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Molecular Regulation of Iron Homeostasis in Plants

Nathalie Berger, Agustin J Marin, Max J J Stassen, Tiago Lourenço, Meijie Li, Shunsuke Watanabe, Herlander Azevedo, Pedro Humberto Castro, Ioannis A Stringlis, Daniel Marino, et al.

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17 **Abstract** Iron (Fe) is a micronutrient that is essential for plant growth and devel-
18 opment as well as for crop productivity and the quality of their derived products.
19 Despite its high abundance in Earth's crust, Fe is poorly available to plants in
20 one-third of the cultivated land. This is because at neutral to alkaline pH, Fe is
21 mostly present in the form of oxides/hydroxides that are not readily available for
22 plants. Nevertheless, not only Fe deficiency, but also Fe excess is detrimental to the
23 plant. This is due to the capacity of Fe to interact with oxygen in aerobic conditions,
24 leading to the generation of reactive oxygen species via the Fenton reaction.
25 Therefore, to maintain optimal Fe levels in plant cells, Fe homeostasis must be
26 tightly regulated. To this end, plants have evolved several molecular mechanisms
27 modulating Fe uptake, partitioning, and assimilation. Within this chapter, the main
28 strategies evolved by plants to take up Fe from soil will be first described. Then, the
29 main molecular mechanism regulating this process will be summarized. Last, an
30 outline will be given on how abiotic (i.e., other micro- and macronutrients) and
31 biotic factors affect Fe homeostasis in plants.

32 **Keywords** Arabidopsis, Beneficial microbes, bHLH, Iron homeostasis, Rice,
33 Transcription

35 1 Introduction

36 Iron (Fe) is a micronutrient that is essential for plant growth and development. In
37 addition, Fe availability affects not only the yield of crops but also the quality of their
38 derived products (Briat et al. 2015a). This is because Fe acts as a cofactor for several
39 metalloproteins involved in numerous physiological processes such as the respira-
40 tion, the photosynthesis, the assimilation of macronutrients (e.g., sulfur and nitro-
41 gen), or the biosynthesis of branched-chain amino acids (Przybyla-Toscano et al.
42 2021; Touraine et al. 2019).

43 Despite the fact that Fe is the fourth most abundant element in Earth's crust,
44 plants suffer from Fe deficiency in one-third of the cultivated land. This is because in
45 these soils, Fe is mostly present in the form of poorly soluble oxides/hydroxides,

especially at neutral to alkaline pH, that are not readily available for plants. If Fe deficiency is detrimental to the plants, Fe excess also has deleterious effects. This is due to its capacity to interact with oxygen, generating reactive oxygen species via the Fenton reaction. Therefore, to maintain optimal Fe levels in plant cells, Fe homeostasis must be tightly regulated. To this end, plants have evolved several molecular mechanisms to modulate Fe uptake, partitioning, and assimilation.

Within this chapter, the main strategies evolved by plants to take up Fe from soil will be first described. Then the main molecular mechanism regulating this process will be summarized. Last, an outline will be given on how abiotic (i.e., other micro- and macronutrients) and biotic factors affect Fe homeostasis in plants.

2 Plant Iron Uptake Strategies

In order to cope with poor soil Fe availability, plants have evolved different strategies to take up Fe. From pioneering work, two main types of Fe uptake strategies have emerged, mostly discriminating grass and non-grass species, the so-called Strategy I and Strategy II (Marschner and Romheld 1994; Romheld and Marschner 1986).

Strategy I is also called the “reduction-based strategy” (Fig. 1). This strategy essentially relies on the combined action of three types of proteins. The activity of the first protein aims at increasing the solubility of Fe. This is achieved via the P-type ATPase-dependent secretion of protons into the rhizosphere. Then, solubilized Fe^{3+} is reduced into Fe^{2+} by FERRIC REDUCTION OXIDASES whose activity is promoted at acidic pH (Susin et al. 1996). Last, Fe^{2+} is taken up into the plant roots via the activity of high-affinity Fe transporters belonging to the ZIP (ZINC REGULATED TRANSPORTER/IRON-REGULATED TRANSPORTER LIKE PROTEIN) family (Rodrigues et al. 2023). It is noteworthy that the expression of the genes encoding these proteins is induced when Fe availability is low. In the model plant *Arabidopsis thaliana* (hereafter *Arabidopsis*), these three steps rely on the activity of AHA2 (H^+ -ATPase 2), FRO2 (FERRIC REDUCTION OXIDASE 2), and IRT1 (IRON-REGULATED TRANSPORTER 1), respectively (Gao and Dubos 2021). Interestingly, it was recently shown that AHA2, FRO2, and IRT1 form a protein complex at the root plasma membrane, on the side facing the rhizosphere (Martin-Barranco et al. 2020). It is proposed that such a complex might create a local environment of pH and Fe^{2+} concentration optimizing Fe uptake and avoiding Fe^{2+} oxidation into Fe^{3+} when reacting with the oxygen present in soils (Martin-Barranco et al. 2020). In *Arabidopsis*, the low affinity Fe^{2+} transporter NRAMP1 (NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 1) also participates in Fe uptake, but to a lower extent than IRT1 (Castaings et al. 2016; Tergemina et al. 2022). Interestingly, it was recently demonstrated that IRT1 also plays a role in the Fe root-to-shoot partitioning (Quintana et al. 2022).

In some instances (e.g., *Medicago* species), Strategy I response to Fe deficiency also relies on flavin compounds whose biosynthesis is induced under these

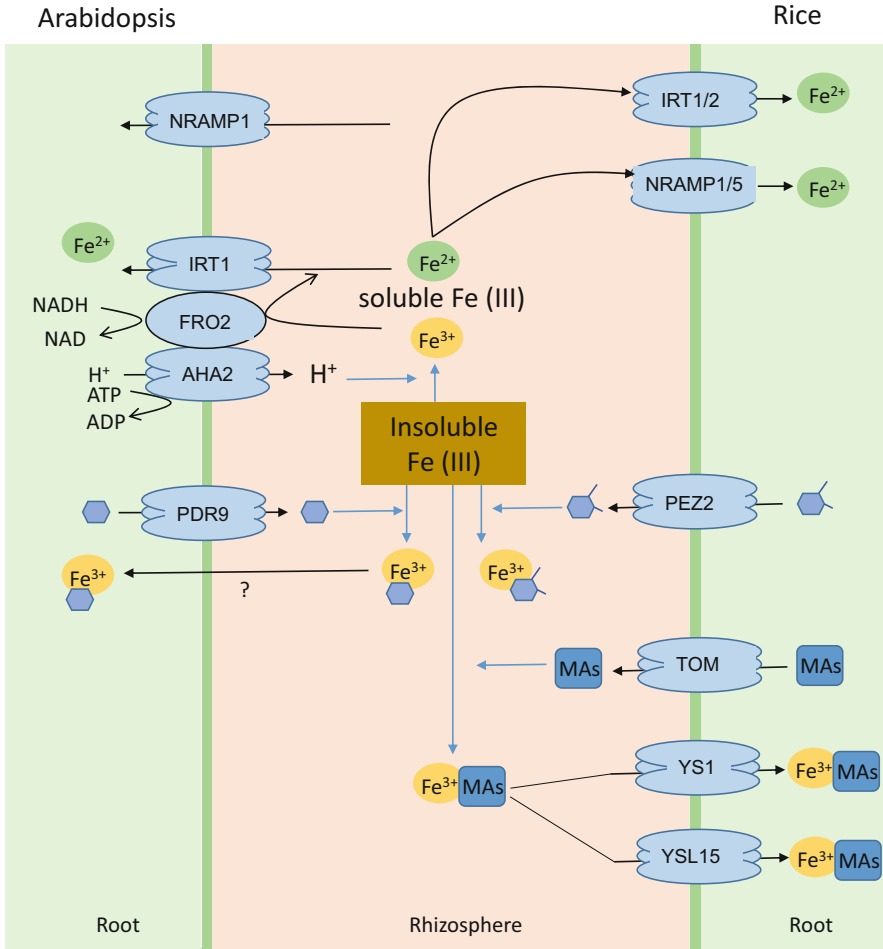


Fig. 1 Schematic representation of iron uptake in *Arabidopsis thaliana* and rice (*Oryza sativa*) roots. In *Arabidopsis*, AHA2 allows the secretion of protons that drive the solubilization of Fe^{3+} and PDR9 secretes the sideretin and fraxetin Fe^{3+} -mobilizing coumarins leading to the entry of Fe into the roots in the form of coumarin-Fe complexes via a mechanism that is still to be determined. Fe^{3+} present in the rhizosphere can also be reduced by FRO2 into Fe^{2+} and enters into the root through IRT1 transporter activity and NRAMP1 in a lesser extent. In rice, Fe^{2+} is directly taken up into the root via the activity of IRT1, IRT2, NRAMP1, and NRAMP5. Phenolic compounds can be secreted by the PEZ2 transporter to chelate Fe^{3+} allowing its entry into the root. Rice can also secrete mugineic acids (MAs) in the rhizosphere that bind to Fe^{3+} and the Fe-MA complexes are taken up into the root via YS1 and YSL15 transporters

87 conditions (Gheshlaghi et al. 2021; Rodriguez-Celma et al. 2013). Once synthesized,
 88 flavins either accumulate in the root epidermis cells or are secreted into the rhizo-
 89 sphere. It is proposed that during this process, flavins act as electron shuttles for FRO
 90 enzymes, enhancing the ferric reduction activity of the plants (Wang et al. 2022b).

Strategy II is also called the “Fe chelation strategy” (Fig. 1). This strategy relies on the secretion of phytosiderophores (PS) into the rhizosphere. PS are organic compounds with metal chelation properties. The main PS released by Strategy II plants are from the mugineic acid (MA) family. MAs are synthesized from the successive conversion of S-adenosyl-L methionine into nicotianamine (NA) by NAS (NICOTIANAMINE SYNTHASE) enzymes. NA is then converted into 2'-deoxymugineic acid (DMA) by the NAAT (NA AMINOTRANSFERASE) enzyme. It is the hydroxylation of DMA that gives rise to MAs (i.e., 3-hydroxymugineic acid, 3-epihydroxy-2'-deoxymugineic acid, 3-epihydroxymugineic acid). In rice (*Oryza sativa*), it has been shown that once MAs are synthesized, in response to Fe deficiency, they are secreted into the rhizosphere via the TOM1 (TRANSPORTER OF MA 1) PS transporter (Nozoye et al. 2011, 2015). Once secreted, MAs generate coordination bonds with Fe³⁺ via their tricarboxylic groups resulting in the formation of Fe–MA complexes that are taken up into the epidermis root cells by transporters of the YS1/YSL (YELLOW STRIPE1/YSL-LIKE) family.

Strategy II was until very recently considered as being specific to grass species. However, recent findings indicate that a similar mechanism occurs in dicot species, but relies on a different type of chelating molecules. For instance, it was shown that Arabidopsis plants secrete into the rhizosphere Fe-mobilizing coumarins (FMC) – compounds derived from the phenylpropanoid pathway – to promote Fe uptake when Fe availability is low (Robe et al. 2021a). The main secreted FMC are fraxetin, sideretin, and esculetin.

The biosynthesis of FMC is overall well described and understood. The first specific step leading to the biosynthesis of FMC is catalyzed by the F6'H1 (FERULOYL-CoA 6'-HYDROXYLASE 1) enzyme that converts feruloyl-coenzyme A (CoA) into 6'-hydroxyferuloyl-CoA that is then converted into scopoletin mostly via the activity of COSY (COUMARIN SYNTHASE). The hydroxylation of scopoletin by the SCOPOLETIN 8-HYDROXYLASE (S8H) leads to the formation of fraxetin, the main FMC secreted at alkaline pH. The cytochrome P450 enzyme CYP82C4 ensures the conversion of fraxetin into sideretin, the main FMC secreted at acidic pH. To date, the biosynthesis of esculetin is still an open question. Once synthesized, and prior to their storage into the vacuoles, FMC are glycosylated by UDP-GLYCOSYLTRANSFERASE encoded by UGT72E cluster genes (Wu et al. 2022). Prior to their secretion into the rhizosphere via the PDR9/ABCG37 (PLEIOTROPIC DRUG RESISTANCE/ATP-BINDING CASSETTE G SUBFAMILY 37) transporter, stored FMC are deglycosylated by β -glucosidases such as BGLU42 (Fourcroy et al. 2014; Stringlis et al. 2018; Zamioudis et al. 2014).

FMC are characterized by a catechol moiety that is essential for their interaction with and mobilization of insoluble Fe. Initially, FMC Fe mobilization activity was thought to be their main role in plant Fe nutrition (Fourcroy et al. 2016). However, more recent studies showed that fraxetin directly binds Fe³⁺ and forms Fe–fraxetin complexes that are taken up by the plant roots in a manner similar to that of grass PS (Li et al. 2023; Robe et al. 2021b). The fact that fraxetin biosynthesis occurs at high

136 pH, when ferric-chelate reductase activity and Fe availability are low, suggests that
 137 dicot plants have evolved a Strategy II like mechanism to cope with such growth
 138 conditions.

139 Conversely, it was also evidenced that Strategy I might not be restricted to
 140 non-grass species. For instance, it was shown that the rice *OsIRT1*, *OsIRT2*,
 141 *OsNRAMP1*, and *OsNRAMP5* genes are involved in Fe²⁺ uptake (Kobayashi et al.
 142 2014; Takahashi et al. 2011). In this case, the reduction of Fe³⁺ is not insured by the
 143 secretion of protons into the rhizosphere but by releasing phenolic compounds such
 144 as the protocatechuic and caffeic acids via the PEZ2 (PHENOLICS EFFLUX ZERO
 145 2) transporter (Ishimaru et al. 2011).

146 Taken together, these studies indicate that the initial dichotomy of Fe uptake
 147 strategies between grass and non-grass species is fainting and suggest that plants
 148 select Fe uptake modules to adapt to local environments. For instance, in support of
 149 this assertion, it was shown that wild dicot plants adapted to alkaline grassland –
 150 whose FRO activity is inhibited by the soil pH – have evolved an alternate Strategy I
 151 (Wang et al. 2022a). Here the plants secrete into the rhizosphere secondary metabo-
 152 lites (e.g., phenolic compounds) with Fe³⁺ reducing activities prior to taking up
 153 Fe²⁺. In addition, the Fe³⁺ reducing activity of these plants is not inhibited when the
 154 concentration of Fe present in the media increases, unlike what is observed with the
 155 activity of FRO proteins or the secretion of FMC of plants that are not adapted to
 156 such environment.

157 It is noteworthy that Fe uptake is not only regulated at the local level (i.e., roots)
 158 by the Fe status. Systemic signal from the shoot also modulates the Fe uptake
 159 activity (García et al. 2013). For instance, disruption of the Arabidopsis OPT3
 160 (OLIGO PEPTIDE TRANSPORTER 3) transporter impairs Fe loading into the
 161 phloem-companion cells that is perceived in roots as a Fe deficiency signal (Men-
 162 doza-Cózatl et al. 2014; Zhai et al. 2014, Chia et al. 2023). Additional transporters
 163 such as those of the YSL family (i.e., YSL1 and YSL3) are also involved in this
 164 process (Kumar et al. 2017).

165 3 Transcriptional Regulation of Iron Homeostasis

166 Over the past two decades, several teams have investigated how plants control Fe
 167 homeostasis. These studies suggested that the transcriptional machinery plays a
 168 central role in this process (Gao and Dubos 2021; Gao et al. 2019; Kobayashi
 169 et al. 2019; Li et al. 2023; Schwarz and Bauer 2020). More specifically, it was
 170 found that several transcription factors belonging to at least 13 different families
 171 (e.g., ABI3/VP1, ARF, bHLH, bZIP, B3, C2H2, EIL, ERF, MYB, NAC, NFY,
 172 WRKY, YABBY) were involved. Additionally, it was shown that the bHLH family
 173 of transcription factor plays a preponderant role. For instance, in Arabidopsis, 10%
 174 of the encoded bHLH transcription factors are involved in the regulation of Fe
 175 homeostasis (17 out of 169 members) (Fig. 2). It is noteworthy that the role of
 176 bHLH transcription factors is not restricted to non-grass species but also extends to

AU1

AU2

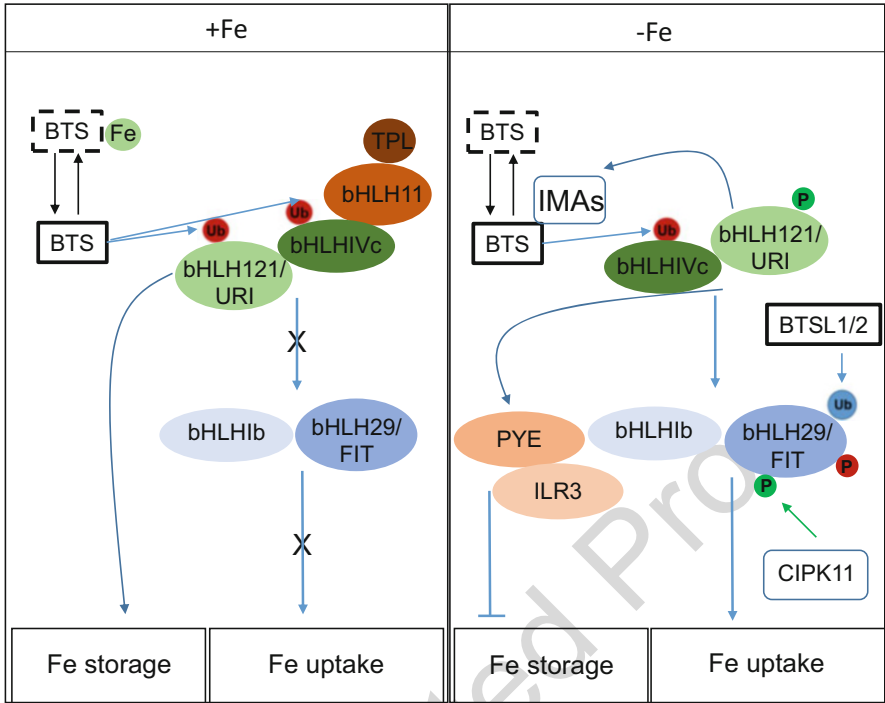


Fig. 2 Phylogenetic tree of bHLH TFs involved in the regulation of iron homeostasis in plants (color-coded genes). Remaining genes (in black) represent automated gene family assignment of gene family members in the Arabidopsis, rice, and Amborella genomes (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v5_dicots/). The full-length bHLH amino acid sequences from different species were aligned using MAFFT on XSEDE (7.505) software, trimmed using Phyutility (v.2.2.6), and the phylogenetic tree was constructed using RAxML-HPC2 on XSEDE (8.2.12). The bootstrap analysis was carried out with 1,000 replicates. The different bHLH clades are designated as previously reported (Heim et al. 2003). Species abbreviations used in the analysis: At, *Arabidopsis thaliana*; ATR, *Amborella trichopoda*; Cm, *Chrysanthemum morifolium* (for CmbHLH1) or *Cucumis melo* (for CmbHLH38); Gm, *Glycine max*; Md, *Malus domestica*; Mx, *Malus xiaojinensis*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Pt, *Populus tremula*; Sl, *Solanum lycopersicum*

grasses, such as rice (Li et al. 2023). These bHLH transcription factors constitute the backbone of the intricate regulatory network that controls Fe homeostasis that can be divided into two interconnected modules (Figs. 3 and 4) (Gao et al. 2020a; Li et al. 2023).

The first module is composed of bHLH transcription factors that directly regulate the expression of structural genes involved in Fe uptake, transport, and storage. In Arabidopsis, it relies on the activity of FIT/bHLH29 (clade IIIe) that directly induces the expression of *FRO2* and *IRT1* when Fe availability is scarce (Wang et al. 2013). FIT activity depends on its dimerization with bHLH38, bHLH39, bHLH100, and bHLH101 (clade Ib). These interactions allow for the nuclear localization of clade Ib

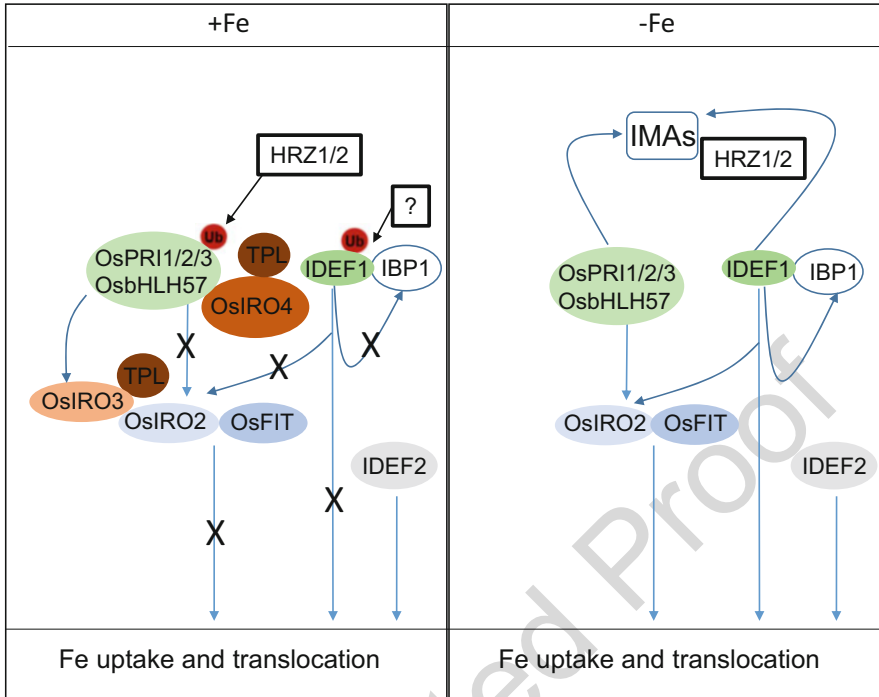


Fig. 3 Regulation of iron uptake and storage in *Arabidopsis thaliana*. In iron sufficient condition (+Fe), *FIT* and *clade Ib bHLHs* are not expressed in the root and consequently the transcription of Fe uptake actors is not induced. bHLH IVc and bHLH121/URI are transcribed but are supposed to stay inactive: unphosphorylated or ubiquitinated and localized in the stele and the endodermis. In Fe sufficient condition, the expression of the *BTS* E3-ubiquitin is not induced and is destabilized due to Fe binding. Additionally, bHLH11 is able to bind to clade IVc bHLH and allows the recruitment of the TOPLESS (TPL) repressor machinery, inhibiting clade IVc bHLH target genes expression (e.g., clade Ib bHLH). In parallel, heterodimers formed between clade IVc bHLH and bHLH121/URI induce the expression of Fe storage proteins named FERRITINS. Under Fe deficient conditions, the expression of clade Ib bHLH transcription factors is induced directly by URI (phosphorylated form) that is then localized mostly in cortex and epidermis cells. FIT expression is induced by an unknown mechanism and enhances the Fe uptake machinery. To limit the toxic effect of Fe excess in the cell, some feedback mechanisms emerged. *BTS* and *BTSL1/2* are induced and can target to the 26S proteasome clade IVc bHLH and URI, and FIT, respectively. IMA peptides, whose expression is directly induced by URI, compete with clade IVc bHLH to counteract this regulation loop. In parallel, *PYE-ILR3* complex represses the transcription of Fe storage proteins like FERRITINS

187 bHLH (Trofimov et al. 2019). The multiple FIT-dependent heterodimers display
 188 different but overlapping functions. The rice functional ortholog of FIT (i.e., OsFIT/
 189 OsbHLH156) is also a positive regulator of genes involved in Fe uptake (i.e.,
 190 *OsNAS1*, *OsNAS2*, *OsYSL15*, and *OsIRT1*) (Liang et al. 2020; Ogo et al. 2006,
 191 2007; Wang et al. 2020). Like in Arabidopsis, OsFIT activity depends on its
 192 interaction with clade Ib bHLH (i.e., OsIRO2/OsbHLH56) and OsIRO2 nuclear
 193 localization depends on its interaction with FIT (Liang et al. 2020; Wang et al.

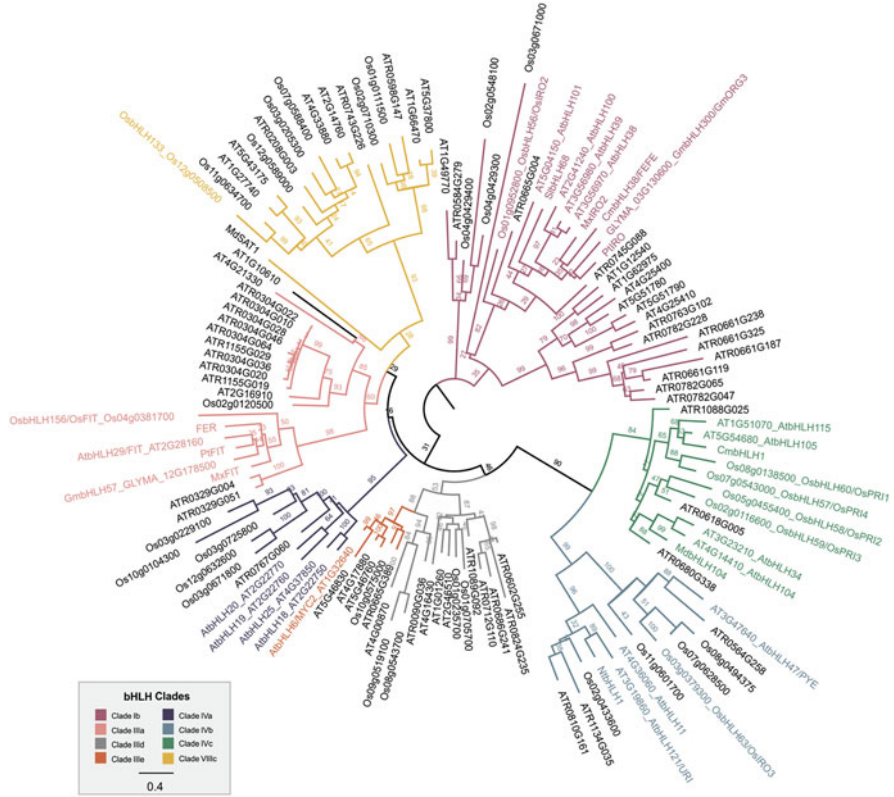


Fig. 4 Regulation of iron uptake in rice. *OsPRI1/OsbHLH60*, *OsPRI2/OsbHLH58*, *OsPRI3/OsbHLH59*, and *OsbHLH57* are positive regulators of Fe uptake machinery in rice. *OsPRI1*, *OsPRI2*, *OsPRI3*, and *bHLH57* induce the expression of *OsIRO2/OsbHLH56* and *OsIRO3/OsbHLH63* that are, respectively, positive and negative transcriptional regulators of genes involved in the Fe chelation strategy. In Fe sufficient condition (+Fe), *OsIRO2* expression is low and thus the activity of the Fe uptake machinery. The low level of expression of *OsIRO2* in +Fe condition is due to the TOPLESS/TOPLESS-RELATED (TPL/TPR)-dependent inhibition of *OsPRI1* and *OsPRI2* activity following their interaction with *OsIRO3/OsbHLH63* and *OsIRO4/OsbHLH61*. In addition, the interaction of *OsPRI1/OsbHLH60*, *OsPRI2/OsbHLH58*, and *OsPRI3/OsbHLH59* with *HRZ1* and *HRZ2* leads to their degradation via the 26S proteasome pathway. In Fe starvation condition (-Fe), *HRZ1*- and *HRZ2*-dependent ubiquitination is decreased thanks to their interaction with IMA peptides that inhibits their activity. In addition, *OsIRO4* is not expressed. Consequently *IRO2* expression increases, as is the expression of genes involved in Fe uptake. In parallel, *IBP1* induction under -Fe condition counteracts the ubiquitination of *IDEF1* and allows the expression of Fe uptake genes. *IDEF2* is a positive regulator of Fe homeostasis

2020). Nonetheless, *OsIRT1* expression does not depend on *OsIRO2*. Whether or not *OsIRT1* expression necessitates the activity of rice clade Ib bHLHs is still to be determined.

The second module regulates the expression of genes encoding regulatory proteins and peptides. In Arabidopsis, it involves homo- and heterodimers composed of

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199 clade IVc bHLH, namely ILR3/bHLH105 (IAA-LEUCINE RESISTANT 3), IDT1/
 200 bHLH34 (IRON DEFICIENCY TOLERANT 1), bHLH104 and bHLH115 (Li et al.
 201 2016; Liang et al. 2017; Zhang et al. 2015). Clade IVc bHLH positively regulates the
 202 expression of clade Ib bHLH (direct) and *FIT* (indirect) when Fe availability is low.
 203 Their rice functional homologs are OsPRI1/OsbHLH60, OsPRI2/bHLH58, OsPRI3/
 204 OsbHLH59 (POSITIVE REGULATOR OF IRON HOMEOSTASIS 1, 2, and 3),
 205 and OsbHLH57 (Kobayashi et al. 2019; Wang et al. 2022c; Zhang et al. 2017, 2020).
 206 Clade IVb bHLHs are also involved in this second module. The Arabidopsis
 207 PYE/bHLH47 and its rice functional homologs OsIRO3/OsbHLH63 and OsIRO4/
 208 OsbHLH61 are transcriptional repressors. Their repressing activity is conferred by
 209 the presence of an EAR motif in their C-terminal region (Kagale and Rozwadowski
 210 2011). Interestingly, when ILR3 interacts with PYE it acts as a transcriptional
 211 repressor (direct) of genes involved in Fe storage such as those encoding FERRI-
 212 TINS (Tissot et al. 2019). The ILR3-PYE heterodimer also directly targets *PYE* in a
 213 negative feedback regulatory loop when Fe is not limiting, most probably allowing
 214 balancing Fe uptake and Fe storage to meet the need of the plant in this micronutrient
 215 (Tissot et al. 2019). A similar mechanism exists in rice (negative feedback regulatory
 216 loop) even if no gene encoding Fe storage protein has been identified, to date, as
 217 direct target of OsIRO3 and OsIRO4 (Li et al. 2022a; Wang et al. 2022d). It has been
 218 demonstrated that EAR-domain containing transcription factors act as negative
 219 regulators by recruiting TOPLESS/TOPLESS RELATED repressors (Kagale and
 220 Rozwadowski 2011). Such mechanism was confirmed for OsIRO3 and OsIRO4
 221 (Li et al. 2022a; Wang et al. 2022d). Whether this is also the case for PYE is still to
 222 be demonstrated. The Arabidopsis bHLH11 is another clade IVb bHLH that contains
 223 an EAR-domain and that recruits TOPLESS/TOPLESS RELATED repressors to
 224 inhibit the expression of its target genes (Li et al. 2022b; Tanabe et al. 2018). Like
 225 PYE, bHLH11 forms heterodimers with clade IVc bHLHs, inhibiting the expression
 226 of both *clade Ib bHLHs* (direct) and *FIT* (indirect). This activity most probably
 227 prevents Fe overaccumulation and thus toxicity when its availability is high. The last
 228 member of the Arabidopsis clade IVb bHLH is URI/bHLH121 (UPSTREAM
 229 REGULATOR OF IRT1) and is also involved in this second module. Unlike the
 230 other members of this clade, URI acts as a transcriptional activator and can
 231 heterodimerize with the four clade IVc bHLH transcription factors (Gao et al.
 232 2020a; Kim et al. 2019; Lei et al. 2020). Under Fe deficiency, URI mainly localizes
 233 in the cortex and the root epidermis cells, where it promotes Fe uptake by activating
 234 the expression of both clade Ib bHLH (direct) and *FIT* (indirect) (Gao et al. 2020a;
 235 Kim et al. 2019). URI positive transcriptional activity extends (directly or indirectly)
 236 to most of the known genes involved in this intricate transcriptional regulatory
 237 network (Gao et al. 2020a, b). Under Fe sufficient condition, URI mainly localizes
 238 in the stele and the endodermis where it promotes the transient storage of Fe into
 239 ferritins by directly inducing their expression. Since URI can activate the expression
 240 of *FERRITIN* genes when Fe is not limiting, it is likely that URI regulates the
 241 expression of some of its target genes independently of clade IVc bHLH transcrip-
 242 tion factors.

4 Post-Translational Regulation of Iron Homeostasis

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Post-translational modifications are also essential mechanisms allowing plants to modulate Fe homeostasis.

Among the post-translational mechanisms regulating Fe homeostasis, ubiquitination is to date the most studied one. In recent years, E3 ubiquitin ligases containing a hemerythrin motif in their N-terminal part have emerged as essential players in regulating Fe homeostasis. These E3 ubiquitin ligases are induced in response to Fe deficiency conditions and are negative regulators of Fe homeostasis. It includes the Arabidopsis BRUTUS (BTS) and BRUTUS LIKE 1 and 2 (BTSL1 and BTSL2) (Hindt et al. 2017; Rodriguez-Celma et al. 2019; Selote et al. 2015) and the rice HEMERYTHRIN MOTIF-CONTAINING REALLY INTERESTING NEW GENE (RING) AND ZINC-FINGER PROTEIN 1 and 2 proteins (HRZ1, HRZ2) (Figs. 3 and 4) (Kobayashi et al. 2013). The identified targets of these E3 ubiquitin ligases are transcription factors of the bHLH family. BTS activity leads to the degradation, via the 26S proteasome pathway, of IDT1, ILR2, and bLH115 (clade IVc bHLH) and of URI (Kim et al. 2019; Selote et al. 2015). Similarly, HRZ1 was shown to ubiquitinate the clade IVc OsPRI1 bHLH transcription factor (Kobayashi et al. 2013). BTS expression and activity is induced in response to Fe deficiency and BTS is stabilized when its hemerythrin domains are not linked to Fe. This is consistent with the role of BTS in preventing clade bHLHI IVc overaccumulation that might lead to Fe excess and toxicity (Selote et al. 2015). In contrast to BTS, HRZ1 and HRZ2, BTSL1 and BTSL2 act partly redundantly to target FIT (Rodriguez-Celma et al. 2019). Interestingly, if the interaction of FIT with BTSL1 and BTSL2 leads to its ubiquitin-dependent degradation, it is suggested that the ubiquitination of FIT is also required for its transcriptional activity. On the other hand, it is speculated that FIT needs ubiquitination to be active considering that the application of the MG132 proteasome inhibitor reduced the expression of direct targets of FIT, such as *IRT1* and *FRO2* (Meiser et al. 2011; Sivitz et al. 2011; Spielmann and Vert 2021).

The genome of Arabidopsis contains eight IRON MAN/FE-UPTAKE-INDUCING PEPTIDES (IMA/FEP) that act at least partly redundantly to regulate Fe homeostasis (Fig. 3). Among them, IMA1/FEP3 and IMA3/FEP1 are the most studied ones (Grillet et al. 2018; Hirayama et al. 2018). IMA1 and IMA3 are positive regulators of Fe uptake and were firstly proposed to act as signaling molecules of shoot Fe status to the roots (Grillet et al. 2018; Hirayama et al. 2018). BTS was recently found to interact in vivo with the eight IMA peptides (Li et al. 2021b). Interestingly it was shown that IMA3 is degraded via the 26S proteasome pathway following its ubiquitination by BTS (Li et al. 2021b). IMA3 would act as an inhibitor of BTS activity and buffer the degradation of ILR3 and bHLH115 proteins by BTS, thereby activating the Fe deficiency response (Li et al. 2021b). Similar function was proposed for rice IMA peptides (Fig. 4) (Kobayashi et al. 2021; Peng et al. 2022). Interestingly, no lysine has been reported in the predicted amino acid sequence of IMA3 (Grillet et al. 2018; Hirayama et al. 2018) suggesting that its ubiquitination

286 might occur through other amino acids than lysine, a mechanism not yet described in
287 plants.

288 In rice, IDEF1, an ABI3/VP1 family transcription factor, is a positive regulator of
289 a large subset of Fe regulated genes (Kobayashi et al. 2012). The stability of IDEF1
290 depends on its ubiquitination (Tan et al. 2016; Zhang et al. 2014). The ubiquitin-
291 dependent degradation of IDEF1 is counteracted by its interaction with IDEF1
292 INTERACTING PROTEIN 1 (IBP1), whose expression is induced in low Fe
293 conditions (Zhang et al. 2014). The stability of IDEF1 protein is also controlled by
294 the CONSTITUTIVE PHOTOMORPHOGENESIS 9 (COP9) signalosome (CSN)
295 (Tan et al. 2016; Zhang et al. 2014). To date, the E3 ubiquitin ligases implicated in
296 these mechanisms are still to be identified.

297 It is noteworthy that the regulation of Fe homeostasis via the ubiquitination of
298 proteins not only occurs at the transcription factors level. For instance, IRT1 activity
299 is also regulated by ubiquitination allowing the fine-tuning of Fe uptake and
300 avoiding the entry of divalent metals (e.g., cobalt, manganese, zinc) into the root
301 cells that could be toxic to the plant (Spielmann and Vert 2021).

302 Phosphorylation also plays a central role in the regulation of Fe homeostasis. In
303 Arabidopsis that is notably by modulating the activity of the two key transcriptional
304 regulators, FIT and URI (Fig. 3).

305 According to in silico studies followed by in vitro kinase assay, FIT was
306 identified as a phosphorylation target for CIPK11, a serine threonine protein kinase
307 that interact with calcineurin B-like (CBL) calcium-binding proteins. CIPK11-
308 dependent phosphorylation of FIT occurs on two serine residues (i.e., Ser221 and
309 Ser272). It is proposed that the phosphorylation of these two serine residues pro-
310 motes FIT interaction with bHLH39 (clade Ib) and its accumulation into the nucleus
311 to promote Fe uptake (Gratz et al. 2019). In contrast, similar experiments highlighted
312 that the phosphorylation of FIT on tyrosine residues (i.e., Tyr238 and Tyr278) has
313 opposite effects (Gratz et al. 2019).

314 The phosphorylated form of URI accumulates in Fe deficiency conditions and is
315 proposed to be necessary for its activity (Kim et al. 2019). It is proposed that the URI
316 phosphorylated form is specifically degraded via BTS under Fe sufficient conditions.

317 In the apple (*Malus xiaojinensis*) rootstocks, the MITOGEN ACTIVATED
318 PROTEIN KINASE 6 (MAPK6) was found to phosphorylate MxbHLH104 (clade
319 IVc bHLH) increasing its activity under Fe deficiency conditions (Li et al. 2021a).

320 Sumoylation process is another post-translational mechanism that regulates tran-
321 scription factors activity by conjugating small ubiquitin-like modifier (SUMO)
322 peptides (Miura et al. 2007). For instance, it was shown that the sumoylation of
323 the apple (*Malus domestica*) MdbHLH104 transcription factor (a functional homo-
324 log of the Arabidopsis bHLH104) by SIZ1 (a SIZ/PIAS-type SUMO E3 ligase) was
325 promoting root plasma membrane H⁺-ATPase activity and thus the acidification of
326 the rhizosphere and Fe uptake. Interestingly, SUMO-conjugation is promoted by Fe
327 deficiency conditions and that stabilizes MdbHLH104 (Zhou et al. 2019). In addi-
328 tion, it was observed that several Arabidopsis bHLH transcription factors are
329 SUMO-targets (Castro et al. 2023). For instance, MYC2 and bHLH3/JAM3 are
330 modified by SUMO1 (Rytz et al. 2018). It is also likely that other Arabidopsis

Fe-regulatory bHLHs can be sumoylated but, as MdbHLH104, might only occur in response to Fe deficiency. Emphasizing that possibility is that sumoylation is a rapid and transient modification and can be enhanced by specific environmental stress conditions (Castro et al. 2012). Having that in mind, it will be highly relevant to verify SUMO-conjugate patterns and specific SUMO-targets in response to unbalanced Fe levels.

5 Epigenetic Regulation of Iron Homeostasis

Epigenetic regulation of gene expression occurs through multiple mechanisms such as histone modifications, DNA methylation, or the expression of small and long non-coding RNA.

Different types of histone modifications have been associated with the regulation of Fe homeostasis in plants.

Trimethylation of histone H3 lysine 4 (H3K4m3) is generally associated with active gene expression (Xiao et al. 2016). Among the factors regulating *FIT* transcription, there is the Arabidopsis 14-3-3 protein GENERAL REGULATORY FACTOR 11 (GRF11), whose expression is regulated in a H3K4m3-dependent manner (Singh et al. 2021). The deposition of H3K4m3 marks to modulate *GRF11* expression occurs in its promoter region via the activity of NON-RESPONSE TO Fe-DEFICIENCY 2/EARLY FLOWERING8 (NRF2/ELF8), a trithorax type (TrxG) methyltransferase (Singh et al. 2021). Similarly, the expression of Arabidopsis *FERRITIN* genes is also associated with H3K4m3 marks in the region of their promoter where URI, ILR3, and PYE were shown to directly bind (Gao et al. 2020b; Tissot et al. 2019).

Trimethylation of histone H3 lysine 27 (H3K27me3) leads in most cases to gene repression. H3K27me3 mark depends on the activity of POLYCOMB REPRESSIVE COMPLEX 2 (PRC2). In Arabidopsis, PRC2 regulation of Fe homeostasis relies on the activity of the CURLY LEAF (CFL) methyl transferase. The *clf* mutant displays an increased expression of *FIT*-target genes (e.g., *FRO2*, *IRT1*) as well as of *FIT* itself compared to wild type plants under Fe deficiency (Park et al. 2019). It is proposed that the deposition of H3K27me3 marks on Fe uptake genes might be a mechanism to avoid Fe toxicity due to Fe overload.

Another histone modification mark associated with the regulation of Fe homeostasis is the symmetric dimethylation of histone4 arginine3 (H4R3me2). The Arabidopsis Shk1 BINDING PROTEIN 1 (SKB1) catalyzes H4R3me2 modification and regulates Fe homeostasis by modulating the expression of clade Ib bHLH (i.e., bHLH38, bHLH39, bHLH100, and bHLH101) transcription factors (Fan et al. 2014). This regulation is direct, and the more Fe is available in the growth media, the higher are the H4R3me2 marks in the promoter of clade Ib bHLH transcription factors and the lower is their expression (Fan et al. 2014).

Histone acetylation is also an important modification that participates in the regulation of gene expression. To date, no correlation has been identified between

372 this type of histone modification and the regulation of transcription factor expression
373 involved in the control of Fe homeostasis. Nonetheless, it was reported that such
374 histone modification was part of the molecular machinery controlling the expression
375 of some Fe transporters, and thus Fe homeostasis (Xing et al. 2015). For instance, the
376 histone acyltransferase GENERAL CONTROL NONREPPRESSED PROTEIN
377 5 (GCN5) directly regulates the expression of *FERRIC REDUCTASE DEFECTIVE*
378 3 (*FRD3*), a key transporter involved in root-to-shoot Fe translocation. GCN5
379 positive regulation of *FRD3* expression occurs via the acetylation of histone 3 at
380 lysine 9 (H3K9ac) and lysine 14 (H3K14ac) present in its promoter (Xing et al.
381 2015). Conversely, it was proposed that HISTONE DEACETYLASE7 (HDA7)
382 negatively regulates *FRD3* expression through *FRD3* promoter histone
383 deacetylation (Xing et al. 2015).

384 To date, asymmetric cytosine methylation at CHH loci (H: adenine or thymine or
385 cytosine) is the sole DNA modification that is associated with the regulation of Fe
386 homeostasis in plants. For instance, it was shown that under Fe starvation conditions,
387 the promoter of *OsIRO2* and *OsBHLH156* was hypermethylated at CHH loci and
388 correlated with an increased expression of these genes (Sun et al. 2021). In this
389 study, the authors suggested that this mechanism could implicate small RNAs.

390 Long non-coding RNAs (lncRNAs) are known to play critical roles in regulating
391 the expression of genes involved in several physiological and developmental pro-
392 cesses. A recent study highlighted that in response to Fe deficiency, a large set of rice
393 lncRNAs were differentially expressed (Wang et al. 2021). It is proposed that several
394 of these differentially expressed lncRNAs could regulate the expression of
395 Fe-related genes by generating miRNAs or acting as endogenous target mimics.
396 However, to date, only one example of lncRNA has been associated with the
397 regulation of Fe homeostasis. This is the Arabidopsis *CAN OF SPINACH (COS)*,
398 whose expression is induced in response to Fe deficiency and that was shown to
399 participate in the regulation of this process (Bakirbas and Walker 2022). The
400 mechanism by which COS regulates the plant response to Fe deficiency is yet
401 unclear, but evidence suggests it could be via the generation of small RNAs
402 (Bakirbas and Walker 2022).

403 6 Iron Interaction with Other Mineral Nutrients

404 Plants must adapt to constantly changing and often stressful or unfavorable envi-
405 ronments, including fluctuations in the availability of essential mineral nutrients. The
406 availability of some nutrients can be significantly altered in the presence or absence
407 of other soil nutrients. Recent research highlighted that nutrients interact with each
408 other, at the molecular level, in complex ways. These interactions can have positive
409 or negative effects on nutrient uptake and may also result in “hidden responses” that
410 are more complex than the sum of individual stress responses (Bouain et al. 2019). In
411 this context, the study of Fe interactions with other mineral nutrients has the potential

to uncover new pathways associated with the uptake and utilization of this micronutrient.

In Arabidopsis, Fe uptake relies on the activity of IRT1 that is a high-affinity Fe transporter but also has low specificity. This means that IRT1 can transport a range of other divalent metal cations including zinc, manganese, cobalt, nickel, and cadmium. For instance, it has been reported that in Arabidopsis plants grown under Fe deficiency, an increase in the accumulation of zinc, manganese, cobalt, or cadmium is observed (Baxter et al. 2008; Hanikenne et al. 2021; Robe et al. 2020). The availability of these non-Fe metals in soils modulates the amount of IRT1 at the plasma membrane. When Fe availability is low, these divalent metal cations will lead to the internalization of IRT1 from the plasma membrane by its ubiquitination followed by either its recycling to the plasma membrane or its degradation in the vacuole (Dubeaux et al. 2018). This mechanism ensures a proper balance between Fe uptake and the toxicity effects of these divalent metals. In addition, zinc availability also regulates FIT activity via its interaction with FIT-BINDING PROTEIN (FBP), which blocks the DNA-binding capacity of FIT (Chen et al. 2018). This mechanism allows fine-tuning Fe and zinc interactions.

The homeostasis of Fe is also tightly connected to that of copper (Cu). For instance, under Fe deficiency, plants overaccumulate Cu and vice versa. In Arabidopsis, the Fe deficiency-induced expression of Cu uptake genes is under the control of FIT and clade Ib bHLHs and the subsequent accumulation of Cu is necessary to improve plant growth (Cai et al. 2021). Such tight cross talk between Fe and Cu occurs also at the systemic level, where the OPT3 transporter plays a major role in this process (Chia et al. 2023).

Phosphate is one of the nutrients that has been extensively studied in relation to its interaction with Fe, and there is strong evidence that the transcriptional regulation of Fe homeostasis in plants can be influenced by the availability of phosphate in the soil. In Arabidopsis, two key transcription factors induced by phosphate deprivation, namely PHOSPHATE-DEFICIENCY RESPONSE 1 (PHR1) and PHR1-LIKE 1 (PHL1), are related to Fe homeostasis. Specifically, these two transcription factors bind to the promoter of *FERRITIN 1* gene, leading to an accumulation of Fe when plants are starved of phosphate. In fact, it is suggested that the inhibition of primary root growth in phosphate limitation condition is related to this regulation (Bournier et al. 2013). Interestingly, when plants are exposed to double Fe-phosphate deficiency, the primary root growth inhibition is recovered via the SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) transcription factor. This is because when plants have a phosphorus-deficient nutrition, STOP1 activates the expression of the *ALUMINUM MALATE TRANSPORTER 1 (ALMT1)* gene, leading to an increase in the malate-exudation-dependent mechanism of Fe relocation in the root apical meristem (Mora-Macias et al. 2017). It is noteworthy that PHR1 might also be involved in the integration of sulfur and zinc nutrition signals (Briat et al. 2015b).

Recently, it was also demonstrated that chlorotic symptoms observed under Fe deficiency in Arabidopsis were a phosphorus-dependent mechanism (Nam et al. 2021). The authors showed that a dual Fe-phosphorus deficiency alleviated the Fe-dependent chlorosis symptoms and restored the photosynthesis activity, a process

457 that was dependent on the bZIP58 transcription factor (Nam et al. 2021). Here,
458 bZIP58 promoted the accumulation of ascorbate into the chloroplast that in turn
459 modulated the homeostasis of ROS present in this cell compartment and therefore
460 adapt photosynthesis to nutrient availability (Nam et al. 2021).

461 The interplay between Fe and sulfur is a critical aspect of the functioning of
462 cellular metabolism. These two elements work together to support a wide range of
463 vital processes within cells by participating in the formation of prosthetic groups in a
464 variety of proteins that are essential for many metabolic processes such as respira-
465 tion, photosynthesis, biosynthesis of primary and secondary metabolites, or the
466 biosynthesis of branch chain amino acids among others (Astolfi et al. 2021; Touraine
467 et al. 2019). Sulfur deficiency has been shown to negatively regulate the Fe
468 deficiency-induced expression of several transcription factors associated with the
469 maintenance of Fe homeostasis (Robe et al. 2020). This process may result in a
470 limitation of the non-specific entry of zinc and manganese through the root via IRT1,
471 limiting the levels of toxicity associated with these elements. In fact, less chlorosis
472 was observed in Arabidopsis plants exposed to double Fe and sulfur deficiency
473 compared to those exposed only to Fe deprivation (Robe et al. 2020).

474 Plants typically obtain nitrogen from the soil in the form of nitrate and ammo-
475 nium. While nitrogen assimilation into biomolecules typically occurs from ammo-
476 nium, many plant species experience stress symptoms when grown in media with
477 high concentrations of ammonium. This stress is thought to be caused by a variety of
478 factors, including pH changes, oxidative stress, and energy imbalances (Britto and
479 Kronzucker 2002).

480 Alteration of Fe homeostasis in plants grown with ammonium as N source has
481 been reported (Coletto et al. 2021; De la Pena et al. 2022; Liu et al. 2022a, b). Indeed,
482 Fe-deficiency related genes are induced in both Strategy I (i.e., Arabidopsis) and
483 Strategy II (i.e., *Brachypodium distachyon*) plants grown under ammonium nutrition
484 (Coletto et al. 2021; De la Pena et al. 2022; Liu et al. 2022a). MYB28 and MYB29
485 transcription factors appeared as a molecular link between ammonium stress and Fe
486 homeostasis. The double loss-of-function mutant *myb28 myb29* was found to be
487 highly sensitive to ammonium stress, displaying symptoms and physiological and
488 genetic responses similar to those of Fe deficiency. Actually, an extra supply of Fe
489 restored the ammonium hypersensitive phenotype of *myb28myb29* mutant by recover-
490 ing its chlorotic and root system architecture phenotype (Coletto et al. 2021).
491 Moreover, LOW PHOSPHATE ROOT 2 (LPR2), but not LPR1, ferroxidase that
492 converts Fe^{2+} to Fe^{3+} , is involved in the inhibition of primary root growth in
493 Arabidopsis under ammonium stress (Liu et al. 2022a). The authors reported an
494 increase of LPR2 expression and activity under ammonium nutrition that provoked
495 Fe^{3+} accumulation leading to ROS production via the Fenton reaction. In conse-
496 quence, ROS produced callose accumulation in the phloem that impaired proper
497 nutrient flow affecting root growth (Liu et al. 2022a).

7 Roles of Microorganisms in the Regulation of Plant Iron Homeostasis

498

499

In addition to the plant's nutrient status, Fe is also a central player in plant–microbe interactions. Improvement of plant Fe uptake by mycorrhiza, plant-beneficial fungi that form a symbiotic relationship with most plant species, can supply the plant directly with Fe, acting as an extended root system (Caris et al. 1998; González-Guerrero et al. 2016; Lehmann and Rilig 2015). Conversely, plants that form intimate symbiotic relationships with nitrogen-fixing rhizobia form specialized organs called nodules to house the bacteria, and supply their symbiotic partners with, amongst other nutrients, Fe to sustain them (Escudero et al. 2020; Liu et al. 2020; Walton et al. 2020). In addition to symbiotic relationships, Fe is a major player in antagonistic plant–microbe interactions.

Fe availability is a well-known determinant of disease outcome in both plant and animal systems. During pathogen infection, both a high and a low plant Fe status can inhibit pathogenicity, and both active Fe withholding and accumulation can be viable plant strategies to combat infection (Deak et al. 1999; Kieu et al. 2012; Sanchez-Sanuy et al. 2022; Verbon et al. 2019; Ye et al. 2014). For example, infection of Arabidopsis with *Dickeya dadantii* leads to Fe redistribution in the plant tissue resulting in low Fe contents in the infected tissues, and high Fe contents in healthy tissues, apparently withholding Fe from the pathogen (Aznar et al. 2015). Infection by *D. dadantii*, but also treatment with its siderophores (Deak et al. 1999; Kieu et al. 2012; Sanchez-Sanuy et al. 2022; Verbon et al. 2019; Ye et al. 2014) leads to the induction of *FERRITIN 1* expression in Arabidopsis around the lesion site (Aznar et al. 2014, 2015; Dellagi et al. 2005). FER1 is an Fe-sequestering protein required for resistance to *D. dadantii* in Arabidopsis (Dellagi et al. 2005). Treatment of Arabidopsis with siderophores (Fe-chelating molecules involved in bacterial Fe acquisition) of *D. dadantii* showed an initial induction of *bHLH100* and *bHLH101* expression, indicative of Fe deficiency, and a later induction of *FER1* expression, normally expressed during Fe excess (Fig. 5). In wheat, instead of sequestration, infection with *Blumeria graminis* f. sp. *tritici* resulted in an accumulation of Fe³⁺ at the infection site causing a ROS burst that suppresses the pathogen (Fig. 5) (Liu et al. 2007). Direct scavenging of Fe through siderophores is not the only way pathogens acquire Fe during infection. It was recently shown that *Pseudomonas syringae* DC3000 produces an effector protein that inactivates BRUTUS in Arabidopsis, leading to a potentiated Fe deficiency response. This results in more Fe in the apoplast, promoting infection (Xing et al. 2021). A recent finding paints Fe deficiency as an integral part of the immune response of Arabidopsis to *Botrytis cinerea*, showing that infection-induced expression of *FIT* and *clade Ib bHLHs* elevate ethylene levels through enhancing the expression of genes encoding S-ADENOSYLMETHIONINE SYNTHETASE (i.e., *SAM1* and *SAM2*), resulting in resistance (Lu and Liang 2022). Additionally, receptor kinase SRF3 emerged as a modulator of both Fe-deficiency and immune-related responses through regulation of callose synthases.

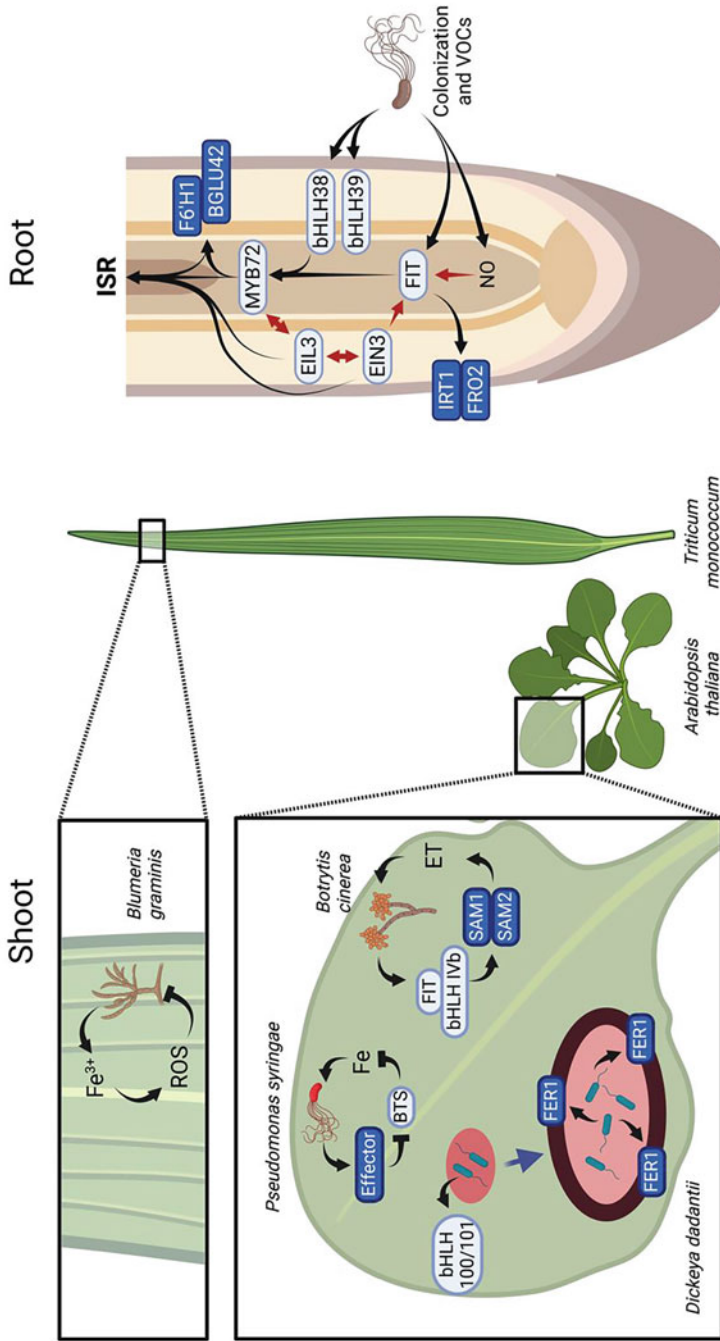


Fig. 5 Transcriptional activation of iron-responsive genes in the shoot and root in response to microbes. In *Arabidopsis* shoots, *Dickeyea dadantii* activates bHLH100 and bHLH101 during early infection, and triggers Fe sequestration around the infection site by FER1 in later infection stages. *Pseudomonas syringae* uses an effector to inhibit the Fe-deficiency repressor BTS, leading to Fe accumulation and enhanced infection. Infection by *Botrytis cinerea* activates FIT and clade IVb bHLH transcription factors, leading to activation of ethylene biosynthesis by SAM1 and SAM2, which results in resistance. Foliar infection of *Triticum monococcum* by *Blumeria graminis* causes Fe^{3+} to accumulate at the infection site, leading to a ROS burst that inhibits the pathogen. In *Arabidopsis*

roots, multiple beneficial microbes and their VOCs can activate the expression of Fe-deficiency genes such as *FIT*, *bHLH38*, *bHLH39*, *MYB72* as well as *IRT1*, *FRD2*, *F6'H1*, and *BGLU42*, of which some have been shown to also be essential during the induced systemic resistance response. Dark blue boxes are proteins, light blue boxes are transcription factors, green boxes denote collections of genes, black arrows denote stimulation or inhibition, red arrows denote stabilization or binding, blue arrows denote progression in time, red colored areas indicate different abundances of Fe (light – low, medium – average, dark – high), ROS, reactive oxygen species; NO, nitric oxide; Fe, iron; Fe³⁺, ferric oxide; ET, ethylene. Figure was designed using Biorender (<https://biorender.com>)

541 Over the last 10 years, it has become clear that beneficial microorganisms hijack
542 the plant's Fe deficiency response to stimulate plant resistance (Martinez-Medina
543 et al. 2017; Verbon et al. 2019; Zamioudis et al. 2015). Induced systemic resistance
544 (ISR) is a phenomenon where root colonization by beneficial microbes triggers a
545 root transcriptional response, after which the foliar tissue becomes "primed,"
546 displaying a broad-spectrum resistance response upon subsequent pathogen attack.
547 This has been extensively studied in the interaction between *Pseudomonas simiae*
548 WCS417 (WCS417) and Arabidopsis (Pieterse et al. 2021). Following root coloni-
549 zation by WCS417, 20% of the genes responding to the bacterium overlap with
550 genes activated during the Fe deficiency response (Zamioudis et al. 2014). Among
551 those genes are classical Fe-uptake genes *FIT*, *bHLH38*, *bHLH39*, *FRO2*, and *IRT1*,
552 but also *MYB72*, which is a central regulator of ISR, and in Fe deficiency has been
553 studied for its role in the regulation of production and secretion of Fe-chelating
554 coumarins through F6'H1 and BGLU42 (Fig. 5) (Palmer et al. 2013; Stringlis et al.
555 2018; Van der Ent et al. 2008). Besides direct colonization, the Fe deficiency
556 responses can also be stimulated by volatile organic compounds produced by
557 WCS417 (Fig. 5) (Zamioudis et al. 2015). Activation of MYB72 by WCS417-
558 VOCs occurs in a FIT-dependent manner, however, FIT alone is not sufficient to
559 activate MYB72 (Zamioudis et al. 2015). Using MYB72 overexpression lines it was
560 discovered that FIT and bHLH38 are both necessary to induce MYB72 (Zamioudis
561 et al. 2015). FIT is stabilized by EIN3, an ethylene-responsive transcription factor
562 involved in the establishment of ISR. EIN3 can dimerize with its paralog EIL3,
563 which is similarly required to mount a successful ISR response by WCS417
564 (Wawrzyńska and Sirko 2016; Zhu et al. 2022). However, while MYB72 interacts
565 with EIL3 in vitro, WCS417 is still able to activate MYB72 in *ein2-1* Arabidopsis
566 loss-of-function mutant plants, which are ISR-deficient and ethylene-insensitive
567 (Van der Ent et al. 2008). In addition to WCS417, additional ISR inducing micro-
568 organisms have been shown to activate Fe deficiency responsive genes, such as
569 *Paenibacillus polymyxa* BFKC01 (Zhou et al. 2016). This bacterium can induce Fe
570 deficiency in Arabidopsis in an auxin-dependent manner. In addition to direct
571 colonization, volatile organic compounds (VOCs) of WCS417, *Bacillus subtilis*,
572 *Pseudomonas* spp., and *Trichoderma* spp. have all been reported to induce Fe uptake
573 machinery (Martinez-Medina et al. 2017; Zamioudis et al. 2015; Zhang et al. 2009).
574 The Fe deficiency response of plant roots to VOCs of WCS417 and the *Trichoderma*
575 species seems to be dependent on NO, which is known to stabilize FIT (Meiser et al.
576 2011; Pescador et al. 2022; Zamioudis et al. 2015).

577 8 Conclusion

578 Because Fe is a cofactor for several metalloproteins involved in diverse physiolog-
579 ical processes (e.g., respiration, photosynthesis, etc.) it is an essential micronutrient
580 for plants. Indeed, this is also the case for almost all living organisms. For instance,
581 Fe homeostasis disorders in humans are associated with health issues such as cancer

risk or neurodegenerative diseases. The most prominent health issue being 582
 Fe-deficiency anemia since it affects about one billion people worldwide. To over- 583
 come the associated symptoms, Fe content and Fe availability in their diet must be 584
 improved in a sustainable manner. Therefore, even if humans preferentially absorb 585
 heme-bound Fe present in large amounts in animal flesh, this is not the solution that 586
 should be followed. In contrast, improving the use of biofortification to improve Fe 587
 content and Fe availability in crops would have a considerable beneficial effect on 588
 human health. Obviously, decrypting the molecular mechanisms that regulate Fe 589
 homeostasis in plants is an important step to reach this goal. Within this chapter, one 590
 can appreciate the tremendous efforts made by the scientific community during the 591
 last decades to decrypt the molecular mechanisms by which plants regulate Fe 592
 homeostasis. These studies have provided several candidate genes to develop 593
 biofortification strategies in plants. Nevertheless, additional efforts are still necessary 594
 to improve our understanding of how plants maintain Fe homeostasis, and therefore 595
 providing new molecular tools/targets for improving Fe content and Fe availability 596
 in staple crops. In particular, special focus should be given on how plant Fe 597
 homeostasis is modulated by other biotic and abiotic stresses. The influence of 598
 predicted global climate change is an important parameter to be taken into account, 599
 considering, for instance, that an increase in atmospheric carbon dioxide concentra- 600
 tion was correlated with drastic reduction in Fe and other micronutrient content in 601
 several crop species (Loladze 2014), via a mechanism that most probably inhibits 602
 their uptake (Cassan et al. 2023). 603

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Uncorrected Proof