



# Metal ligands in micronutrient acquisition and homeostasis

Stephan Clemens

Department of Plant Physiology and Bayreuth Center of Ecology and Environmental Research, University of Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany

## Correspondence

S. Clemens, Department of Plant Physiology, University of Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany.  
Email: stephan.clemens@uni-bayreuth.de

## Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: Cl 152/11-1

## Abstract

Acquisition and homeostasis of micronutrients such as iron (Fe) and zinc (Zn) pose specific challenges. Poor solubility and high reactivity require controlled synthesis and supply of ligands to complex these metals extracellularly and intracellularly. Cytosolic labile pools represent only a minute fraction of the total cellular content. Several low-molecular-weight ligands are known in plants, including sulfur ligands (cysteine and peptides), nitrogen/oxygen ligands (*S*-adenosyl-L-methionine-derived molecules and histidine), and oxygen ligands (phenolics and organic acids). Some ligands are secreted into the extracellular space and influence the phytoavailability of metal ions. A second principal function is the intracellular buffering of micronutrients as well as the facilitation of long-distance transport in xylem and phloem. Furthermore, low-molecular-weight ligands are involved in the storage of metals, predominantly in vacuoles. A detailed molecular understanding is hampered by technical limitations, in particular the difficulty to detect and quantify cellular metal–ligand complexes. More, but still too little, is known about ligand synthesis and the transport across membranes, either with or without a complexed metal. Metal ligands have an immediate impact on human well-being. Engineering metal ligand synthesis and distribution in crops has tremendous potential to improve the nutritional quality of food and to tackle major human health risks.

## KEYWORDS

heavy metals, intermediary metabolism, plant nutrition

## 1 | INTRODUCTION

Micronutrients are essential elements or compounds required by organisms in smaller quantities, relative to macronutrients such as nitrogen, potassium, or phosphorus. According to definitions generally used in biology, micronutrients comprise both organic molecules such as ascorbic acid and other vitamins as well as minerals. Plants, of course, only have to acquire inorganic micronutrients from the environment as they synthesize all organic molecules themselves. Most micronutrients from the plant's perspective belong to the transition

metals and posttransition metals in the fourth and fifth periods of the periodic table, namely, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and nickel (Ni). They are often referred to as trace elements and the subject of this review. Not discussed are the nonmetallic micronutrients boron (B) and chlorine (Cl).

During the course of evolution, the metallic micronutrients were recruited for myriad biochemical functions, albeit to very different extent (Krämer & Clemens, 2005; Zhang & Gladyshev, 2011). Although nearly 10% of all plant proteins are predicted to contain Zn as a cofactor (Andreini, Banci, Bertini, & Rosato, 2006), there is only

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Plant, Cell & Environment published by John Wiley & Sons Ltd

one plant protein known to require Ni, namely, urease. Zn is of major importance as a cofactor in enzymes. In all six Enzyme Commission classes, Zn-dependent proteins are known (Clemens, 2010; Waldron, Rutherford, Ford, & Robinson, 2009). Furthermore, it has structural roles especially in many proteins interacting with nucleic acids, for example, polymerases and transcription factors. The redox activity of Fe and Cu is essential for the major energy-providing processes, photosynthesis and respiration. A photosystem I complex, for example, contains 12 Fe atoms per monomer in Fe-S clusters. Cu is present in the cytochrome c oxidase complex of the respiratory chain and part of mobile electron carriers such as plastocyanin. Mn has a major function in photosynthesis, too. A Mn cluster is at the heart of the oxygen-evolving complex in photosystem II. Mo is found in only few but very important plant proteins including nitrate reductase and the enzyme catalyzing the final step in abscisic acid biosynthesis, aldehyde oxidase (Hänsch & Mendel, 2009). Furthermore, the key enzyme of biological nitrogen fixation, nitrogenase, contains a Mo cofactor.

When compared with a macronutrient such as the alkali metal potassium (K), acquisition and homeostasis of transition metals pose particular challenges for organisms. First, their availability for uptake by plants can be very poor even when they are abundant in soil. The best known example is Fe. In aerated soil, it is mostly present as Fe(III), because it forms complexes of low solubility with various soil anions, especially at alkaline pH. Second, for the very reason transition metals and Zn have such a wide range of biological functions, their activity needs to be tightly controlled inside an organism. Strong interaction with organic molecules, for example, the thiol groups of proteins, or redox activity can damage cellular structures. This is illustrated by the high toxic potential of metallic environmental pollutants without biological function, such as cadmium, mercury, lead, and arsenic. After entering a cell, they are not guided to sites of usage by a homeostatic system. When the limited capacity to bind them is exhausted, they wreak havoc on a wide range of molecules.

Besides transporter proteins mediating membrane passage of metals (Argüello, Raimunda, & González-Guerrero, 2012; Krämer, Talke, & Hanikenne, 2007), low-molecular-weight (LMW) compounds that have the ability to form stable complexes with metals play a key role in metal homeostasis. From now on, they are referred to as LMW ligands. When ligands form multidentate complexes, that is, at least two coordinate bonds with the central metal atom, they are also called chelators. Another important function of some LMW compounds in metal homeostasis is redox activity and here predominantly the reduction of Fe(III) to Fe(II). This review aims to provide an overview about molecules and functions, to describe recent progress in the physiological understanding of LMW ligands, for example, the newly discovered role of coumarin secretion for Fe acquisition (Tsai & Schmidt, 2017), and to discuss the relevance of this knowledge for human nutrition.

## 2 | EXPERIMENTAL CHALLENGES

The focus here is on the molecules that act before, in between, or after transmembrane metal transport steps. Overall, much more is

known about the functions of metal transporters, even though they are predominantly inferred from mutant phenotypes, heterologous expression, and subcellular localization, as direct transport measurements are in most cases missing.

A persistent limitation of experiments aiming at unravelling the exact physiological roles of LMW ligands is the difficulty of detecting metal-ligand complexes in cells (reviewed in Álvarez-Fernández, Díaz-Benito, Abadía, Lopez-Millan, & Abadía, 2014). Visualization with the help of fluorescent tags is not possible as chemical modifications would strongly affect the metal-LMW ligand interaction. Any type of extraction is associated with the risk of changing the metal speciation because alternative binding partners become available when tissue integrity is compromised. Direct detection of most metal-ligand complexes is therefore restricted to accessible plant fluids such as the xylem sap, the phloem sap, or the liquid endosperm of pea seeds. It can be achieved by direct infusion chromatography, for example, hydrophilic interaction chromatography (HILIC) or size-exclusion chromatography (SEC), coupled to electrospray ionization-mass spectrometry (ESI-MS; Flis et al., 2016). Before this became possible, metals and potential ligands were quantified separately, and the existence of complexes was predicted using chemical speciation software and *in vitro* data on the stability constants of various metal-ligand complexes.

A more indirect approach has to be followed when metal-ligand complexes are to be detected in tissues. Mild, ideally metal speciation-preserving extraction is combined with SEC coupled to inductively coupled plasma-MS (ICP-MS; Husted et al., 2011). Fractions containing the metal of interest are then subjected to a second chromatographic step and ESI-MS for the identification of potential ligands that coeluted with the metal in SEC.

Data on the atomic environment and thereby on potential ligands of a metal can be obtained by synchrotron-based X-ray absorption spectroscopy (XAS; Wu & Becker, 2012). Bulk tissue samples or sections are exposed to focused X-ray beams. Absorption of X-rays excites core electrons, which results in the release of element-specific fluorescence. In XAS, absorption over a range of photon energies is recorded, and the resulting spectra provide information on redox state of the metal and the identity of neighbouring atoms. Although the actual ligands cannot be determined, an advantage of XAS lies in the detection *in situ*, that is, without any extraction or separation.

Determining the speciation of an element in a biological system entails data on the distribution and the chemical species, that is, the oxidation state, isotope ratio, and molecular structure of ligands (Templeton et al., 2000). Methods to study the distribution of metals are rapidly progressing (reviewed in Zhao, Moore, Lombi, & Zhu, 2014). Imaging techniques include synchrotron radiation X-ray fluorescence, proton/particle-induced X-ray emission spectrometry, secondary-ion MS (SIMS), and laser ablation ICP-MS (LA-ICP-MS). In recent years, sensitivity and resolution have improved tremendously so that elemental maps are becoming more and more precise. Colocalization of elements can provide indirect evidence as to the nature of molecules complexing metals. For example, high-resolution nano-SIMS of rice nodes localized arsenic in rice phloem companion

cells together with sulfur, suggesting the presence of As–thiol complexes (Moore et al., 2014).

The major bottleneck remains the direct detection of metal–ligand complexes. Thus, the physiological function of LMW ligands in most cases has to be inferred from phenotypes related to the concentrations and/or speciation of metals that are observed after genetic interventions causing changes in the rates of LMW ligand synthesis or in LMW ligand distribution. For example, as discussed in more detail below, *Arabidopsis thaliana* mutants defective in the synthesis of the nonproteinogenic amino acid nicotianamine (NA; Figure 1) showed symptoms of Fe deficiency especially in young leaves, which indicated a role of this LMW chelator in the supply of Fe via the phloem to sink leaves (Schuler, Rellán-Álvarez, Fink-Straube, Abadía, & Bauer, 2012). Similarly, RNAi-mediated knockdown of root NA synthesis reduced the level of Zn hyperaccumulation in leaves of the metallophyte *Arabidopsis halleri*, supporting a role of NA in the root-to-shoot translocation of Zn (Deinlein et al., 2012; Uraguchi, Weber, & Clemens, 2019).

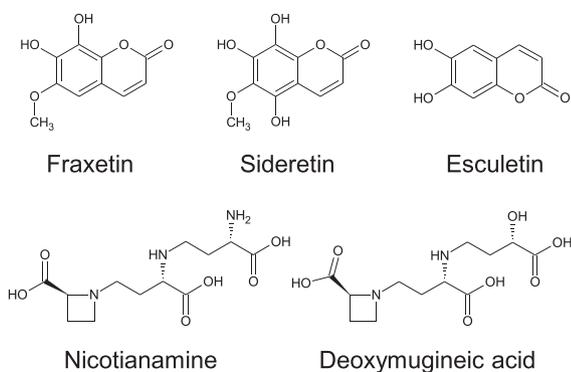
### 3 | PRINCIPAL FUNCTIONS OF LMW LIGANDS IN METAL HOMEOSTASIS

The metal homeostasis functions of LMW ligands lie before in between and after metal transport steps. Accordingly, they can be grouped into different categories (Figure 2). Some LMW ligands are secreted into the extracellular space and influence the phytoavailability of metal ions (Chen, Wang, & Yeh, 2017). Phytoavailability can either be enhanced, as illustrated best by the essentiality of LMW chelator secretion for the acquisition of Fe, or be decreased and in this way be beneficial for the plant. An example for the latter is the immobilization of  $Al^{3+}$  outside of root cells in acidic soil to attenuate toxic effects. This is achieved by secretion of organic acids that can form Al complexes (Kochian, Piñeros, Liu, & Magalhaes, 2015). LMW ligand secretion is certainly most active in root cortical and epidermal cells but not confined to them. Cells surrounding the

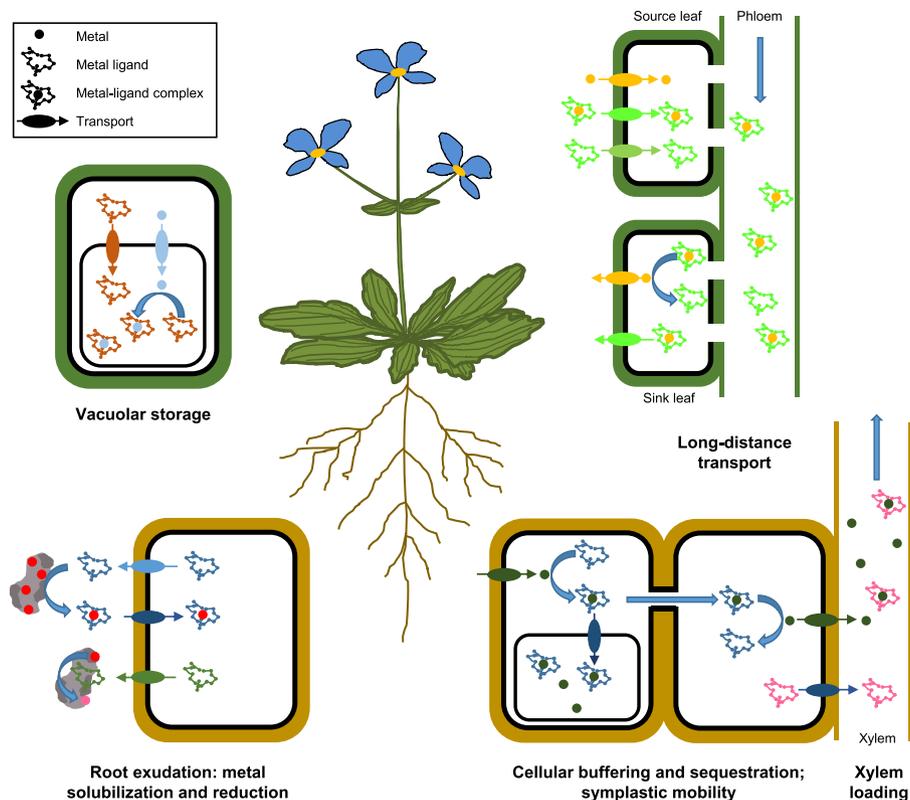
xylem, for example, secrete ligands that function as binding partners for metals in the xylem.

A second principal function is the buffering of metals in the cytosol and other cellular compartments (Figure 2). As introduced above, the interactions of metallic micronutrients with cellular components need to be tightly controlled. This can be illustrated by considering the Irving–Williams series. It describes the stability of complexes between divalent metal ions and organic ligands (Irving & Williams, 1953). Regardless of the chemical nature (be it sulfur, nitrogen, and/or oxygen containing), Cu(II) will always form the most stable complexes followed by Zn(II) and Ni(II). For biological systems, this implies that these metals should practically not be available in order to prevent a displacement of metals forming less stable complexes, such as Fe(II) or Mn(II), from their designated binding sites. This applies especially during protein folding when nascent polypeptides are particularly vulnerable to becoming populated with the wrong metal (Waldron & Robinson, 2009). Evolution solved this problem by maintaining very low concentrations of free metal ions in the cytosol. In the case of Cu(II), this concentration is close to zero. Cu(II) taken up by cells is bound by metallochaperones, which deliver it to target proteins and transporters (Burkhead, Reynolds, Abdel-Ghany, Cohu, & Pilon, 2009). The metal transfer from one protein to the other is in this case enabled by protein–protein interaction and structural changes during the docking. Conformational changes in the acceptor protein or a switch in the redox state can trap the donated metal so that it is no longer available for exchange (reviewed in Waldron & Robinson, 2009). Generally, ligand exchange reactions in cells are not simply determined by absolute affinities. Perhaps more meaningful are relative affinities. A metal further down in the Irving–Williams series, for example, Fe(II), can be complexed by a cellular component, provided the metals further up in the Irving–Williams series, for example, Zn(II), are tightly bound by other molecules with higher affinity.

The metal fraction bound in such a way that it can be exchanged, that is, is available for interaction with cellular molecules, is referred to as the labile pool. The free metal (hydrated ions) and the labile pool together constitute only a minute portion of the total metal in a cell. For Zn(II), experiments with *A. thaliana* roots expressing a range of Förster resonance energy transfer Zn sensors determined a concentration of around 400 pM for free Zn(II), whereas the total cellular Zn concentration is around 100  $\mu$ M (Lanquar et al., 2014). For animal cells, similar values have been obtained (Maret, 2013). Most of the metal is bound as cofactors in proteins and not available. Controlling the labile pool, that is, the exchangeable metal, and the free metal is a major function of LMW ligands. Several abundant molecules in cells, for example, glutathione (GSH), citrate, ATP, or histidine, are potential Zn(II) ligands in cells. Their actual contribution to Zn buffering, however, is not known because of the experimental challenges that preclude detection and quantification of the respective metal ligand complexes in vivo (Krężel & Maret, 2016). In plants, the buffering of Zn, Fe, and other metals also contributes to symplastic mobility and thus enables, for example, the lateral movement of micronutrients across the root towards the vasculature (Figure 2).



**FIGURE 1** Important metal ligands in plants. The top row shows coumarins involved in the Fe acquisition by strategy I plants. The two S-adenosyl-L-methionine-derived metal chelators in the bottom row are involved in the acquisition and homeostasis of several micronutrients



**FIGURE 2** Principal functions of metal ligands in micronutrient acquisition and homeostasis. In strategy I plants, Fe acquisition at neutral or alkaline pH is dependent on the secretion of coumarins and possibly other metabolites that either chelate or reduce poorly soluble Fe(III). Inside cells, ligands have a metal buffering function and can mediate vacuolar sequestration under conditions of metal excess. Furthermore, cytosolic metal–ligand complexes ensure symplastic mobility. Metals and ligands are generally assumed to be loaded into the xylem separately. In the xylem sap, metals can be complexed or remain free as hydrated ions. Most metal atoms in the phloem are bound by ligands. Metal ions, ligands, or metal–ligand complexes can be loaded into the phloem. Sink leaves are supplied with micronutrients by source leaves via the phloem. Micronutrients can be stored in vacuoles either in free form or in complexed form. Please note that the ligand symbol indicates a chelator, that is, a ligand forming multidentate complexes. For reasons of simplicity, one symbol is used for chelators and other ligands

Finally, LMW ligands are involved in the storage of metals, predominantly in vacuoles (Figure 2). Micronutrients are supplied to the offspring via storage in seeds. The main storage sites at least in cereals are vacuoles. Furthermore, when micronutrients or nonessential elements are in excess, LMW ligands can mediate vacuolar sequestration to prevent toxic effects in the more vulnerable cellular compartments. A special case is the extremely high accumulation of Ni, Zn, or Cd in the leaves of metal hyperaccumulating plants. Toxicity of the accumulated metals presumably confers elemental defence against herbivores and pathogens (Kazemi-Dinan, Thomaschky, Stein, Krämer, & Müller, 2014).

All functional categories of LMW ligands are tightly interlaced with metal transport. Adequate understanding of LMW ligand functions requires careful investigation not only of their synthesis and the formation of complexes but also of their transport across membranes, either with or without a complexed metal (Haydon & Cobbett, 2007). Major transporter groups involved in metal ligand and/or metal-complex transport belong to the YELLOW STRIPE 1-like transporters (YSL), the major facilitator superfamily, and ABC transporters. Furthermore, it remains an open question as to whether metal–ligand

complexes deliver metals to transporter proteins in a way analogous to the interaction between Cu chaperones and Cu-ATPases (González-Guerrero & Argüello, 2008).

#### 4 | LMW LIGANDS IN PLANTS

A variety of molecules have been assigned a function as LMW ligands in plants. Many of them are involved in more than one of the principal functions and can be found in various compartments. The list of established components comprises sulfur ligands (cysteine, peptides, and small proteins), ligands containing N- and O-atoms (molecules derived from *S*-adenosyl-L-methionine, histidine, and other amino acids), and O-ligands (phenolics such as coumarins, and organic acids; Figure 1). Most likely, however, our current inventory of LMW ligands is far from complete. Candidate LMW ligands include, for example, flavonoids (Corso et al., 2018) and other specialized metabolites. Many more may await identification.

#### 4.1 | S-Adenosyl-L-methionine-derived metal chelators

Among the best documented functions of LMW ligands is the mobilization of scarcely available soil Fe. Under oxic conditions, most of the Fe is bound as Fe(III) in (hydr)oxides and poorly soluble especially in calcareous soils. Two different strategies are employed by plants to acquire Fe (Marschner & Römheld, 1994). Strategy I of dicots and nongraminaceous monocots comprises acidification of the rhizosphere and reduction of Fe(III) to Fe(II) as described in detail below. An integral part of the strategy II typical for grasses is the secretion of phytosiderophores belonging to the mugineic acid family (reviewed in Kobayashi & Nishizawa, 2012). The most prominent compound in this class is deoxymugineic acid (DMA; Figure 1). Root cells secrete DMA and other more hydroxylated mugineic acids in a diurnal pattern through transporters such as TOM1 in rice and barley (Nozoye et al., 2011). Synthesis is strongly up-regulated under conditions of Fe deficiency. Fe(III) is solubilized, and the stable phytosiderophore-Fe(III) chelate complexes are taken up into root cells by YELLOW STRIPE 1 and YSL transporters.

DMA is detectable not only in roots and root exudates of grasses such as rice but also in shoots and seeds as well as in phloem and xylem (Kobayashi, Nozoye, & Nishizawa, 2019). Indeed, the physiological role of DMA goes beyond Fe(III) acquisition from the soil. DMA is secreted internally as well. The efflux transporter TOM2 is expressed in many tissues and cell types not directly involved in Fe uptake, for example, in the vasculature of roots, shoots, and developing seeds (Nozoye et al., 2015). It may be important for solubilizing Fe bound to negatively charged polymers in the apoplast. Furthermore, DMA is hypothesized to function as a chelator of Fe(III) in xylem and, more importantly, phloem. In rice phloem sap, DMA was detected as the major Fe ligand by SEC and ESI-MS of Fe-containing fractions (Nishiyama, Kato, Nagata, Yanagisawa, & Yoneyama, 2012). Several YSL transporters have been implicated in the transport of Fe(III)-DMA complexes. A recent example is OsYSL9. Mutants lacking functional OsYSL9 show a reduced loading of Fe into the embryo, which illustrates the contribution of DMA complexation to Fe translocation within grasses (Senoura et al., 2017).

DMA may also contribute to the acquisition and distribution of other micronutrients. Early observations associated stronger release of DMA with higher Zn efficiency in wheat (Cakmak et al., 1996). Transport activities with Cu(II)-DMA or Zn(II)-DMA have been reported for YSLs, and Cu(II)-DMA was detected in rice xylem sap (Ando, Nagata, Yanagisawa, & Yoneyama, 2013).

A precursor of mugineic acids is NA (Figure 1). It is produced from three molecules of SAM in a reaction catalysed by NA synthases (NAS; Curie et al., 2009). Members of the Poaceae possess NA aminotransferases and DMA synthases that synthesize DMA from NA (Bashir et al., 2017). Both DMA and NA function in metal homeostasis of grasses. In dicots and nongraminaceous monocots, however, NA is not converted further and acts as the main SAM-derived LMW chelator. It is known to form complexes with Fe(III), Fe(II), Zn(II), Mn(II), Ni(II), and Cu(II) (Rellan-Alvarez, Abadia, & Alvarez-Fernandez, 2008).

Dissociation constants and stability of these complexes vary. For example, NA has a higher affinity for Fe(III) than for Fe(II), but the complex stability is greater for Fe(II)-NA. Metal-NA complexes are usually more stable at a pH around neutral than at an acidic pH. Most available evidence suggests a role in the distribution of these micronutrients within plants and, in accordance with the pH influence on complex stability, mostly in the cytosol and the phloem. *A. thaliana* plants lacking NA develop interveinal chlorosis as a symptom of Fe deficiency especially in young leaves because the transfer of Fe from the phloem to sink tissues is defective (Schuler, Rellán-Álvarez, Fink-Straube, Abadía, & Bauer, 2012). YSL transporters are hypothesized to move Fe-NA complexes from the phloem to surrounding parenchyma (Kumar et al., 2017).

Research into the physiological role of NA was for a long time focused on Fe homeostasis because the Fe deficiency symptoms of NA synthesis mutants are obvious. Today, it is clear that NA complexation serves important functions in the homeostasis of several other micronutrients, too. Defects in NA synthesis or a lowering of the cytosolic NA pool in root cells negatively affect the translocation of Zn from roots to shoots. Overexpression of the vacuolar NA transporter ZIF1 in *A. thaliana* causes a retention of Zn in the roots (Haydon et al., 2012). When the spatial distribution of metals across *A. thaliana* roots was determined by LA-ICP-MS, comparisons of the wild type and an NA biosynthesis mutant directly showed a build-up of Zn in mutant roots, consistent with impaired mobility of Zn towards the xylem (Persson et al., 2016). Accordingly, xylem sap and shoot levels of Zn were significantly lower.

In the Zn hyperaccumulating species *A. halleri*, root NA synthesis is constitutively stronger than in non-hyperaccumulating plants. When NA synthesis is lowered through knockdown of the most highly expressed NAS gene, Zn accumulation in leaves is significantly reduced regardless of Zn availability in the soil (Deinlein et al., 2012; Uruguchi, Weber, & Clemens, 2019). Apparently, high NA concentrations maintain Zn mobility in the root symplast and facilitate the loading of Zn into the xylem. Whether or not NA serves as a major Zn chelator in the xylem as well remains to be determined. The existence of Zn-NA complexes is considered less likely because of the lower pH. Still, Zn was detected in pea xylem sap chelated by NA (Flis et al., 2016). As discussed further below, organic acids represent the best alternative Zn ligand candidates under acidic conditions and were indirectly shown to chelate Zn in the xylem of *A. halleri* (Cornu et al., 2015). Chelation of Zn in the phloem likely represents another function of NA in Zn homeostasis. Zn-NA complexes were detected in rice phloem sap (Nishiyama, Kato, Nagata, Yanagisawa, & Yoneyama, 2012).

The most comprehensive analysis of metal-LMW ligand complexes in plant liquids published to date detected several metal-NA complexes in xylem and/or embryo sac liquid of pea via HILIC coupled to dual detection by ICP-MS and high-resolution ESI-MS (Flis et al., 2016). For Cu(II) and Zn(II), complexes with NA were dominant, accounting for more than half of the total metal signal, and for Co(II) and Ni(II), they were the most abundant. NA complexes with Cu(II), Zn(II), Co(II), and Ni(II) were detected in embryo sac liquid and xylem, whereas Mn(II) and Fe(II) complexes were restricted to the embryo.

Fe(III)-NA was not detected. Detection of Cu-NA in xylem is consistent with the suspected function of NA as a Cu ligand in this compartment (Irtelli, Petrucci, & Navari-Izzo, 2009). NA is also an important binding partner for Cu in the phloem. The phloem-expressed OsYSL16 is required for efficient transfer of Cu from older to younger leaves and was found to transport Cu-NA in yeast (Zheng, Yamaji, Yokosho, & Ma, 2012).

## 4.2 | Phenylpropanoid-derived ligands

The reduction-based Fe acquisition strategy I of dicots and nongraminaceous monocots comprises acidification of the rhizosphere through the pumping of protons, the reduction of Fe(III) in soluble Fe(III) chelates by plasma membrane-localized ferric reductases such as FRO2 and the uptake of Fe(II) by IRT1 and related transporters (Brumbarova, Bauer, & Ivanov, 2015). It had long been hypothesized that the secretion of phenolic compounds and other LMW ligands contributes to strategy I as well (Marschner & Römheld, 1994). First direct evidence was obtained when it could be demonstrated for red clover that removal of phenolics from the external medium caused Fe deficiency (Jin et al., 2007). Only recently, however, were the first phenolics involved in Fe acquisition molecularly identified (reviewed in Clemens & Weber, 2016; Tsai & Schmidt, 2017). Synthesis and secretion of coumarins such as scopoletin, fraxetin, or esculetin (Figure 1) are essential for growth of *A. thaliana* under alkaline conditions either in soil or in hydroponics. Mutants lacking a functional feruloyl-CoA 6'-hydroxylase, a key enzyme in the coumarin biosynthesis pathway, are severely Fe deficient when Fe availability is low (Rodríguez-Celma et al., 2013; Schmid et al., 2014; Schmidt et al., 2014). The same applies to plants carrying loss-of-function mutations in the ABC-type transporter ABCG37, which mediates the export of coumarins into the rhizosphere (Fourcroy et al., 2014). Later, sideretin (Figure 1) was identified as a major coumarin secreted by Fe-deficient roots of *A. thaliana* (Rajniak et al., 2018). In fact, many other dicot species were found to secrete sideretin as well, and the biosynthetic pathway apparently arose early in angiosperm evolution. Thus, it appears likely that the secretion of coumarins or functionally equivalent metabolites is a widespread and essential component of strategy I especially under neutral to alkaline conditions when the classic trio of proton pump-FRO2-IRT1 is barely active (Grillet & Schmidt, 2017).

A key question emerged with the discovery of the role that coumarins play in Fe acquisition: Are the beneficial effects of secretion attributable to the reduction or the chelation of Fe(III) by coumarins (Schmid et al., 2014; Schmidt et al., 2014)? Fe(III) chelates could mobilize insoluble Fe, facilitate ferric reductase-dependent reduction, or in addition be taken up by plant roots analogous to phytosiderophores. The physiologically active coumarins are catecholic coumarins carrying neighbouring hydroxyl groups on the aromatic ring, for example, fraxetin (Figure 1). They can solubilize Fe(III) from Fe hydroxide precipitates (Schmid et al., 2014). Experiments with strategy I pathway mutants showed that the beneficial effects of coumarins secretion are dependent on functionality of both FRO2 and IRT1 (Fourcroy,

Tissot, Gaymard, Briat, & Dubos, 2016). This was interpreted as evidence for Fe(III) chelation as the predominant activity and for the absence of Fe(III) chelate uptake systems in *A. thaliana*. However, fraxetin and sideretin can reduce Fe(III) (Rajniak et al., 2018), making it likely that both chelation and reduction are important, especially when considering that in soil, localized concentrations of secreted coumarins may be much higher than in a hydroponic medium (Tsai & Schmidt, 2017). Furthermore, several plant species secrete redox-active molecules under Fe deficiency (Rajniak et al., 2018). An example is the secretion of flavins by *Beta vulgaris* (Sisó-Terraza, Rios, Abadía, Abadía, & Álvarez-Fernández, 2016). They were shown to reductively dissolve Fe(III). Thus, the picture emerges that the secretion of structurally diverse LMW reductants is a common element of strategy I in higher plants (Rajniak et al., 2018). How widespread the mechanism is and which molecules are involved remain to be addressed. Other important questions concern the responsible biosynthetic pathways, that is, the identification of additional enzymes (Siwinska et al., 2018; Tsai et al., 2018), their regulation, and the metabolism of secreted compounds in the rhizosphere.

A contribution of phenolic compounds to Fe nutrition is not restricted to strategy I plants. In grasses, the secretion of compounds such as protocatechuic acid and caffeic acid aids in solubilizing Fe(III) precipitated in the apoplast. A defect in the efflux transporter PEZ1, which is predominantly expressed in the stele of rice roots, resulted in higher Fe concentrations in the root apoplast and lower Fe concentrations in xylem sap (Ishimaru et al., 2011).

## 4.3 | Thiols

The major LMW thiols in plant cells are cysteine, GSH, and phytochelatins (PCs), the latter being peptides synthesized from GSH. According to the Irving-Williams series, sulfur ligands have a particularly strong affinity for metal ions, making thiols potential LMW ligands. Because of the roughly 100-fold higher intracellular concentrations relative to cysteine, GSH is far more relevant in this context (reviewed in Seth et al., 2012). PC concentrations vary strongly. In cells not exposed to any metal excess, they are small but can reach millimolar levels when PC synthases become activated by metals (Kühnlenz, Schmidt, Uraguchi, & Clemens, 2014).

The metal-binding activity of thiols has been mostly implicated in cytosolic buffering, vacuolar sequestration, and long-distance transport in the phloem (Figure 2). Direct detection of thiol-metal complexes in the cytosol, however, is extremely challenging, the exception being complexes with As(III), which are much more stable (Liu et al., 2010). Thus, most of the current knowledge on thiols as LMW ligands is inferred from mutant phenotypes, indirect biochemical evidence for the existence of thiol-metal complexes, and data on in vitro stability constants.

Mostly based on in vitro data and theoretical considerations, GSH was proposed to function as the main Fe(II) ligand in the labile Fe pool, which is thought to be less tightly controlled than the Cu and Zn pools, meaning that the concentration of labile Fe(II) is much higher (Hider &

Kong, 2013). Nonetheless, GSH was suggested to represent an important cytosolic buffer of Zn as well (Krężel & Maret, 2016). Experimental evidence for these well-substantiated hypotheses is still scarce. The combination of SEC-ICP-MS and ESI-MS applied to root extracts of *A. halleri* identified GSH as a potential Zn ligand besides NA and PCs (Deinlein et al., 2012). *A. thaliana* lines with elevated GSH levels in the leaves were found to translocate Zn more efficiently from roots to shoots (Wongkaew et al., 2019), consistent with a role of GSH as a symplastic Zn ligand.

PCs may serve a similar function because PC-deficient *A. thaliana* mutants accumulated less Zn in leaves when grown in regular lab soil, that is, in the absence of any metal excess (Kühnlenz et al., 2016). Most data on PCs as metal ligands, however, are related to conditions when external concentrations of essential or nonessential metals/metalloids are so high that growth inhibition occurs. The ability of plants to tolerate exposure to the environmental pollutants Cd, As, and Pb is strongly dependent on PCs (Fischer, Kühnlenz, Thieme, Schmidt, & Clemens, 2014; Mendoza-Cozatl, Jobe, Hauser, & Schroeder, 2011). In addition, PC synthesis helps plants to survive Zn excess conditions (Kühnlenz et al., 2016). The second essential component of detoxification via the PC pathway is transport of PC-metal complexes into the vacuole (Figure 2). Responsible proteins are ABCC transporters (reviewed in Martinoia, 2018). Detection of metal-PC complexes, Cd-PC in particular, in phloem sap of plants exposed to toxic metal concentrations suggests, however, that they are not quantitatively sequestered in vacuoles (Mendoza-Cozatl, Jobe, Hauser, & Schroeder, 2011).

#### 4.4 | Organic acids

The role of organic acids as metal ligands is largely confined to acidic environments because the stability of complexes with metals is too low at neutral or alkaline pH (Álvarez-Fernández, Díaz-Benito, Abadía, Lopez-Millan, & Abadía, 2014). Thus, apoplast, xylem sap, and vacuoles represent the main compartments where such complexes occur. Concentrations of citrate or malate can reach the millimolar range there, which is high enough to enable metal-complex formation. Citrate is well established as a ligand for Fe(III) in the xylem. The existence of respective complexes was directly demonstrated (Rellán-Álvarez et al., 2010), and *A. thaliana* mutants with a defect in FRD3, a transporter mediating secretion of citrate into xylem and apoplast, showed various Fe deficiency symptoms (Roschztardt, Séguéla-Arnaud, Briat, Vert, & Curie, 2011). The recently published inventory of metal complexes in plant fluids found Fe speciation in pea xylem to be dominated by citrate and malate (Flis et al., 2016). At least a fraction of total Zn in pea xylem sap appears to be chelated by organic acids as well. This is consistent with the indirect detection of Zn-organic acid complexes in the xylem of *A. halleri* via SEC-ICP-MS (Cornu et al., 2015). Fittingly, the overexpression of FRD3 in rice can enhance the mobility of both Fe and Zn, resulting in elevated levels of these important micronutrients in the endosperm (Wu, Gruitsem, & Bhullar, 2018).

Evidence for citrate, malate, and also oxalate as metal ligands in vacuoles is much less abundant. Genetic interventions are difficult given the multiple roles organic acids play in metabolism. Thus, data are largely confined to Zn hyperaccumulating plants, where Zn concentrations in leaves are up to three orders of magnitude higher than in normal plants and the binding environment of Zn can be studied more easily. In *A. halleri*, a large fraction of total leaf Zn was estimated to be present in complexes with malate and citrate based on X-ray fluorescence data (Sarret et al., 2009). For another hyperaccumulator model species, *Noccaea caerulescens*, citrate and malate were proposed to function as Zn ligands in epidermal cells where most of the Zn accumulates, whereas in mesophyll cells, NA appeared to chelate most of the Zn (Schneider et al., 2013).

Regarding the presence of Fe-organic acid complexes in the extracellular space, a surprising finding was reported for pea seeds (Grillet, Ouedane, et al., 2014). XANES data indicated that Fe was present as Fe(III) in embryo sac liquid and mostly complexed with citrate. This was further substantiated by HILIC coupled to ICP-MS and ESI-MS. Two major Fe complexes, both consisting of four organic acid molecules (citrate and malate) and three Fe atoms, were detected. Prior to uptake into the embryo, however, Fe(III) has to be reduced to Fe(II). Unlike in Fe uptake by roots, this reduction was not mediated by a ferric reductase but chemically by ascorbic acid, secreted into the embryo sac liquid. A corresponding transport activity was found in *A. thaliana* as well, suggesting a general function of ascorbic acid in modulating the redox state of Fe.

An important LMW ligand not belonging to the carboxylic acids is phytate, that is, inositol hexakisphosphate. In cereal grains, phytate is a major binding partner of micronutrients and especially Fe (Grillet, Mari, & Schmidt, 2014). The presence of Fe-phytate complexes restricts the bioavailability of Fe in food as discussed further below.

#### 4.5 | Amino acids

The above-mentioned inventory of metal complexes in plant fluids contained several amino acids as ligands, namely, asparagine, aspartate, glutamine, phenylalanine, tryptophan, and histidine (Flis et al., 2016). This group plus cysteine overlaps well with the typical metal ligands in proteins. The side chains of cysteine, histidine, asparagine, aspartate, glutamine, and glutamate all contain donor atoms that can engage in metal binding (Bertini, Gray, Valentine, & Stiefel, 2007). Although myriad structures of metalloproteins, and with that the amino acid ligand environments of their metallic cofactors have been resolved, very little is known about the role of free amino acids as metal ligands. Again, this is explained by the inability to capture intact complexes of metals with LMW ligands. Relative to SAM-derived chelators or organic acids, amino acids appear to play only a minor role as metal ligands in plant fluids (Flis et al., 2016). Inside the cytosol, thiols are the more prominent ligands. One exception is the special case of Ni hyperaccumulation. Histidine can form stable complexes with Ni(II), a fact that has been widely used for the affinity purification of recombinant proteins. For Ni hyperaccumulators, which represent the vast

majority of the >700 plant species that show metal hyperaccumulation (Reeves et al., 2018), it was shown that elevated levels of histidine enhance symplastic mobility and xylem loading of Ni (reviewed in Krämer, 2010). Owing to its three potential binding sites, the carboxylate, amino, and imidazole groups, histidine is a potent chelator for a range of metals and is a candidate especially for binding Cu in phloem sap (Ando, Nagata, Yanagisawa, & Yoneyama, 2013).

## 5 | LMW LIGANDS AND HUMAN NUTRITION

The challenges associated with acquiring metallic micronutrients in sufficient quantities do not apply to plants alone, of course. Poor solubility or high-affinity binding to molecules in the extracellular milieu limits the bioavailability of Fe or Zn even in heterotrophic organisms. In humans, between less than 1% and about 20%, with an average of 5–8%, of the nonheme Fe contained in the diet is actually entering the body (Collings et al., 2013). Bioavailability of Zn is considered to be higher with around 25% but still low. An important factor influencing bioavailability is composition of the diet, specifically the ligand environment of micronutrients in plant tissues, changes possibly occurring during processing or storage, and the fate of respective complexes during the digestive process. A ligand such as phytate is considered an antinutrient because it is abundant in cereal grains, binds Fe and Zn very tightly, and thus severely limits the uptake of Fe and Zn by gut epithelial cells.

Increasing the bioavailable Fe and Zn in staple crops is a major research goal because deficiencies in these micronutrients belong to most prevalent health risks globally (World Health Organization, 2009). One of the promising approaches is to engineer the synthesis of plant metal chelators in order to enhance both the total content in edible organs and the bioavailability. A key element of most studies with this goal has been the overexpression of NAS (Grillet, Mari, & Schmidt, 2014; Clemens, 2014). In rice, it was shown that NA overproduction can change the ligand environment of Fe and Zn in the endosperm and positively influence bioavailability, at least according to feeding studies with mice (Lee et al., 2009, 2011). A recent example is the overexpression of OsNAS2 in wheat. Transgenic plants accumulated more NA and DMA as well as Fe and Zn in the endosperm (Beasley et al., 2019). After grains obtained from field-grown plants were milled, Fe bioavailability was enhanced according to studies employing Caco-2 cells, human epithelial colorectal adenocarcinoma cells that are widely used as a model. Such studies indicate the potential of engineering metal ligand synthesis and distribution in plants. It is conceivable that several other metal ligands may be used to favourably influence the bioavailability of micronutrients in plant-derived food, once we know more about their exact impact on metal mobility or storage.

### ACKNOWLEDGEMENT

The work in the author's laboratory on metal ligands in plants is supported by the Deutsche Forschungsgemeinschaft (CI 152/11-1).

### ORCID

Stephan Clemens  <https://orcid.org/0000-0003-0570-1060>

### REFERENCES

- Álvarez-Fernández, A., Díaz-Benito, P., Abadía, A., Lopez-Millan, A.-F., & Abadía, J. (2014). Metal species involved in long distance metal transport in plants. *Frontiers Plant Sci*, 5, 105.
- Ando, Y., Nagata, S., Yanagisawa, S., & Yoneyama, T. (2013). Copper in xylem and phloem saps from rice (*Oryza sativa*): The effect of moderate copper concentrations in the growth medium on the accumulation of five essential metals and a speciation analysis of copper-containing compounds. *Functional Plant Biology*, 40, 89–100. <https://doi.org/10.1071/FP12158>
- Andreini, C., Banci, L., Bertini, I., & Rosato, A. (2006). Zinc through the three domains of life. *Journal of Proteome Research*, 5, 3173–3178. <https://doi.org/10.1021/pr0603699>
- Argüello, J. M., Raimunda, D., & González-Guerrero, M. (2012). Metal transport across biomembranes: Emerging models for a distinct chemistry. *Journal of Biological Chemistry*, 287, 13510–13517. <https://doi.org/10.1074/jbc.R111.319343>
- Bashir, K., Nozoye, T., Nagasaka, S., Rasheed, S., Miyauchi, N., Seki, M., ... Nishizawa, N. K. (2017). Paralogs and mutants show that one DMA synthase functions in iron homeostasis in rice. *Journal of Experimental Botany*, 68, 1785–1795. <https://doi.org/10.1093/jxb/erx065>
- Beasley, J. T., Bonneau, J. P., Sánchez-Palacios, J. T., Moreno-Moyano, L. T., Callahan, D. L., Tako, E., ... Johnson, A. A. T. (2019). Metabolic engineering of bread wheat improves grain iron concentration and bioavailability. *Plant Biotechnology Journal*. <https://doi.org/10.1111/pbi.13074>
- Bertini, G., Gray, H. B., Valentine, J. S., & Stiefel, E. I. (2007). *Biological inorganic chemistry: Structure and reactivity*. Sausalito, California: University Science Books.
- Brumbarova, T., Bauer, P., & Ivanov, R. (2015). Molecular mechanisms governing *Arabidopsis* iron uptake. *Trends in Plant Science*, 20, 124–133. <https://doi.org/10.1016/j.tplants.2014.11.004>
- Burkhead, J. L., Reynolds, K. A. G., Abdel-Ghany, S. E., CoHu, C. M., & Pilon, M. (2009). Copper homeostasis. *New Phytologist*, 182, 799–816. <https://doi.org/10.1111/j.1469-8137.2009.02846.x>
- Cakmak, I., Sari, N., Marschner, H., Ekiz, H., Kalayci, M., Yilmaz, A., & Braun, H. J. (1996). Phytosiderophore release in bread and durum wheat genotypes differing in zinc efficiency. *Plant and Soil*, 180, 183–189. <https://doi.org/10.1007/BF00015301>
- Chen, Y.-T., Wang, Y., & Yeh, K.-C. (2017). Role of root exudates in metal acquisition and tolerance. *Current Opinion in Plant Biology*, 39, 66–72. <https://doi.org/10.1016/j.pbi.2017.06.004>
- Clemens, S. (2010). Zn—A versatile player in plant cell biology. (eds R. Hell, R. R. Mendel). *Cell Biology of Metals*. Heidelberg Germany: Springer.
- Clemens, S. (2014). Zn and Fe biofortification: The right chemical environment for human bioavailability. *Plant Science*, 225, 52–57. <https://doi.org/10.1016/j.plantsci.2014.05.014>
- Clemens, S., & Weber, M. (2016). The essential role of coumarin secretion for Fe acquisition from alkaline soil. *Plant Signaling & Behavior*, 11, e1114197. <https://doi.org/10.1080/15592324.2015.1114197>
- Collings, R., Harvey, L. J., Hooper, L., Hurst, R., Brown, T. J., Ansett, J., ... Fairweather-Tait, S. J. (2013). The absorption of iron from whole diets: A systematic review. *American Journal of Clinical Nutrition*, 98, 65–81. <https://doi.org/10.3945/ajcn.112.050609>
- Cornu, J.-Y., Deinlein, U., Höreth, S., Braun, M., Schmidt, H., Weber, M., ... Clemens, S. (2015). Contrasting effects of nicotianamine synthase

- knockdown on zinc and nickel tolerance and accumulation in the zinc/cadmium hyperaccumulator *Arabidopsis halleri*. *New Phytologist*, 206, 738–750. <https://doi.org/10.1111/nph.13237>
- Corso, M., Schwartzman, M. S., Guzzo, F., Souard, F., Malkowski, E., Hanikenne, M., & Verbruggen, N. (2018). Contrasting cadmium resistance strategies in two metalcolous populations of *Arabidopsis halleri*. *New Phytologist*, 218, 283–297. <https://doi.org/10.1111/nph.14948>
- Curie, C., Cassin, G., Couch, D., Divol, F., Higuchi, K., Jean, M., ... Mari, S. (2009). Metal movement within the plant: Contribution of nicotianamine and yellow stripe 1-like transporters. *Annals of Botany*, 103, 1–11. <https://doi.org/10.1093/aob/mcn207>
- Deinlein, U., Weber, M., Schmidt, H., Rensch, S., Trampczynska, A., Hansen, T. H., ... Clemens, S. (2012). Elevated nicotianamine levels in *Arabidopsis halleri* roots play a key role in zinc hyperaccumulation. *Plant Cell*, 24, 708–723. <https://doi.org/10.1105/tpc.111.095000>
- Fischer, S., Kühnlenz, T., Thieme, M., Schmidt, H., & Clemens, S. (2014). Analysis of plant Pb tolerance at realistic submicromolar concentrations demonstrates the role of phytochelatin synthesis for Pb detoxification. *Environmental Science & Technology*, 48, 7552–7559. <https://doi.org/10.1021/es405234p>
- Flis, P., Ouerdane, L., Grillet, L., Curie, C., Mari, S., & Lobinski, R. (2016). Inventory of metal complexes circulating in plant fluids: A reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. *New Phytologist*, 211, 1129–1141. <https://doi.org/10.1111/nph.13964>
- Fourcroy, P., Sisó-Terraza, P., Sudre, D., Savirón, M., Reyt, G., Gaymard, F., ... Briat, J.-F. (2014). Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by *Arabidopsis* roots in response to iron deficiency. *New Phytologist*, 201, 155–167. <https://doi.org/10.1111/nph.12471>
- Fourcroy, P., Tissot, N., Gaymard, F., Briat, J.-F., & Dubos, C. (2016). Facilitated Fe nutrition by phenolic compounds excreted by the *Arabidopsis* ABCG37/PDR9 transporter requires the IRT1/FRO2 high-affinity root Fe<sup>2+</sup> transport system. *Molecular Plant*, 9, 485–488. <https://doi.org/10.1016/j.molp.2015.09.010>
- González-Guerrero, M., & Argüello, J. M. (2008). Mechanism of Cu<sup>+</sup>-transporting ATPases: Soluble Cu<sup>+</sup> chaperones directly transfer Cu<sup>+</sup> to transmembrane transport sites. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 5992–5997. <https://doi.org/10.1073/pnas.0711446105>
- Grillet, L., Mari, S., & Schmidt, W. (2014). Iron in seeds—Loading pathways and subcellular localization. *Frontiers in Plant Science*, 4, 535.
- Grillet, L., Ouerdane, L., Flis, P., Hoang, M. T. T., Isaure, M.-P., Lobinski, R., ... Mari, S. (2014). Ascorbate efflux as a new strategy for iron reduction and transport in plants. *Journal of Biological Chemistry*, 289, 2515–2525. <https://doi.org/10.1074/jbc.M113.514828>
- Grillet, L., & Schmidt, W. (2017). The multiple facets of root iron reduction. *Journal of Experimental Botany*, 68, 5021–5027. <https://doi.org/10.1093/jxb/erx320>
- Hänsch, R., & Mendel, R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology*, 12, 259–266. <https://doi.org/10.1016/j.pbi.2009.05.006>
- Haydon, M. J., & Cobbett, C. S. (2007). Transporters of ligands for essential metal ions in plants. *New Phytologist*, 174, 499–506. <https://doi.org/10.1111/j.1469-8137.2007.02051.x>
- Haydon, M. J., Kawachi, M., Wirtz, M., Hillmer, S., Hell, R., & Krämer, U. (2012). Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in *Arabidopsis*. *Plant Cell*, 24, 724–737. <https://doi.org/10.1105/tpc.111.095042>
- Hider, R. C., & Kong, X. (2013). Iron speciation in the cytosol: An overview. *Dalton Transactions*, 42, 3220–3229. <https://doi.org/10.1039/C2DT32149A>
- Husted, S., Persson, D. P., Laursen, K. H., Hansen, T. H., Pedas, P., Schiller, M., ... Schjoerring, J. K. (2011). Review: The role of atomic spectrometry in plant science. *Journal of Analytical Atomic Spectrometry*, 26, 52–79. <https://doi.org/10.1039/C0JA00058B>
- Irtelli, B., Petrucci, W. A., & Navari-Izzo, F. (2009). Nicotianamine and histidine/proline are, respectively, the most important copper chelators in xylem sap of *Brassica carinata* under conditions of copper deficiency and excess. *Journal of Experimental Botany*, 60, 269–277. <https://doi.org/10.1093/jxb/ern286>
- Irving, H., & Williams, R. J. P. (1953). The stability of transition-metal complexes. *Journal of the Chemical Society*, 3192–3210. <https://doi.org/10.1039/jr9530003192>
- Ishimaru, Y., Kakei, Y., Shimo, H., Bashir, K., Sato, Y., Sato, Y., ... Nishizawa, N. K. (2011). A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. *Journal of Biological Chemistry*, 286, 24649–24655. <https://doi.org/10.1074/jbc.M111.221168>
- Jin, C. W., You, G. Y., He, Y. F., Tang, C., Wu, P., & Zheng, S. J. (2007). Iron deficiency-induced secretion of phenolics facilitates the reutilization of root apoplastic iron in red clover. *Plant Physiology*, 144, 278–285. <https://doi.org/10.1104/pp.107.095794>
- Kazemi-Dinan, A., Thomaschky, S., Stein, R. J., Krämer, U., & Müller, C. (2014). Zinc and cadmium hyperaccumulation act as deterrents towards specialist herbivores and impede the performance of a generalist herbivore. *New Phytologist*, 202, 628–639. <https://doi.org/10.1111/nph.12663>
- Kobayashi, T., & Nishizawa, N. K. (2012). Iron uptake, translocation, and regulation in higher plants. *Annual Review of Plant Biology*, 63, 131–152. <https://doi.org/10.1146/annurev-arplant-042811-105522>
- Kobayashi, T., Nozoye, T., & Nishizawa, N. K. (2019). Iron transport and its regulation in plants. *Free Radical Biology & Medicine*, 133, 11–20. <https://doi.org/10.1016/j.freeradbiomed.2018.10.439>
- Kochian, L. V., Piñeros, M. A., Liu, J., & Magalhaes, J. V. (2015). Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annual Review of Plant Biology*, 66, 571–598. <https://doi.org/10.1146/annurev-arplant-043014-114822>
- Krämer, U. (2010). Metal hyperaccumulation in plants. *Annual Review of Plant Biology*, 61, 517–534. <https://doi.org/10.1146/annurev-arplant-042809-112156>
- Krämer, U., & Clemens, S. (2005). Functions and homeostasis of zinc, copper and nickel in plants. *Topics in Current Genetics*, 14, 215–271. [https://doi.org/10.1007/4735\\_96](https://doi.org/10.1007/4735_96)
- Krämer, U., Talke, I. N., & Hanikenne, M. (2007). Transition metal transport. *FEBS Letters*, 581, 2263–2272. <https://doi.org/10.1016/j.febslet.2007.04.010>
- Krežel, A., & Maret, W. (2016). The biological inorganic chemistry of zinc ions. *Archives of Biochemistry and Biophysics*, 611, 3–19. <https://doi.org/10.1016/j.abb.2016.04.010>
- Kühnlenz, T., Hofmann, C., Uraguchi, S., Schmidt, H., Schempp, S., Weber, M., ... Clemens, S. (2016). Phytochelatin synthesis promotes leaf Zn accumulation of *Arabidopsis thaliana* plants grown in soil with adequate Zn supply and is essential for survival on Zn-contaminated soil. *Plant & Cell Physiology*, 57, 2342–2352. <https://doi.org/10.1093/pcp/pcw148>
- Kühnlenz, T., Schmidt, H., Uraguchi, S., & Clemens, S. (2014). *Arabidopsis thaliana* phytochelatin synthase 2 is constitutively active in vivo and can rescue the growth defect of the PCS1-deficient *cad1-3* mutant on Cd-contaminated soil. *Journal of Experimental Botany*, 65, 4241–4253. <https://doi.org/10.1093/jxb/eru195>

- Kumar, R. K., Chu, H.-H., Abundis, C., Vasques, K., Rodriguez, D. C., Chia, J.-C., ... Walker, E. L. (2017). Iron-nicotianamine transporters are required for proper long distance iron signaling. *Plant Physiology*, 175, 1254–1268. <https://doi.org/10.1104/pp.17.00821>
- Lanquar, V., Grossmann, G., Vinkenborg, J. L., Merckx, M., Thomine, S., & Frommer, W. B. (2014). Dynamic imaging of cytosolic zinc in *Arabidopsis* roots combining FRET sensors and RootChip technology. *New Phytologist*, 202, 198–208. <https://doi.org/10.1111/nph.12652>
- Lee, S., Jeon, U. S., Lee, S. J., Kim, Y. K., Persson, D. P., Husted, S., ... An, G. (2009). Iron fortification of rice seeds through activation of the nicotianamine synthase gene. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 22014–22019. <https://doi.org/10.1073/pnas.0910950106>
- Lee, S., Persson, D. P., Hansen, T. H., Husted, S., Schjoerring, J. K., Kim, Y.-S., ... An, G. (2011). Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase. *Plant Biotechnology Journal*, 9, 865–873. <https://doi.org/10.1111/j.1467-7652.2011.00606.x>
- Liu, W.-J., Wood, B. A., Raab, A., McGrath, S. P., Zhao, F.-J., & Feldmann, J. (2010). Complexation of arsenite with phytochelatins reduces arsenite efflux and translocation from roots to shoots in *Arabidopsis*. *Plant Physiology*, 152, 2211–2221. <https://doi.org/10.1104/pp.109.150862>
- Maret, W. (2013). Zinc biochemistry: From a single zinc enzyme to a key element of life. *Advances in Nutrition*, 4, 82–91. <https://doi.org/10.3945/an.112.003038>
- Marschner, H., & Römheld, V. (1994). Strategies of plants for acquisition of iron. *Plant and Soil*, 165, 261–274. <https://doi.org/10.1007/BF00008069>
- Martinoia, E. (2018). Vacuolar transporters—Companions on a longtime journey. *Plant Physiology*, 176, 1384–1407. <https://doi.org/10.1104/pp.17.01481>
- Mendoza-Cozatl, D. G., Jobe, T. O., Hauser, F., & Schroeder, J. I. (2011). Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. *Current Opinion in Plant Biology*, 14, 554–562. <https://doi.org/10.1016/j.pbi.2011.07.004>
- Moore, K. L., Chen, Y., van de Meene, A. M. L., Hughes, L., Liu, W., Geraki, T., ... Zhao, F.-J. (2014). Combined nanoSIMS and synchrotron X-ray fluorescence reveal distinct cellular and subcellular distribution patterns of trace elements in rice tissues. *New Phytologist*, 201, 104–115. <https://doi.org/10.1111/nph.12497>
- Nishiyama, R., Kato, M., Nagata, S., Yanagisawa, S., & Yoneyama, T. (2012). Identification of Zn-nicotianamine and Fe-2'-deoxymugineic acid in the phloem saps from rice plants (*Oryza sativa* L.). *Plant & Cell Physiology*, 53, 381–390. <https://doi.org/10.1093/pcp/pcr188>
- Nozoye, T., Nagasaka, S., Kobayashi, T., Sato, Y., Uozumi, N., Nakanishi, H., & Nishizawa, N. K. (2015). The phytosiderophore efflux transporter TOM2 is involved in metal transport in rice. *Journal of Biological Chemistry*, 290, 27688–27699. <https://doi.org/10.1074/jbc.M114.635193>
- Nozoye, T., Nagasaka, S., Kobayashi, T., Takahashi, M., Sato, Y., Sato, Y., ... Nishizawa, N. K. (2011). Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *Journal of Biological Chemistry*, 286, 5446–5454. <https://doi.org/10.1074/jbc.M110.180026>
- Persson, D. P., Chen, A., Aarts, M. G., Salt, D. E., Schjoerring, J. K., & Husted, S. (2016). Multi-element bioimaging of *Arabidopsis thaliana* roots. *Plant Physiology*, 172, 835–847.
- Rajniak, J., Giehl, R. F., Chang, E., Murgia, I., Wirén, N., & Sattely, E. S. (2018). Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nature Chemical Biology*, 14, 442–450. <https://doi.org/10.1038/s41589-018-0019-2>
- Reeves, R. D., Baker, A. J. M., Jaffré, T., Erskine, P. D., Echevarria, G., & van der Ent, A. (2018). A global database for plants that hyperaccumulate metal and metalloids trace elements. *New Phytologist*, 218, 407–411. <https://doi.org/10.1111/nph.14907>
- Rellán-Alvarez, R., Abadía, J., & Alvarez-Fernandez, A. (2008). Formation of metal-nicotianamine complexes as affected by pH, ligand exchange with citrate and metal exchange. A study by electrospray ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 22, 1553–1562. <https://doi.org/10.1002/rcm.3523>
- Rellán-Álvarez, R., Giner-Martínez-Sierra, J., Orduna, J., Orera, I., Rodríguez-Castrillón, J. Á., García-Alonso, J. I., ... Álvarez-Fernández, A. (2010). Identification of a tri-iron (III), tri-citrate complex in the xylem sap of iron-deficient tomato resupplied with iron: New insights into plant iron long-distance transport. *Plant and Cell Physiology*, 51, 91–102. <https://doi.org/10.1093/pcp/pcp170>
- Rodríguez-Celma, J., Lin, W.-D., Fu, G.-M., Abadía, J., López-Millán, A.-F., & Schmidt, W. (2013). Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by *Arabidopsis* and *Medicago truncatula*. *Plant Physiology*, 162, 1473–1485. <https://doi.org/10.1104/pp.113.220426>
- Roschztardt, H., Séguéla-Arnaud, M., Briat, J.-F., Vert, G., & Curie, C. (2011). The FRD3 citrate effluxer promotes iron nutrition between symplastically disconnected tissues throughout *Arabidopsis* development. *The Plant Cell Online*, 23, 2725–2737. <https://doi.org/10.1105/tpc.111.088088>
- Sarret, G., Willems, G., Isaure, M. P., Marcus, M. A., Fakra, S. C., Frerot, H., ... Saumitou-Laprade, P. (2009). Zinc distribution and speciation in *Arabidopsis halleri* x *Arabidopsis lyrata* progenies presenting various zinc accumulation capacities. *New Phytologist*, 184, 581–595. <https://doi.org/10.1111/j.1469-8137.2009.02996.x>
- Schmid, N. B., Giehl, R. F. H., Doll, S., Mock, H.-P., Strehmel, N., Scheel, D., ... von Wiren, N. (2014). Feruloyl-CoA 6'-hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in *Arabidopsis*. *Plant Physiology*, 164, 160–172. <https://doi.org/10.1104/pp.113.228544>
- Schmidt, H., Günther, C., Weber, M., Spörlein, C., Loscher, S., Böttcher, C., ... Clemens, S. (2014). Metabolome analysis of *Arabidopsis thaliana* roots identifies a key metabolic pathway for iron acquisition. *PLoS ONE*, 9, e102444. <https://doi.org/10.1371/journal.pone.0102444>
- Schneider, T., Persson, D. P., Husted, S., Schellenberg, M., Gehrig, P., Lee, Y., ... Meyer, S. (2013). A proteomics approach to investigate the process of Zn hyperaccumulation in *Noccaea caerulea* (J & C. Presl) F. K. Meyer. *Plant Journal*, 73, 131–142. <https://doi.org/10.1111/tbj.12022>
- Schuler, M., Rellán-Álvarez, R., Fink-Straube, C., Abadía, J., & Bauer, P. (2012). Nicotianamine functions in the phloem-based transport of iron to sink organs, in pollen development and pollen tube growth in *Arabidopsis*. *Plant Cell*, 24, 2380–2400. <https://doi.org/10.1105/tpc.112.099077>
- Senoura, T., Sakashita, E., Kobayashi, T., Takahashi, M., Aung, M. S., Masuda, H., ... Nishizawa, N. K. (2017). The iron-chelate transporter OsYSL9 plays a role in iron distribution in developing rice grains. *Plant Molecular Biology*, 95, 375–387. <https://doi.org/10.1007/s11103-017-0656-y>
- Seth, C. S., Remans, T., Keunen, E., Jozefczak, M., Gielen, H., Opendakker, K., ... Cuypers, A. (2012). Phytoextraction of toxic metals: A central role for glutathione. *Plant, Cell & Environment*, 35, 334–346. <https://doi.org/10.1111/j.1365-3040.2011.02338.x>
- Sisó-Terraza, P., Rios, J. J., Abadía, J., Abadía, A., & Álvarez-Fernández, A. (2016). Flavins secreted by roots of iron-deficient *Beta vulgaris* enable

- mining of ferric oxide via reductive mechanisms. *New Phytologist*, 209, 733–745. <https://doi.org/10.1111/nph.13633>
- Siwinska, J., Siatkowska, K., Olry, A., Grosjean, J., Hehn, A., Bourgaud, F., ... Ilnatowicz, A. (2018). Scopoletin 8-hydroxylase: A novel enzyme involved in coumarin biosynthesis and iron-deficiency responses in *Arabidopsis*. *Journal of Experimental Botany*, 69, 1735–1748. <https://doi.org/10.1093/jxb/ery005>
- Templeton, D. M., Ariese, F., Cornelis, R., Danielsson, L.-G., Muntau, H., Leeuwen, H., & van & Lobinski R. (2000). Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). *Pure and Applied Chemistry*, 72, 1453–1470. <https://doi.org/10.1351/pac200072081453>
- Tsai, H.-H., Rodríguez-Celma, J., Lan, P., Wu, Y.-C., Vélez-Bermúdez, I. C., & Schmidt, W. (2018). Scopoletin 8-hydroxylase-mediated fraxetin production is crucial for iron mobilization. *Plant Physiology*, 177, 194–207.
- Tsai, H. H., & Schmidt, W. (2017). Mobilization of iron by plant-borne coumarins. *Trends in Plant Science*, 22, 538–548. <https://doi.org/10.1016/j.tplants.2017.03.008>
- Uraguchi, S., Weber, M., & Clemens, S. (2019). Elevated root nicotianamine concentrations are critical for Zn hyperaccumulation across diverse edaphic environments. *Plant, Cell & Environment*, 42, 2003–2014. <https://doi.org/10.1111/pce.13541>
- Waldron, K. J., & Robinson, N. J. (2009). How do bacterial cells ensure that metalloproteins get the correct metal? *Nature Reviews Microbiology*, 7, 25–35. <https://doi.org/10.1038/nrmicro2057>
- Waldron, K. J., Rutherford, J. C., Ford, D., & Robinson, N. J. (2009). Metalloproteins and metal sensing. *Nature*, 460, 823–830. <https://doi.org/10.1038/nature08300>
- World Health Organization (2009). *Global health risks: Mortality and burden of disease attributable to selected major risks*. Geneva, Switzerland: World Health Organization.
- Wongkaew, A., Nakamura, S., Suzui, N., Yin, Y.-G., Ishii, S., Kawachi, N., ... Ohkama-Ohtsu, N. (2019). Elevated glutathione synthesis in leaves contributes to zinc transport from roots to shoots in *Arabidopsis*. *Plant Science*, 283, 416–423. <https://doi.org/10.1016/j.plantsci.2018.11.003>
- Wu, B., & Becker, J. S. (2012). Imaging techniques for elements and element species in plant science. *Metallomics*, 4, 403–416. <https://doi.org/10.1039/c2mt00002d>
- Wu, T.-Y., Grisse, W., & Bhullar, N. K. (2018). Facilitated citrate-dependent iron translocation increases rice endosperm iron and zinc concentrations. *Plant Science*, 270, 13–22. <https://doi.org/10.1016/j.plantsci.2018.02.002>
- Zhang, Y., & Gladyshev, V. N. (2011). Comparative genomics of trace element dependence in biology. *Journal of Biological Chemistry*, 286, 23623–23629. <https://doi.org/10.1074/jbc.R110.172833>
- Zhao, F.-J., Moore, K. L., Lombi, E., & Zhu, Y.-G. (2014). Imaging element distribution and speciation in plant cells. *Trends in Plant Science*, 19, 183–192. <https://doi.org/10.1016/j.tplants.2013.12.001>
- Zheng, L., Yamaji, N., Yokosho, K., & Ma, J. F. (2012). YSL16 is a phloem-localized transporter of the copper-nicotianamine complex that is responsible for copper distribution in rice. *Plant Cell*, 24, 3767–3782. <https://doi.org/10.1105/tpc.112.103820>

**How to cite this article:** Clemens S. Metal ligands in micronutrient acquisition and homeostasis. *Plant Cell Environ.* 2019;42:2902–2912. <https://doi.org/10.1111/pce.13627>