Romheld 1994). In non-graminaceous plants, the IRT/FRO system is a major component of the Fe uptake system (Qin et al. 2017). The main transporter in graminaceous plants responsible for uptake of Fe from siderophore-Fe complexes is YSL (Thomine and Vert 2013). Apart from the transporters involved in both these strategies, the NRAMP family represents another transporter family associated with Fe uptake and transport (Thomine and Vert 2013). The presence of plant NRAMP genes in these two groups of plants suggests their involvement in metal uptake from the soil solution and metal homeostasis. NRAMP genes also affect the remobilization of intracellular Cd (Cailliatte et al. 2009), which might also help to increase tolerance to toxic heavy metals in plants.

The current knowledge regarding the properties and functional aspects of the plant NRAMP is presented in this chapter. The plant NRAMP family of transporters has been reported in both plant genomic and plant EST databases, indicating that genes from this family are present in all the plants studied at the molecular level. However, there is a huge gap in the understanding of the molecular and physiological functions of these groups of proteins. Here, we provide an overview of the plant NRAMP systems that are thought to be involved in the acquisition, distribution, and redistribution of transition metals. Specific focus would be paid to their location, function, and known substrate specificity considering the overall view of transition metal nutrition in plants.

5.2 Genomic Analysis

The NRAMP family of transporters in yeast is represented by three genes, namely *SMF1*, *SMF2*, and *SMF3*, in fly by *MVL* (*malvolio*), and by *NRAMP1* and *NRAMP2* in mammals. The rat isoform of the human NRAMP2 is DCT1 (*d*ivalent *c*ation *t*ransporter 1 or *d*ivalent *m*etal *t*ransporter DMT1) (Gunshin et al. 1997). One of the characteristics of plant *NRAMP* genes is the comparatively high number of *NRAMP* gene homologs per species. The rice genome contains seven distinct genes which encode NRAMP homologous proteins (Bennetzen 2002). Additionally, several ESTs with homology to NRAMP in several species like *Medicago truncatula*, soya bean, maize, cotton, pine, tomato, and barley have been identified.

In higher plants like *Arabidopsis*, rice, soybean, *Medicago*, barley, peanut, mustard, tomato, and apple, the identified NRAMPs are divided into two large groups, namely, subfamily I and subfamily II, with both dicots and monocots represented in each of the groups (Qin et al. 2017). The *Arabidopsis* genome encodes six *NRAMP* homologs; it has four members in subfamily I and two members in subfamily II. The rice genome encodes seven members, two in subfamily I and five in subfamily II and correspondingly four and three from *Medicago*. In soya bean (*Glycine max*), 13 *GmNRAMP* homologs have been identified to be encoded by the genome. Of the 13 NRAMP members in soya bean, eight belong to subfamily I and five to subfamily II. While in mustard, NRAMP proteins are grouped into subfamily I alone, those in peanut, barley, and apple are grouped into subfamily II (Qin et al. 2017). There are 11 CsNRAMPs, in tea which are divided into group I (CsNRAMP3, CsNRAMP4, CsNRAMP5, and CsNRAMP8) and group II (the remaining CsNRAMPs) (Jinqiu Li et al. 2021).

The members of the group II family of NRAMPs appear to be more related to the reported animal NRAMP homologs. It has been reported that both groups of NRAMPs are required for a regulated metal homeostasis in both the plant and animal kingdoms (Thomine and Vert 2013).

5.3 Structural Analysis

The plant NRAMPs have been highly conserved throughout evolution. In rice, OsNRAMPs are 518–550 amino acid residues long and are predicted to have 11–12 transmembrane domains (Mani and Sankaranarayanan 2018a). In *Phaseolus vulgaris* (common bean), PvNRAMPs have 12 transmembrane domains, and they are 507–554 amino acid residues long (Ishida et al. 2018). In *Camellia sinensis*, CsNRAMP proteins are 279–1373 amino acid residues long and contain 3–12 transmembrane regions. This is believed to be due to a broken NRAMP domain (Jinqiu Li et al. 2021). In *Brassica napus*, BnNRAMPs are only 100–200 amino acid residues long (Meng et al. 2017).

Members of the NRAMP family have considerable protein sequence similarity of 28% (yeast), 40% (plant), and 55% (fly) with the mammalian proteins (46%, 58%, and 73% similarity, respectively) (Cellier et al. 1995). The rat isoform DCT1 shares

92% identity with NRAMP2 and 73% identity to NRAMP1 in humans (Gunshin et al. 1997). In the plant kingdom, NRAMP proteins show high amino acid sequence similarity than NRAMP proteins from other kingdoms. *Arabidopsis* NRAMP proteins share 40–50% amino acid sequence identity with NRAMP1 in mouse and about 30% with SMF1 the yeast NRAMP. The sequence similarity of the bacterial consensus transport sequence found between transmembrane domains TM8 and TM9 and the predicted transmembrane domains is even greater (Kerppola and Ames 1992).

NRAMPs generally have 11 or 12 transmembrane domains. The first ten TMs form a LeuT-fold, like in bacterial homolog structures and the model *Deinococcus radiodurans* (Dra) NRAMP (Bozzi et al. 2016b). Conserved residues in TM1 and TM6 like asparagine, methionine, and aspartate coordinate transition metal substrates (Ehrnstorfer et al. 2014). In between TM8 and TM9, there is a characteristic "consensus transport motif" (CTM) (Williams et al. 2000). This CTM has similarities with the previously studied conserved regions of numerous transport proteins in bacteria and particularly with the highly conserved part of the permeation pore of the *Shaker*-type K^+ channel in animals (Belouchi et al. 1997).

It has been reported that NRAMP proteins carry consensus residues between TMD8 and TMD9. GQSSTITGT YAG QY(/F)V(/I)MQGFLD(/E/N) is the consensus transport motif (CTM) commonly present among NRAMP proteins. In OsNRAMP1, OsNRAMP2, OsNRAMP3, OsNRAMP5, and OsNRAMP7, it is highly conserved, and in other members of the OsNRAMP family of transporter proteins, it is partially conserved. In the OsNRAMP6 protein sequence, it is least conserved (Mani and Sankaranarayanan 2018b). In *Arabidopsis*, the AtNRAMP proteins have GQSSTITGTY AGQXXMXGFLX as CTM, while in *Phaseolus vulgaris*, PvNRAMP proteins have GQSSTITGTYAGQFIMGGFLN (Ishida et al. 2018). The CsNRAMP proteins in *Camellia sinensis* also have similar consensus residues, that is, GQSSTxTGTYAGQFIMxGFLxLxxKKW (Jinqiu Li et al. 2021). For the NRAMP transporter family, the signature sequence is DPGN, and any mutation in these residues leads to defects in the transporter function (Mani and Sankaranarayanan 2018a).

X-ray crystallography which is one of the primary means of elucidating the structures of transporter proteins has the potential to provide snapshots of their structures at different stages of its functioning. Knowledge about the conformational changes taking place in the NRAMP transporter structure as it transports metal ions and protons can help us to understand its functioning. Using X-ray crystallography, Bozzi et al. (2019) studied the structure of the NRAMP transporters from bacterium *Deinococcus radiodurans*. The results hint at four distinct conformations that the protein adopts during different stages of metal transport. A unique feature of this mechanism is that it transports metal ions and protons by different pathways that has not been previously reported in transporter proteins with similar structures.

5.4 Functional Characterization

NRAMPs are a class of amino acid-polyamine-organocation (APC) superfamily transition metal transporters that are involved in the uptake of micronutrients like Mn^{2+} in plants, bacteria, and Fe^{2+} in animals (Cellier 2012). The APC superfamily of secondary transporters consists of several related transporters that transport various substrates like metabolites, neurotransmitters, and transition metals in all living organisms (Vastermark et al. 2014). Essential divalent metals like Mn^{2+}, Ni^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, and Zn^{2+} and toxic elements like Pb^{2+}, Cd^{2+}, and Hg^{2+} are transported by NRAMPs. However alkaline metals like Mg^{2+} and Ca^{2+} which are widely available in soil are not transported by them (Bozzi et al. 2016a). Generally, metal uptake by the NRAMP transporter is triggered by acidic pH and followed by an influx of proton (Ehrnstorfer et al. 2017). No studies to date have confirmed that NRAMP is a thermodynamically coupled secondary transporter capable of using the desired gradient of metal or proton to power electrochemical uphill transport of the other substrate.

The transporters of NRAMP family have been reported in various plant species with diversified functions (Ishimaru et al. 2012). In numerous plant species like rice, soya bean, and *Arabidopsis*, several members of the NRAMP family have been identified and functionally characterized (Qin et al. 2017). Various *NRAMP* genes also have been recognized in plant species, like *TjNRAMP4* in *Thlaspi japonicum* (Mizuno et al. 2005), *MbNRAMP1* in *Malus baccata* (Xiao et al. 2008), *MtNRAMP1* in *Medicago truncatula* (Tejada-Jiménez et al. 2015), *GmNRAMPs* in soybean (Qin et al. 2017), and *NtNRAMP5* in tobacco (Tang et al. 2017).

Starting from the NRAMPs found in yeast SMF1, all the NRAMP proteins have been found to function as metal transporters. In yeast, SMF1 was first identified as a part of the Mn uptake system (Supek et al. 1996). Later in the mammalian duodenum, NRAMP2 was mainly identified as the Fe uptake transporter and was found to transport a range of heavy metals (Gunshin et al. 1997). NRAMP2 in mammalian cells plays an important role in iron uptake and recycling. Currently, it is assumed that all the *NRAMP* genes encode metal transporters with broad specificity. Plant NRAMP proteins have been demonstrated to be metal transporters by complementation studies in yeast mutants compromised in metal uptake (Thomine and Vert 2013). MtNRAMP1 in *Medicago truncatula* is the only transporter that was characterized in *M. truncatula*, as a Fe uptake protein (Tejada-Jiménez et al. 2015).

The first plant NRAMP genes were cloned from rice (Belouchi et al. 1997). In rice, there are seven Nramp transporters, namely, OsNRAMP1–OsNRAMP7. But not all of them have been functionally characterized (Nevo and Nelson 2006). In the NRAMP family, many of the transporter proteins are involved in Fe transport. Under Fe deficiency, *OsNRAMP1* is highly upregulated. The OsNRAMP1 transporter is localized on the plasma membrane and is involved in the transport of Fe and Cd. *OsNRAMP1* is involved in the cellular uptake of Cd thus leading to high Cd accumulation in rice (Takahashi et al. 2011) grown in cadmium-rich soils. OsNRAMP3 functions as a Mn-influx transporter and is involved in Mn translocation from old leaves via the phloem cells to young leaves. OsNRAMP3 is regulated

posttranslationally in response to environmental nutrient availability. OsNRAMP3 is not involved in Mn uptake but is involved in Mn translocation (Yang et al. 2013; Mani and Sankaranarayanan 2018a).

OsNRAMP4 is also known as NRAMP aluminum transporter 1 (Nrat1). It is the first transporter in the NRAMP family to be identified as the trivalent Al ion transporter (Xia et al. 2010). OsNRAMP4 in rice does not transport other divalent metal ions, like Zn, Mn, and Fe. It also shares relatively low similarity with the other OsNRAMP members in rice (Xia et al. 2010). In rice Al tolerance, NRAT1 plays an important role by reducing the toxic Al level in the root cell wall and sequesters Al into the vacuole in root cells. OsNRAMP4 plays an important role in Al tolerance; hence, rice is the most Al tolerant of all the cereal crops (Famoso et al. 2010).

OsNRAMP5 is a plasma membrane protein involved in Mn and Fe transport (Ishimaru et al. 2012). When plants are under Fe or Zn deficiency, OsNRAMP5 gene expression increases slightly in the roots, but varying levels of Mn in the surrounding do not affect it (Sasaki et al. 2012). During flowering and seed development in rice plant, OsNRAMP5 plays a role in Fe and Mn transport as well and is constitutively involved in the uptake of Fe and Mn (Ishimaru et al. 2012; Mani and Sankaranarayanan 2018b). OsNRAMP5 is highly expressed in the hulls. It is also expressed in leaves, but the expression levels decline as the leaf ages. *OsNRAMP5* transporters have been reported to be expressed in the rice root and shoot vascular bundles, specifically in the parenchyma cells surrounding the xylem. It plays an important role in xylem-mediated root-to-shoot transport as it is highly expressed in stele cells especially in the xylem region. Thus OsNRAMP5 in rice plants plays a crucial role in Mn uptake, translocation, and distribution. *OsNRAMP5* is highly expressed in stele cells especially in the xylem region and plays an important role in the xylem-mediated root-to-shoot transport (Yang et al. 2014). Cd uptake in rice is also majorly orchestrated by OsNRAMP5 (Sasaki et al. 2012).

OsNRAMP6 is a plasma membrane-localized protein and has been identified to be involved in the uptake of Fe and Mn. Rice plant immunity is negatively regulated by it as loss of its function will result in increased resistance against *M. oryzae* (Peris-Peris et al. 2017).

In *Arabidopsis*, there are six NRAMP transporters (Williams et al. 2000). Heterologous expression studies of transporters AtNRAMP1, AtNRAMP3, and AtNRAMP4 in yeast mutants indicated that these proteins transport Fe, Mn, and Cd (Curie et al. 2000; Thomine et al. 2000). AtNRAMP3 and AtNRAMP4 are involved in the mobilization of Fe stores from vacuoles (Thomine et al. 2003; Lanquar et al. 2005). In adult plants, they also function in vacuolar Mn export into photosynthetic tissues (Lanquar et al. 2010). AtNRAMP6 is an intracellular Cd transporter (Cailliatte et al. 2009). AtNRAMP1 has been shown to be essential for the uptake of Mn from the soil and thus is a high-affinity Mn transporter (Cailliatte et al. 2010). It has been reported recently that the AtNRAMP2 protein is involved in the remobilization of Mn^{2+} in Golgi for root growth instead of Mn^{2+} uptake through roots (Gao et al. 2018).

AtNramp3 is also associated with sensitivity and uptake of Fe and Cd (Thomine et al. 2000). AtNramp3 is thought to function in long-distance metal transport. In

Arabidopsis, AtNRAMP3 and AtNRAMP4, to some extent, are involved in resistance against *E. chrysanthemi* a bacterial pathogen (Thomine et al. 2003). AtNRAMP3 and AtNRAMP4 function in vacuolar Fe mobilization (Mary et al. 2015). AtNRAMP6 plays an important role in intracellular Fe homeostasis and is located in the Golgi network (Li et al. 2019). AtNRAMP3 and AtNRAMP4 contribute to Fe mobilization and are located on vacuolar membranes (Mary et al. 2015).

Five NRAMP genes in cacao (*Theobroma cacao*) have been identified, namely, TcNRAMP1, TcNRAMP2, TcNRAMP3, TcNRAMP5, and TcNRAMP6. TcNRAMP5 transports essential metal ions and additionally transports Cd^{2+}. TcNRAMP3 and TcNRAMP5 are involved in the transport of Fe^{2+} and Mn^{2+}. TcNRAMP6 is specific for Mn^{2+} transport. It is suggested that TcNRAMP2 may be involved in the remobilization of metal cations rather than their uptake (Ullah et al. 2018).

MbNRAMP1 in the fruit tree *Malus baccata* is involved in the transfer of Fe, Mn, and Cd (Xiao et al. 2008). Under Fe deficiency in tomato plants (*Solanum lycopersicum*), LeNRAMP1 is believed to play a role in the distribution of Fe in the vascular parenchyma (Bereczky et al. 2003). NRAMP1 and NRAMP3 in tomato have also been suggested to be involved in Mn transport (Bereczky et al. 2003). The *NRAMP* gene *AhNRAMP1* in peanuts plays a role in Fe nutrition (Xiong et al. 2012). In wheat, *TpNRAMP3* is a Mn, Co, and Cd transporter and is potentially responsible for the accumulation of Mn, Co, and Cd (Peng et al. 2018). *NRAMP* genes in soybean are widely involved in the uptake, homeostasis regulation, and transport of Fe, Mn, Cu, and Cd metal ions. In *Phaseolus vulgaris* (common bean), *PvNRAMP1*, *PvNRAMP2*, *PvNRAMP3*, *PvNRAMP4*, and *PvNRAMP5* might be required for general metal homeostasis during all the developmental stages. While *PvNRAMP6* and *PvNRAMP7* might play a role in symbiotic associations with beneficial microorganisms (Ishida et al. 2018).

In the CSS reference genome database, 11 NRAMP genes have been identified and characterized as CsNRAMPs. CsNRAMP3, CsNRAMP4, CsNRAMP5, and CsNRAMP8 participate in the absorption of metal ions. Both CsNRAMP2 and CsNRAMP5 fusion proteins located in the plasma membrane may function in the transmembrane transport of metal ions (Jinqiu Li et al. 2021).

Members of the NRAMP family of transporters located on separate organelles show various functions. In plants, the transport selectivity of NRAMP protein is independent of the group (group I or II) they belong to in the phylogenetic tree (Thomine and Vert 2013). Therefore, the biological function of the *NRAMP family of transporters* is diverse in different plant species and needs to be further studied.

The metal uptake functions of plant NRAMP homologs have been demonstrated in mutant yeasts deficient in metal uptake; however, their roles in metal homeostasis in plants have not been fully interpreted yet. The presence of a large number of *NRAMP* genes in plant genomes has made this study very complex as it may cause functional redundancy within the NRAMP gene family.

5.5 Expression Pattern and Regulation

NRAMP proteins in plants mostly localize on intracellular membranes like the vacuolar membrane and plastid envelope (Thomine and Vert 2013). Fig. 5.1 shows different regions of cell where NRAMP proteins are localised (Jogawat et al., 2021).

AtNRAMP1 and *AtNRAMP2*, *LeNRAMP1* and *LeNRAMP3*, and *OsNRAMP1* are few members of the NRAMP family which are preferentially expressed in roots, and *AtNRAMP3* and *AtNRAMP4* and *OsNRAMP2* and *OsNRAMP3* are expressed in the shoots. *OsNRAMP3* and *AtNRAMP1* and *LeNRAMP1* despite being in the same group (group I) are preferentially expressed in different parts of the plant. In shoot, *OsNRAMP3* expression is stronger, while in the root, *AtNRAMP1* and *LeNRAMP1*

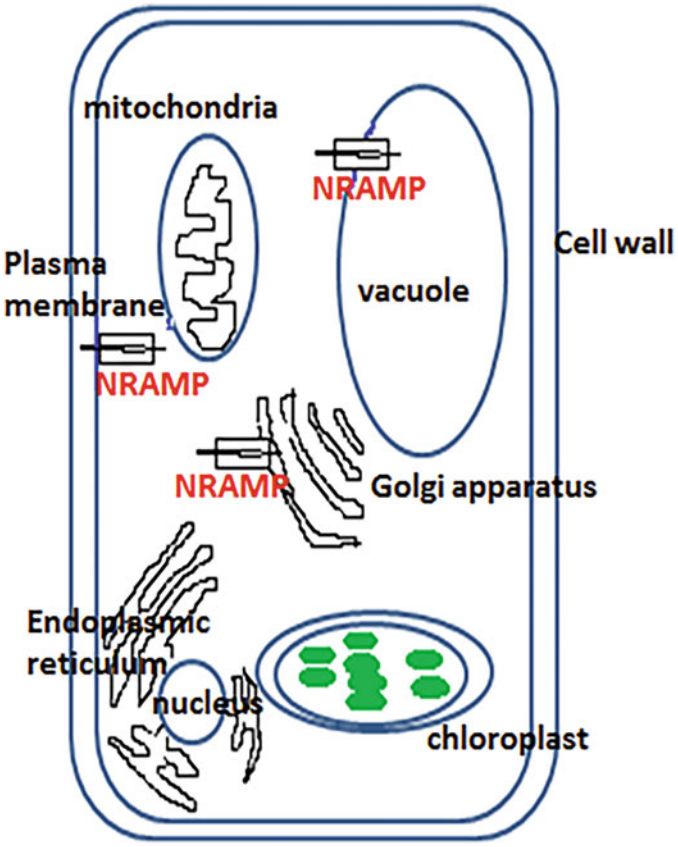

Fig. 5.1 NRAMP metal transporters expressed in different regions of the cell (plasma membrane, Golgi apparatus, vacuole) in different plant species

expressions are stronger (Curie et al. 2000; Bereczky et al. 2003). In *Arabidopsis*, the expression of AtNRAMP5 is restricted to the reproductive organs. In the vascular tissues of root and shoot, *AtNRAMP3* and *AtNRAMP4* are expressed (Thomine and Vert 2013). Thus the preferential expression of plant *NRAMP* genes in the roots or shoots does not depend on whether they belong to group I or group II of the phylogenetic tree.

During the vegetative state, *OsNRAMP1* expression is observed mainly in roots (Takahashi et al. 2011). The difference in *OsNRAMP1* expression levels in root affects Cd accumulation among different rice cultivars (Takahashi et al. 2011). During the reproductive stage, *OsNRAMP1* expression is higher in leaf blade and stem. OsNRAMP1 transporter is expressed on the plasma membrane of endodermis and pericycle cells, thus helping in the mobilization of metals from root to shoot. OsNRAMP3 is expressed in the plasma membrane and specifically in vascular bundles, particularly in phloem companion cells. In the rice node, *OsNRAMP3* is constitutively expressed (Yamaji et al. 2013). In rice plants, the expression of OsNRAMP3 in leaves slightly increases as the leaves age. To meet its minimal growth requirement during Mn deficiency, Mn from the enlarged vascular bundles to the younger tissues and panicles is transported by OsNRAMP3. However, OsNRAMP3 is internalized in vesicles and rapidly degraded when Mn is in excess. To protect the developing tissues from Mn toxicity, Mn is preferentially loaded into the older leaves and is directly connected to the enlarged vascular bundles. OsNRAMP3 undergoes post translational regulation in response to the nutrient availability in the environment (Yang et al. 2013; Mani and Sankaranarayanan 2018b).

In roots, OsNRAMP5 gene expression increases slightly when plants are under Fe or Zn deficiency but is not affected by variation in Mn level in the surrounding (Sasaki et al. 2012). It is expressed in the plasma membrane of the exodermal and endodermal layers at the mature root zone (Ishimaru et al. 2012; Sasaki et al. 2012). In rice hull, OsNRAMP5 is highly expressed. It is expressed in leaves also, but as leaves age, its expression decreases. In rice, OsNRAMP5 transporter is present in root and shoot vascular bundle, particularly the parenchyma cells around the xylem. *OsNRAMP5 is* highly expressed in stele cells especially in the xylem region and thus plays an important role in the xylem-mediated root-to-shoot transport. In rice plants, OsNRAMP5 plays an important role in the process of uptake, translocation, and distribution of Mn. *OsNRAMP5* plays an important role in the xylem-mediated root-to-shoot transport as it is highly expressed in stele cells particularly in the xylem region (Yang et al. 2014; Mani and Sankaranarayanan 2018a).

In *Arabidopsis* root, under conditions of Fe deficiency, expression of AtNramp1, AtNramp3, and AtNramp4 is upregulated (Curie et al. 2000; Thomine et al. 2000). AtNRAMP1 is located in the root plasma membrane (Meng et al. 2017). AtNRAMP3 is expressed in the vascular bundle of root, stem, and leaves (Thomine et al. 2003). It is upregulated and localized to the vacuolar membrane under conditions of Fe starvation (Thomine et al. 2003).

In tomato, *LeNramp1* is specifically expressed in the root epidermis and the cortex behind the root tip; it localizes to the root vascular parenchyma of the root hair zone and is upregulated by Fe deficiency (Bereczky et al. 2003); under conditions of Fe deficiency, *LeNramp1* is believed to mobilize Fe in the vascular tissue. In barley, when nitrogen (N) is adequate in the presence of Cd, Nramp transcript is downregulated, but under N-deficiency, it is strongly upregulated (Finkemeier et al. 2003). Some members of the NRAMP family are involved in Fe and Cd uptake and homeostasis, and other members may have different physiological functions.

In peanuts, the expression of *AhNRAMP1* is specifically higher in the roots and increased further under Fe deficiency (Xiong et al. 2012). Under Fe deficiency *LeNRAMP1*, *AtNRAMP1*, *MbNRAMP1*, and *OsNRAMP1* genes in tomato, *Arabidopsis*, *M. baccata*, and rice show higher expression in the roots (Takahashi et al. 2011). Thus these *NRAMP* genes are believed to have a conserved function in Fe homeostasis and to belong to a subclass of this family of proteins that are induced by Fe deficiency.

Expression of *TcNRAMP1* and *TcNRAMP5* in cacao root is high when compared to their expression levels in flower buds and beans. The *TcNRAMP6* gene is widely expressed in root and unopened flower buds. *TcNRAMP2* and *TcNRAMP3* are uniformly expressed across various organs and are constitutively expressed in the leaf and root tissues (Ullah et al. 2018).

In the tea plant (*Camellia sinensis*), 11 CsNRAMP genes' expression has been detected in different tissues (Jinqiu Li et al. 2021). In the root, CsNRAMP3, CsNRAMP4, and CsNRAMP5 are highly expressed, while in the leaf, CsNRAMP1, CsNRAMP2, CsNRAMP10, and CsNRAMP11 are highly expressed, and in the stem, CsNRAMP6 and CsNRAMP9 are highly expressed. CsNRAMP7 and CsNRAMP8 are highly expressed in both leaf and shoot tissues. CsNRAMP proteins are thought to play different roles in metal transport. Upon Pb treatment, expressions of CsNRAMP1, CsNRAMP2, CsNRAMP9, and CsNRAMP10 are upregulated in leaves. CsNRAMP3, CsNRAMP4, CsNRAMP5, CsNRAMP7, and CsNRAMP9 are thought to play a role in Pb transportation as their expression levels increased in the root when exposed to Pb. The expression of CsNRAMP3 increased greatly under Pb treatment (Jinqiu Li et al. 2021).

In *Arabidopsis*, AtNRAMP6 functions in young leaves and lateral roots. AtNRAMP3 and AtNRAMP4 are localized in the vacuolar membrane (Lanquar et al. 2005; Mary et al. 2015). AtNRAMP1 regulates Fe homeostasis (Curie et al. 2000) and functions as a high-affinity transporter for Mn uptake (Cailliatte et al. 2010). During seed germination, AtNRAMP3 and AtNRAMP4 participate in vacuolar Fe mobilization as both are localized on the vacuolar membrane (Lanquar et al. 2005). AtNRAMP6 functions as an intracellular metal transporter and is targeted to vesicular-shaped endomembrane compartments, and it is believed to be involved in Cd tolerance (Cailliatte et al. 2009).

A native Chinese plant species, *Sedum alfredii*, is a metal hyperaccumulator that can accumulate large amounts of Cd and Zn in the shoot without any significant

effect on its growth and metabolism (Zhang et al. 2020). SaNRAMP1 transports Cd, Mn, and Zn and is highly expressed in the young shoots (Zhang et al. 2020).

TpNRAMP3 is a member of the NRAMP family identified in Polish wheat (*Triticum polonicum L.*). *TpNRAMP3* is localized on chromosome 7BL. At the jointing and booting stages, the expression of *TpNRAMP3* is very high in leaf blades and root and the first nodes during the grain filling stage. It is a plasma membrane-localized protein. The expression of *TpNRAMP3* in seedling roots is upregulated by Fe and Cu and downregulated by Mg and Ni (Peng et al. 2018).

In soybean root, under high concentration of Cu and Cd, the expressions of *GmNRAMP1a*, *GmNRAMP5a*, and *GmNRAMP3a* are highly increased, and *GmNRAMP5a* expression is increased significantly by toxic levels of metals like Cd, Cu, and Mn (Qin et al. 2017). GmNRAMP7 is the only NRAMP protein in soya bean which is expressed mainly in roots along with other monocot NRAMPs (like OsNRAMP5, OsNRAMP1, and HvNRAMP5) (Sasaki et al. 2012; Wu et al. 2016). Members of the GmNRAMPs which belong to subfamily I are predicted to localize to vacuoles, while members belonging to subfamily II localize to the plasma membrane (Table 5.1).

5.6 Conclusion

For normal plant growth and development, transition metals are essential. These metal ions are absorbed from the soil via their roots, distributed throughout the plant, and their concentrations in the organelles in specific cells of various tissues are regulated. Metal transporters play an important role in the uptake and transport of these metals. In the last decade, our knowledge regarding these metal transporters has increased tremendously, and a number of transporter families have been identified.

Similar to yeast and animal NRAMPs, *NRAMP* genes in plants also encode transition metal transporters with a broad selectivity. We have summarized the general structural information, genomic and functional analysis, and the expression pattern of these NRAMP transporters in various plant species. However, the existing knowledge regarding the functioning of NRAMP transporters in plant remains finite. Much remains to be understood specifically on the cofactors and other physiological/environmental conditions in plant cells which determine the selectivity of the NRAMP transporters in vivo. It would be fascinating to identify the variations in the substrate for each NRAMP transporter and the structural features which result in the difference in substrate selectivity. A combination of several studies like analyses of the structure-function using heterologous expression systems, molecular genetic studies in plant, cell biological and biochemical studies regarding structure stability, and imaging studies for observing metal localization will give a clear picture of the function of NRAMP transporters and their connection with other transporters and chelators associated with metal homeostasis in plants. This understanding is pivotal in harnessing the tools of genetics to develop superior varieties of heavy metal-

Table 5.1 Summary of the NRAMP transporters mentioned in the chapter

SI no.	Plant	Name of NRAMP	No. of members predicted/ identified	Predicted/identified functional role
1	Rice	OsNRAMP	7(Nevo et al., 2006)	*OsNRAMP1*—Cd uptake (Takahashi et al., 2011) OsNRAMP3—Mn translocation (Yang et al., 2013, Mani and Sankaranarayanan, 2018a, b) OsNRAMP4—Al ion transporter (Xia et al., 2010) OsNRAMP5—Mn and Fe transport (Ishimaru et al., 2012) OsNRAMP6—uptake of Fe and Mn (Peris-Peris et al., 2017)
2	*Arabidopsis*	*AtNRAMP*	6 (Williams et al., 2000).	AtNRAMP1—Mn uptake. (Cailliatte et al., 2010). AtNRAMP2—remobilization of Mn^{2+} (Gao H. et al., 2018). AtNRAMP3 and AtNRAMP4—Fe mobilization (Thomine et al., 2003; Lanquar et al., 2005) AtNRAMP6—intracellular Fe homeostasis (Cailliatte et al., 2010)
3	Glycine max	*GmNRAMP*	13	*GmNRAMP7*—acquisition of Fe in the root (Qin, L et al., 2017)
4	*Camellia sinensis*	CsNRAMP	11	CsNRAMP3, CsNRAMP4, CsNRAMP5, and CsNRAMP8—absorption of metal ions CsNRAMP2 and CsNRAMP5—transmembrane transport of metal ions (Jinqiu et al., 2021)
5	*Phaseolus vulgaris*	PvNRAMP	7	*PvNRAMP1*, *PvNRAMP2*, *PvNRAMP3*, *PvNRAMP4*, and *PvNRAMP5*—general metal homeostasis during all developmental stages of the common bean *PvNRAMP6* and *PvNRAMP7*—symbiosis with beneficial microorganism (Ishida et al., 2018)
6	*Brassica napus*	BnNRAMP	22	BnNRAMP1—transport of Cd (Meng et a., 2017)
7	*Theobroma cacao*	TcNRAMP	5	TcNRAMP2—remobilization of metal ions TcNRAMP3—transport of Fe^{2+} and Mn^{2+} TcNRAMP5—transport of Fe^{2+}, Mn^{2+}, and Cd^{2+} TcNRAMP6—Mn^{2+} transporter (Ullah et al., 2018)

(continued)

Table 5.1 (continued)

SI no.	Plant	Name of NRAMP	No. of members predicted/ identified	Predicted/identified functional role
8	Wheat	*TpNRAMP*	NA	*TpNRAMP3*—accumulation of Mn, Co, and Cd (Peng et al., 2018)
9	Tomato	*LeNRAMP*	NA	LeNRAMP1—distribution of Fe LeNramp1 and LeNramp3—Mn transport (Bereczky et al., 2003)
10	Peanut	*AhNRAMP*	NA	*AhNRAMP1—Fe nutrition* (Xiong et al., 2012)
11	*Sedum alfredii*	SaNRAMP	NA	SaNRAMP1—which transports Cd, Mn, and Zn (Zhang et al., 2020)
12	*Hordeum vulgare*	HvNRAMP	NA	HvNramp5—uptake of Mn and Cd (Wu et al., 2016)
13	*Malus baccata*	MbNRAMP	NA	MbNRAMP1—which transfers Fe, Mn, and Cd (Xiao et al., 2008)

NA clear data not available

resistant crop plants by plant breeding techniques and also in heavy metal hyperaccumulation.

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