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Review

Getting a sense for signals: Regulation of the plant iron deficiency response ☆

Maria N. Hindt, Mary Lou Guerinot *

Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

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ABSTRACT

Understanding the Fe deficiency response in plants is necessary for improving both plant health and the human diet, which relies on Fe from plant sources. In this review we focus on the regulation of the two major strategies for iron acquisition in plants, exemplified by the model plants *Arabidopsis* and rice. Critical to our knowledge of Fe homeostasis in plants is determining how Fe is sensed and how this signal is transmitted and integrated into a response. We will explore the evidence for an Fe sensor in plants and summarize the recent findings on hormones and signaling molecules which contribute to the Fe deficiency response. This article is part of a Special Issue entitled: Cell Biology of Metals.

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1. Introduction

Fe is essential for both plant growth and crop yields and most importantly, humans rely on dietary Fe from plant sources. According to the World Health Organization, the current most common nutritional disorder in the world is Fe deficiency, with over 30% of the world's population affected [http://www.who.int/nutrition/topics/ida/en/index.html]. Due to the limited solubility of Fe in most neutral or basic soils, there is not a readily accessible supply of Fe in the rhizosphere and plants are often limited in Fe content. Thus, increasing the ability of plants to acquire and store Fe could have significant effects on plant and human nutrition. With this goal in mind, it is important to uncover the mechanisms of how plants sense and respond to Fe availability.

When faced with Fe limitation, plants employ a set of responses to boost Fe mobilization and uptake from soil so they can ensure there is enough Fe for critical cellular processes [1]. Fe is an essential cofactor in metabolic processes such as the respiratory electron transport chain. Additionally, as photosynthetic organisms, plants require Fe for chlorophyll biosynthesis and for the reactions of photosynthesis. There are two main strategies plants use for Fe acquisition. First, Strategy I, based on reduction of Fe, is used by non-grasses such as *Arabidopsis*. Second, grasses (also known as graminaceous plants) such as rice use Strategy II, a chelation based strategy. We will briefly outline the basic components of each of these strategies and then discuss in detail the regulation of each of these strategies. For a more comprehensive overview of the two strategies as well as discussion of how our knowledge about these strategies has led to transgenic crops with enhanced tolerance to iron deficiency or with increased iron content, the reader is referred to Kobayashi and Nishizawa [2]. This review will also explore the possibilities for how Fe is sensed and how different signals are integrated into the response, with particular attention to the recent advances in the field.

2. The reduction strategy

Upon Fe limitation, plants which use the reduction strategy release protons via root plasma membrane H^+-ATPases belonging to the AHA family [3,4]. As exemplified by *Arabidopsis*, several AHAs are induced in Fe-deficient roots, but analysis of loss of function mutants suggests that AHA2 is the chief player [5]. This acidification by proton release serves to increase the solubility of Fe in the soil. One unit drop in pH increases the solubility of Fe by 1000 fold [6]. Following acidification, Fe^{3+} is reduced to Fe^{2+} by a membrane-bound ferric-chelate reductase enzyme, i.e. by AtFRO2 in *Arabidopsis* or PsFRO1 in pea [7,8]. Reduction seems to be a rate-limiting step in Fe uptake because transgenic overexpression of ferric chelate reductases in *Arabidopsis*, rice, tobacco, and soybeans increases tolerance to low iron [9–12]. The reduced form of Fe is transported into the root by the plasma-membrane divalent cation transporter IRT1 [13,14], the founding member of the ZIP family [15]. IRT1 is an essential gene because *irt1* mutants are severely chlorotic and seedling-lethal unless supplied with large amounts of exogenous Fe [16–18]. Expression of *IRT1* and *FRO2* indicates that Fe uptake occurs predominantly in epidermal layers [16,19].

Besides these physiological mechanisms, plants respond to Fe deficiency through morphological changes that result in increased root

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* Corresponding author. Tel.: +1 603 646 2527; fax: +1 603 646 1347.
E-mail address: mary.lou.guerinot@dartmouth.edu (M.L. Guerinot).

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surface area for the reduction and uptake of Fe. Examples include increased formation and branching of root hairs, root-tip swelling, and enhanced lateral root formation [20,21].

3. The chelation strategy

Grasses release phytosiderophores (PSs), such as mugineic acids (MAs), which bind Fe^{3+} with high affinity, in order to acquire Fe from the rhizosphere in Fe-limited conditions [22]. Phytosiderophores are synthesized from nicotianamine (NA), a non-proteinogenic amino acid formed by condensation of three molecules of S-adenosyl methionine. Although all plants can synthesize NA, which serves as a transition metal chelator, only the grasses go on to convert NA to PS. The chelated complexes of Fe(III)–PS are subsequently transported into the roots through yellow stripe (YS)/yellow stripe-like (YSL) family transporters, named for YS1 of maize [23,24]. For example, OsYSL15 is the major transporter responsible for Fe(III)–PS uptake in rice [25,26]. Other members of the YSL family transport metal–NA complexes in both grasses and non-grasses.

Although the biosynthetic pathway and the uptake transporters have been well studied [2], the mechanism by which PS are released remained unknown. The missing piece was recently identified: two transporters of the major facilitator superfamily (MFS), TOM1 and HvTOM1 from rice and barley respectively, were shown to be involved in the efflux of the PS deoxymugineic acid [27]. *Xenopus* oocytes expressing either transporter were able to release ^{14}C-labeled deoxymugineic acid but not ^{14}C-labeled NA, suggesting that TOM1 and HvTOM1 are PS efflux transporters.

In the same study, two other rice MFS members, ENA1 and ENA2, were identified as NA transporters by their ability to transport ^{14}C-labeled NA, but not ^{14}C-labeled deoxymugineic acid [27]. ENA1 is similar to AtZIF1, which localizes to the vacuolar membrane and was shown to be involved in Zn detoxification [28]. Although originally thought to be a Zn transporter given its localization and the zinc sensitive phenotype of an *atzif1* loss of function mutant, its similarity to ENA1 suggested that AtZIF1 might be a NA transporter. Recently, overexpression of *ZIF1* has been shown to enhance NA accumulation in vacuoles [29]. Additionally, heterologous expression of ZIF1 increases NA content in yeast cells expressing nicotianamine synthase, but does not complement a Zn-hypersensitive mutant that lacks vacuolar Zn transport activity. Similarly, ENA1 may participate in metal detoxification by transporting NA into the vacuole.

Despite being a Strategy II plant by uptake of Fe(III)–PS, rice possesses a ferrous transporter, OsIRT1, and can take up Fe^{2+} [30,31]. Evidence in support of the importance of being able to take up Fe^{2+} comes from a study of rice that cannot synthesize PS due to a mutation in the *NICOTIANAMINE AMINOTRANSFERASE (NAAT)* gene. This mutant can still grow normally when supplied with Fe^{2+} [30]. Furthermore, lines carrying a T-DNA insertion in the YSL15 transporter gene are viable. Strategy II plants, however, do not have an inducible ferric-chelate reductase activity that is a classic part of the Strategy I response. This is likely the result of an adaptation to waterlogged rice paddies, in which Fe^{2+} is more prevalent than Fe^{3+} due to reduced oxygen levels [31].

4. Regulation of the reduction strategy

There have been a number of transcriptomic and proteomic studies aimed at determining gene expression and protein profile changes upon Fe deficiency. Proteomic studies are beyond the scope of this review, but for a recent discussion of the Fe deficient protein profile in *Arabidopsis*, refer to Schmidt and Buckhout [32]. Several recent transcriptomic studies have aimed at identifying regulatory networks involved in Fe homeostasis in *Arabidopsis* [32–35]. Here, we will discuss two main networks that are currently at the forefront of the field: the FIT network and the POPEYE network. For another recent review on regulation of the reduction strategy, refer to Ivanov et al. [36]. Both Ivanov et al. [36] and the study by Long et al. [37] use co-expression analysis to show regulatory networks involved in Fe deficiency responses.

4.1. The FIT network

In *Arabidopsis*, FIT (FER-like iron-deficiency-induced transcription factor), is required for regulation of the Strategy I Fe-deficiency response [38–41]. FIT is the functional ortholog of FER, a bHLH transcription factor essential for the Fe-deficiency response in tomato [38–41]. In addition to *IRT1* and *FRO2*, *FIT* is induced in the epidermis of the root upon Fe-deficiency and like the *irt1* mutant, *fit* is seedling lethal unless watered with supplemental Fe [38]. FIT controls the Fe uptake machinery at multiple levels: *FRO2* is transcriptionally regulated by FIT, while *IRT1* is both transcriptionally and posttranscriptionally regulated by FIT [38,39]. Recent studies have examined how FIT itself is regulated [42–44]. Sivitz et al. [44] demonstrated a dual regulation of FIT by Fe starvation. At the transcriptional level, *FIT* is induced by Fe starvation and FIT protein subsequently accumulates. The second mode of regulation is post-transcriptional. Using proteasomal and translational inhibitors, they showed that in Fe-limited conditions, FIT is actively destabilized and turned over by 26S proteasomal degradation. The authors suggest that in Fe deficiency, FIT binds to its target promoters and then this “exhausted” FIT is rapidly degraded. “Fresh” FIT is synthesized to allow for subsequent transcriptional cycles and amplification of FIT target gene transcription. This idea, that a limited half life of transcriptional activators may promote continuous gene expression by delivery of “fresh” activator after “fatigued” activator is spent, has previously been described [45]. Alternatively, a more simple explanation is that the decreased stability and increased proteasomal degradation of FIT in Fe-deficient conditions is to ensure that once synthesized, FIT can be rapidly removed from the cell. The function of this would be to prevent FIT from activating gene expression when it is no longer needed (i.e. when Fe supply increases).

Regulated turnover and activity of FIT strengthen the notion that FIT is a major transcriptional regulator of the Fe deficiency response. Evidence suggests that FIT is a key regulator in integrating incoming hormonal and other intracellular signals. For example, ethylene signaling effects FIT abundance via two transcription factors in the ethylene signaling pathway [42]. This model for FIT turnover will be discussed in Section 7.1.3. Another signaling molecule, NO, has also been implicated in posttranslational regulation of FIT [43]. It is proposed to play a role in stabilization of FIT protein. These findings will be discussed with further detail in Section 7.1.5. Overall, control of FIT turnover and activity may be the major way plant roots respond to changing Fe availability in the rhizosphere and meet the nutritional demand for Fe.

Overexpression of *FIT* does not affect *FRO2* and *IRT1* expression in the root, suggesting that FIT may act with a binding partner to form a heterodimer [38–40]. qRT-PCR experiments have shown that mRNA for four additional bHLH genes (*bHLH38*, *bHLH39*, *bHLH100*, and *bHLH101*) are induced by Fe-deficiency [40,46,47]. Using bimolecular fluorescence complementation, FIT was shown to interact with both bHLH38 and bHLH39 [47]. In plants constitutively overexpressing both *FIT* and *bHLH38* or *bHLH39*, *FRO2* and *IRT1* expression are high and plants exhibit greater Fe accumulation than WT [47]. These data support a role for FIT heterodimer formation with either bHLH38 or bHLH39 for induction of the Strategy I response.

In a recent report [48], co-overexpression of *FIT* and *bHLH38* or *bHLH39* was shown to increase expression of *HMA3* and *IRT2* in addition to *MPT3* and *IREG2*/*FPN2*, which are previously published FIT targets [38]. These genes encode proteins that function in sequestration of heavy metals in vacuoles and vesicles. One result of such overexpression is increased Cd sequestration in roots and therefore less Cd

accumulation in the shoots. Additionally, the overexpression lines exhibit increased *NICOTIANAMINE SYNTHASE 1* and *NICOTIANAMINE SYNTHASE 2* (*NAS1* and *NAS2*) expression, which results in enhanced NA accumulation. The increase in NA causes an increase in transport of Fe from root to shoot. Both the increase in Cd storage in the roots and an increase in shoot Fe content may serve to alleviate Cd toxicity.

4.2. The POPEYE network

High resolution expression profiling has been used by the Benfey laboratory to report how each cell layer in the root responds to Fe deficiency [19]. Although expression of most genes related to Fe uptake are increased in the epidermal tissue and are represented by the FIT regulatory network, another set of transcriptional changes upon Fe deficiency occurs in the vasculature and may represent another transcriptional network. Two genes from this additional regulatory system have been investigated in more detail. These are the recently identified bHLH transcription factor (*bHLH047*) named *POPEYE* (*PYE*) and the putative E3-ubiquitin ligase called *BRUTUS* (*BTS*) that are both induced in Fe deficiency with their highest expression in the pericycle of root [37]. However, PYE protein was localized to the nuclei of all cells within Fe-deficient roots, suggesting that after induction by Fe-deficiency within the pericycle, PYE may move throughout the root. *pye-1* exhibits chlorosis and root growth inhibition in Fe-deficient conditions, suggesting that it is an important regulator of the Fe deficiency response.

ChIP-on-chip analysis reveals that PYE targets key genes implicated in metal homeostasis including *NAS4*, *FRO6*, and *ZIF1*, which encode a nicotianamine synthase, a plasma membrane-localized ferric chelate reductase from the same family as FRO2, and a vacuolar-localized transporter important for Zn tolerance [28,49,50]. In *pye-1*, expression of these genes is significantly increased and prolonged upon Fe deficiency, suggesting that PYE normally acts to repress the activity of these metal homeostasis genes [37].

bHLH proteins, like FIT, often form dimers that interact with downstream targets [47]. Therefore, it is likely that the bHLH protein PYE may act with a binding partner. Yeast-two-hybrid studies suggest that PYE indirectly interacts with BTS through PYE homologs, notably two additional bHLHs: ILR3 and bHLH115 [37].

Analysis of the partial loss-of-function *bts-1* mutant, which exhibits increased root length and increased rhizosphere acidification in Fe deficient conditions compared to WT, suggests that BTS is a negative regulator of the Fe deficiency response [37]. This study contains the only reported data on BTS to date and it is clear that we need more information to understand how BTS is functioning. The authors suggest that PYE, PYE homologs, and BTS form a regulatory network for maintaining Fe homeostasis in low Fe conditions, but this hypothesis requires in planta confirmation.

5. Regulation of the chelation strategy

5.1. Positive regulation by OsIRO2 and OSIDEF1/2

Using microarray expression profiling in rice, the bHLH protein *OsIRO2* was identified as a transcription factor that is upregulated upon Fe deficiency [51]. Unlike FIT/FER, of which there appears to be no counterpart in rice, IRO2 expressed in both roots and shoots in Fe deficient conditions. When *OsIRO2* is overexpressed, plants grow better than WT under Fe-deficient conditions while RNAi knocked-down *OsIRO2* plants do worse than WT. Correspondingly, the overexpression lines have enhanced expression of genes involved in PS synthesis and transport while the RNAi lines have diminished expression of the same genes. In these plants, *OsIRT1* levels remain unchanged, suggesting that OsIRO2 is responsible for regulation of PS-mediated Fe uptake, but not Fe(II) uptake. Additionally, not all genes regulated by OsIRO2 have the characteristic consensus IRO2 binding sequence (CACGTGG), so IRO2 may act by regulating other transcription factors. In another recent study, promoter-GUS lines demonstrated that the expression pattern of *OsIRO2* is very similar to downstream genes such as *OsNAS1* and *OsNAS2* in embryos during seed development and germination and in vascular bundles in roots and leaves during vegetative stages [52]. As previously reported, *OsIRO2* overexpression lines exhibited improved tolerance to Fe deficiency. Additionally, seeds accumulated higher levels of Fe compared to WT when plants were grown in alkaline soil. The results of this study suggest that *OsIRO2* is synchronously expressed with other genes involved in Fe homeostasis and plays a key role in Fe uptake and Fe transport in both germination and seed development.

OsIDEF1 and 2 (IDE binding Factor), members of the ABI3/VP1 and NAC transcription factor families, respectively, have also been identified as positive regulators of the Fe deficiency response based on their ability to bind to sequences that confer Fe regulation on the barley root-specific *IDS2* promoter [53]. These sequences are called iron-deficiency-responsive elements 1 and 2, or IDE1 and IDE2 [53]. Interestingly, sequences homologous to IDE1 are also found in other Fe deficiency inducible promoters, such as OsNAS1, OsNAS2, OsIRT1, AtIRT1, and AtFRO2. OsIDEF1 and OsIDEF2 bind to specific sequences within IDE1 and IDE2: CATGC and CA[A/C]G[T/C][T/C/A] [T/C/A], respectively. *OsIDEF1* transcripts are constitutively expressed and levels do not depend on Fe status [54]. Rice plants with RNAi knocked-down *OsIDEF1* are susceptible to early-stage Fe deficiency, while rice overexpressing *OsIDEF1* are more tolerant to Fe-deficiency [55,56]. Time course expression analysis revealed that on the onset of Fe starvation, *OsIDEF1* positively regulates the induction of several known Fe uptake and utilization genes in rice, such as *OsIRO2*, *OsYSL15*, *OsYSL2*, *OsIRT1*, *OsNAS1*, *OsNAS2*, and *OsNAS3* [55]. As Fe deficiency progressed, activation of these genes by OsIDEF1 was limited. As for *OsIDEF2*, reduction of expression by RNAi results in change in only one gene previously implicated in Fe homeostasis: *OsYSL2*, which encodes a metal–NA transporter [57]. Unlike OsIDEF1, OsIDEF2 does not appear to change its effects on downstream targets as Fe deficiency progresses [56]. These observations indicate a differential response to early and subsequent Fe deficiency. Taken together, the data suggest that OsIDEF1 may be a very upstream regulator in the Fe-deficiency response. Kobayashi et al. [58] suggest the possibility that OsIDEF1 may function to sense the cellular Fe status at the beginning of Fe deficiency. We will report their findings in Section 6 of this review.

5.2. Negative regulation by bHLH OsIRO3

Through analysis of transcriptome data from Fe-deficient rice, another bHLH TF has just been identified for its role in Fe homeostasis [59]. This TF, *OsIRO3*, is greatly induced both in root and shoot in Fe deficiency. Rice plants overexpressing *OsIRO3* were hypersensitive to Fe deficiency and accumulated less Fe in shoots than WT. Additionally, genes normally induced in Fe deficiency such as *OsNAS1*, *OsNAS2*, *OsIRO2*, *OsIRT1*, *OsYSL15*, and *OsNRAMP1* are no longer induced in *OsIRO3* overexpressing plants. Together, these results indicate that OsIRO3 is a negative regulator of the Fe deficiency response in rice and acts upstream of *OsIRO2*.

Interesting, phylogenetic analysis shows that *OsIRO3* is the most similar of three rice orthologs to *POPEYE* in *Arabidopsis* [59] (refer to Fig. 1). Both PYE and OsIRO3 seem to be involved in negatively regulating gene expression. In *pye-1*, several Fe homeostasis genes are upregulated including *bHLH39*, *bHLH101*, *OPT3*, *FRD3*, *NRAMP4* and as mentioned previously, *NAS4*, *FRO3*, and *ZIF1* [37]. For *OsIRO3*, only overexpression lines have been analyzed [59]. Knocking down *OsIRO3* in rice would allow us to more directly compare the effects of *OsIRO3* and *POPEYE*. It will be interesting to see if these two orthologous genes play similar roles in the Fe deficiency response in rice and *Arabidopsis*.

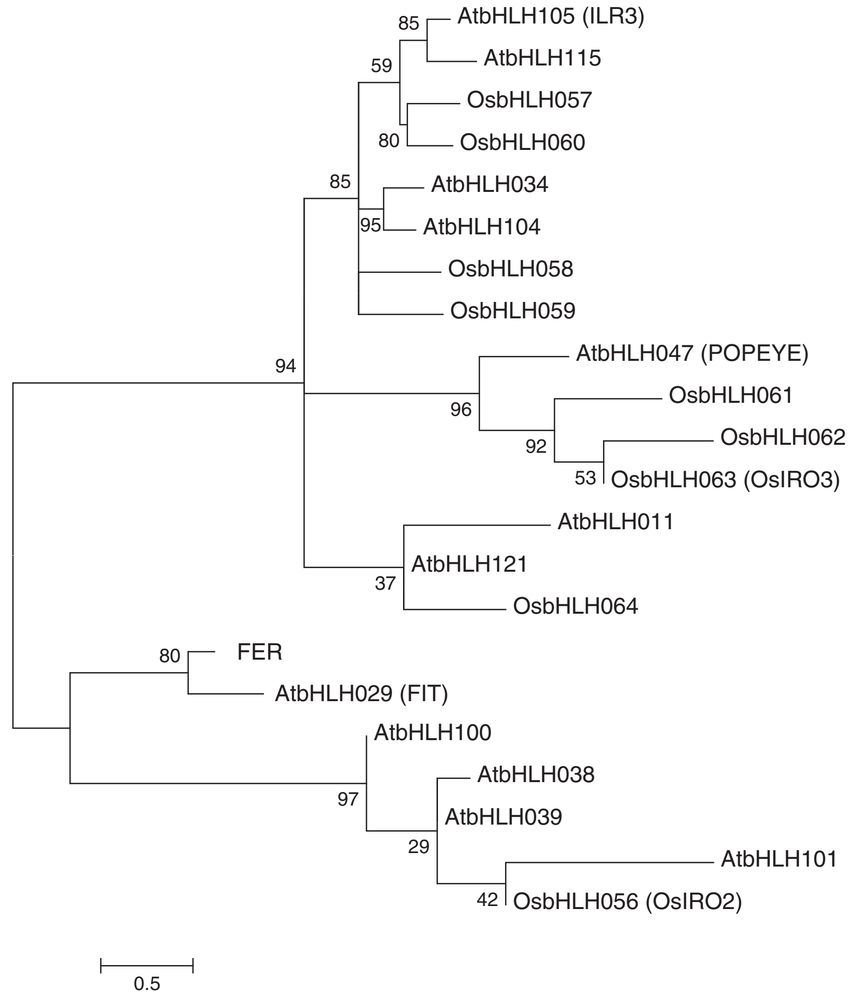

Fig. 1. Phylogenetic tree of bHLH transcription factors important for the Fe deficiency response in *Arabidopsis* and rice. The bHLH transcription factor family is greatly expanded in plants (162 bHLH genes in *Arabidopsis* and 111 in rice compared to 8 in *Chlamydomonas*; [107]) and several members are implicated in Fe homeostasis as discussed in this review. Evolutionary history was inferred using maximum likelihood analysis conducted in MEGA5 [108]. The bootstrap consensus tree displayed here was inferred from 1000 replicates. Values represent percentage of replicate trees in which the associated proteins are clustered together. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

6. The Fe sensor

Grafting and split root experiments suggest that there are both local and systemic signals involved in the Fe deficiency response in plants [60–62]. Signals must be then transmitted by a signal transduction cascade that ultimately results in transcriptional regulation of downstream effector genes of the Fe uptake system. Although there are several candidates for a long distance Fe signal in plants, including the metal chelator NA [63], the identity of a local Fe sensor remains unknown.

In other systems, there is evidence for such a master Fe sensor. For example, in many species of bacteria, the Fe-binding ferric uptake regulator (Fur) protein is responsible for Fe^{2+}-dependent transcriptional repression of Fe-regulated genes [64]. Because Fur directly binds Fe, it serves as an Fe sensor. The Fe sensor in yeast is unknown, but evidence supports a role for mitochondrial Fe–S cluster formation and cytosolic monothiol glutaredoxins in the sensing mechanism [65–67].

In mammals, Fe regulatory proteins (IRPs) govern cellular Fe homeostasis by regulation of translation and stability of mRNAs involved in Fe homeostasis [68]. In a state of low Fe, IRPs bind to Fe response elements (IREs) in the 5′ or 3′ untranslated regions of mRNA. Binding in the 5′ region causes translational repression, while binding in the 3′ region causes mRNA stabilization. A well-characterized example is the binding of IRP to the 5′ region of ferritin mRNA under low Fe conditions. This results in decreased synthesis of ferritin, which halts sequestration of Fe. In general, in low Fe conditions, IRPs bind to IREs to increase bioavailable Fe by increasing uptake and decreasing Fe utilization and storage. Conversely, when cells have excess Fe, IRPs do not bind to IREs, reducing Fe levels by decreasing uptake and increasing utilization and storage. There are two IRPs: IRP1 and IRP2. Fe governs the activity of each of these IRPs in a different manner. In Fe-sufficient conditions, Fe binding to IRP1 leads to formation of an Fe–S cluster and IRP1 acts as a cytosolic aconitase. This Fe–S cluster inhibits IRP1 from binding to IREs in mRNA. In Fe deficient conditions, IRP1 is free to act on mRNAs. IRP2, other hand, does not form an Fe–S cluster in Fe sufficient conditions but rather is ubiquitinated and degraded by the proteasome. Until the recent work of two independent labs [68,69], the identity of the E3 ligase responsible for IRP2 degradation was unknown. Each group elucidated the function of FBXL5 in a different manner. Vashisht et al. focused on uncovering new roles for mammalian F-box proteins [69]. An F-box protein is a member of an SCF (Skp1, Cullin1, and RBX1)-type E3 ligase and is responsible for tethering a target protein to an E3 ligase complex that tags a protein for ubiquitination and degradation by the proteasome. In this study, the F-box domain of FBXL5 was deleted. This deletion construct was expressed in cells and because it

could avoid degradation without an F-box domain, it was used to trap its targets. IRP1 and IRP2 were then identified by mass-spectrometry as FBXL5 binding partners. Saluheeden et al. used RNA interference to decrease expression of specific E3 ligase components in cell culture [68]. In cells treated with Fe, IRP2 degradation occurred. However, in cells lacking FBXL5 or any SCF components, IRP2 was spared from degradation.

Both groups identified a conserved hemerythrin domain in FBXL5 [68,69]. Hemerythrin domains are cation binding motifs known to bind Fe and oxygen at their center [70]. Previously hemerythrin domain-containing proteins were identified in invertebrates and bacteria, but were not known to exist in higher life forms [71]. Both groups demonstrate that binding of Fe to the hemerythrin domain stabilizes FBXL5, while lack of Fe destabilizes FBXL5, resulting in its degradation [68,69]. This leads to accumulation of IRPs and their downstream translational activity that increases Fe uptake and decrease Fe storage. Although both IRP1 and IRP2 can be targeted by FBXL5, as mentioned previously, IRP1 has the ability to form Fe–S clusters to prevent it from Fe-dependent degradation. This work has identified FBXL5 as the mammalian Fe sensor.

In plants, although it has been long sought, no Fe sensor has been identified. Fe–S cluster formation as in IRP1 has not so far been demonstrated in plants [72]. However, putative Fe-binding domains have been identified in both *Arabidopsis* and rice [37,58]. The proteins containing these domains warrant further study as they may act as local sensors that bind Fe to transmit the cellular Fe status for downstream transcriptional regulation. In *Arabidopsis*, the putative RING-type E3 ligase BTS contains hypothetical hemerythrin domains [37]. Therefore, it is likely there is an ubiquitin-dependent proteolysis system involved in Fe regulation. However, it is clear that BTS is not acting in an identical manner to FBXL5. First, BTS belongs to a different family of E3 ligases and contains 6 hemerythrin domains, rather than the one identified in FBXL5. Probably more importantly, *BTS* is induced in Fe-deficient conditions [37] whereas FBXL5 mRNA accumulation is independent of Fe status [68,69]. There are presently no published data on BTS protein accumulation as a function of Fe status, but as mentioned previously, FBXL5 is stabilized by binding of Fe to the hemerythrin domain in Fe-sufficient conditions. In Fe-deficient conditions, the hemerythrin domain and thus FBXL5 are unstable. If BTS protein accumulation corresponds to *BTS* expression in Fe-deficient conditions, then BTS and FBXL5 accumulation would be opposite for plants and mammals. If this is the case and BTS is only present in Fe-deficient conditions, it is unlikely that Fe binding can stabilize hemerythrin domains and BTS itself as it occurs with FBXL5.

The data that *bts-1* performs better in Fe-deficient conditions than does WT suggests it may be a negative regulator of the Fe deficiency response [37]. Therefore, its mode of action as an E3 ligase may be the ubiquitin-dependent degradation of proteins positively regulating the Fe-deficiency response. However, BTS' activity as an E3 ligase and its hemerythrin domains remain largely uncharacterized. Further analysis of BTS may provide important insight into how Fe status is perceived in *Arabidopsis*.

In rice, OsIDEF1 may be a candidate Fe sensor. As mentioned previously, *OsIDEF1* mRNA levels are not regulated by Fe status and OsIDEF1 positively regulates a set of Fe uptake and utilization genes early in the Fe deficiency response [55,56]. In rice, histidine-asparagine (His-Asn) repeats and proline-rich regions were identified in both OsIDEF1 and its ortholog in barley, HvIDEF1 [58]. Affinity chromatography demonstrated that these domains in OsIDEF1 bind to Fe^{2+} and other divalent metals such as Ni^{2+}, Zn^{2+}, and Cu^{2+} with no particular specificity. Some impairment of transcriptional regulation in the early Fe-deficiency response occurred as a result of deleting these regions, suggesting that OsIDEF1 binds metals and senses changes in Fe availability. The authors propose a model in which OsIDEF1 binding of metals by His-Asn and proline-rich domains exists in an equilibrium between binding Fe, binding other metals, and in a metal-free form [58]. They suggest that OsIDEF1 may detect the ratio of Fe to other metals to sense the progression of Fe deficiency. Basically, under Fe-sufficient conditions, the ratio of Fe to other metals would be high. Upon the onset of Fe deficiency, the ratio would decrease and OsIDEF1 target gene regulation may change. Subsequently, as Fe deficiency progresses, the ratio decreases further. Again, such a change may alter the influence OsIDEF1 has on its target promoters. The question remains how OsIDEF1 transmits a metal-binding signal to its downstream pathways. Deletion analysis revealed that the His-Asn repeats and proline-rich regions were not necessary for DNA-binding and trans-activation. Although these classic functions of transcription factors are unaffected, the authors suggest that regulation may occur through another mechanism such as protein modification or degradation.

7. Signaling molecules of the Fe deficiency response

While it is becoming increasingly clear that hormones and other small molecules such as nitric oxide (NO) play crucial roles in modulating gene expression and in altering root system architecture in response to changes in Fe availability, such control appears to be particularly complex: one hormone can affect the synthesis of another and there is likely to be cross talk among the intricate signaling pathways through which hormones work.

First, we will examine positive regulation of the Fe-deficiency response by auxin, ethylene, and NO, all of which are found at increased levels in Fe deficient conditions [73]. We will then review the data implicating both cytokinin and jasmonate as negative regulators of the Fe deficiency response [74,75]. Refer to Table 1 for a summary of what is known about the role of each hormone or signaling molecule in the Strategy I Fe deficiency response and Fig. 2 for a graphical model.

7.1. Positive regulators: auxin, ethylene, and NO

7.1.1. Auxin: Strategy I plants

Plant organ formation has been shown to depend on dynamic gradients of the hormone auxin [76]. Cross talk between auxin and Fe homeostasis had been suggested earlier with clear evidence that auxin plays a role in the root morphology response to Fe deficiency [77] but there have been some new important findings regarding the role of auxin in regulating the physiological response to Fe

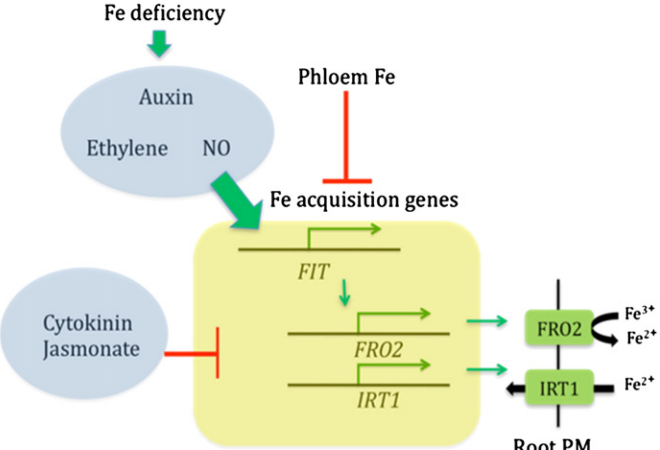

Fig. 2. Model of hormone and small molecule effects on regulation of the Strategy I Fe deficiency response. Auxin, ethylene, and NO have been implicated as positive regulators of the Fe acquisition genes *FIT*, *FRO2*, and *IRT1*, while cytokinin and jasmonate have been demonstrated to negatively regulate *FRO2* and *IRT1* in a FIT-independent manner. Fe from the phloem is thought to serve as a negative regulator of Fe deficiency gene expression.

Table 1
Summary of hormonal and small molecule signaling involvement in the Strategy I Fe deficiency response.

Signal	Regulation on *IRT1*/*FRO2*	Does action depend on FIT?	Mechanistic detail	Proposed reason for link to Fe homeostasis	References
Auxin	Positive	Yes	Fe effects AUX-1	Modification of root architecture to meet nutrient demand	[77,78]
Ethylene	Positive	Yes	FIT interacts with EIN3/EIL1	Reduction of photooxidative damage	[42,84,85,87]
Nitric oxide	Positive	Yes	NO stabilizes FIT		[43,92,93]
Cytokinin	Negative	No		Modification of root architecture to meet nutrient demand	[74]
Jasmonate	Negative	No		Stress adaptation i.e. tolerance to herbivores	[75]

deficiency. Fe deficiency leads to increased auxin synthesis in *Arabidopsis*, which results in enhanced expression of *FIT* and *FRO2* [34]. Exogenous auxin addition further stimulated transcription of these genes, while auxin inhibition decreased expression. Additionally, in an auxin overproducing mutant, *yucca*, *FIT* and *FRO2* expression were increased in Fe deficiency compared to WT [34].

The most recent connection of auxin to the Fe deficiency response was the discovery that localized Fe supply regulates lateral root growth by affecting the AUX-1 transporter mediated auxin distribution [78]. Root system architecture, such as lateral root formation, is known to depend on nutrient availability [79]. The authors wanted to better understand how nutrient signals are integrated into developmental pathways by studying how Fe affects the morphological root response [78]. Relative to homogenous Fe distribution in roots, localized Fe supply strongly induced lateral root growth. In low Fe conditions, lateral root growth was severely diminished in the *irt1* mutant, but Fe addition to shoots could rescue this effect, suggesting that symplastic Fe is responsible for triggering local lateral root elongation. The Fe-induced root development effects depended on rootward auxin flow, with a critical role for AUX1, indicated by Fe-responsive AUX1 promoter activity and failure of mutants defective in auxin transport to exhibit lateral root elongation with localized Fe. The authors propose a model in which root uptake of local Fe is mediated by IRT1, which leads to accumulation of symplastic Fe. This pool of Fe can be replenished by Fe movement from the shoot or from other parts of the root and works to establish a local Fe gradient towards Fe deficient root tissues. The local symplastic Fe pool induces AUX1 expression and this facilitates auxin transport to further induce AUX1 expression and formation and elongation of lateral roots. This model demonstrates how hormone signaling can act with developmental pathways to allow for an increase in efficiency of micronutrient acquisition.

7.1.2. Auxin: Strategy II plants

As with all plants, optimal root architecture in rice is important for efficient nutrient uptake [79] and auxin has been demonstrated to mediate developmental processes in root architecture [73]. However, the mechanism for how root architecture is controlled is largely uncharacterized. A study by Qi et al. [80] demonstrates the involvement of OsARF12, an auxin response factor. OsARF12 is a transcription factor which can facilitate the expression of DR5-GFP, an auxin-inducible promoter construct [81]. *osarf12* mutant rice plants have altered root architecture and are insensitive to auxin [80]. Additionally, in *osarf12*, Fe and other essential micronutrient content were lower than in WT and expression of *OsIRT1* was reduced. These results indicate that OsARF12 plays a role in the crosstalk between the auxin response and Fe signaling. Understanding how root architecture is altered to maximize nutrient availability in plants is important for optimizing crop ability to cope with nutrient shortages.

7.1.3. Ethylene: Strategy I plants

Ethylene, a gaseous plant hormone, has been implicated in a regulation of a diverse number of plant processes from seed germination to fruit ripening and senescence [82].

Several studies have indicated a physiological link between ethylene and Fe deficiency signaling. Upon Fe deficiency, ethylene is produced [83] and by affecting *FIT* or *FER* expression, ethylene can regulate the Fe-deficiency response in *Arabidopsis* and tomato [84,85]. Treatment of Fe-deficient *Arabidopsis* or tomato plants with ACC (an ethylene precursor) enhanced expression of *FIT* or *FER*, and consequently the *FRO* and *IRT* genes. Conversely, treatment of plants grown in low Fe with ethylene inhibitors repressed expression of these genes. Because these effects are only seen in Fe-deficient conditions, as is the case with auxin, the authors propose a model in which ethylene is an activator for *FIT* or *FER* and Fe, possibly from the phloem, acts as an inhibitor.

Until very recently, ethylene signaling and the Fe deficiency response were only physiologically linked. Lingam et al. [42] uncovered the molecular basis of this observation by showing that ethylene signaling pathway transcription factors ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 (EIN3/EIL1) directly interact with FIT, are necessary for WT-level FIT accumulation, and are important for expression of FIT downstream target genes. In accordance with previous findings, they suggest that production of ethylene upon Fe deficiency is a positive signal for FIT regulation. Subsequently, by its physical interaction with EIN3/EIL1, the proteosomal degradation of FIT is reduced, which results in an increased level of Fe acquisition gene expression. According to their model, EIN3/EIL1 do not act with FIT to induce *IRT1* and *FRO2* expression, but rather serve to increase Fe acquisition by stabilizing FIT. The authors pose the question of why FIT interacts with EIN3/EIL1 specifically and in turn what is the function of EIN3/EIL1? The authors note that among the targets of EIN3/EIL1 in Fe-deficiency are shoot-expressed genes involved in remodeling of photosynthetic machinery and response to reactive oxygen species (ROS) [86]. This type of gene expression may reflect a response to photooxidative damage [87]. Although complete support is missing for this hypothesis, EIN3/EIL1 may function to reduce photooxidative damage in Fe deficient conditions and do so simply in part by increasing Fe uptake in roots via enhanced FIT accumulation [42].

7.1.4. Ethylene: Strategy II plants

Recently, the first suggestion that ethylene is involved in Fe homeostasis in Strategy II plants comes from a study which demonstrates an involvement of ethylene in rice, the only graminaceous plant characterized which possesses a Strategy I-type system for Fe-regulation in addition to its standard Strategy II response [88]. Ethylene production of rice roots was increased when grown in Fe-deficient conditions and treatment with ACC conferred tolerance to Fe deficiency. By contrast, in another grass (barley), no induction of ethylene upon Fe deficiency or ethylene-mediated effects could be detected. Expression analysis showed that *OsIRO2*, *OsNAS1*, *OsNAS2*, *OsYSL15*, and *OsIRT1* transcript abundance was increased, demonstrating that ethylene is involved in positive regulation of both Fe(II) and Fe(III)–PS uptake systems. Analysis of an RNAi line for OsIRO2 indicates that ethylene positively regulates this transcription factor to activate the expression of *OsNAS1*, *OsNAS2*, *OsYSL15*, and interestingly, *OsIRT1*. As mentioned previously, the central transcription factor upstream of *OsIRO2* and *OsIRT1* is *OsIDEF1*. Although *OsIDEF1* is constitutively expressed [54], this study finds that *OsIDEF1* expression is slightly induced by ACC, mainly in Fe-deficient conditions [88]. It remains to be determined whether this induction is relevant to the Fe deficiency response and tolerance to Fe deficiency. The authors also suggest that ethylene may directly regulate OsIRO2 and its downstream genes by interaction with other transcription factors such as *EILS* or *EREBPs*. They report that promoters of these genes contain ethylene *cis*-acting elements, supporting this

hypothesis. If OsIRO2 indeed interacts with an EIL transcription factor as part of the involvement of ethylene in the regulation of the Fe deficiency response, this would be an analogous result to the recent interaction demonstrated between FIT and EIN3/EIL1 in *Arabidopsis* [42].

7.1.5. Nitric oxide (NO)

The signaling molecule NO has been implicated in a huge array of plant processes related to development and response to abiotic and biotic stresses [89]. Like auxin, NO has a role in controlling lateral root development [90,91]. However, NO does not usually appear to be the key player regulating these processes and more often seems to play a supportive role. Understanding how NO is connected to other hormonal networks may be a complicated task.

Like auxin and ethylene, NO is also proposed as an Fe-deficiency signaling molecule. In maize mutants defective in Fe uptake, NO can revert chlorosis [92]. In tomato, NO accumulation is triggered by Fe limitation [93]. As with auxin and ethylene, NO is a positive regulator of Fe uptake. Application of a NO donor to Fe-deficient tomato roots induces *FRO1*, *IRT1*, and *FER*, while NO scavenger treatment leads to repression of these same genes.

Very recently, NO has also been identified as a stabilizing stimulus for FIT protein abundance [43]. Application of NO inhibitors caused a decrease in FIT protein levels and FIT activity. This effect was reversed by using a proteasome inhibitor, supporting the hypothesis that NO works to decrease proteosomal degradation of FIT. At this point it is unknown if NO is involved in stabilizing and activating FIT directly or indirectly. The authors suggest that a direct role for NO could be the nitrosylation of Cys residues present in FIT. Indeed, *S-nitrosylation* of protein thiol residues has been shown to be an important mechanism for transduction of NO bioactivity [94–96]. It will be interesting to see if this modification occurs with FIT.

7.1.6. Interplay between auxin, ethylene, and NO

Because we know that an increase in auxin, ethylene, and NO all precede induction of Fe acquisition genes, the question that follows is which signaling molecule acts upstream of the other or whether they act in concert. Chen et al. [34] present a model in which, upon Fe deficiency, an increase in auxin acts upstream to induce NO which in turn acts to upregulate Fe acquisition machinery. Support for this model comes from the fact that FCR activity, NO level, and expression of *FIT* and *FRO2* are increased in an auxin-over producing mutant while dramatically decreased in an auxin transport mutant. These results indicate that auxin acts upstream of NO.

As for ethylene and NO, recent work suggests these two signaling molecules work together to regulate the Fe-deficiency response. Garcia et al. [97] aimed to study the role of ethylene and NO in the expression of genes in *Arabidopsis* up-regulated in Fe deficiency beyond the three previously known genes; *FIT*, *IRT1*, and *FRO2*. By the use of microarray analysis and RT-PCR to determine which genes are both upregulated by Fe deficiency, repressed by ethylene inhibitors, and responsive to NO, this study extends the role of ethylene to 16 new genes. Notably, this list includes known Fe homeostasis genes such as *bHLH38*, *bHLH39*, *NAS1*, and *NAS2*. It is clear from this work that NO and ethylene upregulate many of the same genes.

Garcia et al. [98] point out that previous studies demonstrate that NO enhances ethylene production, but they wondered if the converse was true. Indeed, they found this to be the case. Additionally, they show that the action of ethylene and NO in Fe homeostasis requires low Fe and thus Fe sufficiency serves as an inhibitory signal on these processes. They propose a model for Strategy I plants in which under Fe deficiency (and the absence of Fe likely from the phloem), roots increase production of ethylene and NO, with each one positively influencing the production of the other. They propose that this activity generates an activating signal for the transcription of Fe acquisition genes.

7.2. Negative regulators

7.2.1. Cytokinin

Cytokinins, or CKs, are hormones which are known to control growth and developmental processes like cell proliferation, seed germination, and nutrient mobilization [99]. In addition to their negative effect on the Fe deficiency response, which we will discuss here, CKs have been shown to inhibit responses to nitrate, phosphate, and sulfate deficiencies in *Arabidopsis* [100–102].

CKs have the opposite effect as the positive regulators of the Fe deficiency response we have mentioned so far. Addition of exogenous cytokinin results in repression of *IRT1*, *FRO2*, and *FIT* which depends on cytokinin receptors [74]. To determine if *FIT* was responsible for mediating the CK-dependent downregulation of *IRT1* and *FRO2*, *IRT1* and *FRO2* levels were examined in response to CK in WT and in the *fit1* mutant. In WT, *IRT1* mRNA is reduced by only two-fold in Fe-deficient conditions, while *FRO2* is almost completely repressed. Both genes are further repressed with CK treatment. In *fit-1*, CK treatment strongly represses both *IRT1* and *FRO2*, as in WT, suggesting that FIT is not necessary for CK-mediated *IRT1* repression. Therefore, the CKs must act through a distinct signaling pathway from FIT.

CK treatment results in decreased primary root elongation [74]. Thus, it is suggested that altered root growth due to CK treatment may be a signal for the plant to limit root nutrient uptake due to the decrease in demand. Indeed, this paper shows that root growth inhibitory conditions, caused either by hormonal control or osmotic stress, lead to repression of *IRT1* expression regardless of Fe supply. Therefore, the authors propose that CKs control root Fe uptake machinery in a growth-dependent manner to achieve a level of nutrient uptake that fits the demand of the plant.

7.2.2. Jasmonate

Jasmonates are oxylipin-based mobile plant hormones which act systemically and belong to a category of hormones called stress hormones that are known to act in response to various stimuli; i.e. wounding, insect attack, or UV light [103,104].

Maurer et al. [75] showed that jasmonate is a negative regulator of Fe deficiency gene expression: *FRO2*, *IRT1*, and *FIT* were downregulated by application of methyl-jasmonate. Additionally, in mutants defective in jasmonate signaling activity, *IRT1* and *FRO2* mRNA levels were greater than in WT in Fe-deficient conditions. To determine if the jasmonate-mediated effects on the Fe-deficiency response depend on FIT, jasmonate treatments in the *fit* mutant were analyzed. In the mutant, though not to the degree as in WT, *FRO2* and *IRT1* are still partially induced in Fe-deficiency. However, upon jasmonate treatment, this partial induction is lost, with *FRO2* and *IRT1* gene expression at very low levels. These results show that, like with cytokinin, jasmonate inhibition of *FRO2* and *IRT1* is not dependent on *FIT*. The authors thus suggest that jasmonate serves as a subtle inhibitor for fine-tuning the Fe deficiency response, but does not systemically down-regulate Fe deficiency. Thus, the question arises as to what is the function of the jasmonate-mediated inhibition of Fe acquisition response. Since jasmonate belongs to the stress hormones, one possibility is that inhibition of acquisition mediated by jasmonate might be the result of a specific stress adaptation. One example given is the ability of jasmonate to confer tolerance to insect herbivores and necrotic pathogens. It is suggested that decreasing Fe uptake may aid to reduce cell death because necrosis involves Fe-dependent enzymes. As Maurer et al. mention [75], it is important to note that the positive regulators of Fe uptake, ethylene and NO, have been suggested as potential antagonists of jasmonate signaling [105,106]. Clearly, the Fe deficiency response is controlled by a series of complex interactions among hormones.

8. Conclusion

In the past several years, there has been significant progress in understanding how the Fe deficiency response in plants is controlled. Here we have described the key players of the major transcriptional networks that control Fe homeostasis in both grasses and non-grasses. It is evident that within these networks, there are multiple levels of control, i.e. transcriptional and post-transcriptional. A major goal in the field of Fe homeostasis in plants will be to uncover a potential master Fe sensor. We need to understand how the Fe status of a plant is sensed and how this signal is in turn relayed to the transcriptional networks controlling genes for Fe acquisition.

We have also summarized some of the major recent findings of how hormones and other signaling molecules are tied to the Fe deficiency pathway. Auxin, NO, ethylene, cytokinin, jasmonate and other signaling molecules not characterized or mentioned in this review may act in linear pathways and/or in a concerted manner. Further characterization and understanding of how these signals are involved in both the Fe deficiency response and other plant physiological processes will allow us to elucidate how they are integrated into a broader pathway.

The work of this field will help us achieve our overarching goals of improving plant growth and crop yields in less than optimal soils and addressing the problem of human Fe deficiency. We seek to achieve a food-based solution to this problem because it offers a sustainable approach to solving malnutrition.

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References

[1] N. Grotz, M.L. Guerinot, Molecular aspects of Cu, Fe and Zn homeostasis in plants, Biochim. Biophys. Acta 1763 (2006) 595–608.
[2] T. Kobayashi, N.K. Nishizawa, Iron uptake, translocation, and regulation in higher plants, Annu. Rev. Plant Biol. 63 (2012) 1–22.
[3] S. Santi, S. Cesco, Z. Varanini, R. Pinton, Two plasma membrane H+-ATPase genes are differentially expressed in iron-deficient cucumber plants, Plant Physiol. Biochem. 43 (2005) 287–292.
[4] S. Santi, W. Schmidt, Laser microdissection-assisted analysis of the functional fate of iron deficiency-induced root hairs in cucumber, J. Exp. Bot. 59 (2008) 697–704.
[5] S. Santi, W. Schmidt, Dissecting iron deficiency-induced proton extrusion in *Arabidopsis* roots, New Phytol. 183 (2009) 1072–1084.
[6] M.L. Guerinot, Y. Yi, Iron: nutritious, noxious, and not readily available, Plant Physiol. 104 (1994) 815–820.
[7] N.J. Robinson, C.M. Procter, E.L. Connolly, M.L. Guerinot, A ferric-chelate reductase for iron uptake from soils, Nature 397 (1999) 694–697.
[8] B.M. Waters, D.G. Blevins, D.J. Eide, Characterization of FRO1, a pea ferric-chelate reductase involved in root iron acquisition, Plant Physiol. 129 (2002) 85–94.
[9] E.L. Connolly, N. Campbell, N. Grotz, C.L. Prichard, M.L. Guerinot, Overexpression of the FRO2 iron reductase confers tolerance to growth on low iron and uncovers post-transcriptional control, Plant Physiol. 133 (2003) 1102–1110.
[10] Y. Ishimaru, S.A. Kim, T. Tsukamoto, H. Oki, T. Kobayashi, S. Watanabe, S. Matsuhashi, M. Takahashi, H. Nakanishi, S. Mori, N.K. Nishizawa, Mutational reconstructed ferric chelate reductase confers enhanced tolerance in rice to iron deficiency in calcareous soil, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7373–7378.
[11] H. Oki, S. Kim, H. Nakanishi, M. Takahashi, H. Yamaguchi, S. Mori, N.K. Nishizawa, Directed evolution of yeast ferric reductase to produce plants with tolerance to iron deficiency in alkaline soils, Soil Sci. Plant Nutr. 50 (2004) 1159–1165.
[12] M. Vasconcelos, H. Eckert, V. Arahana, G. Graef, M.A. Grusak, T. Clemente, Molecular and phenotypic characterization of transgenic soybean expressing the *Arabidopsis* ferric chelate reductase gene, *FRO2*, Planta 224 (2006) 1116–1128.
[13] D. Eide, M. Broderius, J. Fett, M.L. Guerinot, A novel iron-regulated metal transporter from plants identified by functional expression in yeast, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5624–5628.
[14] U. Eckhardt, A.M. Marques, T.J. Buckhout, Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants, Plant Mol. Biol. 45 (2001) 437–448.
[15] M.L. Guerinot, The ZIP family of metal transporters, Biochim. Biophys. Acta 1465 (2000) 190–198.
[16] G. Vert, N. Grotz, F. Dedaldechamp, F. Gaymard, M.L. Guerinot, J.-F. Briat, C. Curie, IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and plant growth, Plant Cell 14 (2002) 1223–1233.
[17] C. Varotto, D. Maiwald, P. Pesaresi, P. Jahns, S. Francesco, D. Leister, The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*, Plant J. 31 (2002) 589–599.
[18] R. Henriques, J. Jásik, M. Klein, E. Martinoia, U. Feller, J. Schell, M.S. Pais, C. Koncz, Knock-out of *Arabidopsis* metal transporter gene *IRT1* results in iron deficiency accompanied by cell differentiation defects, Plant Mol. Biol. 50 (2002) 587–597.
[19] J.R. Dinneny, T.A. Long, J.Y. Wang, J.W. Jung, D. Mace, S. Pointer, C. Barron, S.M. Brady, J. Schiefelbein, P.N. Benfey, Cell identity mediates the response of *Arabidopsis* roots to abiotic stress, Science 320 (2008) 942–945.
[20] W. Schmidt, Mechanisms and regulation of reduction-based iron uptake in plants, New Phytol. 141 (1999) 1–26.
[21] M. Muller, W. Schmidt, Environmentally induced plasticity of root hair development in *Arabidopsis*, Plant Physiol. 134 (2004) 409–419.
[22] S.S. Conte, E.L. Walker, Transporters contributing to iron trafficking in plants, Mol. Plant 4 (2011) 464–476.
[23] C. Curie, Z. Panaviene, C. Loulergue, S.L. Dellaporta, J.-F. Briat, E.L. Walker, Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake, Nature 409 (2001) 346–349.
[24] C. Curie, G. Cassin, D. Counch, F. Divol, K. Higuchi, M. Le Jean, J. Misson, A. Schikora, P. Czernic, S. Mari, Metal movement within the plant: contribution of nictotianamine and yellow stripe 1-like transporters, Ann. Bot. 103 (2009) 1–11.
[25] S. Lee, J.C. Chiecko, S.A. Kim, E.L. Walker, Y. Lee, M.L. Guerinot, G. An, Disruption of OsYSL15 leads to iron inefficiency in rice plants, Plant Physiol. 150 (2009) 786–800.
[26] H. Inoue, T. Kobayashi, T. Nozoye, M. Takahashi, Y. Kakei, K. Suzuki, M. Nakazono, H. Nakanishi, S. Mori, N.K. Nishizawa, Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings, J. Biol. Chem. 284 (2009) 3470–3479.
[27] T. Nozoye, S. Nagasaka, T. Kobayashi, M. Takahashi, Y. Sato, Y. Sato, N. Uozumi, H. Nakanishi, N.K. Nishizawa, Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants, J. Biol. Chem. 286 (2011) 5446–5454.
[28] M.J. Haydon, C.S. Cobbett, A novel major facilitator superfamily protein at the tonoplast influences zinc tolerance and accumulation in *Arabidopsis*, Plant Physiol. 143 (2007) 1705–1719.
[29] M.J. Haydon, M. Kawachi, M. Wirtz, S. Hillmer, R. Hell, U. Kramer, Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in *Arabidopsis*, Plant Cell 2 (2012) 724–737.
[30] L. Cheng, F. Wang, H. Shou, F. Huang, L. Zheng, F. He, J. Li, F.J. Zhao, D. Ueno, J.F. Ma, P. Wu, Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice, Plant Physiol. 145 (2007) 1647–1657.
[31] Y. Ishimaru, M. Suzuki, T. Tsukamoto, K. Suzuki, M. Nakazono, T. Kobayashi, Y. Wada, S. Watanabe, S. Matsuhashi, M. Takahashi, H. Nakanishi, S. Mori, N.K. Nishizawa, Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}, Plant J. 45 (2006) 335–346.
[32] W. Schmidt, T.J. Buckhout, A hitchhiker's guide to the *Arabidopsis* ferrome, Plant Physiol. Biochem. 49 (2011) 462–470.
[33] R.J. Stein, B.M. Waters, Use of natural variation reveals core genes in the transcriptome of iron-deficient *Arabidopsis thaliana* roots, J. Exp. Bot. 63 (2011) 1039–1055.
[34] W.W. Chen, J.L. Yang, C. Qin, C.W. Jin, J.H. Mo, T. Ye, S.J. Zheng, Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis*, Plant Physiol. 154 (2010) 810–819.
[35] M. Schuler, A. Keller, C. Backes, K. Philippar, H.P. Lenhof, P. Bauer, Transcriptome analysis by GeneTrail revealed regulation of functional categories in response to alterations of iron homeostasis in *Arabidopsis thaliana*, BMC Plant Biol. 11 (2011) 87.
[36] R. Ivanov, T. Brumbarova, P. Bauer, Fitting into the harsh reality: regulation of iron-deficiency responses in dicotyledonous plants, Mol. Plant 5 (2011) 27–42.
[37] T.A. Long, H. Tsukagoshi, W. Busch, B. Lahner, D.E. Salt, P.N. Benfey, The bHLH transcription factor POPEYE regulates response to iron deficiency in *Arabidopsis* roots, Plant Cell 22 (2010) 2219–2236.
[38] E.P. Colangelo, M.L. Guerinot, The essential bHLH protein FIT1 is required for the iron deficiency response, Plant Cell 16 (2004) 3400–3412.
[39] M. Jakoby, H.-Y. Wang, W. Reidt, B. Weissharr, P. Bauer, FRU(BHLH029) is required for induction of iron mobilization genes in *Arabidopsis thaliana*, FEBS Lett. 577 (2004) 528–534.
[40] Y.X. Yuan, J. Zhang, D.W. Wang, H.Q. Ling, AtbHLH29 of *Arabidopsis thaliana* is a functional ortholog of tomato FER involved in controlling iron acquisition in Strategy I plants, Cell Res. 15 (2005) 613–621.
[41] H.-Q. Ling, P. Bauer, Z. Bereczky, B. Keller, M. Ganal, The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in roots, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13938–13943.

[42] S. Lingam, J. Mohrbacher, T. Brumbarova, T. Potuschak, C. Fink-Straube, E. Blondet, P. Genschik, P. Bauer, Interaction between the bHLH transcription factor FIT and ETHYLENE INSENSITIVE3/ETHYLENE INSENSITIVE3-LIKE1 reveals molecular linkage between the regulation of iron acquisition and ethylene signaling in *Arabidopsis*, Plant Cell 23 (2011) 1815–1829.

[43] J. Meiser, S. Lingam, P. Bauer, Posttranslational regulation of the iron deficiency basic helix-loop-helix transcription factor FIT is affected by iron and nitric oxide, Plant Physiol. 157 (2011) 2154–2166.

[44] A. Sivitz, C. Grinvalds, M. Barberon, C. Curie, G. Vert, Proteasome-mediated turnover of the transcriptional activator FIT is required for plant iron-deficiency responses, Plant J. 66 (2011) 1044–1052.

[45] G.A. Collins, W.P. Tansey, The proteasome: a utility tool for transcription? Curr. Opin. Genet. Dev. 16 (2006) 197–202.

[46] H.-Y. Wang, M. Klatte, M. Jacoby, H. Baumlein, B. Weisshaar, P. Bauer, Iron deficiency-mediated stress regulation of four subgroup Ib bHLH genes in *Arabidopsis thaliana*, Planta 226 (2007) 897–908.

[47] Y. Yuan, H. Wu, N. Wang, J. KLi, W. Zhao, J. Du, D. Wang, H.-Q. Ling, FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in *Arabidopsis*, Cell Res. 18 (2008) 385–397.

[48] H. Wu, C. Chen, J. Du, H. Liu, Y. Cui, Y. Zhang, Y. He, J. Li, Z. Feng, Y. Wang, C. Chu, H.Q. Ling, Co-overexpression FIT with AtbHLH38 or AtbHLH39 in *Arabidopsis* enhanced cadmium tolerance via increased cadmium sequestration in roots and improved iron homeostasis of shoots, Plant Physiol. 158 (2011) 790–800.

[49] C. Curie, G. Cassin, D. Couch, F. Divol, K. Higuchi, M. Le Jean, J. Misson, A. Schikora, P. Czernic, S. Mari, Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters, Ann. Bot. 103 (2009) 1–11.

[50] J. Jeong, C. Cohu, L. Kerkeb, M. Piln, E.L. Connolly, M.L. Guerinot, Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 10619–10624.

[51] Y. Ogo, R.N. Itai, H. Nakanishi, H. Inoue, T. Kobayashi, M. Suzuki, M. Takahashi, S. Mori, N.K. Nishizawa, Isolation and characterization of IRO2, a novel iron regulated bHLH transcription factor in graminaceous plants, J. Exp. Bot. 57 (2006) 2867–2878.

[52] Y. Ogo, R.N. Itai, T. Kobayashi, M.S. Aung, H. Nakanishi, N.K. Nishizawa, OsIRO2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil, Plant Mol. Biol. 75 (2011) 593–605.

[53] T. Kobayashi, Y. Nakayama, R.N. Itai, H. Nakanishi, T. Yoshihara, S. Mori, N.K. Nishizawa, Identification of novel cis-acting elements, IDE1 and IDE2, of the barley IDS2 gene promoter conferring iron-deficiency-inducible, root-specific expression in heterologous tobacco plants, Plant J. 36 (2003) 780–793.

[54] T. Kobayashi, Y. Ogo, R.N. Itai, H. Nakanishi, M. Takahashi, S. Mori, N.K. Nishizawa, The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants, Proc. Natl. Acad. Sci. U. S. A. (2007) 19150–19155.

[55] T. Kobayashi, R.N. Itai, Y. Ogo, Y. Kakei, H. Nakanishi, M. Takahashi, N.K. Nishizawa, The rice transcription factor IDEF1 is essential for the early response to iron deficiency, and induces vegetative expression of late embryogenesis abundant genes, Plant J. 60 (2009) 948–961.

[56] T. Kobayashi, Y. Ogo, M.S. Aung, T. Nozoye, R.N. Itai, H. Nakanishi, T. Yamakawa, N.K. Nishizawa, The spatial expression and regulation of transcription factors IDEF1 and IDEF2, Ann. Bot. 105 (2010) 1109–1117.

[57] Y. Ogo, T. Kobayashi, R. Nakanishi Itai, H. Nakanishi, Y. Kakei, M. Takahashi, S. Toki, S. Mori, N.K. Nishizawa, A novel NAC transcription factor, IDEF2, that recognizes the iron deficiency-responsive element 2 regulates the genes involved in iron homeostasis in plants, J. Biol. Chem. 283 (2008).

[58] T. Kobayashi, R.N. Itai, M.S. Aung, T. Senoura, H. Nakanishi, N.K. Nishizawa, The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status, Plant J. 69 (2012) 81–91.

[59] L. Zheng, Y. Ying, L. Wang, F. Wang, J. Whelan, H. Shou, Identification of a novel iron regulated basic helix-loop-helix protein involved in Fe homeostasis in *Oryza sativa*, BMC Plant Biol. 10 (2011) 166.

[60] A. Schikora, W. Schmidt, Iron stress-induced changes in root epidermal cell fate are regulated independently from physiological responses to low iron availability, Plant Physiol. 125 (2001) 1679–1687.

[61] M.A. Grusak, S. Pezeshgi, Shoot-to-root signal transmission regulates root Fe(III) reductase activity in the *dgl* mutant of pea, Plant Physiol. 110 (1996) 329–334.

[62] G. Vert, J.-F. Briat, C. Curie, Dual regulation of the *Arabidopsis* high affinity root iron uptake system by local and long-distance signals, Plant Physiol. 132 (2003) 796–804.

[63] C. Curie, J.-F. Briat, Iron transport and signaling in plants, Annu. Rev. Plant Biol. 54 (2003) 183–206.

[64] B.M. Carpenter, J.M. Whitmire, D.S. Merrell, This is not your mother's repressor: the complex role of Fur in pathogenesis, Infect. Immun. 77 (2009) 2590–2601.

[65] O.S. Chen, R.J. Crisp, M. Valachovic, M. Bard, D.R. Winge, J. Kaplan, Transcription of the yeast iron regulon does not respond directly to iron but rather to iron–sulfur cluster biosynthesis, J. Biol. Chem. 279 (2004) 29513–29518.

[66] A. Kumanovics, O.S. Chen, L. Li, D. Bagley, E.M. Adkins, H. Lin, N.N. Dingra, C.E. Outten, G. Keller, D. Winge, D.M. Ward, J. Kaplan, Identification of *FRA1* and *FRA2* as genes involved in regulating the yeast iron regulon in response to decreased mitochondrial iron–sulfur cluster synthesis, J. Biol. Chem. 283 (2008) 10276–10286.

[67] J.C. Rutherford, L. Ojeda, J. Balk, U. Mulenhoff, R. Lill, D.R. Winge, Activation of the iron regulon by yeast Aft1/Aft2 transcription factors depends on mitochondrial but not cytosolic iron–sulfur protein biogenesis, J. Biol. Chem. 280 (2005) 10135–10140.

[68] A.A. Salahudeen, J.W. Thompson, J.C. Ruiz, H.W. Ma, L.N. Kinch, Q. Li, N.V. Grishin, R.K. Bruick, An E3 ligase possessing an iron-responsive hemerythrin domain is a regulator of iron homeostasis, Science 326 (2009) 722–726.

[69] A.A. Vashisht, K.B. Zumbrennen, X. Huang, D.N. Powers, A. Durazo, D. Sun, N. Bhaskaran, A. Persson, M. Uhlen, O. Sangfelt, C. Spruck, E.A. Leibold, J.A. Wohlschlegel, Control of iron homeostasis by an iron-regulated ubiquitin ligase, Science 326 (2009) 718–721.

[70] S. Sheriff, W.A. Hendrickson, J.L. Smith, Structure of myohemerythrin in the azidomet state at 1.7/1.3 A resolution, J. Mol. Biol. 197 (1987) 273–296.

[71] R.E. Stenkamp, L.C. Sieker, L.H. Jensen, J.D. McCallum, J. Sanders-Loehr, Active site structures of deoxyhemerythrin and oxyhemerythrin, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 713–716.

[72] N. Arnaud, K. Ravet, A. Borlotti, B. Touraine, J. Boucherez, J.F. Briat, F. Cellier, F. Gaymard, The iron responsive element (IRE)/iron regulatory protein1 (IRP1)-cytosolic aconitase iron-regulatory switch does not operate in plants, Biochem. J. 405 (2007) 523–531.

[73] F.J. Romera, M.J. Garcia, E. Alcantara, R. Perez-Vicente, Latest findings about the interplay of auxin, ethylene and nitric oxide in the regulation of Fe deficiency responses by Strategy I plants, Plant Signal. Behav. 6 (2011) 167–170.

[74] M. Seguela, J.-F. Briat, G. Vert, C. Curie, Cytokinins negatively regulate the root iron uptake machinery in *Arabidopsis* through a growth-dependent pathway, Plant J. 55 (2008) 289–300.

[75] F. Maurer, S. Mueller, P. Bauer, Suppression of Fe deficiency gene expression by jasmonate, Plant Physiol. Biochem. 49 (2011).

[76] E. Benkova, M. Michniewicz, M. Sauer, T. Teichmann, D. Seifertova, G. Jurgens, J. Friml, Local, efflux-dependent auxin gradients as a common module for plant organ formation, Cell 115 (2003) 591–602.

[77] W. Schmidt, J. Tittel, A. Schikora, Role of hormones in the induction of iron deficiency responses in *Arabidopsis* roots, Plant Physiol. 122 (2000) 1109–1118.

[78] R.F. Giehl, J.E. Lima, N. von Wiren, Localized iron supply triggers lateral root elongation in *Arabidopsis* by altering the AUX1-mediated auxin distribution, Plant Cell 1 (2012) 33–49.

[79] J. Lopez-Bucio, A. Cruz-Ramirez, L. Herrera-Estrella, The role of nutrient availability in regulating root architecture, Curr. Opin. Plant Biol. 6 (2003) 280–287.

[80] Y. Qi, S. Wang, C. Shen, S. Zhang, Y. Chen, Y. Xu, Y. Liu, Y. Wu, D. Jiang, OsARF12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (*Oryza sativa*), New Phytol. 193 (2011) 109–120.

[81] T. Ulmasov, G. Hagen, T.J. Guilfoyle, ARF1, a transcription factor that binds to auxin response elements, Science 276 (1997) 1865–1868.

[82] Q. Zhao, H.W. Guo, Paradigms and paradox in the ethylene signaling pathway and interaction network, Mol. Plant 4 (2010) 626–634.

[83] F.J. Romera, E. Alcantara, Ethylene involvement in the regulation of Fe-deficiency stress responses by Strategy I plants, Funct. Plant Biol. 31 (2004) 315–328.

[84] C. Lucena, B.M. Waters, F.J. Romera, M.J. Garcia, M. Morales, E. Alcantara, R. Perez-Vicente, Ethylene could influence ferric reductase, iron transporter, and H^+-ATPase gene expression by affecting *FER* (or *FER-like*) gene activity, J. Exp. Bot. 57 (2006) 4145–4154.

[85] B.M. Waters, C. Lucena, F.J. Romera, G.G. Jester, A.N. Wynn, C.L. Rojas, E. Alcántara, R. Pérez-Vicente, Ethylene involvement in the regulation of the H(+)-ATPase *CsHA1* gene and of the new isolated ferric reductase *CsFRO1* and iron transporter *CsIRT1* genes in cucumber plants, Plant Physiol. Biochem. 45 (2007) 293–301.

[86] J.L. Moseley, T. Allinger, S. Herzog, P. Hoerth, E. Wehinger, S. Merchant, M. Hippler, Adaptation to Fe-deficiency requires remodeling of the photosynthetic apparatus, EMBO J. 21 (2002) 6709–6720.

[87] C. Triantaphylides, M. Havaux, Singlet oxygen in plants: production, detoxification and signaling, Trends Plant Sci. 14 (2009) 219–228.

[88] J. Wu, C. Wang, L. Zheng, L. Wang, Y. Chen, J. Whelan, H. Shou, Ethylene is involved in the regulation of iron homeostasis by regulating the expression of iron-acquisition-related genes in *Oryza sativa*, J. Exp. Bot. 62 (2011) 667–674.

[89] D. Wendehenne, J. Durner, D.F. Klessig, Nitric oxide: a new player in plant signalling and defence responses, Curr. Opin. Plant Biol. 7 (2004) 449–455.

[90] N. Correa-Aragunde, M. Graziano, L. Lamattina, Nitric oxide plays a central role in determining lateral root development in tomato, Planta 218 (2004) 900–905.

[91] A. Mendez-Bravo, J. Raya-Gonzalez, L. Herrera-Estrella, J. Lopez-Bucio, Nitric oxide is involved in alkamide-induced lateral root development in *Arabidopsis*, Plant Cell Physiol. 51 (2010) 1612–1626.

[92] M. Graziano, L. Lamattina, Nitric oxide and iron in plants: an emerging and converging story, Trends Plant Sci. 10 (2005) 4–8.

[93] M. Graziano, L. Lamattina, Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots, Plant J. 52 (2007) 949–960.

[94] C. Lindermayr, J. Durner, S-nitrosylation in plants: pattern and function, J. Proteomics 73 (2009) 1–9.

[95] J. Astier, S. Rasul, E. Koen, H. Manzoor, A. Besson-Bard, O. Lamotte, S. Jeandroz, J. Durner, C. Lindermayr, D. Wendehenne, S-nitrosylation: an emerging post-translational protein modification in plants, Plant Sci. 181 (2011) 527–533.

[96] M.C. Terrile, R. Paris, L.I. Calderon-Villalobos, M.J. Iglesias, L. Lamattina, M. Estelle, C.A. Casalongue, Nitric oxide influences auxin signaling through S-nitrosylation of the *Arabidopsis* transport inhibitor response 1 auxin receptor, Plant J. (2011), doi: 10.1111/j.1365-313X.2011.04885.x.

[97] M.J. Garcia, C. Lucena, F.J. Romera, E. Alcantara, R. Perez-Vicente, Ethylene and nitric oxide involvement in the up-regulation of key genes related to iron acquisition and homeostasis in *Arabidopsis*, J. Exp. Bot. 61 (2010) 3885–3899.

[98] M.J. Garcia, V. Suarez, F.J. Romera, E. Alcantara, R. Perez-Vicente, A new model involving ethylene, nitric oxide and Fe to explain the regulation of Fe-acquisition genes in Strategy I plants, Plant Physiol. Biochem. 49 (2011) 537–544.

[99] H. Sakakibara, Cytokinins: activity, biosynthesis, and translocation, Annu. Rev. Plant Biol. 57 (2006) 431–449.

[100] W.G. Brenner, G.A. Romanov, I. Kollmer, L. Burkle, T. Schmulling, Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades, Plant J. 44 (2005) 314–333.
[101] J.M. Franco-Zorrilla, A.C. Martin, A. Leyva, J. Paz-Ares, Interaction between phosphate-starvation, sugar, and cytokinin signaling in *Arabidopsis* and the roles of cytokinin receptors CRE1/AHK4 and AHK3, Plant Physiol. 138 (2005) 847–857.
[102] A. Maruyama-Nakashita, Y. Nakamura, T. Yamaya, H. Takahashi, A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation, Plant J. 38 (2004) 779–789.
[103] J. Shah, Plants under attack: systemic signals in defence, Curr. Opin. Plant Biol. 12 (2009) 459–464.
[104] C. Wasternack, E. Kombrink, Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development, ACS Chem. Biol. 5 (2010) 63–77.
[105] E. Adams, J. Turner, COI1, a jasmonate receptor, is involved in ethylene-induced inhibition of *Arabidopsis* root growth in the light, J. Exp. Bot. 61 (2011) 4373–4386.
[106] M.L. Orozco-Cardenas, C.A. Ryan, Nitric oxide negatively modulates wound signaling in tomato plants, Plant Physiol. 130 (2002) 487–493.
[107] A. Feller, K. Machemer, E.L. Braun, E. Grotewold, Evolutionary and comparative analysis of MYB and bHLH plant transcription factors, Plant J. 66 (2011) 94–116.
[108] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.

Web reference

http://www.who.int/nutrition/topics/ida/en/index.html. Last accessed 30 Jan 2012.