Plant Communications
Review Article

CellPress
Partner Journal

Iron uptake, signaling, and sensing in plants

Gang Liang[1,*]

[1]CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Kunming, Yunnan 650223, China

*Correspondence: Gang Liang (lianggang@xtbg.ac.cn)

https://doi.org/10.1016/j.xplc.2022.100349

ABSTRACT

Iron (Fe) is an essential micronutrient that affects the growth and development of plants because it participates as a cofactor in numerous physiological and biochemical reactions. As a transition metal, Fe is redox active. Fe often exists in soil in the form of insoluble ferric hydroxides that are not bioavailable to plants. Plants have developed sophisticated mechanisms to ensure an adequate supply of Fe in a fluctuating environment. Plants can sense Fe status and modulate the transcription of Fe uptake-associated genes, finally controlling Fe uptake from soil to root. There is a critical need to understand the molecular mechanisms by which plants maintain Fe homeostasis in response to Fe fluctuations. This review focuses on recent advances in elucidating the functions of Fe signaling components. Taking *Arabidopsis thaliana* and *Oryza sativa* as examples, this review begins by discussing the Fe acquisition systems that control Fe uptake from soil, the major components that regulate Fe uptake systems, and the perception of Fe status. Future explorations of Fe signal transduction will pave the way for understanding the regulatory mechanisms that underlie the maintenance of plant Fe homeostasis.

Keywords: iron, metal homeostasis, Fe uptake, Fe signaling, Fe sensor, plant nutrition

Liang G. (2022). Iron uptake, signaling, and sensing in plants. Plant Comm. **3**, 100349.

INTRODUCTION

Iron (Fe) is one of the nutrients that are indispensable for plant growth and development. Fe takes part in many chemical reactions, including photosynthesis and respiration, as it is redox active owing to its ability to easily accept and donate electrons. Fe deficiency usually leads to a reduction in plant viability. Plants mainly absorb Fe from the soil (Zelazny and Vert, 2014). Because of its high redox activity, Fe is readily oxidized in aerobic or alkaline soils. The oxidized Fe is insoluble and hence unavailable to plants. Approximately one-third of the world's cultivated lands are calcareous (Mori, 1999), making Fe deficiency a limiting factor for crop yield and quality. However, excess Fe is toxic to cells, as Fe can cause the formation of highly reactive hydroxyl radicals through the Fenton reaction (Dixon and Stockwell, 2014). Plants have evolved sophisticated mechanisms for tightly controlling the influx of Fe to maintain Fe homeostasis. Thus, elucidating the mechanisms by which plants control Fe uptake and transport is essential for breeding of crops that are rich in Fe or tolerant to Fe-deficient soils.

This review discusses how plants integrate different components into the Fe deficiency response, with a focus on research in *Arabidopsis* and rice, and highlights the most recently characterized molecular components involved in Fe sensing, Fe signaling, and downstream Fe uptake processes. Fe translocation from roots to shoots through vascular tissues and Fe allocation from pools to sinks are not the aims of this review (for review, see Curie et al., 2009; Kobayashi and Nishizawa, 2012; and Jeong et al., 2017). There are many excellent reviews about the Fe-deficiency response that may also interest readers (Grillet and Schmidt, 2019; Gao and Dubos, 2021; Riaz and Guerinot, 2021; Vélez-Bermúdez and Schmidt, 2022).

FE ACQUISITION SYSTEMS

Plants use two different strategies for Fe uptake to adapt to Fe-deficient environments, "strategy I" and "strategy II" (Römheld and Marschner, 1986).

Strategy I is a reduction-based mechanism that involves acidification of the rhizosphere by root-released protons, reduction of Fe^{3+} to Fe^{2+} by a membrane-localized reductase, and root uptake of Fe^{2+} by a membrane-localized high-affinity Fe transporter (Figure 1). In *Arabidopsis thaliana*, AHA2 (Arabidopsis H^+-ATPase 2), AtFRO2 (ferric reduction oxidase 2) and AtIRT1 (iron-regulated transporter 1) are involved in these three steps (Robinson et al., 1999; Henriques et al., 2002; Varotto et al., 2002; Vert et al., 2002; Santi and Schmidt, 2009), and they interact with one another to form an iron-acquisition protein complex that optimizes Fe uptake at the cell surface of *Arabidopsis* root epidermal cells (Figure 1; Martín-Barranco et al., 2020). Notably, Fe^{3+} bio-availability decreases 1000-fold with an

Published by the Plant Communications Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and CEMPS, CAS.

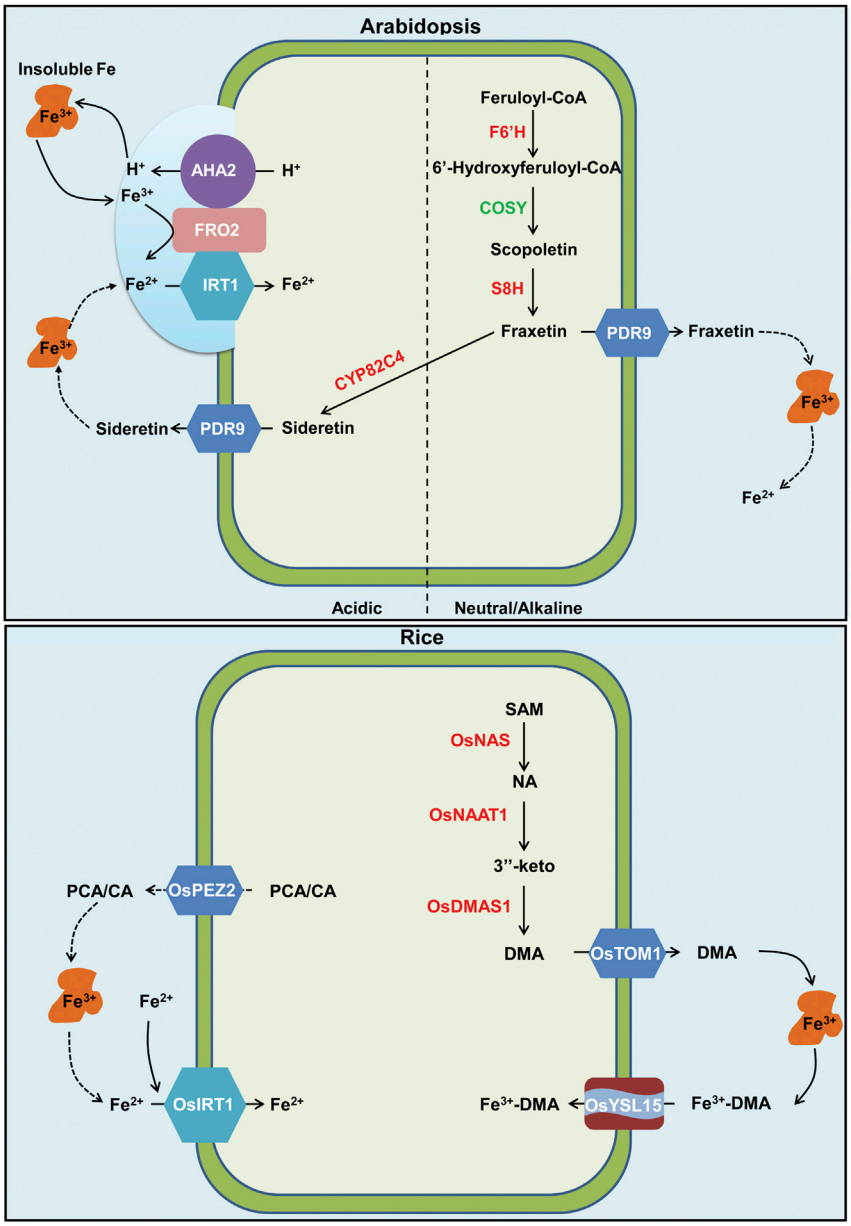

Figure 1. Fe acquisition systems in *Arabidopsis* and rice.

Arabidopsis plants absorb Fe from soil using strategy I, which involves a protein complex consisting of AHA2, FRO2, and IRT1. AHA2 secretes protons to acidify rhizosphere soil and mobilize Fe^{3+}. After reduction by FRO2, Fe^{2+} enters roots with the assistance of IRT1. In *Arabidopsis*, coumarins also contribute to the reduction strategy, probably by converting Fe^{3+} to Fe^{2+}. Sideretin is the major coumarin at acidic pH, whereas fraxetin is the major coumarin at neutral or alkaline pH. PDR9 mediates the efflux of coumarins from roots. Rice plants use 2 different strategies. In strategy I, PEZ2 may be responsible for the secretion of PCA/CA, which can reduce Fe^{3+} to Fe^{2+}, and IRT1 directly transports Fe^{2+}, which is abundant in paddy fields. In strategy II, DMA is excreted by TOM1 to chelate Fe^{3+}, and Fe^{3+}-DMA is transferred into roots by YSL15. Names in red indicate that their transcripts are inducible in response to Fe deficiency. *COSY* is not inducible in response to Fe deficiency. The dotted line indicates that it has not been validated experimentally.

increase of one pH unit between 4 and 9 (Lindsay, 1997). The low efficiency of AtFRO2 for reduction of Fe^{3+} in alkaline soil results in the failure of strategy I. Coumarins were found to be crucial for the adaptation of *Arabidopsis* plants to alkaline soils (Rodríguez-Celma et al., 2013; Schmid et al., 2014; Siwinska et al., 2018; Tsai et al., 2018). To cope with inadequate soluble Fe in alkaline soil, *Arabidopsis* plants secrete Fe-mobilizing coumarins into the rhizosphere (Robe et al., 2021; Figure 1). In *Arabidopsis*, AtF6′H1 (feruloyl-coenzyme A [CoA] 6′-hydroxylase 1), a 2-oxoglutarate-dependent dioxygenase enzyme, is responsible for the conversion of feruloyl CoA to 6′-hydroxyferuloyl CoA, which is the common precursor for the synthesis of coumarins (Kai et al., 2006; Rodríguez-Celma et al., 2013; Schmid et al., 2014). The conversion of 6′-hydroxyferuloyl-CoA into scopoletin can occur spontaneously, but coumarin synthase (COSY) improves the reaction efficiency (Vanholme et al., 2019). With the addition of a catechol group, coumarins possess the ability to bind Fe (Rajniak et al., 2018). Fraxetin and sideretin are two main catechol coumarins that are biosynthesized by AtS8H (scopoletin 8-hydroxylase), a 2-oxoglutarate-dependent dioxygenase, and AtCYP82C4, a P450-dependent monooxygenase, respectively (Siwinska et al., 2018; Tsai et al., 2018). The expression of *AtCYP82C4* is reduced at alkaline pH but increased at acidic pH under Fe-deficient conditions, explaining why alkaline pH and acidic pH favor the biosynthesis of fraxetin and sideretin, respectively (Figure 1; Tsai and Schmidt, 2020; Gautam et al., 2021). Fraxetin is a major type of coumarin that dissolves and binds Fe to form stable complexes at neutral to alkaline pH. It has been established that the ABCG transporter AtPDR9 (pleiotropic drug resistance 9) mediates the secretion of coumarins from root to rhizosphere (Fourcroy et al., 2014; Figure 1). It is hypothesized that Fe-mobilizing coumarins promote the reduction of Fe^{3+} to Fe^{2+}, contributing to reduction-based Fe uptake (Rodríguez-Celma et al., 2013; Rajniak et al., 2018). Similar to coumarins, flavins also facilitate reduction-based Fe uptake (Rodríguez-Celma et al., 2013; Sisó-Terraza et al., 2016). Some species synthesize and secrete large quantities of flavins in response to Fe deficiency (Rodríguez-Celma et al., 2011). Moreover, the induction of coumarins and flavins under Fe-deficient conditions is mutually exclusive in *Arabidopsis* and *Medicago truncatula* (Rodríguez-Celma et al., 2013).

Strategy II is a chelation-based mechanism, well known in graminaceous plants (Figure 1). This strategy involves release of the mugineic acid (MA) family of phytosiderophores from roots to solubilize and chelate Fe^{3+} in soil (Takagi et al., 1984). The

synthesis of MA derives from methionine. Methionine is activated to SAM (S-adenosyl methionine) by SAM, and three SAM molecules are combined by NAS (nicotianamine synthase) to form NA (nicotianamine) (Mori and Nishizawa, 1987). NA is the direct precursor for MA synthesis and is converted into a 3″-keto intermediate by OsNAAT1 (NA amino transferase 1) (Cheng et al., 2007). The reduction of the keto intermediate by OsDMAS1 (deoxymugineic acid synthase 1) (Bashir et al., 2017) results in the production of deoxymugineic acid (DMA), which is excreted into the rhizosphere via OsTOM1 (transporter of mugineic acid 1) (Nozoye et al., 2011). DMA chelates Fe^{3+} to form the Fe^{3+}-DMA complex, which is transported into roots by OsYSL15 (yellow stripe-like 15) (Inoue et al., 2009; Lee et al., 2009).

Although the genes that encode IRT1 and FRO2 exist in barley and maize, the Fe-deficiency-inducible Fe^{2+} uptake system is absent (Zaharieva and Römheld, 2000). The ferric-chelate reductase activity of rice roots is very low and not induced by Fe deficiency (Ishimaru et al., 2006). However, rice plants possess a partial strategy I (Figure 1). Rice as a specific graminaceous species grows better under anaerobic and Fe^{2+}-rich conditions such as paddy fields. As a result of the pressures of surviving under submerged conditions with abundant Fe^{2+}, rice plants have retained their ability to directly absorb Fe^{2+} by OsIRT1 (Ishimaru et al., 2006). Similar to *Arabidopsis* plants, which secrete various phenolic compounds, rice plants also secret protocatechuic acid (PCA) and caffeic acid (CA) into the rhizosphere under low Fe availability conditions. OsPEZ2 (phenolics efflux zero 2), a phenolic efflux transporter, is expressed in the plasma membrane of the root epidermis and exodermis and may account for the secretion of PCA and CA (Bashir et al., 2011). PCA and CA possess the chemical properties to chelate and reduce Fe^{3+} *in vitro* (Yoshino and Murakami, 1998), hence improving Fe uptake by strategy I. However, the expression of *OsPEZ2* is not inducible in response to Fe deficiency (Kobayashi et al., 2014), implying that this effect is limited.

REGULATION OF FE ACQUISITION SYSTEMS

Plants have evolved an intricate network for the regulation of their Fe acquisition systems. The basic helix-loop-helix (bHLH) transcription factor LeFER was the first Fe signaling regulator identified in plants. LeFER is crucial for Fe acquisition in tomato (*Lycopersicon esculentum*), as the *fer* mutant has no ability to activate strategy I under Fe-deficient conditions (Ling et al., 2002). Later, its *Arabidopsis* ortholog AtFIT (Fer-like Fe deficiency-induced transcription factor)/AtbHLH29 was found to positively regulate the expression of *AtIRT1* and *AtFRO2* (Colangelo and Guerinot, 2004; Jakoby et al., 2004; Yuan et al., 2005). Loss-of-function *fit* mutants display extreme Fe-deficiency symptoms and cannot survive in soil without Fe supplementation (Colangelo and Guerinot, 2004). Correspondingly, rhizosphere acidification ability, Fe reductase activity, and Fe content are considerably lower in the *fit* mutants. However, the overexpression of *AtFIT* alone is insufficient to constitutively stimulate the expression of *AtIRT1* and *AtFRO2* (Colangelo and Guerinot, 2004). It has been established that AtFIT interacts with each of the four members of the bHLH Ib clade (AtbHLH38, AtbHLH39, AtbHLH100, and AtbHLH101) to form heterodimers. The overexpression of both *AtFIT* and AtbHLH Ib is sufficient for constitutive activation of *AtIRT1* and *AtFRO2*, irrespective of Fe status (Yuan et al., 2008; Wang et al., 2013). Recently, Cai et al. (2021) revealed that the quadruple mutant (*bhlh4x*) of bHLH Ib displays Fe-deficiency symptoms similar to those of *fit*. However, it is still unclear how AtFIT and AtbHLH Ib interdependently regulate the expression of *AtIRT1* and *AtFRO2*. Comparative analysis of phenotypes and transcriptomes between *fit* and *bhlh4x* will provide insights into the molecular functions of AtFIT and AtbHLH Ib.

The synthesis of coumarins is also controlled by the AtFIT-AtbHLH Ib module, as induction of *AtS8H* and *AtCYP82C4* is dependent on AtFIT (Schwarz and Bauer, 2020). The transcription factor AtMYB72 is crucial for *Arabidopsis* growth in alkaline soil (Palmer et al., 2013) and is a key regulator of coumarin synthesis (Stringlis et al., 2018; Robe et al., 2021). AtMYB72 tightly controls the expression of *AtF6′H1*, and the loss-of-function *myb72* mutant can produce almost no scopoletin (Stringlis et al., 2018). By contrast, scopoletin can still accumulate in the *fit* mutant (Chutia et al., 2019), in line with the fact that expression of *AtMYB72* is partially regulated by AtFIT (Colangelo and Guerinot, 2004; Zamioudis et al., 2015). *AtMYB72* is a typical Fe-deficiency-inducible gene. Interestingly, the expression of *AtMYB72* is also regulated by the AtFIT-AtbHLH Ib module because the transcript abundance of *AtMYB72* is higher in *AtFIT*/AtbHLH Ib dual overexpression plants than in the wild type (Zamioudis et al., 2015). Notably, *AtMYB72* is still induced in *fit* mutants in response to Fe deficiency, implying that other regulators mediate the upregulation of *AtMYB72* in an AtFIT-independent manner. Recent studies reveal that AtbHLH121/AtURI (upstream regulator of IRT1) directly regulates AtbHLH Ib and *AtMYB72* (Kim et al., 2019; Gao et al., 2020; Lei et al., 2020). Consistent with this finding, mutation of *AtbHLH121* results in very low levels of two main catechol coumarins, fraxetin glycoside and sideretin glycoside (Gao et al., 2020). Unlike *AtMYB72* and *AtS8H*, both of which are induced under high-pH conditions, *AtCYP82C4* is not induced under these conditions (Gautam et al., 2021), implying that the expression of *AtCYP82C4* is independent of AtMYB72. Taken together, AtFIT-bHLH Ib and AtMYB72 coordinate the synthesis of coumarins according to changes in Fe availability and environmental pH.

A regulatory module similar to AtFIT-bHLH Ib also exists in rice and governs its Fe acquisition systems. OsFIT/OsbHLH156 is an ortholog of AtFIT (Wang et al., 2020a; Liang et al., 2020), and the rice bHLH Ib member OsIRO2 (iron-related bHLH transcription factor 2/OsbHLH056) corresponds to the four AtbHLH Ib members (Supplemental Figure 1; Ogo et al., 2007). OsFIT and OsIRO2 interact with each other to form a functional transcription complex (Wang et al., 2020a; Liang et al., 2020). Owing to functional redundancy among the four AtbHLH Ib members, the Fe-deficiency symptoms of their double or triple mutants are not as severe as those of the *fit* mutants (Wang et al., 2013; Maurer et al., 2014), whereas their quadruple mutants phenocopy the *fit* mutants (Cai et al., 2021). In rice, OsIRO2 is the only member corresponding to AtbHLH Ib, and this conclusion is also supported by genetic evidence that the *iro2* mutant is similar to the *fit* mutants in terms of Fe-deficiency symptoms and expression of Fe-deficiency-inducible genes (Liang et al.,

2020). The strategy II genes (*OsNAS1*, *OsNAS2*, *OsNAAT1*, *OsDMAS1*, *OsTOM1*, and *OsYSL15*) are under the control of the OsFIT-OsIRO2 module because their induction is almost completely blocked in roots of *iro2* and *fit* mutants under Fe-deficient conditions (Wang et al., 2020a; Liang et al., 2020). By contrast, the strategy I gene *OsIRT1* is only weakly regulated by OsFIT-OsIRO2 (Wang et al., 2020a; Liang et al., 2020) or not regulated by OsIRO2 (Ogo et al., 2007). There are no counterparts of *AtMYB72* and the coumarin synthesis-associated genes among the Fe-deficiency-inducible genes in rice, suggesting that the coumarin-based Fe acquisition strategy is not employed in rice.

In *Arabidopsis*, AtbHLH39 is mainly localized in the cytoplasm, but it accumulates in the nucleus in the presence of AtFIT (Trofimov et al., 2019). Similarly, the cytoplasmic fraction of OsIRO2 decreases and its nuclear fraction increases in the presence of OsFIT (Wang et al., 2020a; Liang et al., 2020). Although *Arabidopsis* and rice have evolved different Fe acquisition systems, they have retained the same regulatory mechanism to control them. Close homologs of FIT and bHLH Ib have also been identified in plant species beyond *Arabidopsis* and rice (Du et al., 2015; Li et al., 2018), suggesting that the Fe acquisition systems of plants are controlled by the conserved FIT/bHLH Ib module. Monocot plants (e.g., rice, maize) have only one bHLH Ib gene, whereas dicot plants (e.g., *Arabidopsis*, tomato, soybean, *Populus*) have more than three (Huang and Dai, 2015; Kurt and Filiz, 2018; Li et al., 2018), implying that the regulatory module evolved further after the monocot-dicot divergence.

TRANSCRIPTIONAL REGULATION OF THE FIT-bHLH IB MODULE

Although AtbHLH Ib genes are positive regulators of Fe uptake genes, they themselves are also dramatically induced by Fe deficiency. It has been established that the upregulation of bHLH Ib genes is dependent on the AtbHLH IVc subgroup, which consists of AtbHLH34/IDT1 (iron deficiency tolerant 1), AtbHLH104, AtbHLH105/ILR3 (IAA-leucine-resistant 3), and AtbHLH115 (Supplemental Figure 1). A yeast one-hybrid screen with the *AtbHLH101* promoter as a bait against an *Arabidopsis* cDNA library identified AtbHLH34 (Li et al., 2016). As a close homolog of bHLH34, AtbHLH104 also binds to the *AtbHLH101* promoter in yeast one-hybrid assays. *bhlh34* and *bhlh104* mutants are sensitive to Fe-deficient conditions and have low levels of AtbHLH Ib transcripts (Li et al., 2016). Zhang et al. (2015) found that BHLH104 and ILR3 are required for upregulation of AtbHLH Ib genes and activation of the Fe-deficiency response. Similarly, *bhlh115* loss-of-function mutants also develop Fe-deficiency symptoms similar to those of *bhlh34*, *bhlh104*, and *ilr3-2* (Liang et al., 2017). Correspondingly, the overexpression of each of the AtbHLH IVc members enhances the expression of AtbHLH Ib genes and rescues the *bhlh115-1* mutant (Zhang et al., 2015; Li et al., 2016; Liang et al., 2017). This suggests that these four members share similar molecular functions. Although the four members of the AtbHLH IVc subgroup have different expression patterns, their expression overlaps in some regions (Liang et al., 2017). Thus, AtbHLH IVc proteins have not only their own independent functions but also interdependent functions, a conclusion that is further supported by the fact that AtbHLH IVc members interact with one another to form homodimers or heterodimers, and their high order mutants develop more severe Fe-deficiency symptoms than their single mutants (Li et al., 2016; Liang et al., 2017). The expression of both AtbHLH Ib and *AtFIT* decreases in single and high-order mutants of AtbHLH IVc. However, unlike AtbHLH Ib members, which are the direct target genes of AtbHLH IVc, *AtFIT* is not a direct target gene of AtbHLH IVc, as its promoter is not associated with AtbHLH IVc (Zhang et al., 2015; Li et al., 2016; Liang et al., 2017; Tissot et al., 2019).

The AtbHLH IVb subgroup contains three members, AtbHLH121, AtbHLH11, and AtPYE (POPEYE/AtbHLH47) (Supplemental Figure 1; Heim et al., 2003). Three different groups have reported that AtbHLH121 interacts with AtbHLH IVc in the nucleus and is required for the induction of AtbHLH Ib and *AtFIT* under Fe-deficient conditions (Kim et al., 2019; Gao et al., 2020; Lei et al., 2020). All three groups established that bHLH121 associates with the promoters of AtbHLH Ib genes. It is still controversial whether AtbHLH121 directly regulates *AtFIT*. Lei et al. (2020) proposed that AtbHLH121 directly targets the *AtFIT* promoter, but the other two groups did not support this conclusion. It is noteworthy that *AtFIT* is expressed mainly in the root and is slightly induced by Fe deficiency. Lei et al. (2020) used roots in ChIP (chromatin immunoprecipitation) assays, **Error! Hyperlink reference not valid.**whereas the other groups used whole seedlings, which may have produced different results. AtbHLH11 is the closest homolog of AtbHLH121, and its overexpression causes a decrease in *AtFIT* but an increase in bHLH Ib (Tanabe et al., 2019). By analyzing *bhlh11* mutants and transgenic plants conditionally overexpressing *AtbHLH11*, Li et al. (2022) confirmed that AtbHLH11 negatively regulates the expression of AtbHLH Ib. They revealed that AtbHLH11 interacts with AtbHLH IVc members and inhibits the transactivation activity of the latter toward AtbHLH Ib genes by its two ethylene response factor-associated amphiphilic repression (EAR) motifs (LxLxL), which recruit the co-repressors topless/topless-related (AtTPL/AtTPRs). They also proposed that the severe Fe-deficient status caused by *AtbHLH11* overexpression indirectly results in the upregulation of AtbHLH Ib genes. AtPYE is a negative regulator of Fe homeostasis. Although the *pye-1* loss-of-function mutant displays severe Fe-deficiency symptoms during Fe limitation, expression of bHLH Ib genes (*bHLH39* and *bHLH101*) is increased in *pye-1* (Long et al., 2010). AtPYE interacts with AtbHLH105, and both of them negatively regulate several structural genes involved in the control of Fe homeostasis, such as *AtFERs* (*Ferritins*) and *AtNAS4* (Tissot et al., 2019). AtPYE contains another type of EAR motif (DLNxxP) that was also predicted to interact with the co-repressors AtTPL/AtTPRs (Kagale et al., 2010; Causier et al., 2012). However, this protein interaction has not been validated experimentally. It is noteworthy that the loss of function of *AtPYE* results in upregulation of AtbHLH Ib genes but causes a reduction in both Fe chelate reductase activity and rhizosphere acidification ability (Long et al., 2010). Extensive exploration is required to clarify how AtPYE negatively regulates Fe acquisition systems.

Expression of AtbHLH Ib genes is significantly higher in *fit* mutants than in the wild type, suggesting that AtFIT is not required for upregulation of AtbHLH Ib genes (Wang et al., 2007). By contrast, expression of *AtFIT* is higher in *AtbHLH39*-overexpressing plants than in the wild type (Naranjo-Arcos

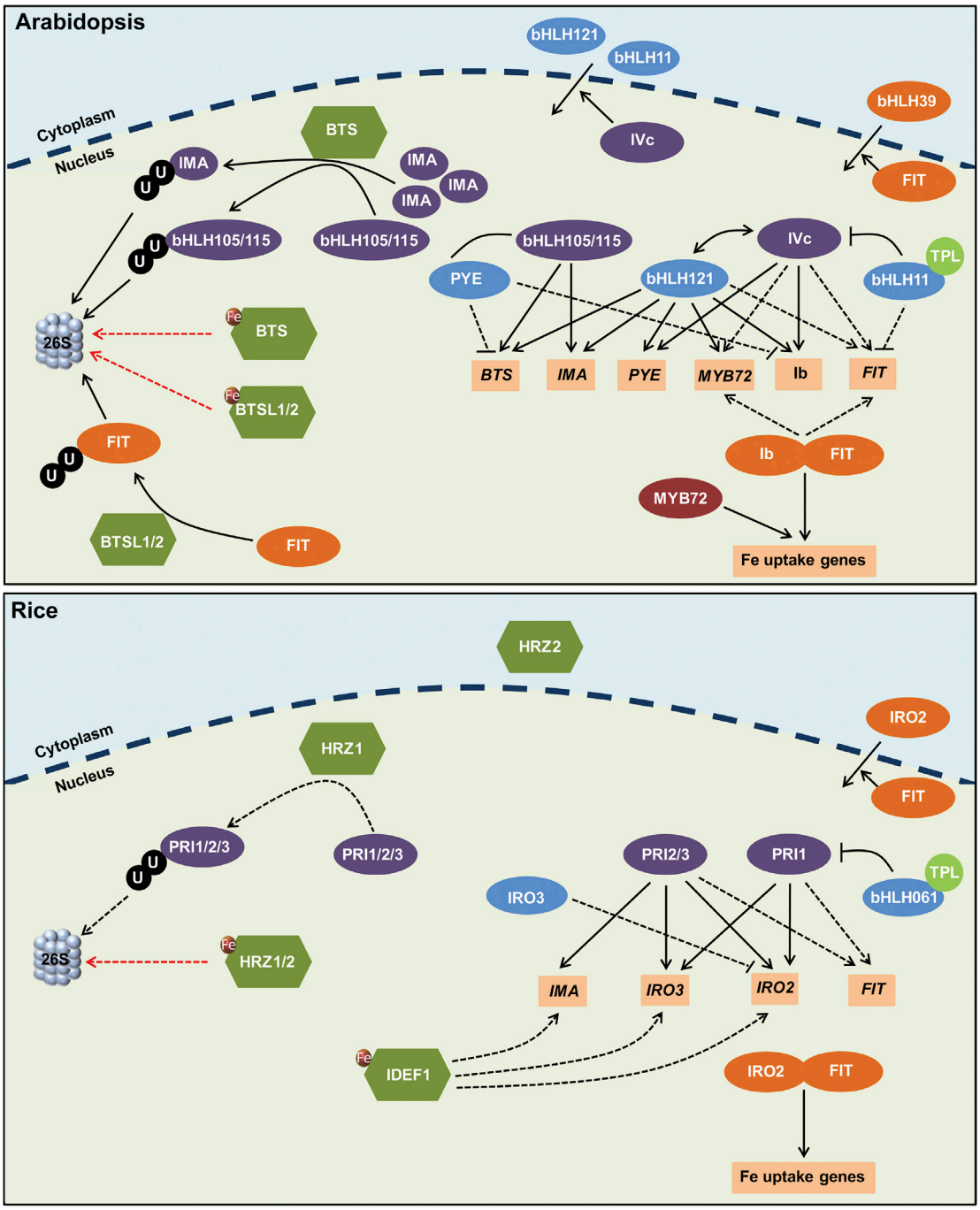

Figure 2. Fe perception and the regulation of Fe acquisition systems.
Hexagon (Fe sensor); oval (protein); and rectangle (transcript). IVc (bHLH34, bHLH104, bHLH105, and bHLH115) and Ib (bHLH38, bHLH39, bHLH100, and bHLH101). The arrow indicates a positive effect, the blunt arrow indicates a negative effect, the black dotted line indicates an indirect regulation, and the red dotted line indicates a hypothesis. The letter U in the black circle indicates ubiquitin.

et al., 2017), suggesting that AtbHLH Ib proteins positively regulate the expression of *AtFIT*. When AtFIT is absent, β-glucuronidase activity decreases in transgenic plants expressing an *AtFIT* promoter-driven β-glucuronidase, implying that *AtFIT* is crucial for its own upregulation under Fe-deficient conditions (Wang et al., 2007). It is possible that the expression of *AtFIT* is also controlled by the AtFIT-AtbHLH Ib complex (Figure 2).

In rice, the expression of *OsIRO2* is regulated by the OsbHLH IVc and OsbHLH IVb proteins. The OsbHLH IVc subgroup contains four members, positive regulator of iron homeostasis1 (OsPRI1)/OsbHLH060, OsPRI2/OsbHLH058, OsPRI3/OsbHLH059, and OsbHLH057 (Supplemental Figure 1). Loss of function or gene silencing of any of OsPRI1/2/3 causes downregulation of *OsIRO2* and enhanced sensitivity to Fe deficiency (Zhang et al., 2017,

2020; Kobayashi et al., 2019). OsPRI1/2/3 can bind to and activate the promoter of *OsIRO2* (Zhang et al., 2017, 2020). Despite its uncharacterized function in the Fe-deficiency response, OsbHLH057 may also regulate the expression of *OsIRO2* based on its sequence similarity to the other members (Zhang et al., 2020). There are four members in the OsbHLH IVb subgroup: OsbHLH061, OsbHLH062, OsIRO3/OsbHLH063, and OsbHLH064 (Supplemental Figure 1). OsIRO3 is a negative regulator of the Fe-deficiency response. Overexpression of *OsIRO3* causes reduced expression of Fe-deficiency-inducible genes, including *OsIRO2*, and severe Fe-deficiency symptoms (Zheng et al., 2010). By contrast, loss of function of *OsIRO3* results in elevated expression of Fe-deficiency-inducible genes and necrotic leaf spots under Fe-deficient conditions (Wang et al., 2020b, 2020c; Carey-Fung et al., 2022). Similar to *Arabidopsis AtbHLH11*, *OsbHLH061* is inducible in response to high Fe, and *bhlh061* mutants are sensitive to excess Fe (Wang et al., 2022). Wang et al. (2022) revealed that OsbHLH061 also has two EAR motifs, one of which recruits the co-repressors OsTPL/OsTPRs, and OsbHLH061 interacts with and inhibits the transcription activation of OsPRI1 toward *OsIRO2* (Figure 2). Thus, OsbHLH061 is also a negative regulator of Fe homeostasis. The transcript abundance of *OsFIT* increases under Fe-deficient conditions (Wang et al., 2020a; Liang et al., 2020). Liang et al. (2020) found that expression of *OsFIT* is reduced in the *pri1-1*, *pri2-1*, and *pri3-1* mutants, suggesting that OsPRI1/2/3 positively regulate *OsFIT* (Figure 2). It is still unclear whether OsbHLH062 and OsbHLH064 are involved in the regulation of the Fe-deficiency response.

INTERACTION BETWEEN bHLH IVb AND bHLH IVc

Among the three AtbHLH IVb members, only *AtbHLH11* is repressed by Fe deficiency (Tanabe et al., 2019; Li et al., 2022). However, the upstream regulators of *AtbHLH11* have not yet been identified. *AtbHLH121* is slightly responsive or unresponsive to Fe deficiency (Kim et al., 2019; Gao et al., 2020; Lei et al., 2020). *AtPYE* is a typical Fe-deficiency-inducible gene. AtbHLH IVc members have been reported to mediate the upregulation of *AtPYE* by directly binding to its promoter (Zhang et al., 2015; Liang et al., 2017; Tissot et al., 2019). AtPYE protein can directly bind to its own promoter, suggesting that *AtPYE* transcription is also under the control of its own protein (Tissot et al., 2019). Among the OsbHLH IVb members, only *OsIRO3* has been reported to be induced by Fe deficiency (Zheng et al., 2010). Three of the OsbHLH IVc members, OsPRI1/2/3, have been confirmed to directly regulate the expression of *OsIRO3* (Zhang et al., 2017, 2020). It is possible that the fourth member, OsbHLH057, also directly regulates *OsIRO3* as do OsPRI1/2/3.

Unlike AtbHLH IVc proteins, which are predominantly localized in the nucleus, AtbHLH IVb proteins are preferentially localized in both the nucleus and cytoplasm. AtbHLH11 and AtbHLH121 physically interact with all four AtbHLH IVc proteins, and AtPYE also interacts with all except AtbHLH34 (Long et al., 2010; Kim et al., 2019; Gao et al., 2020; Lei et al., 2020; Li et al., 2022). Interestingly, the nuclear fractions of AtbHLH11 and AtbHLH121 proteins dramatically increase in the presence of their interaction partners, the AtbHLH IVc members (Lei et al., 2020; Li et al., 2022). By contrast, OsIRO3 is constitutively expressed in the nucleus (Zheng et al., 2010). It will be interesting to investigate whether the other OsbHLH IVb members are preferentially localized in the cytoplasm. AtbHLH11 and OsbHLH061 inhibit the transcription activation of bHLH IVc (Li et al., 2022; Wang et al., 2022). By contrast, AtbHLH121 promotes the transcription activation of AtbHLH IVc (Lei et al., 2020). OsbHLH064 is a counterpart of AtHLH121 in rice, but it remains an open question whether it plays a similar role to AtbHLH121.

STABILITY OF bHLH PROTEINS IN RESPONSE TO FE DEFICIENCY

In addition to the induction of *AtFIT* transcripts under Fe-deficient conditions, AtFIT protein levels also increase (Meiser et al., 2011; Sivitz et al., 2011; Cui et al., 2018; Lei et al., 2020). Two groups found that AtFIT proteins undergo 26S proteasome-mediated degradation under Fe-deficient conditions (Meiser et al., 2011; Sivitz et al., 2011). Native AtFIT proteins increase in response to Fe deficiency, whereas AtFIT proteins derived from a transgene decrease. The authors proposed that a small pool of active AtFIT is sufficient for the induction of Fe uptake genes, and degradation of inactive AtFIT from the target gene promoters allows fresh AtFIT to reinitiate further transcription cycles. AtBTSL1 (BTS like1) and AtBTSL2 are two Fe sensors (see the next section) that negatively regulate Fe homeostasis (Hindt et al., 2017). Rodríguez-Celma et al. (2019) found that AtFIT protein abundance is higher in the *btsl1 btsl2* double mutant than in the wild type. They used AtFIT as a bait to conduct pull-down assays and found AtBTSL2 among the prey. Further investigation suggested that both AtBTSL1 and AtBTSL2 physically interact with AtFIT and mediate its ubiquitination (Rodríguez-Celma et al., 2019).

In *AtbHLH11*-overexpressing plants, protein abundance of AtbHLH11 increases under Fe-excessive conditions and decreases under Fe-deficient conditions, implying that AtbHLH11 proteins may be degraded when Fe availability is low (Li et al., 2022). Fe-deficient plants benefit from the degradation of AtbHLH11 because it is a repressor of Fe uptake. Kim et al. (2019) found that phosphorylated AtbHLH121 undergoes 26S proteasome-mediated degradation in an AtBTS (BRUTUS, an Fe sensor; see the next section)-dependent manner. However, yeast two-hybrid assays showed that AtBTS does not interact with AtbHLH121 (Gao et al., 2020). There is no report about whether AtPYE proteins undergo degradation in response to Fe deficiency. Further identification of the E3 ligases that interact with and ubiquitinate the AtbHLH IVb proteins will enhance our understanding of the Fe signaling pathway.

The transcription of AtbHLH IVc members is not inducible or slightly inducible in response to Fe deficiency (Zhang et al., 2015; Li et al., 2016; Liang et al., 2017). Tissot et al. (2019) found that protein abundance of AtbHLH105 increases in response to Fe deficiency. Protein interaction assays suggest that both AtbHLH105 and AtbHLH115 interact with AtBTS, which possesses E3 ligase activity (Long et al., 2010; Selote et al., 2015). The protein abundance of AtbHLH105 and AtbHLH115 is increased in the *bts-1* mutant, and AtBTS facilitates 26S proteasome-mediated degradation of AtbHLH105 and AtbHLH115 (Selote et al., 2015; Xing et al., 2021). Both AtbHLH34 and AtbHLH104 are required for the full

activation of Fe uptake genes under Fe-deficient conditions (Zhang et al., 2015; Li et al., 2016), implying that their protein activity or abundance may increase under these conditions. Indeed, analysis of *GFP-IDT1*-overexpressing plants suggests that GFP-IDT1 protein abundance is higher under Fe-deficient conditions than under Fe-sufficient conditions (Sharma and Yeh, 2020). When a mutated version of *GFP-IDT1*^{A320V} with the last amino acid changed from A to V is overexpressed, GFP intensity no longer responds to Fe deficiency (Sharma and Yeh, 2020). These data suggest that AtbHLH34 protein abundance responds to Fe deficiency. AtbHLH104 is also an interaction partner of AtBTS; however, unlike AtbHLH105 and AtbHLH115, which are degraded by AtBTS, AtbHLH104 is insensitive to AtBTS (Selote et al., 2015; Xing et al., 2021). Although the abundance of AtbHLH104 protein is expected to increase in response to Fe deficiency, experimental evidence is still lacking. AtBTSL1/2 are homologs of AtBTS, raising the possibility that protein stability of AtbHLH104 is under the control of AtBTSL1/2. Further investigation is required to reveal how plants modulate the protein abundance of AtbHLH34 and AtbHLH104 according to Fe status. Although AtbHLH105 and AtbHLH115 are degraded by AtBTS, they directly promote the transcription of *AtBTS* under Fe-deficient conditions. This feedback loop may be crucial for the maintenance of Fe homeostasis, as reduced expression of *AtBTS* in *bts-1* or overexpression of AtbHLH105/115 causes Fe overaccumulation (Long et al., 2010; Zhang et al., 2015; Liang et al., 2017). In addition, AtBTS was reported to facilitate the degradation of VOZ1 (vascular plant one-zinc finger 1) and VOZ2, which negatively regulate drought and cold stress responses in plants (Selote et al., 2018).

In rice, OsHRZ1 (hemerythrin motif-containing really interesting new gene (RING)- and zinc-finger protein 1) and OsHRZ2 are two Fe sensors (see the next section). Yeast two-hybrid, pull-down, and co-immunoprecipitation assays indicated that OsHRZ1 interacts with the three bHLH IVc members OsPRI1/2/3 (Zhang et al., 2017, 2020). By contrast, Kobayashi et al. (2019) showed that OsHRZs interact with OsPRI1/2, but not with OsPRI3, in yeast two-hybrid assays. Both OsHRZ1 and OsHRZ2 have E3 ligase activity (Kobayashi et al., 2013). Zhang et al. (2017) confirmed that OsHRZ1 mediates ubiquitination of OsPRI1. They also found that OsHRZ1 promotes the degradation of OsPRI1/2/3 (Zhang et al., 2017, 2020). However, Kobayashi et al. (2019) showed that OsHRZs do not ubiquitinate OsPRI1/2/3. Recently, Kobayashi et al. (2022) found that the basic leucine zipper transcription factor OsbZIP83 and the glutaredoxins OsGRX6 and OsGRX9 facilitate rice Fe utilization under the control of OsHRZ1/2. In addition, OsHRZ2 has been reported to ubiquitinate the Gγ protein GS3 and OsPHR2 (phosphate starvation response 2) (Yang et al., 2021; Guo et al., 2022).

FE SENSORS

Because expression of Fe uptake genes is tightly controlled in an Fe-dependent manner, cells must be able to sense changes in Fe bioavailability. Kobayashi and Nishizawa (2014) proposed three criteria for an Fe sensor: (1) binds Fe or an intimately related molecule(s); (2) changes function in response to Fe status; and (3) regulates Fe homeostasis.

In mammals, Fe homeostasis is maintained by IRP1 (iron-regulatory protein 1) and IRP2, both of which coordinate the post-transcriptional regulation of Fe homeostasis genes by binding to IREs (iron-response elements) within their transcripts (Anderson et al., 2012). When Fe is sufficient, IRP1 binds a 4Fe-4S cluster (criterion 1), loses its affinity for IREs (criterion 2), and functions as a cytosolic ACO (aconitase), whereas IRP2 is degraded by the proteasome. IRP1 complies with the three criteria and is thus an Fe sensor in mammals. FBXL5 (F-box and leucine-rich repeat protein 5) was characterized as an Fe sensor in mammals and also conforms to all three criteria. FBXL5 possesses an iron-responsive hemerythrin domain capable of binding Fe (criterion 1) and an F-box domain that mediates the ubiquitination of IRP2 (criterion 3) (Salahudeen et al., 2009; Vashisht et al., 2009). FBXL5 protein abundance increases under Fe-sufficient conditions and decreases under Fe-deficient conditions (criterion 2). When the hemerythrin domain is mutated or removed, FBXL5 protein is no longer responsive to changes in Fe concentration. Thus, FBXL5 senses Fe status by its hemerythrin domain and accordingly modulates its own protein stability.

AtBTS/AtBTSL1/AtBTSL2 and OsHRZ1/OsHRZ2 were thought to be potential Fe sensors in *Arabidopsis* and rice (Kobayashi and Nishizawa, 2014). They contain hemerythrin domains in the N-terminal region that are capable of binding Fe (criterion 1). Their C-terminal region has a RING domain that contributes to E3 ligase activity for ubiquitination, just like the function of the F-box domain in FBXL5. As mentioned above, AtBTS and OsHRZ1 target bHLH IVc proteins for degradation by their RING domains, thus regulating Fe homeostasis (criterion 3). AtBTS is unstable in the presence of sufficient Fe *in vitro*, and mutation of its hemerythrin domains makes AtBTS insensitive to Fe fluctuations (Selote et al., 2015), indicating that AtBTS can sense Fe and correspondingly adjust its own stability (criterion 2). OsHRZ2 is also more stable under Fe-deficient conditions (criterion 2) (Guo et al., 2022). Therefore, both AtBTS and OsHRZ2 are Fe sensors. AtBTSL1 and AtBTSL2 are two close homologs of AtBTS in *Arabidopsis*. Loss of function of *AtBTSL1* and *AtBTSL2* causes the constitutive activation of Fe-deficiency-responsive genes, just as the loss of *AtBTS* function does (Hindt et al., 2017; Rodríguez-Celma et al., 2019). OsHRZ1 and OsHRZ2 also share many similarities in protein sequences, domains, and functions (Kobayashi et al., 2013). Evidence therefore suggests that AtBTS/AtBTSL1/AtBTSL2 and OsHRZ1/OsHRZ2 are Fe sensors. AtBTS preferentially localizes to the nucleus (Selote et al., 2015). Similarly, OsHRZ1 is a nuclear-localized protein (Kobayashi et al., 2013; Zhang et al., 2017). However, OsHRZ2 is preferentially localized to the cytoplasm (Kobayashi et al., 2013). Further investigation is needed to clarify whether the cytoplasmic fraction of OsHRZ2 has specific functions that have not been explored.

ACOs, homologs of mammalian IRPs, have been characterized in *Arabidopsis* and rice (Arnaud et al., 2007; Senoura et al., 2020). Senoura et al. (2020) revealed that OsACO1 is a positive regulator of the Fe-deficiency response, as knockdown of the *OsACO1* gene leads to downregulation of many Fe-deficiency-inducible genes. Interestingly, they also found that OsACO1 has RNA-binding activity with affinity to the plant ACO-interacting RNA element (PAIR), similar to mammalian IRE, raising the possibility that OsACO1 senses Fe status and transmits the Fe signal, as does IRP1. Arnaud et al. (2007) analyzed the functions of three *Arabidopsis* ACOs and concluded that they are not involved in Fe homeostasis. However, they only examined the expression

of Fe storage genes, not Fe-deficiency-inducible genes. Further investigation is required to confirm whether ACOs function as Fe sensors in plants.

An IDE1 (iron-deficiency-responsive *cis*-acting element 1) *cis*-acting element was identified in the promoter of the barley Fe-deficiency-inducible gene *iron-deficiency specific clone no. 2* (Kobayashi et al., 2003). The IDE1 element is similar to the RY element, which is specifically recognized by the ABI3/VP1 family transcription factors. OsIDEF1 (IDE-binding factor 1) from the ABI3/VP1 family can activate the expression of *OsIRO2*, *OsIRO3*, and *OsIMAs* (Kobayashi et al., 2007, 2021; Wang et al., 2020b). Kobayashi et al. (2009) further revealed that OsIDEF1 is a positive regulator of the early Fe-deficiency response. The N-terminal region of OsIDEF1 contains a metal-binding domain that can bind Fe^{2+} and other metal ions, and the removal of this domain causes impairment of its transcriptional regulation (Kobayashi et al., 2012). However, evidence supporting criterion 2 is still lacking. Therefore, OsIDEF1 is thought to be a potential Fe sensor (Kobayashi and Nishizawa, 2014). Notably, orthologs of OsIDEF1 exist only in graminaceous plants. It is possible that grass plants have developed this specific regulator to modulate the early Fe-deficiency response.

IRON MANs

IRON MAN (IMA)/Fe-uptake-inducing peptide is a ubiquitous family of peptides with an Asp stretch in the C-terminal region (Grillet et al., 2018; Hirayama et al., 2018). Experiments *in vitro* showed that IMA peptides can bind Fe^{2+} and that saturation of the binding sites triggers precipitation of the peptides (Grillet et al., 2018). However, it is unclear whether IMA stability responds to Fe excess in plants. Plants overexpressing *IMA* overaccumulate Fe and display enhanced tolerance to Fe deficiency, whereas loss of function of *IMA* genes causes the opposite phenotypes (Grillet et al., 2018; Hirayama et al., 2018; Kobayashi et al., 2021). The overexpression of *IMA* in *Arabidopsis* and rice results in the upregulation of many Fe-deficiency-responsive genes (Grillet et al., 2018; Li et al., 2021a; Kobayashi et al., 2021), implying that IMA functions upstream of Fe-deficiency response signaling.

Li et al. (2021a) revealed that AtIMA interacts with and inhibits AtBTS to regulate Fe homeostasis. There are eight IMA members in *Arabidopsis*, and six of them interact with AtBTS and are functional. The C-terminal AtBTS interaction domain of AtIMA accounts for its interaction with AtBTS. AtbHLH105 and AtbHLH115 also contain an AtBTS interaction domain in their C-terminal region. AtIMA1 and AtIMA3 were found to compete with AtbHLH105 and AtbHLH115 for interaction with AtBTS and to alleviate degradation of the latter by AtBTS. Thus, AtIMA functions as an inhibitor of AtBTS. Li et al. also revealed that AtIMA3 peptides are ubiquitinated by AtBTS. It is noteworthy that AtIMA3 does not possess a lysine amino acid that is classically necessary for ubiquitination. In their ubiquitination assays, GFP-tagged AtIMA3 was used, and the lysine amino acids in the GFP protein may account for the ubiquitination. It is still an open question whether AtIMA3 itself can be ubiquitinated by AtBTS.

Two *OsIMA* genes have been identified in rice (Grillet et al., 2018; Kobayashi et al., 2021). An *IMA* gene was also identified in tomato, and fruits of tomato plants overexpressing *AtIMA1* accumulated more Fe than those of control plants (Grillet et al., 2018). Both Fe sensors and IMA peptides are conserved across different species (Grillet et al., 2018; Rodríguez-Celma et al., 2019). It is likely that the regulatory mechanism of IMA inhibiting the Fe sensor is conserved across different species. Further investigation is needed to confirm this hypothesis.

IMA genes are inducible in response to Fe deficiency (Grillet et al., 2018; Kobayashi et al., 2021). Li et al. (2021a) revealed that the transcription of *AtIMA1–3* is positively regulated by AtbHLH105 and AtbHLH115, both of which directly bind to the promoter of *AtIMA3*. Thus, there is a positive feedback loop, in which AtbHLH105 and AtbHLH115 promote the expression of *AtIMA3*, and AtIMA3 then stabilizes AtbHLH105 and AtbHLH115 by inhibiting AtBTS. Meanwhile, *AtIMA1* and *AtIMA2* are also positively regulated by AtbHLH121, and the latter binds to the promoters of *AtIMA1–3* (Gao et al., 2020). Similarly, the transcription induction of *OsIMA1* and *OsIMA2* is regulated positively by OsPRI2 and OsPRI3 (Kobayashi et al., 2021). However, it remains unknown whether OsIMAs stabilize OsPRI2 and OsPRI3 proteins.

FUTURE PERSPECTIVES

In the past decade, significant progress has been made in understanding the regulation of Fe uptake in plants. However, our understanding of the mechanisms by which plants sense Fe status and transmit the Fe signal is still incomplete. The first step in Fe signaling is Fe sensing. There is therefore an urgent need for research to clarify how plants sense Fe status and coordinate Fe uptake systems. It will be helpful to analyze the conformation and activity of Fe sensors with or without Fe ions. The protein stability of AtBTS and OsHRZ2 increases in response to Fe deficiency, but it remains unclear whether the protein stability of AtBTSL1/2 and OsHRZ1 is also affected by Fe status. A challenge for the characterization of Fe sensors is the instability of full-length Fe sensor proteins. At least four different domains exist in Fe sensors, and functional analysis of each domain will provide insights into Fe sensing. Moreover, their functions diverge in the Fe-deficiency response. Careful in-depth characterization of each Fe sensor will provide us with a detailed view of how Fe sensors coordinate and integrate Fe uptake.

There has been remarkable progress in our understanding of Fe uptake regulation, particularly the characterization of bHLH IVc, bHLH IVb, bHLH Ib, FIT, and AtMYB72. These proteins are involved in the transcriptional control of Fe acquisition system genes. Although loss of function of each bHLH IVc member results in disruption of Fe homeostasis, their Fe-deficiency symptoms differ from one another. Each member is likely to have evolved its own specific functions. For example, AtbHLH105 and AtPYE specifically mediate photoprotection under Fe-deficient conditions (Akmakjian et al., 2021). Exploring their spatiotemporal expression differences and globally identifying their specific target genes will shed further light on the functional specificity of each member.

Once plants are exposed to Fe deficiency, bHLH IVc proteins initiate the transcription of downstream Fe-deficiency-inducible genes. It is unknown how the bHLH IVc transcription factors

are activated. Although IMAs can protect bHLH IVc from degradation, the transcription induction of *IMAs* depends on bHLH IVc. Thus, bHLH IVc must be activated before the transcription induction of *IMAs* under Fe-deficient conditions. The bHLH IVc protein activity may be modulated by phosphorylation. An apple bHLH IVc member was reported to promote the expression of Fe-deficiency-responsive genes after phosphorylation by MAP kinase 6 (Li et al., 2021b). Phosphoproteomics research will be helpful for the characterization of many changes in protein phosphorylation that may contribute to addressing this question.

Both AtbHLH11 and AtbHLH121 are responsive to Fe deficiency at the post-translational level. Moreover, AtbHLH121 protein undergoes phosphorylation under Fe-deficient conditions. Further investigation is required to verify their interacting E3 ligases and kinases. It is still an open question whether bHLH IVb proteins play similar roles in rice. The protein modification of AtFIT involves ubiquitination and phosphorylation. Although AtFIT protein can be ubiquitinated by AtBTSL1/2, it still undergoes degradation in the *btsl1 btsl2* mutant after Fe supply (Rodríguez-Celma et al., 2019). It is plausible that unknown E3 ligases mediate the degradation of AtFIT. Several phosphorylation sites exist in AtFIT, and S^{272} is phosphorylated by CBL-interacting protein kinase 11 (Gratz et al., 2019, 2020). Future investigation should aim to identify the kinases responsible for the phosphorylation of these bHLH proteins and clarify the impact of phosphorylation on their protein activity.

The subcellular localization of AtBTS and OsHRZ1 is different from that of OsHRZ2. Work on bHLH IVb and Ib also highlights the relevance of subcellular localization to the Fe-deficiency response. IMAs are also localized in both the cytoplasm and nucleus. Does their subcellular localization change in response to Fe status? Do they undergo protein modification in the cytoplasm? Do the interactions between Fe sensors and bHLH proteins occur in the cytoplasm or nucleus? These questions are important for understanding how Fe sensors transmit subcellular Fe status for downstream transcriptional regulation.

During the past decade, the identification of Fe sensors and many key transcription factors has helped us to build a basic regulatory network of the Fe-deficiency response. Further dissecting the functions of each component will make a substantial contribution to plant biology, agriculture, and human nutrition.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

FUNDING

This work was supported by the National Natural Science Foundation of China (32070278) and the Youth Talent Support Program of Yunnan Province (YNWR-QNBJ-2018-134). No conflict of interest declared.

Received: March 12, 2022
Revised: May 19, 2022
Accepted: June 9, 2022
Published: June 14, 2022

REFERENCES

Anderson, C.P., Shen, M., Eisenstein, R.S., and Leibold, E.A. (2012). Mammalian iron metabolism and its control by iron regulatory proteins. Biochim. Biophys. Acta **1823**:1468–1483. https://doi.org/10.1016/j.bbamcr.2012.05.010.

Akmakjian, G.Z., Riaz, N., and Guerinot, M.L. (2021). Photoprotection during iron deficiency is mediated by the bHLH transcription factors PYE and ILR3. Proc. Natl. Acad. Sci. USA **118**, e2024918118. https://doi.org/10.1073/pnas.2024918118.

Arnaud, N., Ravet, K., Borlotti, A., Touraine, B., Boucherez, J., Fizames, C., Briat, J.F., Cellier, F., and Gaymard, F. (2007). The iron-responsive element (IRE)/iron-regulatory protein 1 (IRP1)-cytosolic aconitase iron-regulatory switch does not operate in plants. Biochem. J. **405**:523–531. https://doi.org/10.1042/bj20061874.

Bashir, K., Ishimaru, Y., Shimo, H., Kakei, Y., Senoura, T., Takahashi, R., Sato, Y., Sato, Y., Uozumi, N., Nakanishi, H., et al. (2011). Rice phenolics efflux transporter 2 (PEZ2) plays an important role in solubilizing apoplasmic iron. Soil Sci. Plant Nutr. **57**:803–812. https://doi.org/10.1080/00380768.2011.637305.

Bashir, K., Nozoye, T., Nagasaka, S., Rasheed, S., Miyauchi, N., Seki, M., Nakanishi, H., and Nishizawa, N.K. (2017). Paralogs and mutants show that one DMA synthase functions in iron homeostasis in rice. J. Exp. Bot. **68**:1785–1795. https://doi.org/10.1093/jxb/erx065.

Cai, Y., Li, Y., and Liang, G. (2021). FIT and bHLH Ib transcription factors modulate iron and copper crosstalk in Arabidopsis. Plant Cell Environ. **44**:1679–1691. https://doi.org/10.1111/pce.14000.

Carey-Fung, O., O'Brien, M., Beasley, J.T., and Johnson, A.A.T. (2022). A model to incorporate the bHLH transcription factor OsIRO3 within the rice iron homeostasis regulatory network. Int. J. Mol. Sci. **23**:1635. https://doi.org/10.3390/ijms23031635.

Causier, B., Ashworth, M., Guo, W., and Davies, B. (2012). The TOPLESS interactome: a framework for gene repression in Arabidopsis. Plant Physiol. **158**:423–438. https://doi.org/10.1104/pp.111.186999.

Cheng, L., Wang, F., Shou, H., Huang, F., Zheng, L., He, F., Li, J., Zhao, F.J., Ueno, D., Ma, J.F., et al. (2007). Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. Plant Physiol. **145**:1647–1657. https://doi.org/10.1104/pp.107.107912.

Chutia, R., Abel, S., and Ziegler, J. (2019). Iron and phosphate deficiency regulators concertedly control coumarin profiles in Arabidopsis thaliana roots during iron, phosphate, and combined deficiencies. Front. Plant Sci. **10**:113. https://doi.org/10.3389/fpls.2019.00113.

Colangelo, E.P., and Guerinot, M.L. (2004). The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. Plant Cell **16**:3400–3412. https://doi.org/10.1105/tpc.104.024315.

Cui, Y., Chen, C.-L., Cui, M., Zhou, W.-J., Wu, H.-L., and Ling, H.-Q. (2018). Four IVa bHLH transcription factors are novel interactors of FIT and mediate JA inhibition of iron uptake in Arabidopsis. Mol. Plant **11**:1166–1183. https://doi.org/10.1016/j.molp.2018.06.005.

Curie, C., Cassin, G., Couch, D., Divol, F., Higuchi, K., Le Jean, M., Misson, J., Schikora, A., Czernic, P., and Mari, S. (2009). Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. Ann. Bot. **103**:1–11. https://doi.org/10.1093/aob/mcn207.

Dixon, S.J., and Stockwell, B.R. (2014). The role of iron and reactive oxygen species in cell death. Nat. Chem. Biol. **10**:9–17. https://doi.org/10.1038/nchembio.1416.

Du, J., Huang, Z., Wang, B., Sun, H., Chen, C., Ling, H.-Q., and Wu, H. (2015). SlbHLH068 interacts with FER to regulate the iron-deficiency response in tomato. Ann. Bot. **116**:23–34. https://doi.org/10.1093/aob/mcv058.

Fourcroy, P., Sisó-Terraza, P., Sudre, D., Savirón, M., Reyt, G., Gaymard, F., Abadía, A., Abadia, J., Álvarez-Fernández, A., and Briat, J.F. (2014). Involvement of the ABCG37 transporter in secretion

of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency. New Phytol. **201**:155–167. https://doi.org/10.1111/nph.12471.

Gao, F., and Dubos, C. (2021). Transcriptional integration of plant responses to iron availability. J. Exp. Bot. **72**:2056–2070. https://doi.org/10.1093/jxb/eraa556.

Gao, F., Robe, K., Bettembourg, M., Navarro, N., Rofidal, V., Santoni, V., Gaymard, F., Vignols, F., Roschzttardtz, H., Izquierdo, E., et al. (2020). The transcription factor bHLH121 interacts with bHLH105 (ILR3) and its closest homologs to regulate iron homeostasis in Arabidopsis. Plant Cell **32**:508–524. https://doi.org/10.1105/tpc.19.00541.

Gautam, C.K., Tsai, H.-H., and Schmidt, W. (2021). IRONMAN tunes responses to iron deficiency in concert with environmental pH. Plant Physiol. **187**:1728–1745. https://doi.org/10.1093/plphys/kiab329.

Gratz, R., Brumbarova, T., Ivanov, R., Trofimov, K., Tünnermann, L., Ochoa-Fernandez, R., Blomeier, T., Meiser, J., Weidtkamp-Peters, S., Zurbriggen, M.D., et al. (2020). Phospho-mutant activity assays provide evidence for alternative phospho-regulation pathways of the transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR. New Phytol. **225**:250–267. https://doi.org/10.1111/nph.16168.

Gratz, R., Manishankar, P., Ivanov, R., Köster, P., Mohr, I., Trofimov, K., Steinhorst, L., Meiser, J., Mai, H.J., Drerup, M., et al. (2019). CIPK11-dependent phosphorylation modulates FIT activity to promote Arabidopsis iron acquisition in response to calcium signaling. Dev. Cell **48**:726–740.e10. https://doi.org/10.1016/j.devcel.2019.01.006.

Grillet, L., Lan, P., Li, W., Mokkapati, G., and Schmidt, W. (2018). IRON MAN is a ubiquitous family of peptides that control iron transport in plants. Nat. Plants **4**:953–963. https://doi.org/10.1038/s41477-018-0266-y.

Grillet, L., and Schmidt, W. (2019). Iron acquisition strategies in land plants:not so different after all. New Phytol. **224**:11–18. https://doi.org/10.1111/nph.16005.

Guo, M., Ruan, W., Zhang, Y., Zhang, Y., Wang, X., Guo, Z., Wang, L., Zhou, T., Paz-Ares, J., and Yi, K. (2022). A reciprocal inhibitory module for Pi and iron signaling. Mol. Plant **15**:138–150. https://doi.org/10.1016/j.molp.2021.09.011.

Heim, M.A., Marc, J., Martin, W., Cathie, M., Bernd, W., and Bailey, P.C. (2003). The basic helix–loop–helix transcription factor family in plants:A genome-wide study of protein structure and functional diversity. Mol. Biol. Evol. **20**:735–747. https://doi.org/10.1093/molbev/msg088.

Henriques, R., Jásik, J., Klein, M., Martinoia, E., Feller, U., Schell, J., Pais, M.S., and Koncz, C. (2002). Knock-out of Arabidopsis metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. Plant Mol. Biol. **50**:587–597. https://doi.org/10.1023/a:1019942200164.

Hindt, M.N., Akmakjian, G.Z., Pivarski, K.L., Punshon, T., Baxter, I., Salt, D.E., and Guerinot, M.L. (2017). BRUTUS and its paralogs, BTS LIKE1 and BTS LIKE2, encode important negative regulators of the iron deficiency response in Arabidopsis thaliana. Metallomics **9**:876–890. https://doi.org/10.1039/c7mt00152e.

Hirayama, T., Lei, G.J., Yamaji, N., Nakagawa, N., and Ma, J.F. (2018). The putative peptide gene FEP1 regulates iron deficiency response in Arabidopsis. Plant Cell Physiol. **59**:1739–1752. https://doi.org/10.1093/pcp/pcy145.

Huang, D., and Dai, W. (2015). Molecular characterization of the basic helix-loop-helix (bHLH) genes that are differentially expressed and induced by iron deficiency in Populus. Plant Cell Rep. **34**:1211–1224. https://doi.org/10.1007/s00299-015-1779-8.

Inoue, H., Kobayashi, T., Nozoye, T., Takahashi, M., Kakei, Y., Suzuki, K., Nakazono, M., Nakanishi, H., Mori, S., and Nishizawa, N.K. (2009). Rice OsYSL15 is an iron-regulated iron (III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J. Biol. Chem. **284**:3470–3479. https://doi.org/10.1074/jbc.m806042200.

Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T., Wada, Y., Watanabe, S., Matsuhashi, S., Takahashi, M., et al. (2006). Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J. **45**:335–346. https://doi.org/10.1111/j.1365-313x.2005.02624.x.

Jakoby, M., Wang, H.Y., Reidt, W., Weisshaar, B., and Bauer, P. (2004). FRU (BHLH029) is required for induction of iron mobilization genes in Arabidopsis thaliana. FEBS Lett. **577**:528–534. https://doi.org/10.1016/j.febslet.2004.10.062.

Jeong, J., Merkovich, A., Clyne, M., and Connolly, E.L. (2017). Directing iron transport in dicots: regulation of iron acquisition and translocation. Curr. Opin. Plant Biol. **39**:106–113. https://doi.org/10.1016/j.pbi.2017.06.014.

Kagale, S., Links, M.G., and Rozwadowski, K. (2010). Genome-wide analysis of ethylene-responsive element binding factor-associated amphiphilic repression motif-containing transcriptional regulators in Arabidopsis. Plant Physiol. **152**:1109–1134. https://doi.org/10.1104/pp.109.151704.

Kai, K., Shimizu, B.-i., Mizutani, M., Watanabe, K., and Sakata, K. (2006). Accumulation of coumarins in Arabidopsis thaliana. Phytochemistry **67**:379–386. https://doi.org/10.1016/j.phytochem.2005.11.006.

Kim, S.A., LaCroix, I.S., Gerber, S.A., and Guerinot, M.L. (2019). The iron deficiency response in Arabidopsis thaliana requires the phosphorylated transcription factor URI. Proc. Natl. Acad. Sci. USA **116**:24933–24942. https://doi.org/10.1073/pnas.1916892116.

Kobayashi, T., Nakayama, Y., Itai, R.N., Nakanishi, H., Yoshihara, T., Mori, S., and Nishizawa, N.K. (2003). Identification of novel cis-acting elements, IDE1 and IDE2, of the barley IDS2 gene promoter conferring iron-deficiency-inducible, root-specific expression in heterogeneous tobacco plants. Plant J. **36**:780–793. https://doi.org/10.1046/j.1365-313x.2003.01920.x.

Kobayashi, T., Ogo, Y., Itai, R.N., Nakanishi, H., Takahashi, M., Mori, S., and Nishizawa, N.K. (2007). The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants. Proc. Natl. Acad. Sci. USA **104**:19150–19155. https://doi.org/10.1073/pnas.0707010104.

Kobayashi, T., Itai, R.N., Ogo, Y., et al. (2009). The rice transcription factor IDEF1 is essential for the early response to iron deficiency, and induces vegetative expression of late embryogenesis abundant genes. Plant J **60**:948–961. https://doi.org/10.1111/j.1365-313X.2009.04015.x.

Kobayashi, T., and Nishizawa, N.K. (2012). Iron uptake, translocation, and regulation in higher plants. Annu. Rev. Plant Biol. **63**:131–152. https://doi.org/10.1146/annurev-arplant-042811-105522.

Kobayashi, T., Itai, R.N., Aung, M.S., Senoura, T., Nakanishi, H., and Nishizawa, N.K. (2012). The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status. Plant J. **69**:81–91. https://doi.org/10.1111/j.1365-313x.2011.04772.x.

Kobayashi, T., Nagasaka, S., Senoura, T., Itai, R.N., Nakanishi, H., and Nishizawa, N.K. (2013). Iron-binding haemerythrin RING ubiquitin ligases regulate plant iron responses and accumulation. Nat. Commun. **4**:2792. https://doi.org/10.1038/ncomms3792.

Kobayashi, T., Nakanishi Itai, R., and Nishizawa, N.K. (2014). Iron deficiency responses in rice roots. Rice **7**:27. https://doi.org/10.1186/s12284-014-0027-0.

Kobayashi, T., and Nishizawa, N.K. (2014). Iron sensors and signals in response to iron deficiency. Plant Sci. **224**:36–43. https://doi.org/10.1016/j.plantsci.2014.04.002.

Kobayashi, T., Ozu, A., Kobayashi, S., An, G., Jeon, J.-S., and Nishizawa, N.K. (2019). OsbHLH058 and OsbHLH059 transcription factors positively regulate iron deficiency responses in rice. Plant Mol. Biol. **101**:471–486. https://doi.org/10.1007/s11103-019-00917-8.

Kobayashi, T., Shinkawa, H., Nagano, A.J., and Nishizawa, N.K. (2022). The basic leucine zipper transcription factor OsbZIP83 and the glutaredoxins OsGRX6 and OsGRX9 facilitate rice iron utilization under the control of OsHRZ ubiquitin ligases. Plant J **110**:1731–1750. https://doi.org/10.1111/tpj.15767.

Kobayashi, T., Nagano, A.J., and Nishizawa, N.K. (2021). Iron deficiency-inducible peptide-coding genes OsIMA1 and OsIMA2 positively regulate a major pathway of iron uptake and translocation in rice. J. Exp. Bot. **72**:2196–2211. https://doi.org/10.1093/jxb/eraa546.

Kurt, F., and Filiz, E. (2018). Genome-wide and comparative analysis of bHLH38, bHLH39, bHLH100 and bHLH101 genes in Arabidopsis, tomato, rice, soybean and maize: insights into iron (Fe) homeostasis. Biometals **31**:489–504. https://doi.org/10.1007/s10534-018-0095-5.

Lee, S., Chiecko, J.C., Kim, S.A., Walker, E.L., Lee, Y., Guerinot, M.L., and An, G. (2009). Disruption of OsYSL15 leads to iron inefficiency in rice plants. Plant Physiol. **150**:786–800. https://doi.org/10.1104/pp.109.135418.

Lei, R., Li, Y., Cai, Y., Li, C., Pu, M., Lu, C., Yang, Y., and Liang, G. (2020). bHLH121 functions as a direct link that facilitates the activation of FIT by bHLH IVc transcription factors for maintaining Fe homeostasis in Arabidopsis. Mol. Plant **13**:634–649. https://doi.org/10.1016/j.molp.2020.01.006.

Li, X., Zhang, H., Ai, Q., Liang, G., and Yu, D. (2016). Two bHLH transcription factors, bHLH34 and bHLH104, regulate iron homeostasis in Arabidopsis thaliana. Plant Physiol. **170**:2478–2493. https://doi.org/10.1104/pp.15.01827.

Li, L., Gao, W., Peng, Q., Zhou, B., Kong, Q., Ying, Y., and Shou, H. (2018). Two soybean bHLH factors regulate response to iron deficiency. J. Integr. Plant Biol. **60**:608–622. https://doi.org/10.1111/jipb.12651.

Li, Y., Lu, C.K., Li, C.Y., Lei, R.H., Pu, M.N., Zhao, J.H., Peng, F., Ping, H.Q., Wang, D., and Liang, G. (2021a). IRON MAN interacts with BRUTUS to maintain iron homeostasis in Arabidopsis. Proc. Natl. Acad. Sci. USA **118**, e2109063118. https://doi.org/10.1073/pnas.2109063118.

Li, D., Sun, Q., Zhang, G., Zhai, L., Li, K., Feng, Y., Wu, T., Zhang, X., Xu, X., Wang, Y., et al. (2021b). MxMPK6-2-bHLH104 interaction is involved in reactive oxygen species signaling in response to iron deficiency in apple rootstock. J. Exp. Bot. **72**:1919–1932. https://doi.org/10.1093/jxb/eraa547.

Li, Y., Lei, R., Pu, M., Cai, Y., Lu, C., Li, Z., and Liang, G. (2022). bHLH11 inhibits bHLH IVc proteins by recruiting the TOPLESS/TOPLESS-RELATED corepressors. Plant Physiol. **188**:1335–1349. https://doi.org/10.1093/plphys/kiab540.

Liang, G., Zhang, H., Li, X., Ai, Q., and Yu, D. (2017). bHLH transcription factor bHLH115 regulates iron homeostasis in Arabidopsis thaliana. J. Exp. Bot. **68**:1743–1755. https://doi.org/10.1093/jxb/erx043.

Liang, G., Zhang, H., Li, Y., Pu, M., Yang, Y., Li, C., Lu, C., Xu, P., and Yu, D. (2020). Oryza sativa FER-LIKE FE DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (OsFIT/OsbHLH156) interacts with OsIRO2 to regulate iron homeostasis. J. Integr. Plant Biol. **62**:668–689. https://doi.org/10.1111/jipb.12933.

Lindsay, W.L. (1997). Chemical Equilibria in Soils (John Wiley & Sons), pp. 78–104.

Ling, H.-Q., Bauer, P., Bereczky, Z., Keller, B., and Ganal, M. (2002). The tomato fer gene encoding a bHLH protein controls iron-uptake responses in roots. Proc. Natl. Acad. Sci. USA **99**:13938–13943. https://doi.org/10.1073/pnas.212448699.

Long, T.A., Tsukagoshi, H., Busch, W., Lahner, B., Salt, D.E., and Benfey, P.N. (2010). The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. Plant Cell **22**:2219–2236. https://doi.org/10.1105/tpc.110.074096.

Martín-Barranco, A., Spielmann, J., Dubeaux, G., Vert, G., and Zelazny, E. (2020). Dynamic control of the high-affinity iron uptake complex in root epidermal cells. Plant Physiol. **184**:1236–1250. https://doi.org/10.1104/pp.20.00234.

Maurer, F., Naranjo Arcos, M.A., and Bauer, P. (2014). Responses of a triple mutant defective in three iron deficiency-induced basic helix-loop-helix genes of the subgroup Ib (2) to iron deficiency and salicylic acid. PLoS One **9**:e99234. https://doi.org/10.1371/journal.pone.0099234.

Meiser, J., Lingam, S., and Bauer, P. (2011). Posttranslational regulation of the iron deficiency basic helix-loop-helix transcription factor FIT is affected by iron and nitric oxide. Plant Physiol. **157**:2154–2166. https://doi.org/10.1104/pp.111.183285.

Mori, S., and Nishizawa, N. (1987). Methionine as a dominant precursor of phytosiderophores in Graminaceae plants. Plant Cell Physiol. **28**:1081–1092.

Mori, S. (1999). Iron acquisition by plants. Curr. Opin. Plant Biol. **2**:250–253. https://doi.org/10.1016/s1369-5266(99)80043-0.

Naranjo-Arcos, M.A., Maurer, F., Meiser, J., Pateyron, S., Fink-Straube, C., and Bauer, P. (2017). Dissection of iron signaling and iron accumulation by overexpression of subgroup Ib bHLH039 protein. Sci. Rep. **7**:10911. https://doi.org/10.1038/s41598-017-11171-7.

Nozoye, T., Nagasaka, S., Kobayashi, T., Takahashi, M., Sato, Y., Sato, Y., Uozumi, N., Nakanishi, H., and Nishizawa, N.K. (2011). Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. J. Biol. Chem. **286**:5446–5454. https://doi.org/10.1074/jbc.m110.180026.

Ogo, Y., Nakanishi Itai, R., Nakanishi, H., Kobayashi, T., Takahashi, M., Mori, S., and Nishizawa, N.K. (2007). The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. Plant J. **51**:366–377. https://doi.org/10.1111/j.1365-313x.2007.03149.x.

Palmer, C.M., Hindt, M.N., Schmidt, H., Clemens, S., and Guerinot, M.L. (2013). MYB10 and MYB72 are required for growth under iron-limiting conditions. PLoS Genet. **9**:e1003953. https://doi.org/10.1371/journal.pgen.1003953.

Rajniak, J., Giehl, R.F.H., Chang, E., Murgia, I., von Wirén, N., and Sattely, E.S. (2018). Biosynthesis of redox-active metabolites in response to iron deficiency in plants. Nat. Chem. Biol. **14**:442–450. https://doi.org/10.1038/s41589-018-0019-2.

Riaz, N., and Guerinot, M.L. (2021). All together now: regulation of the iron deficiency response. J. Exp. Bot. **72**:2045–2055. https://doi.org/10.1093/jxb/erab003.

Robe, K., Izquierdo, E., Vignols, F., Rouached, H., and Dubos, C. (2021). The coumarins: secondary metabolites playing a primary role in plant nutrition and health. Trends Plant Sci. **26**:248–259. https://doi.org/10.1016/j.tplants.2020.10.008.

Robinson, N.J., Procter, C.M., Connolly, E.L., and Guerinot, M.L. (1999). A ferric-chelate reductase for iron uptake from soils. Nature **397**:694–697. https://doi.org/10.1038/17800.

Rodríguez-Celma, J., Lattanzio, G., Grusak, M.A., Abadía, A., Abadía, J., and López-Millán, A.F. (2011). Root responses of Medicago truncatula plants grown in two different iron deficiency

conditions: changes in root protein profile and riboflavin biosynthesis. J. Proteome Res. **10**:2590–2601. https://doi.org/10.1021/pr2000623.

Rodríguez-Celma, J., Lin, W.D., Fu, G.M., Abadía, J., López-Millán, A.F., and Schmidt, W. (2013). Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by Arabidopsis and Medicago truncatula. Plant Physiol. **162**:1473–1485. https://doi.org/10.1104/pp.113.220426.

Rodríguez-Celma, J., Connorton, J.M., Kruse, I., Green, R.T., Franceschetti, M., Chen, Y.-T., Cui, Y., Ling, H.-Q., Yeh, K.-C., and Balk, J. (2019). Arabidopsis BRUTUS-LIKE E3 ligases negatively regulate iron uptake by targeting transcription factor FIT for recycling. Proc. Natl. Acad. Sci. USA **116**:17584–17591. https://doi.org/10.1073/pnas.1907971116.

Römheld, V., and Marschner, H. (1986). Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol. **80**:175–180. https://doi.org/10.1104/pp.80.1.175.

Salahudeen, A.A., Thompson, J.W., Ruiz, J.C., Ma, H.-W., Kinch, L.N., Li, Q., Grishin, N.V., and Bruick, R.K. (2009). An E3 ligase possessing an iron-responsive hemerythrin domain is a regulator of iron homeostasis. Science **326**:722–726. https://doi.org/10.1126/science.1176326.

Santi, S., and Schmidt, W. (2009). Dissecting iron deficiency-induced proton extrusion in Arabidopsis roots. New Phytol. **183**:1072–1084. https://doi.org/10.1111/j.1469-8137.2009.02908.x.

Schmid, N.B., Giehl, R.F.H., Döll, S., Mock, H.P., Strehmel, N., Scheel, D., Kong, X., Hider, R.C., and von Wirén, N. (2014). Feruloyl-CoA 6'-Hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in Arabidopsis. Plant Physiol. **164**:160–172. https://doi.org/10.1104/pp.113.228544.

Schwarz, B., and Bauer, P. (2020). FIT, a regulatory hub for iron deficiency and stress signaling in roots, and FIT dependent and independent gene signatures. J. Exp. Bot. **71**:1694–1705. https://doi.org/10.1093/jxb/eraa012.

Selote, D., Samira, R., Matthiadis, A., Gillikin, J.W., and Long, T.A. (2015). Iron-binding E3 ligase mediates iron response in plants by targeting basic helix-loop-helix transcription factors. Plant Physiol. **167**:273–286. https://doi.org/10.1104/pp.114.250837.

Selote, D., Matthiadis, A., Gillikin, J.W., Sato, M.H., and Long, T.A. (2018). The E3 ligase BRUTUS facilitates degradation of VOZ1/2 transcription factors. Plant Cell Environ. **41**:2463–2474. https://doi.org/10.1111/pce.13363.

Senoura, T., Kobayashi, T., An, G., Nakanishi, H., and Nishizawa, N.K. (2020). Defects in the rice aconitase-encoding OsACO1 gene alter iron homeostasis. Plant Mol. Biol. **104**:629–645. https://doi.org/10.1007/s11103-020-01065-0.

Sharma, R., and Yeh, K.C. (2020). The dual benefit of a dominant mutation in Arabidopsis IRON DEFICIENCY TOLERANT1 for iron biofortification and heavy metal phytoremediation. Plant Biotechnol. J. **18**:1200–1210. https://doi.org/10.1111/pbi.13285.

Sisó-Terraza, P., Rios, J.J., Abadía, J., Abadía, A., and Álvarez-Fernández, A. (2016). Flavins secreted by roots of iron-deficient Beta vulgaris enable mining of ferric oxide via reductive mechanisms. New Phytol. **209**:733–745. https://doi.org/10.1111/nph.13633.

Sivitz, A., Grinvalds, C., Barberon, M., Curie, C., and Vert, G. (2011). Proteasome-mediated turnover of the transcriptional activator FIT is required for plant iron-deficiency responses. Plant J. **66**:1044–1052. https://doi.org/10.1111/j.1365-313x.2011.04565.x.

Siwinska, J., Siatkowska, K., Olry, A., Grosjean, J., Hehn, A., Bourgaud, F., Meharg, A.A., Carey, M., Lojkowska, E., and Ihnatowicz, A. (2018). Scopoletin 8-hydroxylase: a novel enzyme involved in coumarin biosynthesis and iron-deficiency responses in Arabidopsis. J. Exp. Bot. **69**:1735–1748. https://doi.org/10.1093/jxb/ery005.

Stringlis, I.A., Yu, K., Feussner, K., de Jonge, R., Van Bentum, S., Van Verk, M.C., Berendsen, R.L., Bakker, P.A.H.M., Feussner, I., and Pieterse, C.M.J. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proc. Natl. Acad. Sci. USA **115**:5213. https://doi.org/10.1073/pnas.1722335115.

Takagi, S.i., Nomoto, K., and Takemoto, T. (1984). Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr. **7**:469–477. https://doi.org/10.1080/01904168409363213.

Tanabe, N., Noshi, M., Mori, D., Nozawa, K., Tamoi, M., and Shigeoka, S. (2019). The basic helix-loop-helix transcription factor, bHLH11 functions in the iron-uptake system in Arabidopsis thaliana. J. Plant Res. **132**:93–105. https://doi.org/10.1007/s10265-018-1068-z.

Tissot, N., Robe, K., Gao, F., Grant-Grant, S., Boucherez, J., Bellegarde, F., Maghiaoui, A., Marcelin, R., Izquierdo, E., Benhamed, M., et al. (2019). Transcriptional integration of the responses to iron availability in Arabidopsis by the bHLH factor ILR3. New Phytol. **223**:1433–1446. https://doi.org/10.1111/nph.15753.

Trofimov, K., Ivanov, R., Eutebach, M., Acaroglu, B., Mohr, I., Bauer, P., and Brumbarova, T. (2019). Mobility and localization of the iron deficiency-induced transcription factor bHLH039 change in the presence of FIT. Plant Direct **3**:e00190. https://doi.org/10.1002/pld3.190.

Tsai, H.-H., Rodríguez-Celma, J., Lan, P., Wu, Y.-C., Vélez-Bermúdez, I.C., and Schmidt, W. (2018). Scopoletin 8-hydroxylase-mediated fraxetin production is crucial for iron mobilization. Plant Physiol. **177**:194–207. https://doi.org/10.1104/pp.18.00178.

Tsai, H.-H., and Schmidt, W. (2020). pH-dependent transcriptional profile changes in iron-deficient Arabidopsis roots. BMC Genom. **21**:694. https://doi.org/10.1186/s12864-020-07116-6.

Varotto, C., Maiwald, D., Pesaresi, P., Jahns, P., Salamini, F., and Leister, D. (2002). The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in Arabidopsis thaliana. Plant J. **31**:589–599. https://doi.org/10.1046/j.1365-313x.2002.01381.x.

Vanholme, R., Sundin, L., Seetso, K.C., Kim, H., Liu, X., Li, J., De Meester, B., Hoengenaert, L., Goeminne, G., Morreel, K., et al. (2019). COSY catalyses trans-cis isomerization and lactonization in the biosynthesis of coumarins. Nat. Plants **5**:1066–1075. https://doi.org/10.1038/s41477-019-0510-0.

Vashisht, A.A., Zumbrennen, K.B., Huang, X., Powers, D.N., Durazo, A., Sun, D., Bhaskaran, N., Persson, A., Uhlen, M., Sangfelt, O., et al. (2009). Control of iron homeostasis by an iron-regulated ubiquitin ligase. Science **326**:718–721. https://doi.org/10.1126/science.1176333.

Vélez-Bermúdez, I.C., and Schmidt, W. (2022). How plants recalibrate cellular iron homeostasis. Plant Cell Physiol. **63**:154–162. https://doi.org/10.1093/pcp/pcab166.

Vert, G., Grotz, N., Dédaldéchamp, F., Gaymard, F., Guerinot, M.L., Briat, J.-F., and Curie, C. (2002). IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell **14**:1223–1233. https://doi.org/10.1105/tpc.001388.

Wang, H.Y., Klatte, M., Jakoby, M., Bäumlein, H., Weisshaar, B., and Bauer, P. (2007). Iron deficiency-mediated stress regulation of four subgroup Ib BHLH genes in Arabidopsis thaliana. Planta **226**:897–908. https://doi.org/10.1007/s00425-007-0535-x.

Wang, N., Cui, Y., Liu, Y., Fan, H., Du, J., Huang, Z., Yuan, Y., Wu, H., and Ling, H.-Q. (2013). Requirement and functional redundancy of Ib subgroup bHLH proteins for iron deficiency responses and uptake in

Arabidopsis thaliana. Mol. Plant **6**:503–513. https://doi.org/10.1093/mp/sss089.

Wang, S., Li, L., Ying, Y., Wang, J., Shao, J.F., Yamaji, N., Whelan, J., Ma, J.F., and Shou, H. (2020a). A transcription factor OsbHLH156 regulates Strategy II iron acquisition through localising IRO2 to the nucleus in rice. New Phytol. **225**:1247–1260. https://doi.org/10.1111/nph.16232.

Wang, F., Itai, R.N., Nozoye, T., Kobayashi, T., Nishizawa, N.K., and Nakanishi, H. (2020b). The bHLH protein OsIRO3 is critical for plant survival and iron (Fe) homeostasis in rice (Oryza sativa L.) under Fe-deficient conditions. Soil Sci. Plant Nutr. **66**:579–592. https://doi.org/10.1080/00380768.2020.1783966.

Wang, W., Ye, J., Ma, Y., Wang, T., Shou, H., and Zheng, L. (2020c). OsIRO3 plays an essential role in iron deficiency responses and regulates iron homeostasis in rice. Plants **9**:1095. https://doi.org/10.3390/plants9091095.

Wang, W., Ye, J., Xu, H., Liu, X., Fu, Y., Zhang, H., Rouached, H., Whelan, J., Shen, Z., and Zheng, L. (2022). OsbHLH061 links TOPLESS/TOPLESS-RELATED repressor proteins with POSITIVE REGULATOR OF IRON HOMEOSTASIS 1 to maintain iron homeostasis in rice. New Phytol. **234**:1753–1769. https://doi.org/10.1111/nph.18096.

Xing, Y., Xu, N., Bhandari, D.D., Lapin, D., Sun, X., Luo, X., Wang, Y., Cao, J., Wang, H., Coaker, G., et al. (2021). Bacterial effector targeting of a plant iron sensor facilitates iron acquisition and pathogen colonization. Plant Cell **33**:2015–2031. https://doi.org/10.1093/plcell/koab075.

Yang, W., Wu, K., Wang, B., et al. (2021). The RING E3 ligase CLG1 targets GS3 for degradation via the endosome pathway to determine grain size in rice. Mol. Plant. **14**:1699–1713. https://doi.org/10.1016/j.molp.2021.06.027.

Yoshino, M., and Murakami, K. (1998). Interaction of iron with polyphenolic compounds: application to antioxidant characterization. Anal. Biochem. **257**:40–44. https://doi.org/10.1006/abio.1997.2522.

Yuan, Y.X., Zhang, J., Wang, D.W., and Ling, H.Q. (2005). AtbHLH29 of Arabidopsis thaliana is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy I plants. Cell Res. **15**:613–621. https://doi.org/10.1038/sj.cr.7290331.

Yuan, Y., Wu, H., Wang, N., Li, J., Zhao, W., Du, J., Wang, D., and Ling, H.Q. (2008). FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in Arabidopsis. Cell Res. **18**:385–397. https://doi.org/10.1038/cr.2008.26.

Zamioudis, C., Korteland, J., Van Pelt, J.A., van Hamersveld, M., Dombrowski, N., Bai, Y., Hanson, J., Van Verk, M.C., Ling, H.Q., Schulze-Lefert, P., et al. (2015). Rhizobacterial volatiles and photosynthesis-related signals coordinate MYB72 expression in Arabidopsis roots during onset of induced systemic resistance and iron-deficiency responses. Plant J. **84**:309–322. https://doi.org/10.1111/tpj.12995.

Zaharieva, T., and Römheld, V. (2000). Specific Fe^{2+} uptake system in strategy I plants inducible under Fe deficiency. J. Plant Nutr. **23**:1733–1744. https://doi.org/10.1080/01904160009382137.

Zelazny, E., and Vert, G. (2014). Plant nutrition: root transporters on the move. Plant Physiol. **166**:500–508. https://doi.org/10.1104/pp.114.244475.

Zhang, H., Li, Y., Pu, M., Xu, P., Liang, G., and Yu, D. (2020). Oryza sativa POSITIVE REGULATOR OF IRON DEFICIENCY RESPONSE 2 (OsPRI2) and OsPRI3 are involved in the maintenance of Fe homeostasis. Plant Cell Environ. **43**:261–274. https://doi.org/10.1111/pce.13655.

Zhang, H., Li, Y., Yao, X., Liang, G., and Yu, D. (2017). Positive regulator of iron homeostasis1, OsPRI1, facilitates iron homeostasis. Plant Physiol. **175**:543–554. https://doi.org/10.1104/pp.17.00794.

Zhang, J., Liu, B., Li, M., Feng, D., Jin, H., Wang, P., Liu, J., Xiong, F., Wang, J., and Wang, H.-B. (2015). The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis. Plant Cell **27**:787–805. https://doi.org/10.1105/tpc.114.132704.

Zheng, L., Ying, Y., Wang, L., Wang, F., Whelan, J., and Shou, H. (2010). Identification of a novel iron regulated basic helix-loop-helix protein involved in Fe homeostasis in Oryza sativa. BMC Plant Biol. **10**:166. https://doi.org/10.1186/1471-2229-10-166.