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# Gene networks involved in iron acquisition strategies in plants

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**Abstract** – Iron uptake is strictly controlled in all organisms. Iron deficiency induces a well-defined series of physiological events, either based on iron reduction (strategy I, dicots and monots except grasses) or iron chelation (strategy II, grasses). Genes encoding the structural components of the strategies I and II have been described recently using molecular, genetic and biochemical techniques. The major participants of the strategy I are the ferric reductase and the Fe II transporter encoded by *Atfro2* and *Atirt1* homologs, respectively. The strategy II comprises the enzymes for phyto siderophore biosynthesis encoded by the genes for nicotianamine synthase, nicotianamine aminotransferase and others. Transcriptional and post-transcriptional regulatory mechanisms control the strategy I and II genes. The tomato *fer* gene is among the first identified regulator genes for iron uptake. The identification and characterisation of the regulatory mechanisms is an important step towards designing new strategies for improving the iron content of crop plants for human health.

**iron uptake / strategy I genes / strategy II genes / regulation / *fer***

**Résumé** – Les réseaux de gènes impliqués dans les stratégies d'absorption de fer par les plantes. L'absorption de fer est contrôlée de manière rigoureuse dans tous les organismes. La carence en fer entraîne des séries bien connues d'incidents physiologiques, qu'ils soient basés sur la réduction de fer (stratégie I, dicotylédones et monocotylédones à l'exception des plantes herbacées) ou la chélation de fer (stratégie II, plantes herbacées). Les gènes codant pour les composants structuraux des stratégies I et II ont été décrits récemment en utilisant des techniques moléculaires, génétiques et biochimiques. Les principaux participants de la stratégie I sont la réductase ferrique et le transporteur Fe II codés respectivement par les homologues de *Atfro2* et *Atirt1*. La stratégie II comporte les enzymes pour la biosynthèse de phyto siderophore codées par les gènes de la nicotianamine synthase, de la nicotianamine aminotransférase et d'autres. Les mécanismes de régulation transcriptionnels et post-transcriptionnels contrôlent les gènes des stratégies I et II. Le gène *fer* de la tomate figure parmi les premiers gènes régulateurs pour l'absorption de fer à avoir été identifiés. L'identification et la caractérisation des mécanismes régulateurs est une avancée considérable pour envisager de nouvelles stratégies visant à améliorer le contenu en fer des plantes cultivées pour la santé humaine.

**absorption de fer / stratégie I gènes / stratégie II gènes / régulation / *fer***

## 1. INTRODUCTION

Iron is essential for cellular redox reactions due to its ability to undergo reversible valence changes from Fe II to Fe III. Iron is, for example, required for electron transport during respiration and photosynthesis, and the catalytical reactions of metalloproteins that utilise oxygen, hydrogen or water as substrates [26]. Due to its high redox reactivity, free cellular iron can generate hydroxyl radicals in the Fenton reaction. To find the balance between iron requirement and toxicity, the uptake and distribution of iron is strictly controlled in all organisms.

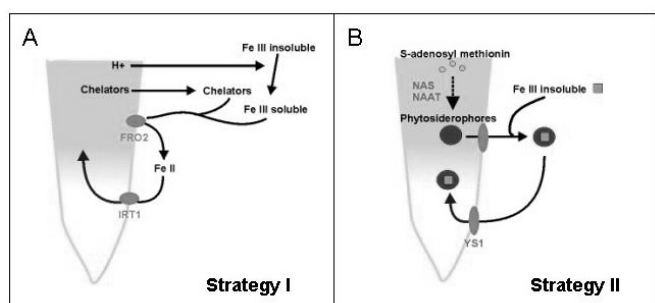
The identification of iron signals, signal transduction components and regulators for iron homeostasis is a major

issue in iron plant biology today. Metal homeostasis can be regulated during entry of iron into the root epidermis, transport to the xylem, transport throughout the plant, intracellular distribution and storage. Disturbed iron homeostasis may cause leaf chlorosis, growth inhibition and a decreased yield. Roots explore the available iron in the environment, trap iron and take it up in response to the needs of the shoot for iron. Iron assimilation can be controlled by altering the amount and activity of iron mobilisation and uptake components. At low nutrient supply, organisms tend to express high-affinity uptake systems to transport the nutrients with higher efficiency. In contrast, if nutrient supply is sufficient, low-affinity transport mechanisms are usually employed [14, 19, 57]. High- and low-affinity iron transport in plant roots have only been partially analysed [52].

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**Figure 1.** Schematic representation of iron acquisition in roots or root hairs upon iron deficiency. (A) Strategy I of dicot and monocot plants with the exception of grasses (modified according to [45]). Iron deficiency causes proton extrusion ( $H^+$ ), secretion of small organic chelating molecules (chelators) to solubilise Fe III. Fe III is reduced by FRO2 to Fe II [42], and subsequently taken up by the Fe II transporter IRT1 present in the root epidermis [13, 52]. Iron mobilisation and uptake may also take place in the root apoplast in addition to the rhizosphere as represented. (B) Strategy II of the *Gramineae* (modified according to [45]). Phytosiderophores are synthesised from S-adenosylmethionine via the action of nicotianamine synthase, nicotianamine aminotransferase and other enzymes summarised in [35] and secreted into the rhizosphere. After chelation of Fe III, phytosiderophore-Fe III complexes are reimported into the root via YSI [11].

Iron acquisition is currently the best studied system for metal uptake in plants. The reason is that iron limitation induces a well-defined series of physiological responses which can be easily monitored in various laboratory assays to isolate iron uptake components at a molecular level. Iron deficiency is often caused by the low solubility of Fe III rather than the low amounts of iron in the soil, especially on calcareous and alkaline soils. Plant strategies to cope with iron deficiency therefore aim at solubilising Fe III to facilitate its uptake by the plant roots. Dicots and monocots except grasses acidify the apoplast and rhizosphere due to the action of  $H^+$ -ATPases, secrete small chelating organic molecules like citrate and reduce iron. Fe II is taken up by the root epidermis via a transporter for Fe II (strategy I, [45], Fig. 1A).

Grasses secrete phytosiderophores, potent chelators for Fe III. These phytosiderophores are derived from the non-proteinogenic tripeptide-like molecule nicotianamine, a condensation product of methionine. Fe III-phytosiderophore complexes are reimported into the root (strategy II, [45], Fig. 1B).

Both strategies are induced upon iron limitation and switched off after sufficient iron supply, as established physiologically [45]. This suggests that the strategy I and II components are only required for the uptake of iron upon an inadequate iron nutritional status, presumably high-affinity uptake components. Upon sufficient iron supply, different mechanisms for the uptake and transport of iron presumably exist which have not been described in detail for plants.

In the following overview we describe our current approaches to isolating the genes involved in iron mobilisation and uptake. A number of observations indicate that iron assimilation is controlled at several levels. Molecular gene identifi-

cation allows us to study the regulation of iron acquisition more precisely.

## 2. GENES ENCODING IRON MOBILISATION COMPONENTS OF THE STRATEGY I AND STRATEGY II

In recent years genetic, biochemical and molecular approaches and their combination have successfully contributed to the identification of the genes mediating iron acquisition (Tab. I).

### 2.1. Ferric chelate reductase genes

Root iron reductase activity can be visualised using BPDS (Bathophenanthroline disulfonate) or ferrozine which turn red or pink upon binding of reduced Fe II. This colour reaction was used for a forward genetic screen to identify *Arabidopsis* mutants devoid of iron reductase activity [58]. In this way, three allelic mutations were identified, *frd1-1*, *frd1-2* and *frd1-3* (ferric reductase deficient mutants) [58]. Mapping of *frd1-1* together with a candidate gene approach for identifying ferric reductase homologs from *Arabidopsis* revealed that the candidate gene *Atfro2* cosegregated with *frd1-1* [42]. *Atfro2* encodes a predicted eight membrane-spanning protein, harbouring a FAD cofactor binding site and two sites for haem coordination [42]. *Atfro2* is induced upon iron limitation in roots [42]. A ferric reductase homolog has been identified in pea via a PCR-based approach using degenerate oligonucleotides, *Psfro1* [53]. PSFRO1 confers increased ferric chelate reductase activity in yeast [53]. *Psfro1* is expressed in iron-starved roots and shoots as well as in nodules in the infection and nitrogen-fixing zones [53]. Symbiotic nitrogen fixation requires iron for the activity of nitrogenase and leghemoglobin. The presence of *PSFRO1* transcripts in leaf mesophyll and parenchyma cells was explained as the need for iron reduction to retrieve Fe III, which is presumably complexed with citrate [53]. *fro* genes represent a gene family in plants with specific expression patterns [42]. The identification of *Atfro2* as the strategy I ferric reductase seems to indicate that despite the apparent redundancy, some *fro* family members have acquired specific functions.

### 2.2. Transporter genes

The family of ZIP metal transporters (ZIP stands for ZRP, IRT-like proteins, ZRP = zinc-regulated transporter, IRT = iron-regulated transporter) was found to be involved in metal uptake in eukaryotes and some bacteria [15, 20, 22]. ZIP proteins harbour mostly eight predicted transmembrane-spanning regions and a postulated metal ion binding site in the intracellular loop between helices III and IV [15]. The first zip cDNA from plants, *Atirt1*, was identified in a functional yeast complementation assay to restore growth of the iron uptake-deficient yeast strain *fet3fet4* [13]. *Atirt1* has a broad substrate range, including iron (Fe II is preferred over Fe III), zinc, manganese and cadmium [13, 29, 43]. *Atirt1* and a close relative *Atirt2* were found to be induced by iron limitation in the root where they are expressed in the root epidermis of the

**Table I.** Genes involved in iron uptake mobilisation responses in the root.

Function of gene product	Name	Organism	Role of gene product in iron uptake	Approaches for gene identification	Gene regulation in response to iron supply	References
Ferric chelate reductase	<i>Atfro2</i>	<i>Arabidopsis thaliana</i>	Strategy I iron reductase	Forward genetics, candidate gene	Induction in –Fe roots	[42]
	<i>Psfro1</i>	<i>Pisum sativum</i>	Iron reductase	Candidate approach, yeast complementation	Induction in –Fe roots and –Fe shoots	[53]
ZIP transporter	<i>Atirt1</i>	<i>Arabidopsis thaliana</i>	Strategy I Fe II transporter in root epidermis	Yeast complementation, reverse genetics	Up-regulation in –Fe roots	[13, 52]
	<i>Atirt2</i>	<i>Arabidopsis thaliana</i>	Iron (metal) transporter	Candidate gene identification, yeast complementation	Up-regulation –Fe roots	[51]
	<i>Leirt1</i>	<i>Lycopersicon esculentum</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Up-regulation –Fe roots	[12]
	<i>Leirt2</i>	<i>Lycopersicon esculentum</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Similar expression in +/- roots	[12]
	<i>Psrit1</i>	<i>Pisum sativum</i>	Iron (metal) transporter	Candidate gene	Induction in –Fe roots	[8]
	<i>Osirt1</i>	<i>Oryza sativa</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Induction in –Fe roots	[6]
NRAMP transporter	<i>Atnramp1</i>	<i>Arabidopsis thaliana</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Up-regulation –Fe roots, low expression with up-regulation in –Fe leaves	[10, 50]
	<i>Atnramp3</i>	<i>Arabidopsis thaliana</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Up-regulation –Fe roots and –shoots	[50]
	<i>Atnramp4</i>	<i>Arabidopsis thaliana</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Up-regulation –Fe roots and –Fe shoots	[50]
	<i>Lenramp1</i>	<i>Lycopersicon esculentum</i>	Putative iron (metal) transporter	Candidate gene	Up-regulation –Fe roots	Berczky and Bauer
	<i>Lenramp3</i>	<i>Lycopersicon esculentum</i>	Putative iron (metal) transporter	Candidate gene	Similar expression in +/- roots and shoots	Berczky and Bauer
	<i>Osnramp1</i>	<i>Oryza sativa</i>	Iron (metal) transporter	Candidate gene, yeast complementation	Not analysed	[2, 10]
	<i>Osnramp3</i>	<i>Oryza sativa</i>	Putative iron (metal) transporter	Candidate gene	Not analysed	[2]
YSL transporter	<i>Zmys1</i>	<i>Zea mays</i>	Strategy II Fe III phytosiderophore transporter	Forward genetics, yeast complementation	Induction –Fe roots and –leaves	[11]
Transporter MATE	<i>Atfrd3</i>	<i>Arabidopsis thaliana</i>	Putative transporter	Forward genetics	Weak up-regulation in –Fe roots	[44]
Nicotianamine synthase	<i>LechlN</i>	<i>Lycopersicon esculentum</i>	Nicotianamine synthase	Forward genetics, Enzyme assay	Similar expression in +/- Fe shoots and roots	[31]
	<i>HvnaS</i>	<i>Hordeum vulgare</i>	Strategy II nicotianamine synthase	Enzyme purification	Induction in –Fe roots	[24]
	<i>Osnas</i>	<i>Oryza sativa</i>	Nicotianamine synthase	Candidate approach	Induction in –Fe leaves and roots, basal expression	[25]
Phytosiderophore enzyme	<i>Hvnaata</i> <i>Hvnaatb</i>	<i>Hordeum vulgare</i>	Strategy II nicotianamine aminotransferase	Enzyme purification	Induction in –Fe roots	[48]
	<i>Hvids2</i> <i>Hvids3</i>	<i>Hordeum vulgare</i>	Putative dioxygenase	Mapping, differential cDNA library screening	Induction in –Fe roots	[33, 34, 36]
Regulator	<i>Lefer</i>	<i>Lycopersicon esculentum</i>	Strategy I regulator, putative transcription factor	Forward genetics	Similar expression in +/- roots	[32]

root hair zone [51, 52]. The IRT1 protein is present in the plasmalemma [52]. Loss of *Atirt1* function leads to chlorosis and reduced growth upon low iron supply [52]. Moreover, IRT1 appears to transport iron with high affinity [13, 52]. *Atirt2* was not capable of rescuing *Arabidopsis irt1-1* mutants [52]. Moreover, *Arabidopsis irt2* mutant plants did not display any iron deficiency phenotypes [55]. Therefore, IRT1 is the high-affinity Fe II transporter acting during the strategy I [52]. The role of IRT2 is not yet fully established in *Arabidopsis*; presumably, it may act in addition to IRT1, in other compartments than IRT1 or in the uptake of other metals. Due to the lack of genetic evidence in other systems we do not know yet whether ATIRT1's function as the main strategy I Fe II transporter is conserved in other strategy I plants. In tomato, *Leirt1* encodes a root-specific metal transporter with enhanced expression upon low iron supply, and a basal level of expression upon sufficient iron nutrition [12]. Induction at mRNA level has also been reported for putative *irt1* genes from pea and rice [6, 8]. *Leirt2* was expressed in roots irrespective of iron supply [12]. Interestingly, the tomato as well as the *Arabidopsis irt1* and *irt2* genes are located in tandem in the genome [12, *Arabidopsis* database].

A second metal transporter family from plants identified through similarity searches using the database is the NRAMP family, comprising six members in *Arabidopsis* [10, 50, <http://plantst.sdsc.edu/>]. *nramp* genes are involved in metal uptake in eukaryotes and also some bacteria [16]. NRAMP proteins were named after the phagosomal protein NRAMP1 (Natural Resistance-Associated Macrophage Protein 1) that functions as a divalent metal efflux pump in the membrane and contributes to the resistance against intracellular bacteria by reducing metal availability [38]. NRAMP proteins have a broad substrate range and can transport manganese, iron, zinc, cobalt, cadmium, copper, nickel and lead ions [38]. Plant NRAMP proteins contain about 12 predicted transmembrane regions and usually a predicted conserved transport motif [10, 50]. ATNRAMP3 and ATNRAMP4 from *Arabidopsis* were able to complement the yeast *fet3fet4* mutant and to mediate iron uptake in yeast [50]. ATNRAMP1, as well as the rice homolog OSNRAMP1, were less efficient than ATNRAMP3 in the *fet3fet4* complementation and iron uptake assays [10, 50]. Possibly, NRAMP1-type transporters in plants serve a different function from NRAMP3 and NRAMP4-type transporters. This assumption seems further supported by the mRNA expression patterns. The related *Atnramp1* and *Osnramp1* genes are predominantly expressed in roots [2, 10]. *Osnramp3*, *Atnramp3* and *Atnramp4* are expressed similarly in roots and shoots [2, 50]. We also found a similar situation for the tomato *Lenramp1* and *Lenramp3* genes (Z. Berezky and P. Bauer). *Atnramp1*, *Atnramp3* and *Atnramp4* show increased expression upon iron limitation [10, 50]. An *Atnramp3* T-DNA insertion mutant was more resistant to cadmium than wild-type plants, whereas an obvious growth reduction or iron chlorosis was not observed [50]. ATNRAMP2 and OSNRAMP2 failed to complement the *fet3fet4* yeast mutant, indicating that these two proteins may not be iron transporters [10]. ATNRAMP5 and ATNRAMP6 have not been further described in the literature yet. ATEIN2, an ethylene signalling component, shows partial similarity to NRAMP proteins, but was devoid of metal transport

function [1]. To really assess the possible roles of *nramp* genes in iron uptake it will be necessary to obtain detailed data on mRNA and protein localisation as well as loss and gain of function effects. It is possible that NRAMP proteins function in a less obvious manner than ATIRT1, for example, for the relocation of iron in specific organelles. Thomine et al. proposed that AtNRAMP3 might regulate the vacuolar-cytosolic metal pools and thereby have a control function upon iron acquisition processes (S. Thomine, F. Lelièvre, E. Debarbieux, J.I. Schröder, H. Barbier-Brygoo, *AtNRAMP3* encodes a vacuolar metal transporter induced by iron deficiency that down-regulates iron acquisition mechanisms in *Arabidopsis*, XI Intern. Symposium in iron nutrition and interactions in plants, Udine, Italy, June 23-28, 2002).

A new family of transporters was discovered with the isolation of *Zmys1* (maize *yellow stripe1*) from the *ys1-m1* mutant using transposon mutagenesis [11]. *ys1* mutant plants are deficient in the uptake of phytosiderophore complexes into roots and suffer from leaf chlorosis [56]. YS1 confers complementation of *fet3fet4* yeast strains if Fe III-phytosiderophore complexes are provided in the growth medium [11]. *Zmys1* is induced or expressed at a higher level upon iron deficiency in maize leaves and roots, suggesting that *Zmys1* may be important for the distribution of iron throughout the plant [11]. Interestingly, the *Arabidopsis* genome contains eight *Zmys1* homologs (called *Atysl* = *ysl-like* genes) [11]. Since strategy I plants do not produce phytosiderophores, this may suggest that YSL-type transporters could have a conserved function in higher plants. It is possible that YSL proteins transport metal-nicotianamine complexes in the plant. Nicotianamine is present in all higher plants tested, where it presumably serves an essential role as metal chelator [47]. LeJean et al. reported that AtYSL1 might mediate iron partitioning in the shoot (M. LeJean, J.F. Briat, C. Curie, Involvement of AtYSL1 in plant resistance to iron excess, XI Intern. Symposium in iron nutrition and interactions in plants, Udine, Italy, June 23-28, 2002).

A gene encoding a putative novel transporter involved in metal uptake was recently identified from the *Arabidopsis frd3-1* mutant [44]. FRD3 is a member of the multidrug and toxin efflux family which can transport small organic molecules [44]. The concrete function or localisation of FRD3 are not described yet. *frd3* is specifically expressed in the root [44]. *frd3* mutant plants display upregulated iron and metal acquisition phenotypes [44].

### 2.3. Gene coding for enzymes involved in nicotianamine and phytosiderophore production

Nicotianamine is a plant-specific compound essential for plant growth [47]. Tomato *chloronerva* (*chl*) mutants, so far the only nicotianamine-free plants described, suffer from severe leaf chlorosis and show induced iron mobilisation and uptake upon sufficient iron supply compared with the wild type [47, Berezky and Bauer]. Presumably, nicotianamine serves as metal chelator in the plant to achieve proper intracellular or intercellular distribution of iron and other metals, and could be involved in the relocation of iron. In strategy II plants, nicotianamine serves the additional function as precursor for phytosiderophores [35]. Two independent approaches led to the isolation of nicotianamine synthase (*nas*) genes. Due

to its enzymatic activity, HvNAS was purified from protein extracts prepared from iron-starved barley roots which showed highly-induced nicotianamine synthase activity [23, 24]. Degenerate oligonucleotides derived from HvNAS peptide sequences were used to amplify the *Hvnas* DNA fragments to isolate the corresponding cDNA clone [23, 24]. *Hvnas* was strongly induced at a mRNA and protein level in barley iron-deficient roots, but hardly expressed in iron-sufficient roots and leaves, suggesting that HvNAS is the strategy II specific nicotianamine synthase [23, 24]. In parallel, map-based isolation of *Lechln* revealed that it encodes a nicotianamine synthase [31]. *Lechln* was found expressed throughout the plant [31]. *nas* genes form multigene families in general. Barley has six genes at least [24], and *Arabidopsis* four apparent *nas* genes (database searches). Tomato appears to be an exception as only one *nas* band was detected in a genomic Southern blot using a *Lechln* probe [31] and only *Lechln* was found expressed in the tomato EST (= expressed sequence tag) database (<http://www.tigr.org/tdb/tgi/lgi/>).

The second critical enzyme in phytosiderophore production is nicotianamine transferase, NAAT, catalysing the conversion of nicotianamine into demugineic acid. NAAT was purified from barley iron-deficient roots and two corresponding cDNAs were obtained [48]. *Hvnaat* genes were only found expressed in iron-deficient roots [48]. *Hvnaat* genes show a similarity with aminotransferase genes, of which many can be found in the *Arabidopsis* genome (*Arabidopsis* database). Two *Arabidopsis* aminotransferases show higher sequence similarity with the HVNAAT proteins and could potentially be involved in nicotianamine metabolism in *Arabidopsis*. So far, metabolised products of nicotianamine have not yet been reported for strategy I plants.

Subsequent phytosiderophore metabolic steps depend on the genetic background in grasses, whereby iron efficiency may correlate with the amount, time course and variety of phytosiderophores released. Barley produces a large variety of phytosiderophores. Genetic *loci* for the conversion of demugineic acid to mugineic acid and mugineic acid to epihydroxymugineic acid have been mapped to the barley chromosome arms 4L and 7L, respectively [33–35]. These *loci* corresponded to the mapping positions of two iron limitation-induced dioxygenase-encoding cDNAs from roots, *Hvids3* and *Hvids2*, respectively, suggesting that these two cDNAs might encode phytosiderophore biosynthetic enzymes [36]. *Hvids3* might encode a strategy II phytosiderophore dioxygenase. Transgenic rice containing *Hvids3* can produce mugineic acid, in contrast to untransformed rice [28].

Using microarray analysis, Negishi et al. (2002) found iron limitation-induced transcripts encoding polar vesicle transport components, supporting the hypothesis that polar vesicle transport might be involved in the diurnal release of phytosiderophores in barley [37].

#### 2.4. Regulatory genes

The tomato *fer* mutant (T3238*fer*) does not display iron reductase activity and proton extrusion, and is highly deficient in the uptake of iron from the soil [4, 5]. *fer* mutant plants exhibit morphological root phenotypes (such as no root hair proliferation at low iron supply) as well as reduced mRNA

expression of certain metal transporter genes [32, Bereczky and Bauer]. *Lefer* is required in the root but not the shoot [4]. Phenotypes of *fer* mutant plants are detectable at low and sufficient iron supply compared with wild-type plants, suggesting that *Lefer* may be involved in controlling root adaptations to iron supply [32]. The tomato *Lefer* gene has been isolated by map-based cloning and found to encode a bHLH protein, a putative regulator protein, possibly acting at transcriptional level as a DNA binding protein [32]. *Lefer* was found expressed in the root but not the shoot, irrespective of iron supply [32]. *LeFER* transcripts were detected in root tips in a cell-specific manner. Close to the root tip, *Lefer* expression occurred in the cortex and epidermis, while in the root hair zone expression was found to be restricted to parenchyma cells of the vascular bundles [32]. Therefore, LEFER may be a master regulator for iron acquisition in tomato roots by integrating signals for the iron status and inducing the appropriate root responses. We are currently investigating how the activity of the regulator LEFER may be controlled by the iron status and which could be the direct or indirect target genes.

Due to the efforts being made in structural and functional genomic approaches, it will only be a matter of time before novel regulators are identified. Interacting partners for regulators may be identified by yeast two hybrid screens, co-immunoprecipitation, pull-down assays or other biochemical techniques available. An alternative approach may rely on mRNA profiling using the array technology in *Arabidopsis* or other species to identify putative transcription factor genes up- or down-regulated in response to iron supply. Nevertheless, a transcription factor gene which is itself regulated at transcriptional level by the availability of iron requires that further upstream regulators may exist, which either induce or repress the gene or influence its mRNA stability. Insertion mutagenesis, tilling and RNAi approaches offer the possibility of isolating the corresponding knockout mutants in the identified genes to further investigate the roles of the newly identified regulator genes in plants. New forward screens and suppressor/enhancer screens using the already existing mutants may reveal further regulatory components in the future.

#### 2.5. Other genes

Other described genes involved in iron homeostasis are not directly part of the strategies I or II. A recently identified gene from castor bean, a member of the *lea* late embryogenesis abundant gene family, encoding an iron transport protein (ITP), may serve the phloem transport of iron from the cotyledons [30]. Ferritins play an important role as storage proteins for iron in all organisms. Plant ferritins are located in plastids and encoded by gene families (in *Arabidopsis* four members) with differentially expressed gene members [3, 40]. Some of the ferritin genes have been found to be repressed by low iron availability [40, 41]. Moreover, modulation of ferritin contents in transgenic plants influenced iron acquisition processes, suggesting that ferritins are involved in regulation of the iron pool [54].

In addition, differential cDNA screening procedures and mRNA array technology led to the identification of a number of iron-regulated genes whose concrete functions in iron uptake are not yet known [for example, 17, 36, 37, 49].

**Table II.** Regulation of strategy I ferric reductase and iron transporter genes in iron uptake mutants upon sufficient iron supply.

Mutant	Function of mutated gene	Species	Ferric reductase activity	<i>fro</i> gene expression	<i>irt1</i> gene expression	References
<i>irt1-1</i>	Fe II transporter	<i>Arabidopsis thaliana</i>	+	+	nd	[52]
<i>frd1</i>	Fe III reductase	<i>Arabidopsis thaliana</i>	-	nd	+	[13, 42, 58]
<i>frd3</i>	Putative MATE-like transporter	<i>Arabidopsis thaliana</i>	+	+	+	[13, 44]
<i>chln</i>	Nicotianamine synthase	<i>Lycopersicon esculentum</i>	+	nd	+	[47], Berczky and Bauer
<i>fer</i>	Putative transcription factor	<i>Lycopersicon esculentum</i>	-	nd	-	[4, 32], Berczky and Bauer
<i>brz</i>	Unknown	<i>Pisum sativum</i>	+	+	nd	[27, 53]
<i>dgl</i>	Unknown	<i>Pisum sativum</i>	+	+	nd	[21, 53]

nd = non determined.

### 3. REGULATORY MECHANISMS INVOLVED IN IRON UPTAKE

The physiological characteristics of the strategy I and strategy II are induced upon low iron supply and switched off after iron resupply. Thus, strategy I and II responses are efficiently regulated by the availability of iron.

A prerequisite for iron regulation is that an iron sensing component must exist with differential activity upon high or low iron supply. In the easiest way, one may assume that iron could be an activator or repressor for this sensor component. Iron may bind to a regulator directly and influence the biochemical activity of the regulator. For example, iron may regulate by altering mRNA stability, DNA binding capacity, the protein-protein interaction surface or activating a receptor-mediated signal transduction chain. An alternative possibility is that iron may bind an internal plant signal component. Nicotianamine could serve as a trigger. Finally, the presence or absence of iron may be sensed by certain physiological components or processes, translated into a secondary signal which then acts on a signal transduction cascade, ultimately arriving at the level of DNA. For example, the translocation of iron might emit a signal. Or iron availability could be sensed in the plant tissues during its incorporation into functional structures, such as the photosynthetic apparatus in the plastids. Lack of iron may induce in these tissues a stress signal, possibly related to plant hormones or signalling peptides. Currently, the exact mechanisms for regulation are purely hypothetical. Presumably different iron signals and different regulatory mechanisms are involved since regulation of the strategy I and II occurs at different levels.

First, the expression of iron mobilisation genes (such as *fro2*, *irt1*, *nramp*, *nas* and *naat*) is induced, suggesting transcriptional regulation by DNA binding transcription factors (Sect. 2, Tab. I). The tomato *fer* gene is to date the only candidate for such a regulator [32]. We still do not know whether the *fer*-dependent iron transporter genes are the direct targets of FER in tomato or whether further regulators act as intermediates. The definition of promoter elements of iron deficiency-induced target genes and concrete interaction of a regulator protein with such elements has yet to be demonstrated. A cis-regulatory element able to bind proteins (IDRS = iron-dependent regulatory sequence) has been identified in the promoters of ferritin genes *Atfer1* and *Zmfer1* for the repression upon low

iron supply [41]. Iron mobilisation genes encoding the Fe II transporter (IRT1-type) and the reductase (FRO-type) could be directly regulated by the availability of iron in the growth medium. Nevertheless, direct iron regulation is not the only way of regulation. The iron mobilisation genes are also induced in various iron uptake mutants in *Arabidopsis*, pea and tomato at sufficient iron supply (Tab. II). In the mutants, an iron-deficient situation could be mimicked, either due to the inefficient uptake of iron into the root (*irt1-1* [52], *frd1* [42]) or due to altered distribution of iron in the leaves (*chln* [44], *brz* [bronze, 27, 53], *dgl* [degenerate leaflet, 21, 53], *frd3* [44]) (Tab. II). Therefore, an iron signal was generated internally in the plant mutants. Schmidt discusses extensively the possible involvement of shoot signals in the induction of iron mobilisation responses in the roots [46]. In the mutants iron mobilisation genes are expressed at higher levels than observed in the wild type (Tab. II). For example, *ATFRD3* mRNA is present in much higher amounts in the roots of *frd3* mutant plants than in wild-type plants in the absence of iron [44]. *Atfro2* is expressed at higher levels in *frd1-3* mutants than normally upon iron limitation [42], and a similar observation was also made for *Atirt2* and *Atfro2* expression in *irt1-1* mutant plants [52]. These effects could be an indication of feedback of the activity of the protein to regulate the expression of its own gene. Alternatively, the lack of iron may influence the uptake of other metals which may exert additional regulatory effects. *frd3* mutants overaccumulate iron, zinc and manganese, which may also explain the high *Atfrd3* expression [44].

Nicotianamine synthase genes are iron-deficiency and root-regulated in barley, whereas the rice homologs show in addition expression in leaves and low expression upon sufficient iron supply [24]. Interestingly, barley genes behave in their expression in a similar way to the rice genes once they are transferred into rice, demonstrating that the regulatory sequences in the barley genes are recognised in rice, and that probably the differences between barley and rice reside, to a certain extent, in the regulation of phytosiderophore synthesis [25].

Another regulatory level interferes with protein stability. ATIRT1 is stable only upon low iron supply in the roots, whereas upon sufficient iron supply ATIRT1 is not detected, even if *Atirt1* is overexpressed in transgenic plants behind the 35S promoter [9]. It was suggested that ATIRT1, similar to the zinc transporter ZIP protein ZRT1 from yeast, may become ubiquitinated and degraded if iron is present in sufficient

amounts [9, 59]. ZRT1 contains within its intracellular loop between helices III and IV a histidine-rich motif which could be involved in metal binding or sensing [59]. Furthermore, a lysine residue within this variable region is required for ubiquitination [18]. Both features, a histidine-rich sequence and lysine residues, are found in AtIRT1 [9, 15].

#### 4. CONCLUSIONS

Most iron uptake components are encoded by gene families in strategy I and strategy II plants. Despite this, it was possible to identify individual genes which have gained a specific function in the mobilisation of iron upon iron deficiency, suggesting that the members of these gene families are only partially redundant. Since the gene families exist in strategy I and strategy II plants, the specific iron mobilisation gene family members must have been recruited for the purpose of enhancing iron uptake (*nas*, *naat* and *ys1* in grasses, and *irt1* and *fro2*-type in other plants). Future analysis may reveal the functions of all gene family members in *Arabidopsis* (<http://www.nsf.gov/pubsys/ods/getpub.cfm?bio0202>). It would certainly be important, however, to see whether the *Arabidopsis* strategy I mechanism is indeed conserved in other plant species (meaning *irt1* and *fro2* should be most similar in sequence and map position in various strategy I families) or whether in fact different plant families may have recruited different members of these or even other families. Studies with hyperaccumulation accessions (for example, the zinc and nickel accumulating lines of *Thlaspi*, *Arabidopsis halleri* or *Alyssum*) showed that indeed certain metal transporter families can be upregulated in these lines to tolerate heavy metals [for example, 7, 39], indicating that probably in a short time differential functions of gene family members can be selected.

The signals, sensors and effectors for iron homeostasis are just being characterised with the tomato *Lefer* gene being among the first identified regulator genes [32]. *Lefer* may allow the control of multiple iron uptake responses at once, and thus the *Lefer* gene could be used to increase the iron content of fruits or leaves in genetically modified plants. However, the current knowledge about iron-regulated systems clearly shows that we first need to learn more about the regulated features of iron mobilisation components. Obviously, the simple overexpression of strategy I and II genes is not the solution to generating iron-rich crops, as for the activity of the genes, we need to control the post-translational regulatory mechanisms as well. Furthermore, iron assimilation is regulated within the context of nutrition in general. It is well established that the availability of certain mineral nutrients influences the uptake of other nutrients. Some of the iron mobilisation responses serve generally increased nutrient uptake, such as citrate secretion, acidification or root hair proliferation. Clearly, the iron homeostasis components do not distinguish exactly between iron and other metals, especially heavy metals. In the future our efforts therefore also have to concentrate on improving the specificity of iron homeostasis components.

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