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Iron Stress in Plants: Dealing with Deprivation and Overload

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ABSTRACT

Iron (Fe) is an essential nutrient for plants and one of the most abundant elements in soils. However, it is nearly inaccessible to plants because of its poor solubility in aerobic conditions at neutral or basic pH, resulting in much lower concentrations than required for the optimal growth of plants. However, when Fe is taken up in excess of cellular needs, it becomes highly toxic, since both Fe^{2+} and Fe^{3+} can act as catalysts in the formation of hydroxyl radicals, which are potent oxidizing agents that may damage DNA, proteins and lipids. Plants must be able to sense and respond to Fe stress in terms of both Fe-deprivation and Fe-overload. Depending on the level of severity, plants are unable to deal with such stress and undergo dramatic changes in cellular metabolism with a sequential dismantling of cellular structures, resulting in growth inhibition and ultimately plant death. Therefore, plants must tightly regulate Fe levels within the cell to ensure that Fe is present at adequate levels. Here, we describe recent progress made in understanding how Fe is sensed by plants, and how plants are affected by and try to deal with non-optimal Fe concentrations.

Keywords: ferritin, iron deprivation, iron overload, oxidative stress, plant death, senescence
Abbreviations: **LHC**, light harvesting complex; **MA**, mugineic acid; **NA**, nicotianamine; **PS**, phytosiderophore; **PSI**, photosystem I; **PSII**, photosystem II; **ROS**, reactive oxygen species; **SAG**, senescence-associated gene; **TSS**, total soluble sugars

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INTRODUCTION

Iron (Fe) is an essential micronutrient for almost all living organisms and represents one of the most versatile metals in biology. Its functions are mostly based on the reversible redox reaction of Fe^{2+} (ferrous) and Fe^{3+} (ferric) ions, its ability to form octahedral complexes with various ligands and to vary its redox potential in response to different ligand environments (Hell and Stephan 2003). Such properties allow Fe to participate as catalytic cofactor in multiple metabolic pathways. Fe is mainly required for photosynthesis, respiration, sulphate assimilation, hormone synthesis, nitrogen fixation, as well as DNA synthesis and repair (Puig *et al*. 2007). The essential role of Fe is evidenced by the disorders that its deficiency promotes in plants, including severe chlorosis and reduction in both yield and nutritional value of crops (Briat *et al*. 1995; Marschner 2002; Curie and Briat 2003). But despite its absolute requirement, when present at elevated concentrations, Fe can react with oxygen and generate noxious reactive oxygen species that damage cells at the level of membranes, proteins and nucleic acids (Halliwell and Gutteridge 1984). Plants must therefore respond to Fe stress in terms of both Fe deprivation and overload. Plants subjected to Fe deprivation/overload respond in different ways (Römheld and Marschner 1991; Abadia 1992). Therefore, Fe homeostasis in the whole organism, as well as in individual cells, must be balanced to supply enough Fe for cell metabolism and to avoid excessive, toxic

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Invited Review

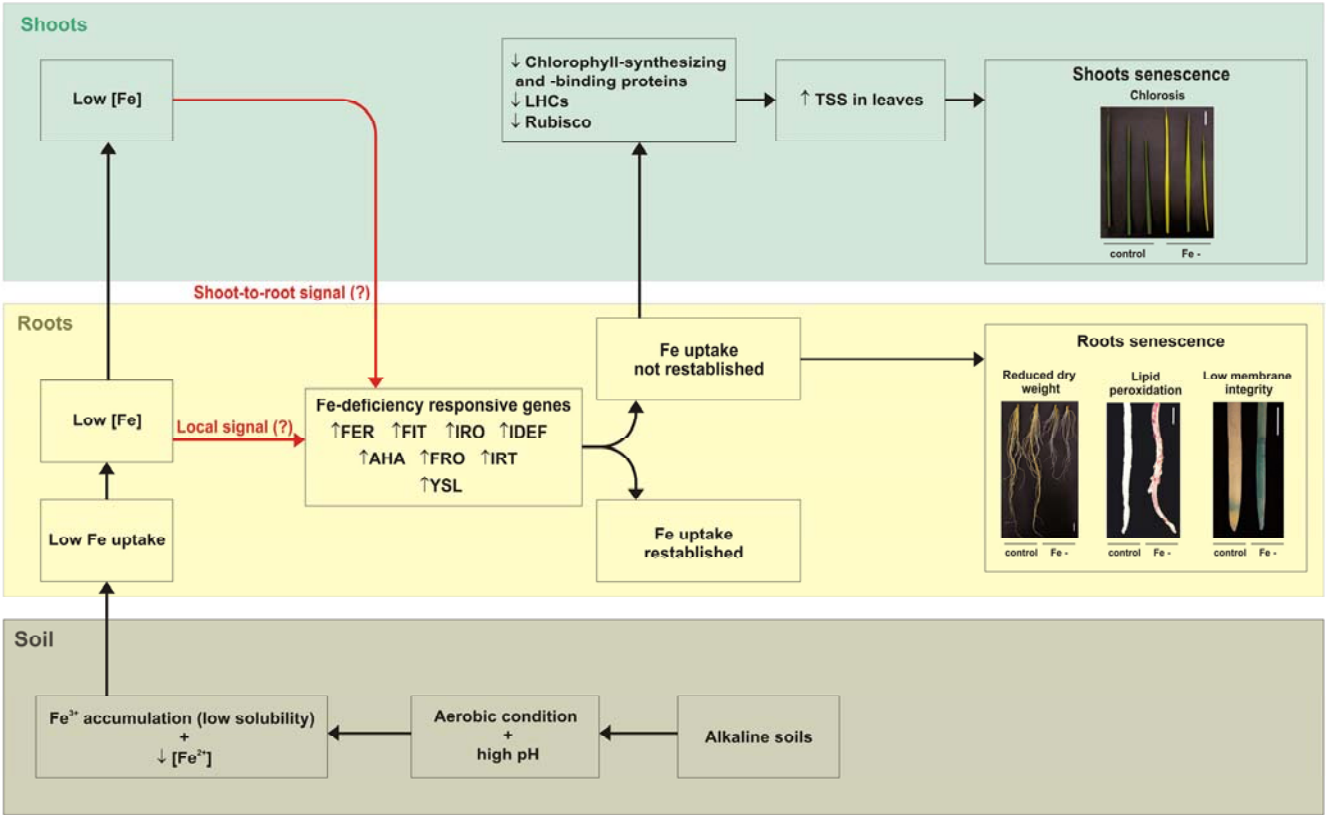

Fig. 1 Schematic representation of plant responses to Fe-deprivation. In well aerated alkaline soils, most Fe is precipitated and unavailable for plant uptake. Both shoot- and root-borne signals could induce transcription factors (*FER*, *FIT*, *IRO*, *IDEF*), Strategy I (*AHA*, *FRO*, *IRT*) and Strategy II (*YSL*) genes to improve Fe uptake in roots of plants with low Fe concentrations. When adequate Fe levels are not achieved, chlorophyll synthesis and several other physiological processes are impaired, leading to both shoot and root senescence. LHCs, light harvesting complexes; TSS, total soluble sugars. Pictures were obtained from **Sperotto RA, Ricachenevsky FK, Fett JP** (2007) Iron deficiency in rice shoots: identification of novel induced genes using RDA and possible relation to leaf senescence. *Plant Cell Reports* **26**, 1399-1411, ©2007 and **Sperotto RA, Boff T, Duarte GL, Fett JP** (2008) Increased senescence-associated gene expression and lipid peroxidation induced by iron deficiency in rice roots. *Plant Cell Reports* **27**, 183-195, ©2008 with kind permission from Springer Science + Business Media (license numbers 2123690403786 and 2123690080509, respectively).

levels (Curie and Briat 2003). To maintain adequate levels of Fe ions in tight homeostasis, plants have evolved complex regulatory mechanisms to modulate Fe uptake, transport and storage (Wintz *et al*. 2002). In this review, we focus on how plants sense and deal with Fe deprivation and overload and also the processes which lead to senescence in plants unable to deal with extreme Fe concentrations. The steps and mechanisms mostly discussed in this review are summarized in **Figs. 1** and **2**.

IRON IN THE ENVIRONMENT AND IN PLANTS

Environmental conditions leading to iron stress

Iron availability is one of the major constraints for crop production. Although Fe is the fourth most abundant element in soils, it is not readily available to plants. Fe^{2+} is relatively soluble but it is readily oxidized by atmospheric oxygen, while Fe^{3+} tends to form insoluble oxyhydroxide polymers (Hell and Stephan 2003). The concentration of free Fe^{3+} in aerobic soils of neutral pH (~10^{-17} M) is much lower than concentrations required for the optimal growth of plants (~10^{-9} to 10^{-4} M) (Guerinot and Yi 1994; Marschner 2002). Thus, Fe-deficiency is an issue for plants grown on calcareous soils (**Fig. 1 -** Soil), which represent approximately 30% of soils worldwide (Mori 1999; Kerkeb and Connolly 2006), causing agricultural problems. This limitation cannot easily be overcome by the use of Fe-containing fertilizers, because Fe availability is a problem of solubility and not of abundance (Guerinot 2001; Hell and Stephan 2003). Fe-deficiency is usually recognized by chlorotic or yellowing of interveinal areas in new leaves and if severe, can lead to reduction in crop yields and sometimes complete crop failure (Guerinot and Yi 1994). In anaerobic and acid sulphate soils, high amounts of reduced iron (Fe^{2+}) become available and soluble because of the reductive environment created by waterlogging (Ponnanperuma *et al.* 1955; Ponnanperuma 1972), leading to the occurrence of Fe toxicity (**Fig. 2 -** Soil). Thus, Fe-overload occurs in flooded soils and hence affects primarily the production of lowland cultivated plants (Becker and Asch 2005). Fe toxicity is the most widely distributed nutritional disorder in lowland-rice production (Dobermann and Fairhurst 2000) and occurs when the rice roots take up a high concentration of Fe^{2+} from the soil and a toxic concentration of Fe accumulates in the leaves (Sahrawat 2004), where an elevated production of toxic oxygen radicals can damage cellular structural components and impair physiological processes (Becker and Asch 2005).

How plants perceive the iron status

One of the most intriguing issues on Fe homeostasis is how plants sense the Fe concentration within tissues. The most discussed question is if Fe status is perceived locally by roots or there is a long-range signal transported from shoots throughout the plant body (**Fig. 1**, red arrows). Romera *et al*. (1992) reported that soaking half of the root system in Fe-deprived medium induced Fe-deficiency responses in the Fe-sufficient half of roots, indicating the existence of a long-distance signal in sunflower and cucumber. Results obtained with two non-allelic pea mutants, *brz* and *dgl*, which show constitutive Fe-deficiency responses in roots, but over-accumulate Fe in shoots, suggested that a "shoot factor" is involved in signaling the Fe status to roots (Grusak *et al*. 1990; Kneen *et al*. 1990; Grusak and Pezeshgi 1996). When mutant shoots were grafted into wild-type

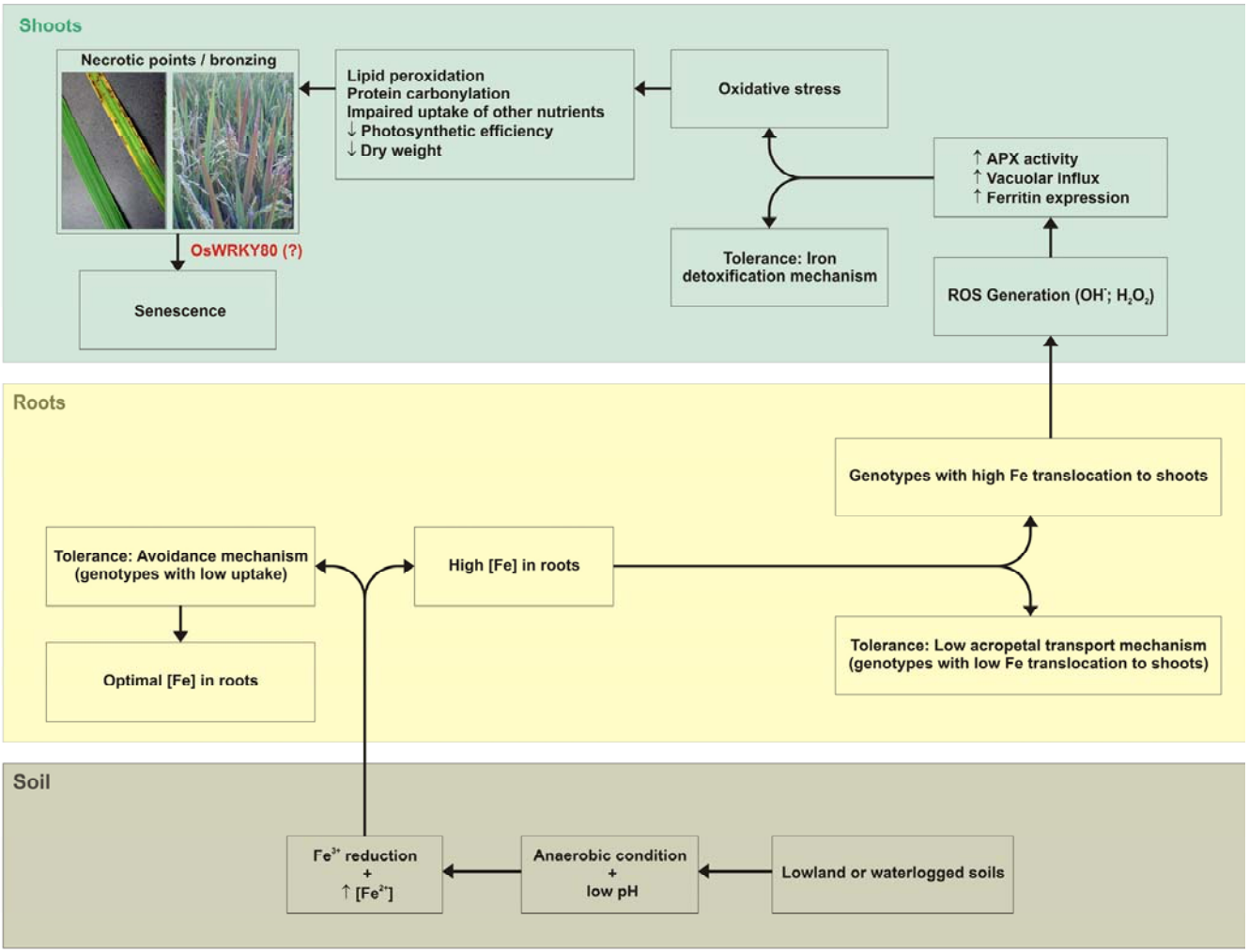

Fig. 2 Schematic representation of plant responses to Fe-overload. In waterlogged soils, low pH and low oxygen availability lead to Fe reduction. Highly available ferrous iron can be precipitated in the root's apoplast (avoidance mechanism) or enter root cells. Some plant genotypes are able to keep most of the Fe in the roots (low acropetal transport), while others translocate high Fe levels to shoots, where it leads to oxidative stress. Diverse plant species and genotypes within a species can take advantage of Fe compartmentalization or chelation, storage inside Ferritin and induction of anti-oxidant enzymes to deal with high leaf Fe concentrations. When such mechanisms are not efficient enough, several physiological processes lead to the typical Fe overload symptoms and to senescence, possibly regulated by a WRKY transcription factor in rice. ROS, reactive oxygen species; APX, ascorbate peroxidase. Pictures obtained by Ricardo J. Stein.

roots and grew under Fe-sufficient conditions, plants still exhibited enhanced Fe^{3+} reductase activity in roots (Grusak and Pezeshgi 1996), a characteristic response to Fe-deficiency. This indicates that the shoot of *brz* and *dgl* mutants fail to correctly signal plant Fe-sufficiency. Grusak (1995) showed that interruption of phloem transport decreases the Fe^{3+} reductase activity in roots of pea, suggesting that the shoot factor is a phloem mobile molecule.

Other works clearly indicate that a local signal is responsible for at least some responses of the root system under Fe-deprivation. Schikora and Schmidt (2001) using the pea mutants *brz* and *dgl* and the tomato mutant *chloronerva* (Becker *et al*. 1998), showed that the formation of root epidermis transfer cells is Fe-regulated, independently of the constitutive Fe^{3+} reductase activity of these mutants. This demonstrates that even mutants with high Fe concentration in shoots can sense the Fe status locally, inducing morphological changes in root epidermal cells only when submitted to Fe starvation (Schikora and Schmidt 2001). When describing an Fe^{3+} reductase gene from pea (*FRO1*), Waters *et al*. (2002) showed that both its mRNA levels and Fe^{3+} reductase activity are regulated by different signals in roots and shoots. In roots, *brz* and *dgl* mutants constitutively expressed *FRO1*, under both Fe-deficiency and Fe-sufficiency conditions. However, expression of *FRO1* in shoots was Fe-responsive, with up-regulation of the mRNA level only under Fe-deficiency in mutants as well as in wild-type. A recent work reporting microarray data from Fe-deprived *Arabidopsis* roots suggests that Fe is sensed internally, as the stele is the root region with the more pronounced general stress-responses (Dinneny *et al*. 2008).

Vert *et al*. (2003) performed split-root experiments with *Arabidopsis* plants. Half of the roots were maintained under Fe-deficiency and the other half supplied with Fe. Intriguingly, mRNA expression of *IRT1* (Eide *et al*. 1996) and *FRO2* (Robinson *et al*. 1999), two Fe-deficiency up-regulated genes, were even higher in Fe-resupplied roots than in Fe-deprived roots after three days. The authors suggested that, after the Fe-deprived status is sensed by the plant, a shoot-borne signal is probably up-regulating the genes in Fe-resupplied roots. Moreover, presence of Fe is necessary for induction of Fe-deficiency responses, as roots which were submitted to removal of the apoplastic Fe pool and then kept in Fe-depleted medium were unable to up-regulate *IRT1* and *FRO2* mRNA expression. This indicates that both long-distance (shoots perceiving the Fe-deficiency status of the plants) and local signals (presence of Fe, although in low concentrations) are necessary to induce the expression of Fe-deficiency genes, explaining why the Fe-resupplied roots up-regulated their *IRT1* and *FRO2* mRNA levels. In conclusion, plants probably have to integrate both signaling pathways to respond to Fe-deficiency (Vert *et al*. 2003).

Enomoto *et al*. (2007) reported that the long-distance

signal is probably a promotive and not a repressive one in tobacco (*Nicotiana tabacum* L.). When leaves of Fe-deficient plants are excised, *NtIRT1* and *NtFRO1* are down-regulated to Fe-sufficient levels, showing that the absence of the source of a promotive signal stops up-regulation of these genes. In agreement with the local signal hypothesis, this work also shows that hairy roots cultured without shoots are still capable of up-regulating *NtIRT1* and *NtFRO1* under Fe-deficiency conditions (Enomoto *et al*. 2007), indicating that both shoot and root signals are important for the induction of correct responses to the Fe status in plants.

The major constraint about this shoot-to-root signaling model is that at least two sensors are necessary. To date, the Fe status-signaling molecules are still unknown. Li CJ *et al*. (2000) suggested that auxin could be a long-distance signal from the shoot Fe status to be sent to the roots, at least in cucumber. Auxin-resistant *Arabidopsis* mutants are still able to respond to Fe-deficiency, indicating that this hormone is not involved in Fe homeostasis (Schmidt *et al*. 2000). Recently, cytokinins were described as repressors of Fe-deficiency responsive genes, but independently from the Fe nutritional status. In fact, cytokinins seem to down-regulate Fe-deficiency genes through its root growth-inhibitory effect, as well as other treatments (Séguéla *et al*. 2008).

It has also been described that ethylene action is necessary for the up-regulation, under Fe-deficiency, of the *AtFRO2*, *AtIRT1*, and *AtFRU* genes in *Arabidopsis* (respectively, Fe reductase, Fe transporter and Fe-deficiency transcription factor); the *LeIRT1*, *LeFRO1* and *LeFER* (*AtFRU* ortholog) genes in tomato (*Lycopersicon esculentum*); and the *CsHA1*, *CsIRT1* and *CsFRO1* genes in cucumber (*Cucumis sativus*) (Lucena *et al*. 2006; Waters *et al*. 2007). The authors showed that the ethylene enhancement of Fe-deficiency gene expression is under the control of the Fe status, as plants under normal Fe concentrations are almost insensitive to added ethylene precursors. A model was proposed, where ethylene production is up-regulated under Fe-deficiency, and Fe could inhibit the up-regulation of Fe uptake genes mediated by ethylene. Low levels of Fe re-circulating in the phloem sap from leaves to roots under Fe-deficiency would allow ethylene to promote up-regulation of *FRU*, *IRT1*, *FRO* and *HA* genes or their homologues (Lucena *et al*. 2006; Waters *et al*. 2007). The phloem Fe concentration is suggested to be a signal for root perception of the Fe status of the plant.

Krüger *et al*. (2002) described an Fe-binding protein localized in phloem exudates of *Riccinus communis*, named iron-transport protein (ITP1). Although the shoot-borne signal is thought to be phloem-transported, there is no evidence that ITP1 participates in signaling processes. Nitric oxide (NO), a small gas molecule, was already described as involved in Fe-deprivation responses (Grazziano and Lamattina 2007). In tomato plants, NO production increases when plants are submitted to low Fe conditions; NO scavenging prevents up-regulation of *LeIRT1* and *LeFRO1* at the mRNA level; and addition of NO enhances the Fe-deprivation responses (Graziano and Lamattina 2007). Moreover, the *fer* mutant, which is defective in FER gene product (a bHLH transcription factor that induces the Fe-deprivation responses), is insensitive to NO (Brumbarova and Bauer 2005). It is clear, therefore, that NO participates in the signaling pathway responsible for sensing local Fe in roots.

IRON DEPRIVATION STRESS

Effects of iron deficiency on chloroplasts and photosynthesis

Chloroplasts are the major Fe sink in plants, accumulating 80% of total Fe from leaf tissues. Fe participates in several chloroplast reactions, including electron transport and chlorophyll synthesis. It has been shown that in maize (*Zea mays*) leaves, Fe-deficiency leads to reduced chlorophyll content, impairment of electron transport on photosystem II (PSII) and mainly on photosystem I (PSI), and lowered CO_2 fixation capacity in Fe-deprived plants when compared to Fe-sufficient plants (Sharma 2007). Similar effects were observed in spinach (*Spinacia oleracea*) leaves under Fe-deficiency, showing photosynthesis inhibition, impairment of photochemical efficiency, increased heat dissipation as well as altered PSI and PSII distribution pattern in the thylakoid membrane and degradation of PSI proteins to a larger extent than of PSII proteins (Timperio *et al*. 2007). Sperotto *et al*. (2007) demonstrated that Fe-deficiency results in decreased chlorophyll content in rice leaves (*Oryza sativa* L.). In sugar beet (*Beta vulgaris*), proteomic approaches were used to show that the thylakoid membrane composition is highly altered by Fe-deficiency, with lower levels of electron transport proteins and increased levels of carbon fixation proteins in leaves (Andaluz *et al*. 2006). A partial disconnection occurs between the internal PSII antenna complex and the reaction center in sugar beet leaves under Fe-deficiency, leading to interruption of energy transfer (Morales *et al*. 2001). Light absorption, PSII and Rubisco carboxylation efficiencies are also coordinately down-regulated in response to Fe-deficiency in sugar beet, optimizing the use of the remaining photosynthetic pigments, electron transport carriers and Rubisco (Larbi *et al*. 2006).

The green algae *Chlamydomonas reinhardtii* has become the model species for the study of Fe-deficiency effects in chloroplasts. It has been demonstrated that, when *C. reinhardtii* is submitted to moderate Fe-deficiency, remodeling of the photosynthetic apparatus is induced (Moseley *et al*. 2002). LHCI antennae proteins are uncoupled from PSI and degraded through a thylakoid membrane-associated protease, and novel LHCI proteins are synthesized in response to Fe-deficiency. This re-adaptation of the photosynthetic apparatus to Fe-deficiency renders *C. reinhardtii* more light-competent under stress conditions. Indeed, the *crd1* mutation, which leads to constitutively uncoupled LHCI, increases growth of intense light-sensitive mutants. This remodeling could function to increase the light-energy dissipation capacity of the LHCI complex (Moseley *et al*. 2002). A keystone to PSI remodeling in *C. reinhardtii* cells submitted to Fe-deficiency is the N-terminal processing of the PSI protein known as Lhca3 (Naumann *et al*. 2005). It is also suggested that two proteins, namely Lhca4 and Lhca9, up-regulated by Fe-deficiency, could be involved in dissipation of light energy (Naumann *et al*. 2005).

The cyanobacteria *Synechocystis* has also been used in Fe-deficiency studies. In this model species, synthesis of the isiA protein is up-regulated under low Fe conditions. Eighteen subunits of the isiA protein are assembled in a ring shape, forming a super complex together with a PSI trimer (Kouril *et al*. 2005). The super complex is capable of light-harvesting, thus increasing efficiency of the remaining PSI after Fe-deficiency stress. Moreover, empty isiA rings were shown to be strong light-energy dissipators, indicating that up-regulation of isiA under Fe-deficiency is important to optimize photosynthesis and protect from photoinhibition, dissipating excessive light energy (van der Weij-de Wit *et al*. 2007). A dissipation activity that resembles isiA functions was already described for a PSII protein from *Arabidopsis* plants, named psbS (Li XP *et al*. 2000), but such photosynthesis optimization and protection mechanisms in higher plants are largely unknown.

Fe-deficiency induces PSI degradation, what results in higher Fe availability. In *C. reinhardtii*, it was demonstrated that the protein ferritin is used to buffer the Fe released by degradation of the photosynthetic complexes and plays a protective role under photo-oxidative stress conditions (Busch *et al*. 2008). Ferritins are ubiquitous, Fe-storage proteins containing 24 subunits organized to form a cavity able to store up to 4,500 Fe atoms in a safe and bio-available form (Briat *et al*. 2006). In plants, ferritin is mainly localized in plastids and found also in mitochondria (Zancani *et al*. 2004). The results obtained in *Chlamydomonas* are in accordance with the function observed for ferritin in *Arabidopsis* plants, where ferritin does not constitute the major Fe pool, but is essential to prevent oxidative damage (Ravet

et al. 2009). Therefore, it is clear that insights from photosynthesis models like *C. reinhardtii* and *Synechocystis* are valuable resources to help understand Fe-deficiency effects on chloroplasts of higher plants.

Chloroplast-Fe-importing mechanisms are not completely understood as well. To date, two transporters have been linked to chloroplast Fe homeostasis (Duy *et al*. 2007). PIC1 is an *Arabidopsis* permease which is imported to the inner envelope of the chloroplast and is capable of complementing yeast mutants defective in Fe transport. The *pic1* mutant only grows heterotrophically and shows chlorosis, dwarfism and impaired chloroplast development, demonstrating that PIC1 is crucial to proper chloroplast function (Duy *et al*. 2007). The *Multiple Antibiotic Resistance 1* (*MAR1*) gene was isolated independently from *Arabidopsis* plants resistant to antibiotics similar to aminoglycosides that inhibit prokaryotic protein synthesis (Ausfatz *et al.* 2009; Conte *et al.* 2009). Further results indicated that MAR1 localizes to the chloroplast and is fairly evenly expressed in most tissue types (Conte *et al.* 2009). Due to its sequence similarity to ferroportins - which are efflux Fe transporters firstly characterized in animals - and because *MAR1* overexpression led to chlorosis, it was suggested that MAR1 could be involved in chloroplast Fe homeostasis, and serves as an opportunistic entrance for antibiotics to that organelle. In fact, the observed chlorosis could be reverted when plants were grown under 300 μM of Fe-EDTA, and *MAR1* mRNA levels decrease under Fe-deficiency, although they were not altered under Fe-excess (Conte *et al*. 2009). Since aminoglycosides mimic polyamines, and NA is a polyamine, MAR1 might transport either Fe or NA. Besides those transporters, an Fe^{3+} reductase from *Arabidopsis*, AtFRO7, was the first *FRO* gene to be localized to chloroplasts (Jeong *et al*. 2008). Mutants defective in AtFRO7 have 75% decrease in chloroplastidic Fe^{3+} reductase activity and 33% reduction in the Fe content inside chloroplasts. These mutants also show severe chlorosis and die in alkaline soils unless treated with high levels of soluble Fe (Jeong *et al*. 2008).

Improving iron uptake

In order to deal with limiting amounts of Fe, plants have evolved two strategies to obtain Fe from the soil. Strategy I is carried out by all vascular plants, except the Gramineae (Poaceae), which use strategy II (Marschner and Römheld 1994). Strategy I plants improve Fe uptake by three processes: secretion of protons via a plasmalemma P-type ATPase to acidify the rhizosphere and thus enhance the solubility of Fe^{3+}; reduction of Fe^{3+} by an Fe(III)-chelate reductase to the more soluble Fe^{2+} form; plasmalemma transport of Fe^{2+} by Fe transporters (Kerkeb and Connolly 2006). All three components of this strategy increase their activities during Fe-deficiency (Hell and Stephan 2003). Proton-ATPases of the AHA (*Arabidopsis* H^+-ATPase) family are involved in this process (Schmidt *et al.* 2003; Kim and Guerinot 2007; Waters *et al.* 2007; Santi *et al*. 2009). The *Arabidopsis* mutant, *ferric-chelate reductase defective 1 (frd1)*, has no inducible root Fe(III)-chelate reductase activity and develops severe chlorosis when Fe is limiting (Yi and Guerinot 1996). The *FRO2* gene was shown to map to the same location as the *frd1* mutation. Subsequently, transformation of the *frd1* mutant with the *FRO2* gene rescued the *frd1* phenotype and proved that *FRO2* encodes the root ferric chelate reductase (Robinson *et al.* 1999). The pea *FRO1* gene was identified and expression of *PsFRO1* in yeast showed that the enzyme function is to reduce Fe^{3+} to Fe^{2+} (Waters *et al.* 2002). PsFRO1 is expressed in many locations throughout the plant, suggesting that PsFRO1 takes part in Fe uptake from the soil and in Fe distribution within the plant (Waters *et al.* 2002). Fe^{2+} is transported into the root by IRT1, a member of the ZIP (ZRT, IRT-like proteins) metal transporter family. The *Arabidopsis IRT1* gene was identified by functional complementation of a yeast Fe uptake mutant (Eide *et al.* 1996). IRT1 expression in roots is induced by Fe starvation (Eide *et al.* 1996; Connolly *et al.* 2002). The *Arabidopsis irt1* mutant exhibits chlorosis, severely impaired growth and dies 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 *Arabidopsis* transporter for Fe uptake from soil (Vert *et al.* 2002; Kim and Guerinot 2007).

Strategy I plants developed additional physiological responses to Fe-deficiency, which can aid Fe uptake from the soil by increasing Fe^{3+} solubility. These responses include, depending on the species, excretion of phenolics and accumulation and/or excretion of flavin compounds (Susín *et al*. 1994) and organic acids (Abadía *et al*. 2002). Phenolic compounds are frequently reported to be the main components of root exudates in response to Fe-deficiency (Jin *et al*. 2007).

In response to Fe-deficiency, strategy II plants produce small compounds known as phytosiderophores (PS), which are secreted to the rhizosphere. Due to their high affinity for Fe, PS efficiently solubilize inorganic Fe^{3+} by chelation, producing Fe(III)-PS complexes that are then taken up by a specific transporter on the root plasmalemma (Römheld and Marschner 1986). Both processes (release of PS and Fe(III)-PS transport) are increased in response to Fe-deficiency via up-regulation of the underlying genes (Hell and Stephan 2003). This chelation strategy is more efficient than the reduction strategy used by the other plants and thus allows grasses to survive in more drastic Fe-deficiency conditions (Curie and Briat 2003). In grasses, PS biosynthesis and secretion, as well as Fe(III)-PS uptake are induced by Fe starvation. Different graminaceous species produce different types and quantities of PS. The amount of PS released into the soil correlates with the plant's ability to tolerate Fe starvation (Kerkeb and Connolly 2006). Plant PS belong to the mugineic acid (MA) family of chelators, which was first described in oats and rice by Takagi (1976). Since then, the biochemical pathway for PS synthesis has been elucidated and many of the essential genes have been cloned (Negishi *et al.* 2002). An Fe-PS transporter was cloned from maize (Curie *et al.* 2001) and named yellow stripe1 (YS1) after the phenotype of a maize mutant deficient in PS uptake. Both the efflux of PS and the steady-state level of *YS1* mRNA are strongly increased by Fe-deficiency in grasses (Mori 1999; Curie *et al.* 2001). Electrophysiological analysis in *Xenopus laevis* oocytes demonstrated that *YS1* encodes a proton-coupled transporter of PS-metal chelates (Schaaf *et al.* 2004). Following the identification of *ZmYS1*, a large number of *YS1* orthologs were noted in several strategy II species such as barley and rice (Murata *et al.* 2006; Inoue *et al.* 2009; Lee *et al.* 2009). In strategy I plants, which cannot synthesize or utilize MAs but only nicotianamine (NA - Curie *et al.* 2001), *Yellow Stripe-Like* genes seem to play a role in the internal transport of Fe^{2+} and Fe^{3+} (Ling *et al.* 1999). NA, a non-proteinogenic amino acid, is the most studied Fe-chelator. NA forms stable complexes with Fe and other divalent transition metal ions (Benes *et al*. 1983; Anderegg and Ripperger 1989) and has a crucial role in the internal transport of Fe and other metals such as Cu and Zn (Stephan *et al*. 1994; Pich and Scholz 1996; Takahashi *et al*. 2003). It has been proposed that the primary function of YSL proteins in strategy I plants is to transport metal-NA complexes (Curie *et al.* 2001; DiDonato Jr. *et al.* 2004; Le Jean *et al.* 2005; Schaaf *et al.* 2005; Gendre *et al.* 2006; Waters *et al.* 2006). In strategy II plants, YSL proteins can also transport metal-NA complexes (Koike *et al.* 2004; Schaaf *et al.* 2004), in addition to their role in the transport of Fe-PS. For a comprehensive review see Curie *et al.* (2009). YSL proteins belong to the oligopeptide transporter (OPT) family, a relatively poorly characterized family of transporter proteins that function in metal homeostasis and movement of Fe to developing seeds of *Arabidopsis* (Stacey *et al.* 2008) and participate in NA-bound Fe transport in rice (Vasconcelos *et al*. 2008).

It was recently shown that rice, in addition to the ability to transport Fe(III)-PS complexes, is able to transport Fe^{2+} (Ishimaru *et al.* 2006). The rice genome encodes two proteins related to the strategy I transporter IRT1 (OsIRT1 and OsIRT2) that are specifically up-regulated in roots of Fe-deficient plants. *OsIRT1* and *OsIRT2* gene expression are localized in the plasma membrane of root epidermal cells, and confer Fe uptake in yeast functional complementation assays (Bughio *et al.* 2002; Ishimaru *et al.* 2006). However, no increases of FRO-like gene expression or Fe(III)-chelate reductase activity were detected in Fe-deficient rice roots. It is possible that rice can compensate for the lack of effective Fe(III)-chelate reductases because of its wetland culture. It was suggested that this may be an adaptation to the soil conditions in flooded, and thus oxygen-poor, rice paddies, in which levels of soluble Fe^{2+} are expected to be high (Ishimaru *et al.* 2006; Walker and Connolly 2008).

Other responses to Fe deprivation

Besides strategy I and II mechanisms, other responses are commonly observed to improve Fe uptake when plants are submitted to Fe-deprivation. Two morphological alterations in roots in response to Fe-deficiency have been characterized, both aiming to increase the surface-to-volume ratio and thus optimize root absorption capacity: initiation of extranumerary root hairs, and transfer cell-like ingrowth depositions.

The most well described alteration is the induction of extranumerary root hair cells. Root hairs are long tubular extensions from rhizodermal trichoblasts controlled by multiple gene loci (Grierson and Schiefelbein 2002; Larkin *et al*. 2003). In fact, absorption in low concentrations of soil-immobile nutrients, such as Fe and phosphate (P), can induce the increase in root surface. In *Arabidopsis* roots, the extra root hairs are generated by such nutrient-deficiencies in root locations where there are no hair cells under normal conditions (Schmidt *et al*. 2000; Schmidt and Schikora 2001). Evidence suggests that the mechanism controlling the induction of root hair differentiation under Fe-deficiency is differentially regulated from the induction under P-deficiency, as defects in ethylene or auxin signaling cascades decrease root hair induction by Fe-deficiency but not by P-deficiency (Schmidt and Schikora 2001). In another work with mutants defective in genes important for normal root hair differentiation, Müller and Schmidt (2004) showed that nutrient-deficiency-induced root hair formation is not a general mechanism, as *Arabidopsis* mutants showed different responses to Fe and P deprivation. Moreover, they showed that extranumerary root hairs of Fe-deprived plants are commonly bifurcated, while P-deprived are only longer in length, when compared to control (Müller and Schmidt 2004). These results strongly suggest that the induction of extranumerary root hairs is a specific adaptation to Fe-deficiency. Using Laser Microdissection (LM), Santi and Schmidt (2008) showed that, in cucumber roots, the differentiation of rhizodermal cells into root hairs in response to Fe-deficiency is accompanied by induction of the H^+ pump *CsHA1*, ferric chelate reductase *CsFRO1* and Fe^{2+} transporter *CsIRT1*, all in a cell-specific manner. The root structural alteration of Fe-deprived plants is thus tightly related to the induction of Fe-acquisition genes, further suggesting the specificity of the developmental alteration.

Transfer cell-like formation is a response specifically found in some taxa, as *Arabidopsis* rhizodermal cells can only differentiate on hair or non-hair cells, while transfer cells are not observed (Schmidt *et al*. 2000). Transfer cells possess extensive labyrinth-like protuberances on the outer cell walls, enrichment for mitochondria and rough endoplasmic reticulum (Schikora and Schmidt 2002). This cell type is not normally observed in plants grown in control conditions. When tomato plants are subjected to Fe-deprivation, about 17% of rhizodermal cells differentiate into transfer cells (Schikora and Schmidt 2002). Addition of the ethylene precursor 1-aminocyclopropane-1 carboxylic acid (ACC) to Fe-sufficient medium induced comparable number of transfer cells, indicating that ethylene may participate in the transfer cell formation process. On the other hand, this hormone is probably not necessary for differentiation, as ethylene antagonists did not show the opposite effect and even increased transfer cell number (Schikora and Schmidt 2002). The same work also showed that Fe and P-deficient tomato plants induced comparable numbers of transfer cells, indicating that, differently from extranumerary root hair induction, a common pathway could be involved in transfer cell induction by these two treatments.

Metabolic changes in response to Fe-deficiency were described in sugar beet (*Beta vulgaris*) plants, which transport organic acids via xylem from roots to shoots, accumulating these compounds in leaves (López-Millán *et al*. 2001). There is increased concentration of organic acids (mostly malate and citrate complexed with Fe) both in the apoplastic compartment and in the xylem sap (López-Millán *et al*. 2000a). It was shown that phosphoenolpyruvate carboxylase (PEPC) activity is enhanced in roots of sugar beet plants submitted to Fe-deficiency, together with other metabolic changes that indicate non-autotrophic, anaplerotic carbon fixation (López-Millán *et al*. 2000b). This carbon can be transported via xylem to leaves for maintenance of basic processes under Fe-deficiency-reduced photosynthetic rates (López-Millán *et al*. 2000b). Working with tomato plants, López-Millán *et al*. (2009) found similar but less pronounced metabolic changes in roots submitted to Fe-deficiency, indicating that these responses are differentially modulated in diverse strategy I species, what could be related to different levels of Fe efficiency.

Molecular regulation of Fe-deprivation responses

The primary response of plants to Fe-deficiency is controlled through coordinated transcriptional activation. Studies with the *fer* tomato mutant have led to the identification of the LeFER basic helix-loop-helix (bHLH) transcription factor, which controls root Fe^{3+} reductase and *LeIRT1* induction upon Fe limitation. The *fer* mutant is unable to induce the Strategy I mechanism in response to Fe-deprivation, leading to chlorosis and lethality under low Fe conditions (Ling *et al.* 2002), but can be completely rescued if supplied with high amounts of easily degradable Fe chelates (Ling *et al.* 2002) or by grafting on wild type rootstock (Brown *et al.* 1971), showing that FER is required in the roots and not in the shoots. *FER* transcripts are detected when plants are grown with either low (0.1 μM) or moderate (10 μM) amounts of Fe. However, FER protein levels are controlled by Fe availability. In transgenic tomato overexpressing FER, transcripts can be detected from the plants grown at 10 μM and 100 μM Fe (Ling *et al.* 2002; Brumbarova and Bauer 2005). FER protein, however, is not detected in plants grown at 100 μM Fe, suggesting FER is down-regulated post-transcriptionally at elevated Fe levels (Brumbarova and Bauer 2005). The *Arabidopsis* LeFER orthologue is the *Fe-deficiency-induced transcription factor 1* (FIT1), also known as bHLH29/FRU (Colangelo and Guerinot 2004; Jakoby *et al.* 2004; Yuan *et al.* 2005). Expression of FIT1 in the *fer* mutant allows the tomato mutant to induce the Fe-deficiency responses and survive under Fe-limiting conditions (Yuan *et al.* 2005). *fit1* mutants are also chlorotic and die under low Fe conditions. 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, no increase in *FRO2* mRNA levels and Fe(III)-chelate reductase activity is observed upon Fe depletion (Colangelo and Guerinot 2004; Jakoby *et al.* 2004). However, whereas *IRT1* mRNA up-regulation is not significantly affected in *fit1* mutants, the IRT1 protein is undetectable (Colangelo and Guerinot 2004; Jakoby *et al.* 2004). These results indicate that *FRO2* is a direct target for transcriptional regulation by FIT1, while a FIT1-dependent mechanism of post-transcriptional regulation controls IRT1 protein levels

(Colangelo and Guerinot 2004; Jakoby *et al.* 2004; Kim and Guerinot 2007; Puig *et al.* 2007). Besides *FRO2*, FIT1 regulates 71 out of the 179 genes whose expression is modified in roots after three days of Fe-deficiency, including many important genes induced upon Fe-deprivation (Colangelo and Guerinot 2004). Overexpression of FIT1 does not alter *FRO2* and *IRT1* expression patterns in roots under Fe-sufficient conditions, suggesting that FIT acts with a binding partner that is expressed only in response to Fe limitation (Colangelo and Guerinot 2004; Puig *et al.* 2007). Additional bHLH family members (bHLH38, bHLH39, bHLH100, and bHLH101) have been identified as key players in the Fe-deficiency response (Yuan *et al.* 2005; Wang *et al.* 2007; Yuan *et al.* 2008). bHLH38 and bHLH39 physically interact with FIT and transgenic plants that constitutively co-express either bHLH38 or bHLH39 with FIT show Fe-independent high-level expression of FRO2 and IRT1 and accumulate more Fe than wild type plants (Yuan *et al.* 2008; Walker and Connolly 2008). It is therefore suggested that FIT interacts with either bHLH38 or bHLH39 to induce expression of the strategy I Fe uptake machinery (Walker and Connolly 2008).

Ogo *et al*. (2006) identified a bHLH transcription factor gene named *OsIRO2* which is up-regulated at the mRNA level by Fe-deficiency in both shoots and roots of rice. The expression of *OsIRO2* was induced exclusively by Fe-deficiency, and not by deficiencies of other metals. Although *IRO2* is well conserved in grasses, it is not closely related to *AtFIT* or *LeFER* (Ogo *et al*. 2007; Walker and Connolly 2008). It was shown that OsIRO2 binds preferentially to the sequence 5′-ACCACGTGGTTTT-3′, and sequences similar to the OsIRO2-binding sequence were found upstream of several genes that are involved in Fe acquisition, suggesting that IRO2 is involved in the regulation of gene expression under Fe-deficient conditions (Ogo *et al*. 2006). Overexpression of IRO2 resulted in improved growth and increased MAs secretion under Fe-deficient conditions, whereas repression of IRO2 resulted in reduced biomass and lower MAs secretion, besides hypersensitivity to Fe-deficiency and lower accumulation of Fe, Zn, Cu and Mn (Ogo *et al*. 2007; Walker and Connolly 2008). Northern blots revealed that the expression of the genes involved in the Fe(III)-PS transport system was dependent on OsIRO2, but the expression of *OsIRT1* was unchanged, indicating that OsIRO2 regulates the PS-mediated Fe uptake system of rice, but not the additional Fe^{2+} uptake mechanism (Ogo *et al*. 2007; Walker and Connolly 2008).

Two *cis*-acting elements named IDE1 and IDE2 were identified by promoter deletion analysis in the barley *IDS2* gene, a dioxygenase involved in PS biosynthesis (Nakanishi *et al*. 2000; Kobayashi *et al*. 2003). IDE1 and IDE2 synergistically induce Fe-deficiency-specific gene expression in tobacco roots (Kobayashi *et al*. 2003). The promoter regions of many Fe-deficiency-inducible genes in barley, rice, and *Arabidopsis* possess IDE-like sequences (Kobayashi *et al*. 2003, 2005), suggesting that gene regulation mechanisms involving IDEs not only are conserved among graminaceous (Strategy II) plants but are also functional in non-graminaceous (Strategy I) plant species (Kobayashi *et al*. 2007). Recently, transcription factors that interact with IDEs have been described (Kobayashi *et al*. 2007; Ogo *et al*. 2008). The rice IDEF1 protein has the ability to bind to the IDE1 sequence. IDEF1 belongs to an uncharacterized branch of the plant-specific transcription factor family ABI3/VP1 and exhibits the sequence recognition property of efficiently binding to the CATGC sequence within IDE1. *IDEF1* transcripts are constitutively present in rice roots and leaves. Transgenic tobacco plants expressing *IDEF1* under the control of the constitutive cauliflower mosaic virus 35S promoter are able to transactivate IDE1-mediated expression only in Fe-deficient roots, indicating that IDEF1 is specifically involved in Fe-regulated gene expression. Overexpression of IDEF1 caused up-regulation of the *OsIRT1* and *OsIRO2* genes and enhances the plant ability to tolerate Fe-deficiency (Kobayashi *et al*. 2007). Recently, Kobayashi *et al.* (2009) showed that the rice transcription factor IDEF1 is essential for the early response to Fe-deficiency, and mediate transactivation of several Fe-related and late embryogenesis abundant genes, just after the onset of Fe starvation. The rice IDEF2 protein has the ability to bind to the IDE2 sequence which belongs to an uncharacterized branch of the NAC transcription factor family and predominantly recognizes CA(A/C)G(T/C)(T/C/A)(T/C/A) within IDE2 as the core-binding site. As well as *IDEF1*, expression of the *IDEF2* transcript is not Fe-regulated, and *IDEF2* mRNA is present in both shoots and roots, although expression is substantially higher in shoots (Ogo *et al*. 2008; Walker and Connolly 2008). Repression of the IDEF2 function caused aberrant Fe homeostasis in rice. Several genes up-regulated by Fe-deficiency, including the Fe(II)-NA transporter gene *OsYSL2*, were less induced by Fe-deficiency in rice plants in which the *IDEF2* gene was silenced by RNAi, suggesting that IDEF2 is involved in the regulation of these genes. The transgenic lines exhibited lower than normal Fe levels in both shoots and roots of plants grown in Fe-deficient conditions, indicating an important role for IDEF2 in maintaining optimal Fe levels in tissues (Ogo *et al*. 2008; Walker and Connolly 2008).

A summary list of Fe-deficiency responsive genes from plants is shown in **Fig. 1 -** Roots, including transcription factors (*FER*, *FIT*, *IRO*, *IDEF*), typical strategy I-induced genes (*AHA*, *FRO*, *IRT*) and the Fe-PS transporters (typical from the strategy II response) from the *YSL* gene family.

IRON OVERLOAD STRESS

Under normal conditions, the transient pool of Fe that could catalyze the formation of active oxygen is very small and plants face Fe-deficiency much more frequently. However, reactions involving free radicals such as lipid peroxidation are largely accentuated in tissues that have sustained physical injury or are in senescence, conditions that could lead to a decompartmentalization of Fe, and a consequent facilitation of activated oxygen formation (Wolff *et al*. 1986).

Wetland plants have evolved and are adapted to anaerobic and anoxic environments – conditions that are prerequisites to the solubilization of large amounts of Fe from the soil. High amounts of soluble Fe (100-1,000 mg L^{-1}) have been reported to occur in acid soils (Ponnanperuma 1972). Thereby, they are a good model to study the toxic effects of Fe in plants. As a wetland plant with high economic and scientific importance (Shimamoto and Kyozuka 2002), rice is known to display a wide variability in tolerance to Fe toxicity, depending on the cultivars (Fageria and Rabelo 1987; Sahrawat *et al*. 1996) and agronomic management used (Benckiser *et al*. 1984; Winslow *et al*. 1989). Fe toxicity symptoms vary, but are generally characterized by a reddish-brown, purple bronzing, yellow or orange discoloration of the lower leaves, with rice yield reductions from 12 to 100%, depending on the Fe tolerance of the genotype, intensity of Fe toxicity stress and soil fertility status (Sahrawat 2004).

Physiological impacts of Fe-overload stress

When free and in excessive levels inside the cell, Fe can act as a pro-oxidant agent, reacting with H_2O_2, generating the hydroxyl radical through the Fenton Reaction (Becana *et al*. 1998). The hydroxyl radical is extremely toxic to the cellular metabolism, leading to the oxidation of macromolecules, including nucleic acids (Halliwell and Gutteridge 1984). The regeneration of Fe^{2+} can occur through the Haber-Weiss reaction, with the reduction of Fe^{3+} by the superoxide anion, turning the Fe-mediated ROS (reactive oxygen species) generation into a cyclic process (Floyd 1983).

Due to its properties, Fe-excess is closely related to oxidative stress. The excessive accumulation of Fe has been linked to oxidative damage to proteins, lipids and losses in

chlorophyll content (Gallego *et al*. 1996; Fang *et al*. 2001; Stein *et al*. 2009), causing photooxidative damage and oxidative stress. Excessive amounts of Fe have been reported to cause root cell death in rice seedlings, stimulating a rapid induction of a MBP (myelin basic protein) kinase, dependent of ROS (Tsai and Huang 2006).

In leaves, most of the cellular Fe is located in chloroplasts (Terry and Low 1982). Thereby, any adverse event at the cellular level caused by Fe overload would occur primarily in these organelles. Fe-excess has been reported to cause severe photooxidative damage, leading to decrease in photosynthetic activity, accompanied by photoinhibition, increased reduction of PSII and higher thylakoid energization (Kampfenkel *et al*. 1995). The mechanism of Fe toxicity in leaf cells was precisely detailed by Suh *et al*. (2002), using de-rooted pea plants. Upon excessive Fe supply (0.9 mM), positive correlations are seen between thylakoid Fe content, the rate of $^1O_2$ photoproduction in thylakoids and the severity of photoinhibition damage to PSII (Suh *et al*. 2002), indicating that the toxic effect of Fe-excess on the chloroplast metabolism is probably due to excessive production of $^1O_2$.

Avoiding excessive iron accumulation

The capacity to retain Fe at the root level is known as a tolerance mechanism used by some plants to avoid excessive accumulation in shoots. This capacity is absent or restricted in wet-intolerant plants (Wheeler *et al*. 1985).

Bartlett *et al*. (1961) noted that oxidized Fe deposits were common in roots of hydrophytes and showed that the root oxidizing activity was specifically correlated with the ability to tolerate waterlogged soils. The Fe-oxide deposits are commonly observed on the roots of wetland and aquatic plants (Mendelssohn *et al*. 1995), and are generally named as "Fe plaque". The Fe plaque is formed by the active oxidation of Fe at the root surface. Whether the formation of such barrier can cause nutrient imbalances, acting as a physical barrier to the active capture of other important nutrients, remains as a matter of debate, depending on plant genotype and soil characteristics (Sahrawat 2004).

The ability to regulate Fe uptake and therefore to avoid its excessive accumulation could also be a mechanism for plants to deal with Fe-excess. Several genes involved in the uptake and internal transport of Fe have been described in plants (Briat *et al*. 2007). However, due to practical implications with the experimental procedures, only Fe transporters involved in the response to low Fe availability and with high Fe affinity have been identified. Indeed, the expression of known Fe transporters, such as the *YSL* and *IRT/ZIP* genes, are positively regulated only by Fe-deficiency, being negatively regulated by Fe-excess in rice (Stein *et al*., unpublished data). Other transporters, with lower affinity for the metal, could act as the main Fe transporters under Fe-excess, but their identification remains as an unachieved goal. The avoidance mechanism shown in **Fig. 2 -** Roots, represents both the "Fe plaque" formation and lower Fe uptake rates, since both result in reduced intracellular root Fe concentrations.

The ability to reduce Fe translocation from the root to the aerial parts, leading to accumulation of Fe in roots, could also be an important feature in the avoidance of excessive accumulation in leaves (**Fig. 2 -** Roots, Low Acropetal Transport Mechanism). One example is the rice EPAGRI 108 cultivar, studied in detail by our group. Iron concentration in shoots was 2.5 times lower in plants from this cultivar than in plants susceptible to Fe overload, from the BR-IRGA409 cultivar (Silveira *et al.* 2007). Becker and Asch (2005) pointed that Fe that has entered the xylem stream follows the transpiration-driven acropetal long-distance transport, and under Fe-excess (e.g. waterlogged conditions) plants probably regulate its translocation. Organic acids (e.g. citrate) are the main Fe-chelators found in xylem exudates (Cataldo *et al*. 1988). A recently identified rice citrate transporter, OsFRDL1, is responsible for the translocation of Fe and is localized at the root pericycle cells (Yokosho *et al*. 2008).

Storing high iron levels

The capacity to store Fe at high levels in plants may rely on the capacity to sequester and keep Fe at a safe and bioavailable form. Fe capable of ROS generation (especially the hydroxyl radical) is known to be bound to several small chelators (such as carboxylic acids, di- and tri-phosphate nucleotides - Floyd 1983; Baker and Gebicki 1986). Complexation of Fe with non-Fenton reactive molecules plays an important role in the protection of the cell. One of these Fe-chelators is NA, which was proposed to have a central role in the regulation of Fe uptake and in its internal transport (Stephan and Scholz 1993), based on the phenotype of the NA-auxotroph tomato mutant *chloronerva* (Scholz *et al*. 1985). Although displaying interveinal chlorosis, a typical symptom of Fe-deficiency, *chloronerva* mutants over-accumulate Fe and other trace metals when grown at normal Fe levels (Pich and Scholz 1991), indicating the importance of NA in Fe transport. Despite the role of NA in the internal Fe trafficking, the Fe-NA complexes are relatively poor Fenton reagents (von Wirén *et al*. 1999) and NA concentration is increased in response to Fe-excess (Pich *et al*. 2001). Plants overexpressing *nicotianamine synthase* (*NAS*) genes accumulate higher NA levels in the plant body, which leads to increased accumulation of Fe, Zn and Mn in leaves, improving the Fe use efficiency and leading to nickel tolerance (Douchkov *et al*. 2005).

Storage and buffering of Fe at the sub-cellular level are crucial mechanisms that allow plants to cope with Fe-deficiency and also with toxicity (Briat *et al*. 2007). The transporter proteins encoded by genes from the *YSL* family are probably responsible for the transport of Fe-NA complexes across plant cell membranes (Curie *et al*. 2009). Along with YSL proteins, metal transporters localized in the tonoplast, like VIT1 (Kim *et al*. 2006), AtNRAMP3 and AtNRAMP4 (Lanquar *et al*. 2005) could contribute to the Fe storage.

Ferritins also appear as important players in the storage and protection of the cell to Fe-mediated oxidative damage. Fe overload was shown to be a strong *ferritin* mRNA inductor in all plant systems tested (Lescure *et al*. 1991; Lobreaux *et al*. 1993; Petit *et al*. 2001a; Majerus *et al*. 2007). In *Arabidopsis* and maize, a *cis*-acting element named IDRS (iron-dependent regulatory sequence) is responsible for the transcriptional repression of *AtFer1* and *ZmFer1* under low Fe supply (Petit *et al*. 2001b). The exposure to high Fe concentration led to the degradation of the repressor via ubiquitination, leading to the accumulation of *ferritin* mRNA (Arnaud *et al*. 2006).

Plant ferritins are also induced under oxidative stress, and are regulated by pro-oxidant treatments such as H_2O_2 (Savino *et al*. 1997), NO donors and scavengers (Murgia *et al*. 2002; Murgia *et al*. 2007) and ozone applications (Murgia *et al*. 2001). Plants overexpressing ferritin were shown to be more resistant to oxidative stress (Deák *et al*. 1999). The importance of ferritin in the protection of Fe-mediated oxidative stress was recently demonstrated by Ravet *et al*. (2009), who showed that ferritins are essential to protect cells from Fe-derived oxidative damage, and the lack of ferritin leads to reduced growth and strong defects in flower development, probably due to Fe-excess toxicity.

To our knowledge, no specific Fe hyperaccumulator plants have been described. Metal hyperaccumulators are plants which, in their native habitats, accumulate high concentrations of arsenic, cadmium, cobalt, manganese, nickel, selenium or zinc, depending on the species (Reeves and Baker 2000). Interestingly, some of the genes known to be involved in Fe homeostasis are overexpressed in hyperaccumulators. These include *NAS2* and *3*, *IRT1* and *FRO2* in *Arabidopsis halleri* and *Thlaspi caerulescens* (Lombi *et al*. 2002; Becher *et al*. 2004; Weber *et al*. 2004). Such observations argue that selective pressures have co-opted part of the Fe-response mechanisms in hyperaccumulators to play

an as yet unknown role in the metal hyperaccumulation process (Salt 2006).

Fe complexation with non-Fenton reactive molecules, compartmentalization in the vacuole and storage into ferritin molecules are all represented by the Fe detoxification mechanism shown in **Fig. 2 -** Shoots, which also refers to the enzymatic activities described below.

Trying to detoxify oxidative molecules

To minimize the cell injury caused by ROS, plants have evolved a complex and interconnected antioxidative defense pathway, composed of both non-enzymatic and enzymatic mechanisms. The scavenging of superoxide radicals (O_2^-) is mediated by superoxide dismutase (SOD) (Rabinowitch and Fridovich 1983), while H_2O_2 is scavenged by ascorbate peroxidase (APX) (Asada 1992) and catalase (CAT) (Willekens *et al*. 1997). The antioxidant compounds ascorbate and glutathione are also directly (through ROS scavenging) or indirectly involved, taking part in the Ascorbate-Glutathione cycle (Noctor and Foyer 1998).

Plant exposure to high levels of Fe was shown to induce the activity of several enzymes involved in the detoxification of ROS generated by the excessive amounts of Fe (Kampfenkel *et al*. 1995; Fang *et al*. 2000, 2001). Indeed, Wu *et al*. (1998) proposed that the antioxidative capacity of the leaf tissue plays an essential role in rice tolerance to Fe toxicity.

There are several reports showing that Fe and oxygen metabolism are closely related. In *Saccharomyces cerevisiae*, a cytosolic catalase (CTT1) and an Fe^{3+} reductase are both regulated by the same transcription factor, MAC1 (Jungmann *et al*. 1993). In plants, Fe-excess induced the expression of cytosolic *APX* genes, but treatment with glutathione completely abolished the observed induction (Pekker *et al*. 2002; Fourcroy *et al*. 2004). The same regulatory pathway appears to operate in the regulation of *AtFer1*, with the ferritin induction preceded by a NO burst, as an early event in the signal transduction pathway (Arnaud *et al*. 2006).

DEALING WITH SENESCENCE: LIVE OR LET DIE

Fe-deprivation-induced senescence

Senescence and subsequent death are terminal phases in the development of all plant organs including leaves, stems, flowers and roots. Although senescence occurs in an age-dependent manner, the initiation and progression of senescence can be influenced by a variety of environmental conditions such as shading, low temperature, high light, nutrient deficiency, dehydration and pathogen infection. During senescence, plant cells undergo dramatic changes in cellular metabolism and a sequential degradation of cellular structures (Lim *et al*. 2003). The products of this degradation are translocated as nutrients to younger or reproductive organs. Senescence is therefore an active process, which is required for plant survival and adaptation to unfavorable environmental conditions, rather than a passive process that simply leads to death (Yoshida 2003).

Induction of senescence in plants submitted to Fe-deficiency is not unexpected. It is well known that severe Fe-deficiencies can lead to lower productivity and even to plant death, resulting in complete crop failure (Guerinot and Yi 1994). However, there are only two reports (published by our research group) about the establishment of senescence after Fe-deficiency treatment (Sperotto *et al*. 2007, 2008). In rice shoots, we were able to detect 32 sequences activated by Fe-deficiency (Sperotto *et al*. 2007). Twenty eight of these genes had not been previously related to Fe-deficiency responses in plants, and several classical senescence-related sequences were identified. There was higher accumulation of total soluble sugars prior to the decrease of total chlorophyll content in Fe-deficient leaves, indicating that sugar accumulation may be one of the factors leading to premature leaf senescence induced by Fe-deficiency (Sperotto *et al*. 2007; **Fig. 1 -** Shoots, high TSS in leaves). Sugar content typically increases during *Arabidopsis* leaf senescence (Diaz *et al*. 2005) and Pourtau *et al*. (2006) showed increased accumulation of the hexoses glucose and fructose in senescent leaves, concluding that *Arabidopsis* leaf senescence is induced by sugars. In rice roots, we were able to detect 28 sequences activated by Fe-deficiency (Sperotto *et al*. 2008). None of them had been previously related to Fe-deficiency responses in plants, except in previous experiments performed by our group with Fe-deficiency-induced senescence in rice shoots. From the 28 sequences identified in our experiments, 11 sequences (39%) have already been related to senescence processes. Root growth reduction started after nine days of low Fe treatment, and was evident after 30 days. After the same period of time, there was increased root lipid peroxidation, a typical physiological event during senescence. There were high levels of plasma membrane deterioration, an indicator of increased cell death, in rice roots submitted to 50 days of Fe-deficiency (**Fig. 1 -** Roots). Plants exposed to Fe-deficiency died 70 days after stress imposition (Sperotto *et al*. 2008).

Fe-overload-induced senescence

Our group identified 24 genes that are up-regulated in response to Fe-excess in rice. Several genes are associated with stress response and senescence. Among them, we characterized the expression pattern of *OsWRKY80*, a new senescence-associated gene (SAG) which is up-regulated at the later stages of senescence (**Fig. 2 -** Shoots). The onset of the senescence program in some plant organs may allow remobilization of nutrients (minerals, amino acids and carbohydrates) to less-affected tissues (Ricachenevsky *et al*. 2010). Liu *et al*. (2007) also suggested that Fe is possibly a mediator of the pathogen attack defense signaling in wheat, being secreted to the apoplast and inducing an oxidative burst mediated by H_2O_2 after *Blumeria graminis* invasion. This indicates that Fe could be involved in the crosstalk between multiple stress pathways and senescence.

PERSPECTIVES

A significantly larger amount of work is available about Fe-deficiency in plants than about Fe-overload. Nonetheless, a few key points about Fe-deficiency still need clarification. One important goal for future research would be the identification of the molecules responsible for the Fe status signaling (both root- and shoot-borne signals). Although several plant Fe homeostasis genes have been identified, they are mostly related to Fe-deficiency responses. It is not clear yet whether Fe uptake by roots under Fe-sufficiency or excess conditions is mediated by the same transporters responsible for Fe uptake under Fe-deficiency. Typical strategy I and strategy II genes are down regulated by Fe-excess, but small amounts of the corresponding proteins could be enough to carry Fe into root cells in those conditions. On the other hand, the existence of both high- and low-affinity Fe transporters in plants, as is the case in yeast (Eide *et al.* 1996), was never discarded nor confirmed. If they exist, low-affinity Fe transporters could have important roles when plants face Fe-overload. The transcription factors which regulate the genes responsive to Fe-overload also need to be identified. One candidate for this function, OsWRKY80, is under investigation by our group. It would also be interesting to identify key genes responsible for the different mechanisms that can lead to tolerance to Fe-excess in plants. For that, microarray analyses comparing susceptible and tolerant genotypes could be of help.

Understanding Fe stresses (deficiency and overload) in plants is an important step into assembling the complete puzzle of plant Fe homeostasis. This is an essential area of research, with several implications to plant biology, agriculture and human nutrition.

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