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Minireview

Mining iron: Iron uptake and transport in plants

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Abstract **Iron uptake in plants is highly regulated in order to supply amounts sufficient for optimal growth while preventing excess accumulation. In response to iron deficiency, plants induce either reduction-based or chelation-based mechanisms to enhance iron uptake from the soil. Genes involved in each mechanism have been identified from various model plants including Arabidopsis and rice. Iron transport within plants is also tightly controlled. New information has emerged on transporters that play a role in xylem loading and phloem loading/unloading of iron, and on the iron chelators involved in iron homeostasis. Some of the components regulating iron deficiency responses also have been elucidated, demonstrating that iron dependent gene regulation occurs at both the transcriptional and post-transcriptional levels.**
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1. Introduction

Iron (Fe) is an essential nutrient for plants. It is required for life-sustaining processes from respiration to photosynthesis, where it participates in electron transfer through reversible redox reactions, cycling between Fe^{2+} and Fe^{3+}. Insufficient Fe uptake leads to Fe-deficiency symptoms such as interveinal chlorosis in leaves and reduction of crop yields. Plants need to maintain Fe in the concentration of 10^{-9}–10^{-4} M to achieve optimal growth, however Fe acquisition is challenging due to the low solubility of Fe in soil solution [1]. Although Fe is the fourth most abundant element in the earth's crust, it is not readily available to plants. In well-aerated soils at physiological pH, the concentrations of free Fe^{3+} and Fe^{2+} are less than 10^{-15} M, a value far below that required for optimal growth [2]. Thus, Fe-deficiency often limits plant growth causing agricultural problems. In fact, one third of the world's cultivated soils are calcareous and considered Fe deficient [3]. In addition to the low solubility, the properties of Fe require plant cells to place limitations on its accumulation. Superoxide and hydrogen peroxide, that are produced in the cells during the reduction of molecular oxygen, are catalyzed by Fe^{2+} and Fe^{3+} to form highly reactive hydroxyl radicals [4]. The hydroxyl radical can damage most cellular components such as DNA, proteins, lipids and sugars. Thus, Fe uptake in plants is highly regulated to prevent excess accumulation.

Once Fe has entered the symplast, Fe is bound to various chelators, facilitating it remaining in solution and preventing it from participating in the generation of hydroxyl radicals. Organic acids, such as citrate, are known to bind Fe^{3+}; nicotianamine (NA) forms stable complexes with both Fe^{2+} and Fe^{3+} [5]. Fe-chelator complexes also play roles in short- and/or long-distance transport of Fe.

Over the last 10 years, many Fe uptake and transport related components have been identified at the molecular level. In this review, we will follow the pathway of Fe movement from the soil to the seed and describe the suggested roles of identified gene products in an effort to understand how plants acquire Fe from the soil, how plants partition Fe among different tissues and subcellular organelles, and how plants regulate their Fe-deficiency responses (Fig. 1).

2. Fe uptake

Plants have evolved two strategies to take up Fe from the soil. Non-grasses activate a reduction-based Strategy I when starved for Fe whereas the grasses activate a chelation-based strategy.

2.1. Reduction-based Strategy I

2.1.1. Proton release. Under Fe-deficiency, Strategy I plants extrude protons into the rhizosphere, lowering the pH of the soil solution and increasing the solubility of Fe^{3+}. This works because for every one unit drop in pH, Fe^{3+} becomes a 1000-fold more soluble [6]. The responsible proton-ATPases are not yet identified at a molecular level, but several proton-ATPases of the *AHA* (*A*rabidopsis *H*+-*A*TPase) family are suggested to be involved this process. For example, *AHA7* is up-regulated in response to Fe-deficiency and its expression is dependent *on FIT1* (Fe-deficiency induced transcription factor 1), implicating AHA7 as part of the Fe-deficiency response [7]. Cucumber also has at least one Fe-regulated proton ATPase, CsHA1, whose expression is induced in Fe-deficient roots [8].

2.1.2. Fe(III) chelate reduction. Fe becomes more available by reducing Fe^{3+} to the more soluble Fe^{2+}. The reduction step, prior to Fe^{2+} uptake, has been shown to be critical for Fe uptake from Fe-deficient soil. The Arabidopsis mutant, *ferric-chelate reductase defective 1* (*frd1*), has no inducible root Fe(III) chelate reductase activity and develops severe chlorosis

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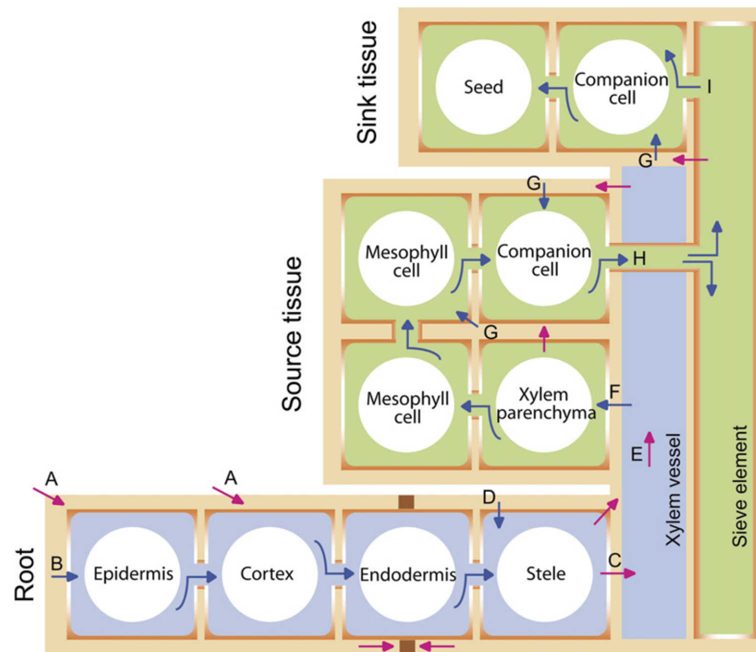

Fig. 1. Fe transport from the soil to the seed. Red arrows represent speculative flow of apoplastic Fe; blue arrows indicate Fe movement into symplastic space. After initial diffusion from the soil (A), Fe is imported into epidermal or cortex cells (B). Once in the cytoplasm, Fe moves through symplastic passages to the stele and then is exported into a xylem vessel (C). Apoplastic leakage is blocked by the Casparian strip (shown in brown boxes). Fe precipitates in root apoplast is re-absorbed under Fe-deficiency (D). Fe is transported to the shoot through the transpiration stream (E) and imported into the leaf cytoplasm (F). Fe precipitates in shoot apoplast are remobilized on demand of sink tissues (G). Fe moves through symplastic passages from a source tissue to a sink tissue via phloem loading (H) and unloading (I).

when Fe is limiting [9]. The corresponding Arabidopsis gene, *FRO2*, was identified based on sequence similarity to a subunit of the human respiratory burst oxidase, gp91phox, and the yeast Fe(III) chelate reductase, Fre1 [10]. *FRO2* mapped to the same location as *frd1*, and is able to complement the *frd1* phenotype. *FRO2* is expressed in the epidermal cells of Fe-deficient roots and is thought to be the main Fe(III) chelate reductase in roots. Plants overexpressing *FRO2* are resistant to low Fe growth conditions [11]. The FRO family of metal reductases contains seven additional members in Arabidopsis [12,13]. The expression of *FRO* genes in various locations suggests different sets of FRO proteins are participating in Fe uptake in different plant tissues. For example, like FRO2, FRO3 and FRO5 are expressed in roots. However FRO3 is predominantly expressed in the vascular cylinder of roots, suggesting a role in Fe re-absorption from the root apoplast (Fig. 1D). The shoot-specific *FRO* genes are FRO6, FRO7, and FRO8. The promoter of *FRO6* contains multiple light-responsive elements and a *FRO6* promoter driven reporter gene is activated upon exposure to light [14].

Fe(III) chelate reductases have also been identified in pea and tomato. Like *FRO2* in Arabidopsis, *PsFRO1* mRNA accumulates in Fe deficient roots; however, the mRNA is also seen throughout the root, suggesting that PsFRO1 may also play a role in Fe transport within the plant [15] (Fig. 1B and D). *LeFRO1* mRNA is detected both in roots and shoots, indicating a role in Fe mobilization in the shoots (Fig. 1B and G). *LeFRO1* localizes to the plasma membrane in onion epidermal cells and confers Fe(III) reductase activity when expressed in yeast [16].

2.1.3. Fe^{2+} transport. Fe^{2+} is transported into the root by IRT1, a member of the ZIP (*Z*RT, *I*RT-like *p*roteins) metal transporter family. The Arabidopsis *IRT1* gene was identified by functional complementation of an Fe uptake mutant of yeast [17]. The Arabidopsis *irt1* mutant exhibits chlorosis and severely impaired growth [18–20]. Indeed, *irt1* plants die before setting seed unless supplied with high levels of soluble Fe. IRT1 is expressed in the epidermal cells of Fe-deficient roots and localizes to the plasma membrane. Taken together, these data suggest that IRT1 is the major transporter for Fe uptake from soil [18]. Although only Fe can rescue the survival of *irt1*, metal uptake and growth assays in yeast showed that IRT1 can transport multiple divalent metals (Fe, Zn, Mn and Cd), and in keeping with these observations, *irt1* plants have reduced levels of Fe, Mn, Zn and Co.

There are 15 additional ZIP metal transporters in Arabidopsis: IRT2, IRT3, ZIP1 through ZIP12 [21] and the distantly related IAR1 [22]. IRT2 is most similar in amino acid sequence to IRT1 and is expressed in the external layers of Fe-deficient roots [23]. However, *irt2* plants show no symptoms of Fe-deficiency and overexpression does not appear to substitute for the loss of IRT1. Thus, although IRT2 complements the growth defect of Fe uptake mutant yeast, it must be playing a different role than IRT1. It will be of interest to examine the subcellular localization of IRT2 in plants.

IRT1 orthologs have been found in other Strategy I plants as well as in rice, which is a Strategy II plant. We will discuss OsIRT1 and OsIRT2 in the following section. In tomato, *LeIRT1* and *LeIRT2* are specifically expressed in roots. However, unlike Arabidopsis, *LeIRT1* and *LeIRT2* are expressed in both Fe sufficient and Fe deficient roots, with *LeIRT1* showing induction under Fe-deficiency [24]. RIT1 (*R*oot *I*ron *T*ransporter 1) from pea is expressed in Fe-deficient roots [25]. When

expressed in yeast, RIT1 showed high-affinity Fe transport and low-affinity Zn and Cd transport.

2.2. Strategy II uptake

Grasses, such as corn, wheat and rice, use the chelation-based Strategy II. In response to Fe-deficiency, grasses release small molecular weight compounds known as the mugineic acid (MA) family of phytosiderophores (PS). PS have high affinity for Fe^{3+} and efficiently bind Fe^{3+} in the rhizosphere. Fe^{3+}–PS complexes are then transported into the plant roots via a specific transport system. The chelation strategy is more efficient than the reduction strategy and thus allows grasses to survive under more drastic Fe-deficiency conditions [3].

2.2.1. Synthesis of phytosiderophores. The family of mugineic acids includes MA, 2′-deoxymugineic acid (DMA), 3-epi-hydroxymugineic acid (epi-HMA), and 3-epihydroxy 2′-deoxymugineic acid (epi-HDMA). Each grass produces its own sets of MAs and increases the production and secretion of MAs in response to Fe-deficiency. Thus, tolerance to Fe-deficiency is correlated with the amounts and the types of PS secreted [2]. For example, rice, wheat, and corn secret only DMA in relatively low amounts and thus, are susceptible to low Fe availability. In contrast, barley secretes large amounts of many types of PS, including MA, HMA, and epi-HMA and is therefore more tolerant of low Fe availability [26]. In the generation of MA, nicotianamine (NA) is the key intermediate that is produced from the condensation of three molecules of *S*-adenosyl methionine by nicotianamine synthase (NAS). NA is present not only in grasses but in non-grasses as well. NA can bind various metals including Fe^{2+} and Fe^{3+}, but is not secreted, suggesting a role for NA in intra- and intercellular metal transport in both for Strategy I and Strategy II plants. In Strategy II plants, grass-specific nicotianamine aminotransferase (NAAT) converts NA to the intermediate 3′-keto DMA [27]. Subsequent reduction of the 3′-keto intermediate produces DMA, the common precursor of all other MAs [26]. DMA then undergoes hydroxylation and produces other types of MAs. Two barley genes, *Ids2* and *Ids3*, encode dioxygenases and are thought to catalyze the formation of epi-HMA and epi-HDMA in Fe-deficient roots [28].

In an attempt to enhance Fe nutrition, two barley genes, *NaatA* and *NaatB*, encoding NAAT were introduced under the control of their native promoters into rice [29]. These transgenic rice plants produced fourfold more grain than control plants when they were grown in alkaline soils with limited Fe availability. NAAT activity is therefore a limiting step in the production of MAs in rice. However, despite the high levels of NAAT activity, the increase in MAs released was not striking. One may expect that with more substrates provided to the NAAT enzymes by co-expressing higher levels of NAS, a more impressive change in the levels of MAs produced.

2.2.2. Uptake of Fe–PS complexes. Following Fe^{3+} chelation by PS, a high-affinity uptake system specific for Fe–PS complexes transports Fe–PS into the epidermal cells of Fe-deficient roots. The *yellow-stripe 1* (*ys1*) maize mutant showed a defect in uptake of Fe–PS, resulted in Fe-deficiency, and plants developed interveinal chlorosis (yellow-stripe) [30]. Ac transposon tagging of the *YS1* gene enabled its molecular cloning. *YS1* encodes an Fe–PS transporter, an integral membrane protein with 12 putative transmembrane domains that belongs to the oligopeptide transporter (OPT) superfamily. YS1 can restore the growth of an Fe uptake mutant of yeast when supplied with Fe(III)–DMA, but not when supplied with Fe(III)–citrate. Electrophysiological analysis in *Xenopus* oocytes demonstrated that YS1 encodes a proton-coupled transporter for phytosiderophores and NA metal chelates [31]. *YS1* mRNA accumulates in response to Fe-deficiency, further suggesting that YS1 function in Fe uptake from the soil. In addition, *YS1* is also expressed in the shoots, suggesting a role for YS1 in the intercellular transport of Fe in the plant shoots. A recently characterized barley ortholog, HvYS1, is only expressed in the roots and seems to be specific for uptake of Fe(III)–PS [32].

2.2.3. Mix and match. Strategy II plants can also take up Fe^{2+} like Strategy I plants. Rice, for example, in addition to having the ability to transport Fe–PS complexes, is able to transport Fe^{2+} via OsIRT1 [33]. Like *IRT1* in Arabidopsis, the *OsIRT1* and the *OsIRT2* genes are expressed predominantly in roots and are induced in response to Fe deficiency. However no increases of FRO-like gene expression or Fe(III) chelate reductase activity were detected in Fe-deficient rice roots. It is plausible that rice can compensate for the lack of effective Fe(III) chelate reductases because of its wetland culture. In paddy fields, the equilibrium of Fe^{3+}/Fe^{2+} is shifted in the direction of Fe^{2+} due to the deficiency of oxygen in the soil. The adoption of a Fe^{2+} acquisition strategy can be especially advantageous for rice, since rice plants are not very efficient at Fe^{3+} uptake via Strategy II.

3. Long-distance Fe transport

Once Fe enters the root symplast, Fe is required to be bound by chelating compounds. Fe–chelator complexes then move through intercellular connections into the stele along the diffusion gradient. The release of Fe into the xylem vessels requires Fe efflux from the symplast into the apoplastic space (Fig. 1C). The mechanism of Fe efflux is not yet clearly understood. In mammals, a duodenal protein encoded by the *IREG1* gene is an Fe-regulated transporter involved in the basolateral Fe efflux from epithelial cells into the circulation [34]. In Arabidopsis, there are three proteins predicted to be similar to IREG1. AtIREG2 (also referred to as FPT2) is shown to localize to the vacuolar membrane of root epidermal cells and is involved in Fe-dependent nickel detoxification [35]. FPT1/AtIREG1 is localized to the plasma membrane of stele cells, suggesting a possible role in Fe release into the xylem vessels (Guerinot, unpublished data).

It is generally agreed that Fe is present as Fe(III)–citrate complexes in the xylem where the pH is around 5.5–6 [5] (Fig. 1E). The Arabidopsis mutant, *frd3* (*man1*), has provided molecular evidence for the role of citrate in long-distance Fe transport. *frd3* has deregulated Strategy I responses, showing constitutive expression regardless of the external Fe supply [36]. As a result, the *frd3* mutant accumulates more Fe in its roots. Despite this, *frd3* xylem exudate contains approximately half as much Fe and significantly less citrate than the exudate from wild-type plants. The *FRD3* gene encodes a transmembrane protein belonging to the multidrug and toxin efflux (MATE) family of small molecule transporters and is therefore suggested to transport citrate. Two-electrode voltage clamp analysis has revealed that FRD3 expression in Xenopus

oocytes mediates currents when exposed to citrate, supporting a role for FRD3 in citrate efflux [37]. Supplementation of growth media with citrate rescues the chlorotic appearance of *frd3* plants. FRD3 is expressed in the root pericycle and vascular cylinder, indicating a role for FRD3 in citrate efflux into xylem vessels [38]. *frd3* accumulates more Fe in the shoot apoplast, despite reduced levels of Fe (regardless of the form of the Fe-complexes) in the xylem sap. This raises an interesting question on the role of bypasses in long-distance Fe transport. It is expected that increases in both apoplastic movement from root to shoot and the xylem-to-phloem Fe movement through transfer cells might compensate for xylem-mediated Fe transport.

The mechanism of Fe uptake from the xylem vessels into leaf tissues (xylem unloading to symplast and re-absorption to apoplast) is not clear (Fig. 1F). However, it is believed that components of Strategy I uptake play a role when Fe moves across the plasma membrane of the leaf cells. Several *FRO* genes are expressed in shoots as described earlier in this review. Several Arabidopsis *ZIP* genes are also expressed in shoots (Guerinot, unpublished data). The *IRT1* gene is expressed in the basal part of flowers, suggesting its role in Fe uptake in aerial tissues in addition to roots [18].

Fe must also be transported through the phloem, because the transpiration flow in the xylem vessels is inefficient in developing organs such as the apex, seeds and root tips. Fe remobilization from older leaves to younger leaves also takes place via phloem transport. The pH in the phloem sap is >7, thus Fe needs to be bound to chelators in order to remain soluble. Studies with the castor bean *Ricinus communis* have identified an 11 kDa Fe transport protein (ITP) as an Fe-chelator in the phloem [39]. ITP specifically binds Fe^{3+} as shown by in vivo labeling experiments. Thus, it is presumed that Fe is transported as a Fe(III)–ITP complex in the phloem. ITP belongs to a large family of late embryogenesis proteins known as dehydrins. There is a gene in Arabidopsis similar to the *ITP* gene, so it would be interesting to see whether this mechanism of phloem transport exists in other plants. In addition to ITP, NA has been proposed to function in Fe transport in the phloem, based on its ubiquitous presence in plant tissues and its ability to form stable complexes with Fe^{2+} at neutral and weakly alkaline pH [40]. The tomato mutant *chln* (*chloronera*) demonstrates the role of NA in long-distance Fe transport. *chln* was identified due to its interveinal leaf chlorosis, especially in young leaves. However, *chln* has increased Fe uptake in roots independent of external Fe supply and accumulates high concentrations of Fe in its shoots and roots. *chln* phenotypes are rescued by grafting a *chln* shoot onto wild-type rootstock, suggesting a mobile, normalizing compound. The responsible compound was purified and identified as NA; exogenous application of NA complements *chln* phenotypes. The *chln* gene encodes NAS.

The presence of a small amount of Fe^{2+} in the phloem sap has lead to the idea that NA can act as a shuttle by chelating Fe^{2+} from Fe(III)-ITP during phloem loading and unloading. This hypothesis requires the participation of an oxido-reduction system for Fe^{2+}/Fe^{3+} conversion and specific Fe(II)–NA transporters within the phloem. Yellow-stripe 1 like (YSL) transporters, that share sequence similarity with maize YS1, are likely involved in the transport of Fe(II)–NA complexes. YS1 transports Fe(III)–PS and Fe(II)–NA complexes as mentioned earlier. Indeed, among 8 *YSL* genes in Arabidopsis, most of AtYSLs (AtYSL1 and AtYSL2, AtYSL4 through AtYSL8) are able to complement the Fe uptake mutant of yeast when Fe(II)–NA is exogenously supplied [41,42]. Rice has 18 putative *YSL* genes in its genome. OsYSL2 is capable of mediating the transport of Fe(II)–NA and Mn(II)–NA but not of Fe(III)–DMA or Mn(II)–DMA when expressed in *Xenopus* oocytes [43]. YSL3 from *Thlaspi* complemented the Fe uptake mutant of yeast and mediated NA dependent Ni or Fe uptake [44].

The *YSL* genes are expressed in various tissues, suggesting roles in Fe uptake at diverse locations. *AtYSL1* mRNA is expressed in the vasculature of roots and shoots; more specifically in the xylem parenchyma surrounding xylem tubes [42,45]. AtYSL1 is also detected in young siliques and in the chalazal zone of the embryo, indicating its role in Fe loading of the seed. *ysl1* seeds contain two- to fourfold less NA (and less Fe) than wild-type seeds, while *ysl1* shoots contain elevated levels of NA. The germination of *ysl1* seeds under Fe deficient conditions is slower than that of wild-type seeds, a defect that can be rescued by Fe supply. Interestingly, the levels of *AtYSL1* are increased in response to high Fe, reflecting increased demand for Fe loading. *AtYSL3* has a similar expression pattern to *AtYSL1* and is expressed in the vasculature of shoots and reproductive organs. A more drastic phenotype is observed in *ysl1ysl3* double mutants compared to *ysl1*. *ysl1ysl3* plants exhibit interveinal chlorosis in leaves and reduced fertility, presumably due to defective anther and embryo development. These phenotypes are partially rescued by exogenous Fe supply. Interestingly, similar phenotypes were also observed in previously reported NA-defective tobacco plants [46]. When endogenous NA in tobacco plants was depleted by expressing a barley NAAT, the transgenic plants displayed interveinal chlorosis and defects in reproductive growth and fertility. The *AtYSL2* gene is expressed in differentiated roots and in xylem-associated cells within the vasculature of expanded leaves [47]. A more restricted localization in root endodermis and pericycle cells has also been reported [41]. AtYSL2 localizes to the plasma membrane, more specifically the lateral zone toward meta-xylem tubes, suggesting its role in the lateral transport of metals in veins. However, the *ysl2* mutant does not show any obvious growth defect or altered metal accumulation under various metal conditions.

The *YSL* genes in Strategy II plants also play a role in long-distance Fe transport. The maize *YS1* gene is expressed both in roots and shoots [30]. Several rice *YSL* genes (*OsYSL6*, *OsYSL14*, and *OsYSL16*) are expressed both in roots and shoots; *OsYSL2* and *OsYSL13* are expressed preferentially in shoots [43]. More specifically, *OsYSL2* is expressed in the companion cells of the phloem and the vascular bundles in flowers and in developing seed. Together with the localization to the plasma membrane, OsYSL2 is suggested to play a role in Fe(II)–NA transport in phloem and rice grains.

4. Intracellular Fe transport

Mutants with constitutively active Fe-deficiency responses in their roots can be divided into two groups by their leaf phenotype: one group, including *chln* and *frd3*, shows interveinal chlorosis (Fe deficiency), despite Fe overaccumulation and the other group, such as the *brz* and *dgl* mutants of pea, dis-

play necrotic or degenerative leaves (Fe toxicity) due to Fe overaccumulation [48,49]. This illustrates that Fe homeostasis at a cellular level is highly regulated and plants can develop either Fe-deficiency or Fe toxicity in normal soils depending on where they lose control. Our knowledge is limited concerning how plants regulate cellular Fe homeostasis and intracellular Fe transport, but several observations have suggested that vacuoles play a role in accumulating excess Fe and releasing Fe into the cytosol when external Fe supply is sub-optimal. Upon Fe overload, the concentrations of NA are increased and the bulk of NA is found in the vacuoles of tomato and pea, while NA is detected in the cytosol under normal or Fe-deficient conditions [50]. The *brz* and *dgl* mutants also showed high levels of NA in their vacuoles, similar to what is seen in Fe overloaded plants [50]. It is not yet known whether Fe translocates into the vacuole as Fe–NA complexes or whether specific transporters for NA are present and the Fe–NA complexes then form in the vacuole. In the NA-free *chln* mutant, insoluble Fe(III)-phosphate precipitates are detected in the vacuoles of leaf cells, suggesting NA is required to maintain vacuolar Fe in a soluble form [51]. It is tempting to speculate that some members of *YSL* gene family might localize to the vacuolar membrane and be involved in re-translocation of Fe–NA into the cytosol.

The Arabidospis VIT1 (*V*acuolar *I*ron *T*ransporter 1) was recently identified as an Fe^{2+} transporter that functions in vacuolar Fe storage [52]. VIT1 is 62% similar to its yeast ortholog CCC1p, a transporter that can mediate Fe and Mn efflux into vacuoles [53]. Yeast overexpressing *CCC1* accumulate more Fe in their vacuoles, and conversely, deletion mutants accumulate less Fe and are sensitive to elevated levels of Fe. VIT1 complements the sensitivity of *ccc1* yeast mutants to Fe toxicity. VIT1 localizes to the vacuolar membrane, and it is expressed in the vasculature with increased expression seen during embryo and seed development. When visualized by synchrotron X-ray fluorescence microtomography, Fe localizes to the provascular strands of wild-type seeds. In *vit1*, this Fe distribution is completely abolished, suggesting VIT1-mediated vacuolar Fe transport plays an important role in Fe localization in seeds. Furthermore, *vit1* plants grow poorly in Fe-limiting soils, emphasizing the critical role of vacuolar Fe storage for the growth of germinating seedlings.

Members of the Nramp (*n*atural *r*esistance *a*ssociated *m*acrophage *p*roteins) gene family mediate the uptake of a variety of divalent cations. In Arabidopsis, *AtNramp1*, *AtNramp3* and *AtNramp4* can complement the Fe uptake mutant of yeast, revealing that these proteins can mediate Fe transport [54,55]. *Nramp1* is expressed preferentially in roots and is induced by Fe-deficiency, implicating it in Fe uptake from the soil. However, overexpression of *Nramp1* leads to resistance to toxic levels of Fe, suggesting that Nramp1 may be targeted to an intracellular membrane and may play a role in Fe remobilization into the cytosol upon Fe-deficiency [56]. Indeed, its tomato orthologs, LeNramp1 and LeNramp3, are localized to membranes of intracellular vesicles and vacuoles in yeast in addition to the plasma membrane [57]. Both Nramp3 and Nramp4 are localized to the vacuolar membrane in Arabidopsis [55,58]. The mRNAs of *Nramp3* and *Nramp4* are up-regulated in response to Fe-deficiency. In *Nramp3* overexpressing plants, the mRNAs of *IRT1* and *FRO2* are down-regulated, further indicating that Nramp3 remobilizes vacuolar Fe into cytosol, thereby down-regulating Fe uptake genes. Studies of *atnramp3 atnramp4,* the double knockout *Arabidopsis* mutant, demonstrate that Fe mobilization mediated by Nramp3 and Nramp4 is crucial during early seedling development [58]. Mutant seeds contain wild-type levels of Fe; however, the mutant displays retarded root growth and cotyledon greening during seed germination under low Fe. Electron microscopy showed the disappearance of Fe-associated globoids in wild-type vacuoles during germination while globoids of the mutant remained unaltered, suggesting mutant seeds fail to retrieve Fe from vacuolar globoids. Interestingly, like the *VIT1* gene, both *Nramp3* and *Nramp4* are expressed in the vasculature. The seedling growth of *atnramp3 atnramp4* and *vit1* mutants are arrested on Fe-limiting soil. Taken together, these data suggest that vacuoles in the vascular cells are an important site of Fe storage and Fe remobilization during germination is crucial for the seedling development when the Fe supply is low.

More than 90% of the Fe in leaf cells is located in the chloroplasts [59]. Chloroplasts have a high Fe requirement in order to maintain the structural and functional integrity of the thylakoid membranes, and thus the chloroplast is highly sensitive to Fe-deficiency [2]. Fe can be stored in plant cells in the stroma of plastids as ferritin. Ferritin is a Fe storage protein with the ability to store up to 4500 atoms of Fe. Arabidopsis contains four genes that encode ferritin (*AtFer1–4*). All four proteins are predicted to contain transit peptides for delivery to the plastid. mRNA of AtFer1, AtFer3 and AtFer4 were increased upon excess Fe treatment in both roots and leaves [60]. Despite its abundance, the mechanism of Fe uptake into the chloroplasts is not well understood. Fe uptake studies with isolated barley chloroplasts indicated that this process is light-dependent, and requires Fe(III) chelate reductase activity [61]. In Arabidopsis, FRO6 shows light-dependent gene expression and localization in green tissues, fulfilling the prerequisite characteristics for chloroplast Fe uptake [14]. Most recently, a presumptive Fe transporter, PIC1 (permease in chloroplasts), has been identified that localizes to the chloroplast [62]. Although the function of PIC1 orthologs were annotated as unknown, the cyanobacterial orthologs belonged to COGs (clusters of orthologous groups) that are generally involved in ion or solute transport. PIC1, a homolog of cyanobacterial sll1656, complemented a yeast mutant defective in Fe uptake and *pic1* mutants showed severe chlorosis and only grew heterotrophically.

5. Regulation of Fe-deficiency responses

The tomato *fer* (T3238*fer*) mutant and the cloning of the corresponding gene, FER, have offered the first clues as to how plants regulate their Fe-deficiency responses [63]. The *fer* mutant is unable to induce the Strategy I mechanism in response to Fe-deficiency, leading to chlorosis and lethality under low Fe conditions. The *FER* gene encodes a basic helix-loop-helix (bHLH) transcription factor, suggesting that it may be involved in directly regulating gene expression of Strategy I components. The *FER* mRNA is expressed in the root epidermis, the outer cortical layer of root tips and in the vascular cylinder of the mature root-hair zone. This suggests that FER plays a role in regulating Fe uptake genes from the soil and genes related to Fe redistribution as well. In the *fer* mutant, the induction of *LeIRT1* mRNA in response to Fe-deficiency is abolished and *LeNramp1* expression in the vascular parenchyma in the root hair zone is abolished [16,57,63]. The

expression of *FER* gene itself is rather constitutive regardless of Fe supply (0.1 and 10 μM Fe), or slightly down-regulated in response to supra-optimal Fe (100 μM Fe). However, FER protein levels are controlled by Fe availability. In transgenic tomato expressing *FER* under the control of the constitutive CaMV 35S promoter, *FER* mRNA can be detected from the plants grown at 10 μM and 100 μM Fe. FER protein, however, is not detected from the plants grown at 100 μM Fe, suggesting FER is down-regulated post-transcriptionally at elevated Fe levels.

FIT1 (also known as bHLH29/FRU) is the Arabidopsis ortholog of FER [64,65]. Expression of FIT1 in the *fer* mutant allows the mutant tomato to induce the Fe-deficiency responses and survive under Fe-limiting conditions [65]. *fit1* mutants, like *fer* mutants, are chlorotic and die at the seedling stage unless supplied with Fe. *FIT1* is expressed in the root epidermal cells and is induced under Fe-deficient conditions, suggesting that FIT1 regulates Fe uptake genes in response to Fe-deficiency. In *fit1* mutants, the induction of *FRO2* mRNA is not observed in response to Fe-deficiency. In contrast, up-regulation of *IRT1* mRNA is still observed. In plants expressing *FIT1* driven by the 35S promoter, mRNA expression of *FRO2* is not altered compared to wild-type [7]. Thus it will be interesting to investigate whether FIT1 protein is regulated post-transcriptionally, like FER, and is present only under Fe deficiency.

The overexpression of *IRT1* and *FRO2* in plants has revealed post-transcriptional regulation. *IRT1* mRNA is detected in the roots and shoots of 35S-*IRT1* plants regardless of Fe status, however, IRT1 protein can only be detected in Fe-deficient roots [66]. Likewise, 35S-*FRO2* plants show increased *FRO2* mRNA levels but Fe(III) chelate reductase activity is elevated only when plants are Fe-deficient [11]. The mechanism of this regulation is currently not known, but an FIT1-dependent factor is suggested to control IRT1 protein levels by post-transcriptional regulation. In *fit1* mutants, the IRT1 protein is not detected although *IRT1* mRNA is induced upon Fe deficiency [7].

6. Final remarks

A wealth of information has been obtained in recent years on Fe uptake components in plants. Of particular importance has been the cloning of *FRO2*, *IRT1*, and *YS1* genes, enabling Fe uptake from the soil to be described at the molecular level. Characterization of additional members from these gene families also has enriched our knowledge of Fe transport and distribution within the plant. The future challenge will be to elucidate the specific contribution of each family member by addressing their subcellular localization, tissue specificity, and gene regulation in response to Fe status.

We still know relatively little about how the Fe-deficiency responses are regulated. The cloning of *FIT1* and *FER*, encoding the essential transcription factors of Arabidopsis and tomato, helped us to understand the FIT1-dependent regulation of Strategy I components; FIT1 is necessary for the induction of *FRO2* mRNA and the maintenance of increased levels of IRT1 protein in response to Fe-deficiency. FIT1 regulates subsets (71 out of the 179) of Fe-deficiency inducible genes, indicating that FIT1-independent regulatory mechanisms also operate in Fe-deficiency responses [7]. *FIT1* mRNA itself is up-regulated by Fe-deficiency, thus upstream regulatory components also remain to be discovered. Ethylene is suggested to participate in the regulation of *FIT1* and *FER* expression; treatment of plants with an ethylene inhibitor decreases the levels of *FIT1* and *FER* mRNA induction in response to Fe-deficiency [67].

Among the least understood mechanisms is how plants sense Fe status and formulate the Fe-deficiency signal. It is a well-established idea that long-range signals are involved in the regulation of nutrient uptake genes in roots [68,69]. The growing shoot communicates its requirement to the root and the uptake in the root matches the demand for nutrients. Reciprocal grafting between the pea mutants, *brz* and *dgl*, and their parental genotypes reveals that the Fe(III) chelate reductase activity in roots is determined by the shoot genotype, indicating a shoot-derived signal regulates the root response [49]. In split root experiments, the Fe(III) chelate reductase activity was increased in half of the root supplied with Fe, suggesting a long-distance signaling from Fe-deprived half to the other half [69]. Interestingly, Fe(III) chelate reductase activity was decreased in the Fe-deprived portion of the root, implying the local presence of Fe is also involved in the induction of the Fe uptake activity. Recently, the expression of *IRT1* and *FRO2* was tested using split roots. When plants were grown under Fe-deficiency and transferred into a split root system, *IRT1* and *FRO2* mRNAs were shown to be more abundant in the roots supplied with Fe, further supporting the inductive effect of local Fe [70]. Identifying the components for the local- and long-range signaling and deciphering how these signaling events are integrated with the Fe deficiency responses will enable us to fully understand the molecular basis of Fe uptake and transport in plants.

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References

[1] Guerinot, M.L. and Yi, Y. (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol. 104, 815–820.
[2] Marschner, H. (1995) Mineral Nutrition of Plants, Academic Press, Boston.
[3] Mori, S. (1999) Iron acquisition by plants. Curr. Opin. Plant Biol. 2, 250–253.
[4] Halliwell, B. and Gutteridge, J.M.C. (1992) Biologically relevant metal ion-dependent hydroxyl radical generation. FEBS Lett. 307, 108–112.
[5] Hell, R. and Stephan, U.W. (2003) Iron uptake, trafficking and homeostasis in plants. Planta 216, 541–551.
[6] Olsen, R.A., Clark, R.B. and Bennett, J.H. (1981) The enhancement of soil fertility by plant roots. Am. Scientist 69, 378–384.
[7] Colangelo, E.P. and Guerinot, M.L. (2004) The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. Plant Cell 16, 3400–3412.
[8] Santi, S., Cesco, S., Varanini, Z. and Pinton, R. (2005) Two plasma membrane H+-ATPase genes are differentially expressed in iron-deficient cucumber plants. Plant Physiol. Biochem. 43, 287–292.
[9] Yi, Y. and Guerinot, M.L. (1996) Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency. Plant J. 10, 835–844.
[10] Robinson, N.J., Proctor, C.M., Connolly, E.L. and Guerinot, M.L. (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397, 694–697.

[11] Connolly, E.L., Campbell, N., Grotz, N., Prichard, C.L. and Guerinot, M.L. (2003) Overexpression of the FRO2 iron reductase confers tolerance to growth on low iron and uncovers post-transcriptional control. Plant Physiol. 133, 1102–1110.

[12] Wu, H., Lihua, L., Du, J., Yuan, Y., Cheng, X. and Ling, H.Q. (2005) Molecular and biochemical characterization of the Fe(III) chelate reductase gene family in *Arabidopsis thaliana*. Plant Cell Physiol. 46, 1505–1514.

[13] Mukherjee, I., Campbell, N.H., Ash, J.S. and Connolly, E.L. (2006) Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper. Planta 223, 1178–1190.

[14] Feng, H., An, F., Zhang, S., Ji, Z., Ling, H.-Q. and Zuo, J. (2006) Light-regulated, tissue- and cell differentiation-specific expression of the Arabidopsis Fe(III)-chelate reductase gene AtFRO6. Plant Physiol. 140, 1345–1354.

[15] Waters, B.M., Blevins, D.G. and Eide, D.J. (2002) Characterization of FRO1, a pea ferric-chelate reductase involved in root iron acquisition. Plant Physiol. 129, 85–94.

[16] Li, L., Cheng, X. and Ling, H.-Q. (2004) Isolation and characterization of Fe(III)-chelate reductase gene LeFRO1 in tomato. Plant Mol. Biol. 54, 125–136.

[17] Eide, D., Broderius, M., Fett, J. and Guerinot, M.L. (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc. Natl. Acad. Sci. USA 93, 5624–5628.

[18] Vert, G., Grotz, N., Dedaldechamp, F., Gaymard, F., Guerinot, M.L., Briat, J.-F. and Curie, C. (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and plant growth. Plant Cell 14, 1223–1233.

[19] Varotto, C., Maiwald, D., Pesaresi, P., Jahns, P., Francesco, S. and Leister, D. (2002) The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*. Plant J. 31, 589–599.

[20] Henriques, R., Jásik, J., Klein, M., Martinoia, E., Feller, U., Schell, J., Pais, M.S. and Koncz, C. (2002) Knock-out of Arabidopsis metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. Plant Mol. Biol. 50, 587–597.

[21] Mäser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K., Sze, H., Talke, I.N., Amtmann, A., Matthuis, F.J.M., Sanders, D., Harper, J.F., Tchieu, J., Gribskov, M., Persans, M.W., Salt, D.E., Kim, S.A. and Guerinot, M.L. (2001) Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol. 126, 1646–1667.

[22] Lasswell, J., Rogg, L.E., Nelson, D.C., Rongey, C. and Bartel, B. (2000) Cloning and characterization of IAR1, a gene required for auxin conjugate sensitivity in Arabidopsis. Plant Cell 12, 2395–2408.

[23] Vert, G., Briat, J.-F. and Curie, C. (2001) Arabidopsis IRT2 gene encodes a root-periphery transporter. Plant J. 26, 181–189.

[24] Eckhardt, U., Marques, A.M. and Buckhout, T.J. (2001) Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. Plant Mol. Biol. 45, 437–448.

[25] Cohen, C.K., Garvin, D.F. and Kochian, L.V. (2004) Kinetic properties of a micronutrient transporter from *Pisum sativum* indicate a primary function in Fe uptake from the soil. Planta 218, 784–792.

[26] Bashir, K., Inoue, H., Nagasaka, S., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2006) Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. J. Biol. Chem. 281, 32395–32402.

[27] Kanazawa, K., Higuchi, K., Nishizawa, N., Fushiya, S., Chino, M. and Mori, S. (1994) Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient conditions in Gramineae. J. Exp. Biol. 46, 1903–1906.

[28] Nakanishi, H., Yamaguchi, H., Sasakuma, T., Nishizawa, N.K. and Mori, S. (2000) Two dioxygenase genes, Ids3 and Ids2, from *Hordeum vulgare* are involved in the biosynthesis of mugineic acid family phytosiderophores. Plant Mol. Biol. 44, 199–207.

[29] Takahashi, M.T., Nakanishi, H., Kawasaki, S., Nishizawa, N.K. and Mori, S. (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nat. Biotechnol. 19, 466–469.

[30] Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S.L., Briat, J.-F. and Walker, E.L. (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409, 346–349.

[31] Schaaf, G., Ludewig, U., Erenoglu, B.E., Mori, S., Kitahara, T. and von Wiren, N. (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J. Biol. Chem. 279, 9091–9096.

[32] Murata, Y., Ma, J.F., Yamaji, N., Ueno, D., Nomoto, K. and Iwashita, T. (2006) A specific transporter for iron(III)-phytosiderophore in barley roots. Plant J. 46, 563–572.

[33] Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T., Wada, Y., Watanabe, S., Matsuhashi, S., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J. 45, 335–346.

[34] McKie, A.T., Marciana, P., Rolfs, A., Brennan, K., Wehr, K., Barow, D., Miret, S., Bomford, A., Peters, T.J., Farzaneh, F., Hediger, M.A., Hentze, M.W. and Simpson, R.J. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5, 299–309.

[35] Schaaf, G., Honsbein, A., Meda, A.R., Kirchner, S., Wipf, D. and von Wiren, N. (2006) AtIREG2 encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. J. Biol. Chem. 281, 25532–25540.

[36] Rogers, E.E. and Guerinot, M.L. (2002) FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. Plant Cell 14, 1787–1799.

[37] Durrett, T.P., Gassmann, W. and Rogers, E.E. (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiol., doi:10.1104/pp.107.097162.

[38] Green, L.S. and Rogers, E.E. (2004) FRD3 controls iron localization in *Arabidopsis thaliana*. Plant Physiol. 136, 2523–2531.

[39] Kruger, C., Berkowitz, O., Stephan, Udo W. and Hell, R. (2002) A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. J. Biol. Chem. 277, 25062–25069.

[40] von Wiren, N., Klair, S., Bansal, S., Briat, J.-F., Khodr, H., Shioiri, T., Leigh, R.A. and Hider, R.C. (1999) Nicotianamine chelates both Fe(III) and Fe(II). Implications for metal transport in plants. Plant Physiol. 119, 1107–1114.

[41] DiDonato, R.J., Roberts, L., Sanderson, T., Eisley, R. and Walker, E. (2004) Arabidopsis *Yellow Stripe-Like2* (YSL2): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine–metal complexes. Plant J. 39, 403–414.

[42] Waters, B.M., Chu, H.H., Didonato, R.J., Roberts, L.A., Eisley, R.B. and Walker, E.L. (2006) Mutations in Arabidopsis Yellow Stripe-Like1 and Yellow Stripe-Like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. Plant Physiol. 141, 1446–1458.

[43] Koike, S., Inoue, H., Mizuno, D., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N. (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J. 39, 415–424.

[44] Gendre, D., Czernic, P., Conejero, G., Pianelli, K., Briat, J.-F., Lebrun, M. and Mari, S. (2007) TcYSL3, a member of the YSL family from the hyperaccumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. Plant J. 49, 1–15.

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

[46] Takahashi, M., Terada, Y., Nakai, I., Nakanishi, H., Yoshimura, E., Mori, S. and Nishizawa, N. (2003) Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell 15, 1263–1280.

[47] Schaaf, G., Schikora, A., Haberle, J., Vert, G., Ludewig, U., Briat, J.F., Curie, C. and von Wiren, N. (2005) A putative function for the Arabidopsis Fe–phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. Plant Cell Physiol. 46, 762–774.

[48] Kneen, B.E., LaRue, T.A., Welch, R.M. and Weeden, N.F. (1990) Pleiotropic effects of brz. A mutation in *Pisum sativum* (L.) cv.

'Sparkle' conditioning decreased nodulation and increased iron uptake and leaf necrosis. Plant Physiol. 93, 717–722.
[49] Grusak, M.A. and Pezeshgi, S. (1996) Shoot-to-root signal transmission regulates root Fe(III) reductase activity in the dgl mutant of pea. Plant Physiol. 110, 329–334.
[50] Pich, A., Manteuffel, R., Hillmer, S., Scholz, G. and Schmidt, W. (2001) Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration? Planta 213, 967–976.
[51] Becker, R., Fritz, E. and Manteuffel, R. (1995) Subcellular localization and characterization of excessive iron in the nicotianamine-less tomato mutant *chloronerva*. Plant Physiol. 108, 269–275.
[52] Kim, S.A., Punshon, T., Lanzirotti, A., Li, L., Alonzo, J.M., Ecker, J.R., Kaplan, J. and Guerinot, M.L. (2006) Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. Science 314, 1295–1298.
[53] Li, L., Chen, O.S., Ward, D.M. and Kaplan, J. (2001) CCC1 is a transporter that mediates vacuolar iron storage in yeast. J. Biol. Chem. 276, 29515–29519.
[54] Curie, C., Alonso, J.M., Le Jean, M., Ecker, J.R. and Briat, J.-F. (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. Biochem. J. 347, 749–755.
[55] Thomine, S., Lelievre, F., Debarbieux, E., Schroeder, J.I. and Barbier-Bygoo, H. (2003) AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J. 34, 685–695.
[56] Thomine, S., Wang, R., Ward, J.M., Crawford, N.M. and Schroeder, J.I. (2000) Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to *Nramp* genes. Proc. Natl. Acad. Sci. USA 97, 4991–4996.
[57] Bereczky, Z., Wang, H.-Y., Schubert, V., Ganal, M. and Bauer, P. (2003) Differential regulation of *nramp* and irt metal transporter genes in wild type and iron uptake mutants of tomato. J. Biol. Chem. 278, 24697–24704.
[58] Lanquar, V., Lelievre, F., Bolte, S., Hames, C., Alcon, C., Neumann, D., Vansuyt, G., Curie, C., Schroder, A., Kramer, U., Barbier-Brygoo, H. and Thomine, S. (2005) Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. EMBO J. 24, 4041–4051.
[59] Terry, N. and Abadia, J. (1986) Function of iron in chloroplasts. J. Plant Nutr. 9, 609–646.
[60] Petit, J.M., Briat, J.-F. and Lobréaux, S. (2001) Structure and differential expression of the four members of the *Arabidopsis thaliana* ferritin gene family. Biochem. J. 359, 575–582.
[61] Bughio, N., Takahashi, M., Yoshimura, E., Nishizawa, N.K. and Mori, S. (1997) Light-dependent iron transport into isolated barley chloroplasts. Plant Cell Physiol. 38, 101–105.
[62] Duy, D., Wanner, G., Meda, A.R., von Wiren, N., Soll, J. and Philippar, K. (2007) PIC1, an ancient permease in Arabidopsis chloroplasts, mediates iron transport. Plant Cell, doi:10.1105/tpc.106.047407.
[63] Ling, H.-Q., Bauer, P., Bereczky, Z., Keller, B. and Ganal, M. (2002) The tomato fer gene encoding a bHLH protein controls iron-uptake responses in roots. Proc. Natl. Acad. Sci. USA 99, 13938–13943.
[64] Jakoby, M., Wang, H.-Y., Reidt, W., Weissharr, B. and Bauer, P. (2004) FRU(BHLH029) is required for induction of iron mobilization genes in *Arabidopsis thaliana*. FEBS Lett. 577, 528–534.
[65] Yuan, Y.X., Zhang, J., Wang, D.W. and Ling, H.Q. (2005) AtbHLH29 of *Arabidopsis thaliana* is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy I plants. Cell Res. 15, 613–621.
[66] Connolly, E.L., Fett, J.P. and Guerinot, M.L. (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. Plant Cell 14, 1347–1357.
[67] Lucena, C., Waters, B.M., Romera, F.J., Garcia, M.J., Morales, M., Alcantara, E. and Perez-Vicente, R. (2006) Ethylene could influence ferric reductase, iron transporter, and H+-ATPase gene expression by affecting FER (or FER-like) gene activity. J. Exp. Bot. 57, 4145–4154.
[68] Forde, B.G. (2002) Local and long-range signaling pathways regulating plant responses to nitrate. Annu. Rev. Plant Biol. 53, 203–224.
[69] Schikora, A. and Schmidt, W. (2001) Iron stress-induced changes in root epidermal cell fate are regulated independently from physiological responses to low iron availability. Plant Physiol. 125, 1679–1687.
[70] Vert, G., Briat, J.-F. and Curie, C. (2003) Dual regulation of the Arabidopsis high affinity root iron uptake system by local and long-distance signals. Plant Physiol. 132, 796–804.