Shedding light on iron nutrition: exploring intersections of transcription factor cascades in light and iron deficiency signaling

Ksenia Trofimov ,Samriti Mankotia ,Mary Ngigi ,Dibin Baby ,Santosh B Satbhai ,Petra Bauer

Suggested Citation:
Trofimov, K., Mankotia, S., Ngigi, M., Baby, D., Satbhai, S. B., & Bauer, P. (2024). Shedding light on iron nutrition: exploring intersections of transcription factor cascades in light and iron deficiency signaling. Journal of Experimental Botany, 76(3), 787–802. https://doi.org/10.1093/jxb/erae324

Journal of Experimental Botany, Vol. 76, No. 3 pp. 787–802, 2025
https://doi.org/10.1093/jxb/erae324 Advance Access Publication 8 August 2024

REVIEW PAPER

Shedding light on iron nutrition: exploring intersections of transcription factor cascades in light and iron deficiency signaling

Ksenia Trofimov[1,†], **Samriti Mankotia**[2], **Mary Ngigi**[1,3], **Dibin Baby**[1], **Santosh B. Satbhai**[2], and **Petra Bauer**[1,3,*]

[1] Institute of Botany, Heinrich-Heine-University, D-40225 Düsseldorf, Germany
[2] Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Mohali, SAS Nagar, Punjab 140406, India
[3] Cluster of Excellence on Plant Science (CEPLAS), Heinrich-Heine-University, D-40225 Düsseldorf, Germany

[†] Present address: Center for Advanced Imaging, Heinrich-Heine-University, D-40225 Düsseldorf, Germany.
[*] Correspondence: Petra.Bauer@hhu.de

Received 10 April 2024; Editorial decision 18 July 2024; Accepted 7 August 2024

Editor: Kishore Panigrahi, National Institute of Science Education and Research, India

Abstract

In the dynamic environment of plants, the interplay between light-dependent growth and iron nutrition is a recurring challenge. Plants respond to low iron levels by adjusting growth and physiology through enhanced iron acquisition from the rhizosphere and internal iron pool reallocation. Iron deficiency response assays and gene co-expression networks aid in documenting physiological reactions and unraveling gene-regulatory cascades, offering insight into the interplay between hormonal and external signaling pathways. However, research directly exploring the significance of light in iron nutrition remains limited. This review provides an overview on iron deficiency regulation and its cross-connection with distinct light signals, focusing on transcription factor cascades and long-distance signaling. The circadian clock and retrograde signaling influence iron uptake and allocation. The light-activated shoot-to-root mobile transcription factor ELONGATED HYPOCOTYL5 (HY5) affects iron homeostasis responses in roots. Blue light triggers the formation of biomolecular condensates containing iron deficiency-induced protein complexes. The potential of exploiting the connection between light and iron signaling remains underutilized. With climate change and soil alkalinity on the rise, there is a need to develop crops with improved nutrient use efficiency and modified light dependencies. More research is needed to understand and leverage the interplay between light signaling and iron nutrition.

Keywords: bHLH, biomolecular condensate, blue light, BTS, circadian clock, FIT, HY5, iron, long-distance signaling, SR45.

Introduction

Iron is among the most abundant elements in the Earth's crust, where it is mostly present in the form of oxidized ferric iron in non-soluble iron oxide and oxyhydroxide compounds of mineral rocks, hence representing large stores of non-bioavailable iron. The levels of atmospheric oxygen substantially increased due to the emergence of oxygen-producing photosynthetic organisms >2 billion years ago. This so-called Great Oxidation Event had profound effects on the Earth's environment and the evolution of life. It significantly impacted the bioavailability of iron, and the resulting race for iron has become a strong selection pressure for the new evolving life forms (Wade *et al.*, 2021). For terrestrial plants, iron bioavailability is heavily influenced by soil pH and moisture levels, which are in turn dictated by climatic conditions. The transition between dry and wet climates with mostly either alkaline or acidic soils occurs abruptly, leading to corresponding shifts between zones with bioavailable and non-bioavailable iron (Slessarev *et al.*, 2016). Global warming and anthropogenic activities may contribute to an alkalinization of soils, even though this phenomenon seems far more complex, involving additional factors such as radiation and soil depth (Sun *et al.*, 2023). Clearly, land plants live in an ever-increasing harsh environment with regard to mineral availability. The increase of alkaline soil and drought will also change vegetation and expose plants more frequently to direct sunlight. Hence, the strategies that plants use to deal with critically low amounts of iron in the rhizosphere, while also reacting to light, will be even more important with climate change.

Light is pivotal for plants as it facilitates biochemical redox and electron transfer reactions. Iron cofactor-containing enzymes and proteins (e.g. those utilizing heme–iron, iron–sulfur clusters, or free iron) play roles in nutrient assimilation (e.g. for reduction of carbon, nitrogen, and sulfur), and occur in many biochemical pathways in plants, particularly in mitochondria and chloroplasts. Photosynthesis is among the most noteworthy biochemical reactions that depends on iron and light. The importance of iron in light-controlled nutrient assimilation is explained by its high reactivity as a redox reagent to shuffle between ferric Fe^{3+} and ferrous Fe^{2+} states. One potential drawback for plant health is that ferrous iron is a strong Fenton reagent. In an oxygenated environment, Fenton reagents catalyze the reduction of oxygen species. As a result, various types of reactive oxygen species (ROS) can be generated in the presence of ferrous iron, including hydroxyl radicals, that are the most potent ROS (Le *et al.*, 2019). Oxidative stress also occurs in chloroplasts under high light or when the substrates for electron transfer are overloaded, as it is frequently the consequence of abiotic stresses linked with water deprivation (Choudhury *et al.*, 2017; Kanwar *et al.*, 2021). Hence, it is important for plants to adjust iron acquisition and homeostasis with light during plant growth and upon environmental stresses.

Plants have distinct iron and light signaling pathways, whereby recent studies uncover intricate connections between them. Yet limited research directly explores the significance of light in iron acquisition, which underscores the necessity for further investigation to develop sustainable, nutrient-efficient crops by focusing in particular on understanding and utilizing the interplay between light signaling and iron nutrition. Here, we summarize recent evidence indicating the multiple interconnections between light and iron signaling.

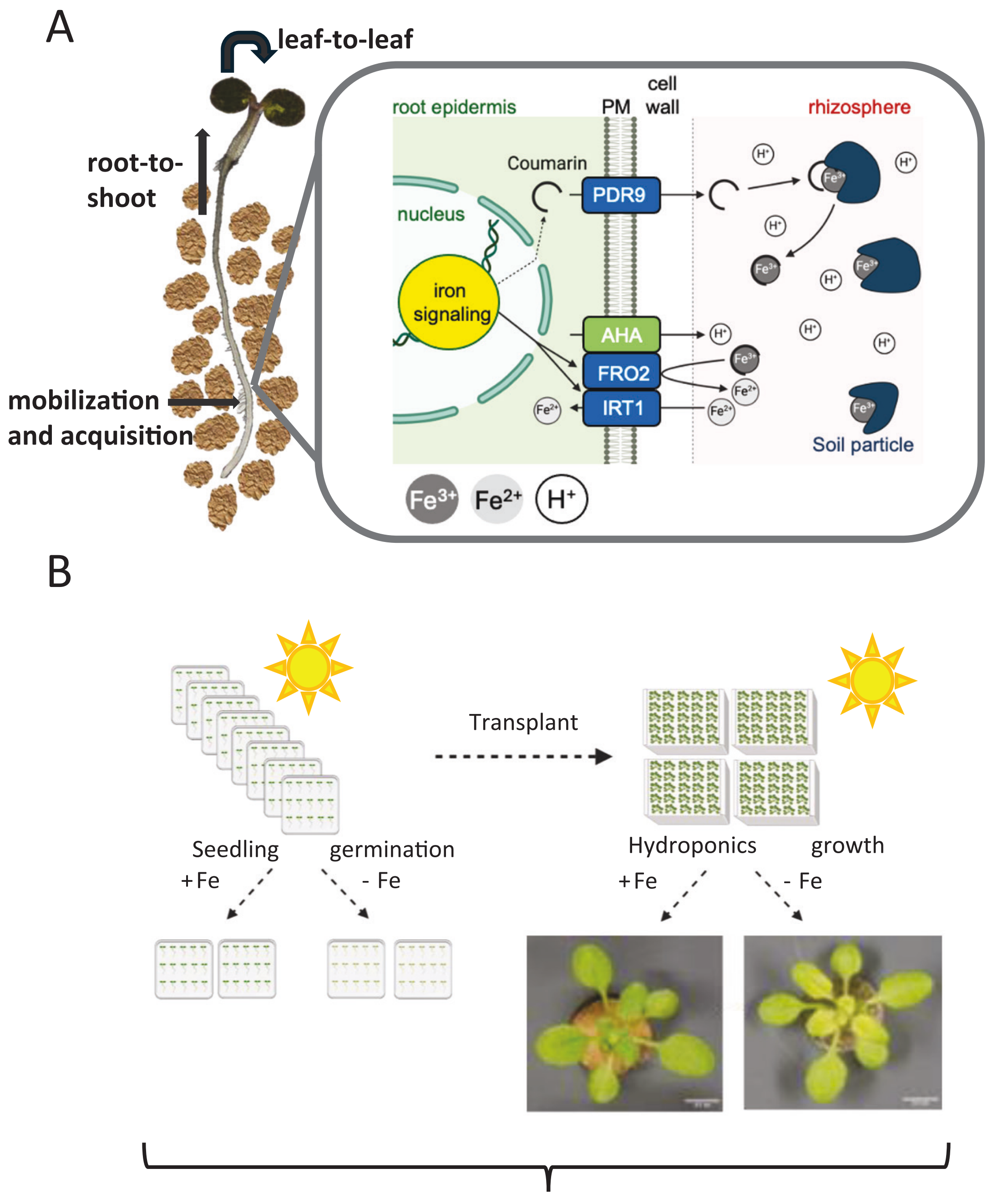
Iron acquisition and allocation under iron deficiency

Light can affect multiple aspects of iron homeostasis. To investigate light and iron signaling interconnections, it is important to understand the key processes of iron uptake and distribution in plants. Plants that experience iron deficiency can mobilize iron in the rhizosphere and increase their capacity to take up iron efficiently, and/or they can re-allocate iron within the plant (typical iron deficiency responses of seedlings are summarized in Fig. 1A).

Plants acquire iron primarily in the root differentiation zone above the root tip. They can mobilize iron in various ways in the rhizosphere depending on genetic and environmental factors. In the past, plants have been divided into two major groups. One group has been defined by the capacity to acidify the soil, and reduce and acquire ferrous iron (Strategy I, non-graminaceous plants, represented in Fig. 1A). The other group has been characterized based on secretion and uptake of phytosiderophores, which are chelators that form a complex with ferric iron (Strategy II, grasses) (Römheld and Marschner, 1986; Brumbarova *et al.*, 2015; Connorton *et al.*, 2017). Meanwhile, the general importance of coumarins and flavins has been clearly stated. These compounds are secreted under iron deficiency to enhance iron mobilization and reduction depending on ecological constraints of the habitat, such as pH, and on the species (Rodríguez-Celma *et al.*, 2011; Clemens and Weber, 2016; Lefèvre *et al.*, 2018; Li *et al.*, 2023). It has emerged that coumarins play a role in plant–microbe communication as microbes and endophytes play important roles in helping plants mobilize iron through microbial siderophores and plant growth-promoting molecules (Stassen *et al.*, 2021). Iron acquisition is also modulated by changing root cell barriers along the root (Zandi *et al.*, 2023; Grünhofer *et al.*, 2024) and altering root cell differentiation and root architecture (López-Bucio *et al.*, 2003).

In parallel to acquiring new iron, plants liberate iron from internal stores of roots and shoots. Iron is bound to cell wall material, such as pectin, and modifying pectin structure allows iron to accumulate (Zhu *et al.*, 2024). Iron is stored inside cell vacuoles, a process affected by altered vacuolar iron transport (Bastow *et al.*, 2018; Ram *et al.*, 2021). Iron stored in ferritin complexes, that are macromolecular assemblies for iron storage, or iron present in the electron transport chains in plastids and mitochondria can be released through autophagy, a form of targeted degradation of compartments under nutrient deficiency (Wan and Ling, 2022). Iron is also detected in the nucleolus (Roschzttardtz *et al.*, 2011). Plants control the amount of iron that is allocated to different plant parts. Iron is transported upwards in the inner stele of the root via xylem, dependent on transpiration, and from source to sink in the phloem. These types of allocation require different soluble iron-binding compounds and chelators (Schuler *et al.*, 2012; Kovács *et al.*, 2016).

Plant responses can be recorded by performing iron deficiency response assays which can provide evidence of the



Response assays and measurements

Shoot: leaf size, chlorophyll, photosynthesis efficiency, elemental analysis, iron staining, ferritin detection, gene expression markers

Root: architecture and root length, iron reductase activity, rhizosphere acidification, exudate analysis, elemental analysis, iron staining, ferritin detection, gene expression markers

Fig. 1. Overview of iron acquisition and allocation and iron deficiency response assays (example of *Arabidopsis thaliana*). (A) Iron is mobilized in the rhizosphere, acquired by roots, and transported from root to shoot. *A. thaliana* is a Strategy I plant, that secretes coumarins, acidifies the rhizosphere, reduces ferric iron (Fe^{3+}), and takes up ferrous iron (Fe^{2+}). PM, plasma membrane; FRO2, FERRIC REDUCTASE OXIDASE2; IRT1, IRON-REGULATED

TRANSPORTER1. (B) Iron deficiency responses are studied by growing plants on agar plates or in hydroponic medium and exposing plants to iron-sufficient and -deficient conditions. Various responses can be qualitatively and quantitatively analyzed in shoots and roots. The figure has been created with Biorender.com. Iron-deficient and -sufficient plant growth phenotypes are shown for hydroponic growth.

degree of iron deficiency and the responsiveness of plants to mobilize iron (Fig. 1B). Such assays have been informative to interpret phenotypes of mutants harboring defects in the acquisition and distribution of iron (Fig. 1B).

Overall, plants exhibit a multifaceted array of reactions in response to iron deficiency, aimed at fine-tuning their growth and physiology. These iron-dependent responses can be meticulously documented, allowing for thorough exploration of their interplay with other abiotic factors, such as variations in light quality.

Iron deficiency response signaling

As in response to light, plant responses to iron supply are triggered by signaling systems involving E3 ligases and basic helix–loop–helix (bHLH) transcription factors. Thus, numerous crossing links may exist due to genetic and biochemical interactions. Therefore, it is important to understand the players that regulate iron deficiency response signaling. When iron deficiency is sensed by plants, thousands of genes become differently regulated during the following 3 days. A small subset of iron deficiency-induced genes is directly involved in the acquisition and allocation of iron upon iron deficiency stress (Schwarz and Bauer, 2020; Schwarz *et al.*, 2020). These genes encode proteins for iron mobilization, iron transport, and iron chelation or storage, and they can be integrated in regulatory iron-responsive networks (Fig. 2). The remaining differentially expressed genes reflect the broad metabolic, hormonal, and physiological spectrum of plant responses to iron deficiency (Brady *et al.*, 2007).

Iron availability is sensed by plants; however, concrete iron sensor proteins still need to be identified and confirmed for plants. Among the candidates for sensing iron are the *Arabidopsis thaliana* proteins BRUTUS (BTS) and BTS-LIKE proteins BTSL1 and BTSL2, which are E3 ligases (Rodríguez-Celma *et al.*, 2019). Based on mutant studies, BTS/BTSLs can be classified as negative regulators of iron acquisition and homeostasis (Long *et al.*, 2010; Hindt *et al.*, 2017). They share some similarity in structure with the human iron-sensing F-box and leucine-rich repeat protein 5 (FBXL5), which is a substrate adaptor of an E3 ubiquitin ligase complex. FBXL5 stability and target protein interaction are controlled by oxygen and iron (Mayank *et al.*, 2019; Wang *et al.*, 2020). BTS/BTSL proteins possess hemerythrin domains for iron–oxygen binding, and their protein stability characteristics are linked with the presence of iron binding to this site (Selote *et al.*, 2015). A current model of the mode of action is that BTS/L proteins target the top layer of regulation (Li *et al.*, 2021; Lichtblau *et al.*, 2022) (Fig. 2A). This layer is represented by the bHLH transcription factors that activate the iron deficiency response, among them in particular the bHLH subgroup IVb and IVc transcription factors. For example, bHLH IVc transcription factors, such as bHLH034, bHLH104, bHLH105/ILR3, and bHLH115, promote iron deficiency responses (Zhang *et al.*, 2015; Li *et al.*, 2016; Liang *et al.*, 2017; Samira *et al.*, 2018; Tissot *et al.*, 2019). bHLH IVb factors, such as bHLH121/URI (Gao *et al.*, 2019; Kim *et al.*, 2019), can activate iron deficiency responses, or others such as bHLH11 (Liang *et al.*, 2017; Tanabe *et al.*, 2019; Li *et al.*, 2022) can deactivate these responses. In the absence of the promoting transcription factors, plants have reduced capabilities for iron uptake, while iron accumulates when they are overactivated (Liang, 2022). The interactions with BTS/L proteins might lead to ubiquitination and destabilization of the bHLH transcription factors. BTS/L proteins may be involved in iron signaling considering that their stability depends on the presence of iron cofactors. Clearly, more research is needed to confirm in plants whether and how BTS/L protein stability is iron dependent. The BTS/L–bHLH protein interaction can be counteracted by small proteins (~5 kDa) of the IRON MAN (IMA) family that are positive regulators of iron uptake (Li *et al.*, 2021; Lichtblau *et al.*, 2022; Peng *et al.*, 2022). A possible scenario is that IMA protein effectors can block the degradation of bHLH transcription factors by themselves becoming targets of ubiquitination via BTS/Ls (Li *et al.*, 2021) (Fig. 2A).

bHLH IVb and IVc transcription factors control a second level of bHLH proteins (Fig. 2B). They comprise two types, in *A. thaliana*, the subgroup IVb transcription factor POPEYE (PYE) (Long *et al.*, 2010) and the subgroup Ib transcription factors bHLH38, bHLH39, bHLH100, and bHLH101 (Wang *et al.*, 2007, 2013; Naranjo Arcos *et al.*, 2017). PYE has negative effects on the intracellular and intercellular redistribution of iron. It can down-regulate *FERRIC REDUCTASE OXIDASE3* (*FRO3*), *NICOTIANAMINE SYNTHASE4* (*NAS4*), and *ZINC-INDUCED FACILITATOR1* (*ZIF1*) (Long *et al.*, 2010), encoding components for iron allocation, and the subgroup Ib *BHLH* genes (Pu and Liang, 2023). The *pye-1* loss-of-function mutant is chlorotic, presumably because PYE controls the proper redistribution of iron in a cell-specific manner in the root stele and vasculature (Muhammad *et al.*, 2022). bHLH Ib transcription factors up-regulate root iron uptake, but they are only able to do so together with the subgroup IIIa bHLH transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT). Together, they form an active nuclear transcription factor complex that up-regulates iron acquisition (Yuan *et al.*, 2008; Wang *et al.*, 2013; Trofimov *et al.*, 2019). FIT has been

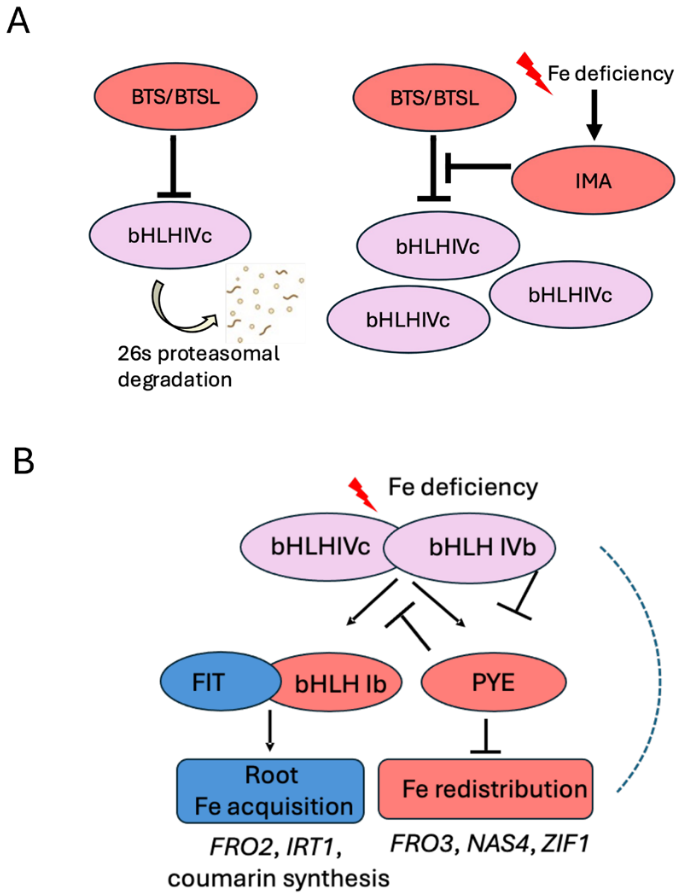


Fig. 2. bHLH transcription factor cascade regulating plant iron homeostasis. The iron homeostasis pathway in plants is tightly regulated by bHLH transcription factors, E3 ligases, and other proteins. (A) E3 ligases, such as BTS (BRUTUS) and BTSL (BTS-like) proteins, can control the protein levels of bHLH IVc transcription factors by targeting them for proteasomal degradation. During iron deficiency, IMA (IRON MAN) proteins may repress BTS/L function by direct binding and serving as an alternative target for degradation, thereby increasing the abundance of bHLH IVc proteins. (B) Transcriptional cascade leading to iron deficiency responses. Interaction of bHLH IVc and IVb transcription factors leads to downstream targeting of another bHLH subgroup IVb member PYE (POPEYE) and the bHLH subgroup Ib (bHLH38, bHLH39, bHLH100, and bHLH101) for transcriptional regulation; for example, bHLH IVc plus URI (UPSTREAM REGULATOR OF IRT1)/bHLH121 leads to activation, while bHLH IVc plus bHLH11 leads to de-activation. Active PYE protein represses the expression of certain target genes, *FRO3* (*FERRIC REDUCTASE OXIDASE3*), *NAS4* (*NICOTIANAMINE SYNTHASE4*), *ZIF1* (*ZINC FACILITATOR1*), and *BHLH IB*, which causes iron redistribution. On the other hand, bHLH Ib transcription factors activate root iron acquisition by heterodimerization with a central regulator, FIT (FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR), leading to the activation of root-induced genes *FRO2* (*FERRIC REDUCTASE OXIDASE2*) and *IRT1* (*IRON-REGULATED TRANSPORTER1*). The dashed line indicates that regulation of target genes by bHLH IVc and IVb is also possible. The color code indicates distinct patterns of gene regulation: 'blue' color, iron deficiency-induced and FIT-dependent, acting primarily in seedling roots; 'red' color, iron deficiency-induced, but not dependent on FIT, acting in seedling roots and shoots; 'rose' color, not induced by iron deficiency, not dependent on FIT, acting in seedling roots and shoots. References are mentioned in the text. The figure has been created with Biorender.com.

found to be responsive to environmental and hormonal cues as it integrates various signaling pathways to adjust iron acquisition accordingly, as discussed in Brumbarova *et al.* (2015) and Gratz *et al.* (2019). Genetic studies have been very informative in dissecting regulatory networks and dividing iron deficiency response genes into co-regulated gene expression networks that can be distinguished by their regulatory expression patterns (Fig. 2B) (Schwarz and Bauer, 2020; Schwarz *et al.*, 2020). The FIT-dependent 'blue' group comprises genes for iron acquisition and transport from soil into roots. 'Blue' components are induced in response to iron deficiency in seedling roots, requiring FIT. The FIT-independent 'red' group of genes and proteins is induced in response to iron deficiency in roots and shoots of seedlings and switched on in the absence of a functional FIT. These genes include targets of bHLH IVb and IVc factors (Schwarz and Bauer, 2020). 'Rose-colored' genes that activate iron deficiency are the above-mentioned *BHLHIVc* and *BHLHIVb* genes, and *BHLH11* that is up-regulated by sufficient iron. These genes are expressed in seedling roots and shoots and encode the upper regulatory level of the iron deficiency cascade (Fig. 2B). Some genes are down-regulated under iron deficiency. They include genes encoding components for iron storage and chelation or components that prevent circulation of excessive iron. Typical target genes in this 'iron sufficiency' group are the ferritin gene *FER1* and genes encoding nicotianamine synthase and YELLOWSTRIPE-LIKE transporters for translocation of nicotianamine–iron complexes (*NAS3*, *YSL1*, and *YSL3*). *FER1* is negatively controlled by the bHLH IVb transcription factor ILR3 (Tissot *et al.*, 2019).

The concept of a hierarchical organization of iron deficiency target genes and a discrimination into subnetworks and co-expression clusters ('blue', 'red', and 'rose' iron sufficiency clusters) has been very helpful for integrating new genetic components into the network and assessing positive and negative effects of the new components (Kim *et al.*, 2019; Lichtblau *et al.*, 2022; Muhammad *et al.*, 2022). This same concept can also become instrumental to decode light signaling cues that interfere with the process of iron deficiency response regulation.

Influence of light and the circadian clock on iron uptake

Leaves play major roles in light perception and in the regulation of iron homeostasis, thereby offering potential cross-control. Iron is transported towards the shoot inside the xylem, and hence the transpiration stream is of prime relevance to iron allocation. Stomatal opening and transpiration depend on guard cell signaling, a process directly stimulated by blue and red light (Shimazaki *et al.*, 2007; Inoue and Kinoshita, 2017). Leaf xylem differentiation directly affects iron homeostasis as it is also relevant for water conductivity (Eichert *et al.*, 2010). Importantly, the leaf vasculature contains the tissues that respond first to an iron deficiency stimulus (Khan *et al.*, 2018). Hence, light-mediated control of iron homeostasis can be exerted in leaves.

Light stimulates iron deficiency response genes such as *BHLH39*, *FERRIC REDUCTASE-OXIDASE2* (*FRO2*), and *IRON REGULATED TRANSPORTER1* (*IRT1*) and root iron reductase activity, while at dawn expression of ferritin genes such as *FER1* increases (Vert *et al.*, 2003; Duc *et al.*, 2009; Trofimov *et al.*, 2024) (Fig. 3). *FIT* is an exception as it is regulated differently from 'blue' and 'red' genes. FIT is also induced by low iron in darkness (Trofimov *et al.*, 2024). In continuous darkness, on the other hand, etiolated leaves can still acquire iron (Sági-Kazár *et al.*, 2023). Studies on the nature of the mechanism controlling light-induced iron homeostasis have indicated that photoreceptor-mediated signaling, the circadian clock, and chloroplast functionality can be involved (Tissot *et al.*, 2014).

Different photoreceptors enable plants to perceive differing light spectra during the day and season (Paik and Huq, 2019). The circadian clock plays a crucial role in plants in co-ordinating various physiological and developmental processes in response to daily and seasonal changes in the environment (Xu *et al.*, 2019). Circadian rhythms control thousands of genes in *A. thaliana* (Tissot *et al.*, 2014). The circadian clock also synchronizes iron acquisition and iron allocation with the external day–night cycle, and hence is fundamental for optimizing iron resource allocation during growth (Fig. 3). The circadian clock of *A. thaliana* has two types of central components that form a double-negative feedback loop (Davis *et al.*, 2022). These are on one hand a set of morning components, MYB transcription factors CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY1), and on the other hand a set of evening components, TIMING OF CAB EXPRESSION1 (TOC1) and PSEUDO-RESPONSE REGULATOR (PRR) proteins. CCA1/LHY1 and TOC1/PRR bind to and negatively regulate each other's promoters. The stability of these proteins is controlled through protein–protein interaction, protein phosphorylation, and degradation via the proteasome. The continued feedback regulation ensures that the protein activities are oscillating in a circadian rhythm (Davis *et al.*, 2022). Promoter activities of *IRT1*, *BHLH39*, and *FER1* depend on clock components. *FER1* and other iron excess genes are under negative control exerted by the nuclear regulator and clock component TIME FOR COFFEE (TIC), as are downstream responses such as iron accumulation and ferritin protein formation. The promoter activities of *IRT1*, *BHLH39*, and *FER1* cycled together with those of *TOC1* and *CCA1* (Hong *et al.*, 2013). Interestingly, the circadian period was lengthened under iron deficiency compared with sufficiency (Chen *et al.*, 2013; Hong *et al.*, 2013). Under iron-deficient conditions, clock components also showed a lengthened circadian period. In addition to CCA1 and LHY, this observation was made for PRR5, PRR7, PRR9, GIGANTEA (GI), TOC1, and the nuclear protein and clock component EARLY FLOWERING4 (ELF4) (Salomé *et al.*, 2013). Additionally, the lengthened circadian period showed dependency on the amount of supplied iron by being longest when iron was least available and shortest under moderate iron supply (Salomé *et al.*, 2013). Other tested metal ions such as zinc, copper, and manganese did not influence the circadian period length in this way (Chen *et al.*, 2013; Salomé *et al.*, 2013). Hence, it is beneficial for plants to prolong iron acquisition during the day when iron supply is limited. It has also been described that expression of iron deficiency response genes is directly controlled by CCA1 binding to the respective iron deficiency response promoters (Xu *et al.*, 2019). The fact that iron acquisition and allocation depend on the circadian clock raises questions as to whether crops can be further improved in their abilities to use iron efficiently by better synchronizing the utilization of iron with day length.

Chloroplasts represent a major iron sink with 80% of total cellular iron content (Fig. 3). Lack of iron affects PSI, PSII, the cytochrome b_6f complex, and ferredoxin, and the major phenotype is leaf chlorosis under iron-deficient conditions (Nouet *et al.*, 2011; Hantzis *et al.*, 2018). Iron excess can lead to oxidative damage as the photosynthetic electron transport chain is the source of ROS that can react with iron (Conte and Walker, 2011). Chloroplasts must have a way to signal iron status. In leaves, *FER1* is negatively controlled by bHLH subgroup IVc transcription factors, such as ILR3 and PYE, while the same transcription factors control iron distribution in leaves and up-regulate iron deficiency responses (Tissot *et al.*, 2019). They are also relevant for photoprotection upon high light intensity by preventing oxidative stress in leaves (Akmakjian *et al.*, 2021). Hence, there is a connection between high light, ROS, and iron responses in leaves.

Retrograde signaling may control iron homeostasis in leaves. This term refers to communication pathways that inform the nucleus about the metabolic state of organelles generating energy (chloroplasts and mitochondria) to influence nuclear gene expression. This can be achieved by metabolites produced in the organelles, that are translocated towards the cytosol and nucleus and ultimately interfere with the control of gene expression. One potential retrograde signal is the phosphonucleotide 3′-phosphoadenosine 5′-phosphate (PAP) which accumulates under stress such as drought and high light. PAP levels are negatively controlled by the enzyme SAL1, located in chloroplasts and mitochondria (Estavillo *et al.*, 2011). PAP may move from the chloroplast to the cytoplasm or nucleus to inhibit 5′–3′ exoribonucleases (XRNs), which then can cause stress-related mRNAs to increase (Estavillo *et al.*, 2011). *Arabidopsis thaliana sal1* and *xrn4* mutant plants had enhanced levels of iron along with increased *FER1*, *FER3*, and *FER4* transcript amounts and signs of different post-transcriptional regulation of iron deficiency components (Estavillo *et al.*, 2011). The iron uptake machinery was also increased in the mutants, suggesting that components of the PAP/SAL1 retrograde signaling pathway

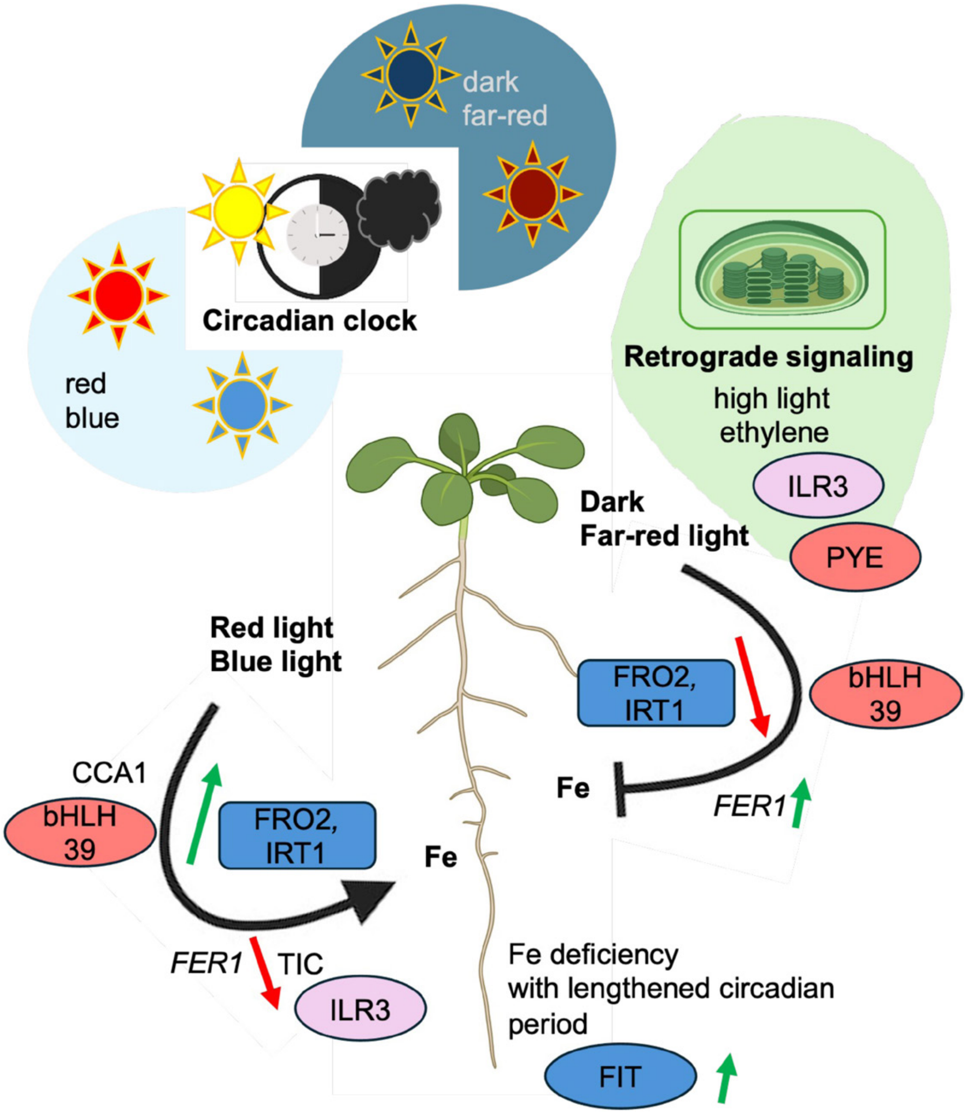


Fig. 3. Light-dependent regulation of iron homeostasis. Different light conditions or darkness can influence iron acquisition and allocation positively or negatively, as indicated by black arrows. Red and blue light, in contrast to darkness and far-red light, can up- and down-regulate genes as represented by green and red arrows. The color code of iron deficiency components is as in Fig. 2. The circadian clock regulates iron homeostasis. Iron deficiency results in lengthening of the circadian period. The circadian clock components, CCA1 (CIRCADIAN CLOCK ASSOCIATED1) and TIC (TIME FOR COFFEE), may directly interfere with promoters of iron deficiency response genes or the iron sufficiency marker *FER1* (*FERRITIN1*). Retrograde signaling in chloroplasts, high light, and ethylene may intersect and control iron homeostasis. References are mentioned in the text. The figure has been created with Biorender.com.

control iron homeostasis (Balparda *et al.*, 2020, 2021). The cytosolic XRN4 (also known as ETHYLENE INSENSITIVE5, EIN5) is required for ethylene signaling (Potuschak *et al.*, 2006). Interestingly, the photoprotection gene *EARLY LIGHT-INDUCED PROTEIN* (*ELIP*) is up-regulated in *sal1* and *xrn4* mutants (Estavillo *et al.*, 2011). *ELIP* is also up-regulated in iron-deficient seedlings with a functional EIN3/EIN3-LIKE (EIL)1 as compared with *ein3 eil1* mutants (Bauer and Blondet, 2011; Lingam *et al.*, 2011). *ein3 eil1* mutants have iron deficiency response phenotypes, that had suggested that perhaps one role for EIN3/EIL1 might be to enhance iron acquisition to prevent oxidative stress under iron deficiency (Lingam *et al.*, 2011). It had been speculated that ethylene may promote iron acquisition as one way to deal with oxidative stress (Lingam *et al.*, 2011). Ethylene may act to enhance iron acquisition through signaling in leaves, potentially involving components of the PAP/SAL1 pathway. Finally, it should be noted that XRN4 is linked with circadian rhythm, and removing a functional XRN4 causes a lengthening of the circadian period, similar to under iron deficiency (Litthauer *et al.*, 2018). Hence, it is also a possibility that the effects of retrograde signaling and the circadian clock merge to control iron deficiency responses.

Furthermore, iron-related signals can be linked with heme- or nitric oxide-derived metabolites (Shekhawat and Verma, 2010; Santos *et al.*, 2019; Gracheva *et al.*, 2022; Romera *et al.*, 2023). Etiolated leaves are also competent to acquire iron and signal iron-related cues (Sági-Kazár *et al.*, 2022, 2023). Etiolation of green plants may also liberate iron stores.

Taken together, multiple leaf, chloroplast, and light signals are linked with iron signaling. More detailed studies are needed to better resolve how the various potential signaling routes converge and which concrete iron signaling molecules directly or indirectly control iron acquisition. One possibility to start with could be to better assess the circadian responses of iron allocation mutants and dissect the capabilities of individual leaves to act as sinks and sources for iron allocation and root iron uptake.

Long-distance iron signaling

Root physiology can be dependent on systemic shoot-derived and light signals, and this provides a possibility for how light can affect iron uptake. This became evident from studying plant mutants affected in long-distance shoot-to-root regulation of iron acquisition (Fig. 4). Some of these mutants accumulate iron in the root stele or vasculature, but the high amounts of iron seem not to be readily bioavailable inside the plant to be allocated to various tissues (Rogers and Guerinot, 2002; Schuler *et al.*, 2012; Nguyen *et al.*, 2022; Chia *et al.*, 2023). As a consequence, such mutants may experience iron deficiency despite iron overload, and display deregulated iron signaling. Root iron uptake can be promoted through shoot-to-root long-distance iron deficiency signaling, as revealed through grafting experiments, for example with the pea *degenerate leaves* (*dgl*) and *bronze* (*brz*) mutants, probably carrying mutations in *OLIGOPEPTIDE TRANSPORTER3* (*OPT3*) and *BTS* genes (Welch and Larue, 1990; Grusak and Pezeshgi, 1996; Harrington *et al.*, 2024) (Fig. 4A). Foliar iron application experiments provided evidence for an iron sufficiency signal acting shoot to root and being transmissible via OPT3 (Garcia *et al.*, 2013) (Fig. 4B). Split-root experiments with one-half of the root system being exposed to iron-rich media, and the other half to iron-depleted conditions demonstrated that the need for iron mobilization is sensed both systematically and locally. Iron was preferentially mobilized in the root halves exposed to iron in the split-root situation while iron acquisition responses were abolished on the iron-deficient root halves (Vert *et al.*, 2003; De Nisi *et al.*, 2012). These experiments indicated that both root sides had been responsive to systemic iron deficiency signals while the presence of iron in the rhizosphere had a positive effect on mobilization (Fig. 4C). Finally, leaf excision experiments supported that leaves, but not the shoot apex, provided systemic information to instruct roots in mobilizing iron (Enomoto *et al.*, 2007). In sum, root iron uptake can be suppressed by shoot-derived long-distance iron sufficiency signals, while it can also be induced by long-distance iron deficiency signals. The exact nature of the systemic iron signals still needs to be revealed. *OPT3* is among the earliest iron-responsive genes, responding with an altered level of expression in the leaf phloem within 2–4 h of iron deficiency or iron resupply in comparison with roots which only respond from 24 h onwards (Zhai *et al.*, 2014; Khan *et al.*, 2018). IMA small proteins may be mobile signals to promote iron acquisition in roots (Grillet *et al.*, 2018; Tabata *et al.*, 2022). Recently, the ELONGATED HYPOCOTYL5 (HY5) transcription factor came to attention. HY5 is a shoot-to-root mobile protein that may control iron acquisition as a long-distance signal in the light.

HY5 as a long-distance regulator for iron uptake

HY5 is activated by light signaling in the shoot and upregulates light-mediated developmental processes, also in roots, and is linked with iron and micronutrient supply. In the absence of HY5, seedlings display elongated hypocotyls, a typical etiolation response (Oyama *et al.*, 1997). HY5 also regulates processes related to nutrient acquisition (Mankotia *et al.*, 2024). An example for that is the blue-light-controlled primary root growth inhibition in response to phosphate starvation, which includes iron accumulation at the tip, a response partly dependent on cryptochrome photoreceptors and HY5 (Gao *et al.*, 2021). This same example also indicates a positive effect of HY5 and blue light on nutrient and iron allocation in the root. Furthermore, different scenarios can explain how HY5 can affect iron nutrition. In the following, we introduce HY5 and summarize evidence that both direct and indirect effects speak in favor of HY5-promoted iron acquisition.

HY5 is a basic-leucine zipper (bZIP) transcription factor. HY5 protein stability is light regulated and its abundance is closely linked with the extent of photomorphogenesis (Osterlund *et al.*, 2000). *HY5* mRNA levels rise in response to light exposure, and the protein levels are further controlled post-translationally (Fig. 4D). There are typically two isoforms of HY5, phosphorylated and unphosphorylated. Studies have shown that the non-phosphorylated mutant form of HY5 has a higher binding to its target promoters, and higher transcriptional activity as compared with the phospho-mimicking mutant of HY5. The non-phosphorylation mutant also showed enhanced photomorphogenesis as it was found to have shorter hypocotyl length as compared with the wild type (Hardtke *et al.*, 2000; Wang *et al.*, 2021). Thus, the non-phosphorylated version of HY5 is the physiologically most active form for regulating photomorphogenesis, and perhaps it may also be the one that controls the regulation of nutrient homeostasis (Fig. 4D). HY5 can be phosphorylated by photoregulatory protein kinase PPK1 that has been recently described (Zhang *et al.*, 2024). By controlling the phosphorylation status of HY5, the activity of downstream targets of HY5 are fine-tuned, and HY5 levels are controlled through ubiquitination mediated by SUPPRESSOR OF PHYTOCHROME

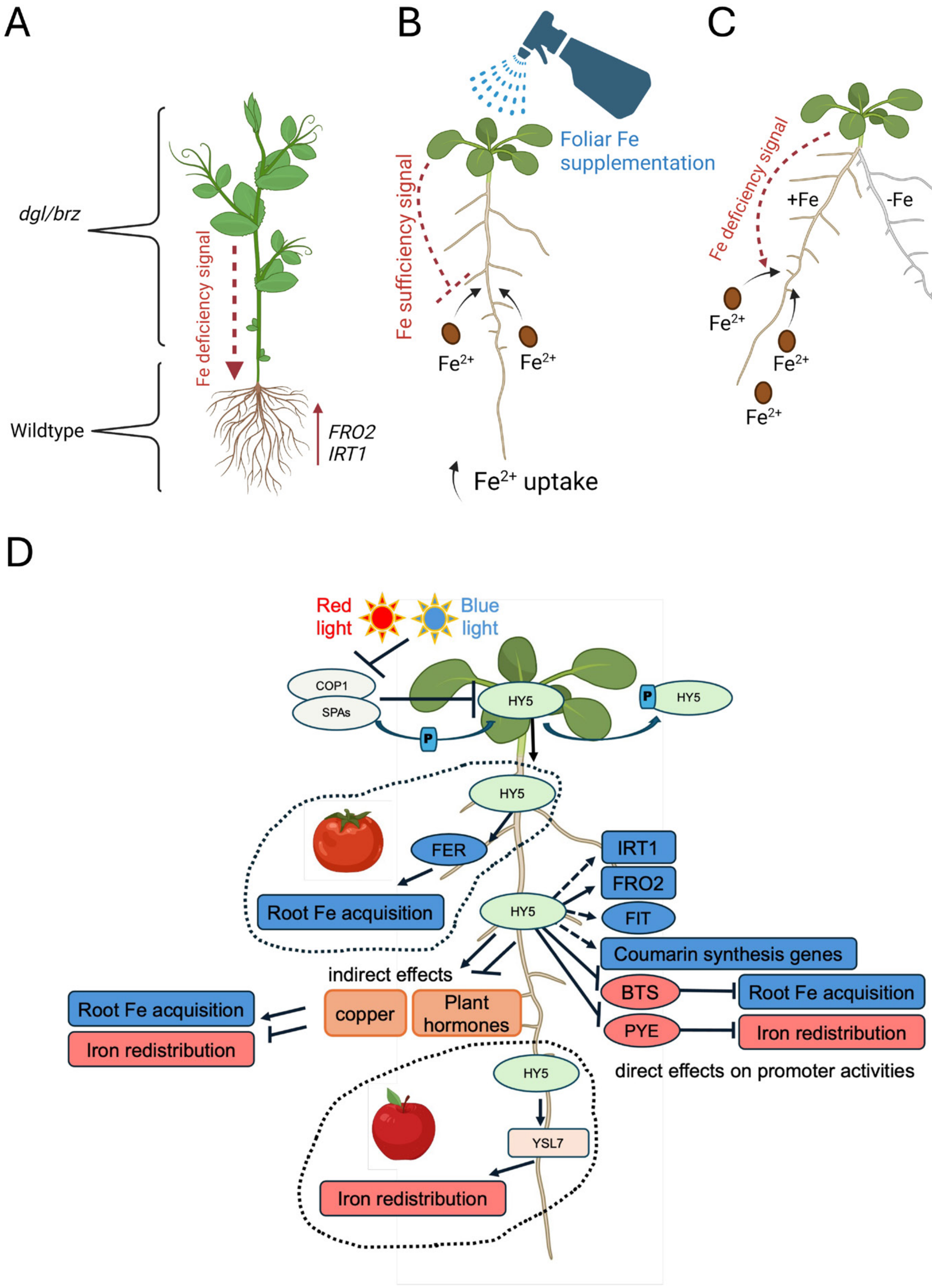


Fig. 4. Long-distance signaling of the iron status, indicating the presence of diverse systemic iron signals. (A–C) Examples of three methods employed to reveal the importance of systemic shoot-to-root iron signals. (A) Reciprocal grafting using leaf iron-overaccumulating mutants such as *brz* (*bronze*) and *dgl* (*degenerate leaflet*) of pea (*Pisum sativum*) showed the existence of a long-distance shoot-to-root iron deficiency signal constitutively promoting root iron uptake and allocation towards leaves. (B) Foliar iron application to iron-deficient plants leads to a down-regulation of root iron uptake

responses due to iron sufficiency signals. However, foliar application does not rescue the pea *dgl* and *Arabidopsis thaliana opt3* mutant defective in OLIGOPEPTIDE TRANSPORTER3, a component involved in the transmission of the long-distance iron sufficiency signal in the phloem. (C) Split root assays have demonstrated that iron uptake can be increased in response to local and systemic iron deficiency signals in root halves exposed to iron but not in root halves maintained in iron deficiency conditions. (D) HY5 (ELONGATED HYPOCOTYL5) is a regulator of iron homeostasis in roots. Light leads to activation of various photoreceptors, for example in response to red and blue light. Active photoreceptors inhibit the COP1 (CONSTITUTIVE PHOTOMORPHOGENIC1)–SPA (SUPPRESSOR OF PHYTOCHROME A) complex, thereby preventing degradation of HY5. A small pool of HY5 remains phosphorylated under the activity of SPA and is inactive even in the light. PPK1 is a photoregulatory protein kinase acting upon HY5. Active non-phosphorylated HY5 accumulates in light conditions and acts as a mobile signal in roots to induce various responses related to iron, as shown by studies in *A. thaliana*, tomato (*Solanum lycopersicum*), and apple (*Malus baccata*). In *A. thaliana*, HY5 can directly control expression of some genes for iron acquisition and allocation. Alternatively, HY5 may also indirectly control iron homeostasis as a consequence of its functioning in hormone and copper homeostasis. In tomato, HY5 can interfere with the action of FER, an ortholog of FIT. In apple, HY5 acts on a YELLOWSTRIPE-LIKE transporter gene (*YSL7*). References are mentioned in the text. The figure has been created with Biorender.com.

A (SPA) proteins (Wang *et al.*, 2021) and CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) protein, which are negative regulators of photomorphogenesis under dark conditions (Osterlund *et al.*, 2000; Wang *et al.*, 2021). The nuclear levels of the COP1–SPA complex are negatively regulated by light, which is mediated by the activity of multiple photoreceptors in response to various light wavelengths, thus allowing HY5 accumulation under light (Podolec and Ulm, 2018) (Fig. 4D). Since light is needed for iron acquisition to occur in roots, it can be assumed that the regulatory mechanisms that control HY5 also control iron uptake. To date, experimental evidence is lacking as to whether the photoregulatory protein kinases or the COP–SPA complex affects iron nutrition in response to iron deficiency. A study based on gene co-expression analysis suggested that PHYTOCHROME-INTERACTING TRANSCRIPTION FACTOR4 (PIF4) and HY5 might be involved in global transcriptome reprogramming to maintain iron homeostasis (Brumbarova and Ivanov, 2019).

One possibility is that HY5 acts directly upon the promoters of iron deficiency response genes (Fig. 4D). bZIP transcription factors such as HY5 are characterized by a basic region which enables DNA binding and an adjacent leucine zipper allowing bZIP dimerization. Interestingly, bZIP transcription factor-binding motifs are enriched in the FIT-dependent and -independent (‘blue’ and ‘red’) target gene promoters like those of bHLH transcription factors (Schwarz *et al.*, 2020). The presence of putative *cis*-regulatory elements suggests that bZIP proteins such as HY5 could indeed be involved in controlling expression of iron deficiency target genes. A recent study has revealed the role of HY5 in regulation of iron deficiency response in *A. thaliana*. A *hy5* mutant showed impaired growth and increased chlorosis under iron deficiency conditions as compared with the wild type (Mankotia *et al.*, 2023). The expression of ‘blue’ genes involved in iron uptake such as *FIT*, *IRT1*, *FRO2*, and coumarin biosynthesis genes were found to be positively regulated by HY5. *FRO2* was shown to be directly regulated by HY5. On the other hand, *BTS* and *PYE* were found to be directly negatively regulated by HY5 (Mankotia *et al.*, 2023). HY5 can also regulate iron uptake in tomato (*Solanum lycopersicum* L.). Red light activates SlHY5 through phytochrome B (PHYB); SlHY5 then can move from shoot to root and activate iron uptake by activating *SlFER* expression (Guo *et al.*, 2021). *SlFER* is the ortholog of *AtFIT* (Ling *et al.*, 2002). In apple (*Malus baccata*), MbHY5 may alleviate iron deficiency-induced chlorosis and promote iron transport by positively regulating the expression of *MbYSL7*, encoding a prospective nicotianamine–metal ion chelate transport protein (Sun *et al.*, 2022). Hence, it is possible that HY5 acts as a long-distance signal. It may be translocated inside the phloem from shoot to root and directly control the promoter activities of iron deficiency response genes that contain bZIP-binding sites.

Another possibility is that HY5 regulates nutrient or hormone signaling pathways and thereby indirectly affects iron nutrition (Fig. 4D). HY5 lacks its own activation or repressor domain and its ability to activate or repress transcription depends on the transcription factor with which it interacts (Burko *et al.*, 2020). Several studies have highlighted the crucial involvement of HY5 in the regulation of hormone signaling pathways. Several hormone signaling pathways such as those involving auxin, gibberellic acid, brassinosteroids, and ethylene can be suppressed by HY5 (Sibout *et al.*, 2006; Lee *et al.*, 2007; Weller *et al.*, 2009; Li *et al.*, 2011; Shi *et al.*, 2011; Li and He, 2016). HY5 promotes abscisic acid (ABA) signaling and regulates the crosstalk between light and ABA signaling by physically interacting with ABA-INSENSITIVE5 (ABI5) (Chen *et al.*, 2008; Bhagat *et al.*, 2021). It is of interest to note that the various processes controlled by HY5 also indirectly intersect with the regulation of iron acquisition. For example, light has a positive effect on iron acquisition. Hormones such as auxin, ethylene, and gibberellic acid can regulate iron uptake via affecting expression of *FIT*, *FRO2*, and *IRT1* (García *et al.*, 2010; Lingam *et al.*, 2011; Giehl *et al.*, 2012; Brumbarova *et al.*, 2015; Wild *et al.*, 2016; Kanwar *et al.*, 2021). ABA alleviates iron deficiency stress by promoting the transport and reutilization of iron (Lei *et al.*, 2014; Zhang *et al.*, 2020). Brassinosteroids can negatively regulate iron deficiency responses (Wang *et al.*, 2012, 2015; Kour *et al.*, 2021). Therefore, HY5 could be indirectly involved in controlling iron uptake via hormone pathways. Additionally, several studies have reported that iron accumulation is enhanced under copper deficiency, and vice versa (Bernal *et al.*, 2012; Waters and Armbrust, 2013; Kastoori Ramamurthy *et al.*, 2018; Kroh and Pilon, 2020;

Rai *et al.*, 2021). HY5 positively regulates copper homeostasis by interacting with SQUAMOSA PROMOTER BINDING PROTEIN-LIKE7 (SPL7) (Zhang *et al.*, 2014; Araki *et al.*, 2018). SPL7 can negatively regulate the expression of some of the iron deficiency-responsive genes (subgroup Ib *BHLH* and *IMA*) under iron deficiency (Kastoori Ramamurthy *et al.*, 2018). Hence, HY5 might indirectly regulate some of the iron homeostasis genes.

Overall, HY5 can act in multiple circumstances to control iron deficiency responses by directly and/or indirectly regulating key genes involved in iron deficiency signaling pathways. Further studies are required to fully understand the role of HY5 in regulation of iron homeostasis. The role of HY5 in regulation of iron transport or storage is not clear yet. The effects of photoreceptors and COP1–SPA1 complexes on HY5 and downstream iron acquisition responses remain to be investigated. HY5 protein needs to be visualized in plant roots, and it is of interest to understand tissue-specific localization and regulatory aspects. Indirect and direct effects can be disentangled by dissecting the hormone- and copper-mediated responses via HY5.

Biomolecular condensates containing transcription factors of light and iron signaling

Cryptochromes, phytochromes, and iron signaling components can accumulate in subnuclear foci termed biomolecular condensates, and these may represent regulatory hubs for cross-connecting the regulation of iron uptake in response to light. In plants, condensation of nuclear proteins can be triggered, among other factors, by light and temperature. Photobodies are the subnuclear structures for which this phenomenon was first observed and is best studied in the plant field (Pardi and Nusinow, 2021). Condensates form as a result of protein interaction. The ability of proteins to engage in condensates such as photobodies depends on the presence of certain protein domains and in particular on the presence of intrinsically disordered regions. These allow proteins to be multivalent and engage in protein aggregation so that large protein complexes consisting of primary and secondary interacting proteins can form and separate into a dense phase from the remaining less concentrated nucleoplasm during phase separation (Kim *et al.*, 2023). The coalescing proteins form biomolecular condensates (Emenecker *et al.*, 2021; Pardi and Nusinow, 2021). A number of different proteins in addition to the blue and red light perception system are detected in photobodies, including HY5 and PIF bHLH transcription factors, indicating that photobodies are locations of regulatory protein interaction assemblies that can serve protein stability control, and transcriptional and post-transcriptional control (Pardi and Nusinow, 2021). Depending on the intensity and quality of light and temperature, photobodies can vary from being numerous and small to becoming large subnuclear entities (Pardi and Nusinow, 2021; Willige *et al.*, 2024).

It was recently found that an iron deficiency response regulator, namely FIT, can accumulate in biomolecular condensates in subnuclear membraneless compartments in the root epidermis of iron-deficient plants (Trofimov *et al.*, 2024). In several aspects, the FIT-containing nuclear bodies resembled photobodies. They were blue light inducible, reversible, highly dynamic, and the likely result of liquid–liquid phase separation (Fig. 5). FIT was able to form nuclear bodies containing active FIT–bHLH039 transcription factor complexes as well as homomeric FIT complexes (Trofimov *et al.*, 2024). FIT and bHLH subgroup Ib transcription factors have intrinsically disordered regions and undergo protein interactions in multiple ways via the N- or C-terminus or via their HLH domains (Yuan *et al.*, 2008; Wild *et al.*, 2016; Gratz *et al.*, 2019; Schwarz and Bauer, 2020; Lichtblau *et al.*, 2022). FIT also formed nuclear bodies with speckle components and splicing factors such as ARGININE/SERINE-RICH45 (SR45) (Trofimov *et al.*, 2024). This was interesting because mutation of SR45 splicing factor resulted in disturbed iron homeostasis. *sr45* mutants accumulated iron in the root stele, indicating that mutants were not able to use iron properly. This misutilization of iron might have been caused by aberrant iron deficiency response gene

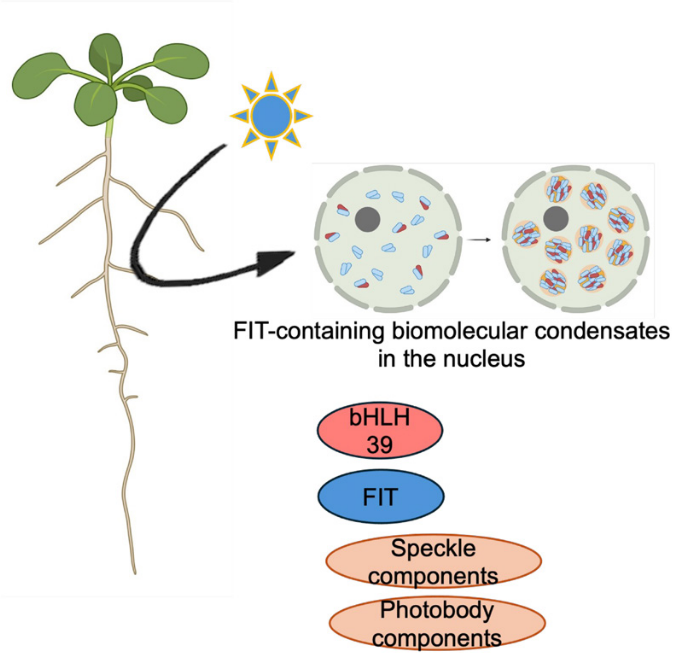


Fig. 5. Blue light-dependent accumulation of iron deficiency response regulators in subnuclear biomolecular condensates. FIT and bHLH39 can form condensates, which are light inducible, reversible, highly dynamic, and probably formed due to liquid–liquid phase separation. The subnuclear bodies may contain speckle components, such as SR45 (ARGININE/SERINE-RICH45), and photobody components such as PIF (PHYTOCHROME-INTERACTING TRANSCRIPTION FACTOR) proteins. The figure has been created with Biorender.com.

expression including differential alternative splicing of some iron response genes (Fanara *et al*., 2022). Alternative splicing occurs in response to low iron (Li *et al*., 2013; Fanara *et al*., 2022; Trofimov *et al*., 2024), and is also regulated by light, for example through phytochromes (Shikata *et al*., 2014). FIT also co-localized with PIF3 and PIF4 bHLH factors in photobodies in particular dynamic patterns, indicating that FIT may be interwoven into photobodies without a blue light cue (Trofimov *et al*., 2024). This behavior of FIT may speak in favor of a close association with PIF proteins in the nucleus (Fig. 5).

Thus, the presence of iron nutrition regulators in blue light-induced nuclear bodies indicates a cross-connection and complex signaling connecting light and iron deficiency signals (Fig. 5). Indeed, light-controlled iron nutrition-related nuclear bodies can be regulatory hubs in which multiple signaling pathways merge. There is clearly a need for future exploration to address the functional roles of protein complexes inside nuclear bodies with FIT protein for light and iron response regulation. The environmental factors for FIT condensation can be further elucidated to find out whether, for example, temperature and other abiotic signals can play a role.

Conclusions

Light, in addition to nutrient availability, is a major environmental cue whose changes are directly registered by plants to redirect growth accordingly. The above analysis shows that light and iron nutrition signaling are interlinked in multiple ways. The concept of subdividing genes into 'red', 'blue', 'rose', and 'iron sufficiency' genes and understanding their regulatory principles is helpful in defining genetic and environment effects and consequences on iron nutrition. Clearly, future research can be intensified to better understand the cross-connection in iron and light signaling. Future endeavors can address the following as yet understudied open questions.

- How is transpiration controlled under low iron conditions? How does iron availability affect vascular differentiation to adjust iron nutrition to abiotic stress?
- How does etiolation affect iron acquisition? Etiolation can occur naturally during early plant development or appear as a consequence of a changing light environment during plant growth. What are the capacities of etiolated versus non-etiolated organs to act as iron sinks? Which effects does iron supply have on the capacities of etiolated versus non-etiolated leaves to acquire iron? Which signals are involved?
- How do changing light conditions, direction of light, shading, and varying light intensities during growth seasons affect the abilities of plants to utilize iron?
- How is internal iron supply controlled by iron signaling? Which sensing mechanisms are important? What are the roles of retrograde signaling via the chloroplasts and autophagy? How are these processes linked with high light regulation and the action of plant hormones, such as ethylene? Can other signaling molecules be relevant here, such as nitric oxide and heme?
- How are systemic leaf iron signals connected with light? What could be the nature of a systemic signal?
- How does HY5 signaling add specificity to the iron deficiency responses? How does HY5 signaling interact with the bHLH signaling controlling iron acquisition? Where is HY5 exerting effects in the root?
- What is the function of FIT nuclear bodies in iron signaling and which conditions favor their appearance? What is the role of alternative splicing of iron deficiency response genes? Is FIT forming protein interactions with light signaling components in roots? Are other iron deficiency response regulators also accumulating in subnuclear condensates following liquid–liquid phase separation?

With rising global temperatures and increasingly alkaline soils for agricultural purposes, we should experiment with and design novel crops. This will probably require improving nutrient use characteristics and modifying the photoperiod dependences so that novel crops can complete their life cycles in new environments. So far, the resulting effects of photoperiod changes on iron nutrition in different soils have been scarcely considered in crop improvements. Because of the need to develop sustainable crops with highly nutritious properties, more research should be dedicated to understanding and exploiting the interconnections between light signaling and iron nutrition.

Author contributions

PB, KT, and SM: wrote the manuscript; PB, KT, MN, DB, and SM: prepared the figures. All authors reviewed/edited the article. PB: acquired funding.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work received funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under GRK F020512056 (NEXT*plant*) and Germany's Excellence Strategy—EXC-2048/1—project ID 390686111.

References

Akmakjian GZ, Riaz N, Guerinot ML. 2021. Photoprotection during iron deficiency is mediated by the bHLH transcription factors PYE and ILR3. Proceedings of the National Academy of Sciences, USA **118**, e2024918118.

Araki R, Mermod M, Yamasaki H, Kamiya T, Fujiwara T, Shikanai T. 2018. SPL7 locally regulates copper-homeostasis-related genes in Arabidopsis. Journal of Plant Physiology **224-225**, 137–143.

Balparda M, Armas AM, Estavillo GM, Roschzttardtz H, Pagani MA, Gomez-Casati DF. 2020. The PAP/SAL1 retrograde signaling pathway is involved in iron homeostasis. Plant Molecular Biology **102**, 323–337.

Balparda M, Armas AM, Gomez-Casati DF, Pagani MA. 2021. PAP/SAL1 retrograde signaling pathway modulates iron deficiency response in alkaline soils. Plant Science **304**, 110808.

Bastow EL, Garcia de la Torre VS, Maclean AE, Green RT, Merlot S, Thomine S, Balk J. 2018. Vacuolar iron stores gated by NRAMP3 and NRAMP4 are the primary source of iron in germinating seeds. Plant Physiology **177**, 1267–1276.

Bauer P, Blondet E. 2011. Transcriptome analysis of *ein3 eil1* mutants in response to iron deficiency. Plant Signaling and Behavior **6**, 1669–1671.

Bernal M, Casero D, Singh V, *et al*. 2012. Transcriptome sequencing identifies SPL7-regulated copper acquisition genes FRO4/FRO5 and the copper dependence of iron homeostasis in Arabidopsis. The Plant Cell **24**, 738–761.

Bhagat PK, Verma D, Sharma D, Sinha AK. 2021. HY5 and ABI5 transcription factors physically interact to fine tune light and ABA signaling in Arabidopsis. Plant Molecular Biology **107**, 117–127.

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318**, 801–806.

Brumbarova T, Bauer P, Ivanov R. 2015. Molecular mechanisms governing Arabidopsis iron uptake. Trends in Plant Science **20**, 124–133.

Brumbarova T, Ivanov R. 2019. The nutrient response transcriptional regulome of Arabidopsis. iScience **19**, 358–368.

Burko Y, Seluzicki A, Zander M, Pedmale UV, Ecker JR, Chory J. 2020. Chimeric activators and repressors define HY5 activity and reveal a light-regulated feedback mechanism. The Plant Cell **32**, 967–983.

Chen H, Zhang J, Neff MM, Hong S-W, Zhang H, Deng X-W, Xiong L. 2008. Integration of light and abscisic acid signaling during seed germination and early seedling development. Proceedings of the National Academy of Sciences, USA **105**, 4495–4500.

Chen YY, Wang Y, Shin LJ, Wu JF, Shanmugam V, Tsednee M, Lo JC, Chen CC, Wu SH, Yeh KC. 2013. Iron is involved in the maintenance of circadian period length in Arabidopsis. Plant Physiology **161**, 1409–1420.

Chia J-C, Yan J, Rahmati Ishka M, *et al*. 2023. Loss of OPT3 function decreases phloem copper levels and impairs crosstalk between copper and iron homeostasis and shoot-to-root signaling in *Arabidopsis thaliana*. The Plant Cell **35**, 2157–2185.

Choudhury FK, Rivero RM, Blumwald E, Mittler R. 2017. Reactive oxygen species, abiotic stress and stress combination. The Plant Journal **90**, 856–867.

Clemens S, Weber M. 2016. The essential role of coumarin secretion for Fe acquisition from alkaline soil. Plant Signaling & Behavior **11**, e1114197.

Connorton JM, Balk J, Rodriguez-Celma J. 2017. Iron homeostasis in plants—a brief overview. Metallomics **9**, 813–823.

Conte SS, Walker EL. 2011. Transporters contributing to iron trafficking in plants. Molecular Plant **4**, 464–476.

Davis W, Endo M, Locke JCW. 2022. Spatially specific mechanisms and functions of the plant circadian clock. Plant Physiology **190**, 938–951.

De Nisi P, Vigani G, Dell'Orto M, Zocchi G. 2012. Application of the split root technique to study iron uptake in cucumber plants. Plant Physiology and Biochemistry **57**, 168–174.

Duc C, Cellier F, Lobréaux S, Briat JF, Gaymard F. 2009. Regulation of iron homeostasis in *Arabidopsis thaliana* by the clock regulator time for coffee. Journal of Biological Chemistry **284**, 36271–36281.

Eichert T, Peguero-Pina JJ, Gil-Pelegrín E, Heredia A, Fernández V. 2010. Effects of iron chlorosis and iron resupply on leaf xylem architecture, water relations, gas exchange and stomatal performance of field-grown peach (*Prunus persica*). Physiologia Plantarum **138**, 48–59.

Emenecker RJ, Holehouse AS, Strader LC. 2021. Sequence determinants of in cell condensate morphology, dynamics, and oligomerization as measured by number and brightness analysis. Cell Communication and Signaling **19**, 65.

Enomoto Y, Hodoshima H, Shimada H, Shoji K, Yoshihara T, Goto F. 2007. Long-distance signals positively regulate the expression of iron uptake genes in tobacco roots. Planta **227**, 81–89.

Estavillo GM, Crisp PA, Pornsiriwong W, *et al*. 2011. Evidence for a SAL1–PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. The Plant Cell **23**, 3992–4012.

Fanara S, Schloesser M, Hanikenne M, Motte P. 2022. Altered metal distribution in the *sr45-1* Arabidopsis mutant causes developmental defects. The Plant Journal **110**, 1332–1352.

Gao F, Robe K, Bettembourg M, *et al*. 2019. The transcription factor bHLH121 interacts with bHLH105 (ILR3) and its closest homologs to regulate iron homeostasis in Arabidopsis. The Plant Cell **32**, 508–524.

Gao Y-Q, Bu L-H, Han M-L, Wang Y-L, Li Z-Y, Liu H-T, Chao D-Y. 2021. Long-distance blue light signalling regulates phosphate deficiency-induced primary root growth inhibition. Molecular Plant **14**, 1539–1553.

García MJ, Lucena C, Romera FJ, Alcántara E, Pérez-Vicente R. 2010. Ethylene and nitric oxide involvement in the up-regulation of key genes related to iron acquisition and homeostasis in Arabidopsis. Journal of Experimental Botany **61**, 3885–3899.

Garcia MJ, Romera FJ, Stacey MG, Stacey G, Villar E, Alcantara E, Perez-Vicente R. 2013. Shoot to root communication is necessary to control the expression of iron-acquisition genes in strategy I plants. Planta **237**, 65–75.

Giehl RF, Lima JE, von Wirén N. 2012. Localized iron supply triggers lateral root elongation in Arabidopsis by altering the AUX1-mediated auxin distribution. The Plant Cell **24**, 33–49.

Gracheva M, Homonnay Z, Singh A, Fodor F, Marosi VB, Solti A, Kovács K. 2022. New aspects of the photodegradation of iron(III) citrate: spectroscopic studies and plant-related factors. Photochemical & Photobiological Sciences **21**, 983–996.

Gratz R, Manishankar P, Ivanov R, *et al*. 2019. CIPK11-dependent phosphorylation modulates FIT activity to promote Arabidopsis iron acquisition in response to calcium signaling. Developmental Cell **48**, 726–740.e10.

Grillet L, Lan P, Li WF, Mokkapati G, Schmidt W. 2018. IRON MAN is a ubiquitous family of peptides that control iron transport in plants. Nature Plants **4**, 953–963.

Grünhofer P, Heimerich I, Pohl S, *et al*. 2024. Suberin deficiency and its effect on the transport physiology of young poplar roots. New Phytologist **242**, 137–153.

Grusak MA, Pezeshgi S. 1996. Shoot-to-root signal transmission regulates root Fe(III) reductase activity in the *dgl* mutant of pea. Plant Physiology **110**, 329–334.

Guo Z, Xu J, Wang Y, Hu C, Shi K, Zhou J, Xia X, Zhou Y, Foyer CH, Yu J. 2021. The phyB-dependent induction of HY5 promotes iron uptake by systemically activating FER expression. EMBO Reports **22**, e51944.

Hantzis LJ, Kroh GE, Jahn CE, Cantrell M, Peers G, Pilon M, Ravet K. 2018. A program for iron economy during deficiency targets specific Fe proteins. Plant Physiology **176**, 596–610.

Hardtke CS, Gohda K, Osterlund MT, Oyama T, Okada K, Deng XW. 2000. HY5 stability and activity in *Arabidopsis* is regulated by phosphorylation in its COP1 binding domain. The EMBO Journal **19**, 4997–5006.

Harrington SA, Franceschetti M, Balk J. 2024. Genetic basis of the historical iron-accumulating *dgl* and *brz* mutants in pea. The Plant Journal **117**, 590–598.

Hindt MN, Akmakjian GZ, Pivarski KL, Punshon T, Baxter I, Salt DE, Guerinot ML. 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.

Hong S, Kim SA, Guerinot ML, McClung CR. 2013. Reciprocal interaction of the circadian clock with the iron homeostasis network in *Arabidopsis*. Plant Physiology **161**, 893–903.

Inoue SI, Kinoshita T. 2017. Blue light regulation of stomatal opening and the plasma membrane H$^+$-ATPase. Plant Physiology **174**, 531–538.

Kanwar P, Baby D, Bauer P. 2021. Interconnection of iron and osmotic stress signalling in plants: is FIT a regulatory hub to cross-connect abscisic acid responses? Plant Biology **23**, 31–38.

Kastoori Ramamurthy R, Xiang Q, Hsieh E-J, Liu K, Zhang C, Waters BM. 2018. New aspects of iron–copper crosstalk uncovered by transcriptomic characterization of Col-0 and the copper uptake mutant *spl7* in *Arabidopsis thaliana*. Metallomics **10**, 1824–1840.

Khan MA, Castro-Guerrero NA, McInturf SA, *et al*. 2018. Changes in iron availability in Arabidopsis are rapidly sensed in the leaf vasculature and impaired sensing leads to opposite transcriptional programs in leaves and roots. Plant, Cell & Environment **41**, 2263–2276.

Kim C, Kwon Y, Jeong J, Kang M, Lee GS, Moon JH, Lee H-J, Park Y-I, Choi G. 2023. Phytochrome B photobodies are comprised of phytochrome B and its primary and secondary interacting proteins. Nature Communications **14**, 1708.

Kim SA, LaCroix IS, Gerber SA, Guerinot ML. 2019. The iron deficiency response in *Arabidopsis thaliana* requires the phosphorylated transcription factor URI. Proceedings of the National Academy of Sciences, USA **116**, 24933–24942.

Kour J, Kohli SK, Khanna K, *et al*. 2021. Brassinosteroid signaling, crosstalk and, physiological functions in plants under heavy metal stress. Frontiers in Plant Science **12**, 608061.

Kovács K, Pechoušek J, Machala L, *et al*. 2016. Revisiting the iron pools in cucumber roots: identification and localization. Planta **244**, 167–179.

Kroh GE, Pilon M. 2020. Micronutrient homeostasis and chloroplast iron protein expression is largely maintained in a chloroplast copper transporter mutant. Functional Plant Biology **47**, 1041–1052.

Le CTT, Brumbarova T, Bauer P. 2019. The interplay of ROS and iron signaling in plants. In: Panda SK, Yamamoto YY, eds. Redox homeostasis in plants, from signalling to stress tolerance, **Vol. 32**. Cham: Springer, 43–66.

Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao H, Lee I, Deng XW. 2007. Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. The Plant Cell **19**, 731–749.

Lefèvre F, Fourmeau J, Pottier M, Baijot A, Cornet T, Abadía J, Álvarez-Fernández A, Boutry M. 2018. The *Nicotiana tabacum* ABC transporter NtPDR3 secretes *O*-methylated coumarins in response to iron deficiency. Journal of Experimental Botany **69**, 4419–4431.

Lei GJ, Zhu XF, Wang ZW, Dong F, Dong NY, Zheng SJ. 2014. Abscisic acid alleviates iron deficiency by promoting root iron reutilization and transport from root to shoot in Arabidopsis. Plant, Cell & Environment **37**, 852–863.

Li M, Watanabe S, Gao F, Dubos C. 2023. Iron nutrition in plants: towards a new paradigm? Plants **12**, 384.

Li Q-F, He J-X. 2016. BZR1 interacts with HY5 to mediate brassinosteroid- and light-regulated cotyledon opening in Arabidopsis in darkness. Molecular Plant **9**, 113–125.

Li W, Lin WD, Ray P, Lan P, Schmidt W. 2013. Genome-wide detection of condition-sensitive alternative splicing in Arabidopsis roots. Plant Physiology **162**, 1750–1763.

Li X, Zhang H, Ai Q, Liang G, Yu D. 2016. Two bHLH transcription factors, bHLH34 and bHLH104, regulate iron homeostasis in *Arabidopsis thaliana*. Plant Physiology **170**, 2478–2493.

Li Y, Lei R, Pu M, Cai Y, Lu C, Li Z, Liang G. 2022. bHLH11 inhibits bHLH IVc proteins by recruiting the TOPLESS/TOPLESS-RELATED corepressors in Arabidopsis. Plant Physiology **188**, 1335–1349.

Li Y, Lu CK, Li CY, Lei RH, Pu MN, Zhao JH, Peng F, Ping HQ, Wang D, Liang G. 2021. IRON MAN interacts with BRUTUS to maintain iron homeostasis in Arabidopsis. Proceedings of the National Academy of Sciences, USA **118**, e2109063118.

Li Z, Zhang L, Yu Y, Quan R, Zhang Z, Zhang H, Huang R. 2011. The ethylene response factor AtERF11 that is transcriptionally modulated by the bZIP transcription factor HY5 is a crucial repressor for ethylene biosynthesis in Arabidopsis. The Plant Journal **68**, 88–99.

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

Liang G, Zhang HM, Li XL, Ai Q, Yu DQ. 2017. bHLH transcription factor bHLH115 regulates iron homeostasis in *Arabidopsis thaliana*. Journal of Experimental Botany **68**, 1743–1755.

Lichtblau DM, Schwarz B, Baby D, Endres C, Sieberg C, Bauer P. 2022. The iron deficiency-regulated small protein effector FEP3/IRON MAN1 modulates interaction of BRUTUS-LIKE1 with bHLH subgroup IVc and POPEYE transcription factors. Frontiers in Plant Science **13**, 930049.

Ling HQ, Bauer P, Bereczky Z, Keller B, Ganal M. 2002. The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in roots. Proceedings of the National Academy of Sciences, USA **99**, 13938–13943.

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

Litthauer S, Chan KX, Jones MA. 2018. 3'-Phosphoadenosine 5'-phosphate accumulation delays the circadian system. Plant Physiology **176**, 3120–3135.

Long TA, Tsukagoshi H, Busch W, Lahner B, Salt DE, Benfey PN. 2010. The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. The Plant Cell **22**, 2219–2236.

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. 2003. The role of nutrient availability in regulating root architecture. Current Opinion in Plant Biology **6**, 280–287.

Mankotia S, Jakhar P, Satbhai SB. 2024. HY5: a key regulator for light-mediated nutrient uptake and utilization by plants. New Phytologist **241**, 1929–1935.

Mankotia S, Singh D, Monika K, Kalra M, Meena H, Meena V, Yadav RK, Pandey AK, Satbhai SB. 2023. ELONGATED HYPOCOTYL 5 regulates BRUTUS and affects iron acquisition and homeostasis in *Arabidopsis thaliana*. The Plant Journal **114**, 1267–1284.

Mayank AK, Pandey V, Vashisht AA, Barshop WD, Rayatpisheh S, Sharma T, Haque T, Powers DN, Wohlschlegel JA. 2019. An oxygen-dependent interaction between FBXL5 and the CIA-targeting complex regulates iron homeostasis. Molecular Cell **75**, 382–393.e5.

Muhammad D, Clark NM, Haque S, Williams CM, Sozzani R, Long TA. 2022. POPEYE intercellular localization mediates cell-specific iron deficiency responses. Plant Physiology **190**, 2017–2032.

Naranjo Arcos MA, Maurer F, Meiser J, Pateyron S, Fink-Straube C, Bauer P. 2017. Dissection of iron signaling and iron accumulation by overexpression of subgroup Ib bHLH039 protein. Scientific Reports **7**, 10911.

Nguyen NT, Khan MA, Castro-Guerrero NA, Chia JC, Vatamaniuk OK, Mari S, Jurisson SS, Mendoza-Cozatl DG. 2022. Iron availability within the leaf vasculature determines the magnitude of iron deficiency responses in source and sink tissues in Arabidopsis. Plant and Cell Physiology **63**, 829–841.

Nouet C, Motte P, Hanikenne M. 2011. Chloroplastic and mitochondrial metal homeostasis. Trends in Plant Science **16**, 395–404.

Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature **405**, 462–466.

Oyama T, Shimura Y, Okada K. 1997. The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes & Development **11**, 2983–2995.

Paik I, Huq E. 2019. Plant photoreceptors: multi-functional sensory proteins and their signaling networks. Seminars in Cell and Developmental Biology **92**, 114–121.

Pardi SA, Nusinow DA. 2021. Out of the dark and into the light: a new view of phytochrome photobodies. Frontiers in Plant Science **12**, 732947.

Peng F, Li C, Lu C, Li Y, Xu P, Liang G. 2022. IRONMAN peptide interacts with OsHRZ1 and OsHRZ2 to maintain Fe homeostasis in rice. Journal of Experimental Botany **73**, 6463–6474.

Podolec R, Ulm R. 2018. Photoreceptor-mediated regulation of the COP1/SPA E3 ubiquitin ligase. Current Opinion in Plant Biology **45**, 18–25.

Potuschak T, Vansiri A, Binder BM, Lechner E, Vierstra RD, Genschik P. 2006. The exoribonuclease XRN4 is a component of the ethylene response pathway in Arabidopsis. The Plant Cell **18**, 3047–3057.

Pu MN, Liang G. 2023. The transcription factor POPEYE negatively regulates the expression of bHLH Ib genes to maintain iron homeostasis. Journal of Experimental Botany **74**, 2754–2767.

Rai S, Singh PK, Mankotia S, Swain J, Satbhai SB. 2021. Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. Plant Stress **1**, 100008.

Ram H, Sardar S, Gandass N. 2021. Vacuolar Iron Transporter (Like) proteins: regulators of cellular iron accumulation in plants. Physiologia Plantarum **171**, 823–832.

Rodríguez-Celma J, Chou H, Kobayashi T, Long TA, Balk J. 2019. Hemerythrin E3 ubiquitin ligases as negative regulators of iron homeostasis in plants. Frontiers in Plant Science **10**, 98.

Rodríguez-Celma J, Vázquez-Reina S, Orduna J, Abadía A, Abadía J, Álvarez-Fernández A, López-Millán AF. 2011. Characterization of flavins in roots of Fe-deficient strategy I plants, with a focus on *Medicago truncatula*. Plant and Cell Physiology **52**, 2173–2189.

Rogers EE, Guerinot ML. 2002. FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. The Plant Cell **14**, 1787–1799.

Romera FJ, García MJ, Lucena C, Angulo M, Pérez-Vicente R. 2023. NO is not the same as GSNO in the regulation of Fe deficiency responses by dicot plants. International Journal of Molecular Sciences **24**, 12617.

Römheld V, Marschner H. 1986. Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition **9**, 695–713.

Roschzttardtz H, Grillet L, Isaure MP, Conéjéro G, Ortega R, Curie C, Mari S. 2011. Plant cell nucleolus as a hot spot for iron. Journal of Biological Chemistry **286**, 27863–27866.

Sági-Kazár M, Sárvári E, Cseh B, Illés L, May Z, Hegedűs C, Barócsi A, Lenk S, Solymosi K, Solti A. 2023. Iron uptake of etioplasts is independent from photosynthesis but applies the reduction-based strategy. Frontiers in Plant Science **14**, 1227811.

Sági-Kazár M, Solymosi K, Solti A. 2022. Iron in leaves: chemical forms, signalling, and in-cell distribution. Journal of Experimental Botany **73**, 1717–1734.

Salomé PA, Oliva M, Weigel D, Krämer U. 2013. Circadian clock adjustment to plant iron status depends on chloroplast and phytochrome function. The EMBO Journal **32**, 511–523.

Samira R, Li B, Kliebenstein D, Li C, Davis E, Gillikin JW, Long TA. 2018. The bHLH transcription factor ILR3 modulates multiple stress responses in Arabidopsis. Plant Molecular Biology **97**, 297–309.

Santos CS, Ozgur R, Uzilday B, Turkan I, Roriz M, Rangel A, Carvalho SMP, Vasconcelos MW. 2019. Understanding the role of the antioxidant system and the tetrapyrrole cycle in iron deficiency chlorosis. Plants **8**, 348.

Schuler M, Rellán-Álvarez R, Fink-Straube C, Abadía J, Bauer P. 2012. Nicotianamine functions in the phloem-based transport of iron to sink organs, in pollen development and pollen tube growth in Arabidopsis. The Plant Cell **24**, 2380–2400.

Schwarz B, Azodi CB, Shiu S-H, Bauer P. 2020. Putative cis-regulatory elements predict iron deficiency responses in Arabidopsis roots. Plant Physiology **182**, 1420–1439.

Schwarz B, Bauer P. 2020. FIT, a regulatory hub for iron deficiency and stress signaling in roots, and FIT-dependent and -independent gene signatures. Journal of Experimental Botany **71**, 1694–1705.

Selote D, Samira R, Matthiadis A, Gillikin JW, Long TA. 2015. Iron-binding E3 ligase mediates iron response in plants by targeting basic helix–loop–helix transcription factors. Plant Physiology **167**, 273–286.

Shekhawat GS, Verma K. 2010. Haem oxygenase (HO): an overlooked enzyme of plant metabolism and defence. Journal of Experimental Botany **61**, 2255–2270.

Shi QM, Yang X, Song L, Xue HW. 2011. Arabidopsis MSBP1 is activated by HY5 and HYH and is involved in photomorphogenesis and brassinosteroid sensitivity regulation. Molecular Plant **4**, 1092–1104.

Shikata H, Hanada K, Ushijima T, Nakashima M, Suzuki Y, Matsushita T. 2014. Phytochrome controls alternative splicing to mediate light responses in Arabidopsis. Proceedings of the National Academy of Sciences, USA **111**, 18781–18786.

Shimazaki K, Doi M, Assmann SM, Kinoshita T. 2007. Light regulation of stomatal movement. Annual Review of Plant Biology **58**, 219–247.

Sibout R, Sukumar P, Hettiarachchi C, Holm M, Muday GK, Hardtke CS. 2006. Opposite root growth phenotypes of *hy5* versus *hy5 hyh* mutants correlate with increased constitutive auxin signaling. PLoS Genetics **2**, e202.

Slessarev EW, Lin Y, Bingham NL, Johnson JE, Dai Y, Schimel JP, Chadwick OA. 2016. Water balance creates a threshold in soil pH at the global scale. Nature **540**, 567–569.

Stassen MJJ, Hsu SH, Pieterse CMJ, Stringlis IA. 2021. Coumarin communication along the microbiome–root–shoot axis. Trends in Plant Science **26**, 169–183.

Sun W, Li S, Zhang G, Fu G, Qi H, Li T. 2023. Effects of climate change and anthropogenic activities on soil pH in grassland regions on the Tibetan Plateau. Global Ecology and Conservation **45**, e02532.

Sun Y, Luo J, Feng P, Yang F, Liu Y, Liang J, Wang H, Zou Y, Ma F, Zhao T. 2022. MbHY5–MbYSL7 mediates chlorophyll synthesis and iron transport under iron deficiency in *Malus baccata*. Frontiers in Plant Science **13**, 1035233.

Tabata R, Kamiya T, Imoto S, *et al*. 2022. Systemic regulation of iron acquisition by Arabidopsis in environments with heterogeneous iron distributions. Plant and Cell Physiology **63**, 842–854.

Tanabe N, Noshi M, Mori D, Nozawa K, Tamoi M, Shigeoka S. 2019. The basic helix–loop–helix transcription factor, bHLH11 functions in the iron-uptake system in *Arabidopsis thaliana*. Journal of Plant Research **132**, 93–105.

Tissot N, Przybyla-Toscano J, Reyt G, Castel B, Duc C, Boucherez J, Gaymard F, Briat JF, Dubos C. 2014. Iron around the clock. Plant Science **224**, 112–119.

Tissot N, Robe K, Gao F, *et al*. 2019. Transcriptional integration of the responses to iron availability in Arabidopsis by the bHLH factor ILR3. New Phytologist **223**, 1433–1446.

Trofimov K, Gratz R, Ivanov R, Stahl Y, Bauer P, Brumbarova T. 2024. FER-like iron deficiency-induced transcription factor (FIT) accumulates in nuclear condensates. Journal of Cell Biology **223**, e202311048.

Trofimov K, Ivanov R, Eutebach M, Acaroglu B, Mohr I, Bauer P, Brumbarova T. 2019. Mobility and localization of the iron deficiency-induced transcription factor bHLH039 change in the presence of FIT. Plant Direct **3**, e00190.

Vert GA, Briat JF, Curie C. 2003. Dual regulation of the Arabidopsis high-affinity root iron uptake system by local and long-distance signals. Plant Physiology **132**, 796–804.

Wade J, Byrne DJ, Ballentine CJ, Drakesmith H. 2021. Temporal variation of planetary iron as a driver of evolution. Proceedings of the National Academy of Sciences, USA **118**, e2109865118.

Wan C, Ling Q. 2022. Functions of autophagy in chloroplast protein degradation and homeostasis. Frontiers in Plant Science **13**, 993215.

Wang B, Li G, Zhang W-H. 2015. Brassinosteroids are involved in Fe homeostasis in rice (*Oryza sativa* L.). Journal of Experimental Botany **66**, 2749–2761.

Wang B, Li Y, Zhang W-H. 2012. Brassinosteroids are involved in response of cucumber (*Cucumis sativus*) to iron deficiency. Annals of Botany **110**, 681–688.

Wang H, Shi H, Rajan M, *et al*. 2020. FBXL5 regulates IRP2 stability in iron homeostasis via an oxygen-responsive [2Fe2S] cluster. Molecular Cell **78**, 31–41.

Wang HY, Klatte M, Jakoby M, Baumlein H, Weisshaar B, Bauer P. 2007. Iron deficiency-mediated stress regulation of four subgroup Ib *BHLH* genes in *Arabidopsis thaliana*. Planta **226**, 897–908.

Wang N, Cui Y, Liu Y, Fan H, Du J, Huang Z, Yuan Y, Wu H, Ling HQ. 2013. Requirement and functional redundancy of Ib subgroup bHLH proteins for iron deficiency responses and uptake in *Arabidopsis thaliana*. Molecular Plant **6**, 503–513.

Wang W, Paik I, Kim J, Hou X, Sung S, Huq E. 2021. Direct phosphorylation of HY5 by SPA kinases to regulate photomorphogenesis in Arabidopsis. New Phytologist **230**, 2311–2326.

Waters BM, Armbrust LC. 2013. Optimal copper supply is required for normal plant iron deficiency responses. Plant Signaling and Behavior **8**, e26611.

Welch RM, Larue TA. 1990. Physiological characteristics of Fe accumulation in the 'Bronze' mutant of *Pisum sativum* L., cv 'Sparkle' E107 (*brz brz*). Plant Physiology **93**, 723–729.

Weller JL, Hecht V, Vander Schoor JK, Davidson SE, Ross JJ. 2009. Light regulation of gibberellin biosynthesis in pea is mediated through the COP1/HY5 pathway. The Plant Cell **21**, 800–813.

Wild M, Daviere JM, Regnault T, Sakvarelidze-Achard L, Carrera E, Lopez Diaz I, Cayrel A, Dubeaux G, Vert G, Achard P. 2016. Tissue-specific regulation of gibberellin signaling fine-tunes Arabidopsis iron-deficiency responses. Developmental Cell **37**, 190–200.

Willige BC, Yoo CY, Saldierna Guzmán JP. 2024. What is going on inside of phytochrome B photobodies? The Plant Cell **36**, 2065–2085.

Xu G, Jiang Z, Wang H, Lin R. 2019. The central circadian clock proteins CCA1 and LHY regulate iron homeostasis in Arabidopsis. Journal of Integrative Plant Biology **61**, 168–181.

Yuan Y, Wu H, Wang N, Li J, Zhao W, Du J, Wang D, Ling HQ. 2008. FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in Arabidopsis. Cell Research **18**, 385–397.

Zandi P, Yang J, Darma A, Bloem E, Xia X, Wang Y, Li Q, Schnug E. 2023. Iron plaque formation, characteristics, and its role as a barrier and/or facilitator to heavy metal uptake in hydrophyte rice (*Oryza sativa* L.). Environmental Geochemistry and Health **45**, 525–559.

Zhai Z, Gayomba SR, Jung HI, *et al*. 2014. OPT3 is a phloem-specific iron transporter that is essential for systemic iron signaling and redistribution of iron and cadmium in Arabidopsis. The Plant Cell **26**, 2249–2264.

Zhang H, Zhao X, Li J, Cai H, Deng XW, Li L. 2014. MicroRNA408 is critical for the HY5–SPL7 gene network that mediates the coordinated response to light and copper. The Plant Cell **26**, 4933–4953.

Zhang J, Liu B, Li M, Feng D, Jin H, Wang P, Liu J, Xiong F, Wang J, Wang HB. 2015. The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis. The Plant Cell **27**, 787–805.

Zhang J-C, Wang X-F, Wang X-N, Wang F-P, Ji X-L, An J-P, Yang K, Zhao Q, You C-X, Hao Y-J. 2020. Abscisic acid alleviates iron deficiency by regulating iron distribution in roots and shoots of apple. Scientia Horticulturae **262**, 109018.

Zhang N, Wei CQ, Xu DJ, Deng ZP, Zhao YC, Ai LF, Sun Y, Wang ZY, Zhang SW. 2024. Photoregulatory protein kinases fine-tune plant photomorphogenesis by directing a bifunctional phospho-code on HY5 in Arabidopsis. Developmental Cell **59**, 1737–1749.e7.

Zhu H, Wang J, Huang R, Yang Z, Fan W, Huang L, Yang J, Chen W. 2024. Epigenetic modification of a pectin methylesterase gene activates apoplastic iron reutilization in tomato roots. Plant Physiology **195**, 2339–2353.