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REVIEW PAPER

Coordinated homeostasis of essential mineral nutrients: a focus on iron

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Abstract

In plants, iron (Fe) transport and homeostasis are highly regulated processes. Fe deficiency or excess dramatically limits plant and algal 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 being uncovered. Although these interactions have profound consequences for our understanding of Fe homeostasis and its regulation, their molecular bases and biological significance 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. Finally, we 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: Algae, ion signaling crosstalk, iron, iron deficiency, iron uptake, nicotianamine, nutrient interaction, 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 into terrestrial and aquatic food webs, 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, algal 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 the World Health Organization (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 amounts in the nucleolus (Roschzttardtz *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, and pollen production (Briat *et al.*, 2015*a*; Bouain *et al.*, 2019*b*). 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, and five 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 have 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 lesser extent sensing and signaling mechanisms. More recently, research has opened into the investigation of interactions between nutrient homeostatic networks at the molecular level, revealing (i) multiple kinds 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 on the uptake of another, and 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.*, 2019*b*). 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 of 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 with higher plants, highlighting originalities found in algae (Box 1). Gaining new insights into 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 Fe excess, in the model plant *Arabidopsis thaliana* (Arabidopsis). *FERRIC REDUCTASE OXIDASE 2* (*FRO2*) was indeed identified as a gene responsible for the regulation of primary root growth in response to Fe deficiency (Satbhai *et al.*, 2017). Primary root growth 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.*, 2019*a*). 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 reinhardtii*, 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) and 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 there 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 grasses (Marschner and Römheld,

Box 1. 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 differences in Fe availability, give ample room for originality and innovation in homeostasis of Fe 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 algal 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 the 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 highly diversified in algae. In *Chlamydomonas reinhardtii* (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 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; Herbik *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 are 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 limitation of the impact of Cd on Fe homeostasis but comes at the cost of higher Cd uptake (Thiriet-Rupert and Hanikenne, unpublished data). This response is in part controlled by the concerted action of a bHLH transcription factor and the main P_i transcriptional regulator, PHOSPHATE STARVATION RESPONSE 1 (PSR1) (Thiriet-Rupert and Hanikenne, unpublished data), 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 Fe^{3+} uptake.

Several other algal species also re-oxidize Fe^{2+} into Fe^{3+} and, in addition, possess, as in animals, transferrin-like proteins responsible for Fe^{3+} 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 with plants that export or secrete siderophores or phenolic compounds to mobilize and/or scavenge Fe^{3+} in the soil, algae also employ diverse strategies to capture Fe from their environment: using extracellular Fe-chelating proteins, such as the secreted Fe-Assimilation (FEA) 1 and FEA2 in Chlamydomonas or the plasmamembrane-anchored Fe Starvation-Inducible Protein (ISIP2a) 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 zinc (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 plactocyanin (Hanikenne *et al.*, 2005*a*). It also lacks MCOs, but possesses multiple Fe permeases (Blaby-Haas and Merchant, 2012; Hanikenne *et al.*, 2005*a*).

Cytosolic calcium (Ca) signaling was shown to be crucial for the response to changes of environmental Fe concentration in the diatom *P. 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 *C. 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 N_2 (Boyd and Peters, 2013). In soil, legumes can, however, form a symbiosis with a diazotrophic bacterium in order to fix N_2 (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 as a 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 medium, but nitrate reduction and N_2 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 concentration and species of Fe 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). Algal species found in these habitats, such as *Chlamydomonas acidophila*, can tolerate an approximately 20 times higher amount of Fe compared with neutrophile species, such as Chlamydomonas (Spijkerman *et al.*, 2018).

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 and *C. reinhardtii* 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 IRON-REGULATED TRANSPORTER 1 (IRT1), a transporter of the ZINC-REGULATED AND IRON-REGULATED-LIKE PROTEINS (ZIP) 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 *HEAVY METAL ATPASE 3* (*HMA3*; Morel *et al.*, 2009), *METAL TOLERANCE PROTEIN 3* (*MTP3*; Arrivault *et al.*, 2006), *MTP8* (Eroglu *et al.*, 2016), and *FERROPORTIN 2* (*FPN2*; 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 FER-LIKE IRON-DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) and a multi-layer combination of additional transcription factors (e.g. more than 14 basic helix–loop–helix (bHLH) proteins, two MYELOBLASTOSIS (MYB)) and regulators (e.g. E3 ubiquitin ligase BRUTUS (BTS) 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, FIT-BINDING PROTEIN (FBP) 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 also plays 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.

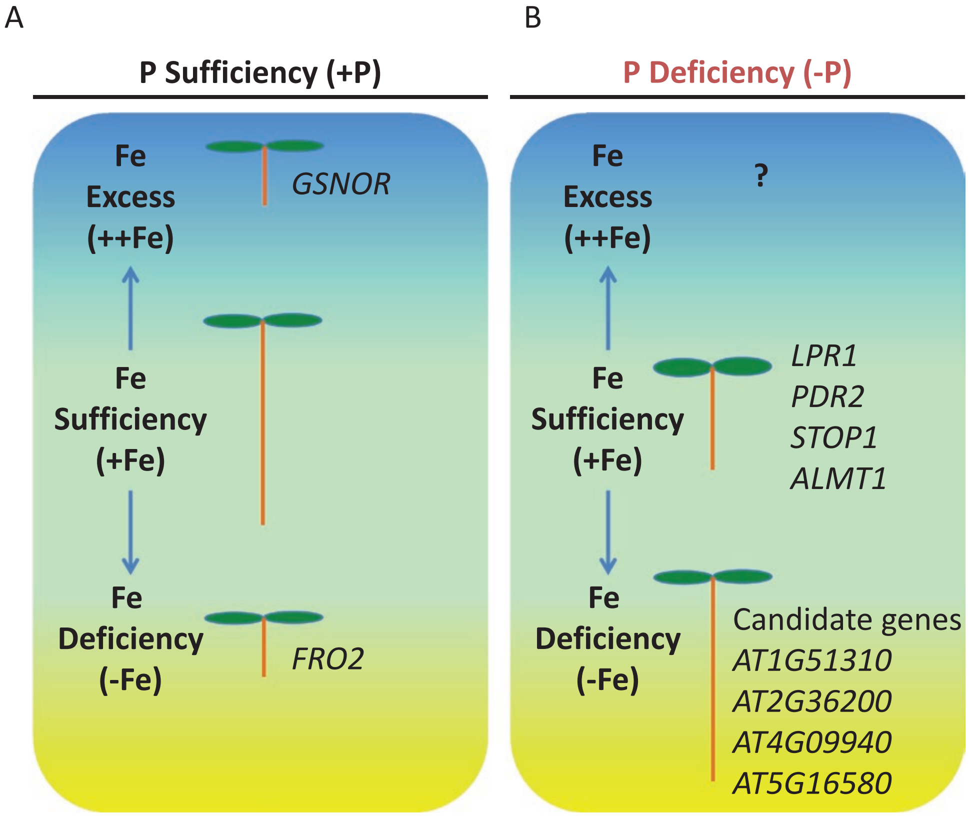

Fig. 1. Primary root growth in Arabidopsis depends on iron and phosphate availability. (A) Under phosphate sufficiency (+P), iron deficiency (–Fe) or excess (++Fe) inhibits primary root growth (PRG) compared with Fe sufficiency (+Fe). The PRG responses to –Fe and ++Fe involve the *FERRIC REDUCTASE OXIDASE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

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; Schvartzman *et al.*, 2018) and *Noccaea caerulescens* (Halimaa *et al.*, 2019), and (ii) Ni shoot accumulation in Ni-hyperaccumulating populations of *N. caerulescens* (Halimaa *et al.*, 2014*a*,*b*) and in *Senecio coronatus* (Meier *et al.*, 2018). More generally, exposure to high metal levels and metal hyperaccumulation itself interfere 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; Schvartzman *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 shoot molybdenum (Mo) accumulation (Baxter *et al.*, 2008). Indeed, the acidification of the medium associated with strategy I Fe uptake limits Mo bioavailability (Marschner, 2012). In addition, several microRNAs initially identified as major players in Cu homeostasis (Pilon 2017) were recently shown to also be linked to Fe homeostasis, by controlling the oxidative stress response and oxidative metabolic processes (Waters *et al.*, 2012; Carrió-Seguí *et al.*, 2019).

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, MAs are secreted into the soil by TRANSPORTER OF MUGINEIC ACID FAMILY PHYTOSIDEROPHORES 1 (TOM1) (Nozoye *et al.*, 2011). MA–Fe(III) complexes are then taken-up by YELLOW-STRIPE 1/YS-LIKE (YS1/YSL) transporters (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 IRON DEFICIENCY-RESPONSIVE ELEMENT BINDING FACTOR 1 (IDEF1) and IDEF2 transcription factors in transcriptional regulation and Fe status sensing in rice (for

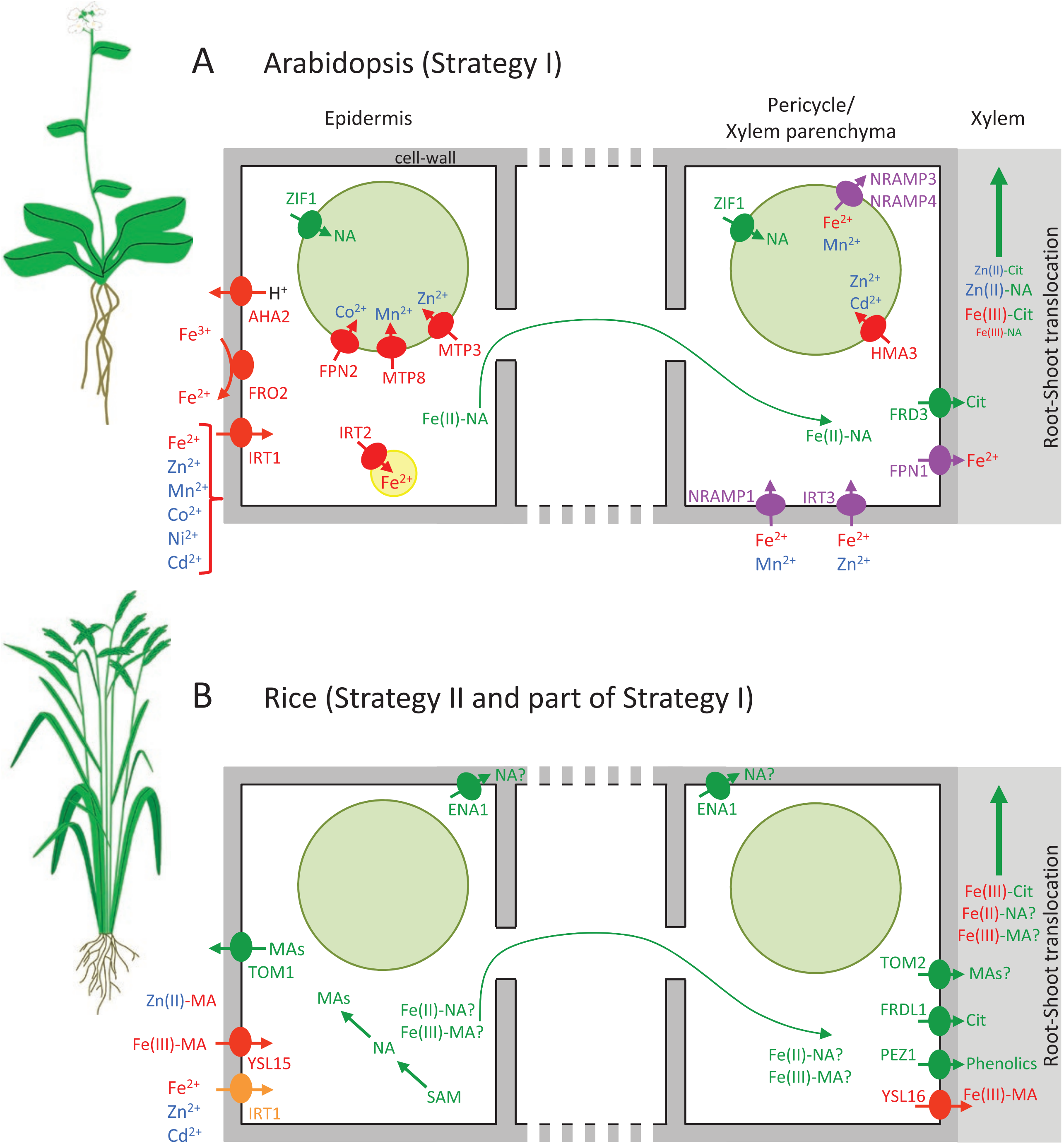

Fig. 2. Iron and micronutrient uptake and trafficking interactions in Arabidopsis and rice roots. Only the interface with the rhizosphere (left) and the xylem (right) is detailed. (A) Arabidopsis. In dicots, iron uptake from the soil relies on a reduction strategy (strategy I). The localization of IRT1 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 sizes, 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 strategy I. The mechanisms shared by all grasses for iron uptake are represented in red, and the function of IRT1 specific to rice is in orange. Note that the biosynthesis of mugineic acids (MAs) from *S*-adenosyl-L-methionine 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 secretion of phenolics 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; HMA3, HEAVY METAL ATPASE 3; IRT, IRON-REGULATED TRANSPORTER; 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.

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 an 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 in 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 synthetize 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 uptake of Mn, 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 with 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 (QTL) 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 NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN (NRAMP) family also tie 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 with 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.*, 2011*a*,*b*) or *N. caerulescens* (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, furthermore, contribute 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. caerulescens*, 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 an F_2 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*b*). 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 for 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 additionally contribute 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 $[\mathrm{FeCitrateOH}]^-$ and $[\mathrm{FeCitrate_2}]^{3-}$ 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 FERRIC REDUCTASE DEFECTIVE 3 (FRD3), a member of the MULTIDRUG AND TOXIC EFFLUX (MATE) family (Durrett *et al.*, 2007), whose expression in Arabidopsis is induced not only 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 depicts not only 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). A 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, PHENOLICS EFFLUX ZERO 1 (PEZ1), 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 EFFLUX TRANSPORTER OF NA 1 (ENA1) (Nozoye *et al.*, 2019) are additionally required for efflux of 2′-deoxymugeneic acid and NA into the apoplasm, respectively, and also contribute to Fe mobilization in this compartment.

NA has a key function in the control of cellular metal 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, 2007*a*). 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, favoring either vacuolar storage or translocation to the shoots, two processes in which Fe and Zn compete as well (Arrivault *et al.*, 2006; Haydon *et al.*, 2012; Chen *et al.*, 2018). NA overaccumulation in Arabidopsis 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 the long distance transport of NA–metal complexes. Two major facilitator superfamily transporters, ZINC-INDUCED FACILITATOR 1 (ZIF1) and ZIF2, 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, 2007*b*), and omission of Zn from the medium 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, 2007*b*) 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–metal 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 eight YSL genes display differential patterns of regulation of 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, ABC TRANSPORTER OF THE MITOCHONDRIA 3 (ATM3) exports GSH-polysulfide out of the mitochondria and is required for the maturation of Fe–S cytosolic enzymes and for Mo cofactor (Moco) biosynthesis (Schaedler *et al.*, 2014; Teschner *et al.*, 2010). *atm3* mutants are moreover sensitive to Cd (Hanikenne *et al.*, 2005*b*; Kim *et al.*, 2006*a*).

Interaction between iron and sulfur homeostasis

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, conditions of Fe deficiency 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 for 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-deficient 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* (Forieri *et al.*, 2013). Interestingly, when challenged with both S and Fe starvation, the expression of *IRT1* is induced but to a lower extent compared with Fe-deficient conditions, which can be explained by a decreased requirement of Fe for Fe–S cluster synthesis under S deficiency (Forieri *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 (Forieri *et al.*, 2013; Briat *et al.*, 2015*b*; Bouain *et al.*, 2019*b*). 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, post-transcriptional, 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 post-transcriptional 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.

Interaction between iron and phosphate homeostasis

The interaction between Fe and phosphate (P_i) is well-recognized in soil as well as in plants (Müller *et al.*, 2007; Thibaud *et al.*, 2010; Briat *et al.*, 2015*b*; Bouain *et al.*, 2019*b*). Indeed, the complexing of Fe by P_i 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 P_i 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 P_i-deficient plants revealed an increase in abundance of transcripts of both P_i 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 P_i starvation conditions (Hirsch *et al.*, 2006). Interestingly, higher *AtFER1* 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 P_i status of the plant (Bournier *et al.*, 2013; Bouain *et al.*, 2019*b*; see also Box 1). Decades of research have helped in understanding how plants respond to P_i 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 those for 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 primary root growth inhibition under P_i deficiencies (Müller *et al.*, 2007; Ward *et al.*, 2008; Ticconi *et al.*, 2009; Thibaud *et al.*, 2010; Briat *et al.*, 2015*b*; Bouain *et al.*, 2019*b*). This is consistent with the fact that simultaneous Fe and P_i deficiencies restore primary root growth in Arabidopsis. This observation reveals that plants respond to the combined Fe and P_i stress in a manner distinct from individual Fe or P_i deficiencies (Bouain *et al.*, 2019*b*). 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.*, 2019*b*). Additional candidate genes to regulate root growth under co-occurring Fe and P deficiency stress have been identified (AT1G51310, AT2G36200, AT4G09940, and AT5G16580) and await functional validation (Bouain *et al.*, 2019*b*). It is worth mentioning that a recent study showed, unexpectedly, that if P_i deficiency stimulates early root growth rate of most accessions, Fe deficiency reduces it (Bouain *et al.*, 2019*a*). The combination of both P_i 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 P_i deficiency (Bouain *et al.*, 2019*a*), 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 aimed 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 P_i, 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 P_i 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 P_i content still show chlorosis (Dekock *et al.*, 1979). This provides further support for the interplay between Fe and P_i homeostasis in shoots. Remarkably, plants grown under a combined limitation of Fe and P_i 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 P_i 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 genetics approaches can be used to screen for the phenotypic diversity of Fe and P_i stress responses across plant ecotypes and accessions, and would help in better understanding 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 P_i 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 limits Fe, and also Zn, absorption in the intestinal tract, thereby contributing to mineral deficiencies (Gibson *et al.*, 2018). Finally, whereas it is known that Fe and P_i 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, furthermore, that Fe homeostatic mechanisms are 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.*, 2019*b*). This is even clearer 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 P_i 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 P_i accumulation, it has been shown that Fe accumulation strongly increases in response to combined nitrogen (N) and P_i deficiencies, as compared with the small increase observed under either single P_i or single 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, and 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, P_i, and other micronutrient signals will help in 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 improving Fe accumulation in crops.

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Author contributions

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.

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