Coordinated homeostasis of essential mineral nutrients: a focus on iron

Marc Hanikenne¹, Sara M. Esteves¹, Steven Fanara¹, Hatem Rouached^{2,3,4}

¹ InBioS - PhytoSystems, Functional Genomics and Plant Molecular Imaging, University of Liège, 4000 Liège, Belgium

² BPMP, Univ Montpellier, CNRS, INRA, Montpellier SupAgro, Montpellier, France

³ Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA

⁴ Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA

To whom correspondence should be addressed: Dr. Marc Hanikenne, InBioS - PhytoSystems, Functional Genomics and Plant Molecular Imaging, University of Liège, Quartier de la Vallée, 1, Chemin de la Vallée, 4 - Bât B22 4000 Liège, Belgium. Telephone: +32-4-3663844; Fax: +32-4-3662960; E-mail: marc.hanikenne@uliege.be

Highlight

Considering how the regulation of nutrient homeostasis networks is interconnected to determine growth in plants is topical. This review focuses on iron and its interactions with other macro- and micronutrients.

Abstract

In plants, iron (Fe) transport and homeostasis are highly regulated processes. Fe deficiency or excess dramatically limit plant and algae productivity. Interestingly, complex and unexpected interconnections between Fe and various macro- and micronutrient homeostatic networks, supposedly maintaining general ionic equilibrium and balanced nutrition, are currently uncovered. Although these phenomena have profound consequences on our understanding of Fe homeostasis and its regulation, the molecular bases and biological significance of these interactions remain poorly understood. Here, we review recent knowledge gained on how Fe interacts with micronutrient (e.g. zinc, manganese) and macronutrient (e.g. sulfur, phosphate) homeostasis, and on how these interactions affect Fe uptake and trafficking. We finally highlight the importance of developing an improved model of how Fe signaling pathways are integrated into functional networks to control plant growth and development in response to fluctuating environments.

Keywords:

Iron, iron deficiency, iron uptake, nutrient interaction, ion signaling crosstalk, nicotianamine, mugineic acid, root growth

Introduction

Iron (Fe) is an essential element for all living organisms (Kobayashi and Nishizawa, 2012). Plants and algae are an important source of Fe entry in the terrestrial and aquatic food web, respectively. However, although it is abundant in the environment, Fe is poorly available to plants in soils (Marschner, 2012), and hence Fe deficiency is a major issue limiting crop productivity, as well as the quality of agricultural products. Similarly, algae primary productivity is severely impaired by low Fe availability in the open ocean (Sunda *et al.*, 1991; Strzepek and Harrison, 2004). Consequently, Fe deficiency threatens human health. Indeed, according to WHO (<http://www.who.int/nutrition/en/>), one third of the human population worldwide is affected by Fe deficiency anemia. Therefore, there is an urgent need to better understand how photosynthetic organisms regulate Fe homeostasis.

In plant cells, while Fe can also be found in substantial amount in the nucleolus (Roschztardt *et al.*, 2011), up to 80% of cellular Fe is found in chloroplasts in leaves (Terry and Abadia, 1986) and Fe represents >60% of micronutrients in plant mitochondria (Tan *et al.*, 2010), where it is found associated to heme and iron-sulfur clusters or as a cation. Photosynthesis and respiration therefore require adequate Fe supply and are highly sensitive to Fe deficiency (Nouet *et al.*, 2011; Hanikenne *et al.*, 2014; Vigani and Hanikenne, 2018). Adequate Fe supply is also required for several developmental processes, including for instance root growth, flowering or pollen production (Briat *et al.*, 2015a; Bouain *et al.*, 2019b). Fe deficiency is thus deleterious for the functioning of plant cells. In contrast, the redox properties that make Fe an essential cofactor of multiple biological processes can be harmful when Fe is delivered to cells in excessive amounts or if its interactions with biological macromolecules are uncontrolled (Stohs and Bagchi, 1995; Marschner, 2012). Therefore, plants possess sophisticated mechanisms, referred to as Fe homeostasis, to adjust, within a range of Fe supply, Fe uptake and distribution to needs throughout their development and upon changes in their environment (Kobayashi and Nishizawa, 2012; Thomine and Vert, 2013; and this special issue).

In addition to Fe, 13 mineral (micro- and macro-)elements are also essential for plant growth and development, whereas 5 others are considered beneficial (Marschner, 2012). Plants require an optimal and properly balanced supply of these elements. Nevertheless, in nature, plants can encounter multiple combinations of nutrient excesses and/or limitations. So far, the bulk of experimental studies mainly focused on the responses of plants to variation in the supply of a single element. This enables the detailed dissection of the molecular homeostasis mechanisms for these elements, identifying transporters, chelators, assimilation pathways, as well as a number of (transcriptional) regulators and to a lower extent sensing and signaling mechanisms. More recently, research opened to the investigation of interactions between nutrient homeostatic networks at the molecular level, revealing (i) multiple ways of nutrient inter-dependency: an element may be

required for the proper uptake of another, deficiency or excess of one element impacts positively or negatively the uptake another, elements share pathways (e.g. transporters or chelators with broad specificity) or regulatory processes and (ii) hidden responses that are more than the addition of single stress responses (Bouain *et al.*, 2019b). Those networks need to be examined in more detail to better comprehend the challenges faced by plants in their natural environments during their lifetime. This will enable the design of integrated and sustainable approaches to ensure plant production and quality.

Here, we present recent findings on the role of Fe in regulating complex developmental processes in plants, with a focus on the regulation of primary root growth. We then review our current knowledge on the molecular mechanisms orchestrating homeostatic interactions of Fe with other micro- and macronutrients in vascular plants, pinpointing whenever possible commonalities and differences between monocots and dicots. Finally, we also present a brief overview of those interactions in algae in comparison to higher plants, highlighting originalities found in algae (Box 1). Gaining new insight on how plants regulate Fe homeostasis will likely help to improve crop production on Fe-poor soils and to meet the challenge of global population growth and increasing demand for biofortified food.

Regulation of primary root growth by iron availability

Fe uptake from the soil is mediated by the root system (Dubeaux *et al.*, 2015). Root-related processes are major targets of responses to changing Fe availability in the rhizosphere (Fig. 1). Fe deficiency or excess imposes drastic changes on the root system architecture, which varies within and between plant species (Gruber *et al.*, 2013). This supports the potential for discovering key genes and signaling pathways by screening for phenotypic diversity in Fe stress responses across plant ecotypes and accessions, using quantitative genetic approaches (Dubeaux *et al.*, 2015; Rouached and Rhee, 2017). Recently, genome-wide association studies (GWAS) were employed in the discovery of genes underpinning the root architecture in response to either Fe limitation or excess, in the model plant *Arabidopsis thaliana* (Arabidopsis). *FRO2* (*FERRIC REDUCTASE OXIDASE 2*) was indeed identified as a gene responsible for the regulation of primary root growth (PRG) in response to Fe deficiency (Satbhai *et al.*, 2017). PRG variation in Fe-deficient conditions is explained by sequence variation of the *FRO2* locus that causes differential gene expression, as well as ferric-chelate reductase activity (Satbhai *et al.*, 2017). The same GWAS approaches were used more recently to identify additional genes involved in the control of root growth rate in response to Fe deficiency in Arabidopsis. Several candidate genes were identified, although their functional

validation and the discovery of causal polymorphisms await future study (Bouain *et al.*, 2019a). GWAS has also been employed to identify loci associated with the natural variation of root growth in response to Fe toxicity. In this context, a causal gene named *S-NITROSOGLUTATHIONE-REDUCTASE* (*GSNOR*) was identified and characterized as essential in determining root growth in high Fe conditions (Li *et al.*, 2019). Plants with a non-functional *GSNOR* gene display higher Fe tolerance than plants with functional alleles. This mechanism appears to be related to the activities of a gaseous molecule, nitric oxide (NO). *GSNOR* likely plays a central role in NO metabolism and regulates the plant's ability to respond to cellular stress and damage (Li *et al.*, 2019). It has been proposed that in response to high Fe concentrations, the NO levels increase, impairing the tolerance of the plant roots (Li *et al.*, 2019). This mechanism is likely conserved between monocots and dicots, but it remains to be confirmed if this mechanism can be generalized to all higher plants. *GSNOR* and NO signaling contribute to salt stress response in the green alga *Chlamydomonas*, but have not been tied to Fe homeostasis (Chen *et al.*, 2016).

Iron and micronutrient interactions: root uptake

The interactions of Fe homeostasis with other micronutrients [e.g. zinc (Zn), manganese (Mn)] or toxic and non-essential metal cations [e.g. cadmium (Cd)] are strongly dependent on the Fe uptake mechanisms, which can come in two different flavors in plants (Fig. 2) and are even more diversified in algae (Box 1). In dicot plants such as *Arabidopsis* exists a reduction-based strategy (or Strategy I) where Fe^{2+} is taken-up by cells, whereas chelation and uptake of chelated Fe^{3+} (Strategy II) is found in graminaceous monocots, i.e. grasses (Marschner and Römheld, 1994). Both strategies however share many molecular actors (see below and Grillet and Schmidt, 2019). Unchelated Fe^{3+} is also taken up directly in many algae (Box 1) (Blaby-Haas and Merchant, 2017). Both strategies of Fe^{2+} or Fe^{3+} uptake coexist in several species, rice or *Chlamydomonas* for instance (see below and Box 1) (Blaby-Haas and Merchant, 2017; Kobayashi *et al.*, 2019). Divalent metal cations (Zn^{2+} , Mn^{2+} , Cd^{2+}) will therefore have a higher impact on Fe homeostasis in plants using the reduction strategy and Fe^{2+} uptake. For instance, Zn excess in *Arabidopsis* results in shoot chlorosis, reduced shoot Fe accumulation and more generally in the induction of a secondary Fe deficiency. These Zn toxicity symptoms result from competition for uptake as well as for Fe binding sites in proteins (e.g. involved in respiration and photosynthesis), and are alleviated by elevated Fe supply (Fukao *et al.*, 2011; Shanmugam *et al.*, 2011; Zargar *et al.*, 2015; Lešková *et al.*, 2017). Conversely, Fe deficiency results in increased shoot accumulation of Zn, but also Mn and cobalt (Co) (Baxter *et al.*, 2008). Such interactions were also documented in multiple dicot species in addition to *Arabidopsis* (Foy *et al.*, 1978; Broadley *et al.*, 2007), for instance *Brassica rapa* (Li *et al.*, 2014) or Poplar (Ariani *et al.*, 2015).

In *Arabidopsis*, upon acidification of the soil (Santi and Schmidt, 2009) and reduction of Fe^{3+} into Fe^{2+} by FRO2 (Robinson *et al.*, 1999), Fe^{2+} is taken up in root epidermal cells by IRT1 (IRON-REGULATED TRANSPORTER 1), a transporter of the ZIP (ZINC-REGULATED AND IRON-REGULATED-like PROTEINS) family (Fig. 2A). IRT1 is a high affinity transporter but has a low specificity: in addition to Fe^{2+} , it transports a range of other divalent metal cations, including Zn^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} and Cd^{2+} (Eide *et al.*, 1996; Vert *et al.*, 2002; Nishida *et al.*, 2011). IRT1 is a major contributor to the interaction of Fe homeostasis with other metal cations, in such a way that back-up systems are implemented in the Fe deficiency response to accommodate the IRT1-mediated non-specific uptake of non-Fe metal cations. Hence, up-regulation of *HMA3* (HEAVY METAL ATPASE 3, Morel *et al.*, 2009), *MTP3* (METAL TOLERANCE PROTEIN 3, Arrivault *et al.*, 2006), *MTP8* (Eroglu *et al.*, 2016) and *FPN2* (FERROPORTIN2, Schaaf *et al.*, 2006; Morrissey *et al.*, 2009) upon Fe deficiency ensures increased vacuolar storage capacity of, respectively, Zn/Cd, Zn, Mn and Co/Ni in root vacuoles, thus effectively limiting their toxicity in roots and their root-to-shoot translocation, whereas *IRT2* ensures buffering of Fe itself in intracellular vesicles (Vert *et al.*, 2009). This back-up system is coordinated by the complex action of the master Fe deficiency regulator FIT (FER-like IRON-DEFICIENCY-INDUCED TRANSCRIPTION FACTOR) and a multi-layer combination of additional transcription factors [e.g. more than 14 bHLH (BASIC HELIX-LOOP-HELIX) proteins, 2 MYB (MYELOBLASTOSIS)] and regulators [e.g. E3 Ubiquitin Ligases BTS (BRUTUS) and BTS-like targeting proteins for proteasome degradation], as well as hormonal control (e.g. by jasmonic acid, ethylene or gibberellins) and NO signaling. These processes have been extensively detailed in recent reviews (see for instance Brumbarova *et al.*, 2015; Buet and Simontacchi, 2015; Rodríguez-Celma *et al.*, 2019; Kobayashi *et al.*, 2019; Wu and Ling, 2019 and this special issue). Recently, FBP (FIT-BINDING PROTEIN) was shown to control FIT activity, and to play an important role in fine-tuning Fe and Zn interactions (Chen *et al.*, 2018). In addition, the IRT1 protein itself is also subjected to a complex regulation by not only Fe but also by non-Fe metals (Zn, Mn, Co), at the transcriptional and post-transcriptional levels (Connolly *et al.*, 2002; Fukao *et al.*, 2011; Shanmugam *et al.*, 2011). Internalization of IRT1 from the plasma membrane upon ubiquitination followed by its recycling to the plasma membrane or its targeting for degradation in vacuoles is regulated by non-Fe metal (Zn, Mn, Co) availability to ensure proper balance between Fe uptake and the hazardous effects of excessive uptake of other divalent metals (Barberon *et al.*, 2011, 2014; Dubeaux *et al.*, 2018).

Evidence is accumulating that the broad specificity of IRT1 plays also a role in metal uptake in so-called metal hyperaccumulator plants (Krämer, 2010; Hanikenne and Nouet, 2011; Merlot *et al.*, 2018) and that IRT1 may be a major determinant of the intraspecific variation of the trait. Indeed, variation of *IRT1* gene expression or function is linked to (i) variation in Zn and/or Cd shoot

accumulation among populations of the Zn/Cd hyperaccumulators *Arabidopsis halleri* (Corso *et al.*, 2018; Schwartzman *et al.*, 2018) and *Nocca caerulescens* (Halimaa *et al.*, 2019); and (ii) Ni shoot accumulation in Ni-hyperaccumulating populations of *N. caerulescens* (Halimaa *et al.*, 2014a,b) and in *Senecio coronatus* (Meier *et al.*, 2018). More generally, exposure to high metal levels and metal hyperaccumulation itself interferes with Fe homeostasis (Talke *et al.*, 2006; Krämer *et al.*, 2007; Lin *et al.*, 2009; Willems *et al.*, 2010; Sinclair and Krämer, 2012; Charlier *et al.*, 2015), and maintaining Fe homeostasis is thus key in hyperaccumulator species. This is achieved, in at least some populations or species, by inducing an Fe deficiency response, which mobilizes specific parts of the multi-layer regulation pathway found in *Arabidopsis* (Shanmugam *et al.*, 2011; Corso *et al.*, 2018; Schwartzman *et al.*, 2018).

If Fe deficiency results in Zn, Co and Cd accumulation in *Arabidopsis* shoots (Col-0 accession), it has the opposite effect on molybdenum (Mo) shoot accumulation (Baxter *et al.*, 2008). Indeed, the acidification of the medium associated to the Fe uptake strategy I limits Mo bioavailability (Marschner, 2012). In addition, several micro-RNAs initially identified as major players on Cu homeostasis (Pilon 2017) were recently shown to also be linked to Fe homeostasis, by controlling the oxidative stress response and oxidative metabolic processes (Carrió-Seguí *et al.*, 2019; Waters *et al.*, 2012).

In grasses, Fe acquisition involves the secretion of mugineic acid family (MA) phytosiderophores, high affinity Fe^{3+} chelators able to solubilize Fe from the soil (Fig. 2B) (Marschner and Römheld, 1994; Kobayashi and Nishizawa, 2012). Upon synthesis from S-adenosyl-L-methionine (SAM), with nicotianamine (NA, see below) as intermediate, MA are secreted into the soil by TOM1 (TRANSPORTER OF MUGINEIC ACID FAMILY PHYTOSIDEROPHORES 1) (Nozoye *et al.*, 2011). MA-Fe(III) complexes are then taken-up by YS1/YSL transporters (YELLOW-STRIPE 1/YSL-Like) (Curie *et al.*, 2001; Inoue *et al.*, 2009). This strategy is particularly advantageous on calcareous soils where high pH reduces strongly Fe availability (Marschner and Römheld, 1994). Many commonalities have been found between the regulation of the Fe deficiency response in dicots and grasses, including the involvement of bHLH transcription factors and E3 Ubiquitin Ligases, but with also some differences, including the key role of IDEF1 and IDEF2 (IRON DEFICIENCY-RESPONSIVE ELEMENT BINDING FACTOR) transcription factors in transcriptional regulation and Fe status sensing in rice (for detailed reviews, see Kobayashi and Nishizawa, 2012, 2015; Kobayashi *et al.*, 2019).

Rice (*Oryza sativa*) has been the most used model to uncover metal homeostasis mechanisms in grasses. However, in contrast to other graminaceous plants, it uses a Fe uptake strategy, which possibly evolved prior to rice domestication (Wairich *et al.*, 2019), combining elements of both reduction and chelation strategies and this may have biased our view on how Fe homeostasis takes

place in grasses. Indeed, OsIRT1 and OsIRT2, two proteins related to the Arabidopsis IRT1 protein (Evens *et al.*, 2017), are involved in Fe^{2+} uptake, in the absence of both acidification by H^+ -ATPase and ferric-chelate reductase activity. The two corresponding genes are highly induced upon Fe deficiency. In addition to the chelation strategy, direct uptake of Fe^{2+} comes handy in paddy fields, which have low oxygen and high Fe^{2+} levels (Ishimaru *et al.*, 2006; Cheng *et al.*, 2007). It possibly comes at a cost of specificity as overexpression of OsIRT1 in rice not only results in increased accumulation of Fe, but also of Zn and Cd in root and shoot tissues, and in higher sensitivity to high Zn and Cd exposure (Lee and An, 2009). OsIRT1 and OsIRT2 were suggested to contribute to higher Cd accumulation upon Fe deficiency (Nakanishi *et al.*, 2006). In contrast to its Arabidopsis homolog, however, the *OsIRT1* transcript level is not induced by Zn excess, suggesting a distinct impact of Zn in the two species (Ishimaru *et al.*, 2008). The combined strategy of Fe uptake is limited to rice and a few relatives (Ishimaru *et al.*, 2008; Wairich *et al.*, 2019). Although its genome contains an *IRT1* homolog (Li *et al.*, 2013; Evens *et al.*, 2017) and genes putatively encoding an H^+ -ATPase and a ferric-chelate reductase (Li *et al.*, 2018), maize (*Zea mays*) is indeed strictly dependent on MA-Fe(III) uptake for Fe acquisition and survival, whereas rice plants unable to synthesize MAs are healthy when supplied with Fe^{2+} (Cheng *et al.*, 2007). An IRT1 homolog is also found in barley (Evens *et al.*, 2017), but is important for Mn uptake, and not Fe (Pedas *et al.*, 2008).

Grasses are more sensitive to Zn toxicity than dicots, especially on calcareous soils. This observation can be linked to the Fe deficiency-induced production of phytosiderophores by roots that also strongly solubilizes Zn (Chaney, 1993). MAs were indeed shown to contribute to chelation and uptake of non-Fe metals, including Zn upon Zn deficiency in the form of MA-Zn(II), in barley and maize (von Wirén *et al.*, 1996; Suzuki *et al.*, 2006). In rice, secretion of MAs upon Zn deficiency is low compared to other grasses (Suzuki *et al.*, 2008), but appears to be sufficient to account for Zn uptake in these conditions (Arnold *et al.*, 2010; Ptashnyk *et al.*, 2011; Marković *et al.*, 2017). MAs are also involved in Zn translocation to shoot tissues in rice (Suzuki *et al.*, 2008). In many cases, overexpression of elements of the MA synthesis, secretion, uptake or translocation pathways results in an increased Fe but also Zn content in rice grains (Bashir *et al.*, 2013).

Iron and micronutrient interactions: intracellular and long distance trafficking

In addition to uptake, Fe and micronutrient intracellular trafficking and long distance distribution within the plant is mediated by common metal chelators and (chelated) metal transporters (Fig. 2). For instance, the ZIP transporter IRT3 is a plasma-membrane located Zn and Fe transporter in Arabidopsis, and was therefore suggested to contribute to cellular uptake of Fe and Zn (Lin *et al.*, 2009). The *IRT3* gene is induced by Zn deficiency (Talke *et al.*, 2006) but not by Fe deficiency (Yang *et al.*, 2010), and its expression level, together with that of *ZIP4*, has been linked to variation of Zn

deficiency tolerance among *Arabidopsis* accessions (Campos *et al.*, 2017). *IRT3* is also associated with a quantitative trait locus for Fe accumulation in shoots of *A. halleri*, suggesting that it may contribute to maintenance of Fe homeostasis in the Zn/Cd hyperaccumulator (Willems *et al.*, 2010). Several other *Arabidopsis* ZIP transporters were shown to transport Fe and Zn (ZIP3) or to be regulated by both the Fe and Zn status of the plant (ZIP10, ZIP11, ZIP12) (Shanmugam *et al.*, 2013), but as for most ZIP transporters in plants, their individual contributions to metal homeostasis remain largely unknown (Ricachenevsky *et al.*, 2015).

Transporters of the NRAMP (NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN) family are also tying Fe to other microelements, such as Mn and Zn. Indeed, the *Arabidopsis* NRAMP1 was shown to transport Mn and Fe in yeast (Thomine *et al.*, 2000; Curie *et al.*, 2000), and to be the main Mn uptake system in plant roots (Cailliatte *et al.*, 2010; Bian *et al.*, 2018). NRAMP1 also contributes to Fe uptake together with IRT1 (Castaings *et al.*, 2016). As IRT1 (see above), NRAMP1 undergoes intracellular trafficking, involving a phosphatidylinositol 3-phosphate-binding protein, with a localization on endomembranes or the plasma membrane, possibly enabling the fine-tuning of Fe, and Mn, uptake (Agorio *et al.*, 2017). Higher expression of NRAMP1 has been associated with higher shoot Cd accumulation among rice (Takahashi *et al.*, 2011a,b) or *N. caerulea* (Milner *et al.*, 2014) accessions. In rice and possibly other monocots, NRAMP5, primarily a Mn transporter, is a major determinant of Cd accumulation in grains (reviewed in Clemens, 2019).

NRAMP3 and NRAMP4 are two additional Fe and Mn transporters, localized in the vacuolar membrane in *Arabidopsis*. Together, they are essential for Fe mobilization from vacuoles during seed germination (Lanquar *et al.*, 2005), and for providing sufficient Mn for the photosynthetic apparatus in mature leaves (Lanquar *et al.*, 2010). Both proteins are furthermore contributing to Zn and Cd tolerance by mediating appropriate Fe and Mn supply to chloroplasts, thus maintaining the photosynthetic function when exposed to Zn excess or Cd (Molins *et al.*, 2013). NRAMP3 is overexpressed in *A. halleri* and *N. caerulea*, suggesting a contribution to Zn/Cd hypertolerance or hyperaccumulation (Talke *et al.*, 2006; van de Mortel *et al.*, 2006; Oomen *et al.*, 2009). The NRAMP3 gene is further associated with a Zn tolerance QTL for photosynthetic yield in a F2 progeny of an intraspecific cross in *A. halleri* (Karam *et al.*, 2019). The counterpart of NRAMP3 and NRAMP4 in *Arabidopsis* is VIT1, which is key for Fe vacuolar storage in seeds (Kim *et al.*, 2006). In rice, two VIT1 homologs (OsVIT1 and OsVIT2) are vacuolar transporters of Fe and Zn, and are responsible for vacuolar storage of these metals in flag leaf blade and sheath, and consequently controlling the transfer of both elements to grains (Zhang *et al.*, 2012).

Both inter- and intra-cellular movements of Fe and other metals (e.g. Zn, Mn) are facilitated by the production of common chelating compounds such as the organic acid citrate and NA, with which

these metals form soluble complexes (Morrissey and Guerinot, 2009; Sinclair and Krämer, 2012; Flis *et al.*, 2016). In grasses, MA phytosiderophores are additionally contributing to these processes (Suzuki *et al.*, 2008; Kobayashi and Nishizawa, 2012). The production and subcellular distribution of these metal chelator molecules are affected by metal availability. It is indeed well known that Fe deficiency and Zn excess both result in increased concentration of citrate in roots (i.e. in the xylem sap) (López-Millán *et al.*, 2000; Sarret *et al.*, 2002). Citrate has an optimum chelating capacity and forms more stable complexes at acidic pH (ranging from 5.5 to 6), a pH similar to that of the xylem sap (López-Millán *et al.*, 2000; Ryan *et al.*, 2001; Yoneyama *et al.*, 2015). At this pH range, Fe is transferred from cytoplasmic NA to citrate upon loading in the xylem sap as $[\text{FeCitrateOH}]^{-1}$ and $[\text{FeCitrate}_2]^{-3}$ complexes (López-Millán *et al.*, 2000; Ryan *et al.*, 2001; Yoneyama *et al.*, 2015). Similarly, Zn-citrate complexes are also largely present in the xylem sap (Cornu *et al.*, 2015; Flis *et al.*, 2016). Citrate loading into the xylem results from the activity of the citrate effluxer FRD3 (FERRIC REDUCTASE DEFECTIVE 3), a member of the MATE (MULTIDRUG AND TOXIC EFFLUX) family (Durrett *et al.*, 2007), whose expression in Arabidopsis is not only induced upon Fe deficiency but also upon Zn excess in roots and Zn deficiency in shoots (Pineau *et al.*, 2012; Charlier *et al.*, 2015). *frd3* mutant plants are small and chlorotic, and display constitutive overexpression of the strategy I Fe uptake machinery (see above) as well accumulation of Fe, Mn and Zn in tissues (Delhaize, 1996; Rogers and Guerinot, 2002; Green and Rogers, 2004; Durrett *et al.*, 2007). In addition to severe Fe deficiency in shoot tissues, the growth defect of the *frd3* mutant results from Mn toxicity as well as Fe accumulation-induced reactive oxygen species production and biotic stress response (Scheepers *et al.*, 2020). Zn excess partially restores growth of the mutant, by abolishing Mn accumulation in shoot and Fe accumulation in the cell-wall in roots (Scheepers *et al.*, 2020). This not only depicts the cross-talk between Fe and Zn homeostasis but also the role of FRD3 as an essential regulator of this interplay (Pineau *et al.*, 2012; Scheepers *et al.*, 2020). An homolog of FRD3 has been characterized in rice (OsFRDL1, FRD-Like 1) and shares a similar function in citrate efflux to the root xylem for Fe translocation to the shoots and in Fe distribution in panicles (Inoue *et al.*, 2004; Yokosho *et al.*, 2009, 2016; Yoneyama *et al.*, 2015). However, *osfrdl1* mutants do not display severe growth phenotypes as an Arabidopsis *frd3* mutant (Yokosho *et al.*, 2009), suggesting that chelators, in addition to citrate, are important for Fe translocation to shoot in rice. Hence, PEZ1 (PHENOLICS EFFLUX ZERO 1), a MATE transporter, is responsible for phenolic compound release into the xylem in rice roots contributing to Fe mobilization and long distance Fe translocation (Ishimaru *et al.*, 2011). TOM2 (Nozoye *et al.*, 2015) and ENA1 (EFFLUX TRANSPORTER OF NA 1) (Nozoye *et al.*, 2019) are additionally required for efflux of DMA and NA into the apoplasm, respectively, and also contribute to Fe mobilization in this compartment.

NA has a key function in the control of metal cellular levels in both monocots and dicots. The formation and the stability of the NA-metal complexes are optimal at pH above 6.5, suggesting that NA serves as a chelator predominately enriched within cells and phloem sap where it contributes to compartmentalization of metals and long distance transport (von Wirén *et al.*, 1999). NA is synthesized from SAM by NA SYNTHASE (NAS) enzymes (Pianelli *et al.*, 2005; Haydon and Cobbett, 2007a). The Arabidopsis genome contains four NAS genes (*AtNAS1-4*), whose expression is upregulated upon Fe or Zn deficiency, as well as Zn excess (Colangelo and Guerinot, 2004; van de Mortel *et al.*, 2006; Klatte *et al.*, 2009; Chen *et al.*, 2018). *nas4x-1* and *nas4x-2* quadruple mutants are sensitive to Fe deficiency but not Zn deficiency and are unable to properly distribute Fe and Zn to flowers due to the drastic reduction in NA levels, thereby resulting in sterility (Klatte *et al.*, 2009; Schuler *et al.*, 2011, 2012). NA levels are a key element controlling radial movements of metals in the roots, either favoring vacuolar storage or translocation to the shoots, two processes where Fe and Zn compete as well (Arrivault *et al.*, 2006; Chen *et al.*, 2018; Haydon *et al.*, 2012). NA overaccumulation in *A. thaliana* results in increased sensitivity to Fe deficiency, but Ni resistance, as well as decreased levels of Fe in roots resulting from enhanced translocation of Fe to shoots, and Zn elevation in both roots and shoots (Pianelli *et al.*, 2005; Kim *et al.*, 2005; Cassin *et al.*, 2009; Chen *et al.*, 2018). It was also shown that an elevated production of NA is a key factor for Zn hyperaccumulation in *A. halleri* (Weber *et al.*, 2004; Uraguchi *et al.*, 2019).

Numerous proteins are involved in both the partitioning and long distance transport of NA-metal complexes. Two major facilitator superfamily transporters, ZIF1 and ZIF2 (ZINC-INDUCED FACILITATOR), localize to the tonoplast where they control the vacuolar storage of NA-chelated metals (i.e. Fe^{2+} , Fe^{3+} , Zn^{2+}) in Arabidopsis (Beneš *et al.*, 1983; von Wirén *et al.*, 1999). ZIF1 plays a critical role in Zn detoxification under Fe deficiency (Haydon and Cobbett, 2007b), and omission of Zn from the media partially suppresses the growth defect of the Fe-deficient *zif1-3* mutant (Haydon *et al.*, 2012). ZIF1 expression is induced upon Zn excess (Haydon and Cobbett, 2007b) or Fe limitation (Haydon *et al.*, 2012). Increased vacuolar partitioning of NA through ZIF1 overexpression leads to vacuolar Zn sequestration in roots and ultimately to decreased Zn shoot levels as a result of the impairment of both Zn symplastic mobility and xylem loading (Haydon *et al.*, 2012). ZIF1 overexpressing plants also display reduced leaf cell-to-cell mobility of Fe, leading to constitutive symptoms of Fe deficiency (Haydon *et al.*, 2012). Higher expression of ZIF2 upon Zn excess leads to increased Zn tolerance thanks to favored root vacuolar Zn sequestration and prevention of NA-Zn translocation to shoots (Remy *et al.*, 2014). In contrast to non-accumulator species that achieve root vacuolar sequestration of excessive metals when exposed to increased amounts of chelating

compounds, hyperaccumulator species tend to favor the mobility of NA-chelated metal (i.e. Zn) towards the xylem (Deinlein *et al.*, 2012; Cornu *et al.*, 2015).

Involved in the transport of both NA- and MA-metal complexes in monocots, the YSL function is restricted to the mobilization of NA-chelated metals (NA-Fe, -Zn, -Ni or -Cu) in dicots (Schaaf *et al.*, 2004; Waters *et al.*, 2006; Sinclair and Krämer, 2012). In Arabidopsis, the 8 YSL genes display differential patterns of regulation Fe, Zn and Cu deficiency and/or excess (DiDonato *et al.*, 2004; Schaaf *et al.*, 2005; Le Jean *et al.*, 2005; Waters *et al.*, 2006; Conte *et al.*, 2013). Single (*ysl1*) or double mutant (*ysl1ysl3* and *ysl4ysl6*) plants mainly display alterations of NA and metal concentrations in tissues, characterized by increased levels of Zn, Mn and Cu, but reduced amount of Fe, in leaves, as well as a subsequent deficiency of Fe, Zn and Cu in seeds, leading to a low fertility (Le Jean *et al.*, 2005; Waters *et al.*, 2006; Chu *et al.*, 2010; Conte *et al.*, 2013).

In addition to citrate and NA, glutathione (GSH) is known to contribute to the crosstalk between Zn and Fe homeostasis in Arabidopsis. GSH is an antioxidant contributing to the ascorbate-glutathione cycle that plays a role in Fe-mediated Zn tolerance. Indeed, a loss-of-function mutation in *GSH1*, which encodes a γ -glutamylcysteine synthetase involved in the biosynthesis of GSH, leads to increased sensitivity to Zn excess in the concomitant presence of additional Fe (Shanmugam *et al.*, 2012). GSH also links Fe and Mo homeostasis. Indeed, the ATM3 (ABC TRANSPORTER OF THE MITOCHONDRIA 3) exports GSH-polysulfide out of the mitochondria and is required for the maturation of Fe-S cytosolic enzymes and for molybdenum cofactor (Moco) biosynthesis (Schaedler *et al.*, 2014; Teschner *et al.*, 2010). *atm3* mutants are moreover sensitive to Cd (Hanikenne *et al.*, 2005b; Kim *et al.*, 2006).

Iron and sulfur homeostasis interaction

Evidence at the molecular and morphological levels in support of the interplay between Fe and sulfur (S) homeostasis is constantly growing. It has been shown that despite the presence of sufficient S in the medium, Fe deficiency conditions lead to a S deficiency response at the molecular level in durum wheat (*Triticum turgidum* L.; Ciaffi *et al.*, 2013). Grasses (Strategy II plants) respond to Fe deficiency by secreting phytosiderophores into the rhizosphere, which are required for chelation as well as Fe uptake (see above). Interestingly, the release of these molecules is modulated by S availability: S deficiency reduces their release, whereas sulfate resupply enhances it in barley (*Hordeum vulgare* L.) (Astolfi *et al.*, 2010; Ciaffi *et al.*, 2013). On the other hand, Fe nutrition appears to affect S transport in plants, with Fe starvation increasing the expression of sulfate transporters, which enhances S uptake from the soil and influences S remobilization from the vacuole (Schuler *et al.*, 2011; Couturier *et al.*, 2013; Paolacci *et al.*, 2014; Kaur *et al.*, 2019). Fe also affects the S distribution between roots and shoots. In leaves, Fe and S are known to interact in the building of

Fe–S clusters, which are a major sink for Fe and are known to be essential for photosynthetic electron transfer, chlorophyll metabolism, respiration, and many cellular enzymatic reactions (Couturier *et al.*, 2013; Kaur *et al.*, 2019). This suggests a tight coordination between the metabolisms of these two nutrients in support of vital plant biological processes. However, the mechanisms regulating the integrated homeostasis of these two elements remains to be deciphered.

While Fe limitation strongly reduces the total S content in both shoots and roots of tomato (*Solanum lycopersicum*) plants (Zuchi *et al.*, 2015), S limitation causes a decrease in the Fe concentration in leaves. For instance, tomatoes grown under –S conditions display a reduction in Fe concentrations in comparison to plants grown under S-sufficient conditions. This low Fe accumulation could be explained by the reduction in Fe uptake capacity associated with a decrease in the expression and activity of the Fe uptake machinery. In dicots such as Arabidopsis, S deficiency strongly represses the expression of the high affinity Fe transporter *IRT1* (Frieri *et al.*, 2013). Interestingly, when challenged with both S and Fe starvation, the expression of *IRT1* is induced but to a lower extent compared to Fe deficiency conditions, which can be explained by a decreased requirement of Fe for Fe–S cluster synthesis under –S (Frieri *et al.*, 2013). Finally, in tomato plants, the activity of Fe transporters is reduced suggesting that S deficiency prevents the typical responses to Fe deficiency (Zuchi *et al.*, 2009).

Beyond ion transport activity, Fe nutrition profoundly impacts S assimilation in both graminaceous and non-graminaceous plants (Frieri *et al.*, 2013; Briat *et al.*, 2015b; Bouain *et al.*, 2019b). For instance, in Arabidopsis, gene expression profiling has revealed that a number of genes involved in the S assimilation pathway are co-expressed with Fe-deficient genes in response to Fe deficiency (Schuler *et al.*, 2011), suggesting that Fe availability partially controls the S assimilation-related genes. This control is visible at the transcriptional, posttranscriptional, and protein activity levels. Altogether, these findings provide physiological and molecular evidence for a crosstalk between the Fe and S pathways in different plant species, with marked species specificities. These findings also reveal the presence of many transcriptional and posttranscriptional regulatory mechanisms that plants can use to adapt to Fe fluctuation by modulating their S homeostasis, and *vice versa*. Further investigations are now needed to decipher the molecular details of these mechanisms.

Iron and phosphate homeostasis interaction

The interaction between Fe and phosphate (Pi) is well-recognized in soil as well as in plants (Müller *et al.*, 2007; Thibaud *et al.*, 2010; Briat *et al.*, 2015b; Bouain *et al.*, 2019b). Indeed, the complexation of Fe by Pi in the soil leads to the formation of precipitates, which decreases the availability of these two elements for plants (Briat *et al.*, 2020). While low Pi availability in soil leads to the Fe overaccumulation in plants, the converse is also true (Misson *et al.*, 2005; Hirsch *et al.*, 2006; Müller *et al.*, 2007; Thibaud *et al.*, 2010). Indeed, plants have evolved a coordinated gene expression network in order to respond jointly to element deficiencies and/or excesses. For instance, gene expression profiling of Pi-deficient plants revealed an increase in abundance of transcripts of both Pi starvation-related and Fe excess-responsive genes (Misson *et al.*, 2005; Müller *et al.*, 2007; Thibaud *et al.*, 2010). Such examples can be seen in the remarkable induction of expression of *AtFER1-4* mRNAs, which encode the Fe storage protein ferritin, accurately reflecting the increase in available Fe under Pi starvation conditions (Hirsch *et al.*, 2006). Interestingly, *AtFER1* higher expression appears to be mediated by PHOSPHATE RESPONSE 1 (PHR1) and PHR1-LIKE 1 (PHL1), key regulators of the phosphate starvation response (PSR) in plants, thus revealing a role for these transcription factors in the overall control of Fe homeostasis that is integrated with the Pi status of the plant (Bournier *et al.*, 2013; Bouain *et al.*, 2019b) (see also Box 1). Decades of research have helped to understand how plants respond to Pi deficiency, mainly in the roots, leading to the identification of a mechanism that is tightly linked to variation in Fe accumulation. Key genes involved in this process include the cell wall-targeted FERROOXIDASE (LPR1), a P5-type ATPase (PDR2), as well as the SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) transcription factor and its target *ALUMINUM ACTIVATED MALATE TRANSPORTER 1* (*ALMT1*) (Fig. 1). PDR2 controls LPR1 activity, which mediates Fe accumulation in the root tip. STOP1 upregulates *ALMT1*, which modulates exudation of malate (Müller *et al.*, 2007; Thibaud *et al.*, 2010; Balzergue *et al.*, 2017; Mora-Macías *et al.*, 2017), consequently promoting Fe deposition in the root. Thus these genes could be considered as Fe-responsive genes, and they form two modules that act in concert to provoke Fe overaccumulation in roots, which is likely a primary cause of PRG inhibition under Pi deficiencies (Müller *et al.*, 2007; Ward *et al.*, 2008; Ticconi *et al.*, 2009; Thibaud *et al.*, 2010; Briat *et al.*, 2015b; Bouain *et al.*, 2019b). This is consistent with the fact that simultaneous Fe and Pi deficiencies restore PRG in Arabidopsis. This observation reveals that plants respond to the combined Fe and Pi stress in a manner distinct from individual Fe or Pi deficiencies (Bouain *et al.*, 2019b). Key genes associated with this phenotype were recently identified and include *VARIANT IN METHYLATION 1*, *FORMIN-LIKE-PROTEIN-6* and *VOLTAGE-DEPENDENT ANION-SELECTIVE CHANNEL PROTEIN 3* (Fig. 1) (Bouain *et al.*, 2019b). Additional candidate genes to regulate root growth under co-occurring Fe and P deficiency stress

have been identified (AT1G51310; AT2G36200; AT4G09940; AT5G16580) and await functional validation (Bouain *et al.*, 2019b). It is worth mentioning that a recent study showed, unexpectedly, that if Pi deficiency stimulates early root growth rate of most accessions, Fe deficiency reduces it (Bouain *et al.*, 2019a). The combination of both Pi and Fe deficiency leads to a suppression of the growth inhibition exerted by Fe deficiency alone. Surprisingly, the Arabidopsis accession Columbia (Col-0) is not representative of the species under Pi deficiency (Bouain *et al.*, 2019a), and therefore used inappropriately as reference for these stresses in the literature. Both discoveries go against the main stream of thinking and will have an impact on future research aiming at understanding plant response to nutrient deficiency. Finally, it is clear that Fe content plays not only a nutritional role in plants but also a central “signaling” role in controlling different aspects of plant growth under other nutrient deficiencies. For example, recent work has revealed a role for Fe in regulating the number of lateral roots in response to potassium deficiency via a complex mechanism involving auxin and changes in DNA methylation (Shahzad *et al.*, 2020). In short, Arabidopsis roots exhibit increased numbers of lateral roots in response to this stress, and this response is dependent on the Fe status (Shahzad *et al.*, 2020).

Once taken up by the roots, Fe can interact with Pi, leading to a reduction in their translocation to the shoots (Cumbus *et al.*, 1977; Mathan and Amberger, 1977). In shoots, these nutrients are stored in vacuoles or distributed among organelles such as the chloroplast. It is well known that photosynthesis is severely affected by either Fe or Pi deficiency (Chen and Barak, 1982; Carstensen *et al.*, 2018). For instance, leaf Fe content correlates well with photosynthetic capacity (Terry and Abadia, 1986; Shikanai *et al.*, 2003) and Fe deficiency causes chlorosis. Intriguingly, even if the leaf Fe level is sufficient, leaves with high Pi content still show chlorosis (Dekock *et al.*, 1979). This provides further support for the interplay between Fe and Pi homeostasis in shoots. Remarkably, plants grown under a combined limitation of Fe and Pi do not show any chlorotic phenotype (Saenchai *et al.*, 2016; Shi *et al.*, 2018). Despite the importance of this phenomenon in agronomy, the characterization of Fe and Pi interactions involved in regulating photosynthesis is still in its infancy, and more research is needed to understand the complexity of how the various pathways involved are integrated. Systems genetic approaches can be used to screen for the phenotypic diversity of Fe and Pi stress responses across plant ecotypes and accessions, and would help to better understand how plants integrate nutrient deficiency signals to control photosynthesis (Rouached and Rhee, 2017). At the end of the plant life cycle, Fe is accumulated in the seed vacuoles, where it is mainly chelated and stored via its close interaction with Pi ions from phytate (Lanquar *et al.*, 2005). Phytate content is one of the key factors in Fe remobilization during germination and seedling establishment. It has been shown that transgenic plants overexpressing

bacterial phytase, an enzyme that degrades phytate, remobilize Fe faster during germination than wild type plants (Belgaroui *et al.*, 2014). From a dietary point of view for humans, phytate is limiting Fe, and also Zn, absorption in the intestinal tract, therefore contributing to mineral deficiencies (Gibson *et al.*, 2018). Finally, whereas it is known that Fe and Pi interact throughout the plant life cycle, our understanding of how this interaction, and the underlying molecular basis, varies between stages of plant growth and development remains limited.

Conclusion and perspectives

Fe deficiency or excess impacts plant metabolic functions, resulting in major physiological disorders that can adversely affect growth and development. We have discussed here that Fe homeostatic mechanisms are furthermore engaged in a wide range of interactions with other nutrients. The reductionist view of ion homeostasis regulation, in which each ion's level is controlled by its own mechanisms and signaling pathways, therefore needs to be revised (Bouain *et al.*, 2019b). This is even more clear when we consider the mitigated success rate of research efforts focused on improving Fe content in plants (Shahzad *et al.*, 2014). Investigating the precise molecular mechanisms coordinating Fe response pathways that shape plant developmental programs (i.e. root and shoot growth) in response to single or multiple nutrient deficiencies is topical. In this context, the existence of a tight coordination between the homeostasis of Fe and other mineral nutrients offers the potential to identify new mechanisms for Fe uptake and transport. A good example is the recent discovery of a new route for Pi transport and accumulation by the activation of a Zn deficiency signaling pathway (Bouain *et al.*, 2014; Khan *et al.*, 2014; Pal *et al.*, 2017; Kisko *et al.*, 2018). Similar to the effect of Zn deficiency on Pi accumulation, it has been shown that Fe accumulation strongly increases in response to combined nitrogen (N) and Pi deficiencies, as compared to the small increase observed under either single Pi or N starvation stress (Kellermeier *et al.*, 2014). Moreover, other micronutrient deficiencies (Zn or Mn for instance) often cause increased Fe uptake (and *vice versa*), likely because micronutrients share transport and chelation systems with lax specificities, and mechanisms ensuring the tight coordination of micronutrient homeostasis are only starting to be uncovered. In addition to proteins involved in Fe uptake, transport, and storage, genetic manipulation of mechanisms recently shown to play a role in Fe dynamics in plants is of equal importance. For example, autophagy, i.e. the recycling of cytoplasmic components via encapsulation in vesicles and subsequent degradation in the vacuole (recently reviewed in Chen *et al.*, 2019), influences Fe (and also N, Zn, Mn) remobilization to seeds during leaf senescence (Pottier *et al.*, 2014, 2019; Shinozaki *et al.*, 2020). Hence, genetic inactivation of *AuTophagy 5* (*ATG5*) results in a 30% reduction of Fe, as well as a 10-40% reduction of N, in Arabidopsis seeds (Pottier *et al.*,

2019; Chen *et al.*, 2019). Taken together, it is clear that designing research strategies to examine how Fe accumulation in plants is concomitantly influenced by the crosstalk of N, Pi and other micronutrient signals will help identifying new players involved in Fe uptake and transport. Future research efforts need to focus on the molecular basis of the coordinated Fe, micro- and macronutrient homeostatic mechanisms, which will enable integrated approaches to improve Fe accumulation in crops.

Accepted Manuscript

Acknowledgements

Research in the author's laboratories is funded by the "Fonds de la Recherche Scientifique–FNRS" (MIS-F.4511.16, CDR J.0009.17 and PDR-T0120.18 to MH), the University of Liège (ARC GreenMagic to MH) and the "Agence Nationale de la Recherche (ANR)" (ANR-19-CE13-0007 to HR). The Michigan State University also supports HR. MH is Senior Research Associate of the F.R.S.-FNRS and SF was doctoral fellow of the FRIA.

Author Contribution

MH and HR conceptualized the manuscript. MH, HR, SE and SF wrote the draft manuscript. MH, HR and SF made the Figures. MH and HR reviewed and edited the manuscript.

Accepted Manuscript

References

- Agorio A, Giraudat J, Bianchi MW, Marion J, Espagne C, Castaings L, Lelièvre F, Curie C, Thomine S, Merlot S.** 2017. Phosphatidylinositol 3-phosphate-binding protein AtPH1 controls the localization of the metal transporter NRAMP1 in Arabidopsis. *Proceedings of the National Academy of Sciences* **114**, E3354–E3363.
- Allen MD, del Campo JA, Kropat J, Merchant SS.** 2007. FEA1, FEA2, and FRE1, encoding two homologous secreted proteins and a candidate ferriredutase, are expressed coordinately with FOX1 and FTR1 in iron-deficient *Chlamydomonas reinhardtii*. *Eukaryotic Cell* **6**, 1841–1852.
- Ariani A, Di Baccio D, Romeo S, Lombardi L, Andreucci A, Lux A, Horner DS, Sebastiani L.** 2015. RNA sequencing of *Populus x canadensis* roots identifies key molecular mechanisms underlying physiological adaption to excess zinc. *PLoS One* **10**, e0117571.
- Arnold T, Kirk GJD, Wissuwa M, Frei M, Zhao F-J, Mason TFD, Weiss DJ.** 2010. Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. *Plant, Cell & Environment* **33**, 370–381.
- Arrivault S, Senger T, Krämer U.** 2006. The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *The Plant Journal* **46**, 861–879.
- Astolfi S, Zuchi S, Hubberten H-M, Pinton R, Hoefgen R.** 2010. Supply of sulphur to S-deficient young barley seedlings restores their capability to cope with iron shortage. *Journal of Experimental Botany* **61**, 799–806.
- Balzergrue C, Darteville T, Godon C, et al.** 2017. Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nature Communications* **8**, 15300.
- Barberon M, Dubeaux G, Kolb C, Isono E, Zelazny E, Vert G.** 2014. Polarization of IRON-REGULATED TRANSPORTER 1 (IRT1) to the plant-soil interface plays crucial role in metal homeostasis. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 8293–8298.
- Barberon M, Zelazny E, Robert S, Conéjéro G, Curie C, Friml J, Vert G.** 2011. Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) transporter controls iron uptake in plants. *Proceedings of the National Academy of Sciences of the United States of America* **108**, E450–8.
- Bashir K, Takahashi R, Nakanishi H, Nishizawa NK.** 2013. The road to micronutrient biofortification of rice: progress and prospects. *Frontiers in Plant Science* **4**, 15.
- Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE.** 2008. The leaf ionome as a multivariable system to detect a plant's physiological status. *Proceedings of the National Academy of Sciences* **105**, 12081–12086.
- Belgaroui N, Zaidi I, Farhat A, et al.** 2014. Over-expression of the bacterial phytase US417 in Arabidopsis reduces the concentration of phytic acid and reveals its involvement in the regulation of sulfate and phosphate homeostasis and signaling. *Plant & Cell Physiology* **55**, 1912–1924.
- Beneš I, Schreiber K, Ripberger H, Kircheiss A.** 1983. Metal complex formation by nicotianamine, a

possible phytosiderophore. *Experientia* **39**, 261–262.

Benitez-Nelson CR. 2000. The biogeochemical cycling of phosphorus in marine systems. *Earth-Science Reviews* **51**, 109–135.

Bian B, Kageshima S, Yano K, Fujiwara T, Kamiya T. 2018. Screening *Arabidopsis thaliana* mutants for low sensitivity to manganese identifies novel alleles of NRAMP1 and PGSIP6. *Journal of Experimental Botany* **69**, 1795–1803.

Blaby-Haas CE, Merchant SS. 2012. The ins and outs of algal metal transport. *Biochimica Biophysica Acta* **1823**, 1531–1552.

Blaby-Haas CE, Merchant SS. 2014. Lysosome-related organelles as mediators of metal homeostasis. *The Journal of Biological Chemistry* **289**, 28129–28136.

Blaby-Haas CE, Merchant SS. 2017. Regulating cellular trace metal economy in algae. *Current Opinion in Plant Biology* **39**, 88–96.

Bouain N, Korte A, Satbhai SB, Nam H-I, Rhee SY, Busch W, Rouached H. 2019a. Systems genomics approaches provide new insights into *Arabidopsis thaliana* root growth regulation under combinatorial mineral nutrient limitation. *PLoS Genetics* **15**, e1008392.

Bouain N, Krouk G, Lacombe B, Rouached H. 2019b. Getting to the Root of Plant Mineral Nutrition: Combinatorial Nutrient Stresses Reveal Emergent Properties. *Trends in Plant Science* **24**, 542–552.

Bouain N, Shahzad Z, Rouached A, Khan GA, Berthomieu P, Abdelly C, Poirier Y, Rouached H. 2014. Phosphate and zinc transport and signalling in plants: toward a better understanding of their homeostasis interaction. *Journal of Experimental Botany* **65**, 5725–5741.

Bournier M, Tissot N, Mari S, Boucherez J, Lacombe E, Briat J-F, Gaymard F. 2013. *Arabidopsis* ferritin 1 (*AtFer1*) gene regulation by the phosphate starvation response 1 (*AtPHR1*) transcription factor reveals a direct molecular link between iron and phosphate homeostasis. *The Journal of Biological Chemistry* **288**, 22670–22680.

Boyd ES, Peters JW. 2013. New insights into the evolutionary history of biological nitrogen fixation. *Frontiers in Microbiology* **4**, 201.

Boyd PW, Jickells T, Law CS, Blain S, Boyle EA, Buesseler KO, Coale KH, Cullen JJ, de Baar HJW, Follows M, Harvey M, Lancelot C, Levasseur M, Owens NPJ, Pollard R, Rivkin RB, Sarmiento J, Schoemann V, Smetacek V, Takeda S, Tsuda A, Turner S, Watson AJ. 2007. Mesoscale Iron Enrichment Experiments 1993–2005: Synthesis and Future Directions. *Science* **315**, 612–617.

Briat J-F, Dubos C, Gaymard F. 2015a. Iron nutrition, biomass production, and plant product quality. *Trends in Plant Science* **20**, 33–40.

Briat J-F, Gojon A, Plassard C, Rouached H, Lemaire G. 2020. Reappraisal of the central role of soil nutrient availability in nutrient management in light of recent advances in plant nutrition at crop and molecular levels. *European Journal of Agronomy* **116**, 126069.

- Briat J-F, Rouached H, Tissot N, Gaymard F, Dubos C.** 2015b. Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana*: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). *Frontiers in Plant Science* **6**, 290.
- Broadley MR, White PJ, Hammond JP, Zelko I, Lux A.** 2007. Zinc in plants. *New Phytologist* **173**, 677–702.
- Brodie J, Chan CX, De Clerck O, et al.** 2017. The Algal Revolution. *Trends in Plant Science* **22**, 726–738.
- Brumbarova T, Bauer P, Ivanov R.** 2015. Molecular mechanisms governing *Arabidopsis* iron uptake. *Trends in Plant Science* **20**, 124–133.
- Buet A, Simontacchi M.** 2015. Nitric oxide and plant iron homeostasis. *Annals of the New York Academy of Sciences* **1340**, 39–46.
- Cailliatte R, Schikora A, Briat J-F, Mari S, Curie C.** 2010. High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. *The Plant Cell* **22**, 904–917.
- Campos ACAL, Kruijer W, Alexander R, Akkers RC, Danku J, Salt DE, Aarts MGM.** 2017. Natural variation in *Arabidopsis thaliana* reveals shoot ionome, biomass, and gene expression changes as biomarkers for zinc deficiency tolerance. *Journal of Experimental Botany* **68**, 3643–3656.
- Cardol P, Bailleul B, Rappaport F, et al.** 2008. An original adaptation of photosynthesis in the marine green alga *Ostreococcus*. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 7881–7886.
- Carrió-Seguí À, Ruiz-Rivero O, Villamayor-Belinchón L, Puig S, Perea-García A, Peñarrubia L.** 2019. The Altered Expression of microRNA408 Influences the *Arabidopsis* Response to Iron Deficiency. *Frontiers in Plant Science* **10**.
- Carstensen A, Herdean A, Schmidt SB, Sharma A, Spetea C, Pribil M, Husted S.** 2018. The Impacts of Phosphorus Deficiency on the Photosynthetic Electron Transport Chain. *Plant Physiology* **177**, 271–284.
- Cassin G, Mari S, Curie C, Briat J-F, Czernic P.** 2009. Increased sensitivity to iron deficiency in *Arabidopsis thaliana* overaccumulating nicotianamine. *Journal of Experimental Botany* **60**, 1249–1259.
- Castaigns L, Caquot A, Loubet S, Curie C.** 2016. The high-affinity metal Transporters NRAMP1 and IRT1 Team up to Take up Iron under Sufficient Metal Provision. *Scientific Reports* **6**, 37222.
- Chaney RL.** 1993. Zinc Phytotoxicity. *Zinc in Soils and Plants*, 135–150.
- Charlier J-B, Polese C, Nouet C, Carnol M, Bosman B, Krämer U, Motte P, Hanikenne M.** 2015. Zinc triggers a complex transcriptional and post-transcriptional regulation of the metal homeostasis gene *FRD3* in *Arabidopsis* relatives. *Journal of Experimental Botany* **66**, 3865–3878.
- Chen Y, Barak P.** 1982. Iron Nutrition of Plants in Calcareous Soils. In: Brady NC, ed. *Advances in Agronomy*. Academic Press, 217–240.

- Chen C-L, Cui Y, Cui M, Zhou W-J, Wu H-L, Ling H-Q.** 2018. A FIT-binding protein is involved in modulating iron and zinc homeostasis in *Arabidopsis*. *Plant, Cell & Environment* **41**, 1698–1714.
- Chen X, Tian D, Kong X, Chen Q, E.F AA, Hu X, Jia A.** 2016. The role of nitric oxide signalling in response to salt stress in *Chlamydomonas reinhardtii*. *Planta* **244**, 651–669.
- Cheng L, Wang F, Shou H, et al.** 2007. Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. *Plant Physiology* **145**, 1647–1657.
- Chen Q, Shinozaki D, Luo J, et al.** 2019. Autophagy and Nutrients Management in Plants. *Cells* **8**, 1426.
- Chu H-H, Chiecko J, Punshon T, Lanzirotti A, Lahner B, Salt DE, Walker EL.** 2010. Successful reproduction requires the function of *Arabidopsis* Yellow Stripe-Like1 and Yellow Stripe-Like3 metal-nicotianamine transporters in both vegetative and reproductive structures. *Plant Physiology* **154**, 197–210.
- Ciaffi M, Paolacci AR, Celletti S, Catarcione G, Kopriva S, Astolfi S.** 2013. Transcriptional and physiological changes in the S assimilation pathway due to single or combined S and Fe deprivation in durum wheat (*Triticum durum* L.) seedlings. *Journal of Experimental Botany* **64**, 1663–1675.
- Clemens S.** 2019. Metal ligands in micronutrient acquisition and homeostasis. *Plant, Cell & Environment* **42**, 2902–2912.
- Colangelo EP, Guerinot ML.** 2004. The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. *The Plant Cell* **16**, 3400–3412.
- Connolly EL, Fett JP, Guerinot ML.** 2002. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell* **14**, 1347–1357.
- Conte SS, Chu HH, Rodriguez DC, Punshon T, Vasques KA, Salt DE, Walker EL.** 2013. *Arabidopsis thaliana* Yellow Stripe1-Like4 and Yellow Stripe1-Like6 localize to internal cellular membranes and are involved in metal ion homeostasis. *Frontiers in Plant Science* **4**, 283.
- Cornu J-Y, Deinlein U, Höreth S, Braun M, Schmidt H, Weber M, Persson DP, Husted S, Schjoerring JK, 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.
- Corso M, Schwartzman MS, Guzzo F, Souard F, Malkowski E, Hanikenne M, Verbruggen N.** 2018. Contrasting cadmium resistance strategies in two metallicolous populations of *Arabidopsis halleri*. *New Phytologist* **218**, 283–297.
- Couturier J, Touraine B, Briat J-F, Gaymard F, Rouhier N.** 2013. The iron-sulfur cluster assembly machineries in plants: current knowledge and open questions. *Frontiers in Plant Science* **4**, 259.
- Cumbus IP, Hornsey DJ, Robinson LW.** 1977. The influence of phosphorus, zinc and manganese on absorption and translocation of iron in watercress. *Plant and Soil* **48**, 651–660.
- Curie C, Alonso JM, Le Jean M, Ecker JR, Briat JF.** 2000. Involvement of NRAMP1 from *Arabidopsis*

thaliana in iron transport. *Biochemical Journal* **347 Pt 3**, 749–755.

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL. 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature* **409**, 346–349.

De Clerck O, Kao S-M, Bogaert KA, et al. 2018. Insights into the Evolution of Multicellularity from the Sea Lettuce Genome. *Current Biology* **28**, 2921–2933.e5.

Deinlein U, Weber M, Schmidt H, et al. 2012. Elevated nicotianamine levels in *Arabidopsis halleri* roots play a key role in zinc hyperaccumulation. *The Plant Cell* **24**, 708–723.

Dekock PC, Hall A, Inkson RHE. 1979. Active Iron in Plant Leaves. *Annals of Botany* **43**, 737–740.

Delhaize E. 1996. A metal-accumulator mutant of *Arabidopsis thaliana*. *Plant Physiology* **111**, 849–855.

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

Docampo R, de Souza W, Miranda K, Rohloff P, Moreno SNJ. 2005. Acidocalcisomes ? conserved from bacteria to man. *Nature Reviews Microbiology* **3**, 251–261.

Dubeaux G, Neveu J, Zelazny E, Vert G. 2018. Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its Own Degradation and Plant Metal Nutrition. *Molecular Cell* **69**, 953–964.e5.

Dubeaux G, Zelazny E, Vert G. 2015. Getting to the root of plant iron uptake and cell-cell transport: Polarity matters! *Communicative & Integrative Biology* **8**, e1038441.

Durrett TP, Gassmann W, Rogers EE. 2007. The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiology* **144**, 197–205.

Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 5624–5628.

Eroglu S, Meier B, von Wirén N, Peiter E. 2016. The Vacuolar Manganese Transporter MTP8 Determines Tolerance to Iron Deficiency-Induced Chlorosis in *Arabidopsis*. *Plant Physiology* **170**, 1030–1045.

Escudero V, Abreu I, Tejada-Jiménez M, et al. 2020. *Medicago truncatula* Ferroportin2 mediates iron import into nodule symbiosomes. *New Phytologist* doi: 10.1111/nph.16642.

Evens NP, Buchner P, Williams LE, Hawkesford MJ. 2017. The role of ZIP transporters and group F bZIP transcription factors in the Zn-deficiency response of wheat (*Triticum aestivum*). *The Plant Journal* **92**, 291–304.

Falciatore A, d'Alcalà MR, Croot P, Bowler C. 2000. Perception of environmental signals by a marine diatom. *Science* **288**, 2363–2366.

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.

Forieri I, Wirtz M, Hell R. 2013. Toward new perspectives on the interaction of iron and sulfur metabolism in plants. *Frontiers in Plant Science* **4**.

Foy CD, Chaney RL, White MC. 1978. The Physiology of Metal Toxicity in Plants. *Annual Review of Plant Physiology* **29**, 511–566.

Fukao Y, Ferjani A, Tomioka R, Nagasaki N, Kurata R, Nishimori Y, Fujiwara M, Maeshima M. 2011. iTRAQ analysis reveals mechanisms of growth defects due to excess zinc in *Arabidopsis*. *Plant Physiology* **155**, 1893–1907.

Gibson RS, Raboy V, King JC. 2018. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutrition Reviews* **76**, 793–804.

González-Guerrero M, Matthiadis A, Sáez Á, Long TA. 2014. Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. *Frontiers in Plant Science* **5**, 45.

Green LS, Rogers EE. 2004. FRD3 controls iron localization in *Arabidopsis*. *Plant Physiology* **136**, 2523–2531.

Grillet L, Schmidt W. 2019. Iron acquisition strategies in land plants: not so different after all. *New Phytologist* **224**, 11–18.

Gruber BD, Giehl RFH, Friedel S, von Wirén N. 2013. Plasticity of the *Arabidopsis* root system under nutrient deficiencies. *Plant Physiology* **163**, 161–179.

Halimaa P, Blande D, Aarts MGM, Tuomainen M, Tervahauta A, Kärenlampi S. 2014a. Comparative transcriptome analysis of the metal hyperaccumulator *Noccaea caerulescens*. *Frontiers in Plant Science* **5**, 213.

Halimaa P, Blande D, Baltzi E, et al. 2019. Transcriptional effects of cadmium on iron homeostasis differ in calamine accessions of *Noccaea caerulescens*. *The Plant Journal* **97**, 306–320.

Halimaa P, Lin Y-F, Ahonen VH, et al. 2014b. Gene expression differences between *Noccaea caerulescens* ecotypes help to identify candidate genes for metal phytoremediation. *Environmental Science & Technology* **48**, 3344–3353.

Hanikenne M, Bernal M, Urzica E-I. 2014. Ion homeostasis in the Chloroplast. *Plastid Biology*, 465–514.

Hanikenne M, Krämer U, Demoulin V, Baurain D. 2005a. A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae*. *Plant Physiology* **137**, 428–446.

Hanikenne M, Merchant SS, Hamel P. 2009. Transition Metal Nutrition. *The Chlamydomonas Sourcebook*, 333–399.

- Hanikenne M, Motte P, Wu MCS, Wang T, Loppes R, Matagne RF.** 2005b. A mitochondrial half-size ABC transporter is involved in cadmium tolerance in *Chlamydomonas reinhardtii*. *Plant Cell & Environment* **28**, 863–873.
- Hanikenne M, Nouet C.** 2011. Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. *Current Opinion in Plant Biology* **14**, 252–259.
- Haydon MJ, Cobbett CS.** 2007a. Transporters of ligands for essential metal ions in plants. *New Phytologist* **174**, 499–506.
- Haydon MJ, Cobbett CS.** 2007b. A novel major facilitator superfamily protein at the tonoplast influences zinc tolerance and accumulation in Arabidopsis. *Plant Physiology* **143**, 1705–1719.
- Haydon MJ, 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. *The Plant Cell* **24**, 724–737.
- Herbik A, Bölling C, Buckhout TJ.** 2002. The involvement of a multicopper oxidase in iron uptake by the green algae *Chlamydomonas reinhardtii*. *Plant Physiology* **130**, 2039–2048.
- Hirsch J, Marin E, Floriani M, Chiarenza S, Richaud P, Nussaume L, Thibaud MC.** 2006. Phosphate deficiency promotes modification of iron distribution in Arabidopsis plants. *Biochimie* **88**, 1767–1771.
- Hopkinson BM, Morel FMM.** 2009. The role of siderophores in iron acquisition by photosynthetic marine microorganisms. *Biometals* **22**, 659–669.
- Howe CJ, Schlarb-Ridley BG, Wastl J, Purton S, Bendall DS.** 2006. The novel cytochrome c6 of chloroplasts: a case of evolutionary bricolage? *Journal of Experimental Botany* **57**, 13–22.
- Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK.** 2009. Rice OsYSL15 is an iron-regulated iron^(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. *The Journal of Biological Chemistry* **284**, 3470–3479.
- Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK.** 2004. A rice *FRD3-like* (*OsFRDL1*) gene is expressed in the cells involved in long-distance transport. *Soil Science and Plant Nutrition* **50**, 1133–1140.
- Ishimaru Y, Kakei Y, Shimo H, Bashir K, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK.** 2011. A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. *The Journal of Biological Chemistry* **286**, 24649–24655.
- Ishimaru Y, Suzuki M, Ogo Y, Takahashi M, Nakanishi H, Mori S, Nishizawa NK.** 2008. Synthesis of nicotianamine and deoxymugineic acid is regulated by OsIRO2 in Zn excess rice plants. *Soil Science and Plant Nutrition* **54**, 417–423.
- Ishimaru Y, Suzuki M, Tsukamoto T, et al.** 2006. Rice plants take up iron as an Fe³⁺-phytosiderophore and as Fe²⁺. *The Plant Journal* **45**, 335–346.

- Karam M-J, Souleman D, Schwartzman MS, Gallina S, Spielmann J, Poncet C, Bouchez O, Pauwels M, Hanikenne M, Frérot H.** 2019. Genetic architecture of a plant adaptive trait: QTL mapping of intraspecific variation for tolerance to metal pollution in *Arabidopsis halleri*. *Heredity* **122**, 877–892.
- Kaur G, Shukla V, Kumar A, et al.** 2019. Integrative analysis of hexaploid wheat roots identifies signature components during iron starvation. *Journal of Experimental Botany* **70**, 6141–6161.
- Keeling PJ.** 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annual Review of Plant Biology* **64**, 583–607.
- Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A.** 2014. Analysis of the Root System Architecture of *Arabidopsis* Provides a Quantitative Readout of Crosstalk between Nutritional Signals. *The Plant Cell* **26**, 1480–1496.
- Khan GA, Bouraine S, Wege S, Li Y, de Carbonnel M, Berthomieu P, Poirier Y, Rouached H.** 2014. Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1;H3 in *Arabidopsis*. *Journal of Experimental Botany* **65**, 871–884.
- Kim DY, Bovet L, Kushnir S, Noh EW, Martinoia E, Lee Y.** 2006. AtATM3 is involved in heavy metal resistance in *Arabidopsis*. *Plant Physiology* **140**, 922–932.
- Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML.** 2006. Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. *Science* **314**, 1295–1298.
- Kim S, Takahashi M, Higuchi K, Tsunoda K, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK.** 2005. Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. *Plant & Cell Physiology* **46**, 1809–1818.
- Kisko M, Bouain N, Safi A, et al.** 2018. LPCAT1 controls phosphate homeostasis in a zinc-dependent manner. *eLife* **7**, e32077.
- Klatte M, Schuler M, Wirtz M, Fink-Straube C, Hell R, Bauer P.** 2009. The analysis of *Arabidopsis* nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses. *Plant Physiology* **150**, 257–271.
- Kobayashi T, Nishizawa NK.** 2012. Iron Uptake, Translocation, and Regulation in Higher Plants. *Annual Review of Plant Biology* **63**, 131–152.
- Kobayashi T, Nishizawa NK.** 2015. Intracellular iron sensing by the direct binding of iron to regulators. *Frontiers in Plant Science* **6**, 155.
- Kobayashi T, Nozoye T, Nishizawa NK.** 2019. Iron transport and its regulation in plants. *Free Radical Biology and Medicine* **133**, 11–20.
- Krämer U.** 2010. Metal hyperaccumulation in plants. *Annual Review of Plant Biology* **61**, 517–534.
- Krämer U, Talke IN, Hanikenne M.** 2007. Transition metal transport. *FEBS Letters* **581**, 2263–2272.

La Fontaine S, Quinn JM, Nakamoto SS, Page MD, Göhre V, Moseley JL, Kropat J, Merchant S. 2002. Copper-dependent iron assimilation pathway in the model photosynthetic eukaryote *Chlamydomonas reinhardtii*. *Eukaryotic Cell* **1**, 736–757.

Lanquar V, Lelièvre F, Bolte S, et al. 2005. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO Journal* **24**, 4041–4051.

Lanquar V, Ramos MS, Lelièvre F, Barbier-Brygoo H, Krieger-Liszkay A, Krämer U, Thomine S. 2010. Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is required for optimal photosynthesis and growth under manganese deficiency. *Plant Physiology* **152**, 1986–1999.

Lee S, An G. 2009. Over-expression of *OsIRT1* leads to increased iron and zinc accumulations in rice. *Plant, Cell & Environment* **32**, 408–416.

Le Jean M, Schikora A, Mari S, Briat J-F, Curie C. 2005. A loss-of-function mutation in *AtYSL1* reveals its role in iron and nicotianamine seed loading. *The Plant Journal* **44**, 769–782.

Lelandais G, Scheiber I, Paz-Yepes J, et al. 2016. *Ostreococcus tauri* is a new model green alga for studying iron metabolism in eukaryotic phytoplankton. *BMC Genomics* **17**, 319.

Lešková A, Giehl RFH, Hartmann A, Fargašová A, von Wirén N. 2017. Heavy Metals Induce Iron Deficiency Responses at Different Hierarchic and Regulatory Levels. *Plant Physiology* **174**, 1648–1668.

Li J, Liu B, Cheng F, Wang X, Aarts MGM, Wu J. 2014. Expression profiling reveals functionally redundant multiple-copy genes related to zinc, iron and cadmium responses in *Brassica rapa*. *New Phytologist* **203**, 182–194.

Lin Y-F, Liang H-M, Yang S-Y, Boch A, Clemens S, Chen C-C, Wu J-F, Huang J-L, Yeh K-C. 2009. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. *New Phytologist* **182**, 392–404.

Li B, Sun L, Huang J, Göschl C, Shi W, Chory J, Busch W. 2019. GSNOR provides plant tolerance to iron toxicity via preventing iron-dependent nitrosative and oxidative cytotoxicity. *Nature Communications* **10**, 3896.

Liu J, Tan K, He L, Qiu Y, Tan W, Guo Y, Wang Z, Sun W. 2018. Effect of limitation of iron and manganese on microalgae growth in fresh water. *Microbiology* **164**, 1514–1521.

Li S, Zhou X, Chen J, Chen R. 2018. Is there a strategy I iron uptake mechanism in maize? *Plant Signaling & Behavior* **13**, e1161877.

Li S, Zhou X, Huang Y, Zhu L, Zhang S, Zhao Y, Guo J, Chen J, Chen R. 2013. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. *BMC Plant Biology* **13**, 114.

López-Millán AF, Morales F, Abadía A, Abadía J. 2000. Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. *Plant Physiology* **124**, 873–884.

- Maldonado MT, Allen AE, Chong JS, Lin K, Leus D, Karpenko N, Harris SL.** 2006. Copper-dependent iron transport in coastal and oceanic diatoms. *Limnology and Oceanography* **51**, 1729–1743.
- Marković T, Manzoor S, Humphreys-Williams E, Kirk GJ, Vilar R, Weiss DJ.** 2017. Experimental Determination of Zinc Isotope Fractionation in Complexes with the Phytosiderophore 2'-Deoxymugeneic Acid (DMA) and Its Structural Analogues, and Implications for Plant Uptake Mechanisms. *Environmental Science & Technology* **51**, 98–107.
- Marschner H.** 2012. *Marschner's Mineral Nutrition of Higher Plants*. Academic Press.
- Marschner H, Römheld V.** 1994. Strategies of plants for acquisition of iron. *Plant and Soil* **165**, 261–274.
- Mathan KK, Amberger A.** 1977. Influence of iron on the uptake of phosphorus by maize. *Plant and Soil* **46**, 413–422.
- Meier SK, Adams N, Wolf M, Balkwill K, Muasya AM, Gehring CA, Bishop JM, Ingle RA.** 2018. Comparative RNA-seq analysis of nickel hyperaccumulating and non-accumulating populations of *Senecio coronatus* (Asteraceae). *The Plant Journal* **95**, 1023–1038.
- Merchant SS.** 2007. Trace Metal Utilization in Chloroplasts. *Advances in Photosynthesis and Respiration*, 199–218.
- Merchant SS, Allen MD, Kropat J, Moseley JL, Long JC, Tottey S, Terauchi AM.** 2006. Between a rock and a hard place: trace element nutrition in *Chlamydomonas*. *Biochimica et Biophysica Acta* **1763**, 578–594.
- Merlot S, de la Torre VSG, Hanikenne M.** 2018. Physiology and Molecular Biology of Trace Element Hyperaccumulation. *Agromining: Farming for Metals*, 93–116.
- Milner MJ, Mitani-Ueno N, Yamaji N, Yokosho K, Craft E, Fei Z, Ebbs S, Clemencia Zambrano M, Ma JF, Kochian LV.** 2014. Root and shoot transcriptome analysis of two ecotypes of *Noccaea caerulea* uncovers the role of NcNramp1 in Cd hyperaccumulation. *The Plant Journal* **78**, 398–410.
- Misson J, Raghothama KG, Jain A, et al.** 2005. A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences* **102**, 11934–11939.
- Molins H, Michelet L, Lanquar V, Agorio A, Giraudat J, Roach T, Krieger-Liszkay A, Thomine S.** 2013. Mutants impaired in vacuolar metal mobilization identify chloroplasts as a target for cadmium hypersensitivity in *Arabidopsis thaliana*. *Plant, Cell & environment* **36**, 804–817.
- Mora-Macías J, Ojeda-Rivera JO, Gutiérrez-Alanís D, Yong-Villalobos L, Oropeza-Aburto A, Raya-González J, Jiménez-Domínguez G, Chávez-Calvillo G, Rellán-Álvarez R, Herrera-Estrella L.** 2017. Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E3563–E3572.
- Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P.** 2009. AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. *Plant Physiology* **149**, 894–904.

- Morrissey J, Baxter IR, Lee J, Li L, Lahner B, Grotz N, Kaplan J, Salt DE, Guerinot ML.** 2009. The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*. *The Plant Cell* **21**, 3326–3338.
- Morrissey J, Bowler C.** 2012. Iron Utilization in Marine Cyanobacteria and Eukaryotic Algae. *Frontiers in Microbiology* **3**.
- Morrissey J, Guerinot ML.** 2009. Iron uptake and transport in plants: the good, the bad, and the ionome. *Chemical Reviews* **109**, 4553–4567.
- Morrissey J, Sutak R, Paz-Yepes J, et al.** 2015. A novel protein, ubiquitous in marine phytoplankton, concentrates iron at the cell surface and facilitates uptake. *Current Biology* **25**, 364–371.
- van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Ver Loren van Themaat E, Koornneef M, Aarts MGM.** 2006. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **142**, 1127–1147.
- Moseley JL, Chang C-W, Grossman AR.** 2006. Genome-based approaches to understanding phosphorus deprivation responses and PSR1 control in *Chlamydomonas reinhardtii*. *Eukaryotic Cell* **5**, 26–44.
- Müller R, Morant M, Jarmer H, Nilsson L, Nielsen TH.** 2007. Genome-wide analysis of the *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiology* **143**, 156–171.
- Mus F, Crook MB, Garcia K, et al.** 2016. Symbiotic Nitrogen Fixation and the Challenges to Its Extension to Nonlegumes. *Applied and Environmental Microbiology* **82**, 3698–3710.
- Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa NK.** 2006. Iron deficiency enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1 and OsIRT2 in rice. *Soil Science and Plant Nutrition* **52**, 464–469.
- Nishida S, Tsuzuki C, Kato A, Aisu A, Yoshida J, Mizuno T.** 2011. AtIRT1, the primary iron uptake transporter in the root, mediates excess nickel accumulation in *Arabidopsis thaliana*. *Plant & Cell Physiology* **52**, 1433–1442.
- Nouet C, Motte P, Hanikenne M.** 2011. Chloroplastic and mitochondrial metal homeostasis. *Trends in Plant Science* **16**, 395–404.
- Nozoye T, Nagasaka S, Kobayashi T, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK.** 2015. The Phytosiderophore Efflux Transporter TOM2 Is Involved in Metal Transport in Rice. *The Journal of Biological Chemistry* **290**, 27688–27699.
- Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK.** 2011. Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *The Journal of Biological Chemistry* **286**, 5446–5454.
- Nozoye T, von Wirén N, Sato Y, Higashiyama T, Nakanishi H, Nishizawa NK.** 2019. Characterization of the Nicotianamine Exporter ENA1 in Rice. *Frontiers in Plant Science* **10**, 502.

- Oomen RJFJ, Wu J, Lelièvre F, Blanchet S, Richaud P, Barbier-Brygoo H, Aarts MGM, Thomine S.** 2009. Functional characterization of NRAMP3 and NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **181**, 637–650.
- Pal S, Kisko M, Dubos C, Lacombe B, Berthomieu P, Krouk G, Rouached H.** 2017. TransDetect Identifies a New Regulatory Module Controlling Phosphate Accumulation. *Plant Physiology* **175**, 916–926.
- Paolacci AR, Celletti S, Catarcione G, Hawkesford MJ, Astolfi S, Ciaffi M.** 2014. Iron deprivation results in a rapid but not sustained increase of the expression of genes involved in iron metabolism and sulfate uptake in tomato (*Solanum lycopersicum* L.) seedlings. *Journal of Integrative Plant Biology* **56**, 88–100.
- Paz Y, Katz A, Pick U.** 2007. A multicopper ferroxidase involved in iron binding to transferrins in *Dunaliella salina* plasma membranes. *The Journal of Biological Chemistry* **282**, 8658–8666.
- Pedas P, Ytting CK, Fuglsang AT, Jahn TP, Schjoerring JK, Husted S.** 2008. Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. *Plant Physiology* **148**, 455–466.
- Penen F, Isaure MP, Dobritzsch D, Bertalan I, Castillo-Michel H, Proux O, Gontier E, Le Coustumer P, Schaumlöffel D.** 2017. Pools of cadmium in *Chlamydomonas reinhardtii* revealed by chemical imaging and XAS spectroscopy. *Metallomics* **9**, 910–923.
- Pianelli K, Mari S, Marquès L, Lebrun M, Czernic P.** 2005. Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. *Transgenic Research* **14**, 739–748.
- Pilon M.** 2017. The copper microRNAs. *New Phytologist* **213**, 1030–1035.
- Pineau C, Loubet S, Lefoulon C, Chalias C, Fizames C, Lacombe B, Ferrand M, Loudet O, Berthomieu P, Richard O.** 2012. Natural variation at the FRD3 MATE transporter locus reveals cross-talk between Fe homeostasis and Zn tolerance in *Arabidopsis thaliana*. *PLoS Genetics* **8**, e1003120.
- Pottier M, Dumont J, Masclaux-Daubresse C, Thomine S.** 2019. Autophagy is essential for optimal translocation of iron to seeds in *Arabidopsis*. *Journal of Experimental Botany* **70**, 859–869.
- Pottier M, Masclaux-Daubresse C, Yoshimoto K, Thomine S.** 2014. Autophagy as a possible mechanism for micronutrient remobilization from leaves to seeds. *Frontiers in Plant Science* **5**, 11.
- Prasanna R, Kaushik BD.** 2010. Evolutionary relationships among cyanobacteria, algae and plants: Revisited in the light of Darwinism. *Nature at Work: Ongoing Saga of Evolution*, 119–140.
- Ptashnyk M, Roose T, Jones DL, Kirk GJD.** 2011. Enhanced zinc uptake by rice through phytosiderophore secretion: a modelling study. *Plant, Cell & Environment* **34**, 2038–2046.
- Qiu Y, Wang Z, Liu F, Wu Z, Chen H, Tang D, Liu J.** 2020. Effect of complex iron on the phosphorus absorption by two freshwater algae. *Environmental Technology*, 1–9.
- Raven JA.** 1988. The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. *New Phytologist* **109**, 279–287.

- Remy E, Cabrito TR, Batista RA, Hussein MAM, Teixeira MC, Athanasiadis A, Sá-Correia I, Duque P.** 2014. Intron retention in the 5'UTR of the novel ZIF2 transporter enhances translation to promote zinc tolerance in arabidopsis. *PLoS Genetics* **10**, e1004375.
- Ricachenevsky FK, Menguer PK, Sperotto RA, Fett JP.** 2015. Got to hide your Zn away: Molecular control of Zn accumulation and biotechnological applications. *Plant Science* **236**, 1–17.
- Robinson NJ, Procter CM, Connolly EL, Lou Guerinot M.** 1999. A ferric-chelate reductase for iron uptake from soils. *Nature* **397**, 694–697.
- Rodríguez-Celma J, Chou H, Kobayashi T, Long TA, Balk J.** 2019. Hemerythrin E3 Ubiquitin Ligases as Negative Regulators of Iron Homeostasis in Plants. *Frontiers in Plant Science* **10**, 98.
- Rogers EE, Guerinot ML.** 2002. FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. *The Plant Cell* **14**, 1787–1799.
- Roschttardt H, Grillet L, Isaure M-P, Conéjéro G, Ortega R, Curie C, Mari S.** 2011. Plant cell nucleolus as a hot spot for iron. *The Journal of Biological Chemistry* **286**, 27863–27866.
- Rouached H, Rhee SY.** 2017. System-level understanding of plant mineral nutrition in the big data era. *Current Opinion in Systems Biology* **4**, 71–77.
- Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J.** 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & Development* **15**, 2122–2133.
- Ryan PR, Delhaize E, Jones DL.** 2001. Function and mechanism of organic anion exudation from plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 527–560.
- Saenchai C, Bouain N, Kisko M, Prom-U-Thai C, Doumas P, Rouached H.** 2016. The Involvement of OsPHO1;1 in the Regulation of Iron Transport Through Integration of Phosphate and Zinc Deficiency Signaling. *Frontiers in Plant Science* **7**, 396.
- Santi S, Schmidt W.** 2009. Dissecting iron deficiency-induced proton extrusion in Arabidopsis roots. *New Phytologist* **183**, 1072–1084.
- Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann J-L, Traverse A, Marcus MA, Manceau A.** 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **130**, 1815–1826.
- Satbhai SB, Setzer C, Freynschlag F, Slovak R, Kerdaffrec E, Busch W.** 2017. Natural allelic variation of FRO2 modulates Arabidopsis root growth under iron deficiency. *Nature Communications* **8**, 15603.
- Schaaf G, Honsbein A, Meda AR, Kirchner S, Wipf D, von Wirén N.** 2006. AtIREG2 encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. *The Journal of Biological Chemistry* **281**, 25532–25540.
- Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N.** 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *The Journal of Biological*

Chemistry **279**, 9091–9096.

Schaaf G, Schikora A, Häberle J, Vert G, Ludewig U, Briat J-F, Curie C, von Wirén N. 2005. A putative function for the Arabidopsis Fe-Phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. *Plant & Cell Physiology* **46**, 762–774.

Schaedler TA, Thornton JD, Kruse I, Schwarzländer M, Meyer AJ, van Veen HW, Balk J. 2014. A Conserved Mitochondrial ATP-binding Cassette Transporter Exports Glutathione Polysulfide for Cytosolic Metal Cofactor Assembly. *Journal of Biological Chemistry* **289**, 23264–23274.

Scheepers M, Spielmann J, Boulanger M, Carnol M, Bosman B, De Pauw E, Goormaghtigh E, Motte P, Hanikenne M. 2020. Intertwined metal homeostasis, oxidative and biotic stress responses in the Arabidopsis *frd3* mutant. *The Plant Journal* **102**, 34–52.

Schoffman H, Lis H, Shaked Y, Keren N. 2016. Iron-Nutrient Interactions within Phytoplankton. *Frontiers in Plant Science* **7**, 1223.

Schuler M, Keller A, Backes C, Philippar K, Lenhof H-P, Bauer P. 2011. Transcriptome analysis by GeneTrail revealed regulation of functional categories in response to alterations of iron homeostasis in *Arabidopsis thaliana*. *BMC Plant Biology* **11**, 87.

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. *The Plant Cell* **24**, 2380–2400.

Schvartzman MS, Corso M, Fataftah N, Scheepers M, Nouet C, Bosman B, Carnol M, Motte P, Verbruggen N, Hanikenne M. 2018. Adaptation to high zinc depends on distinct mechanisms in metallicolous populations of *Arabidopsis halleri*. *New Phytologist* **218**, 269–282.

Shahzad Z, Eaglesfield R, Carr C, Amtmann A. 2020. Cryptic variation in RNA-directed DNA-methylation controls lateral root development when auxin signalling is perturbed. *Nature Communications* **11**, 218.

Shahzad Z, Rouached H, Rakha A. 2014. Combating mineral malnutrition through iron and zinc biofortification of cereals. *Comprehensive Reviews in Food Science and Food Safety* **13**, 329–346.

Shanmugam V, Lo J-C, Wu C-L, Wang S-L, Lai C-C, Connolly EL, Huang J-L, Yeh K-C. 2011. Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana*--the role in zinc tolerance. *New Phytologist* **190**, 125–137.

Shanmugam V, Lo J-C, Yeh K-C. 2013. Control of Zn uptake in *Arabidopsis halleri*: a balance between Zn and Fe. *Frontiers in Plant Science* **4**, 281.

Shanmugam V, Tsednee M, Yeh K-C. 2012. ZINC TOLERANCE INDUCED BY IRON 1 reveals the importance of glutathione in the cross-homeostasis between zinc and iron in *Arabidopsis thaliana*. *The Plant Journal* **69**, 1006–1017.

Shikanai T, Müller-Moulé P, Munekage Y, Niyogi KK, Pilon M. 2003. PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. *The Plant Cell* **15**, 1333–1346.

- Shi R, Melzer M, Zheng S, Benke A, Stich B, von Wirén N.** 2018. Iron Retention in Root Hemicelluloses Causes Genotypic Variability in the Tolerance to Iron Deficiency-Induced Chlorosis in Maize. *Frontiers in Plant Science* **9**, 557.
- Shinozaki D, Merkulova EA, Naya L, Horie T, Kanno Y, Seo M, Ohsumi Y, Masclaux-Daubresse C, Yoshimoto K.** 2020. Autophagy Increases Zinc Bioavailability to Avoid Light-Mediated Reactive Oxygen Species Production under Zinc Deficiency. *Plant Physiology* **182**, 1284–1296.
- Sinclair SA, Krämer U.** 2012. The zinc homeostasis network of land plants. *Biochimica et Biophysica Acta* **1823**, 1553–1567.
- Smetacek V, Klaas C, Strass VH, et al.** 2012. Deep carbon export from a Southern Ocean iron-fertilized diatom bloom. *Nature* **487**, 313–319.
- Spijkerman E, Behrend H, Fach B, Gaedke U.** 2018. Decreased phosphorus incorporation explains the negative effect of high iron concentrations in the green microalga *Chlamydomonas acidophila*. *The Science of the Total Environment* **626**, 1342–1349.
- Stohs SJ, Bagchi D.** 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology & Medicine* **18**, 321–336.
- Strzepek RF, Harrison PJ.** 2004. Photosynthetic architecture differs in coastal and oceanic diatoms. *Nature* **431**, 689–692.
- Sunda WG.** 2012. Feedback Interactions between Trace Metal Nutrients and Phytoplankton in the Ocean. *Frontiers in Microbiology* **3**, 204.
- Sunda WG, Swift DG, Huntsman SA.** 1991. Low iron requirement for growth in oceanic phytoplankton. *Nature* **351**, 55–57.
- Suzuki M, Takahashi M, Tsukamoto T, et al.** 2006. Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *The Plant Journal* **48**, 85–97.
- Suzuki M, Tsukamoto T, Inoue H, Watanabe S, Matsushashi S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK.** 2008. Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. *Plant Molecular Biology* **66**, 609–617.
- Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa NK.** 2011a. Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. *Plant Signaling & Behavior* **6**, 1813–1816.
- Takahashi R, Ishimaru Y, Senoura T, Shimo H, Ishikawa S, Arai T, Nakanishi H, Nishizawa NK.** 2011b. The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. *Journal of Experimental Botany* **62**, 4843–4850.
- Talke IN, Hanikenne M, Krämer U.** 2006. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **142**, 148–167.
- Tan Y-F, O'Toole N, Taylor NL, Millar AH.** 2010. Divalent metal ions in plant mitochondria and their role

in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiology* **152**, 747–761.

Terry N, Abadia J. 1986. Function of iron in chloroplasts. *Journal of Plant Nutrition* **9**, 609–646.

Teschner J, Lachmann N, Schulze J, Geisler M, Selbach K, Santamaria-Araujo J, Balk J, Mendel RR, Bittner F. 2010. A novel role for Arabidopsis mitochondrial ABC transporter ATM3 in molybdenum cofactor biosynthesis. *Plant Cell* **22**, 468–480.

Thibaud M-C, Arrighi J-F, Bayle V, Chiarenza S, Creff A, Bustos R, Paz-Ares J, Poirier Y, Nussaume L. 2010. Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. *The Plant Journal* **64**, 775–789.

Thomine S, Vert G. 2013. Iron transport in plants: better be safe than sorry. *Current Opinion in Plant Biology* **16**, 322–327.

Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI. 2000. Cadmium and iron transport by members of a plant metal transporter family in Arabidopsis with homology to Nramp genes. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 4991–4996.

Ticconi CA, Lucero RD, Sakhonwasee S, Adamson AW, Creff A, Nussaume L, Desnos T, Abel S. 2009. ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 14174–14179.

Tsednee M, Castruita M, Salomé PA, et al. 2019. Manganese co-localizes with calcium and phosphorus in Chlamydomonas acidocalcisomes and is mobilized in manganese-deficient conditions. *Journal of Biological Chemistry* **294**, 17626–17641.

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.

Urzica EI, Casero D, Yamasaki H, et al. 2012. Systems and trans-system level analysis identifies conserved iron deficiency responses in the plant lineage. *The Plant Cell* **24**, 3921–3948.

Vert G, Barberon M, Zelazny E, Séguéla M, Briat J-F, Curie C. 2009. Arabidopsis IRT2 cooperates with the high-affinity iron uptake system to maintain iron homeostasis in root epidermal cells. *Planta* **229**, 1171–1179.

Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat J-F, Curie C. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell* **14**, 1223–1233.

Vigani G, Hanikenne M. 2018. Metal Homeostasis in Plant Mitochondria. *Annual Plant Reviews online*, 111–142.

von Wirén N, Klair S, Bansal S, Briat JF, Khodr H, Shioiri T, Leigh RA, Hider RC. 1999. Nicotianamine chelates both Fe^{III} and Fe^{II}. Implications for metal transport in plants. *Plant Physiology* **119**, 1107–1114.

von Wirén N, Marschner H, Römheld V. 1996. Roots of Iron-Efficient Maize also Absorb Phytosiderophore-Chelated Zinc. *Plant Physiology* **111**, 1119–1125.

Wairich A, de Oliveira BHN, Arend EB, Duarte GL, Ponte LR, Sperotto RA, Ricachenevsky FK, Fett JP. 2019. The Combined Strategy for iron uptake is not exclusive to domesticated rice (*Oryza sativa*). *Scientific Reports* **9**, 16144.

Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG. 2008. The effect of iron on the primary root elongation of *Arabidopsis* during phosphate deficiency. *Plant Physiology* **147**, 1181–1191.

Waters BM, Chu H-H, Didonato RJ, Roberts LA, Easley RB, Lahner B, Salt DE, Walker EL. 2006. Mutations in *Arabidopsis yellow stripe-like1* and *yellow stripe-like3* reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiology* **141**, 1446–1458.

Waters BM, McInturf SA, Stein RJ. 2012. Rosette iron deficiency transcript and microRNA profiling reveals links between copper and iron homeostasis in *Arabidopsis thaliana*. *Journal of Experimental Botany* **63**, 5903–5918.

Weber M, Harada E, Vess C, Roepenack-Lahaye E v., Clemens S. 2004. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. *The Plant Journal* **37**, 269–281.

Willems G, Frérot H, Gennen J, Salis P, Saumitou-Laprade P, Verbruggen N. 2010. Quantitative trait loci analysis of mineral element concentrations in an *Arabidopsis halleri* x *Arabidopsis lyrata petraea* F2 progeny grown on cadmium-contaminated soil. *New Phytologist* **187**, 368–379.

Wu H, Ling H-Q. 2019. FIT-Binding Proteins and Their Functions in the Regulation of Fe Homeostasis. *Frontiers in Plant Science* **10**, 844.

Wykoff DD, Grossman AR, Weeks DP, Usuda H, Shimogawara K. 1999. Psr1, a nuclear localized protein that regulates phosphorus metabolism in *Chlamydomonas*. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 15336–15341.

Yang TJW, Lin W-D, Schmidt W. 2010. Transcriptional profiling of the *Arabidopsis* iron deficiency response reveals conserved transition metal homeostasis networks. *Plant Physiology* **152**, 2130–2141.

Yokosho K, Yamaji N, Ma JF. 2016. OsFRDL1 expressed in nodes is required for distribution of iron to grains in rice. *Journal of Experimental Botany* **67**, 5485–5494.

Yokosho K, Yamaji N, Ueno D, Mitani N, Ma JF. 2009. OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant Physiology* **149**, 297–305.

Yoneyama T, Ishikawa S, Fujimaki S. 2015. Route and Regulation of Zinc, Cadmium, and Iron Transport in Rice Plants (*Oryza sativa* L.) during Vegetative Growth and Grain Filling: Metal Transporters, Metal Speciation, Grain Cd Reduction and Zn and Fe Biofortification. *International Journal of Molecular sciences* **16**, 19111–19129.

Zargar SM, Kurata R, Inaba S, Oikawa A, Fukui R, Ogata Y, Agrawal GK, Rakwal R, Fukao Y. 2015. Quantitative proteomics of *Arabidopsis* shoot microsomal proteins reveals a cross-talk between excess

zinc and iron deficiency. *Proteomics* **15**, 1196–1201.

Zhang Y, Xu Y-H, Yi H-Y, Gong J-M. 2012. Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *The Plant Journal* **72**, 400–410.

Zuchi S, Cesco S, Varanini Z, Pinton R, Astolfi S. 2009. Sulphur deprivation limits Fe-deficiency responses in tomato plants. *Planta* **230**, 85–94.

Zuchi S, Watanabe M, Hubberten H-M, et al. 2015. The Interplay between Sulfur and Iron Nutrition in Tomato. *Plant Physiology* **169**, 2624–2639.

Accepted Manuscript

Figure legends

Figure 1. Primary root growth in Arabidopsis depends on iron and phosphate availability. (A) Under phosphate sufficiency (+P), iron deficiency (-Fe) or excess (++Fe) inhibit primary root growth (PRG) compared to Fe sufficiency (+Fe). The PRG responses to -Fe and ++Fe involve the *FRO2* (*FERRIC REDUCTASE OXIDASE 2*) and *GSNOR* (*S-NITROSOGLUTATHIONE-REDUCTASE*) genes, respectively. (B) Simultaneous P deficiency (-P) and Fe sufficiency (+Fe) inhibits PRG compared to Fe sufficiency (+Fe). This response involves the *LPR1* (cell wall-targeted *FERROOXIDASE*), *PDR2* (P5-type ATPase), *STOP1* (SENSITIVE TO PROTON RHIZOTOXICITY), encoding a transcription factor, and *ALMT1* (*ALUMINUM ACTIVATED MALATE TRANSPORTER 1*) genes. PRG is similar to Fe sufficiency upon simultaneous P (-P) and Fe (-Fe) deficiencies and this response may be controlled by the AT1G51310, AT2G36200, AT4G09940 and AT5G16580 candidate genes (Bouain *et al.*, 2019a). PRG under -P and Fe excess (++Fe) remains to be studied and is shown by a question mark (?).

Figure 2. Iron and micronutrient uptake and trafficking interactions in Arabidopsis and rice roots. Only the interface with the rhizosphere (left) and the xylem (right) is detailed. (A) Arabidopsis. In dicots, iron uptake from the soil relies on a reduction strategy (Strategy I). The localization of IRT1 and NRAMP1 was shown to be dynamic, between plasma membrane and endo-membranes (see text). Metal ligands in the xylem, with their relative abundance represented by different font size, are based on data in *Pisum sativum* (Flis *et al.*, 2016). (B) Rice. In rice, as in grasses, iron uptake from the soil relies on a reduction strategy (Strategy II). Rice is however an exception among grasses as it also uses part of the Strategy I. The mechanisms shared by all grasses for iron uptake are represented in red, whereas the function of IRT1 specific to rice is in orange. Note that the biosynthesis of MAs from SAM may take place in rough endoplasmic reticulum-derived vesicles, which are not represented here (Kobayashi *et al.*, 2019). Red: uptake processes taking place at the root epidermis, including processes related to the IRT1 lack of specificity in Arabidopsis (A); Purple: intracellular and long distance trafficking processes; Green: chelator and chelator transporter-related processes. Vacuoles and vesicles are represented by green and yellow bubbles, respectively. The intracellular green arrow represents symplastic movements of metal chelates. Note that phenolics secretion by roots into the rhizosphere is not represented (Kobayashi *et al.*, 2019). Most of these processes are induced by iron deficiency under the control of complex and multi-layer regulatory mechanisms (see text). Hypothetical processes are labelled with a question mark (?). AHA2: ARABIDOPSIS H⁺-ATPASE 2; Cit: Citrate; ENA1: EFFLUX TRANSPORTER OF NA 1; FRD(L): FERRIC REDUCTASE DEFECTIVE(-LIKE); FPN: FERROPORTIN; FRO2: FERRIC REDUCTASE-OXIDASE 2; IRT: IRON-REGULATED TRANSPORTER; HMA3: HEAVY METAL ATPASE 3; MA: MUGINEIC ACID FAMILY

PHYTOSIDEROPHORES; MTP: METAL TOLERANCE PROTEIN; NA, NICOTIANAMINE; NRAMP: NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN; PEZ1: PHENOLICS EFFLUX ZERO 1; SAM: S-ADENOSYL-L-METHIONINE; TOM: TRANSPORTER OF MUGINEIC ACID FAMILY PHYTOSIDEROPHORES; YSL: YELLOW-STRIPE-LIKE; ZIF1: ZINC-INDUCED FACILITATOR 1.

Box1. Fe and nutrient interplay in eukaryotic algae

Land plants frequently come to mind when talking about photosynthetic organisms and the heterogeneous group known as algae is often overlooked. Algae is an umbrella term used to describe a polyphyletic group of (mostly aquatic and photosynthetic) eukaryotes (Prasanna and Kaushik, 2010; Brodie *et al.*, 2017) that acquired photosynthesis through primary or secondary endosymbiosis (Keeling, 2013; Brodie *et al.*, 2017). Algae produce ~50% of oxygen on earth and provide multiple ecosystem and economic services to humans (Brodie *et al.*, 2017). Their diverse evolutionary origins and diverse habitats, characterized by different Fe availability give ample room for originality and innovation in Fe homeostasis and its interactions with other nutrients. In this box, we will highlight a few examples of the peculiarities found in algae.

Fe is known to limit algae growth in marine environments (Sunda, 2012; Smetacek *et al.*, 2012; Brodie *et al.*, 2017) as well as in freshwater ecosystems (Liu *et al.*, 2018). Fe is a particularly limiting nutrient in open ocean, the so-called high-nutrient, low-carbon areas (Boyd *et al.*, 2007; Morrissey and Bowler, 2012), leading to adaptation of the photosynthetic machinery in algae inhabiting those areas (Strzepek and Harrison, 2004; Cardol *et al.*, 2008). Fe uptake pathways are very diversified in algae. In *Chlamydomonas*, Fe^{3+} is first reduced by ferric chelate reductases, then high affinity Fe uptake is mediated by a multicopper ferroxidase that reoxidizes Fe^{2+} to Fe^{3+} and an Fe permease that transports Fe^{3+} into the cell, as it occurs in yeast and mammals. This re-oxidation step, and subsequent uptake of Fe^{3+} , confers a high metal specificity to this high-affinity Fe uptake system (La Fontaine *et al.*, 2002; Herbiak *et al.*, 2002; Merchant *et al.*, 2006). However, two ZIP homolog proteins, named IRT1 and IRT2, are also induced by Fe deficiency in *Chlamydomonas* and expected to form a parallel pathway of Fe^{2+} transport (Hanikenne *et al.*, 2009; Urzica *et al.*, 2012). Cd exposure results in the strong induction of both uptake pathways, which enables to limit the impact of Cd on Fe homeostasis but comes at the cost of higher Cd uptake (Thiriet-Rupert and Hanikenne, unpublished). This response is in part controlled by the concerted action of a bHLH transcription factor and the main Pi transcriptional regulator, PSR1 (PHOSPHATE STARVATION RESPONSE 1) (Thiriet-Rupert and Hanikenne, unpublished), an ortholog of the

plant PHR1 transcription factor (Wykoff *et al.*, 1999; Rubio *et al.*, 2001; Moseley *et al.*, 2006) (see main text). This indicates that, as in monocots, Fe homeostasis is impacted by other metal cations also in organisms relying partly on Fe³⁺ uptake.

Several other algal species also re-oxidize Fe²⁺ into Fe³⁺ and, in addition, possess, as in animals, transferrin-like proteins responsible for Fe³⁺ uptake (e.g. the halophyte alga *Dunaliella salina* and the sea weed *Ulva mutabilis*) (Paz *et al.*, 2007; Blaby-Haas and Merchant, 2017; De Clerck *et al.*, 2018). As plants that export/secrete siderophores or phenolic compounds to mobilize and/or scavenge Fe³⁺ in the soil, algae also employ diverse strategies to capture Fe from their environment: using extracellular Fe-chelating proteins, such as the secreted FEA1 and FEA2 (Fe-Assimilation) in *Chlamydomonas* or the plasma-membrane anchored ISIP2a (Fe Starvation-Inducible Protein) in *Phaeodactylum tricornutum* (Allen *et al.*, 2007; Morrissey *et al.*, 2015; Blaby-Haas and Merchant, 2017); or relying on bacterial siderophores, as suggested in *U. mutabilis* and other species (Hopkinson and Morel, 2009; De Clerck *et al.*, 2018). FEAs and ISIP2a genes are induced by Fe deficiency (but not by deficiencies of other metals) (Allen *et al.*, 2007; Morrissey *et al.*, 2015), and ISIP2a was actually shown to bind Fe (Morrissey *et al.*, 2015). Whether these Fe scavenging strategies are influenced by interactions with other nutrients has not been examined in details.

Copper (Cu) and Zn also play important roles in Fe homeostasis in algae. In several species, such as *Chlamydomonas*, *Thalassiosira oceanica* and *D. salina*, multicopper oxidases (MCO) take part in high affinity Fe uptake (Maldonado *et al.*, 2006; Paz *et al.*, 2007; Blaby-Haas and Merchant, 2017). In contrast, in the marine alga *Ostreococcus tauri*, where Fe uptake is dependent on an IRT1-like protein, Zn, and not Cu, appears to be required for proper Fe-uptake and its regulation (Lelandais *et al.*, 2016). An interaction between Zn and the regulation of IRT1 also occurs in *Chlamydomonas* where an induction of IRT1 takes place under Zn-deprivation (Blaby-Haas and Merchant, 2017). In addition, Cu-based plastocyanin and Fe-based cytochrome *c*₆ are interchangeable in the photosynthetic electron transfer chain in green algae such as *Chlamydomonas*. Upon Cu starvation, plastocyanin can be replaced by cytochrome *c*₆ as part of a Cu-saving program (Howe *et al.*, 2006; Merchant, 2007). An extreme example of this is the red alga *Cyanidioschizon merolae*, which inhabits acidic Fe-rich low-Cu hot springs and completely lacks plastocyanin (Hanikenne *et al.*, 2005a). It is also lacking MCOs, but possesses multiple Fe permeases (Blaby-Haas and Merchant, 2012; Hanikenne *et al.*, 2005a).

Cytosolic calcium (Ca) signaling was shown to be crucial for the response to changes of environmental Fe concentration in the diatom *Phaeodactylum tricornutum* (Falciatore *et al.*, 2000). Inside the cells, Ca interacts with Fe storage in vacuole-like acidocalcisomes in several algal species, including *Chlamydomonas* and the red alga *Cyanidioschyzon merolae* (Blaby-Haas and Merchant, 2014). In addition to Fe, these dense acidic organelles, made mostly of Ca and polyphosphate complexes, constitute the main storage compartment for divalent cations such as Zn, Mn and Cd (Docampo *et al.*, 2005; Penen *et al.*, 2017; Tsednee *et al.*, 2019).

Although N availability is key for biomass production, most photosynthetic organisms cannot fix N from the massive reservoir that is atmospheric N₂ (Boyd and Peters, 2013). In soil, legumes can however form a symbiosis with a diazotrophic bacteria in order to fix N₂ (Mus *et al.*, 2016). Hence, the plant *Medicago truncatula* provides Fe to the symbiont, ensuring nitrogenase activity in the symbiont (González-Guerrero *et al.*, 2014; Escudero *et al.*, 2020). Algae can use different sources of N and their Fe requirement varies in function of the N source (Raven, 1988). Indeed, diazotrophic cyanobacteria have a higher Fe requirement than algae growing on nitrate, which is superior to those growing on ammonia. Ammonia can be readily used from the media, but nitrate reduction and N₂ fixation require Fe as cofactor and source of reductive power (Schoffman *et al.*, 2016).

Finally, P and Fe also interact both in fresh and marine water (Benitez-Nelson, 2000; Qiu *et al.*, 2020). In freshwater, both Fe concentration and speciation have a significant impact on P uptake in *Scenedesmus quadricauda* (Qiu *et al.*, 2020). This contrasts with very acidic lakes, where pH is usually below 4 rendering Fe more bioavailable. There, by forming less bioavailable Fe-P complexes, Fe promotes P deficiency, even when P concentration is not limiting, possibly contributing to low primary productivity in Fe-rich acidic lakes (Spijkerman *et al.*, 2018). Algae species found in these habitats, such as *Chlamydomonas acidophila*, can tolerate approximately twenty times higher amount of Fe compared to neutrophile species, such as *Chlamydomonas* (Spijkerman *et al.*, 2018).

Figure 1

Figure 2