Molecular Aspects of Iron Nutrition in Plants

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Abstract The plant kingdom comprises 766 gymnosperms and ~350,000 angiosperms, for which iron (Fe) is an essential and highly demanded nutrient. Iron is necessary for plant growth and development, being involved in a multitude of functions within the plant, including chlorophyll biosynthesis. The understanding of the mechanisms that govern Fe uptake, transport and storage has been the subject of numerous studies since the middle of the twentieth century, but it was only in the 1990s, with the advent of molecular genetics, cheaper genome sequencing and associated bioinformatic techniques, that scientists began to really unveil the detailed molecular networks responsible for regulating iron homeostasis within the plant. Homeostasis must be guaranteed in order to prevent Fe overload and toxicity but also to assure sufficient levels within the plant to exert its numerous roles, since the

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unalike consequences of both deficiency and toxicity are equally adverse. In this chapter we explore the current knowledge on the different molecular aspects that regulate Fe metabolism in higher plants, looking at Fe uptake and distribution mechanisms, the known signalling molecules and Fe sensing mechanisms, the part of Fe in plant-bacteria symbiosis (including nodulated and non-nodulated plants) and finally, how the molecular aspects of Fe metabolism impact and are impacted by other metals.

1 Introduction

Dietary iron (Fe) deficiency is a worldwide public health problem affecting more than two billion people, whose primary dietary sources are legumes and grains (Myers et al. 2014). Therefore, any enhancement of Fe concentrations in these primary crops would be valuable. Iron (Fe) is also an essential nutrient for plants, with intermediate phloem mobility, whose concentration within a plant system must be tightly regulated. Under Fe deficiency, plants usually develop yellowing of leaves and reduced growth, since this nutrient is necessary in the electron transport chains of photosynthesis and respiration (Prasad 2003); on the other hand, if accumulated in high levels, Fe can generate hydroxyl radicals, which damage lipids, proteins and DNA (Kampfenkel et al. 1995). Hence, plants must respond to Fe levels in terms of both Fe deficiency and Fe overload (Connolly and Guerinot 2002).

The ascension of the omics era has contributed with vast information on Fe metabolism response and associated mechanisms, essential for the development of plant genotype improvement programmes (Vasconcelos et al. 2017). As of today, the genomes of at least 225 plant species have been completely sequenced, providing a plethora of data which allows scientists to understand the commonalities and particularities of the genes underlying mineral uptake, transport and storage. Most species with whole-genome sequences are crops, with food crops comprising the biggest fraction (57.7%), and include not only cereals but other important crop species grown for food, such as fruits, legume grains and vegetables (Leitch et al. 2017). Whole-genome sequencing and gene functional studies nowadays are not only targeting yield and disease resistance but also are targeting the understanding of the key genes involved in enhancing nutrient concentrations, so that better breeding and plant improvement techniques may be applied. Still, although Fe uptake, transport and accumulation mechanisms have been studied for long and applied in different types of biofortification strategies, from the point of view of Fe relationship with enzymatic activities (Weinstein and Robbins 1955), Fe translocation within the plant (Tiffin 1966) or the complexity of the plant-soil interactions (Chaney and Bell 1987), the genetic data gathered in recent years, still postulates assumptions that require further investigation, such as the classical division of plants according to their Fe uptake mechanism.

Soil properties, such as pH, amount of organic matter, clay minerals or carbonate content, greatly impact Fe availability for plant utilization (Bech et al. 2008). However, as soil is a complex matrix composed by several other nutrients, which are also affected by these properties, it is important to account for nutrient crosstalk and the net formed between them. Further in this chapter, the common genes regulated by Fe and other metals (copper, zinc, manganese, boron, molybdenum and cobalt), as well as between Fe and two non-metal nutrients (phosphorus and sulphur), will be scrutinized.

Fe is also essential for the establishment of plant-bacteria symbiosis. When growing in nitrogen-deficient soils, plants develop symbiotic interactions with nitrogen-fixing microorganism developing a new organ named root nodule. This newly formed plant organ, tightly regulated by the host plant, confers plants' ability to fix atmospheric nitrogen (N_2). In this chapter the importance of iron uptake and mobilization within legume plants for nodulation and establishment of effective symbiotic interactions with soil bacteria at a molecular level are reviewed and discussed. Although a brief summary is presented considering differences between legume and nonlegume root nodules, this section is focused on legume-rhizobia symbiosis. Legumes represent one third of primary crop produced in the world being an important dietary source of protein and minerals for both livestock and humans. On a global scale, biological nitrogen fixation in the legume-rhizobia symbiosis accounts for roughly 200 million tons of fixed nitrogen per year (Graham and Vance 2003; Ferguson et al. 2010) with symbiotic crops requiring 35–60% less fossil-based energy than conventional, *N*-fertilized crops (Jensen et al. 2012). In order to potentiate intensive and sustainable agriculture, the production of nitrogen-fixing legumes should be promoted. In parallel, efforts should also be made to develop biofortified legume crops with increasing levels of iron. Still, to integrate the beneficial environmental and dietary effects resulting from legume nodulation, it is crucial to understand how iron is transported to nodules, utilized by the nodule and mobilized towards the plant. Despite the importance of understanding the mechanisms involved on Fe uptake throughout the plant for future modulation towards increasing grain Fe concentrations in these environmentally friendly nodulated plants, little is known about these processes. In this chapter an overview of current knowledge is given. In the context of current climate changes, understanding these mechanisms is even more urgent. Increasing atmospheric CO_2 levels (predicted to double by the end of the century reaching 800 ppm) are foreseen to negatively impact the nutritional status of several crops (Loladze 2014). The reduced Fe levels found were purported to be related with mechanisms involved on Fe transportation within the plant. Under eCO_2 whereas photosynthesis is generally induced leading to a generally higher sugar and biomass accumulation and better yields (Högy et al. 2009; Köehler et al. 2019), it also reduces stomatal conductance which may decrease Fe uptake through the xylem (Ainsworth and Rogers 2007; Xu et al. 2016; He et al. 2018). Recently, it has been reported that increasing air temperature (foreseen to increase 6°C by 2050, relative to 1961–1990) counteracts the negative effect of eCO_2 in the nutritional quality of soybean grains (Köehler et al. 2019), and this opposite effect was attributed to differences in stomatal conductance regulation (Urban et al. 2017).

After discussing the recent knowledge on Fe uptake mechanisms, in this chapter, the essential genetic factors involved in Fe transport and signalling within the plant will be reviewed.

2 Iron Uptake Mechanisms

Although Fe is present in great abundance in the soils, it exists mainly as Fe^{3+}, which forms insoluble hydroxides and becomes unavailable for plant absorption. Therefore, when the conditions are adverse, e.g. alkaline or upland soils, plants are unable to uptake Fe from the soils. To overcome this problem, plants developed strategies to acquire Fe from the rhizosphere, which are classically divided into two. Strategy I, also referred to as 'reduction strategy', is utilized by all dicotyledonous and non-graminaceous plants. The first engaged step consists of proton release via H^+-ATPases in order to decrease rhizosphere's pH and, consequently, increase Fe solubility (Colangelo and Guerinot 2004). After the acidification step, Fe^{3+} is reduced to Fe^{2+} by a root ferric chelate reductase. In *Arabidopsis*, this enzyme is encoded by ferric reductase oxidase 2 (AtFRO2), which is composed of two intramembrane haem groups, and is induced in the root epidermis to transfer electrons across the plasma membrane (using NAD(P)H as an electron donor), performing the reduction step (Robinson et al. 1999). Genes encoding the FRO enzyme include eight members that are differentially expressed at the tissue levels, being not only important for metal acquisition from soil but also for intracellular distribution of Fe (Jain et al. 2014). More specifically, FRO1 was characterized in pea to have 74% of overall similarity to AtFRO2 (Waters et al. 2002); AtFRO5, AtFRO7 and AtFRO8 do not seem to be Fe-regulated, and AtFRO3 is expressed in Fe-deficient leaves (Jeong and Connolly 2009); AtFRO6 overexpression in tobacco plants enhanced ferric reductase activity in the leaves (Li et al. 2011). Alongside with FRO, other compounds have been proposed to have a key role in the reducing step, such as phenolics, organic acids, sugars and flavins (López-Millán et al. 2000; Rodríguez-Celma et al. 2011), and recent reports identified scopoletins, a class of phenolic-type compounds, to be secreted under Fe-deficient conditions and have an important role in plant Fe nutrition (Fourcroy et al. 2014; Schmid et al. 2014; Tsai et al. 2018).

After Fe^{3+} is reduced, Fe^{2+} is transported into the root by iron-regulated transporter 1 (IRT1), which belongs to the zinc-regulated transporter/IRT-like protein (ZIP) family (Guerinot 2000). IRT1 was described to be expressed only under Fe-deficient conditions (Connolly et al. 2002), but it can also transport other divalent metals, and it has been shown that the overexpression of AtIRT1 induces metal overload (Barberon et al. 2011). Other studies showed that when the peanut AhIRT1 gene was introduced in tobacco and rice, it had a dual function: besides being responsible for Fe absorption, it could also be responsible for Fe translocation, as the transgenic plants increased their tolerance to Fe deficiency and, even under Fe sufficiency, Fe concentration was enhanced in roots and shoots (Xiong et al. 2014).

Graminaceous plants, like barley, rice and maize, utilize Strategy II (a ‘chelation strategy’) for Fe uptake. In order to increase uptake, plants release phytosiderophores (PSs) to the rhizosphere which act as chelators with high affinity for Fe^{3+}. The primary member of the PSs family is deoxymugineic acid (DMA), and nicotianamine (NA) is the main precursor for its synthesis (Morrissey and Guerinot 2009). Constitutive expression of the rice OsNAS2 gene in bread wheat increased the biosynthesis of NA and DMA leading to higher concentrations of grain Fe, Zn, NA and DMA and increased Fe bioavailability of the resulting flours (Beasley et al. 2019). Two transcription factors seem to have an essential role in DMA and NA synthesis, namely, IRO2 that regulates their synthesis by influencing DMA and NA synthase (DMAS and NAS) expression (Ogo et al. 2007) and an Fe deficiency-responsive cis-acting element-binding factor 1 (IDEF1) that intervenes in this synthesis by positively regulating the expression of IRO2 (Kobayashi et al. 2009). Phytosiderophores are effluxed to the rhizosphere via TOM1, a transporter whose expression levels augment under Fe-deficient conditions (Nozoye et al. 2011). Once in the rhizosphere, the complex Fe^{3+}-PS is formed and is taken up into the root cells by transmembrane proteins of the yellow stripe1 (YS1) family (Curie et al. 2001). YS1 transporters have been identified in several grass species, and, interestingly, non-graminaceous plants also have YS1-like (YSL) genes that encode proteins essential in metal-NA complex transporting Fe (Inoue et al. 2009).

Although this classic division is mostly true, there are few studies showing that some Strategy II plants could use Strategy I mechanisms, as is the example of rice (Bughio et al. 2002; Ricachenevsky and Sperotto 2014; Pereira et al. 2014). Evidences suggest the use of a ‘combined strategy’, where rice plants besides absorbing Fe(III) via the chelation strategy also take up Fe(II) directly by the induction of Strategy I transmembrane transporters IRT1/IRT2 (Ishimaru et al. 2006; Sperotto et al. 2012; Pereira et al. 2014). Alike rice, homologs for Strategy I-related genes have been found in maize, such as *ZmNRAMP1* (Zanin et al. 2017); however the physiological role and putative functions of these genes in maize are yet to be explored (Li et al. 2018).

3 Molecular Factors Involved in Fe Distribution Within the Plants

After entering the root cells, Fe can be transported to the aboveground organs via the xylem (Broadley et al. 2012). This transport has for long been associated to the formation of complexes between Fe and citrate, which seemed to be the preferential form for Fe loading in the xylem (Tiffin 1966). In the meantime, studies confirmed this theory (Green and Rogers 2004), and a ferric reductase defective 3 (FRD3) protein, belonging to the multidrug and toxic compound extrusion (MATE) family, has been described to be necessary for efficient Fe translocation (Durrett et al. 2007).

Despite being predominantly transported through the xylem (López-Millán et al. 2000), Fe can also be transported through the phloem, complexed with NA, as this metabolite, although not secreted by non-graminaceous plants, is synthetized and chelates Fe (Stephan and Scholz 1993; Takahashi et al. 2003). In apple, NAS1 expression was shown to increase under Fe deficiency and to facilitate redistribution of Fe in plants (Sun et al. 2018). When Fe reaches the leaves, it is putatively unloaded in the apoplastic space via the YSL transporters (Waters et al. 2006).

Free Fe is toxic; therefore, it must be incorporated in storage structures. Ferritins, for example, store Fe in excess for detoxification and maintain the mineral available for protein synthesis (Briat et al. 2010). Ferritins can be found in most of the cellular compartments, but the main storage organelle is the chloroplast (Briat et al. 2010). It is generally established that under Fe supply, genes of the ferritin family are usually overexpressed (Lescure et al. 1991; Wu et al. 2016). The majority of the Fe pool is mainly located in chloroplasts (Roschzttardtz et al. 2013), and, although the method for influx is still not well described (López-Millán et al. 2016), it is thought to require a reduction-based mechanism, mediated by a member of the FRO family, probably FRO7, both in Strategy I and Strategy II plants (Solti et al. 2014). The other major reservoir for inactive Fe is the vacuole, and Fe is imported via a vacuolar membrane transporter, VIT1, and remobilized by the NRAMP3 and NRAMP4 transporters (Lanquar et al. 2005). Moreover, these proteins have been shown to have a conserved role in Fe transportation and homeostasis in different crops, as is the case of VIT expression in rapeseed (Zhu et al. 2016) and of *Arabidopsis* VIT1 expression in cassava that showed promising results for biofortification programme development (Narayanan et al. 2015). The later results were further improved in 2019 by combining the overexpression of VIT1 with IRT1 and FER1, with cassava plants accumulating iron levels 7–18 times higher and zinc levels 3–10 times higher than those in non-transgenic controls in the field (Narayanan et al. 2019). Other examples include AhNRAMP1 in peanut (Xiong et al. 2012) and MxNRAMP1 in apple (Pan et al. 2015) leading to increased Fe accumulation.

4 Signalling Molecules and Fe Sensing Mechanisms

In plants, the complete network behind Fe sensing and signalling is yet to be described (Kobayashi et al. 2018). However, it is known that Fe homeostasis requires different signals and regulators, having an ultimate implication on photoassimilate partitioning, due to its source-sink control (Marschner et al. 1996; Lemoine et al. 2013). Shoots have always been pointed as the main responsible organ for signalling the need for increased Fe uptake at the root level (Brown et al. 1961; Schmidt 2003). A negative feedback control for Fe uptake has been proposed, where Fe sufficiency represses the synthesis of the ferric chelate reduction system (Maas et al. 1988); however, a positive regulation has also been proven to exist,

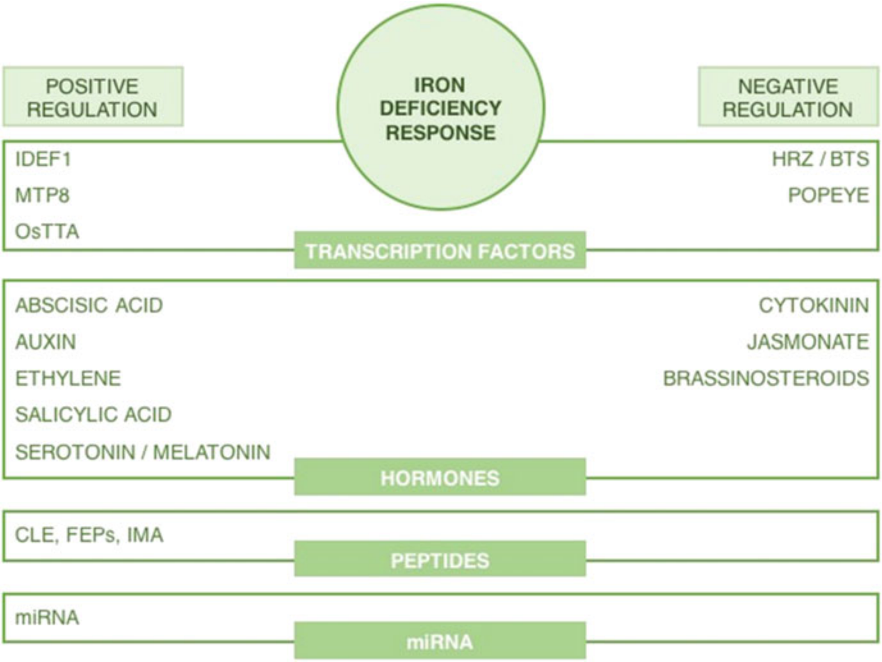

Fig. 1 Positive and negative regulators involved in iron deficiency signalling in plants

where a long-distance signal for Fe deficiency in the shoots induces Fe uptake in the roots (Enomoto et al. 2007). A combined network system for the activation of physiological Fe stress responses has also been suggested (García-Mina et al. 2013). In this model, together with a predominant root-to-shoot signal, which is dependent not on the Fe conditions at the root level, but on the development of Fe stress symptoms in the leaves, a local Fe sensing is also present in the roots, which corresponds to the triggering of FRO and IRT1 genes in response to Fe stress at the root level, independently of the Fe conditions presented by the leaves (Fig. 1).

Molecules with the potential to regulate Fe accumulation have been identified. These include the transcription factor *IDEF1* that positively regulates IRO2 gene in graminaceous plants under Fe deficiency (Kobayashi and Nishizawa 2014); hemerythrin motif-containing really interesting new gene (RING) and zinc-finger proteins (*ZNFs*)/BRUTUS (*BTS*) ubiquitin ligases, which negatively regulate Fe deficiency responses in both graminaceous and non-graminaceous plants, controlling Fe uptake and translocation under Fe sufficiency to prevent Fe excess caused damage (Kobayashi et al. 2013; Matthiadis and Long 2016); a bHLH protein, *POPEYE*, which downregulates genes related to metal translocation (FRO3, NAS4 and ZIF1) under Fe stress conditions (Long et al. 2010); metal tolerance proteins (*MTP*), identified in wheat grains, with a role in divalent metal effluxing out of the cytoplasm and involvement in metal tolerance under Fe deficiency stress (Eroglu et al. 2017; Vatansever et al. 2017); and a putative *Arabidopsis thaliana TITANIA1* ortholog in rice (*OsTTA*) that was shown to be a positive transcriptional regulator of Fe (Tanaka et al. 2018).

Besides the abovementioned proteins and cofactors, phytohormones have been pinpointed for their preponderant role in Fe homeostasis. This is because the concentration and transport of hormones inside the plants seem to dictate shoot responses to nutrient availability in the soil (Kudoyarova et al. 2015). *Cytokinins* act both as root-to-shoot long-distance signal and as a local signal, and they repress IRT1, FRO2 and FIT gene expression (Kiba et al. 2011); *jasmonic acid* also impacts negatively on the expression of Fe uptake genes, IRT1 and FRO2 (Cui et al. 2018; Kobayashi et al. 2016), although some research show that methyl jasmonate foliar application might increase Fe concentration in plant tissues (Ghassemi-Golezani and Farhangi-Abriz 2018; Li et al. 2017); and finally, *brassinosteroids* seem to aggravate Fe deficiency responses by repressing Strategy I response genes, FRO1 and IRT1 (Wang et al. 2012), and phytosiderophore synthesis genes, NAS1, NAS2 and YSL2, in rice (Wang et al. 2015), and by modulating Fe accumulation via a feedback loop (Singh et al. 2018).

Among the phytohormones that positively regulate Fe deficiency responses, it has been demonstrated that *abscisic acid* (ABA) promotes Fe transportation from root-to-shoot and phenolic compound secretion from the roots (Lei et al. 2014) and regulates the maintenance of Fe levels by inducing ferritin expression, as shown in rose petals (Liu et al. 2017a, b); there is a crosstalk between *auxin* and Fe, especially in Strategy II utilizing plants (Garnica et al. 2018; Kabir et al. 2016; Liu et al. 2015; Shen et al. 2016), where auxin application regulates Fe-related genes mainly implicated in phytosiderophore release (NAS, YSL and DMAS), but also in Fe uptake (IRT and FRO), demonstrating its role in root-to-shoot signalling; *ethylene* is an important regulator of Fe deficiency responses (Li and Lan 2017; García et al. 2018) and, more recently, the role of ethylene response factors (ERFs) has been studied (Liu et al. 2017a, b, 2018); *salicylic acid* improves oxidative damage caused by Fe deficiency by affecting the oxidation reaction at the transcriptional level (Kong et al. 2014; Shen et al. 2016), and elevated endogenous levels of this hormone induce other hormone-response genes, such as auxin and ethylene (Shen et al. 2016), implying an interplay in the hormonal signalling; and supplementation with *melatonin* and its precursor *serotonin* alleviates Fe deficiency response by inducing the expression of bHLH protein-encoding genes, FRO2 and IRT1 (Wan et al. 2018), and also by interplaying with abscisic and salicylic acids (Yoon et al. 2019).

However, other molecules are known to also significantly intervene in Fe signalling. Different peptide families have a role in response to Fe stress in plants, for example, the CLAVATA3/ENDOSPERM SURROUNDING REGION (*CLE*) peptides (Araya et al. 2016; Gutiérrez-Alanís et al. 2017) and the FE-UPTAKE-INDUCING PEPTIDEs (*FEPs*) that are induced under Fe-deficient conditions (Hirayama et al. 2018) or a short C-terminal amino-acid sequence consensus motif (IRON MAN; *IMA*) that positively regulates Fe uptake in roots (Grillet et al. 2018). Few studies also point out the role of microRNA (*miRNA*) in Fe homeostasis, whether by up-regulating the expression of Fe deficiency inducible transcription factors and auxin signalling pathways (Kong and Yang 2010) or by post-transcriptionally regulating photosynthetic machinery-related genes (Checovich et al. 2016; Patel et al. 2017) (Fig. 1).

5 The Role of Iron in Plant-Bacteria Symbiosis

Plants are botanically sub-divided into the graminaceous and non-graminaceous families. As mentioned before, these two groups use different pathways to mine iron from the soil. The non-graminaceous plants including the legumes use Strategy I, whereas graminaceous including cereals such as wheat or maize use Strategy II (see Sect. 1). In this section the importance of iron uptake and mobilization within legume plants for nodulation and establishment of effective symbiotic interactions with soil bacteria are reviewed and discussed. A brief summary is presented considering differences between legume and nonlegume root nodules, but considering the importance of legumes for human nutrition, this section is focused on legume-rhizobia symbiosis.

6 Legume and Nonlegume Root Nodules

Root nodules are specialized lateral root organs with 2–5 mm diameter which look like white dots and result from the establishment of an effective mutually beneficial interaction between a plant (the host) and selected soil microorganisms. When plants grow in nitrogen-deficient soils, the formation of root nodules is promoted in order to fulfil the plant nutritional needs. Although nitrogen gas (N_2) is the most abundant gas in the Earth's atmosphere, this molecular form cannot be used by most organisms. Only few bacteria and archaea are capable of fixing N_2 into ammonia that can be assimilated. Nevertheless, many legumes have evolved to establish a symbiosis with gram-negative nitrogen-fixing soil bacteria collectively known as rhizobia. These bacteria belong to the *Rhizobiaceae* family and spread to the alpha- and beta-proteobacteria classes (Moulin et al. 2001) including the genera *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium* (Jordan and Bacteriology 1982), *Mesorhizobium* (Jarvis et al. 1997; Laranjo et al. 2014), *Rhizobium* and *Sinorhizobium* (Chen et al. 1988), among others. *Rhizobia* bacteria grow in the rhizosphere, the narrow soil region in contact with the root, where, due to the organic carbon provided by plant roots in the form of root exudates, the number of microorganism is largely higher (10–100 higher) than in bulk soil (Pii et al. 2015).

Though the majority of plants able to establish this symbiotic relation are in the legume family Fabaceae, there are few exceptions. In the order Rosales, *Parasponia* species belonging to the Cannabaceae family can also establish a symbiotic interaction with *Rhizobia*, constituting the unique nonlegume specie known to be nodulated by *Rhizobia* (Hirsch 1992; Sytsma et al. 2002).

The filamentous gram-positive actinomycete *Frankia* also forms actinorhizal root nodules on a variety of woody shrubs, trees and some herbaceous plants, fixing approximately the same amount of nitrogen as rhizobial symbioses. All actinorhizal species, about 200, belong to the Rosid I clade sharing a common ancestor with legumes (Fabaceae) but differing from them in their wide distribution in numerous botanical families. These species belong to three plant orders, the Fagales which include the Betulaceae, Casuarinaceae and Myricaceae families; the Rosales with the Rosaceae, Elaeagnaceae and Rhamnaceae families; and the Cucurbitales including the Coriariaceae and Datiscaceae families (Santi et al. 2013). Differently from legume nodules which have a peripheral vasculature, the nodules formed by these nonlegume plants possess a central vascular system like roots (Santi et al. 2013; Downie 2014), and bacteria are never released from the infection thread. According to Downie (2014), it may signify that in legumes the oxygen is available for energy production, while in nonlegume the haemoglobin in the cells surrounding the vasculature would tend to bind most of the available oxygen slowing respiration and reducing ATP levels making this symbiosis less efficient than that formed by legume-rhizobia.

7 Legume Root Nodule Formation

Nodulation begins when *Rhizobia* contact the root hairs of the host plant and the roots release flavonoids which are recognized by specific *Rhizobia* bacteria inducing the expression of rhizobial genes encoding enzymes involved in the synthesis and secretion of Nod factors. Nod factors are lipochito-oligosaccharide signalling molecules sensed by the root hair cells through membrane receptor-like kinases, triggering a series of biochemical and morphological changes involved on nodule development (Desbrosses and Stougaard 2011). Root hair deformation (curl) is induced leading to the complete encapsulation of one or more bacteria which multiply, being then transported through an infection thread which grows from the root hair into the basal part of the epidermis cell extending to the root cortex (Hirsch 1992). In the cortical cells *Rhizobia* bacteria are surrounded by a plant-derived membrane named symbiosome membrane forming the symbiosome, an organelle-like structure separated from the plant cell (Verma and Hong 1996). In mature symbiosomes, rhizobia differentiate into a nitrogen-fixing form named bacteroid, which has the ability to absorb dinitrogen gas (N_2) from the atmosphere and convert it to ammonia (NH_3) which is incorporated into organic form before being exported from nodules. For nitrogen fixation the bacteroid requires a constant supply of energy and carbon from the host plant in the form of organic acids. In the nodules, sugars translocated from the shoot are converted to phosphoenolpyruvate through glycolysis which is converted to malate by the action of phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase activities. Malate is considered to be the primary source of carbon transported to bacteroids where it is converted to CO_2 and water to produce ATP and reducing power required for nitrogen fixation (Vance and Gantt 1992; Colebatch et al. 2004; Udvardi and Poole 2013).

Legume nodules formed through these symbiotic interactions can be determinate or indeterminate. Determinate nodules lose meristematic activity shortly after initiation, and nodule growth is due to cell expansion resulting in mature spherical nodules. These nodules develop in tropical legume, such as those of the genera *Glycine* (soybean), *Phaseolus* (bean), *Arachis* (peanut) and *Vigna* (cowpea), and on some temperate legumes such as *Lotus* (e.g. *Lotus japonicus*). Indeterminate nodules maintain an active apical meristem that produces new cells through cell division at the distal end of the nodule becoming elongated. Therefore, these nodules form four zones: meristem (zone I); invasion zone (II) in which rhizobia colonize plant cells, proliferate, and differentiate; the nitrogen fixation zone (III) where bacteroids produce ammonia for the plant; and the senescence zone (IV), which is present only in older nodules and may serve to recycle nutrients (Hirsch 1992). Examples of plants developing indeterminate nodules include temperate legumes such as those from the genera *Pisum* (pea), *Medicago* (alfalfa) and *Trifolium* (clover) (Mao et al. 2015).

8 Impact of Soil Iron Deficiency in Nodulation

Nodulation is regulated by external (heat, soil pH and composition, drought, nitrate) and internal factors (autoregulation of nodulation, ethylene). Autoregulation of nodulation controls nodule numbers per plant through a systemic process involving the leaf (Ferguson et al. 2010). Several studies have shown that Fe starvation limits root nodule bacterial survival and multiplication, as well as host plant growth, nodule initiation and development and nitrogen fixation (Tang et al. 1991a; Slatni et al. 2011, 2014).

Lupinus angustifolius plants supplied with mineral nitrogen have been reported to have lower shoot weight than nodulated plants supplied with increasing Fe levels (Tang et al. 1992). These results demonstrated that nodule formation and function require more Fe than the growth of the host plant.

Nitrogen concentrations and contents in nodulated *Phaseolus vulg*aris L. (Hemantaranjan 1988) and *Arachis hypogaea* L. (Tang et al. 1991b) were reported to decrease under Fe deficiency. In addition, in several legumes, Fe deficiency decreases nodule number and bacteroid nitrogenase activity (Tang et al. 1992). In the nodules of iron-deficient *A. hypogaea*, nitrogenase activity has been shown to be reduced by 10–20% with nitrogen fixation being further delayed in these plants as compared to plants grown under normal conditions (O'Hara et al. 1988; Tang et al. 1991b). The reduced activity may be partly caused by reduced synthesis of leghemoglobin and bacteroid proliferation (Tang et al. 1992). In *L. angustifolius* nodule initiation is impaired by Fe deficiency (Tang et al. 1991a, 1992). Due to the inhibition of rhizobia proliferation in roots, the division of root cortical cells required to form nodule meristems is limited causing the nodulation impairment (Tang et al. 1992).

9 Iron Requirements for Legume-Rhizobia Symbiosis

The establishment of a symbiotic relation between the host plant and the bacteroid requires increased uptake of micronutrients by the plant, since the bacteroids in root nodules are dependent on the plant for all micronutrients (Clarke et al. 2014). Iron is a critical micronutrient for the symbiosis because several symbiotic proteins incorporate iron including the bacterial nitrogen-fixing enzyme (nitrogenase), cytochromes required for phosphorylation in the plant and bacteria, plant leghemoglobin which transports oxygen, ferredoxins involved in transferring electrons and reducing the Fe component of nitrogenase and a variety of other Fe proteins such as hydrogenases (Guerinot 1991; Broadley et al. 2012; Brear et al. 2013). Leghemoglobins are the most abundant plant proteins in nodules representing as much as 25–40% of the total soluble protein in the infected plant cell (Kozlov 2014) and containing about 24% of the soluble iron within the nodule (Ragland and Theil 1993). These proteins are composed of an apoprotein and a haem moiety both synthesized by the plant (O'Brian 1996). Since the bacteroids do not have contact with the pool of leghemoglobin in the cytoplasm of infected cells (Wittenberg et al. 1996), leghemoglobins may not constitute a major source of Fe during symbiosis. However, they have been shown, via reverse genetics, to have a crucial role in symbiotic nitrogen fixation (Ott et al. 2005). Suppression of the expression of the gene encoding leghemoglobin in *Lotus japonicus* led to loss of leghemoglobin in nodules, higher steady-state levels of free oxygen but lower ATP/ADP ratios and a complete absence of nitrogenase activity (Ott et al. 2005). The bacteroid nitrogenase complex consists of six protein subunits (two each of NifH, NifD and NifK), four iron-sulphur clusters (two each of [4Fe–4S] and Fe_8S_7) and two iron-molybdenum cofactors (Fe_7MoS_9N) named FeMoco (Downie 2014). This enzyme is very sensitive to oxygen; therefore, in order to avoid nitrogenase degradation and provide the necessary oxygen for bacterial respiration, the legume host maintains microaerobic oxygen concentrations by controlling the permeability of the nodule cells to oxygen through changes in the proportion of gas and water in the inner cortex of the nodule (Wei and Layzell 2006) or by increasing the expression of leghemoglobin in the infected nodule cells. Leghemoglobin buffers free oxygen content in the nitrogen-fixing zone of the plant nodule reducing it to the nanomolar range avoiding the irreversible inactivation of the oxygen-labile nitrogenase while providing adequate oxygen levels for bacterial respiration (Ott et al. 2005).

10 Iron Uptake to the Nodule and Remobilization

Regardless of the importance of understanding the mechanisms involved on iron uptake to the nodule and translocation from the nodule, little is known about these transport mechanisms (Slatni et al. 2011; Brear et al. 2013).

Legumes are Strategy I plants, reducing Fe by activating the plasma membrane ferric chelate reductase enzyme or by exuding organic acids and protons to the rhizosphere and by taking up reduced Fe using specific Fe transporters. In root nodules of *Phaseolus vulgaris* grown under Fe deficiency, H^+-ATPase and ferric chelate reductase activities have been shown to be induced (Slatni et al. 2009, 2011). In addition it was reported that under Fe deficiency there is an over-accumulation of H^+-ATPase and ZIP transporters (IRT1) in the nodules' cortex cells of plants tolerant to Fe deficiency particularly around the cortex cells of nodules (Slatni et al. 2012). These results suggest that Fe would be transported through the apoplast using H^+-ATPases and ZIP family members to uptake apoplastic Fe. In such case nodule epidermis would play a major role in Fe uptake in tolerant cultivars. This hypothesis was further supported in a subsequent study reporting that under Fe deficiency Fe allocation from the root system to the nodules was preferential in cultivars tolerant to Fe deficiency (Slatni et al. 2014). However, in indeterminate nodules of *Medicago truncatula*, Fe was not observed at the epidermis of the nodule appearing to be delivered into nodules via the vascular system and released into the apoplasm of cells in the invasion zone to the rhizobia-infected cells by an NRAMP plasma membrane transporter before being taken up by plant cells and transported to bacteroids (Rodríguez-Haas et al. 2013; Tejada-Jiménez et al. 2015). These contrasting results suggest that nodule Fe uptake directly from the medium may be a secondary route of Fe acquisition, comprising a mechanism developed by specific cultivars to cope with Fe limitation.

Multidrug and toxic compound extrusion (MATE) proteins transport citrate and can form chelates with ferric Fe facilitating Fe mobilization (Takanashi et al. 2013). In *Arabidopsis* a root citrate transporter (*AtFRD3*), a member of MATE family, has been reported to be required for Fe translocation from roots to shoots (Durrett et al. 2007). And two MATE transporters (*GmFRD3* and *GmFRD3b*) have been shown to mediate Fe translocation in soybean (Rogers et al. 2009). Regarding the possible involvement of MATE transporters on Fe regulation in the nodule, Takanashi et al. (2013) reported that in *Lotus japonicus LjMATE1* is induced during nodule formation, mainly in the infection zone, and suppression of its expression leads to reduced Fe accumulation in infected cells. These observations suggest that *LjMATE1* is a nodule-specific transporter providing citrate for Fe translocation from the root to nodules being involved on Fe accumulation in the nodules (Takanashi et al. 2013) (Fig. 2). In a recent study conducted in *Medicago truncatula*, the *MtMATE67* protein which segregates into a clade that includes the citrate efflux transporters *LjMATE1* and *AtFRD3* was found to be located in the plasma membrane of nodule cells and also in the symbiosome membrane surrounding bacteroids in infected cells. *MtMATE67* has been shown to actively transport citrate out the cells when expressed

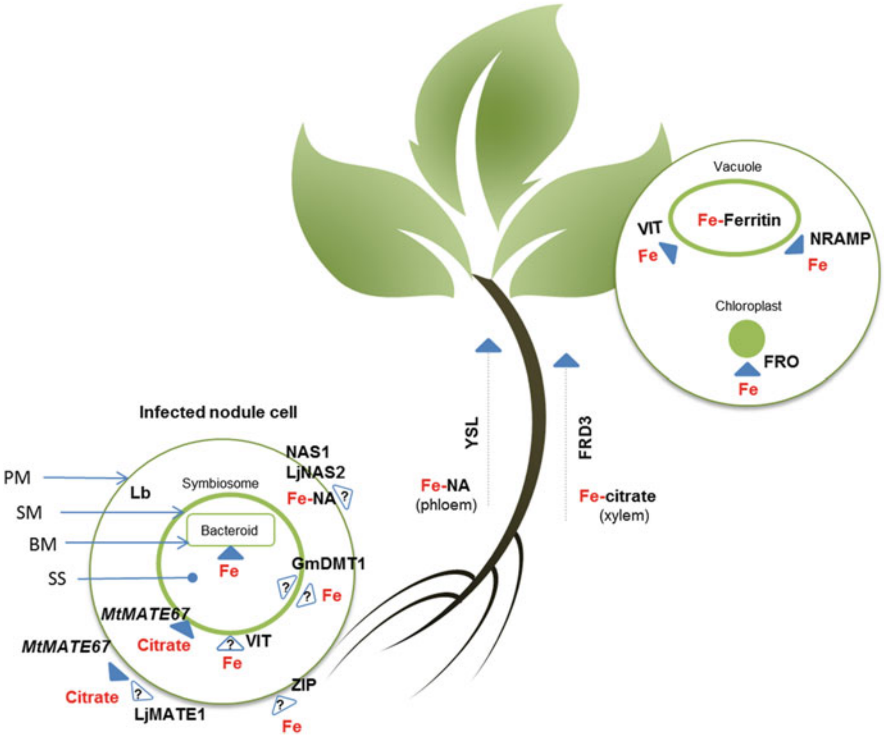

Fig. 2 Main proteins involved in the Fe transport and distribution in the plant and in the nodules. Transport movement is indicated by a blue arrow; all transport proteins and chelators are depicted in black. *PM* plasma membrane, *SM* symbiosome membrane, *SS* symbiosome space, *BM* bacteroid membrane, *Lb* leghemoglobin. Undetermined direction of Fe transport is marked by '?'

in *Xenopus oocytes* with its suppression leading to Fe accumulation in the apoplasm of nodule cells and a significant decrease in nitrogen fixation and plant growth (Kryvoruchko et al. 2018). In this study it became clear that *MtMATE67* plays an important role in the citrate efflux from nodule to cells by increasing Fe(III) solubility and mobility in the apoplasm and uptake into nodule cells. Also at symbiosome level, the citrate transport by *MtMATE67* into the symbiosome space would contribute for pH reduction and increased Fe solubility and availability for bacteroids (Kryvoruchko et al. 2018) (Fig. 2).

In non-graminaceous plants, such as legumes which use Strategy I mechanism for Fe uptake, nicotinamide (NA), a phytosiderophore precursor, is not exuded by the plant to increase Fe uptake, but it chelates metal cations including Fe within the plant. Therefore, NA is believed to play a role as a long-distance signalling molecule (Curie and Briat 2003) possibly acting as a regulator of internal Fe transport, being involved on Fe homeostasis in plants (Takahashi et al. 2003; Hakoyama et al. 2009). When a gene encoding nicotianamine aminotransferase (*HvNAAT*) from barley

(*Hordeum vulgare*, Strategy II plant) was introduced into tobacco plants (Strategy I plants), the plants developed chlorosis symptoms related with NA shortage resulting from the overproduction of the enzyme (NAAT) (Takahashi et al. 2003).

Although in *Lotus japonicus* the suppression of NA synthase 2 (*LjNAS2*) responsible for NA formation did not affect nitrogen fixation, it has been shown to be specifically expressed in the nodules particularly in nodule vascular bundles and further expressed in a later stage of nodule development (maximum reached 24 days after inoculation). In this study the authors hypothesized that *LjNAS2* may be involved on Fe remobilization from the nodules at senescence (Hakoyama et al. 2009) (Fig. 2). During seed filling, the levels of Fe in nodules have been shown to be reduced by about 50%, suggesting that Fe present in nodules may possibly be remobilized at senesce to the seeds as a NA chelate through the phloem (Curie et al. 2009). In soybean plants overexpressing the barley NA synthase 1 (*HvNAS1*) gene, the seeds NA content increased by fourfold and the seeds Fe concentrations by twofold compared with the levels found in the seeds of non-transgenic plants. Furthermore, the transgenic plants showed tolerance to Fe deficiency (Nozoye et al. 2014). In a similar study conducted in sweet potato, transgenic plants overexpressing *HvNAS1* showed 7.9-fold higher levels of leaf NA and accumulated threefold higher levels of Fe than non-transgenic plants. Also, similar to the one observed for soybean, transgenic plants exhibited tolerance to restricted Fe supply which was positively correlated with the *HvNAS1* expression level (Nozoye et al. 2017). These studies further support the hypothesis previously proposed regarding Fe remobilization as Fe-NA chelates from the nodule to the seeds at senescence. If so, this transport would require the involvement of YSL (yellow stripe-like) transporters (Fig. 2).

11 Iron Transport Within the Nodule

A number of transporter families have been implicated in Fe movement across the symbiosome membrane including natural resistance-associated macrophage protein (NRAMP) (Kaiser et al. 2003; Jeong et al. 2017), vacuolar iron transporter (VIT) (Kim et al. 2006; Jeong and Guerinot 2009; Jeong et al. 2017), yellow stripe-like (YSL) (Curie et al. 2009), zinc-iron permease (ZIP) (Moreau et al. 2002; Abreu et al. 2017) and multidrug and toxic compound extrusion (MATE) (Takanashi et al. 2013; Kryvoruchko et al. 2018). The symbiosome membrane is derived from the host plasma membrane but shares properties with the vacuolar membrane (Verma and Hong 1996). It acts as a regulation point under plant control which contains transporters and channels to facilitate the movement of nutrients and metabolites between the host and the symbiosome space (Brear et al. 2013).

The transport of Fe through the symbiosome membrane of soybean has been shown to be faster than Fe transport across the bacteroid membrane (LeVier et al. 1996). Accordingly, ferric Fe chelates are believed to be mainly located in the symbiosome space rather than in the bacteroid. The lower pH of the symbiosome space as compared with that of plant cytosol would promote the stabilization of ferric chelates such as citrate for subsequent uptake by the bacteroid (Moreau et al. 1995). Recent studies suggest that the symbiosome membrane may possess ferric chelate reductase activity (Slatni et al. 2009, 2011). This property would be inherited by the plasma membrane of the infected plant cell of the host plant from which it was formed and would be involved in the reduction of ferric Fe to ferrous Fe for subsequent uptake by the symbiosome. The symbiosome has the ability to uptake ferric and ferrous Fe, but the import of ferrous Fe is much efficient (Moreau et al. 1995; LeVier et al. 1996). Other findings support the hypothesis of ferric chelate accumulation in the symbiosome space. Whereas in the symbiosome space of isolated soybean symbiosomes the levels of leghemoglobin are extremely low, Fe bound to siderophores (compounds binding ferric Fe with high affinity) are present in high concentration in the symbiosome space (Wittenberg et al. 1996).

The ZIP transporter IRT1 was detected in nodule cortex cells of *Phaseolus vulgaris* (Slatni et al. 2012), but the only ZIP family member characterized in this organ, *GmZIP1*, seems to be localized in the symbiosome and has been shown to have a role in Zn uptake (Moreau et al. 2002). Also, a member of the NRAMP family of transporters, the divalent metal transporter 1 (DMT1), has been identified as a ferrous Fe transporter on the soybean symbiosome membrane (Kaiser et al. 2003). However, it is not clear if it transports ferrous Fe across the symbiosome membrane to the symbiosome space or to the cytosol of the infected plant cell or even if this is a bidirectional transporter.

In *Lotus japonicus* a vacuolar iron transporter 1 (VIT1), SEN1, has been shown to be expressed in nodule-infected cells being essential for nitrogen fixation in this host (Hakoyama et al. 2012). It suggests that SEN1 may have a role in the symbiosome membrane as a Fe importer. In *Arabidopsis* VIT1 transports ferrous Fe into the vacuole (Kim et al. 2006), and the symbiosome membrane is known to possess vacuolar membrane properties (Verma and Hong 1996).

YSL transporters belonging to the oligopeptide (OPT) superfamily are mainly located in plasma membrane, transporting ferric and ferrous Fe chelated to nicotinamide (NA) into the phloem or sink cells (Curie et al. 2009). These transporters are mainly found in monocots since they mediate the uptake of Fe(III)-PS complexes from the rhizosphere, but they also exists in dicots where they are thought to be specialized in Fe(II)-NA long-distance transport (Curie and Briat 2003; Nozoye et al. 2011; Nozoye 2018). In a transcriptome study conducted in soybean including the analysis of nodule tissue, it has been shown that 15 YSL transporters were encoded in the genome with one of these transporters (Glyma 11g31870) presenting nodule-specific expression, whereas eight others were also detected (Libault et al. 2010; Severin et al. 2010).

Figure 1 summarizes the main players in the Fe trafficking pathways described above.

Table 1 Common gene regulation and mineral interactions between iron and other metals

Iron interaction with	Common gene regulation	References
Copper	FRO2, IRT1, SOD, oxidoreductases, *redox-responsive transcription factor 1* (*RRTF1*), ethylene response factor (ERF)	Ramamurthy et al. (2018) and Santos et al. (2013)
Zinc	ZIP-like transporters, metallothioneins, metal ion transport and binding, defence response genes	Darbani et al. (2015), Khokhar et al. (2018), Ma et al. (2017), Santos et al. (2013) and Zeng et al. (2018)
Manganese	NRAMP1, NRAMP6, IRT1, MTP8	Cailliatte et al. (2010), Connolly et al. (2002), Eroglu et al. (2017), Long et al. (2018) and Peris-Peris et al. (2017)
Cobalt	IRT1, IREG1/FPN1, IREG2/FPN2	Korshunova et al. (1999), Lange et al. (2017) and Morrissey et al. (2009)
Molybdenum	MOT1, CNX2, CNX3, ABA3, FRO6, FRO7, IREG1	Bittner (2014), Morrissey et al. (2009), Tomatsu et al. (2007) and Vigani et al. (2016)
Nickel	IRT1, IREG2, MTP3, NRAMP1, FRO2	Meier et al. (2018), Mihucz et al. (2012), Nishida et al. (2011) and Schaaf et al. (2006)

12 Molecular Interactions of Fe with Other Nutrients

Since the regulation of Fe homeostasis implies significant responses by plants, other metals essential in the maintenance of optimal plant growth might also be impacted. However, information on the potential interaction and crosstalk among different metal ions and their effect on plant mineral metabolism are still limited (Anjum et al. 2015).

When comparing ionome data from three different plant systems, namely, Strategy I *Glycine max* (Santos et al. 2016), Strategy II *Brassica napus* (Maillard et al. 2016) and *Oryza sativa* (Pereira et al. 2014), that utilizes a combined strategy system, the uptake of micronutrients such as Cu, Zn, Mn, B, Mo and Co was consistently increased by Fe depletion in all plant systems. Hence, it is clear that Fe homeostasis has a serious implication in plants' metal ion regulation, and potential interactions and uptake/transport mechanisms are commonly triggered (Table 1).

Plants utilize the same reduction strategy described for Fe to reduce *Cu*, where FRO2 reduces Cu^{2+} to Cu^{+} at the root surface, although it has been hypothesized that Cu^{2+} could be directly taken up via IRT-like transporters (Jain et al. 2014). Since under Fe deficiency this FRO2/IRT1 system is usually up-regulated (Robinson et al. 1999), it seems probable that, in the absence of Fe substrate and having high affinity for Cu ions, plants increase Cu uptake. A positive correlation between leaf Cu and Fe concentrations was found (Stein et al. 2016), and, in a recent work, a crosstalk between Fe and Cu has been demonstrated (Ramamurthy et al. 2018). Furthermore, this metal is essential for oxidation and reduction reactions, is a constituent of several enzymes with oxidase function and is also a cofactor of Cu/Zn superoxide dismutase (SOD) (Broadley et al. 2012). At a molecular level, Cu deficiency leads to similar gene regulation as Fe, with a particular impact on oxidative stress-related genes, namely, oxidoreductases, *redox-responsive transcription factor 1* (*RRTF1*) and ethylene response factor (ERF) (Ramamurthy et al. 2018; Santos et al. 2013).

On the other hand, *Zn*, which also shares ZIP-like transporters like IRT1, is sometimes found decreased in Fe-deficient roots (Roriz et al. 2014; Santos et al. 2015) but is generally increased in the shoots of different plants. Studies show that the transfer of Zn from the root to the shoot is very fast (Page and Feller 2005); hence, the increase of Zn in the shoot could be representative of increased Zn root uptake by the plants. Low levels of Zn supply are also related to increased Cu concentration in plant shoots (Zeng et al. 2018). This metal is the second most abundant metal in living organisms after Fe, and, in cereals, Zn deficiency is one of the most serious micronutrient deficiencies on calcareous soils, deeply impacting other nutrient accumulation (Broadley et al. 2012; Khokhar et al. 2018). Like Fe, it is typically taken up as a divalent cation (Broadley et al. 2012) and is required for structural and functional activities of several essential proteins (Fox and Guerinot 1998). Molecular studies show that both Fe and Zn homeostasis require the regulation of metallothioneins, metal ion transport and binding and defence response genes (Darbani et al. 2015; Santos et al. 2013; Zeng et al. 2018). With the identification of QTL for levels of Fe and Zn, common markers for these two minerals have been further identified (Ma et al. 2017).

Despite having a wide range of oxidation states, Mn^{2+} is the most soluble form in the soil and consequently the most accumulated form in plants (Broadley et al. 2012). Mn has a major role in activation of enzymes involved in lignin biosynthesis, photosynthesis and detoxification of O_2 free radicals through Mn SOD activity (Page et al. 2006). Its uptake occurs via active diffusion through root epidermal cells, and, like Cu, Mn competes for common transporters and ligands of Fe, namely, NRAMP1 (Cailliatte et al. 2010), NRAMP6 (Peris-Peris et al. 2017) and metal transporters from the ZIP family (IRT1), both in Strategy I plants, like *Arabidopsis* (Connolly et al. 2002), and in Strategy II plants, like barley (Long et al. 2018). Metal tolerance protein 8 (MTP8) was also found to be able to transport both Fe and Mn in *Arabidopsis* plants, with a particular role in regulating Fe and Mn homeostasis in the seeds (Eroglu et al. 2017). Hence, when Fe is present, it has been found a positive correlation between these two metals, particularly regarding root concentrations (Alagic et al. 2018).

Required by legumes for N_2 fixation, *Co* is mainly accumulated in the root system (Page et al. 2006). Reports on the crosstalk between Co and Fe hypothesize that the molecular mechanisms behind Co accumulation might have evolved from Fe homeostasis mechanisms (Lange et al. 2017). Like Fe, Co uptake is mediated by IRT1 (Korshunova et al. 1999), and Co excess is correlated to the decrease of photosynthetic pigments due to the prevention of the incorporation of Fe in the protoporphyrin molecule, the precursor for chlorophyll (Jayakumar et al. 2009). Also in common with Fe metabolism, ferroportins Iron Regulated1 (IREG1/FPN1) and IREG2/FPN2 seem to be important in Co detoxification and translocation to the shoot (Morrissey et al. 2009).

For long, *Mo* and Fe have been correlated with each other (Kannan and Ramani 1978; Bittner 2014), and recent evidence has proven that Fe nutritional status greatly impacts Mo homeostasis in plants (Vigani et al. 2016). Plants absorb Mo essentially as molybdate (MoO_4^{2-}), and this element is a constituent of nitrate reductase enzyme, consequently having an important role in plant metabolism, especially in legumes (Duan et al. 2017). This mineral only has biological activity when complexed by a pterin compound, forming the molybdenum cofactor (Moco) and active Mo enzymes depend on the presence of Fe and Cu (Mendel 2007). Hence, Fe is essential for plant Mo regulatory metabolism since Moco biosynthesis is dependent on Fe availability. Its bioavailability is hindered under acidic conditions (Kaiser et al. 2005), and, given the fact that under Fe deficiency plants usually acidify the rhizosphere, Mo concentration is usually decreased in the leaves (Baxter 2009; Roriz et al. 2014; Vigani et al. 2016) and increased in the roots (Santos et al. 2016; Vigani et al. 2016). Few Mo metabolism-related genes have been identified in plants, but the genes MOT1 (Tomatsu et al. 2007) and MOT2 (Gasber et al. 2011) have been shown to have key role on Mo intracellular and interorgan transport. The MOT1 gene, in particular, was shown to be downregulated under Fe deficiency, while three genes essential for Moco biosynthesis in plants, CNX2, CNX3 and ABA3, seem to be up-regulated (Bittner 2014). On the other hand, Mo deficiency does not impact the main players in Fe reduction, uptake and accumulation (FRO2, IRT1 and ferritin) but impacts other isoforms of the FRO gene, FRO6 and FRO7, as well as the iron-regulated protein-encoding gene IREG1 Morrissey et al. (2009).

Abundantly available in the soils, *Ni* predominant oxidation state is +2, which is the most available for plant uptake through both passive diffusion and active transport (Seregin and Kozhevnikova 2006). This metal is utilized in several physiological processes, being an important component of many metalloenzymes and having a vital role in nitrogen metabolism, mainly in root nodule growth and hydrogenase activation (Yusuf et al. 2011). Studies show that high dosages of Ni increase relative transcription level of AtIRT1 in roots (Nishida et al. 2011) and that Fe and Ni share similar uptake mechanisms (Mihucz et al. 2012), putatively competing for absorption (Boostani et al. 2019). After uptake by IRT1, Ni seems to be internalized in the vacuole by iron-regulated protein 2 (IREG2) (Schaaf et al. 2006). Other Fe-related genes were found to be up-regulated in the presence of Ni, namely, Fe deficiency-induced transcription factor (FIT); two metal transporters, MTP3 and NRAMP1; as well as FRO2 (Meier et al. 2018).

Other, non-metal, nutrients are also correlated to Fe metabolism (Table 2). For example, the concentration of available phosphorus (*P*) modulates Fe^{2+} uptake system (Ward et al. 2008), and P deficiency seems to lead to a common regulation of 579 genes in *Arabidopsis* (Li and Lan 2015). Among this pool of genes, the ones with stronger induction and highly correlated to Fe mechanisms were FRO2 and

Table 2 Common gene regulation and mineral interactions between iron and other non-metal minerals

Fe interaction with	Common gene regulation	References
Phosphorus	FRO2, IRT1, AT3G12900, CYP82C4, AT5G38820, COPT2, AT1G30560, AtOCT1, NRAMP1, PHT1, PHO1, FRO3, IRT1, IRT2, NAS1, FRO6, PHR1	Briat et al. (2015), Li and Lan (2015), Santos et al. (2013) and Zanin et al. (2017)
Sulphur	FRO1, IRT1, SIST1;1, SIST1;2, IRO2, APR, SIR	Astolfi et al. (2006), Ciaffi et al. (2013), Forieri et al. (2013), Grewal et al. (2018), Hantzis et al. (2018), Paolacci et al. (2014) and Zuchi et al. (2009)

IRT1 and AT3G12900, CYP82C4, AT5G38820, COPT2, AT1G30560 and AtOCT1. On a different study, also in *Arabidopsis*, P deficiency decreased the expression of FRO3, IRT1, IRT2, NAS1 and FRO6, but both Fe and P homeostasis were found to be impacted by the regulation of a MYB-like transcription factor, phosphate starvation response 1 (PHR1) (Briat et al. 2015). Moreover, as seen before in soybean plants under Fe deficiency (Santos et al. 2013), P deficiency induces the regulation of flavonoid biosynthetic process (Li and Lan 2015). Strategy II utilizing plants, like maize, also showed up-regulation of transporters for Fe^{2+}, like NRAMP1, PHT1 and PHO1, which resulted in higher accumulation of P in both roots and shoots (Zanin et al. 2017).

Sulphur (*S*) and Fe interaction has been well described from the perspective of the formation of Fe-S clusters, which are essential in photosynthesis, respiration and other cellular reactions (Broadley et al. 2012; Lu 2018). However, the crosstalk between these two minerals at a molecular level is yet to be fully understood. In the last decade, it has been shown that FRO1 and IRT1 gene expression is repressed under S deficiency (Zuchi et al. 2009; Forieri et al. 2013) and that Fe deficiency also modulates S-related gene expression by inducing the up-regulation of S transporters, such as SIST1;1 and SIST1;2 (Paolacci et al. 2014). In Strategy II utilizing plants, S uptake is also related to Fe uptake mechanisms since, when S is deficient, the release of phytosiderophores to the rhizosphere is repressed (Astolfi et al. 2006) and, for example, the expression of Fe-related transcription factor IRO2 is increased (Grewal et al. 2018); on the other hand, when Fe is deficient, S deficiency response is triggered at a molecular level, particularly S transporters and the genes encoding ATP sulphurylase (Ciaffi et al. 2013), adenosine 5′-phosphosulphate reductase (APR) and sulphite reductase (SIR) (Hantzis et al. 2018). It is also interesting to note that S application to the soil acidifies the pH (Ramzani et al. 2017), turning the conditions more appropriate for Fe reduction and uptake mechanisms activation.

13 Conclusion

Since Fe homeostasis regulation is a complex system, much is yet to decipher. Some molecules have been demonstrated to be key in the process of Fe sensing within the plant; however, the positive and negative feedback signals described are not enough to explain this regulation. Local sensing and a long-distance sensing system contribute for the homeostasis maintenance that is strongly supported by the equilibrium of the Fe uptake systems and rapidly triggered/repressed, depending on plant's necessities. Furthermore, since it is difficult to mimic, under controlled conditions, the interactions between various nutrients, studies where two or three different nutrients are assayed at the same time are more frequent. The study of the crosstalk between different nutrients is crucial to understand the variation and plant distribution of different nutrient pools that can impact plants' vigour and health.

Fe is an essential micronutrient for the establishment of effective legume-rhizobia symbiosis, but Fe transport from the root to the nodule and within the nodule of symbiotic legumes is very complex, and little is known on how Fe is transported across a number of membrane layers until reaching the bacteroid. Despite some efforts that have been done to identify Fe transporters involved on Fe uptake by the nodules, it has been challenging to recognize their exact location and their precise function within the nodule.

Considering the importance of nodulation for increasing sustainable nitrogen fixation concerning environmentally friendly agricultural practices and the crucial role of Fe nutrition in the effectiveness of these mutually beneficial associations, it is of utmost importance to further understand the molecular mechanisms involved on Fe uptake in nodulated legumes, and we believe this is an area that warrants further exploration in the future. In the context of current climate changes, understanding these mechanisms is even more urgent. Increasing atmospheric CO_2 levels are foreseen to decrease Fe accumulation in several crops (Loladze 2014), and it is believed to be related with Fe transportation throughout the plant particularly through the xylem since stomatal conductance is generally reduced under eCO2 (Ainsworth and Rogers 2007; Xu et al. 2016; He et al. 2018). Increasing temperature on the contrary induces stomatal conductance (Urban et al. 2017) and has recently been reported to counteract nutritional losses induced by eCO2 (Köehler et al. 2019).

In the frame of current (1) climate changes possibly inducing nutritional losses is several crops, (2) necessity for highly nutritious enhanced food crops capable of coping current Fe deficiencies in populations worldwide, and (3) need of intensive and sustainable agricultural practices to feed an increasing human population, it is crucial to understand the molecular features of Fe uptake and movement within the plant.

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