REVIEW ARTICLES
J. Crop Sci. Biotech. 10 (2) : 64 ~ 72

Rice Iron Metabolism: from Source to Solution

Lívia Scheunemann dos Santos[1], Antonio Costa de Oliveira[1*]

[1]Plant Genomics and Breeding Center, Federal University of Pelotas, P.O. Box 354, Pelotas, RS, Brazil

Abstract

Iron is an important micronutrient for plants. Iron metabolism is a complex mechanism under a delicate balance. Iron metabolism represents two major problems for plants: deficiency as a consequence of solubility problems and toxicity due to excess solubility in anaerobic conditions. In the last few years, new genes have been discovered that influence iron uptake, transport, and storage. Irrigated rice is exposed to high levels of Fe^{II}, normally rare in aerobic soil conditions. The implications of altering iron uptake rates and the effects of newly discovered genes are discussed.

Key words: abiotic stress, genomics, iron metabolism

Mineral nutrition is one of the most important factors involved in plant growth and development. Among the essential mineral elements, iron is of great importance due to its utilization in fundamental processes. The role of iron in plant metabolism is centered around its stable forms Fe^{II} and Fe^{III} (Staiger 2002). Iron-carrying compounds are constantly being oxidized from Fe^{II} to Fe^{III} during the electron transfer and vice-versa. Iron complexes such as Fe-S proteins are key to electron transfer in the respiratory complexes of mitochondria and in the photosynthesis apparatus in the chloroplasts (Balk and Lobreaux 2005). Also, Fe-S clusters participate in nitrogen fixation, DNA repair, and metabolic pathways. Iron also plays an essential role as a component of different enzymes involved in electron transfer (redox reactions), such as cytochromes, both heme and non-heme groups, electron carriers, and ferredoxin, a substance known to be involved in the photosynthesis electron transfer (Barbosa Filho 1994; Briat 1995; Briat and Lobreaux 1997). Iron also acts as an essential enzyme cofactor involved in plant hormone synthesis (Bouzayen 1991; Siedow 1991). Around 75% of leaf iron is present in the chloroplasts as phytoferritin and ferredoxin, which are known to be involved in the photosynthesis electron transfer. In addition, two other important functions require iron: synthesis of ribosomic proteins known as Fe-proteins and the formation of Fe-chelate, which supplies iron to the chlorophyll precursor chain (Barbosa Filho et al. 1994). Although it is not part of the chlorophyll molecule, iron is essential for its synthesis. Under iron deficiency, chlorophyll content and the number of chloroplasts and their grana content decrease in the leaves (Brown et al. 1972; reviewed in Vahl 1991). This review presents some state-of-the-art views on plant iron metabolism and provides insights into the necessity of breeding rice plants tolerant to iron toxicity.

*** To whom correspondence should be addressed**
Antonio Costa de Oliveira
E-mail: acostol@terra.com.br
Tel: 0555332757263

Iron uptake

Iron occurs mainly in the divalent form Fe^{II}. Iron content in the soil is extremely variable, ranging from near zero up to 40% in the form Fe_2O_3. It can be found distributed evenly, in clumps, or nodules. When the distribution is even, iron oxides give the soil a typical red color (Vahl 1991). Iron solubility in aerated soils is controlled by $Fe(OH)_3$. Solubility in reduced soils is controlled by $FeCO_3$. In aerated soils, soluble iron represents just a small fraction of the total iron content. The inorganic forms present in solutions are Fe^{III}, $Fe(OH)^{2+}$, and Fe^{II}. Fe^{II} occurs in extremely low proportions, except at very low pH conditions (Bataglia 1991). Although iron is not a rare element in most soils (around 3-6% of total minerals in the soil), iron deficiency is a serious agricultural problem. Thirty percent of the world's arable land consists of calcareous, and therefore, alkaline soils. This problem is not easily fixed by the use of fertilizers that contain iron, since it is iron availability not its abundance that characterizes the problem (Guerinot 1994; Staiger 2002). Iron availability is dependent on its form and the surrounding environmental conditions. Iron occurs as an insoluble oxyhydroxide polymer, such as goethite (α-FeOOH) or hematite (α-Fe_2O_3). These polymers are generated by weathering. At neutral pH values, free iron ions reach a maximum of 10^{-17} M and ferrous ions that are more soluble are readily oxidized to ferric ions, which precipitate (Guerinot 1994).

In order to cope with the low solubility of ferric ions, plants require an active mechanism to release iron from Fe^{III} oxide hydrates to the soil solution and absorb it. Plants face a range of iron availability in the environment due to their immobility.

Both iron deficiency and toxicity are responsible for severe nutritional dysfunctions greatly affecting their physiology (Chaney et al. 1972; Ponnamperuma et al. 1955).

In general, two strategies have been described for the uptake of iron:

Strategy I

Plants using this strategy release protons into the surrounding rhizosphere via a proton-ATPase. Dicot plants improve iron absorption by three reactions: *i*) proton efflux via ATPase to acidify the medium and therefore increase Fe^{III} solubility; *ii*) reduction of Fe^{III} by a Fe^{III}-reductase to a more soluble form Fe^{II}; *iii*) transport of Fe^{II} by an iron transporter (Römheld and Marschner 1986).

Strategy II

Grasses, similarly to microorganisms, acquire iron without making use of a reductive mechanism. Grass roots release phytosiderophores (PSs) that chelate Fe^{III} at the rhizosphere, allowing specific protein transporters to import the Fe^{III}-PS complexes (Hell and Stephan 2003; Romheld and Marschner 1986). Studies on model organisms for iron uptake have shown that even though yeast does not synthesize or secrete siderophores, bacterial siderophores such as catecholate or hydroxamate can be recognized and taken up by yeast cells (Yun et al. 2000a; Yun et al. 2000b).

Iron transport and signaling

Iron uptake and transport have been described in the model eukaryote *Saccharomyces cerevisiae* (Curie and Briat 2003). Reductases located within the plasma membrane reduce Fe^{III} to Fe^{II}, a more soluble ion. A plasma membrane flavocytochrome (Fre1p) is responsible for Fe^{III} reduction at the cell surface. Paralogs of the *FRE* gene have been found (*FRE2* - *FRE7*) as a result of yeast genome sequencing (Johnston et al. 1997). *FRE2* encodes a protein related to Fre1p while *FRE3* and *FRE4* genes are involved in Fe^{III}-siderophore reduction (Dancis et al. 1990). A low-affinity uptake system is responsible for ferrous iron uptake when the cells are iron replete. Ferrous iron is acquired by a plasma membrane transport protein encoded by the *FET4* gene (Dix et al. 1994; Dix et al. 1997). The genes *FET3* and *FTR1* have been shown to play an important role in high-affinity ferrous uptake, which is induced under iron-deficiency conditions (Askwith et al. 1994; Stearman et al. 1996). *FET3* encodes a transmembrane protein from a family of multicopper oxidases. *FET3* has an oxidase catalytic domain located on the cell surface. The other gene, *FTR1*, encodes a plasma membrane permease containing a REGLE motif. This motif has been identified in the ferritin iron storage protein and seems to be responsible for an iron selective pore. A proposed model for high-affinity iron uptake requires that Fe^{II} produced by the Fe^{III} reductases be oxidized outside the cell by the Fet3p multicopper oxidase into Fe^{III}, which then binds to an Fe^{III} binding site on Ftr1p. A conformational change is caused by this binding, enabling Fe^{III} to be transported to the cytoplasm (Eide 1998).

Other iron transport systems have been suggested, including the NRAMP (Natural Resistance-Associated Macrophage Protein) family of metal transporters NRAMP is conserved from bacteria to mammals. However, these proteins have also been shown to transport Ni, Zn, Cu, Co, and Cd, as well as Fe and Mn (Gunshin et al. 1997). Vascular plants employ inter-organ signaling to avoid imbalances in nutrient supply and to meet the nutritional demands of the entire plant (Schmidt 2003). ITP1, an iron-binding member of the LEA (late embryogenesis abundant) protein family, has been suggested as the signal for systemic regulation of root responses to iron (Krueger et al. 2002). Several transcription factors are induced by iron deficiency, including 14-3-3 and zinc-finger proteins in barley (Negishi et al. 2002). Further, a protein containing a helix-loop-helix domain, FER, was cloned from a tomato mutant (*fer*) unable to switch on the responses to iron deficiency and only able to survive with a heavy supply of iron chelates (Ling et al. 2002). It has been suggested that nitric oxide (NO) is responsible for the translation of the Fe-deficiency signal, a ubiquitous signal in mammals and plants (Wendehenne et al. 2001).

Iron storage

Once iron is transported to the interior of the cell, it is necessary that it be stored in order to avoid possible damage to cellular structures due to the formation of reactive oxygen species. Iron storage takes place in the apoplastic space between the plasmatic membrane and the cell wall of plant cells, in mitochondria (Zancani et al. 2004), plastids (Seckback 1982), and in the vacuole, where the low pH and high concentrations of organic acids provide excellent conditions for iron deposits (Briat and Lobréaux 1997).

The vacuole sequesters iron and a variety of other metals, either as a mechanism of detoxifying the cell or as metal reservoir to enable the cell to grow when challenged by a low iron environment. Exactly how the vacuole contributes to iron metabolism is not clear. Mutations that affect vacuolar function also disturb the assembly of the plasma membranes' high affinity transport system composed of a copper-containing iron oxidase (Urbanowski and Piper 1999).

In both mitochondria and plastids, the specialized iron-storage protein ferritin is used to store iron. Ferritins are a class of widely-distributed iron storage proteins. They consist of hollow spheres composed of 24 subunits which are capable of storing up to 4,500 atoms of iron per molecule in a soluble and bio-available form (Balla et al. 1992; Connoly and Guerinot 2002; Harrison and Arosio 1996). The iron stored inside the ferritin accounts for 92% of the total iron found in mature pea embryos (Marentes and Grusak 1998), indicating an important role in plant development. Ferritin synthesis is highly controlled by iron status inside the cell. When in excess, iron is stored in a nontoxic form in order to avoid reacting with oxygen (Briat et al. 1995; Briat et al. 1999). A substantial amount of iron is stored in pea seeds, and the increase in iron absorption by the root takes place at the early stage of seed development (Lobréaux and Briat 1991). Experiments in soybean and maize have shown that iron controls the transcription and translation of plant ferritins (Fobis-Loisy et al. 1996; Wei and Theil 2000).

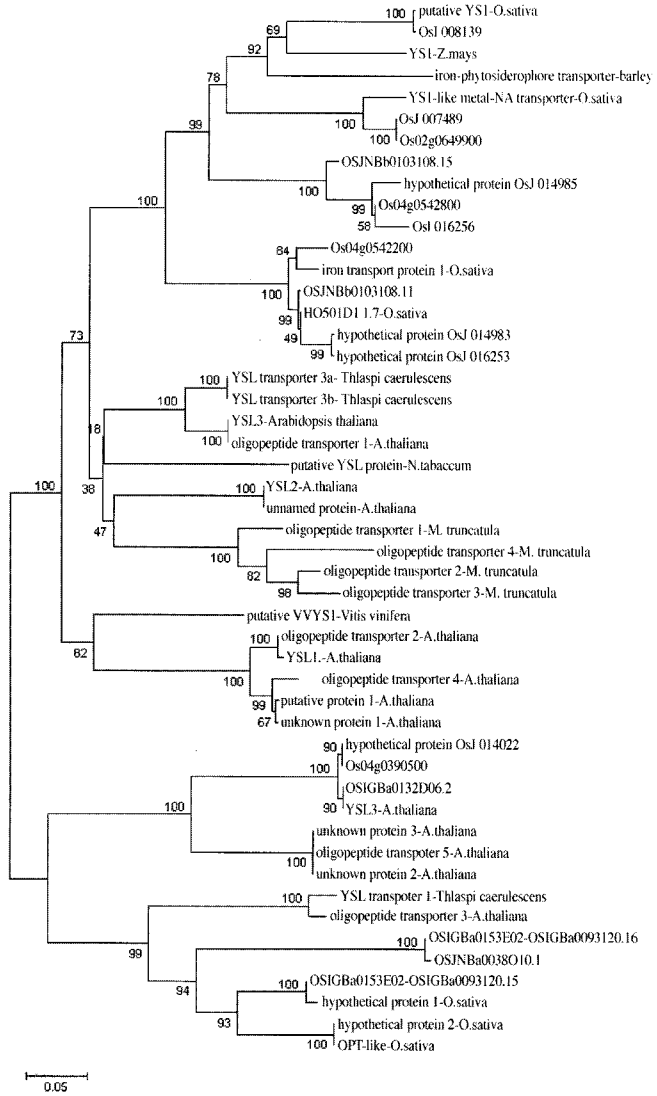

Fig. 1. Comparison of sequences homologous to maize *YS1* gene based on 12 putative membrane-spanning domains predicted by Curie et al. (2001).

Genes involved in plant iron metabolism

Several genes involved in iron transport have been found in Arabidopsis. Two root iron transporters, *IRT1*, responsible for iron uptake from the soil and *IRT2*, have been characterized as members of the ZIP family (Connolly et al. 2002; Eide et al. 1996; Guerinot 2000; Vert et al. 2002). A root iron-chelate reductase, *FRO2* (homologous to *FRE1*, *FRP1*, and *gp91^{phox}*), complements the Arabidopsis *frd1* mutant, deficient in root ferric-reductase activity (Robinson et al. 1999). *NRAMP1*, *3* and *4*, members of the NRAMP family, are divalent metal transporters which tend to show increased mRNA accumulation in Fe deficiency (Curie et al. 2000; Thomine et al. 2000). Another gene, *ITP*, a member of the LEA family, has been suggested by sequence similarity to act as a polypeptide chelating Fe^{III} in the phloem (Krueger et al. 2002). An *NFR* homolog with iron reductase activity in the tonoplast and in the phloem has also been described. This gene belongs to the *cytb5* reductase family (Bagnaresi et al. 2000; Xoconoslte-Cazares et al. 2000). Another group of genes, *IREG1-3*, are iron-efflux transporters belonging to the IREG/Ferroportin family. These genes show sequence similarity to mammalian iron efflux transporters (McKie et al. 2000). *YS1*, an Fe^{III}-phytosiderophore transporter, was cloned in maize from the *ys1* (yellow striped) mutant (Curie et al. 2001). It was reported as a membrane protein that mediates iron uptake. Arabidopsis has eight homologs, *YSL1-8*. Recent reports in Arabidopsis indicate that four genes encode ferritin (*AtFer1-AtFer4*) and that AtFer1 and AtFer3 play important roles in the protection of plant cells from oxidative stress (Petit et al. 2001). Also, there is evidence for the presence of an ABA-dependent pathway for ferritin accumulation in maize. This was confirmed by the expression of the *AtFer2* gene in mature siliquas and dry seeds, and an induction in response to ABA treatment (Briat and Lobreaux 1997). Grasses that utilize strategy II release a low molecular weight chelating compound such as mugineic acid (MA). The phytosiderophore -Fe^{III} complexes are then transported into the plant (Grotz and Guerinot 2002). In this process, two genes are required for the conversion of S-adenosyl methionine to Nicotianamine (nicotianamine synthase - NAS) and Nicotianamine (NA) to deoxymugineic acid (Nicotianamine aminotransferase - NAAT).

In order to investigate possible evolution mechanisms for these genes, a comparison of the conserved regions of the proteins encoded by *YS1*, *IRT1*, and *FRO1* was performed by our group. A total of 48 protein homologs for *YS1* were found in the NCBI database. A phylogenetic analysis was performed using a nearest neighbor joining tree approach (Figure1). Four clusters were identified: one consisted of grass protein coding genes, two consisted of dicot genes, and one cluster consisted of genes from grasses and dicot plants. One possible explanation is that this cluster holds the ortholog genes and the other three clusters show paralogs from grasses and dicots. This would suggest that the dicots shown here have at least three copies of this gene, while rice, representing the grasses, has only two. The genes *IRT1* and *IRT2* have at least 48 homologs deposited in the NCBI database (Figure 2). Three major clusters can be identified. The larger cluster contains only dicot plants. The second cluster consists of five rice homologs and one dicot homolog from *Medicago truncatula*. The third cluster again has protein-coding genes from dicots and monocots. One of the rice iron transporters (Fe^{III}) seems closer to *IRT1* and *IRT2* than the other iron transporter (Fe^{II}). This is consistent with rice's need to transport Fe^{II} ions that are more abundant under flooding or waterlogged soils. This situation is not faced by other plants such as Arabidopsis. The sequence similarity analysis for Arabidopsis *FRO1* revealed that the 30 homologs cluster into three major groups. The first cluster is formed by dicots and monocots, the smallest cluster contains two Arabidopsis homologs, and the third cluster contains both dicots and monocots. Comparing rice sequences, one can predict that rice has two ferric-chelate reductases, one closer to Arabidopsis *FRO1* (ferric reductase 2) and one closer to an oxidoreductase (ferric reductase 1). Possible overlapping functions for these genes remains to be investigated.

The analyses performed on these major genes involved in iron metabolism (*YS1*, *IRT1*, and *FRO1*) suggests that part of the iron metabolism is highly conserved among taxa and that rice

has developed some special strategies to deal with an anaerobic environment. However, further investigations are needed to reveal which proteins/protein domains would be more adequate for transgenic approaches aimed at improving iron tolerance, uptake, and metabolism.

Iron toxicity

Iron excess is found mainly in waterlogged or flooded soils where anaerobic conditions occur, such as irrigated rice fields. Under these conditions, Fe^{III} ions are readily reduced to more soluble Fe^{II} ions. Nutritional disorders associated with iron toxicity have been divided into direct and indirect toxicity (Vahl 1991). Direct toxicity is related to the plant's excessive iron absorption. This is damaging to plant cells. Symptoms appear initially on younger leaves, where the element concentrates in small brown dots. This phenomenon is known as bronzing (Bienfait 1985; Mengel and Kirkby 1982). Under extreme or prolonged deficiency, the leaves may become chlorotic. In advanced toxicity stages necrosis may occurr, i.e. the leaves dry and eventually die. Although the degree of bronzing has been suggested as a good way to measure the degree of toxicity (IRRI 1965; Ota 1968), the mechanism underlying the bronzing phenotype and tolerance is not well understood (Briat and Lobreaux 1997; Ota 1968; Peng and Yamauchi 1993). The leaves become chlorotic because iron is necessary to synthesize some of the chlorophyll-protein complexes in the chloroplast. Iron's low mobility is likely due to its precipitation in older leaves as insoluble oxides or phosphates, or to the formation of complexes with phytoferritin, an iron binding protein (Oh et al. 1996). Iron precipitation decreases the metal's subsequent mobilization inside the phloem. This type of toxicity is less common in Brazilian conditions, but is frequently seen in other climes, where some soils develop extremely high levels of Fe^{2+} when flooded. Indirect toxicity results from the limited absorption of several nutrients such as calcium, magnesium, potassium, phosphorous, and iron itself, due to iron precipitation on rice root epidermis. The formation of an oxide-hydroxide Fe^{3+} layer on the root blocks nutrient absorption, resulting in multiple nutritional deficiencies. Symptoms of this deficiency include plant atrophy, tillering reduction, orange leaves, and the covering of roots by red layers of iron oxides. Toxicity symptoms are usually correlated with iron deposition in the roots (Barbosa Filho et al. 1994; Vahl 1991) but leaf peroxidase activity has been shown to increase (Fang et al. 2000; Peng et al. 1996).

At the cellular level, it is not only insolubility, but iron's high reactivity that can cause severe damage. Reactions involving iron in high concentrations in the interior of the cell may be highly damaging to the plant. These reactions can produce reactive species of oxygen, specifically the hydroxyl radical (OH°), through the Fenton Reaction. The same physical properties that allow iron to act as an efficient cofactor and to catalyze controlled redox reactions also allow it to act as a powerful toxin when not protected from susceptible biomolecules. Numerous intracellular reactions use molecular oxygen as an electron acceptor producing superoxides (O_2^-) or hydrogen peroxide (H_2O_2). These species are not harmful *per se*, but they contribute to the generation of reactive oxygen species, hydroxyl radical (OH°) in this case. Its formation is catalyzed by iron through the Fenton Reaction (Hell and Stephan 2003):

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2^{\cdot-} \rightarrow Fe^{3+} + OH^- + OH^\circ$$

Or:

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^- + OH^\circ$$

Once iron enters the radicular symplast via the membrane transport system, it must be protected once more from oxygen. Protection is necessary in order to avoid precipitation and the generation of reactive oxygen species. While several organic acids and cellular amino acids are able to chelate iron, nicotianamine (NA) seems to be preferred for several reasons: *i*) It forms stable complexes with both oxidation states of iron at neutral and weakly alkaline pH (Stephan et al. 1996). Although the Fe^{3+}-NA complex has a much higher formation constant, the Fe^{2+}-NA complex is kinetically more stable under aerobic conditions (von

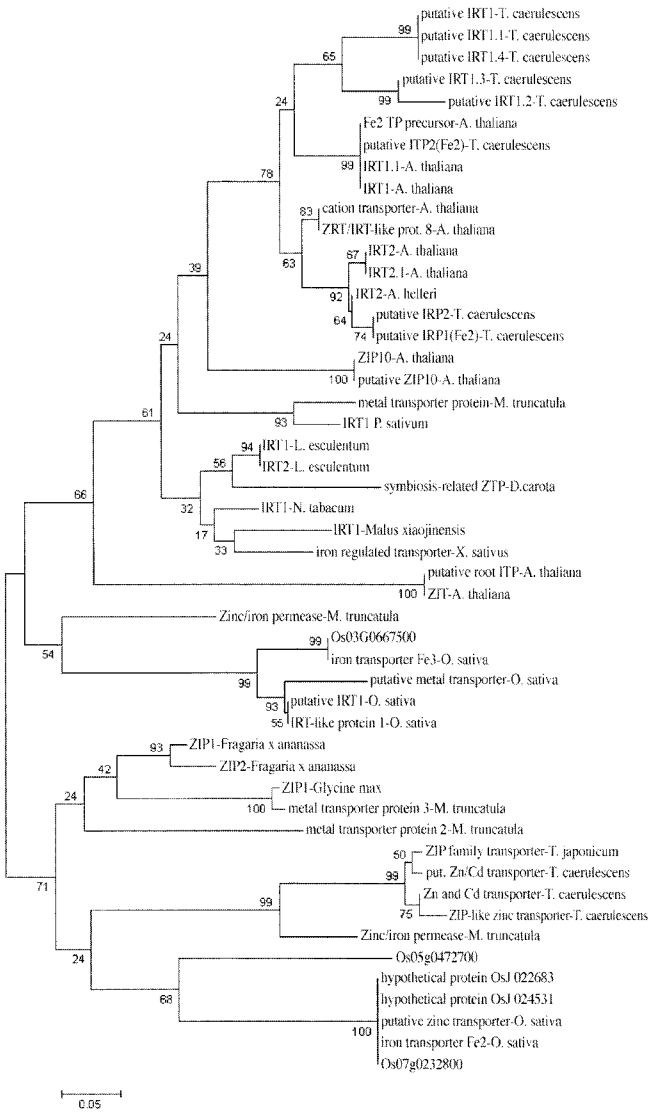

Fig. 2. Comparison of sequences homologous to Arabidopsis *IRT1*, based on conserved protein domains.

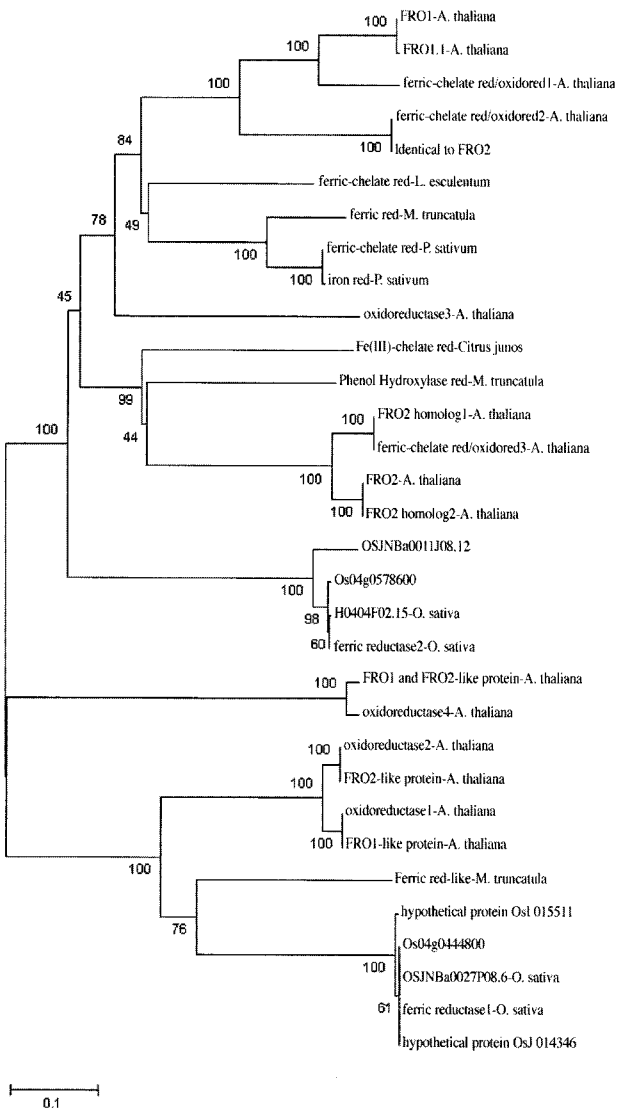

Fig. 3. Comparison of sequences homologous to Arabidopsis *FRO1*, based on conserved protein domains.

Wiren et al. 1999); *ii*) NA appears to be ubiquitous in higher plants and is present in all tissues (Scholz et al. 1992); *iii*) NA-iron complexes are relatively poor Fenton reagents (von Wiren et al. 1999); *iv*) The NA-deficient tomato mutant *chloronerva* shows elevated activities of antioxidant enzymes (Herbik et al. 1996) and precipitation of iron in vacuoles and mitochondria (Liu et al. 1998); *v*). NA concentrations are increased in the root tips in both sunflower and barley in the regions of main iron uptake and radial transport (Stephan and Scholz 1990). NA concentrations correlate with localization and levels of iron in pea and tomato plants (Pich et al. 2001).

Information on nutritional disorders in rice caused by micronutrients in Brazilian soils indicates that, after zinc, iron is the element that most frequently limits rice production, either when cultivated under dry or flooded conditions. In dry situations, the problem is related to the iron's deficiency. Under flooded conditions problems arise due to toxicity. The toxicity observed in flooded plants is due to the accumulation of Fe^{II} in flooded soils. The increase in Fe^{II} concentrations is a consequence of Fe^{III} reduction found in aerated soils under the form of low solubility iron oxides. This reduction, observed when the flooded soil redox potential reaches 100-300mV, may increase soluble iron content up to 600 ppm, versus medium rates of 0.1 ppm in aerated soils (Brennan and Lindsay 1998).

In Brazilian soils commonly cultivated with flooded rice, soluble iron content after flooding does not reach such high levels as registered in other traditional, rice-growing countries. Generally, the iron content in Brazilian soils does not exceed 100 ppm. However, these levels are sufficient to cause iron toxicity in rice (Barbosa Filho et al. 1994). The iron content in which toxicity occurs in the soil and plant ranges is between 10 and 1000 ppm and 50 and 1700 ppm, respectively. Such broad limits illustrate that toxicity development is a complex phenomenon. It does not appear that there is a specific factor in either the soil or the plant that allows a prediction of toxicity (Barbosa Filho et al. 1994).

The predominant and therefore the most important form of toxicity in Brazil is indirect. Toxicity due to the ferric form (Fe^{II}) can cause considerable losses in rice production. This is especially the case in the acid soils of tropical and subtropical areas (Fageria and Rabelo 1987; Wu et al. 1998), as found in southern Brazil. These regions are characterized by their richness in iron and low pH (Silva et al. 2003). Occurrence in rice fields may cause reductions in productivity from 10 to 80% (reviewed in Vahl 1991). Iron toxicity was detected in Brazil during the 1970s. The introduction of modern type rice cultivars, some of which showed sensitivity to the excess of iron in the soil, revealed the problem. The problem was also seen in the states of Santa Catarina, Minas Gerais, Rio de Janeiro, Espírito Santo, Goiás, and Rio Grande do Sul (Reviewed in Vahl 1991; Vieira et al. 1999).

Improving iron tolerance in rice

Although rice is described as a strategy II plant, today we know that it also absorbs iron through strategy I. While it is able to absorb iron via the connection of iron ions to phytosiderophores, it also absorbs iron through the acidification of the medium caused by proton efflux. This leads to the acidification of the medium and increase of the more soluble ferric form, in the soil (Ishimaru et al. 2006). This evidence supports the observation that rice has an advantageous strategy for growth in submerged conditions. Differences in the ability to absorb nutrients, the degree of resistance to toxic elements, and efficiency in the use of absorbed nutrients occurs both within and between many cultivated species (Clark 1983; Furlani 1986).

One strategy that was used to investigate iron tolerance in rice is the development of mapping populations. A DH population consisting of 123 lines was developed from a cross between IR64 and Azucena (Guiderdoni et al. 1992). The parents, 123 DH lines and 100 DHBC1F1 (DH lines backcrossed to Azucena) were used to find markers associated to seedling tolerance for ferrous iron toxicity (Wu et al. 1997). Using 175 cDNA and genomic clones, four marker loci on chromosome 1 were identified to be significantly associated with both segregations of tolerance index value (degree of bronzing) and RDSDW (relative decrease

in shoot dry weight). One marker locus was significantly associated with RDSDW. QTLs explaining 32 and 15% of the tolerance index value and 15, 21, and 10% of the RDSDW were found (Wu et al. 1997). Another population consisting of 96 backcross inbred lines (BILs) derived from a cross Nipponbare/Kasalath/Nipponbare was developed (Wan et al. 2003). The 96 BIL lines in BC1F9 were evaluated for their ferrous iron tolerance toxicity. Using RFLP markers, four QTLs were detected on chromosomes 1 and 3 that were significantly associated with leaf bronze index, stem dry weight, tiller number, and root dry weight.

With the current knowledge of iron metabolism obtained from yeast and Arabidopsis studies, many transgenic approaches have been suggested for improving iron tolerance and biofortification in rice. Expression of the soybean ferritin gene from the rice glutelin gene promoter enhanced the Fe content of rice seed by up to 3-fold (Goto et al. 1999). Any project with this goal must focus on rate-limiting processes in transport, synthesis, and storage of substrate and products. Iron influence in network control of metabolic pathways and allocation must also be taken into account (Hell and Stephan 2003). In transgenic tobacco, constitutive expression of two Fe^{III}-chelate reductases from yeast resulted in a 4-fold increase in iron reductase activity and a 50% increase in leaf iron content (Samuelsen et al. 1998). Constitutive expression of NA synthase from Arabidopsis, resulted in a 2- to 4-fold increase in leaf iron content. Tobacco plants grew faster and performed more efficiently under iron deficient conditions (Douchkov et al. 2001). These observations suggest that improving iron uptake alone is not sufficient because of rate-limiting steps further in the pathway. Alternatively, increasing NA synthesis may be a viable option, although co-suppression has been observed in rice transformed with the barley *NAS* gene (Mori et al. 2001).

It is important to role that rice produces less phytosiderophores than wheat and barley. One could aim to increase its PS production. Indeed, expression of barley NA aminotransferase in rice improved tolerance, achieving higher vigor, and a 4-fold higher grain yield (Takahashi et al. 2001).

Another strategy to obtain improved genotypes for iron toxicity tolerance relies on induced mutation. A collection of rice mutant genotypes (Table 1) showing root morphology polymorphisms derived by gamma ray treatment of indica cultivar Taim was developed by our group (Zimmer et al. 2003). A subset of these mutants has been analyzed for response to iron toxicity (Table1). Seven variables were analyzed: number of roots (NR), main root length (MRL), coleoptile length (CL), shoot length (SL), first leaf insertion (FLI), first leaf length (FLL), and second leaf length (SLL).

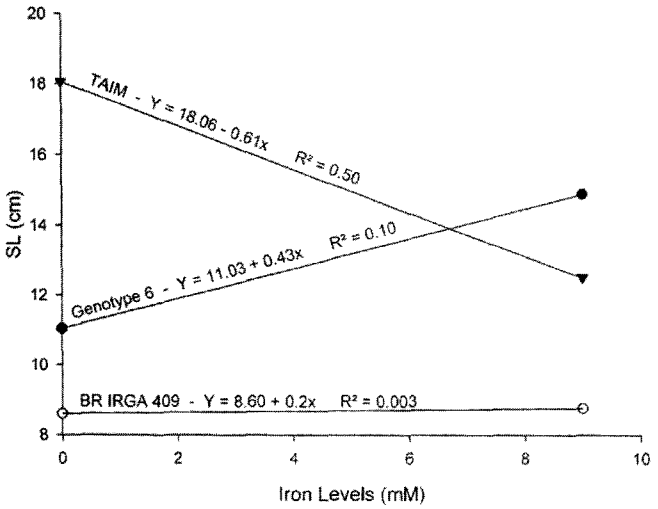

Fig. 4. Display of shoot length responses for mutant 6, parental cultivar Taim and cultivar IRGA 409.

Table 1. List of rice mutant genotypes used for the iron toxicity response analysis.

N	Genotype	N	Genotype	N	Genotype
1	CGF-Z-M05-435	14	CGF-Z-M05-328	27	CGF-Z-M05-65
2	CGF-Z-M05-437	15	CGF-Z-M05-62 ARS2	28	CGF-Z-M05-31 ARS3
3	CGF-Z-M05-78 ARS1	16	CGF-Z-M05-440	29	CGF-Z-M05-168
4	CGF-Z-M05-243	17	CGF-Z-M05-436	30	CGF-Z-M05-59
5	CGF-Z-M05-45	18	CGF-Z-M05-78 ARS	31	CGF-Z-M05-444 P1
6	CGF-Z-M05-188	19	CGF-Z-M05-280	32	CGF-Z-M05-204
7	CGF-Z-M05-42	20	CGF-Z-M05-205	33	CGF-Z-M05-41
8	CGF-Z-M05-44	21	CGF-Z-M05-260 P1	34	CGF-Z-M05-295
9	CGF-Z-M05-79	22	CGF-Z-M05-189	35	CGF-Z-M05-32
10	CGF-Z-M05-121 ARS	23	CGF-Z-M05-167	36	CGF-Z-M05-EPAGRI 108
11	CGF-Z-M05-258	24	CGF-Z-M05-417 ARP	37	CGF-Z-M05-IRGA 409
12	CGF-Z-M05-53	25	CGF-Z-M05-336	38	CGF-Z-M05-TAIM
13	CGF-Z-M05-22 P	26	CGF-Z-M05-192		

Mutant 6 showed one of the best relative performances, being constantly among the three higher values in six of seven evaluated variables (NR, CL, FLI, FLL, SLL, and APL). It also showed the highest values in four variables (FLI, FLL, SLL, and APL), showing great potential as an iron tolerant genotype. Mutants 4 and 7 were also promising, as both were in the top three values of relative performance in four of seven evaluated characters (FLI, FLL, SLL, and APL; CL, FLI, FLL, and APL, respectively). Mutant 26 was among the three highest values of relative performance in three of seven evaluated characters (NR, MRL, and CL). These mutants show promise for studying iron uptake and metabolism and are being further investigated. Figure 4 displays mutant 6 response in SL compared to the parental cultivar Taim under iron toxicity treatment.

Conclusions and perspectives

Improving iron accumulation and bioavailability is the ultimate goal for combating anemia worldwide. However, rice is the major staple food for over half of the world's population. Therefore, it is first necessary to understand how rice tolerates iron in the soil and then drives this excess metal to accumulation in the grain. Dealing with iron is not a simple task, and a better understanding of the mechanisms by which plants absorb, transport, and store iron indicates that a delicate balance exists in its metabolism. Increasing iron uptake sometimes leads to the over absorption of heavy metals such as cadmium. The scientific discoveries of the last decade are promising and will have a clear impact on the manipulation of this important mineral in rice.

Acknowledgement

The authors are thankful to CNPq and CAPES-PROCAD for grants and fellowships. Also the authors are very much indebted to J. DeBarry, Bennetzen's lab, for important comments and suggestions.

References

Askwith C, Eide D, Van HA, Bernard PS, Li L, et al. 1994. The *FET3* gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. Cell 76: 403-10

Bagnaresi P, Mazars-Marty D, Pupillo P, Marty F, Briat JF. 2000. Tonoplast subcellular localization of maize cytochrome b5 reductases. Plant J. 24: 645-54

Balk J, Lobreaux S. 2005. Biogenesis of iron-sulfur proteins in plants. Trends Plant Sci. 10: 324-31

Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM. 1992. Ferritin: a cytoprotective antioxidant stratagem of endothelium. J. Biol. Chem. 267: 18148-18153

Barbosa Filho MP, Dynia JF, Fageria NK. 1994. Zinc and iron in the rice crop (in portuguese). EMBRAPA-SPI , Brasilia. 71pp.

Bataglia OC. 1991. Iron. In: Ferreira ME, Cruz MCP da ,Eds. Micronutrients in agriculture (In portuguese). POTAFOS/ CNPq, Piracicaba. pp 159-172

Bienfait HF. 1985. Regulated redox process at the plasmalemma of plant root cells and their function on iron uptake. J. Bioenerg Biomembr 17: 73-83

Bouzayen M, Felix G, Latché A, Pech JC, Boller T. 1991. Iron: an essential cofactor for the conversion of 1-aminocy-clo propane-1-carboxylic acid to ethylene. Planta 184: 244-247

Brennan EW, Lindsay WL. 1998. Reduction and oxidation effect on the solubility and transformation of iron oxides. Soil Science Society of America Journal, 62: 930-937

Briat J-F, Curie C, Gaymard F. 2007. Iron utilization and metaolism in plants. Curr. Op. Plant Biol. 10: 1-7

Briat J-F, Fobis-Loisy I, Grignon N, Lobréaux S, Pascal N, et al. 1995. Cellular and molecular aspects of iron metabolism in plants. Biol. Cell 84: 69-81

Briat J- F, Lobréaux S. 1997. Iron transport and storage in plants. Trends Plant Sci. 2: 187-193

Briat J-F, Lobréaux S, Grignon N, Vansuyt G. 1999. Regulation of plant ferritin synthesis: how and why. Cell. Mol. Life Sci. 56: 155-166

Brown CJ, Ambler JE, Chaney RL, Foy CD. 1972. Differential responses of plant genotypes to micronutrients. In: Mortvedt JJ, Giordano PM, Lindsay WL, Eds. Micronutrients in agriculture. Soil Science Society of America, Madison. pp 389-418

Chaney RL, Brown JC, Tiffin LO. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiology 50: 208-213

Clark RB. 1983. Plant genotype differences in the uptake, trans location, accumulation and use of mineral elements required for plant growth. Plant and Soil 49-70

Connolly EL, Fett JP, Guerinot ML. 2002. Expression of the *IRT1* metal transporter is controlled by metals at the levels of transcript and protein accumulation. Plant Cell 14: 1347-57

Connolly EL, Guerinot ML. 2002. Iron stress in plants. Genome Biol. 3: 1024.1-1024.4

Curie C, Alonso JM, Le Jean M, Ecker JR, Briat JF. 2000. Involvement of NRAMP1 from Arabidopsis thaliana in iron transport. Biochem. J. 347: 749-55

Curie C, Briat JF. 2003. Iron transport and signaling in plants. Annu Rev Plant Biol. 54: 183-206

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL. 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409: 346-49

Dancis A, Klausner RD, Hinnebusch AG, Barriocanal JG. 1990. Genetic evidence that ferric reductase is required for iron uptake in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 10: 2294-301

Dix DR, Bridgham JT, Broderius MA, Byersdorfer CA, Eide DJ. 1994. The *FET4* gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. J. Biol. Chem. 269: 26092-99

Dix DR, Bridgham J, Broderius M, Eide DJ. 1997. Characterization of the *FET4* protein of yeast. Evidence for a direct role in the transport of iron. J. Biol. Chem. 272: 11770-77

Douchkov D, Hell R, Stephan UW, Baumlein H. 2001. Increased iron efficiency in transgenic plants due to ectopic expression of nicotianamine synthase. In WJ Horst, MK Schenk, A Burkert, N Claasen, H Flessa, WB Frommer, H Goldbach, H Olfs, V Romheld, B Sattelmacher, U Schmidhalter, S Schubert, N v Wiren, L Wittenmayer, eds. Plant Nutrition. Food security and sustainability of agro-ecosystems through basic and applied research. Kluwer, Dordrecht, pp 54-55

Eide DJ. 1998. The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. Annu. Rev. Nutr. 18: 441-69

Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel iron regulated metal transporter from plants identified by functional expression in yeast. Proc. Natl. Acad. Sci. USA 93: 5624-5628

Fageria NK, Rabelo NA. 1987. Tolerance of rice cultivars to iron toxicity. J. Plant Nutr. 10: 653-661

Fang W, Kao CH. 2000. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. Plant Sci. 158: 71-76

Fobis-Loisy I, Aussel L, Briat J-F. 1996. Post-transcriptinal regulation of plant ferritin accumulation in response to iron as observed in the maize mutant *ysI*. FEBS Letters 397: 149-154

Furlani AMC, Bataglia OC, Azzini LE. 1986. Variabilidade entre linhagens de arroz na absorção e utilização de potássioem solução nutritiva. Rev. Brasil. de Ciência do Solo 10: 135-41.

Goto F, Yoshihihara T, Shigemoto N, Toki S, Takaiwa F. 1999. Iron fortification of rice seed by the soybean ferritin gene. Nat. Biotechnol. 17: 282-286

Guerinot ML. 2000. The ZIP family of metal transporters. Biochim. Biophys. Acta 1465: 190-98

Guerinot ML, Yi Y. 1994. Iron: nutritious, noxious and not readily available. Plant Physiol. 104: 815-820

Guiderdoni E, Galinato E, Luistro J, Vergara G. 1992. Anther culture of tropical japonica x indica hybrids of rice (*Oryza sativa* L.). Euphytica 62: 219-224

Grotz N, Guerinot ML. 2002. Limiting nutrients: an old problem with new solutions? Curr Opin Plant Biol. 5: 158-63

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, et al. 1997. Cloning and characterization of a mammalian protoncoupled metal-ion transporter. Nature 388: 482-88

Harrison PM, Arosio P. 1996. The ferritins: molecular properties, iron storage function and cellular regulation. Biochem. Biophys. Acta 1275: 161-203

Hell R, Stephan UD. 2003. Iron uptake, trafficking and homeostasis in plants. Planta 216: 541-551

Herbik A, Giritch A, Horstmann C, Becker R, Balzer HJ, Baumlein H, Stephan UW. 1996. Iron and copper nutrition-dependent changes in protein expression in a tomato wild type and the nicotianamine-free mutant *chloronerva*. Plant Physiol. 111: 533-40

International Rice Research Institute Annual Report 1964, 1965. IRRI, Los Banos, Philippines, pp. 335

Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M et al. 2006. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. The Plant Journal 45: 335-346, 2006.

Johnston M, Hillier L, Riles L, Albermann K, Andre B et al. 1997. The nucleotide sequence of *Saccharomyces cerevisiae* chromosome XII. Nature 387: 87-90

Krueger C, Berkowitz O, Stephan UW, Hell R. 2002. A metalbinding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. J. Biol. Chem. 277: 25062-69

Ling H-Q, Bauer P, Keller B, Ganal M. 2002. The tomato *fer* gene encoding a bHLH protein controls iron uptake responses in roots. Proc. Natl. Acad. Sci. USA 99: 13938-43

Liu DH, Adler K, Stephan UW. 1998. Iron-containing particles accumulate in organelles and vacuoles of leaf and root cells in the nicotianamine-free tomato mutant *chloronerva*. Protoplasma 201: 213-220

Lobréaux S, Briat JF. 1991. Ferritin accumulation and degradation in different organs of pea (*Pisum sativum*) during development. Biochem. J. 274: 601-606

Marentes E, Grusak MA. 1998. Iron transport and storage within the seed coat and embryo of developing seeds of pea (*Pisum sativum* L.). Soil Science Res. 8: 367-375

McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, et al. 2000. A novel duodenal iron-regulated transporter, *IREG1*, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5: 299- 309

Mengel K, Kirkby EA. 1982. Principles of plant nutrition. 3rd ed. International Potash Institute. Switzerland, 655pp.

Mori S, Nakanishi H, Takahashi M, Higuchi K, Nishizawa N-K. 2001. Genetic engineering of transgenic rice with barley strategy-II genes. In WJ Horst, MK Schenk, A Burkert, N Claasen, H Flessa, WB Frommer, H Goldbach, H Olfs, V Romheld, B Sattelmacher, U Schmidhalter, S Schubert, N v Wiren, L Wittenmayer, eds. Plant Nutrition. Food security and sustain-ability of agro-ecosystems through basic and applied research. Kluwer, Dordrecht, pp 54-55

Negishi T, Nakanishi H, Yazaki J, Kishimoto N, Fujii F, et al. 2002. cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. Plant J. 30: 83-94

Oh S-H, Cho S-W, Kwon T-H, Yang M-S. 1996. Purification and characterization of phytoferritin. J. Biochem. Mol. Biol. 29: 540-544

Ota Y. 1968. Studies on the occurrence of the physiological disease called 'bronzing'. Bull. Natl. Inst. Agric. Sci. 18: 31-104

Peng XX, Yamauchi M. 1993. Ethylen production in rice bronzing leaves induced by ferrous iron. Plant and Soil 149: 227-234

Peng XX, Yu XL, Li MQ, Yamauchi M. 1996. Induction of peroxidase by Fe^{+2} in detached rice leaves. Plant and Soil 180: 159-163

Petit JM, van Wuytswinkel O, Briat J-F, Lobréaux S. 2001. Characterization of an iron-dependent regulatory sequence involved in the transcriptional control of *AtFer1* and *ZmFer1* plant ferritin genes by iron. J. Biol. Chem. 276: 5584-90

Pich A, Manteuffel R, Hillmer S, Scholz G, Schmidt W. 2001. Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration? Planta 213: 967-76

Ponnamperuma FN, Bradfield R, Peech M. 1955. Physiological disease of rice attributable to iron toxicity. Nature 175: 275

Robinson NJ, Procter CM, Connolly EL, Guerinot ML. 1999. A ferric-chelate reductase for iron uptake from soils. Nature 397: 694-97

Römheld V, Marschner H. 1986. Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol 80: 175-180

Samuelsen AI, Martin RC, Mok DWS, Mok MC. 1998. Expression of the yeast *FRE* genes in transgenic tobacco. Plant Physiol 118: 51-58

Schmidt W. 2003. Iron solutions: acquisition strategies and signaling pathways in plants. Trends Plant Sci. 8: 188-93

Scholz G, Becker R, Pich A, Stephan UW. 1992. Nicotianamine - a common constituent of strategies I and II of iron acquisition in plants. J Plant Nutr 15: 1649-1665

Seckback JJ. 1982. Ferreting out the secret of plant ferritin - a review. J. Plant Nutr. 5: 369-394

Siedow, JN. 1991. Plant lipoxygenase: structure and function. Annu Rev Plant Physiol Plant Mol Biol 42: 145-188

Silva MM, Vale MG, Damin ACF, Welz B, Mandaji M, Fett JP. 2003. Method development for the determination of iron milligram amounts of rice plants (*Oryza sativa* L.) from cultivation experiments using graphite furnace atomic absorption spectrometry. Anal. Bioanal. Chem. 377: 165-172

Staiger D. 2002. Chemical strategies for iron acquisition in plants. Angew Chem Int Ed Engl. 41: 2259-64

Stearman R, Yuan DS, Yamaguchi-Iwai Y, Klausner RD,

Dancis A. 1996. A permease-oxidase complex involved in high-affinity iron uptake in yeast. Science 271: 1552-57

Stephan UW, Schmidke I, Stephan VW, Scholz G. 1996. The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. Biometals 9: 84-90, 1996.

Stephan UW, Scholz G. 1990. Nicotianamine concentrations in iron sufficient and iron deficient sunflower and barley roots. J Plant Physiol 136: 631-634

Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. 2001. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat. Biotechnol.* 19: 466-69

Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI. 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: 4991-96

Urbanowski JL, Piper RC. 1999. The Iron Transporter Fth1p Forms a Complex with the Fet5 Iron Oxidase and Resides on the Vacuolar Membrane. The Journal Of Biological Chemistry 274: 38061-38070

Vahl LC. 1991. Iron toxicity in rice genotypes irrigated by flooding (In Portuguese with english abstract). Ph.D. Thesis. Federal University of Rio grande do Sul, Porto Alegre.

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML et al. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14: 1223-33

Vieira NRA, Santos AB, Sant'ana EP. 1999. The rice crop in Brazil (In portuguese). Embrapa Rice and Beans, Santo Antônio de Goiás, 633pp.

Von Wiren N, Klair S, Bansal S, Briat J-F, Khodr H, Shioiri T, Leigh RA, Hider RC. 1999. Nicotianamine chelates both Fe^{III} and Fe^{II}. Implications for metal transport in plants. Plant Physiol 119: 1107-1114

Wan J-L, Zhai H-Q, Wan J-M, Ikehashi H. 2003. Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, *Oryza sativa* L. Euphytica 131: 201-206

Wei J, Theil EC. 2000. Identification and characterization of the iron regulatory element in the ferritin gene of a plant (soybean). J Biol Chem. 275: 17488-93

Wendehenne D, Pugin A, Klessig DF, Durner J. 2001. Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends Plant Sci. 6: 177-83

Wu P, Hu B, Liao C, Zhu J, Wu Y, Senadhira D, Paterson, AH. 1998. Characterization of tissue tolerance to iron by molecular markers in different lines of rice. Plant and Soil 203: 217-226, 1998.

Wu P, Luo A, Zhu J, Yang J, Huang N, Senadhira D. 1997. Molecular markers linked to genes underlying seedling tolerance for ferrous iron toxicity. Plant and Soil 196: 317-320

Xoconostle-Cazares B, Ruiz-Medrano R, Lucas WJ. 2000. Proteolytic processing of CmPP36, a protein from the cytochrome b5 reductase family, is required for entry into the phloem translocation pathway. Plant J. 24: 735-47

Yun CW, Ferea T, Rashford J, Ardon O, Brown PO, et al. 2000. Desferrioxamine-mediated iron uptake in *Saccharomyces cerevisiae*. Evidence for two pathways of iron uptake. J. Biol. Chem. 275: 10709-15

Yun CW, Tiedeman JS, Moore RE, Philpott CC. 2000b. Siderophore-iron uptake in *Saccharomyces cerevisiae*. Identification of ferrichrome and fusarinine transporters. J. Biol. Chem. 275: 16354-59

Zancani M, Peresson C, Biroccio A, Federici G, Urbani A, Murgia I, Soave C, Micali F, Vianello A, Macrý F. 2004. Evidence for the presence of ferritin in plant mitochondria. Eur. J. Biochem. 271: 3657-3664

Zimmer PD, Mattos LAT, Oliveira AC, Carvalho FIF, Magalhães Jr A, Köpp MM, Freitas FA. 2003. Identification of rice mutants (*Oryza sativa* L.) for agronomical and root system traits. Rev. Brasil. de Agrociência 9 : 195-199