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Strategies for Iron Mobilization and Uptake in Plant Roots

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Summary

Despite of its abundance in soil, iron (Fe) is still one of the most common nutrients limiting plant growth and development because it exists mostly in low-soluble form that is hardly available for plants. Therefore, plants have evolved two distinct uptake strategies, the reduction (Strategy I) mechanism and the chelation (Strategy II) mechanism. This paper reviews some recent studies in regard to both strategies that are important for iron acquisition, mobilization and uptake in plant roots. These key processes are believed to be relevant for the supply of Fe to plants as well as for supplying human nutritional needs.

Key words : Alkaline soil, Iron nutrition, Mugineic acid, Strategy I, Strategy II

I. Introduction

Iron nutrition in plants

Fe is essential microelement for plant metabolism, growth and crop productivity. Its importance is founded upon its ability to form two stable ions, Fe^{2+} and Fe^{3+}. Accordingly, iron ions are involved in most redox processes in the electron transport chains of photosynthesis and respiration, which serve to transform energy from electron transport in ATP, the energy source of cells. Fe is also involved in the synthesis of chlorophyll and is essential for the maintenance of chloroplast structure and function (Abadia, 1992). Furthermore, it is important, indeed, critical for symbiotic nitrogen fixation in root nodules of legumes due to its role in the activity of both leghemoglobin and nitrogenase (Dakora 1995 ; Kaiser et al., 2003). Fe is contained in the subunits of the nitrogenase enzyme that reduces N_2 to NH_3, as well as in leghemoglobin that binds molecular oxygen

in root nodules (Staiger, 2002).

Fe is also one of the three nutrients (nitrogen and phosphorus are the other) that commonly limit plant growth (Marschner, 1995) in alkaline soils. Though iron is said to be the second most abundant metal in nature and the fourth most abundant element in the Earth's crust, plants still can suffer from iron deficiency chlorosis that results in growth and yield losses and the decreased in nutritional quality of eatable plant parts especially when they are grown in calcareous soils.

Iron availability in soils

Fe is generally present at high quantities in soils where it exists as Fe^{2+} and Fe^{3+}. Fe^{2+} is relatively soluble but is readily oxidized by atmospheric oxygen to ferric ions which again precipitate (Guerinot and Yi, 1994). Solubility of Fe^{3+} on the other hand decreases dramatically with increasing pH values due to hydrolysation, polymerization and finally precipitation with inorganic anions. In aerobic soils with neutral pH, the concentration of soluble Fe^{3+} ranges from 10^{-11} to 10^{-10} M (Lindsay and Schwab, 1982). These concentrations are not sufficient to meet optimal plant growth that requires between 10^{-4} and 10^{-8} M Fe^{3+}. The concentrations of Fe^{3+} in neutral soils are already lower than the optimal concentration for plants (Hell et al., 2003). As a result, iron deficiency in plants occurs and is a major problem in the agriculture, since 30% of the world's arable lands consist of calcareous and alkaline soils (Chen and Barak, 1982; Hell et al., 2003). This phenomenon cannot be easily overcome through the use of iron-containing fertilizers because iron availability problem is not one of abundance but of solubility (Guerinot, 2001).

The iron deficiency problem in plants has long been of interest for plant scientists worldwide for the past decades. Much research particularly on biochemical, molecular and genetic aspects have been conducted to address the perennial problem of iron bioavailability to plants. This paper reviews some of the recent studies on plant physiological mechanisms specifically those on the mobilization and uptake in plant roots under iron shortage condition. These mechanisms are believed to be relevant and indeed are pre-requisite processes in the supply of Fe to plants, especially the edible parts.

II. Overview on Physiological Mechanisms for Iron Acquisition in Plants

Plants have evolved physiological mechanisms/responses to efficiently acquire the scant soluble iron available under different soil conditions and can be categorized as Strategies I and II (Fig. 1) (Romheld and Marschner, 1986; Marschner, et al., 1995).

All plants, except the graminaceous species, use strategy I, also known as the reduction mechanism in response to iron deficiency stress that includes the follow-

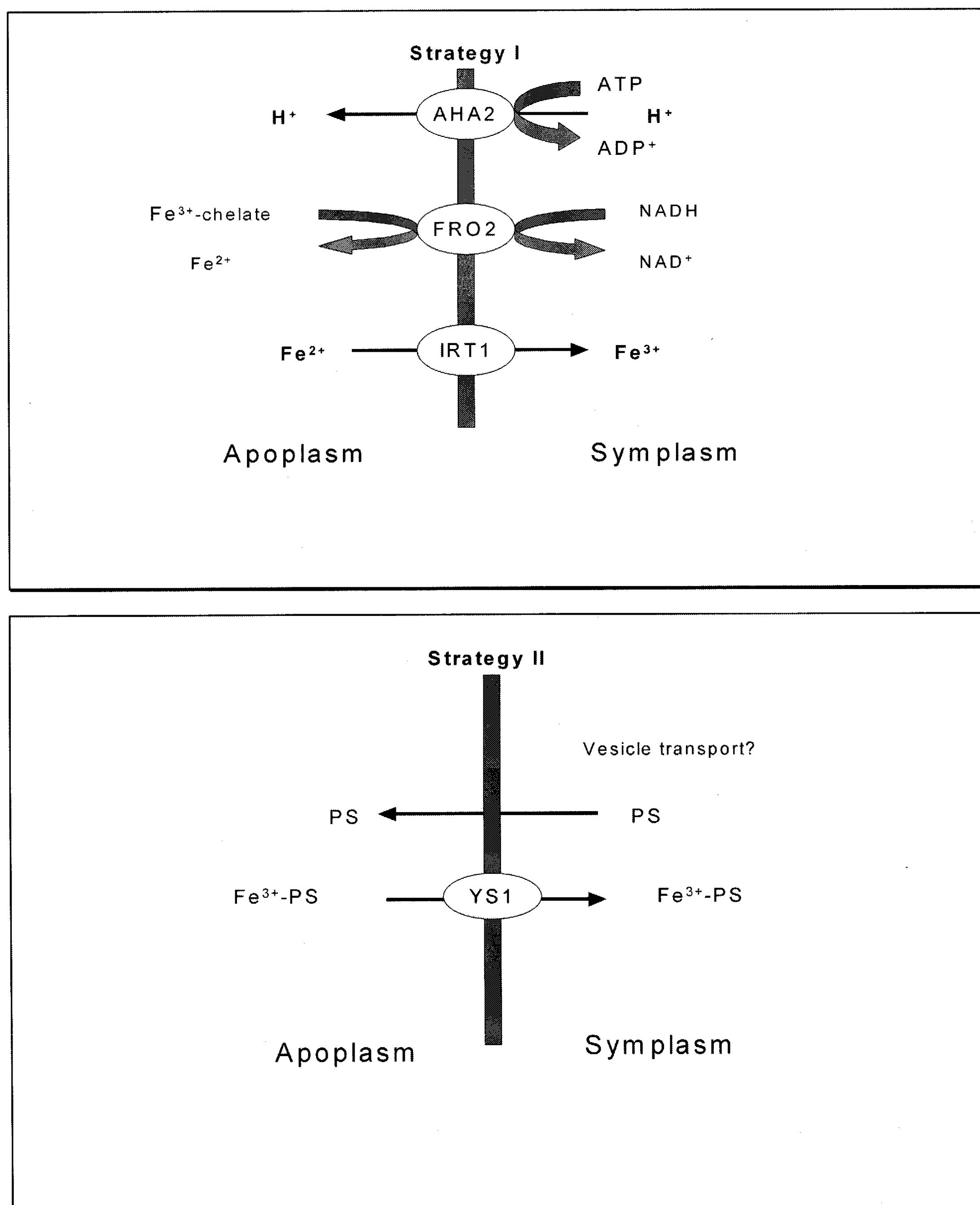

FIG 1. Mechanisms of iron acquisition by higher plants. In strategy I plants (e.g. *Arabidopsis*, pea and tomato), Fe^{3+} chelates are reduced before the Fe^{2+} ion is transported across the plasma membrane. Strategy II plants (e.g. barley, maize and rice) release phytosiderophores capable of solubilizing external Fe^{3+} and then transport the Fe^{3+}-phytosiderophore complex into the cell. AHA2 is a P-type H^+-ATPase, FRO2 is the Fe^{3+} chelate reductase, IRT1 is a Fe^{2+} transporter and YS1 is the transporter of the phytosiderophore (PS)-Fe complex (Schmidt, 2003).

ing: (1) release of protons/H^+ via a plasma membrane P-type ATPase from the roots into the soil to acidify the surrounding solution and thus increase Fe^{3+} solubility, (2) induce the expression of a Fe^{3+} chelate reductase to reduce iron to the more soluble Fe^{2+} form, (3) induce the Fe^{2+} transport system to take in the reduced iron at the root plasma membrane, and (4) accumulation of organic acids (mainly citrate) in root tissues to accelerate translocation of solubilized Fe to shoots (Brown, 1978; Marschner et al., 1986; Brown and Jolley, 1989; Abadia et al., 2002).

Strategy II, or the chelation mechanism, which is exhibited by graminaceous plants and many of the world's main food crops such as wheat, rice, and maize, involves the release of lower-molecular-weight compounds called phytosiderophores (non-proteinogenic amino acids) from roots in response to iron deficiency. These phytosiderophores (PSs) solubilize iron by binding to Fe^{3+}. The Fe^{3+}-PS complex is taken up by the plants via a specific phytosiderophore transporter (Curie et al., 2001). This chelation strategy is more efficient than the reduction strategy used by most other plants and thus allows grasses to survive in more drastic iron-deficiency conditions (Curie and Briat, 2003).

III. Iron Mobilization and Uptake in the Roots

Iron mobilization responses are mainly induced when plants sense an iron deficiency in the soil or when plants sense an increased requirement for iron in their shoots (Bauer et al, 2004). The two strategies for iron uptake require different iron systems for iron uptake from the soil. A closer look at iron mobilization and the uptake involved in each strategy is subsequently discussed.

Dicotyledonous and nongraminaceous monocotyledonous plants

For plants utilizing strategy I, iron mobilization is attained by the combined action of a proton-extruding H^+-ATPase and a ferric chelate reductase, these enzymes being induced by iron deficiency (Robinson et al., 1999; Schmidt et al., 2002). There is a hypothesis that ferric chelate reductase activity is depressed by a high apoplastic pH in the roots, thereby restricting the uptake of Fe^{2+} into the cytosol. This was tested by Kosergarten et al. (2004) with an investigation of the relationship between apoplastic pH and plasma membrane ferric chelate reductase activity in young sunflower roots. Apoplastic pH was recorded by means of the fluorescence ratio in conjunction with video microscopy by covalently tagging fluorescein boronic acid to OH groups of the root cell wall. Fe^{3+} reduction was measured using a similar approach, tagging ferrozine diboronic acid with OH groups of the cell wall. Ferrozine forms an Fe^{2+} complex, thus indicating the reduction of ferric iron. Results revealed that ferric chelate reductase activity in the root apoplast was completely depressed due to increased apoplastic

pH levels of 5.7 directly and ≥ 7 after longer exposure to HCO_3^- and nitrate thus, the quantities of iron translocated to the upper parts of plants are insufficient for plant development. These increased pH levels are due to the H^+-neutralizing effect of HCO_3^- on the protons pumped out of the cytosol. This study confirms that high concentrations of HCO_3^- found in calcareous soils are a main cause of iron deficiency chlorosis. In addition to the above iron responses, iron shortage also characteristically alters the patterning of epidermal root cells thereby increasing the absorptive surface of the roots. For example, root hair density is significantly increased in response to iron unavailability (Schmidt, 2001).

In the past decade, much progress has been achieved studying the molecular mechanisms of strategy I for mobilization and uptake of iron in roots. Many genes have been investigated, identified and isolated from enzymes and transporters of soluble iron into the cytosol via the plasmalemma. To mention just a few, three ferric-chelate reductase genes (*AtFRO2*, *PsFRO1* and *LeFRO1*) were isolated from *Arabidopsis*, pea, and tomato respectively (Robinson et al., 1999 ; Waters et al., 2002 ; Li L et al., 2004). Their transcriptions were induced in roots under conditions of iron deficiency. After reduction of iron, Fe^{2+} is then transported across the plasma membrane mediated by protein transporters. This marks the final step of iron uptake in plants utilizing Strategy I. Many iron-regulated transporters (IRTs) from the zinc- and iron transporter family (ZIP) that are necessary in the process have also been identified and isolated from various plants such as *AtIRT1* and *AtIRT2* from *Arabidopsis thaliana* (Eide et al., 1996 ; Vert et al., 2001), *LeIRT1* and *LeIRT2* from tomato (Eckhardt et al., 2001) and *PsIRT1* from pea (Cohen et al., 1998). This IRT1 is a metal transporter with a broad-ranging substrate specificity when expressed in yeast (Korshunova et al., 1999) and has a tendency to accumulate Cd, Zn, Mn and Co as observed in iron-deficient plants (Curie and Briat, 2003). The IRT1 protein is considered the major root transporter responsible for iron uptake from soil solutions in response to iron deficiency in *Arabidopsis* (Vert et al, 2002) and other nongraminaceous plants. However, other transport systems could play roles in this process such as the IRT2 transporters and another class of metal transporters encoded by the natural resistance-associated macrophage protein (*NRAMP*) gene family. *NRAMP* genes are widely distributed throughout living organisms and are involved in the transport of a broad range of divalent metal cations, including iron (Gunshin et al., 1997). In the case of NRAMP transporters in *Arabidopsis*, no data yet indicate whether these transporters play a role in iron uptake from soil solutions (Curie and Briat, 2003). Table 1 shows genes and gene products involved in iron mobilization and the uptake responses in roots.

Furthermore, the *FER* (iron regulator gene) gene encoding the basic helix-loop-helix (BHLH) protein takes part by controlling iron uptake responses in roots. *FER* isolated from tomato by map-based cloning is proposed as the central

regulatory gene involved in controlling iron deficiency responses and iron uptake in roots of tomato (Ling H-Q et al., 1996 ; Ling H-Q et al., 2002). This *FER* gene in tomato has high similarity to *AtBHLH29* of *Arabidopsis* both genes encoding a BHLH protein are proposed as transcriptional factors functioning in iron deficiency responses and uptake (Ling H-Q et al., 2002 ; Colangelo et al., 2004 ; Jakoby et al., 2004). Just recently, it has been reported that AtBHLH29 of *Arabidopsis* (Fe-deficiency Induced Transcription Factor 1 or FER-like regulator of iron uptake) was required for the iron deficiency responses in *Arabidopsis*. A knockout mutant of *AtBHLH29* displayed typical iron deficiency symptom (chlorosis) and strong growth impairment. The AtBHLH29 (FIT1 or FRU) protein is involved in controlling ferric-chelate reductase AtFRO2 at a transcriptional level and the iron transporter AtIRT1 at a protein level. This was determined using a T-DNA insertion or ethyl-methane sulfonate (EMS)-induced mutants (Colangelo et al., 2004 ; Jakoby et al., 2004). Further analysis of the function of *AtBHLH29* in regulating the iron uptake process in *Arabidopsis thaliana* using RNA interference (RNAi) mutants has been conducted (Zhang et al, 2006). This RNAi technique is a powerful tool for altering the transcript levels of genes in cells and organisms (Hannon, 2002 ; Baulcombe 2004 ; Mello and Conte 2004). Thus, it can be used to more accurately reveal the genetic function of *AtBHLH29*. Zhang et al. (2006) compared the iron deficiency responses of seven RNA strains that contained decreasing amounts of *AtBHLH29* transcripts. The study demonstrates that *AtBLHLH29* is absolutely necessary for plant survival when iron supply is restricted. The transcription of AtBHLH29 was essential for the expression of AtFRO2. In contrast, the expression of AtIRT1 was not so strongly dependent upon the transcription of *AtBHLH29*. In the above study, it has also been found that a stretch of four consecutive arginine residues located at the C-terminal part of the basic region in the BHLH domain is probably required for the nuclear localization of *AtBHLH29*. This suggests that the basic regions in some plant BHLH proteins may also play an important role in nuclear localization (in addition to their role in DNA binding activities). It will be tempting to investigate in the future how basic region affects nuclear localization of *AtBHLH29* in terms of the underlying mechanism, and its contribution to the biochemical function of this protein in regulating the iron-uptake process in *Arabidopsis*.

Colangelo and Guerinot (2004) further suggested that *FER* in tomato and *AtBHLH29* in *Arabidopsis* were two genes governing the effective iron uptake system but in different manner. However, this speculation is disputed by Yuan et al. (2005) who claim that AtBHLH29 of *Arabidopsis* is a functional ortholog of tomato FER and functions in a similar manner as FER in controlling the effective iron uptake system in tomato. In their procedure, *AtBHLH29* was introduced into the genome of the tomato *FER* mutant *T3238fer* mediated by *Agrobacterium*

Table 1. Genes and gene products involved in iron mobilization and uptake in plant roots

Function of gene product	Gene name	Organism	Role of gene product in iron uptake	References
Ferric chelate reductase	*AtFRO2*	*Arabidopsis thaliana*	Strategy I iron reductase	Robinson et al., 1999
	PsFRO1	*Pisum sativum*	Iron reductase	Waters et al., 2002
	LeFRO1	*Lycopersicon esculentum*	*Iron reductase*	Li et al., 2004
ZIP transporter	*AtIRT1*	*Arabidopsis thaliana*	Strategy I Fe^{2+} transporter in root epidermis	Eide et al., 1996 ; Vert et al., 2002
	AtIRT2	*Arabidopsis thaliana*	Iron metal transporter	Vert et al 2001
	LeIRT1	*Lycopersicon esculentum*	Iron metal transporter	Eckhardt et al., 2001
	LeIRT2	*Lycopersicon esculentum*	Iron metal transporter	Eckhardt et al., 2001
	PsIRT1	*Pisum sativum*	Iron metal transporter	Cohen et al., 1998
	OsIRT1	*Oryza sativa*	Iron metal transporter	Bughio et al., 2002
NRAMP	*AtNRAMP 1, 3, 4*	*Arabidopsis thaliana*	Iron metal transporter	Curie et al., 2000 ; Thomine et al., 2000
	LeNRAMP 1, 3	*Lycopersicon esculentum*	Putative iron metal transporter	Bereczky and Bauer, 2003
YSL	*ZmYS1*	*Zea mays*	Strategy II Fe^{3+} phytosiderophore transporter	Curie et al., 2001
Regulator	*LeFER*	*Lycopersicon esculentum*	Strategy I regulator	Ling H-Q et al., 2002
	AtBHLH29/ FIT1	*Arabidopsis thaliana*		Colangelo and Guerinot, 2004 Jakoby et al., 2004

Table modified from Bauer and Bereczky (2003).

tumefacienes. Molecular analysis demonstrated that the expression of *AtBH-LH29* in roots of the *FER* mutant T3238fer enabled it to complement the defect functions of FER. The transgenic plants regained the ability to activate the complete iron deficiency responses and showed normal growth as a wild type under iron-limiting stress. The transformation data demonstrated that AtBHLH29 is a functional ortholog of the tomato FER and can completely replace FER in controlling effective iron acquisition in tomato.

Graminaceous plants

In graminaceous species, mobilization of iron is achieved by the secretion of phytosiderophores (PS) that are composed of the mugineic acid family of phytosiderophores (MAs), including mugineic acid (MA), 2′-deoxymugineic acid (DMA), 3-epihydroxymugineic acid (epi-HMA) and 3-epihydroxy 2′-deoxy-mugineic acid (epi-HDMA), with each graminaceous plant secreting its own specific set of MAs. Plants capable of releasing high amounts of MAs are generally more resistant to Fe-deficiency chlorosis than plants that are not (Jolley and Brown, 1989 ; Romheld and Marschner, 1986). Moreover, secretion of MA occurs in the apical zones of roots as the main regions of MA release. A distinct primary root newly formed on the basal parts of barley has higher activity for releasing MA than other roots with Fe deficiency (Yoshida et al., 2004). The biosynthesis pathway of MA has been extensively studied and has now been almost completely deciphered in barley (Mori, 1999 ; Negishi et al., 2002). Enzymes particularly nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT) play relevant roles in MA biosynthesis. Expressions of these enzymes are increased by an Fe-deficiency, leading to a higher production of MAs under such conditions (Mori, 1999). MA is synthesized from L-methionine via nicotianamine. The initial step in the production of MAs is the condensation of three molecules of S-adenosyl methionine (SAM) to produce one molecule of NA. This process is catalyzed by NAS. NA is produced by both monocotyledonous and dicotyledonous plants but the subsequent steps leading to MAs synthesis are specific to graminaceous plants (Curie and Briat, 2003). The most crucial enzyme in the specific pathway in grasses is the NAAT that catalyzes the transfer of amino residue to NA, and then DMA synthase enzyme hastens the production of DMA, the precursor of all other MAs (Curie and Briat, 2003 ; Kobayashi et al., 2005). The following hydroxylations of DMA result in the formation of other members of the MA family. Two barley cDNA clones particularly expressed in iron-deficient roots, *Ids2* and *Ids3*, were shown to encode dioxygenases involved in hydroxylation of DMA (Nakanishi et al., 2000). While IDS2 is thought to catalyze the hydroxylation that converts MA in epiHMA and DMA in epiHDMA (Nakanishi et al., 2000), IDS3 was shown *in vivo* to be responsible for converting DMA into MA when expressed in rice that normally secretes only DMA (Kobayashi et al., 2001)

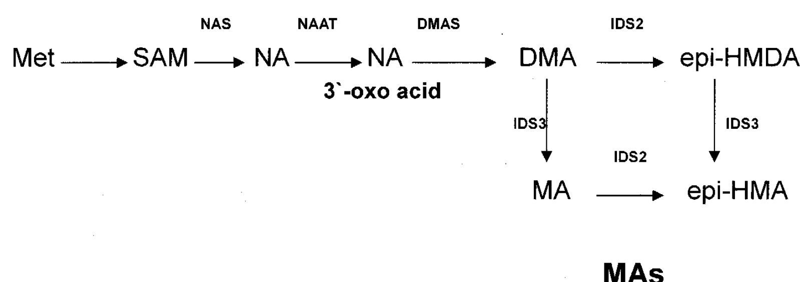

FIG 2. The biosynthetic pathway of MAs in graminaceous plants (Adapted from Negishi et al., 2002 ; Kobayashi et al., 2005).

(see Fig. 2). Secretion of MAs follows a diurnal rhythm particularly in barley (Takagi et al., 1984), and just recently shown in rice (Nozoye et al., 2004). Accordingly, the expression of genes encoding *NAS* and *NAAT* enzymes in the MAs, particularly in DMA biosynthesis, fluctuates diurnally in iron deficient rice roots. It is also hypothesized that MAs synthesized in the rough endoplasmic reticulum (rER)-derived vesicles are localized to cell boundaries facing the rhizosphere through a polar vesicle transport process involving proteins, possibly related to vesicle transport, that lead to the diurnal secretion of MAs (Nozoye et al., 2004).

Chelation of the Fe^{3+} follows after the secretion of phytosiderophores (PS). Phytosiderophore compounds possess a relatively high affinity for the chelation of Fe^{3+}, however, other metals, in particular Cu, Zn, and most likely also Ni are also involved. Therefore these heavy metals represent efficient competitors in the rhizosphere that may finally decrease the amount of solubilized Fe^{3+}. As a result, roots of graminaceous plants will face a mixture of different phytosiderophore-bound heavy metals according to the endogenous metal and chelate composition of the soil and the competivity of the metal to form a stable PS complex. (Schaaf et al, 2004a).

This Fe^{3+}-PS complex is then taken up by the iron uptake system from the soil. A transporter for Fe-PSs has been cloned from maize (Curie, 2001) and is classified as a member of the oligopeptide transporter (OPT) family. The transporter was named yellow stripel (YS1) after the phenotype of a maize mutant deficient in Fe-PSs uptake. Both the efflux of phytosiderophores and the steady-state level of YS1 are strongly enhanced by iron deficiency in grasses (Mori, 1999 ; Curie et al., 2001). It has been reported (Schaaf et al., 2004b) that this YS1 is a H^{+}-Fe^{3+}-phytosiderophore transporter and thus depends on proton cotransport and pH, which allows YS1-mediated transport to occur even at an alkaline pH. In addition, this transporter has broad specificity, and can transport various phytosiderophore-bound metals including Zn, Cu, and Ni, and also Ni, Fe^{2+} and Fe^{3+} complexes with NA. These biochemical properties indicate a novel role of the YS1 transporters for heavy metal homeostasis in plants. However, a contradicting role for this maize Fe-PS or YS1 transporter, which is encoded by

the *Yellow stripe1* (*Ys1*) gene has been reported (Roberts et al., 2004). In that study, YS1 was found to be unable to transport PS bound to Zn, Ni, Mn, Cd or NA complexed with ferric and Cu. This discrepancy may be accounted for by differences in experimental procedures, particularly in the concentrations of substrates used in the two studies as also concluded by Ma (2005). Roberts et al. (2004) used much lower concentrations of metals than Schaaf et al. (2004b), though both investigations used yeast complementation procedures. There is a need then to again verify the experimental procedures and the results thus obtaining a more conclusive statement on the issue of protein transporters. Moreover, differences in the specificity of YS1 between yeast and plants could also be an important focus for a future study.

In spite of being a Strategy II plant, however, rice (*Oryza sativa*) contains a previously identified Fe^{2+} transporter, OsIRT1 (Bughio et al., 2002). A recent study shows that rice has a unique Fe uptake system (Ishimaru et al., 2006). The study isolated the OsIRT2 gene from rice that has a higher homology to OsIRT1. Real-time PCR analysis revealed that OsIRT1 and OsIRT2 are expressed predominantly in roots and that these transporters are induced by low-Fe conditions. Furthermore, analysis using a positron-emitting tracer imaging system (PETIS) showed that rice plants are able to take up both Fe^{3+}-DMA and free Fe^{2+}. The rate of translocation of Fe absorbed as Fe^{3+}-DMA was greater than that for Fe^{2+} suggesting that rice plants would take up Fe^{2+}, although the Fe^{3+}-DMA transport system is more efficient than the Fe^{2+} transport system in Fe-deficient rice plants. However, the study did not detect any increase of Fe^{3+}-chelate reductase activity or the transcript of a FRO-like gene in Fe-deficient roots. These data suggest that rice plants possess a unique Fe^{2+} uptake system that is different from other Strategy I and Strategy II plants, and that OsIRT1 and OsIRT2 might be related to this system. This novel Fe-uptake system in rice, thus makes significant contribution to growth in submerged conditions.

Conclusions and Future Prospects

The most widespread problem in plants affecting human nutrition worldwide could be that of iron deficiency (WHO, 2003). Increasing the plants ability to provide higher levels of minerals, such as Fe, will have a dramatic impact on human health (Clemens et al., 2002 ; Guerinot, 2001). Thus, increasing the iron uptake from soils is a significant prerequisite for increasing the amount of iron in the edible parts of plants. However, plants cannot take up much soluble iron from the soil, especially from calcareous soils. Therefore, it is very important to focus on the uptake and mobilization of soluble iron from the rhizophere into roots, a system that marks the primary entry of iron into plants.

Recent advances in molecular biology have also allowed us to devise poten-

tially useful biotechnological strategies that should improve iron nutrition in plants and so in the human population as well. Most research focuses on the genes encoding the transport proteins of iron, genes encoding enzymes and the like. It is tempting to focus efforts on work that increase iron solubility in soil by enhancing the activity of ferric chelate reductase enzymes that reduce insoluble form of Fe into a soluble one as conducted by Oki et al. (2004). Another way could be studied by engineered crops to hasten the release of iron-solubilizing chelators in the rhizophere, or by boosting the effectiveness of phytosiderophores for better absorption and mobilization of Fe. This should maximize the uptake of Fe from roots and so alleviate plant intolerance to scarce Fe soil conditions. Uptake of iron from the soil into the roots does not end there, but it must also be transported to the other parts of plants that are consumed by humans. It is important then to emphasize the translocation of iron from the roots and upward to other parts of the plants. This may be a quite complex process but has relevance to the ultimate aim which is to produce fortified crops to meet human nutritional needs in respect of iron. Hence, further studies on increasing the bioavailability of Fe in plant products, as realized in "golden rice" (Lucca et al., 2002) for example are to be much encouraged. To attain this goal, it appears useful to work further on the translocation and especially on the accumulation of Fe in edible parts like leaves and grains; thus solving some of the unanswered questions about the transport processes along with utilizing various new biological techniques and approaches.

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