

Minireview

Iron stress in plants

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Published: 30 July 2002

Genome **Biology** 2002, **3(8)**:reviews1024.1–1024.4

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2002/3/8/reviews/1024>

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Abstract

Although iron is an essential nutrient for plants, its accumulation within cells can be toxic. Plants, therefore, respond to both iron deficiency and iron excess by inducing expression of different gene sets. Here, we review recent advances in the understanding of iron homeostasis in plants gained through functional genomic approaches.

Iron is an essential nutrient for plants. It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration. But iron is toxic when it accumulates to high levels. It can act catalytically via the Fenton reaction to generate hydroxyl radicals, which can damage lipids, proteins and DNA. Plants must therefore respond to iron stress in terms of both iron deficiency and iron overload. Recent advances in our understanding of plant responses to iron stress have resulted from the use of traditional approaches including genetics, biochemistry and cell biology. In addition, the use of the model organisms *Arabidopsis thaliana* and *Saccharomyces cerevisiae* has proved invaluable for the isolation and characterization of genes whose products function in iron stress responses. Here, we describe recent advances in the field that have come through the use of functional genomic approaches made possible, in part, by the completion of the *Arabidopsis* genome sequence and the draft sequences of the rice genome [1-3].

Iron storage

Because of the potential for toxicity associated with high iron levels, cells store iron with the specialized iron-storage protein ferritin. Together, 24 ferritin subunits form a hollow sphere that may store up to 4,500 atoms of iron in its core. Thus, ferritin plays an important role in iron homeostasis. Studies of plant ferritins have revealed several important differences in the structure, localization and regulation of plant

ferritins as compared to animal ferritins. For example, while animal ferritins are found in the cytosol, plant ferritins contain transit peptides for delivery to organelles called plastids [4]. Moreover, while iron-regulated expression of animal ferritin is controlled mainly at the level of translation by a system of iron-responsive elements (IREs) and iron-regulatory RNA-binding proteins (IRPs) [5], experiments in soybean and maize have shown that iron regulates expression of plant ferritins both transcriptionally [6-9] and post-transcriptionally [10]. Importantly, no IRE sequences have been identified in the regulatory regions of plant ferritin genes [11]. Until recently, the significance of these findings was unclear because it was not known whether these features of individual plant ferritins extended to all members of a plant ferritin gene family.

One recent study took advantage of the complete *Arabidopsis* genome sequence to examine the organization and structure of the entire ferritin gene family in *Arabidopsis* [12]. *Arabidopsis* contains four genes that encode ferritin (*AtFer1-AtFer4*); all four of these are known to be expressed, as each is represented in the database of *Arabidopsis* expressed sequence tags (ESTs). Analysis of the organization of the four *AtFer* genes showed that all four (like all previously characterized plant ferritin genes) share identical intron and exon structures, pointing to a common ancestral ferritin gene in plants. In addition, all four proteins are predicted to contain transit peptides for delivery to the plastid [12]. The transit peptide comprises the first portion of an

amino-terminal extension peptide (EP) previously identified in plant ferritins by comparison with animal ferritins. The extension peptide is also implicated in the regulation of ferritin protein stability [13]. An additional feature shared by all four *Arabidopsis* proteins is the presence of specific residues thought to be important for both ferroxidase activity and iron nucleation [12]. Animals express two different ferritin subunits, the H-type that has a ferroxidase site for oxidation of Fe(II) and the L-type that has glutamic acid residues facing the core for nucleation of Fe(III) - whereas each of the four plant ferritins contains both sets of residues. Thus, analysis of the complete set of ferritin genes in a single plant species confirmed previous studies of single members of this family in various plant species.

Northern blot analysis showed that the four *AtFer* genes are differentially expressed. *AtFer1* and *AtFer3* transcripts accumulate in response to high iron treatment in both roots and leaves; the same two transcripts accumulate in seedlings in response to treatment with H₂O₂, although *AtFer1* is induced to a greater extent [12]. It is known that Fe(II) can interact with H₂O₂ to form hydroxyl radicals, so that using ferritin to store iron may protect cells against damage from oxidative stress. Thus, the expression patterns suggest that *AtFer1* and *AtFer3* play important roles in the protection of plant cells from oxidative stress resulting from iron overload. *AtFer4* steady-state mRNA levels also are elevated in response to treatment with high concentrations of iron in leaves but are not elevated following H₂O₂ treatment [12]. Previous studies have established a correlation between iron overload and production of abscisic acid (ABA) [14]. Indeed, ferritin levels are reduced in a maize ABA-deficient mutant, indicating the presence of an ABA-dependent pathway for ferritin accumulation (reviewed in [15]). The *AtFer2* gene is expressed in mature siliques (seed pods) and dry seeds and is induced in response to treatment with ABA. Given that ABA is known to accumulate during seed development, and that genes encoding seed-specific proteins are often ABA-responsive, these observations point to a role for *AtFer2* in the storage of iron in seeds.

The response to iron deficiency

When faced with iron deficiency, all plants except the grasses induce a set of responses termed 'strategy I' [16]. Genes whose products function in this response, such as those encoding Fe(III) chelate reductase (*FRO2*) and the Fe(II) transporter (*IRT1*), have been cloned from *Arabidopsis* and shown to be expressed in response to iron deficiency in roots [17,18]. The *IRT1* protein is the major iron transporter responsible for iron uptake from the soil [19]. In addition, iron-deficiency stress in plants results in an increase in expression of various metabolic enzymes, suggesting that iron deficiency stress may cause dramatic changes in carbon metabolism [20-23]. A recent study [24] examined changes in global gene-expression patterns in *Arabidopsis* in

response to iron deficiency using spotted cDNA microarrays. A set of 16,128 cDNA clones corresponding to at least 6,000 genes was used for the study, representing approximately one quarter of all *Arabidopsis* genes. Expression patterns were examined in roots and shoots of plants subjected to iron deficiency for 1, 3 and 7 days. The largest number of differentially expressed cDNA clones was observed after 3 days of iron deficiency. Also, although shoots showed larger numbers of induced clones as compared to repressed clones at days 3 and 7, roots showed approximately equal numbers of induced and repressed clones at day 3 and many more repressed clones at day 7 [24] (see Table 1). These results indicate that roots and shoots respond differently to iron deficiency. In addition, analysis of the data revealed a correlation between repression of photosynthetic enzymes and increasing time of exposure to iron deficiency conditions [24].

Although neither *FRO2* nor *IRT1* was represented on the microarray, two other *FRO* gene family members were found to be induced in roots exposed to iron deficiency for 1 day. In addition, ferritin expression was repressed in roots after just 1 day and in both roots and shoots after 3 and 7 days of growth on iron-deficient media [24]. Several genes that were induced by iron deficiency encode enzymes involved in glycolysis, the citric acid cycle and the oxidative pentose phosphate pathway. Other induced genes include those that encode products involved in mobilization and export of carbon in the form of sugars and starch from the shoots to the roots via the phloem [24]. Together, these results suggest that the iron-deficiency response necessitates an overall increase in respiration, and an increase in carbon import and anaerobic respiration in the roots of *Arabidopsis* plants.

Barley and rice are grasses and employ the so-called strategy II response for iron acquisition from the soil. In response to iron deficiency, the grasses secrete Fe(III) chelators called phytosiderophores. In barley, phytosiderophores belong to the mugineic acid (MA) family [25]. Fe(III)-MA complexes are transported into the root via specific plasma-membrane transporters [26]. Negishi *et al.* [27] examined changes in gene-expression profiles in response to iron deficiency in barley roots using a microarray that

Table 1

Differential expression of *Arabidopsis* cDNA clones, 1, 3 and 7 days after transfer of plants to iron deficiency

	1 day		3 days		7 days	
	Induced	Repressed	Induced	Repressed	Induced	Repressed
Shoot	143	143	2240	620	847	299
Root	84	76	776	767	142	735

Adapted from Thimm *et al.* [24].

contained 8,987 rice EST clones; this represents approximately one-third of all rice genes. (Cereals, such as barley and rice, are members of the *Poaceae* family; these species display synteny and genes of cereals show a high degree of sequence conservation at the DNA level [28], making it possible to obtain meaningful data using barley mRNA for hybridization to a rice microarray.) In addition, the authors used mRNA from barley (rather than rice) because barley is iron-efficient and secretes large amounts of MAs. Negishi *et al.* [27] showed that 200 of the 8,987 clones were induced in roots of barley plants following two weeks of growth in iron-deficient media. Thus, 2% of clones were induced in barley roots in response to iron deficiency. In comparison, Thimm *et al.* [24] saw 4.8% of clones induced in *Arabidopsis* roots after 3 days of exposure to iron-deficient growth conditions; but just 0.5% and 0.9% of clones were induced after 1 and 7 days of growth in iron-deficient media, respectively. Induced barley clones included seven previously identified as iron-deficiency-induced as well as clones that encode enzymes involved in methionine synthesis (MAs are synthesized from methionine). MA secretion from barley roots is known to follow a diurnal rhythm [29]; Negishi *et al.* [27] observed that a subset of the induced genes (50) displayed diurnal regulation of steady-state mRNA abundance, five of which encode proteins implicated in polar-vesicle transport. They thus hypothesize that polar-vesicle transport plays a role in diurnal secretion of MAs.

The recent studies discussed here have confirmed and extended our knowledge of plant responses to iron overload and iron deficiency. It is likely that the availability of the Affymetrix GeneChip® *Arabidopsis* Genome Array, coupled with improved annotation of the *Arabidopsis* genome, will prove invaluable in future studies of iron-stress responses in plants. In addition, the *Arabidopsis* community has access to large sets of T-DNA insertion-mutagenesis lines [30] along with databases of sequenced insertion sites that allow the user to order a loss-of-function (knockout) line for virtually any gene of interest [31-33]. Finally, the development of vectors for RNA interference or silencing of specific genes should allow researchers to disrupt or decrease expression of specific target genes implicated in iron-stress responses [34]. For example, the identification of loss-of-function lines should provide insight into the role of each *AtFer* gene in the plant; furthermore, functional redundancy may be uncovered through the construction of double- and triple-mutant lines. Thus, while the use of functional genomic approaches to study iron-stress responses in plants already has yielded important information, the continued development of resources promises to yield many more insights in the future. Information gained from studies of this type may allow the development of plants that are capable of growth on the one third of the world's soils that are iron-deficient or that accumulate elevated levels of bioavailable iron in aid of human nutrition.

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