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Coordinated homeostasis of essential mineral nutrients: a focus on iron

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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.

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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 (Roscchtztardtz 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; Morrisey 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; Rodriguez-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 *Noccaea 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/YS-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 IDE1 and IDE2 (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 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 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. 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 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. 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 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 [FeCitrateOH]− and [FeCitrate2]− 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 crosstalk 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 (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 to Fe deficiency conditions, which can be explained by a decreased requirement of Fe for Fe–S cluster synthesis under -S (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., 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.
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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.
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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 FERROXIDASE), 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 mouvements 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-STRIPED-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;
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 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 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 plants that export/secrete siderophores or phenolic compounds to mobilize and/or scavenge Fe$^{2+}$ 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 N$_2$ (Boyd and Peters, 2013). In soil, legumes can however form a symbiosis with a diazotrophic bacteria 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 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 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 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