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Contents

Abstract Iron (Fe) is a micronutrient that is essential for plant growth and development as well as for crop productivity and the quality of their derived products. Despite its high abundance in Earth's crust, Fe is poorly available to plants in one-third of the cultivated land. This is because at neutral to alkaline pH, Fe is mostly present in the form of oxides/hydroxides that are not readily available for plants. Nevertheless, not only Fe deficiency, but also Fe excess is detrimental to the plant. This is due to the capacity of Fe to interact with oxygen in aerobic conditions, leading to the generation of 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 modulating 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.

Keywords Arabidopsis, Beneficial microbes, bHLH, Iron homeostasis, Rice, Transcription

1 Introduction

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

Despite the fact that Fe is the fourth most abundant element in Earth's crust, plants suffer from Fe deficiency in one-third of the cultivated land. This is because in 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

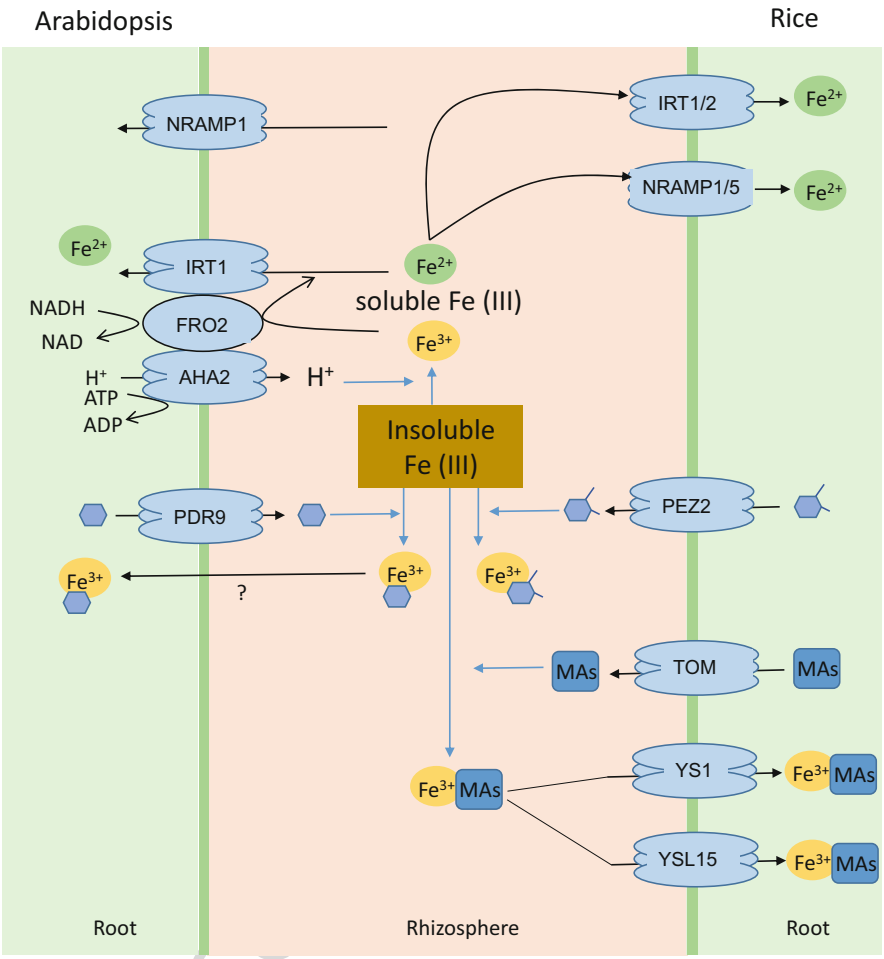

Fig. 1 Schematic representation of iron uptake in *Arabidopsis thaliana* and rice (*Oryza sativa*) roots. In Arabidopsis, AHA2 allows the secretion of protons that drive the solubilization of Fe^{3+} and PDR9 secretes the sideretin and fraxetin Fe^{3+}-mobilizing coumarins leading to the entry of Fe 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 uptaken into the root via YS1 and YSL15 transporters

conditions (Gheshlaghi et al. 2021; Rodriguez-Celma et al. 2013). Once synthesized, flavins either accumulate in the root epidermis cells or are secreted into the rhizosphere. It is proposed that during this process, flavins act as electron shuttles for FRO 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 Fe^{3+} 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/YS1-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 Fe^{3+} 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

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

Conversely, it was also evidenced that Strategy I might not be restricted to non-grass species. For instance, it was shown that the rice *OsIRT1*, *OsIRT2*, *OsNRAMP1*, and *OsNRAMP5* genes are involved in Fe^{2+} uptake (Kobayashi et al. 2014; Takahashi et al. 2011). In this case, the reduction of Fe^{3+} is not insured by the secretion of protons into the rhizosphere but by releasing phenolic compounds such as the protocatechuic and caffeic acids via the PEZ2 (PHENOLICS EFFLUX ZERO 2) transporter (Ishimaru et al. 2011).

Taken together, these studies indicate that the initial dichotomy of Fe uptake strategies between grass and non-grass species is fainting and suggest that plants select Fe uptake modules to adapt to local environments. For instance, in support of this assertion, it was shown that wild dicot plants adapted to alkaline grassland – whose FRO activity is inhibited by the soil pH – have evolved an alternate Strategy I (Wang et al. 2022a). Here the plants secrete into the rhizosphere secondary metabolites (e.g., phenolic compounds) with Fe^{3+} reducing activities prior to taking up Fe^{2+}. In addition, the Fe^{3+} reducing activity of these plants is not inhibited when the concentration of Fe present in the media increases, unlike what is observed with the activity of FRO proteins or the secretion of FMC of plants that are not adapted to such environment.

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

3 Transcriptional Regulation of Iron Homeostasis

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

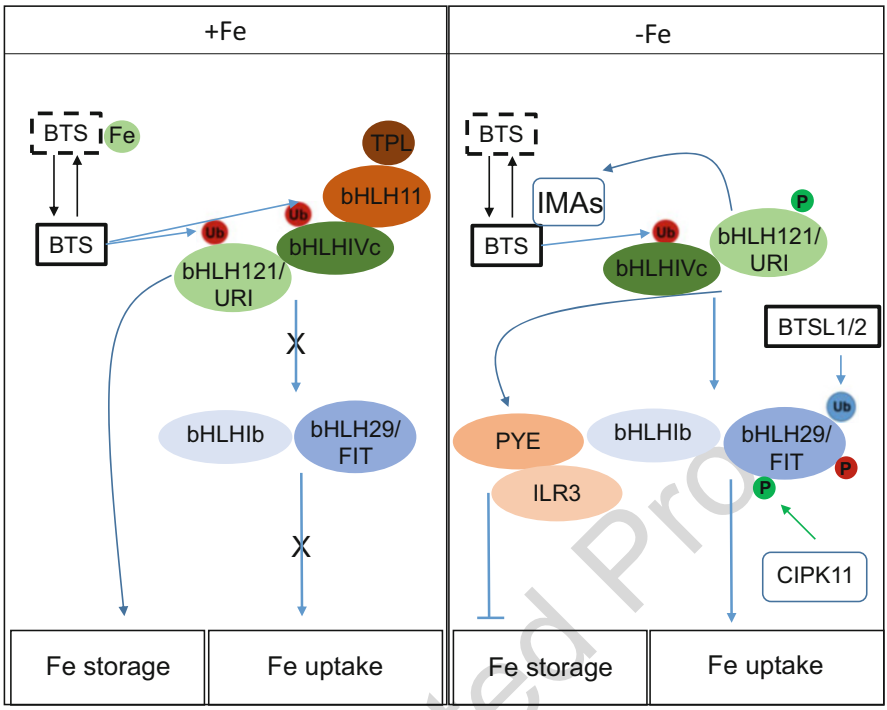

Fig. 2 Phylogenetic tree of bHLH TFs involved in the regulation of iron homeostasis in plants (color-coded genes). Remaining genes (in black) represent automated gene family assignment of gene family members in the Arabidopsis, rice, and Amborella genomes (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v5_dicots/). 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

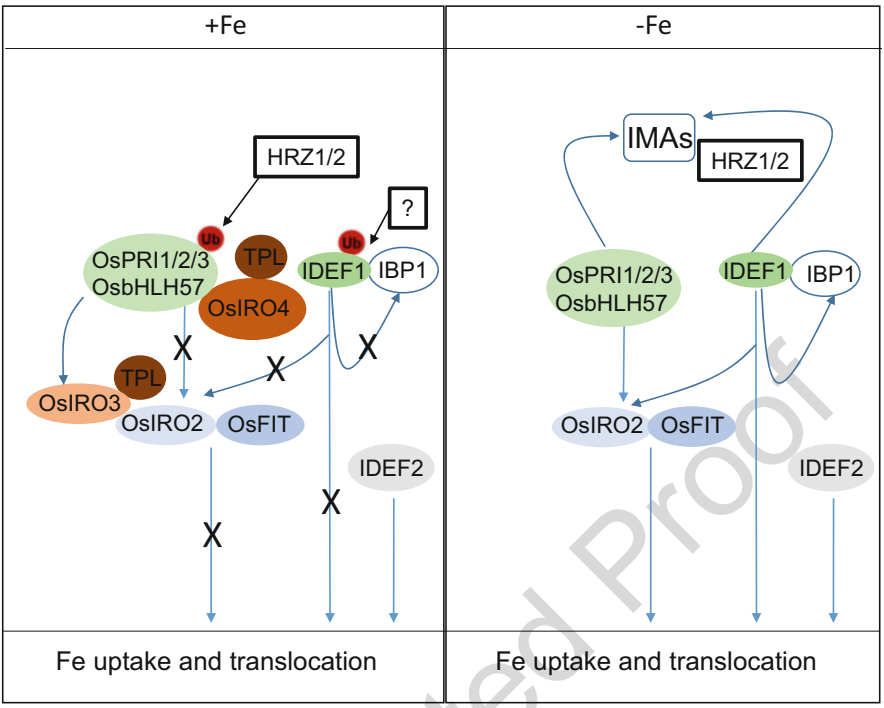

Fig. 3 Regulation of iron uptake and storage in *Arabidopsis thaliana*. In iron sufficient condition (+Fe), *FIT* and *clade Ib bHLHs* are not expressed in the root and consequently the transcription of Fe uptake actors is not induced. bHLH IVc and bHLH121/URI are transcribed but are supposed to stay 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

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

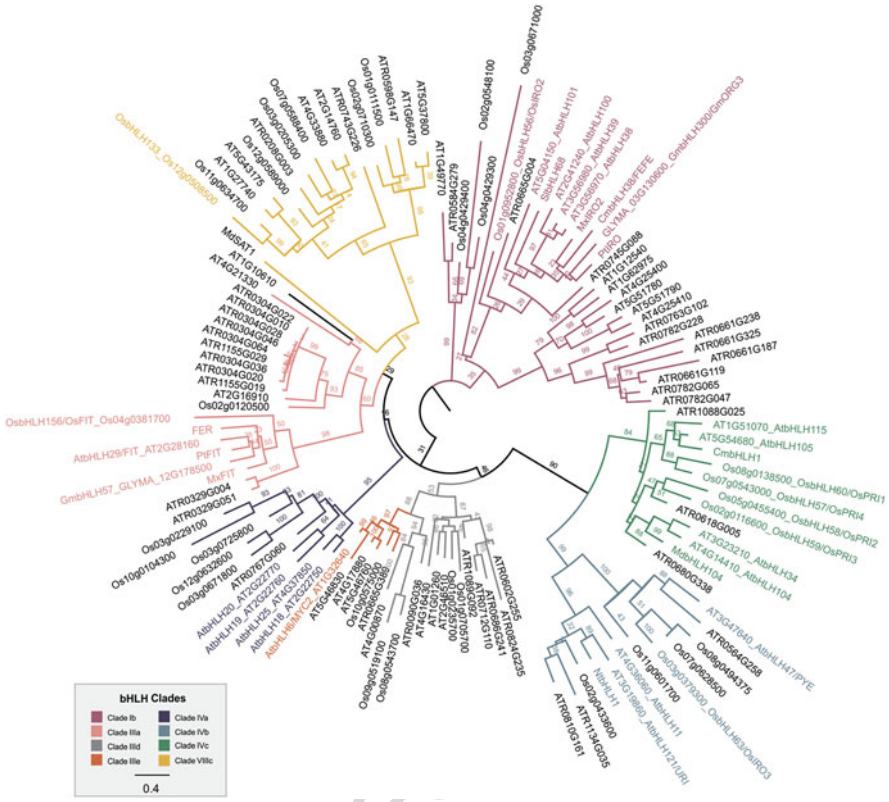

Fig. 4 Regulation of iron uptake in rice. OsPRI1/OsbHLH60, OsPRI2OsbHLH58, OsPRI3/OsbHLH59, and OsbHLH57 are positive regulators of Fe uptake machinery in rice. OsPRI1, OsPRI2, OsPRI3, and bHLH57 induce the expression of *OsIRO2/OsbHLH56* and *OsIRO3/OsbHLH63* that are, respectively, positive and negative 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, OsPRI2OsbHLH58, 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

clade IVc bHLH, namely ILR3/bHLH105 (IAA-LEUCINE RESISTANT 3), IDT1/bHLH34 (IRON DEFICIENCY TOLERANT 1), bHLH104 and bHLH115 (Li et al. 2016; Liang et al. 2017; Zhang et al. 2015). Clade IVc bHLH positively regulates the expression of clade Ib bHLH (direct) and *FIT* (indirect) when Fe availability is low. Their rice functional homologs are OsPRI1/OsbHLH60, OsPRI2/bHLH58, OsPRI3/OsbHLH59 (POSITIVE REGULATOR OF IRON HOMEOSTASIS 1, 2, and 3), and OsbHLH57 (Kobayashi et al. 2019; Wang et al. 2022c; Zhang et al. 2017, 2020).

Clade IVb bHLHs are also involved in this second module. The Arabidopsis PYE/bHLH47 and its rice functional homologs OsIRO3/OsbHLH63 and OsIRO4/OsbHLH61 are transcriptional repressors. Their repressing activity is conferred by the presence of an EAR motif in their C-terminal region (Kagale and Rozwadowski 2011). Interestingly, when ILR3 interacts with PYE it acts as a transcriptional repressor (direct) of genes involved in Fe storage such as those encoding FERRITINS (Tissot et al. 2019). The ILR3-PYE heterodimer also directly targets *PYE* in a negative feedback regulatory loop when Fe is not limiting, most probably allowing balancing Fe uptake and Fe storage to meet the need of the plant in this micronutrient (Tissot et al. 2019). A similar mechanism exists in rice (negative feedback regulatory loop) even if no gene encoding Fe storage protein has been identified, to date, as direct target of OsIRO3 and OsIRO4 (Li et al. 2022a; Wang et al. 2022d). It has been demonstrated that EAR-domain containing transcription factors act as negative regulators by recruiting TOPLESS/TOPLESS RELATED repressors (Kagale and Rozwadowski 2011). Such mechanism was confirmed for OsIRO3 and OsIRO4 (Li et al. 2022a; Wang et al. 2022d). Whether this is also the case for PYE is still to be demonstrated. The Arabidopsis bHLH11 is another clade IVb bHLH that contains an EAR-domain and that recruits TOPLESS/TOPLESS RELATED repressors to inhibit the expression of its target genes (Li et al. 2022b; Tanabe et al. 2018). Like PYE, bHLH11 forms heterodimers with clade IVc bHLHs, inhibiting the expression of both *clade Ib bHLHs* (direct) and *FIT* (indirect). This activity most probably prevents Fe overaccumulation and thus toxicity when its availability is high. The last member of the Arabidopsis clade IVb bHLH is URI/bHLH121 (UPSTREAM REGULATOR OF IRT1) and is also involved in this second module. Unlike the other members of this clade, URI acts as a transcriptional activator and can heterodimerize with the four clade IVc bHLH transcription factors (Gao et al. 2020a; Kim et al. 2019; Lei et al. 2020). Under Fe deficiency, URI mainly localizes in the cortex and the root epidermis cells, where it promotes Fe uptake by activating the expression of both clade Ib bHLH (direct) and *FIT* (indirect) (Gao et al. 2020a; Kim et al. 2019). URI positive transcriptional activity extends (directly or indirectly) to most of the known genes involved in this intricate transcriptional regulatory network (Gao et al. 2020a, b). Under Fe sufficient condition, URI mainly localizes in the stele and the endodermis where it promotes the transient storage of Fe into ferritins by directly inducing their expression. Since URI can activate the expression of *FERRITIN* genes when Fe is not limiting, it is likely that URI regulates the expression of some of its target genes independently of clade IVc bHLH transcription factors.

4 Post-Translational Regulation of Iron Homeostasis

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

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

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

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

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

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

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

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

Sumoylation process is another post-translational mechanism that regulates transcription factors activity by conjugating small ubiquitin-like modifier (SUMO) peptides (Miura et al. 2007). For instance, it was shown that the sumoylation of the apple (*Malus domestica*) MdbHLH104 transcription factor (a functional homolog of the Arabidopsis bHLH104) by SIZ1 (a SIZ/PIAS-type SUMO E3 ligase) was promoting root plasma membrane H^+-ATPase activity and thus the acidification of the rhizosphere and Fe uptake. Interestingly, SUMO-conjugation is promoted by Fe deficiency conditions and that stabilizes MdbHLH104 (Zhou et al. 2019). In addition, it was observed that several Arabidopsis bHLH transcription factors are SUMO-targets (Castro et al. 2023). For instance, MYC2 and bHLH3/JAM3 are 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 H4R3sme2 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 H4R3sme2 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

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

To date, asymmetric cytosine methylation at CHH loci (H: adenine or thymine or cytosine) is the sole DNA modification that is associated with the regulation of Fe homeostasis in plants. For instance, it was shown that under Fe starvation conditions, the promoter of *OsIRO2* and *OsbHLH156* was hypermethylated at CHH loci and correlated with an increased expression of these genes (Sun et al. 2021). In this study, the authors suggested that this mechanism could implicate small RNAs.

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

6 Iron Interaction with Other Mineral Nutrients

Plants must adapt to constantly changing and often stressful or unfavorable environments, including fluctuations in the availability of essential mineral nutrients. The availability of some nutrients can be significantly altered in the presence or absence of other soil nutrients. Recent research highlighted that nutrients interact with each other, at the molecular level, in complex ways. These interactions can have positive or negative effects on nutrient uptake and may also result in "hidden responses" that are more complex than the sum of individual stress responses (Bouain et al. 2019). In 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

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

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

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

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

7 Roles of Microorganisms in the Regulation of Plant Iron Homeostasis

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-sequestrating 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 Fe^{3+} 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.

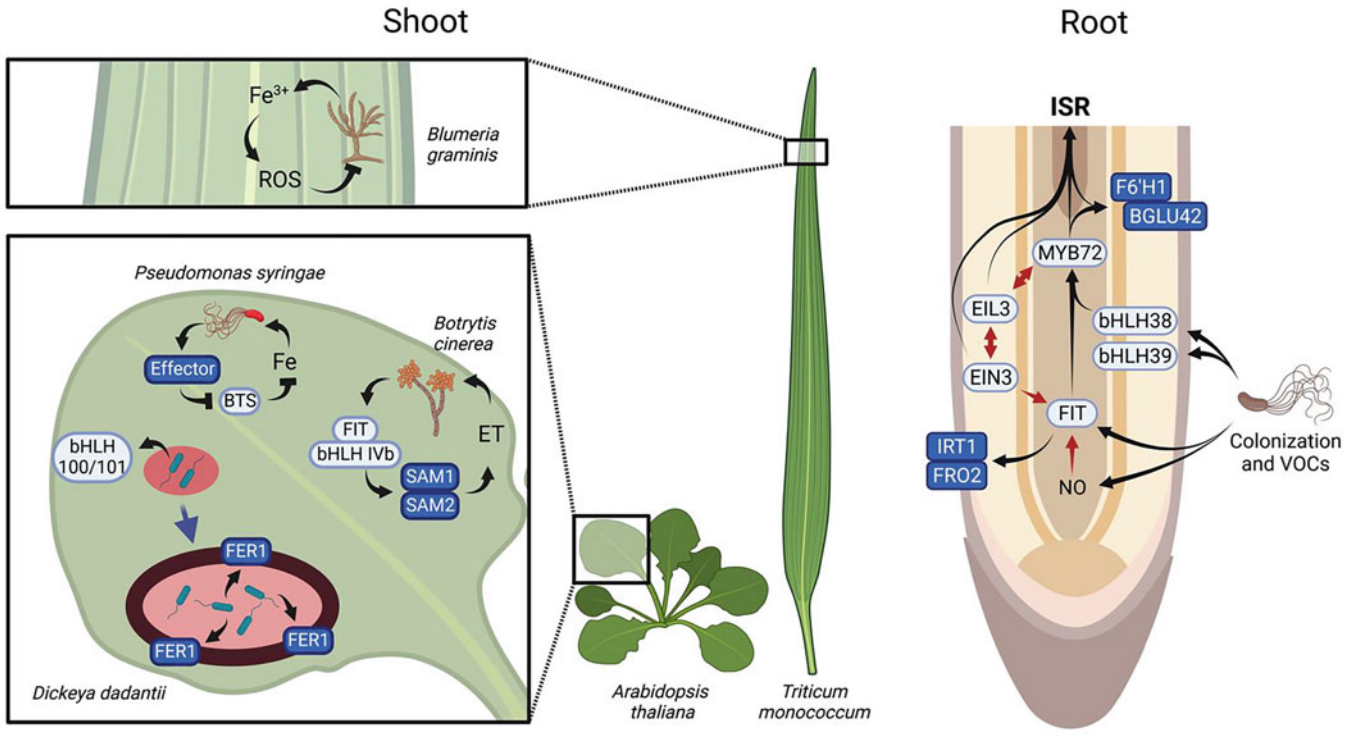

Fig. 5 Transcriptional activation of iron-responsive genes in the shoot and root in response to microbes. In Arabidopsis shoots, *Dickeya dadantii* activates bHLH100 and bHLH101 during early infection, and triggers Fe sequestration around the infection site by FER1 in later infection stages. *Pseudomonas 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*, *FRO2*, *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; Fe^{3+}, ferric oxide; ET, ethylene. Figure was designed using Biorender (https://biorender.com)

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

8 Conclusion

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

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

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