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## Abstract

Plants are the main dietary resource of micronutrients essential for humans. But staple food crops on which people of poor and developing countries feed, do not contain sufficient micronutrient metals and thus results in poor growth, mental disorders and increased mortality of human consumers. Knowledge on mechanism of micronutrient uptake by plant roots, their accumulation in subcellular compartments, long-distance transport in vascular tissues, allocation to economic sinks of crop plant, etc. is, thus, of utmost importance in the biofortification programmes, implemented for nutrient enrichment of plant foods. The present chapter gives an insight on various physiological aspects regulating micronutrient absorption in crop plants. Despite the role of edaphic factors controlling micronutrient availability in soil solution, biological activity of root organs is also determinant of micronutrient metal uptake from rhizosphere. The charged nature of essential micronutrients, which are mostly divalent cations, needs sophisticated transporters for their delivery to respective sinks. Further to achieve metal homeostasis and to reduce their toxicity, root to shoot and shoot to root signalling is in concordance with metal chelators and transporters, which have been discussed in detail in present chapter. Finally, future research avenues have been discussed which can be targeted to enhance the efficacy of crop biofortification.

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## 1 Introduction

Plants are one of the fascinating sessile natural creatures that make aerobic life possible on planet earth. They possess unique property of preparing organic food from simpler inorganic substances, i.e. autotrophic nutrition. The inorganic nutrients which plants use to maintain their proper growth and development are classified in two types:

1. **Macronutrients:** These mineral nutrients are present in higher concentrations (i.e. >100 mg/kg DW) in plant parts.
2. **Micronutrients:** These mineral nutrients are present in low concentrations (i.e. <100 mg/kg DW) in plant parts.

Out of the 14 essential nutrient elements required for maintaining vegetative and reproductive growth of plants, 8 mineral elements (i.e. boron, chlorine, manganese, iron, nickel, copper, zinc and molybdenum) are micronutrients. These micronutrients are involved in primary and secondary metabolism of plant as enzyme cofactors, osmolytes, redox carriers in organic metabolites, signal transducing messengers, structural components of cell walls/membranes, regulators of hormone biosynthesis in addition to role in energy production and defence. The specific role of each of the micronutrients is enlisted in Table 20.1. In addition to essential elements, several other elements (such as selenium (Se), iodine (I), silicon, etc.) have been reported in specific plant taxa at low concentrations (i.e. <100 mg/kg DW). But such elements are not critical to all plants (Kaur et al. 2016). So, these are considered in category of beneficial elements instead of concentration similar to micronutrients. The present chapter will also briefly discuss two of such beneficial elements Se and I, which are well known in biofortification programmes for their crucial importance to humans.

### 1.1 Essentiality of Micronutrients for Plants and Animals

Plants are dietary resource for all 25 essential nutrients required for human growth. But low micronutrient density in edible crops is a serious global concern from the last two decades. Consumption of such low micronutrient food products leads to deficiency disorders in large fraction of humans consuming them. About two third of the world population suffers from 'micronutrient malnutrition' especially iron (Fe) and zinc (Zn) (White and Broadley 2009; Stein 2010). Despite of Fe/Zn, their deficiency leads to impaired physical activity, stunted growth, mental retardness and pregnancy issues (stillbirths and child deaths) (Stein et al. 2005). Fe deficiency (FeD)

**Table 20.1** Role of various essential micronutrients in plants

Micronutrient (symbol)	Ionic form in Rhizosphere/soil	Ionic form for root uptake	Cofactor in enzymes	Part of metabolite/metabolic process	References
Boron (B)	Boric acid or borate $B(OH)_3$	$B(OH)_3$		Cross linked with pectins, polyhydroxyl polymers—cell wall synthesis and stability Plasma membrane integrity Cell division, cytoskeletal polymerization Phenol metabolism Ammonium and nitrogen assimilation	Ahmad et al. (2009) Shireen et al. (2018)
Chlorine (Cl)	$Cl^-$	$Cl^-$	Asparagine synthetase Tonoplast V-type $H^+$ -ATPase	Facilitation of proton flux in PSII Osmoregulation Stabilization of membrane potential and pH gradients	Rognes (1980) Churchill and Sze (1984) Zhang et al. (2014b) White and Broadley (2001) Raven (2017)
Manganese (Mn)	$Mn^{2+}$ , $Mn_2O_3$ , $MnO_2$	$Mn^{2+}$	Arginase Mn SOD Oxalate oxidase Phenylalanine ammonia lyase Phosphoenolpyruvate carboxylase (Mn/Mg)	Oxygen evolving complex of PSII in Photosynthesis Purine and urea catabolism ROS detoxification Pathogen defence Lignin biosynthesis—oxidation of monolignols by $Mn^{3+}$ Tricarboxylic acid cycle-respiration	Alejandro et al. (2020), Bricker et al. (2012) Cao et al. (2010) Ravet and Pilon (2013) Requena and Bornemann (1999) Onnerud et al. (2002), Engelsma (1972) Gregory et al. (2009)

(continued)

Table 20.1 (continued)

Micronutrient (symbol)	Ionic form in Rhizosphere/soil	Ionic form for root uptake	Cofactor in enzymes	Part of metabolite/metabolic process	References
Iron (Fe)	Fe <sup>2+</sup> , Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe-SOD, catalase Nitrate reductase Nitrite reductase Glutamate synthetase APS reductase Sulphite reductase ACC oxidase DNA polymerase, DNA helicase and primase	ROS scavenging Nitrogen assimilation  Sulphate assimilation  Ethylene biosynthesis DNA replication and repair  Cytochrome- photosynthesis and respiration Fe-S cluster in Psa A, Psa B, Psa C of PSI-photosynthesis Ferredoxin in redox homeostasis	Ravet and Pilon (2013) Balk and Lobreaux (2005)  Balk and Lobreaux (2005)  Pech et al. (2003) Zhang (2014)  Ravet and Pilon (2013)
Nickel (Ni)	Ni <sup>2+</sup>	Ni <sup>2+</sup>	SOD Glyoxalases (family I) Ureases Methyl-CoM reductase Peptide deformylases Some hydrogenases	Homeostasis of glutathione ROS scavenging Detoxification of reactive carbonyl species Nitrogen assimilation Biological methanogenesis Protein processing in chloroplasts Biological N fixation	Noctor et al. (2012) Mustafiz et al. (2014) Sousa Silva et al. (2013) Sirko and Brodzik (2000) Su et al. (2019) Hanson et al. (2000) Brito et al. (1994)

Copper (Cu)	Cu <sup>+</sup> , Cu <sup>2+</sup>	Cu <sup>+</sup>	Cu Zn-SOD Cytochrome c oxidase Laccase Amine oxidase Polyphenol oxidases	ROS scavenging Cytochrome complex-ETC of respiration lignin production Oxidation of polyamines Quinone biosynthesis from diphenols (plant defence) Plastocyanin in ETC of photosynthesis Plantacyanins	Ravet and Pilon (2013) Berthet et al. (2012) Puig (2014) Sullivan (2015) Ravet and Pilon (2013) Feng et al. (2013)
Zinc (Zn)	Zn <sup>2+</sup>	Zn <sup>2+</sup>	Zn <sup>2+</sup> —cofactor for >300 enzymes Cu/Zn—SOD Carbonic anhydrase Alkaline phosphatase Alcohol dehydrogenase	ROS scavenging Hydration of CO <sub>2</sub> to release bicarbonate Hydrolysis of phosphate esters Anaerobic respiration/alcoholic fermentation Tryptophan biosynthesis (auxin precursor) Zn, finger proteins; regulation of gene expression Metabolism of carbohydrates, lipids and nucleic acids	Broadley et al. (2007), Gupta et al. (2016) Englbrecht et al. (2004) Palmer and Gueriot (2009)
Molybdenum (Mo)	MoO <sub>4</sub> <sup>2-</sup>	MoO <sub>4</sub> <sup>2-</sup>	Sulphite oxidase Aldehyde oxidase Xanthine oxidoreductase Nitrate reductase Amidoxime-reducing component (ARC)	Sulphite detoxification in to sulphate ABA biosynthesis Purine metabolism Nitrate assimilation Reduction of nitrite to NO	Brychkova et al. (2015) Verma et al. (2016) Maia and Moura (2011) Chamizo-Ampudia et al. (2017) Yang et al. 2015, Chamizo-Ampudia et al. (2016)

ACC 1-aminocyclopropane-1-carboxylic acid; ETC electron transport chain; *PSI* and *PSII* photosystem I and II; *ROS* reactive oxygen species; *SOD* superoxide dismutase

mainly results in anaemia while Zn deficiency (ZnD) often leads to diarrhoea and pneumonia in infants and adults. Zn deficiency also leads to hypogonadism, immune dysfunction, DNA damage and cancer development (Gibson 2006; Prasad 2009). Thus, 'hidden hunger' caused due to micronutrient deficiency is one of the serious global issues. This problem is aggravated in developing countries where cereal-based products are main staple foods that contain antinutritional substances (such as phytate, tannic acid) and possessed low micronutrient density and bioavailability. More than two billion of world population suffers from micronutrient deficiency with approximately 0.8 million deaths are reported annually. In addition to FeD and ZnD, deficiency of Se (SeD) and I also associated with severe health consequences; with about 15 and 30% of world population lacks these nutrients. Iodine deficiency (ID) impairs thyroxin production causing goitre, irreversible mental retardation (autism), reproductive dysfunction and cretinism under severe deficiency (de Benoist et al. 2008). SeD leads to dermatitis, hair loss and garlicky breath, male infertility and increased incidence of cancers, respiratory failure, myocardial infarction and renal failure under severe selenosis (Fordyce 2013). These dietary mineral intakes are of extreme importance to pregnant women, where deficiency of any above micronutrients (Fe, Zn, I and Se) can lead to irreversible brain damage, permanent foetal developmental disorders and cognitive decline in developing foetus (Fordyce 2013; World Health Organization 2007).

## 1.2 Micronutrients Involved in Biofortification Programmes for Crop Improvement

Almost one in three people worldwide suffers from micronutrient malnutrition (FAO, IFAD, WFP 2015). Thus, to sustain good health and development, of individuals having limited access to diverse diets, various interventions such as supplementation, industrial food fortification, etc. have been put forward. But among them, the most promising and cost-effective approach is biofortification. This biological process of nutrient enrichment utilizes tools of conventional plant breeding, agronomic management methods and techniques of transgenics to enhance micronutrient density of staple food crops. Due to crucial importance of Fe, Zn, Se and I for human growth and their widespread deficiency, these four nutrients are of main focus in biofortification. The international programme of Harvest Plus addresses a substantial number of researches on biofortification to develop biofortified iron crops, zinc crops, iodine crops, vitamin A crops, etc. in cereals (wheat, rice, maize, barley, pearl millet), pulses (soybeans, common bean, lupines) vegetables (cassava, orange sweet potato, carrot, cauliflower, potato, tomato) and fruits (papaya, banana, etc.) (Bouis and Saltzman 2017). The two main approaches used in biofortification are genetic biofortification and agronomic biofortification.

The genetic biofortification (GB) enhances plant's own inherent potential (genetic potential) of nutrient acquisition from soil. The rich gene pool of wild germplasm provides candidate genomic segments for introgression in cultivated varieties in genetic biofortification to enhance root uptake of micronutrients, their

remobilization to edible sinks and even to maintain metal homeostasis. But if soil is itself nutrient poor, in that case soil and foliar application of nutrient fertilizers is used for nutrient enrichment of crops grown as in case of agronomic biofortification. This approach is also a shotgun approach in those crops where loss/lack of natural wild diversity occurs.

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## **2 Soil as Reservoir of Micronutrients**

It is well known that soil is the main reservoir of mineral elements, for crop plants. The widespread deficiencies of micronutrients in cultivated soils limit not only micronutrients concentration in crop produced but also affected crop yields. Various edaphic factors contribute significantly towards nutrient concentrations in rhizosphere where plant roots communicate with soil environment to favour mineral absorption even under nutrient-deficient conditions (Fig. 20.1). Some of them are given below.

### **2.1 Factors Affecting Availability of Micronutrients in Soil Solution**

#### **2.1.1 Soil Formation**

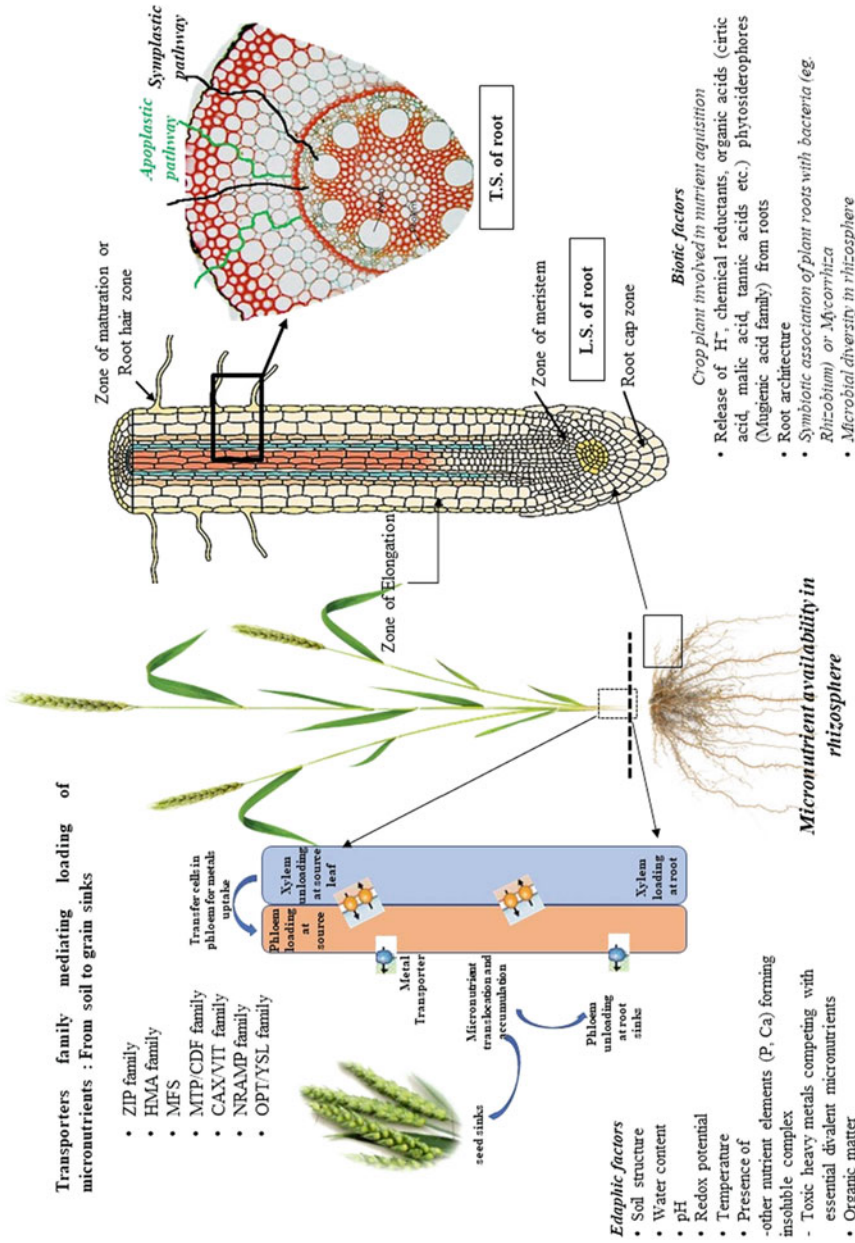
As soil is formed from weathering of rocks, the rocks (e.g. igneous rocks) rich in micronutrients will lead to formation of soils with high concentration of micronutrients. Further, the process of soil formation from parent rock material is another factor which influences micronutrient contents of soil. The non-uniform distribution of micronutrients in different geographical zones is due to differences in parent rock materials and processes that lead to formation of soil. The soil formed after weathering has higher concentration of micronutrients than parent rock due to low mobility of these divalent cations.

#### **2.2 Soil Moisture and Texture**

Soil moisture and texture affects micronutrient concentration in soil solution through diffusion/mass flow. Coarse-textured sandy soils exhibit enhanced leaching of available micronutrients more than dry loam soils.

#### **2.3 Soil pH**

Another determining factor which affects nutrient solubility in soil solution is soil pH. High pH in alkaline/saline soil favours formation of less available micronutrient (particularly Zn, Mn, Fe, etc.) organic complexes and results in their deficiency. In contrast, acidic pH results in decreased adsorption of cationic nutrients to cation



**Fig. 20.1** An overview of micronutrient absorption in plants



exchange sites of soil constituents (such as clay mineral, metal oxides) and makes them available in soil solution. A unit increase in soil pH is found to decline Zn availability by 30- to 45-fold at a pH range of 5.5–7.5. Tight adsorption of  $\text{Fe}^{3+}$  or  $\text{Zn}^{2+}$  as metal oxides, phosphates or carbonates also makes these micronutrients unavailable in rhizosphere for uptake by roots.

## 2.4 Soil Organic Matter

Organic matter is the source of numerous soluble organic compounds which regulate microbial activity in rhizosphere. The wide diversity of microorganisms in soil is responsible for release of various organic acids, chelator compounds, etc. that favours free release of micronutrient cations in soil solution.

## 2.5 Others

In addition to above, redox potential of soil, the presence of similar charged toxic divalent cations (such as  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{2+}$ ,  $\text{Hg}^{2+}$ ), microbiota, etc. also alter mineralization and hence phytoavailability of micronutrients in rhizosphere.

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## 3 Absorption of Micronutrients by Plant as Biological System: Uptake, Transport and Sequestration

In order to enrich food crops with tools of biofortification, there is a need of deep understanding of the micronutrient acquisition, their xylem and phloem transport, sequestration and translocation to edible plant sinks. As most of the essential micronutrients belong to category of heavy metals, there should be a tight regulation in their uptake and metabolism by plant system; otherwise, these will lead to oxidative stress and will be toxic to cellular machinery. Plants maintain metal homeostasis from cellular level to whole plant level in hierarchy through specialized transporters, chelating ligands and sequestration of toxic metal ions in intercellular compartments. These will be discussed in detail below:

### 3.1 Roots-Organs Mediating Nutrient Uptake from Rhizosphere

Plant roots are specialized organs which mediate nutrient absorption from soil solution. The unicellular hairs present on root epidermis perform dual function in nutrient acquisition i.e. (1) enhancing phytoavailability of micronutrients in rhizosphere and (2) subsequent transport of micronutrients through transporters/channel proteins to inner root cells for xylem loading. Roots are not just static organs in rhizosphere; their continuous activity such as release of exudates and mucilage in soil prevents damage to growing apical meristem and allow tight binding of lateral

roots to soil substratum. The activity of root hair cell plasma membrane (RCPM)  $H^+$ -ATPase mediates extracellular acidification in rhizosphere which helps in active uptake of charged micronutrients by declining soil pH. The release of organic acids (such as citric acid, tannic acid, oxalic acid, tartaric acid) in mucilage and exudates of damaged root cap cells also increase solubility of micronutrients by maintaining low soil pH.

The soluble metal cations enter in cytosolic compartment of root hair cell either through transporters/channel proteins. This metal ion uptake can be passive (i.e. along the concentration gradient from higher metal ion concentration in soil solution towards low concentration present in root hair cell) or active (against the concentration gradient) depending upon metabolic essentiality of that metal ion. It is very important to mention here that a wide diversity in uptake mechanism exists among plants for the different metal cations and even for same metal atom with different available forms. For example, Fe existed in  $Fe^{2+}$  form with predominance of  $Fe^{3+}$  in rhizosphere. But plant iron transporters allow uptake of  $Fe^{2+}$  ion. Thus, two specialized mechanisms existed in higher plants to mediate Fe uptake:

1. Strategy I: Reduction of  $Fe^{3+}$  in to  $Fe^{2+}$  by ferric-chelate reductase (Robinson et al. 1999) encoded by FRO gene family. Such reduction mechanism is found in non-graminaceous monocots and dicot plants. These  $Fe^{2+}$  ions are then entered in root cell through iron transporters.
2. Strategy II: Release of phytosiderophores (metal chelators) such as mugineic acids, avenic acid, distichonic acid, etc. to bind with  $Fe^{3+}$  followed by uptake of Fe (III)-ligand chelating complex by specific yellow stripe 1 (YS1) or YS1-like (YSL) transporters. A wide range of phytosiderophores are secreted from cereal family (graminaceous monocots) which play crucial role in metal uptake under Fe/Zn deficiency. Further, amounts of these phytosiderophores released in rhizosphere, determine the tolerance of particular plant species to soils with limited Fe or Zn phytoavailability. For example, roots of barley and wheat secrete large amounts of mugineic acid compounds (MAs) than rice and, thus, confer enhanced tolerance to Fe-limiting soils. Moreover, barley root secretes a range of MA species including mugineic acid (MA), 3-epihydroxymugineic acid, 3-epihydroxy-2'-deoxymugineic acid and 2'-deoxymugineic acid (DMA) than DMA alone by bread wheat, thus, increased tolerance potential of barley to Fe-limited environments (Romheld and Marschner 1990).
3. Combination of both strategy I and strategy II as occur in rice.

### 3.2 Root Uptake of Mineral Nutrients Zn, Se and I

Zn is mainly absorbed in  $Zn^{2+}$  form. Absorption of this divalent cation by root hair cell interior would cause depolarization of plasma membrane and will reduce its further transport. Thus, to maintain  $Zn^{2+}$  influx, RCPM  $H^+$ -ATPase cause efflux of  $H^+$ , resulting in hyperpolarization of RCPM which acts as driving force for Zn uptake by plant roots.  $Zn^{2+}$  ions are mainly transported through specific ZIP family

transporters. However, Zn-chelating complexes formed due to binding of metal chelators (such as MAs, nicotianamine, histidine, etc.) with Zn<sup>2+</sup> ions, are transported through specific YSL transporters in cytoplasmic interior of root cell. In contrast to Zn<sup>2+</sup> and Fe<sup>2+</sup>, Se and I are absorbed as anions. Natural Se exists in various forms as selenate (SeO<sub>4</sub><sup>2-</sup>), selenite (SeO<sub>3</sub><sup>2-</sup>), selenide (Se<sup>2-</sup>), element Se (Se<sup>0</sup>) and organoselenium compounds (selenocysteine (Se Cys) and selenomethionine (Se Met)) in rhizosphere. But plant roots are able to take up only selenate, selenite, SeCys and Se Met from soil solution. Out of all phytoavailable forms, SeO<sub>4</sub><sup>2-</sup> is absorbed readily from soil solution than SeO<sub>3</sub><sup>2-</sup>. Predominance of Se form in soil solution depends upon concentration, pH of soil, redox potential, organic matter, presence of other nutrients particularly sulphur, iron oxides, etc. (Sors et al. 2005). Generally, SeO<sub>4</sub><sup>2-</sup> is mainly present in alkaline soils, while well-drained acidic to neutral soils contain SeO<sub>3</sub><sup>2-</sup>. Due to the presence of negative charge on root cell membrane, SeO<sub>4</sub><sup>2-</sup> and organoselenium compounds are absorbed actively through H<sup>+</sup>/anion symporter, anion channels and amino acid transporters present on RCPM. The sulphate transporters (both high- and low-affinity transporter) present on RCPM mediate SeO<sub>4</sub><sup>2-</sup> uptake in addition to sulphate. This active uptake involves cotransport of three protons for each SeO<sub>4</sub><sup>2-</sup> ion. However, organoselenium compounds are transported through amino acid transporters similar to cysteine and methionine. Selenite is found to be transported passively through phosphate transporters (Li et al. 2008).

The beneficial nutrient iodine is taken up both in inorganic forms as iodide (I<sup>-</sup>) and iodate (IO<sub>3</sub><sup>-</sup>) and organic iodine by plant roots. Till date no iodine transporters have been discovered in plants. But it has been reported that roots cells have iodate reductase/specific nitrate reductases which convert IO<sub>3</sub><sup>-</sup> in to I<sup>-</sup> anion, due to high solubility of iodide than iodate. I<sup>-</sup> anions are effectively absorbed by plant roots (Whitehead 1973) but are susceptible to leaching. These I<sup>-</sup> anions are taken up through chloride channels and further loaded in to xylem through specific anion channels (Blasco et al. 2008; Caffagni et al. 2011; Roberts 2006). Thus, energized transport of micronutrients along with Se and I is under tight metabolic control of transporter proteins. Plants also exhibits an inherent potential to cope the micronutrient deficiency through various mechanisms such as (a) oriented root growth and enhanced lateral branching (b) increased root exudation (c) overexpression of specific high-/low-affinity root membrane transporters (d) release of specific micronutrients from subcellular stores (e) remobilization of micronutrient from senescing tissue, etc. to fulfil the demands for plant growth and metabolism.

### 3.3 Apoplastic and Symplastic Routes in Radial Transport of Nutrients

Micronutrients once acquired by root hair cells can traverse through symplastic (intracellular) or apoplastic (extracellular) pathway for their loading in to xylem (Fig. 20.1). However, suberin deposits on endodermis (i.e. Casparian strips) is the

major barrier to apoplastic transport. This water impermeable layer allows only symplastic transport of charged nutrients from cortex to endodermis. But after this check barrier, nutrient transport can again be apoplastic/symplastic to xylem. The radial transport of nutrients towards root stele increases nutrient concentration in subsequent inner tissue layers due to decreased radii. In this specialized transport, micronutrient sequestration also takes place in different subcellular compartments. Due to charged nature of micronutrients (especially Fe, Zn, Mn, Ni, etc.), these divalent metal cations bind to low molecular weight chelators for facilitating their symplastic transport and intracellular sequestration. A large number of molecules such as organic acids (e.g. citrate, malate), histidine, phytate, mugineic acid, nicotianamine, etc. have been implicated as important cellular ligands for cytosolic and vacuolar sequestration of Zn, Fe, Cd, Ni, etc. (Haydon and Cobbett 2007a; Ma et al. 2005). Such metal ligands not only help in maintenance of cellular homeostasis but also contribute to metal translocation towards storage sinks. Once loaded in xylem, these micronutrients reach to aerial shoots under the influence of transpiration pull and mass flow. The whole process of nutrient uptake and subsequent transport to shoot is dependent upon developmental stage and environmental factors which regulate expression of genetic components mediating nutrient absorption.

After reaching to aerial leaves, xylem unloading and phloem loading of nutrient initiates which is crucial for root to shoot and shoot to root mineral cycling. In phloem tissue, transfer cells play a crucial role in energized loading of micronutrients and thus channelizes them to developing sinks for accumulation. Further, for metal accumulation in aerial tissues, a wide variation of cellular ligands exists in different species for same metal cation. For example, *T. caerulea* utilizes citric acid, while *A. halleri* utilizes malic acid for Zn storage in vacuole (Kupper et al. 2004; Ma et al. 2005). Zn–His complex is generally formed to chelate free Zn<sup>2+</sup> ions in cytosol (Kupper et al. 2004).

In case of beneficial elements such as iodine, xylem transport predominates over phloem transport (Weng et al. 2008) resulting in its less accumulation in sink tissues with undeveloped xylem. Absorption of Se is similar to sulphate, where enzymes of sulphur assimilation pathway convert SeO<sub>4</sub><sup>2-</sup> in to Se Cys in chloroplast which is then further converted in Se Met in cell cytosol. However, in Se hyperaccumulators, selenocysteine is converted into non-protein amino acids like Se-methyl selenocysteine,  $\gamma$ -glutamyl-Se-methylselenocysteine and selenocystathionine for Se detoxification (White 2018).

### 3.4 Transporters and Channel Proteins Involved in Micronutrient Transport

The selective uptake of micronutrients from rhizosphere, their subsequent loading in xylem, storage in intracellular compartments and further translocation to seed/grain sinks in a plant system is only possible due to the presence of specialized membrane transporters which facilitate movement of transition metal ion/micronutrients both intracellularly and intercellularly. Such transporter proteins can be either substrate

specific or may bind to different metal cations with differential affinity. But one thing is sure that the presence of membrane transporters allows selective inflow-outflow of the charged micronutrients. A large number of transporter proteins families such as ZIP family, HMA family, MFS family, MTP family, YSL family, VIT family, CAX family, etc. have been discovered which are involved in regulation of micronutrients (Fe, Zn, Mn, Co, etc.) transport, their accumulation and detoxification under excess, to maintain metal homeostasis (Table 20.2). A few of them are discussed in detail here.

### 3.5 ZR- and IRT-Like Proteins (ZIP) Family

This transporter family gets its name from the first members identified, i.e. zinc-regulated transporter (ZRT) in yeast and iron-regulated transporter (IRT) like proteins identified in *A. thaliana* which mediate influx of  $Zn^{2+}$  and  $Fe^{2+}$  ions in to the cytoplasm. Apart from these micronutrients, some of ZIP transporters have also been shown to transport  $Mn^{2+}$ ,  $Cu^{2+}$  and  $Cd^{2+}$  ions. Most of the members of this family are present either on cell plasma membrane for micronutrient uptake or on integral membranes of intracellular compartments such tonoplast, endoplasmic reticulum, etc. for nutrient translocation to various plant organs (Ajeesh Krishna et al. 2020). Topological studies reveal that ZIP proteins have characteristic eight transmembrane domains (TMD) with their N- and C-termini towards extracellular space. These transporter proteins vary in length from 309 to 476 amino acids. This difference in length of ZIP proteins is due to varied length of potential metal binding domain between TMD III and IV (Guerinot 2000). The conserved sequence present in TMD IV of ZIP members contains histidine residues which play a crucial role in metal transport.

Various orthologs of ZIP proteins have been identified in many crop species such as 12 in barley (Tiong et al. 2014), 14 in wheat (Evens et al. 2017), 17 in rice (Chen et al. 2008) and 23 in common bean (Astudillo et al. 2013). These orthologs differ in their cellular localization, tissue-specific expression, substrate specificity and catalytic potential of metal transport. Among the 15 ZIP family members of *Arabidopsis* (Milner et al. 2013), AtIRT1 is the most well studied for Fe uptake and transport. Recently, Milner et al. (2013) characterized functional ability of 11 At ZIPs in metal transport and revealed involvement of AtZIP1 and AtZIP2 in root to shoot translocation of Zn and Mn. Out of 11 transporters, 6 (ZIP1, ZIP2, ZIP3, ZIP7, ZIP11 and ZIP12) members complement Zn transport, six (ZIP1, ZIP2, ZIP5–7 and ZIP9) mediate Mn, and one (ZIP7) complements Fe transport in yeast mutants. Further, the role of ZAP1 transcriptional factors in transcriptional and post-transcriptional regulation of ZRT1 and ZRT2 has been demonstrated in yeast (Zhao et al. 1998). These transcription factors bind to a zinc-responsive element (ZRE) present in promoter region of ZRT genes to control their expression.

The presence of such zinc deficiency response elements (ZDREs) has also been reported in plant ZIP family such as AtZIP1, AtZIP3, AtZIP4, AtZIP5, AtZIP9 and AtZIP10 (Assuncao et al. 2010) where binding of bZIP (basic leucine zipper

**Table 20.2** Channels and transporter proteins involved in uptake and transport of micronutrients/beneficial elements

Micronutrient	Ionic form for root uptake	Concentration in plants ( $\mu\text{g g}^{-1}$ DW)	Channels and transporter proteins mediating long-distance transport	References
B	$\text{B}(\text{OH})_3$	20–100	Major intrinsic proteins: AtNIP 5.1, AtNIP 6.1, OsNIP3.1 High-affinity active transport: BOR1, BOR2	Takano et al. (2006), Tanaka et al. (2008), Hanaoka et al. (2014) Nakagawa et al. (2007), Takada et al. (2014)
Cl	$\text{Cl}^-$	1–20	CLCs family: At CLCa, AtCLCc, At CLCg CCC family: AtCCC, OsCCC1 CPA2 family: GmSALT3/CHX1 ALMT family: AtALMT9, AtALMT12 SLAC/SLAH family: AtSLAC1, AtSLAH1 NRT/NPF family: AtNPF2.4 and AtNRT1.5	Jossier et al. (2010), Nguyen et al. (2016) Colmenero-Flores et al. (2007), Kong et al. (2011) Liu et al. (2016) De Angeli et al. (2013), Meyer et al. (2010) Negi et al. (2008) Li et al. (2017), Lin et al. (2008)
Mn	$\text{Mn}^{2+}$	30–500	CaCA family: At CAX2, At CAX4, AtCAX5 NRAMP family: At NRAMP1–4, Ah NRAMP1 YSL family: Os YSL2, Os YSL6 ZIP family: At ZIP1–2, At ZIP 5–9 CDF/MTP family: At MTP8–11	Edmond et al. (2009), Mei et al. (2009) Thomine et al. (2000), Lanquar et al. (2010) Yang et al. (2014), Sasaki et al. (2011) Milner et al. (2013) Delhaize et al. (2007)
Fe	$\text{Fe}^{2+}$	50–100	NRAMP family: AtNRAMP3 and AtNRAMP4 OPT family: AtYSL3, OsYSL2, OsYSL15 ZIP family: At IRT1–3 MATE family: FRD3, FRO2 VIT family: At VIT1 MFS family: IREG1–3	Lanquar et al. (2005) Waters et al. (2006), Ishimaru et al. (2010) Korshunova et al. (1999) Rogers and Gueriot (2002), Connolly et al. (2003) Kim et al. (2006) Nino Gonzalez et al. (2019)
Ni	$\text{Ni}^{2+}$	0.05–10	NRAMP family: Ah NRAMP1	Wang et al. (2019)
Zn	$\text{Zn}^{2+}$	2.5–150	YSL/OPT family: TcOPT3, Zm YS1, At YSL2 ZIP family: At IRT1–3, At ZIP1–6, At ZIP9–10	Hu et al. (2012), DiDonato Jr et al. (2004), Korshunova et al. (1999), Pedas and Husted (2009)
Cu	$\text{Cu}^+$ , $\text{Cu}^{2+}$	5–20	COPT family: At COPT1–6 HMA family: AtHMA5–8, Os HMA5 ZIP family: ZIP2 and ZIP4	Puig (2014) Blaby-Haas et al. (2014), Deng et al. (2013) Wu et al. (2015)

(continued)

Table 20.2 (continued)

Micronutrient	Ionic form for root uptake	Concentration in plants ( $\mu\text{g g}^{-1}$ DW)	Channels and transporter proteins mediating long-distance transport	References
Mo	$\text{MoO}_4^{2-}$	0.1–1	$\text{H}^+/\text{Cu}^{2+}$ antiporters YSL family: At YSL1–3, Os YSL16 MFS transporter: TaCTI MOT1 family: AtMOT1, AtMOT1.2 MOT2 family: CrMOT2 Sulphate transporters: SHST1	Parrotta et al. (2015) Chen et al. (2011), Lee et al. (2012) Li et al. (2014) Gasber et al. (2011) Tejada-Jimenez et al. (2011) Fitzpatrick et al. (2008)
Beneficial elements				
Se	$\text{SeO}_4^{2-}$ , selenite ( $\text{SeO}_3^{2-}$ , $\text{HSeO}_3^-$ ) and organoselenium compounds (Se Cys and Se Met)		SULTR transporter: At SULTR1;1, At SULTR1;2 Phosphate transporter: OsPT2, Os PT8	Shibagaki et al. (2002), Rouached et al. (2008) Zhang et al. (2014a), Song et al. (2017)
I	$\text{IO}_3^-$ , $\text{I}^-$		CLC family Voltage gated R and S-type anion channels—iodine transporter not yet discovered	Gonzali et al. (2017), Roberts (2006)

*ALMT* aluminium-activated malate transporters; *CaCA* calcium/chloride cotransporters; *CDC* cation diffusion facilitator; *CHX* cation/H<sup>+</sup> exchanger; *CLC* chloride channels; *COPT* copper transporters; *CPA2* cation proton antiporter 2; *FRD* ferric chelate reductase defective; *FRO* ferric reductase; *HMA* heavy metal ATPases; *MOT* molybdate transporters; *MATE* multidrug and toxic compound extrusion; *MFS* major facilitator superfamily; *MTP* metal tolerance protein; *NRAMP* natural resistance-associated macrophages protein; *NRT/PTF* nitrate transporter/nitrate peptide transporter; *OPT* oligopeptide transporter; *SLAC/SLAH* slow-type anion channel/associated homolog; *SULTR* sulphate transporter; *VIT* vacuolar iron transporter; *ZIP* yellow stripe 1-like; *ZIP* ZRT- and IRT-like proteins

domain) transcription factors, i.e. bZIP19 and bZIP23, enhances the expression of ZIP members under Zn deficiency. But how these bZIP transcription factors sense low cellular Zn<sup>2+</sup> concentration is still to be elucidated. Regarding their functioning, Assuncao et al. (2013) suggested that Zn<sup>2+</sup> ion binds to cysteine-histidine-rich motif of bZIP transcription factor dimers under normal cellular Zn conditions and causes its inactivation. But under low Zn, active bZIP dimer binds to ZDRE motif of ZIPs promoter and results in their increased transcription for enhancing Zn uptake. In addition to Zn, many ZIP members also transport other divalent metal cations (such as Cd<sup>2+</sup>, Cu<sup>2+</sup>, etc.) which are toxic to plants (Tan et al. 2020). Recently, overexpression of OsZIP1 in transgenic rice was found to reduce Zn, Cu and Cd accumulation under excess metal (Liu et al. 2019). Thus tight regulation of ZIP members is essential to maintain metal homeostasis.

### 3.6 Heavy Metal ATPases (HMA) Family

The members of HMA family mediate heavy metal transport across the biological membranes by utilizing ATP as energy source. This family is also known as P<sub>1B</sub>-ATPase family. Depending upon their metal specificity, HMA family is divided in two subgroups: (1) a Cu/silver (Ag) group and (2) a Zn/Co/Cd/lead (Pb) group (Takahashi et al. 2012). HMAs are basically efflux transporters that are ubiquitously present in archaea, prokaryotes and eukaryotes including plants. These transporters play a key role in transition metal detoxification. Plant HMA proteins have basic structure of eight transmembrane helices (TM) with their N- and C-terminal ends towards cytosol. There is the presence of two cytoplasmic loops, i.e. one small between TM 4 and TM5 and other large between TM 6 and TM7. Each member of HMA protein possesses three functionally important domains which are conserved across all P-type ATPases, i.e. cytoplasmic actuator (A) domain located in smaller loop, phosphorylation (P) domain and nucleotide (N) domain present in large cytoplasmic loop responsible for ATP binding. In addition, a specific CPx motif present in TM 6 of all P<sub>1B</sub>-ATPase, is involved in metal translocation. Some putative metal-binding domains (MBD) are present in the N- or C-terminal regions of HMA proteins (Williams and Mills 2005; Arguello et al. 2007). The MBD of N-terminus contains a highly conserved CxxC residue in HMA domain which controls turnover rate of P<sub>1B</sub>-ATPase. The presence of histidine- and cysteine-rich region occurs in MBD of C-terminus which plays a role in metal selectivity of this transporter protein (Lutsenko et al. 2003; Mandal and Arguello 2003).

The members of HMA family are highly diverse in terms of their tissue distribution, subcellular localization, metal specificity and regulation. For instance, *Arabidopsis* contains eight HMA transporters (AtHMA1–8), in which AtHMA1–4 carry out transport of transition metals Zn<sup>2+</sup>, Cd<sup>2+</sup>, etc. (Mills et al. 2005; Eren and Arguello 2004). AtHMA5–8 belongs to subgroup II and is involved in delivery of Cu to chloroplast proteins of thylakoid lumen and stroma.

AtHMA2 and AtHMA4 express in vascular tissues of root, stem and leaves and mediate long-distance transport of Zn. Expression of AtHMA3 on tonoplast results



in sequestration of  $Zn^{2+}$ ,  $Co^{2+}$ ,  $Cd^{2+}$  and  $Pb^{2+}$  ions for detoxification. AtHMA1 is involved in detoxification of excess Zn in chloroplast (Kim et al. 2009). The HMA2 homologues are highly conserved in Poaceae as both OsHMA2 in rice and HvHMA2 in barley are functionally similar in carrying root to shoot transport of Zn and Cd (Mills et al. 2012). So, understanding of regulatory networks controlling HMA transporters will provide opportunities to enhance micronutrient levels in biofortified crop with minimized risk of toxic metals in edible sinks.

### 3.7 Major Facilitator Superfamily (MFS)

This superfamily consists of large group of secondary active membrane transporter proteins that utilize electrochemical potential of proton transport as driving force to carry out import or export of small organic molecules including transition metals  $Zn^{2+}$  and  $Fe^{2+}$ . Most of the MFS proteins contain 12–14 transmembrane  $\alpha$ -helices with a large, cytoplasmic loop between TMD6 and TMD7. A conserved MFS domain is also found between TMD2 and TMD3. The MFS members are ubiquitously present in all living organisms, but in plants ferroportin, drug- $H^+$  antiporter-1 (DHA1) and uncharacterized TET families have been discovered till date as metal transporters. A wide functional diversity and substrate specificity of these MFS transporters reveal their physiological significance in plants (Nino Gonzalez et al. 2019). The ferroportin family of *A. thaliana* includes member IREG1, IREG2 and IREG3 which are involved in efflux of  $Fe^{2+}$  across membrane (Morrissey et al. 2009). Members of DHA-1 family such as ZIF1 (zinc-induced facilitator 1) and ZIF-like 1 (ZIFL1) confer increased tolerance to Zn by sequestering  $Zn^{2+}$  or Zn chelates in vacuole (Haydon and Cobbett 2007a, b).

In addition to above, some MFS transporters are involved in root uptake and phloem transport of divalent micronutrients ( $Zn^{2+}$ ,  $Fe^{2+}$ ,  $Mn^{2+}$  and  $Cu^{2+}$ )-NA/MAs complexes. TOM family, i.e. transporter of mugineic acid family phytosiderophores, is one such example from MFS, which is involved in efflux of DMA to cell exterior. Once released, DMA binds to metal cations (particularly  $Fe^{2+}$ ) in soil solution and helps in its internal *in planta* transport. TOM1 in rice mediates Fe acquisition from rhizosphere. Another homologue TOM2 facilitates metal transport through plant body. TOM2 mediates efflux of DMA in apoplasm which chelates not only  $Fe^{2+}$  but also  $Zn^{2+}$  and  $Cu^{2+}$ . This functionality of TOM2 is demonstrated to enhance metal translocations to sinks under normal plant growth (Nozoye et al. 2015). In addition to TOM family, a recently characterized ENA1 (efflux transporter of NA) transporter maintains Fe homeostasis in rice. ENA1 is found to maintain intracellular trafficking of NA-metal complex for vacuolar detoxification of Fe (Nozoye et al. 2019). Another class of phenolics efflux zero 1 and 2 (PEZ1 and PEZ2) transporter proteins in MFS mediates efflux of protocatechuic acid. They are reported to contribute in long-distance transport of iron through root xylem (Ishimaru et al. 2011; Bashir et al. 2011).

### 3.8 Metal Tolerance Proteins (MTPs) Family

As their name suggests, these transporter proteins are involved in tolerance to micronutrient metals accumulated at toxic levels. Plant MTPs are also known as cation diffusion facilitator (CDF) family. Their main function is efflux of metal cations out of cytosol either in subcellular compartments or to extracellular space. Thus, the role of MTP members in heavy metal homeostasis, its detoxification and hyperaccumulation has been discovered so far in plants (Ricachenevsky et al. 2013). Among them, AtMTP1 and AtMTP3 are the most functionally characterized vacuolar transporters. They preferentially transport  $Zn^{2+}$  but also transport  $Ni^{2+}$ ,  $Mn^{2+}$ ,  $Co^{2+}$ ,  $Cd^{2+}$  and  $Fe^{2+}$  with varied affinity (Arrivault et al. 2006). Structurally plant MTPs possess six TMDs with their N- and C-termini towards cytosol. A conserved CDF signature exists between TMD 2 and 3 (Gustin et al. 2011). A histidine-rich cytoplasmic loop is also present between TMD 4 and 5. This cytoplasmic loop acts as metal sensor to determine its cytoplasmic levels and determines metal selectivity. MTPs function as  $H^+$ -metal cation antiporters with broader substrate affinity. These proteins are generally specified as Zn-CDFs, Fe/Zn CDFs and Mn-CDFs phylogenetically based upon substrate metal ion. But they are also able to transport other heavy metal divalent cations (Montanini et al. 2007). Anuradha et al. (2012) confirmed an increase in expression of OsMTP1 on exposure to metals such as Fe, Cu, Cd, Zn, etc. which accelerated metal accumulation in grain sinks.

### 3.9 CAX and VIT Family

Vacuolar sequestration of micronutrients is one of the mechanisms to maintain micronutrient homeostasis in cytosol and for their precise allocation to desired sinks. To fulfil this function, efflux transporters of CAX (cation exchanger) family and VIT (vacuolar iron transporter) family are present on tonoplast.

#### 3.9.1 CAX Family

CAX are cation/ $H^+$  antiporters which belong to  $Ca^{2+}$ /cation antiporter (CaCA) superfamily (Shigaki and Hirschi 2006). Phylogenetically, CAXs are grouped into three types with plant CAXs belong to category of Type I. Type I CAXs are further divided in two distinct groups—Type IA and Type IB. CAX proteins are encoded by a multigene family and have a structural characteristic of 11 TMDs (Shigaki et al. 2006). The TMDs are divided in three components as TMD1, TMD2–6 and TMD7–11. Among them, TMD1 contains a highly variant nine-amino-acid region which regulates metal cation specificity during transport. Both components TMD2–6 and TMD7–11 are thought to be formed with ancient duplication event. A highly conserved cation-binding region is present between TMD 2 and 3 and TMD7 and 8.

The presence of an N-terminal auto-inhibitory domain has also been detected in a range of plant CAXs. Numerous CAXs have been functionally characterized in *Arabidopsis*, barley, tomato and rice (Edmond et al. 2009; Kamiya et al. 2006). In

*Arabidopsis*, AtCAX2 and AtCAX4 are involved in  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  detoxification under heavy metal stress, while AtCAX5 (ortholog of AtCAX2) regulates only  $\text{Mn}^{2+}$  transport (Korenkov et al. 2007; Zhang et al. 2011) under metal excess. Thus, a wide diversity is present among CAX orthologs for their functional characteristics and broad substrate specificity which alters regulatory mechanism of intracellular sequestration in vacuole.

### 3.9.2 VIT Family

VIT proteins mediate transport of ferrous ions into vacuoles and thus regulate Fe homeostasis in plants. These transporter proteins exhibit high homology to CCC-1 ( $\text{Ca}^{2+}$ -cross-complementer) protein of yeast that catalyses intracellular storage of Fe in vacuoles. The first member of VIT family identified *in planta* is AtVIT1 which is involved in Fe loading in seed. Various VIT-like protein (VTL) transporters have been identified in *Arabidopsis*, rice, wheat, tulip, etc. which exhibit strong selectivity for  $\text{Fe}^{2+}$  but are also able to transport other metal cations, i.e.  $\text{Zn}^{2+}$  and  $\text{Mn}^{2+}$  (Kim et al. 2006; Gollhofer et al. 2014; Eroglu et al. 2017; Zhang et al. 2012; Sharma et al. 2020; Connorton et al. 2017; Momonoi et al. 2009). Differential tissue expressions of these VIT and VTL transporters in plants allow capturing of excess cytoplasmic Fe in vacuolar compartments of different sinks. While AtVIT1 has high expression in provascular tissues of wild-type embryo (Kim et al. 2006), OsVIT1 and OsVIT2 are expressed in flag leaves and regulate partitioning of Fe and Zn in developing grain (Zhang et al. 2012). Due to key role of VIT in Fe distribution of cereal grains, they can act as a potential target in genetic biofortification.

Structurally VIT transporter is a dimeric protein where each monomer consists of 5-TMD and a cytoplasmic MBD (Kato et al. 2019). The respective N- and C-termini of transporter protein are located towards cytoplasm and lumen of vacuole. The MBD of VIT transporter constitutes 3-helical bundles which allow capturing of cytoplasmic  $\text{Fe}^{2+}$  ions from chelating molecule. The ion translocating pathway of transporter protein is present at dimer interface with the presence of conserved methionine and carboxylate residues that facilitate efficient transport of Fe to vacuole. Further, transmembrane kinks due to the presence of proline and glycine residues on TMD1 and 2 are highly conserved among CCC1/VIT1 family transporters (Kato et al. 2019).

### 3.10 Natural Resistance-Associated Macrophages Protein (NRAMP) Family

NRAMP family is a diverse class of integral membrane proteins with members present in bacteria, fungi, animals and plants. They function in both inter- and intracellular trafficking of a wide range of divalent metal ions such as  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Pb}^{2+}$  (Gunshin et al. 1997). Studies on mouse Nramp2 revealed structural similarity of NRAMP protein with Slc 11. NRAMP protein contains 10–12 TMD with a twofold inverted symmetry like LeuT superfamily (Frickey and Lupas 2004). Further, the conserved hydrophobic

core of 10TMD is divided into two direct repeats with TMD1–5 helix repeats have inverted topologies (Cellier 2012; Czachorowski et al. 2009). The presence of Nramp-specific polar residues within TMD1 and TMD6 has also been reported in phylogenetic studies (Gu and Vander Velden 2002; Courville et al. 2008). In higher plants, NRAMPs play an essential role in metal homeostasis. The first plant NRAMP member is identified in *Arabidopsis*, i.e. AtNRAMP1 function as high-affinity  $Mn^{2+}$  transporter, and plays an essential role in Fe homeostasis *in planta* (Curie et al. 2000; Cailliatte et al. 2010). Functional homologs of NRAMP genes have been identified in *Arabidopsis* (At NRAMP 1–6), rice (OsNRAMP 1–7), common bean (Pv NRAMP 1–7) on organellar membrane and cell membrane, which are responsible for translocation of divalent metal ions to various sinks (Thomine et al. 2000; Belouchi et al. 1997; Ishida et al. 2018). But some members such as Nr1 and EIN2 are involved in  $Al^{3+}$  transport and ethylene signal transduction (Lu et al. 2018; Alonso et al. 1999).

### 3.11 OPT/YSL Family

Oligopeptide transporters (OPT) are novel family of transporters which are involved in transport of amino acids and oligopeptides (tri-penta peptides). In addition to peptide substrates, a subfamily of OPT, i.e. yellow stripe 1-like (YSL), mediates transport of metal complexes with peptides/amino acids across cellular membrane. Basically, YSL functions as proton-coupled symporter of metal-chelate complex. Their differential selectivity for metal substrates is dependent on extracellular loop between TMD6 and 7 (Harada et al. 2007).

The first member identified of YSL/OPT family is ZmYS1, mutation of which causes yellow stripes on maize leaves. These yellow stripes signify interveinal chlorosis which is resulting from defective Fe uptake, from which the family gets its name (Curie et al. 2001). Studies on ZmYS1 reveal function of this transporter in transport of Fe-phytosiderophore complexes from root cell exterior to cytoplasmic interior and further its symplastic loading to vasculature. Other metal cations such as  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$ , etc. are also transported by ZmYS1. Multiple YSL genes or their putative homologs have been identified in plants with 18 members in rice (Koike et al. 2004), 8 in *Arabidopsis* (DiDonato Jr et al. 2004), 5 in peanut (Xiong et al. 2013) and 67 in wheat (Kumar et al. 2019) which mediate transport  $Fe^{2+}$ -NA/ $Fe^{3+}$ -MAs or other metal chelates to various tissues and have a key role in metal homeostasis. Among them, AtOPT3, AtYSL1 and AtYSL3. OsYSL2, TcOPT3, etc. are known to be involved in phloem loading of Fe, Zn and other mimic metal cations in sink tissues (such as young leaves, developing fruits and seeds) for their accumulation (Zhai et al. 2014; Waters et al. 2006; Chu et al. 2010; Ishimaru et al. 2010; Hu et al. 2012). In addition to the above, other transporter families such as plant cadmium resistance, multidrug and toxic compound extrusion family, etc. have also been reported to regulate metal ion flux in plants.

### 3.12 Sequestration and Accumulation of Micronutrients in Vacuolar Compartments

The unique ionome composition of different cell types in each plant organ (i.e. root, shoot, leaf, flower, etc.) suggests that cell-specific accumulation and tissue distributions of metal nutrients are under strict regulation to maintain metal homeostasis. It is thought that such regulation contributes to distinct physiology of particular cell type. In this regard, the subcellular organelle vacuole is central for sequestration of metal cations, resulting in maintenance of their plasmatic concentration and further detoxification. This property of vacuolar compartments, to act as metal store house, is necessary for optimum cellular functioning. The compartmentalization of essential micronutrients/heavy metal cations in vacuoles depends upon the functioning of tonoplast transporters and vacuolar pumps (i.e. members of VIT, CAX and NRAMP family). Hyperaccumulators such as *Arabidopsis halleri*, *Thlaspi caerulescens*, *Dichapetalum gelonioides*, etc. are found to accumulate high concentrations of  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ , etc. in root vacuoles just like non-hyperaccumulators, but they differ in metal accumulation by aerial shoots which is significantly higher in hyperaccumulator species due to increased expression of HMA proteins controlling long-distance vascular transport of micronutrient metals.

Sequestration of micronutrient metal ions in leaf vacuoles is one of the tolerance mechanisms to favour hyperaccumulation and detoxification in metal hyperaccumulators. This also maintains nutrient supply in desired sinks at the time of need. Despite of NA, MAs, histidine and organic acids (such as citrate, malate, etc.) form chelating complexes with heavy metal micronutrients in subcellular compartments including vacuole. In addition, cysteine-rich protein entities, phytochelatin (PCs) and metallothioneins (MTs) are best characterized for their metal-binding properties in plants. Despite of ubiquitous presence of MTs in animal and plants, they share a common feature of heavy metal homeostasis with PCs. MTs are gene-encoded low-molecular-weight peptides with a high percentage (20–30%) of cysteine residues responsible for metal binding. In contrast, PCs are enzymatically synthesized peptides having general formula  $\gamma$ -(glutamic acid-cysteine)<sub>n</sub> where  $n = 2-11$  with great affinity for heavy metal ions (Shukla et al. 2016). The sulfhydryl group of cysteine moieties in PCs and MTs reacts with free metal ions in cell cytoplasm and form low-molecular-weight complexes. These PC-metal/MT-metal ion complexes are then transported to vacuolar compartments for their detoxification.

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## 4 Journey of Micronutrients to Seed (Grain) Sinks: Long-Distance Phloem Transport of Micronutrients

The charged nature of micronutrient metal ions results in their specialized vascular transport from root to shoot and then from shoot to other vegetative/reproductive sinks. There is transition in a form of micronutrients absorbed, during the long-

distance transport from root xylem to leaf phloem of minor veins, which affects their mobility in phloem. In contrast to predominance of divalent cationic or metal-organic acid form of micronutrients in xylem sap (pH  $\approx$  5.5), they are generally present in bound chelated form as metal NA, metal-DMA/phytosiderophores in phloem sap (pH  $\approx$  7.3–8.5) to avoid their precipitation during delivery to sink tissues. It is well known that phloem is the main translocating tissue which supplies sugars and mineral nutrients to developing plant sinks irrespective of their location. However, the loading of micronutrients in phloem at site of source (i.e. leaf mesophyll/xylem vessel at minor veins) can be apoplastic or symplastic depending upon the activity of associated companion cell. There can be a direct loading of metal cations from xylem into phloem parenchyma in minor veins due to their close proximity. Transfer cells in phloem mediate such apoplastic loading of micronutrients. The invaginated wall growths and numerous membrane transporters on transfer cells favour greater nutrient fluxes (Sondergaard et al. 2004). The presence of membrane H<sup>+</sup>-ATPase further boosts up secondary active transport of nutrients in apoplastic loaders. Another route is the symplastic loading of metal chelates (metal NA, metal-DMA/phytosiderophores, metal PCs) from mesophyll cells into intermediary cells of sieve element complex via plasmodesmatal connections.

Mutation studies and researches on metal tolerance mechanism of hyperaccumulators revealed that nonproteinogenic amino acid NA is most favoured organic ligand for several micronutrient metals in phloem. NA is also found to form stable complexes with Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup> and Cu<sup>2+</sup> in vitro (Anderegg and Ripperger 1989). Overexpression of NA biosynthetic genes, i.e. nicotianamine synthase (NAS) in soybean (Nozoye et al. 2014), sweet potato (Nozoye et al. 2017), tobacco (Kim et al. 2005), rice (Masuda et al. 2009; Lee et al. 2011), etc. through transgenics, has been found to increase the Fe and Zn concentration of leaves and respective sink organs (i.e. root in sweet potato and seeds in rice and soybean). These investigations also support the role of NA in shoot to root signalling of iron and its remobilization from mature to developing tissues. But NA is the sole player in metal translocation; this assumption has been negated with discovery of OsYSL15 in rice which performs dual function of phloem translocation of Fe<sup>3+</sup>-DMA in addition to its rhizospheric uptake (Inoue et al. 2009). So other metal ligands such as DMA, histidine and phytochelatins have also been associated in phloem translocation of micronutrients.

In plants, the immature organs act as sinks during their early growth and depend upon source for organic and inorganic nutrition. During a particular growth stage, there can be more than one sink for the source leaves, so the allocation/partitioning of nutrients to diverse sinks decides their accumulation in desired sinks under such situation. Thus, in food crops where grains or seeds are economic sinks, total nutrient concentrations in phloem and its distribution towards developing grains are of equal importance. But if the whole shoots are to be consumed as human or animal food, then the total micronutrient contents of shoot matter the most than nutrient allocation. Nutrient remobilization from mature leaves to developing sinks during senescence also boosts the supply of micronutrients. The role of some senescence-

responsive genes in micronutrient loading of seeds has also demonstrated. One such example is NAM genes in wheat whose reduced expression was found to decline nutrient partitioning to grain and lowered grain Fe and Zn contents (Waters et al. 2009).

Another key molecular player in micronutrient transport to the sinks (grains/leaves) is YSL transporter family. As mentioned earlier, YSL transporters are involved in transport of metal chelates especially NA-metal complex. YSLs of *Arabidopsis*, particularly AtYSL1 and AtYSL3, are involved in delivery of Fe, Zn, Cu and Mn to reproductive organs. Mutations in these YSL genes cause impaired vascular transport of metal micronutrients during senescence and thus limit metal accumulation in seed. Similarly, YSL ortholog in rice OsYSL2 mediates phloem transport of Fe and Mn in aerial shoots and metal loading in seeds (Ishimaru et al. 2010). Thus, better understanding on regulation of such metal transporters in long-distance micronutrient signalling will help in bioengineering of staple crops with high micronutrient density.

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## 5 Future Scenario: The Way Ahead

Although recent tools of molecular breeding are continuously harnessing genetic diversity of wild germplasm to enhance micronutrient density of food crops, there is a need to find cell-specific and developmental-stage-specific regulators controlling the metal-oriented circuits to maintain homeostasis. The complex interactions of these essential micronutrients with toxic mimic cations (such as Pb, Cd, Hg, etc.) and other macronutrients (N, P, S, etc.) need to be explored more in order to enhance metal absorption in edible sinks. Inclusion of modern system biology and omics approaches in biofortification studies will be able to enhance our understanding on mechanism of root to shoot signalling with better regulation of metal loading in seed sinks. Understanding transcriptional and post-transcriptional regulation of genes encoding transporter proteins can be another promising research avenue which will help us to increase uptake and translocation micronutrients in seed. In addition to the above, novel senescence-associated genes should be identified to facilitate metal remobilization towards developing sinks.

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