Iron (Fe) is an essential micronutrient for all living organisms, including plants and their associated microbes (1). Iron readily donates and accepts electrons, as it can exist in multiple oxidation states, particularly its ferric (Fe$^{3+}$) and ferrous forms (Fe$^{2+}$). Therefore, iron cofactors such as heme and Fe-sulfur clusters function in all primary metabolic processes, including respiration, DNA synthesis and repair, and cell proliferation and differentiation (1). In plants, iron is also essential for chlorophyll and hormone synthesis and photosynthesis. Despite iron’s essentiality, iron overload can cause damage in any organism. This is because iron’s potent electron chemistry also makes it dangerous when it is in physiological excess. Iron acts as a catalyst with hydrogen peroxide through the Fenton reaction (Table 1), producing more dangerous reactive oxygen species (ROS), including the highly reactive hydroxide ion (2). These potent oxidizers damage lipids, proteins, and nucleic acids (3, 4). When the damage becomes too severe, the cell cannot be saved and undergoes programmed cell death (5). Thus, balance of iron levels is imperative for all organisms. Accordingly, plants tightly regulate iron uptake, localization, transport, and storage. Exciting recent progress has been achieved in understanding how plants acquire and transport biologically active iron from the soil and respond to iron-deficient environments (6, 7).

Along with the challenge of maintaining nutrient homeostasis, plants also must cope with a wide variety of pathogens and pests. Plants have evolved robust mechanisms for perception of detrimental microbes, which in turn trigger physiological responses to impede infection (8). Recent progress on iron homeostasis has been paralleled by progress in the molecular plant-microbe interaction field on understanding plant pathogen surveillance proteins, immune system signaling, and suppression of immunity by pathogen virulence proteins. These foci have provided huge payoffs in understanding how plants and microbes interact at the molecular level (9). The impact of iron on plant-pathogen interactions has been acknowledged for a considerable span of time but has received limited attention; indeed, iron homeostasis and plant immunity are typically studied in isolation from each other. One goal of this review is to highlight recent studies that connect iron and plant-pathogen interactions. We also discuss the implications of iron-immunity cross-talk on efforts to breed iron-fortified crops. We begin with primers on the regulatory networks that mediate plant immunity and plant iron homeostasis.

**Overview of plant immune responses**

Plant immune responses are activated when the plant detects signals that are diagnostic of pathogen invasion. For example, plants recognize a variety of pathogen-associated molecular patterns (PAMPs), initiating pattern-triggered immunity (PTI; Fig. 1C) (10). PAMPs are epitopes such as bacterial flagellin or fungal and oomycete cell wall components. Such epitopes are often evolutionarily conserved, allowing for detection of groups of pathogens (e.g. multiple species) that share the epitope (11). PAMPs can be detected in the apoplast by cell-surface receptors (12). Such recognition initiates cytoplasmic protein kinase cascades, Ca$^{2+}$ influx, and rapid production of ROS (13). As discussed below, iron plays a key role in ROS generation. ROS and hormone signals interact with each other to stimulate diverse molecular and cellular responses that strengthen plant
cells against pathogen attack (14). PAMP perception leads to reprogramming of thousands of genes, including genes for antimicrobial proteins (e.g. iron-sequestering defensins discussed below) and secondary metabolites with antimicrobial activity (13). At the cellular level, pathogens often require access to individual cells or host vasculature; thus, the plant produces callose to reinforce cell walls against hydrolases and pathogen secretion systems (15).
All microbial pathogens produce PAMPs and are therefore vulnerable to PTI. Accordingly, pathogen success depends on evasion of detection and/or suppression of PTI signaling (16). Many pathogens disguise themselves by secreting proteins to bind PAMPs, thereby obscuring recognition, leading to PTI (17). In a second strategy to interfere with activation of host immunity, pathogens secrete virulence proteins called effectors to inhibit critical regulatory components of host immune signaling (18). Effectors from bacteria, fungi, and oomycetes have been shown to target similar hubs in the host immune signaling network (19). The action of these effectors results in an attenuated immune response called effector-triggered susceptibility (20).

To counter the threat of effector-triggered susceptibility, plants have evolved resistance proteins (R proteins) to detect pathogen effectors and initiate effector-triggered immunity (ETI; Fig. 1D) (21). Some R proteins bind directly to the cognate effector, similar to direct binding of PAMP ligands by pattern recognition receptors. However, it is more common for R proteins to indirectly detect effectors by “guarding” immune hubs that effectors target (21). By perceiving the virulence activities of effectors (e.g. proteolytic degradation of an immune signaling protein) rather than the effectors themselves, a single R protein can protect the plant from multiple pathogens that have converged to target the same protein complex (22). ETI and PTI activate many of the same signaling pathways and defense responses. However, ETI is typically faster, its signaling is more resistant to pathogen interference, and the downstream responses are stronger than in PTI (10). Moreover, ETI is often distinguished from PTI by activation of programmed cell death (18). Effectors from bacteria, fungi, and oomycetes have been shown to target similar hubs in the host immune signaling network (19). The action of these effectors results in an attenuated immune response called effector-triggered susceptibility (20).

Plant pathogens typically follow one of three lifestyles: biotrophic, hemibiotrophic, or necrotrophic (Fig. 1C). Biotrophic pathogens can only extract nutrients from living host cells (24). Such pathogens are able to suppress host immunity, extract nutrients, and complete their life cycle without killing host cells. Contrastingly, necrotrophic pathogens kill host cells with toxins and complete their life cycle by feeding from dead or dying plant tissue (25). Hemibiotrophic pathogens begin the infection cycle with an extended period of biotrophy before killing plant tissue (26). These differing pathogen lifestyles hold important implications for predicting whether crop biofortification could impact disease resistance and will be discussed below.

Overview of plant iron metabolism

Iron is highly abundant in soil, but most iron is bound in oxidized and insoluble ferric forms, which are biologically inactive (31). Consequently, the concentration of free iron in most soils is estimated at 10^{-15} to 10^{-17} M, far below what is required for optimal plant growth, 10^{-9} to 10^{-4} M (32). Moreover, iron is considered an immobile mineral; once it is assimilated in older tissues, there is little movement to younger tissues. As a result, plants constantly live on the edge of iron starvation. To maintain homeostasis, plants use two strategies to increase iron solubility and uptake under low-iron conditions (33–36). Strategy I plants, including all non-Poaceae angiosperms, primarily acquire iron by acidifying the rhizosphere via proton ATPases to increase iron solubility and then reducing iron in the soil before direct uptake (7) (Fig. 1A). The Strategy II plants (including Poaceae, such as maize and rice) instead secrete phytosiderophores to bind ferric iron in the rhizosphere for transport back into the root (7, 36). Siderophore is Greek for “iron bearer.” Siderophores are used by diverse organisms, including microbial pathogens of animals and plants, to acquire iron and facilitate its uptake (37–39). The primary plant phytosiderophore for Strategy II uptake from the soil is the methionine derivative mugineic acid (40, 41). The Fe-mugineic acid complex is taken into the root by members of yellow stripe-like (YSL) transporter family (42). Strategy I plants also utilize small iron-binding compounds for acquisition, especially the phenolic coumarins (43). The regulatory pathways that control activation of Strategy I uptake genes have been studied intensively and have been reviewed (44). In the following paragraphs, we summarize pathways with connections to immune signaling or potential roles in iron biofortification.

Basic helix-loop-helix (bHLH) transcription factors (TFs) play a key role in regulating iron homeostasis, characterized by heterodimerization between different clades of the bHLH superfamily. Activation of the Strategy I iron uptake response in the outer cells of the root is primarily regulated by the bHLH TF Fe deficiency–induced transcription factor (FIT) (45). FIT heterodimerizes with clade Ib bHLHs, which facilitate FIT stability upon iron deficiency (46). FIT then promotes transcription of iron mobilization genes, including ferric reduction oxidase 2 (FRO2), which encodes a protein for reduction of ferric iron in the rhizosphere, and iron-regulated transporter 1 (IRT1), which encodes a transporter that delivers reduced ferric iron into the root epidermis (45, 47) (Fig. 1A). Monocots utilize the Strategy II iron uptake mechanism, which occurs through extrusion of mugineic acid family phytosiderophores, such as deoxymugineic acid, via the transporter of mugineic acid 2 (TOM2) (48). Ferric-mugineic acid family phytosiderophore chelates are subsequently transported into the root via YSL transporters and reduced for utilization after uptake. Moreover, the response of rice and other monocots to iron
deficiency differs from that of maize by utilizing aspects of both Strategy I and II for iron uptake (49).

After uptake into the root epidermis from the rhizosphere, small molecules facilitate solubility and transport of iron to the root vasculature (7, 50). In Arabidopsis, iron is chelated to nictianamine (similar to the phytosiderophore mugineic acid referenced above) and transferred from the epidermis to the vasculature. It is likely that iron is actively transported into the xylem via the metal efflux protein ferroporin (FPN1) (51) in the vasculature, where another bHLH TF, called POPEYE (PYE), heterodimerizes with homologous TFs that are members of the bHLH clade IVC, including IAA-leucine-resistant 3 (ILR3), to regulate the expression of genes involved in metal ion storage and translocation (52). In these vascular cells, iron is thought to be sensed by BRUTUS (BTS), an iron-binding E3 ligase that facilitates the degradation of ILR3 and other group IVC TFs, resulting in a decrease in iron uptake (53, 54). Adding to this complexity, another bHLH transcriptional regulator in the same clade as PYE, called upstream regulator of IRT1 (URI) (bHLH121), has been recently shown to interact with ILR3 and other bHLH clade IVC transcription factors, forming heterodimers that presumably transcriptionally up-regulate regulate FIT binding partners (55–57). Furthermore, FIT itself appears to be degraded by BTS-like proteins, BTS1L1 and BTS1L2, in the outer cells of the root (58). Thus, the interplay between BTS proteins and bHLH TF proteins plays an essential role in bridging iron-sensing and transport mechanisms between the outer and inner cell types within the root (59).

After efflux into the xylem, iron is bound to citrate before long distance transport to the shoot (60, 61) (Fig. 1A). Once ferric citrate is translocated to the shoot via the xylem, oligopeptide transporter 3 (OPT3) loads iron into phloem companion cells (62, 63), or iron is bound to nictianamine and loaded into neighboring cells, such as leaf mesophyll cells, by YSL transporters (64–66). In mesophyll cells, various transporters load iron into organelles for storage or metabolism, including vacuolar iron transporter VIT1, which loads iron into the vacuole and is important for iron storage, particularly in developing embryos (67) (Fig. 1A). In addition, permease in chloroplasts 1 (PIC1) and mitochondrial Fe uptake transporter (MIT) have been shown to load Fe into chloroplasts (68) and mitochondria, respectively (69). Plastids also contain ferritin, iron storage proteins that are up-regulated in response to ROS detection to bind iron and thereby mitigate damage from the Fenton reaction (70, 71). Ferritin has been implicated in pathogen responses, as discussed below.

### Plant pathogens use diverse strategies to steal iron from plants

Bacteria and fungi employ varied strategies for iron acquisition that are analogous to Strategy I and II described above for plants (39, 72, 73). The best-studied mechanism of iron acquisition by pathogen of plants and animals is based on secretion of high-affinity iron-binding siderophores to acquire iron from their hosts, analogous to Strategy II (74, 75). These have been reviewed extensively and insightfully for phytopathogenic bacteria and fungi (38, 76, 77); here, we summarize major themes. First, bacteria produce a large diversity of peptide and small-molecule siderophores. These were first validated as critical virulence factors for Erwinia (78) and subsequently studied in several other bacterial genera (76). In pathogenic Pseudomonas, Ralstonia, and Erwinia, the transcriptional regulation of siderophore biosynthesis is mediated by so-called hrp (hypersensitive response and pathogenicity) regulatory factors that also control expression of secreted effectors and other virulence factors (79–81). This molecular association places siderophore synthesis under the control of the pathogen virulence program. Conversely, a major regulator of bacterial iron uptake (ferric uptake regulator, Fur) also regulates genes for other virulence processes, such as toxin production or cell wall degradation, in several genera of bacterial phytopathogens (76). These regulatory connections between siderophore biosynthesis and other virulence processes indicate the importance of siderophores for bacterial success inside the plant host during infection. Phytopathogenic fungi produce nonribosomal peptide synthases that function as siderophores (82). The nonribosomal peptide synthase family is conserved among ascomycete fungi and has been experimentally validated as important for virulence (83). Contrastingly, experiments in basidiomycetes indicate that siderophores are dispensable for virulence, perhaps because of compensatory systems that are summarized in the following paragraph (84, 85).

Alternate mechanisms for iron acquisition have also been shown to be crucial for bacteria and fungi. For example, reduction and subsequent transport of ferrous iron (akin to Strategy I) has been demonstrated for fungal pathogens. Just as in the rhizosphere, iron inside the plant is more soluble and available when reduced. Fungal reductive iron assimilation is a three-part process that was first described in yeast (72). Briefly, ferric reductases are active in reducing ferric iron at the cell membrane (86). Next, the reduced iron is loaded into a protein complex of a ferroxidase (FET) and a ferric permease (FTR) (87). In Saccharomyces, ScFET3 oxidizes the iron before transport into the yeast by ScFTR1. The purpose of this oxidation step has not been clarified. Contrastingly, the FET/FTR complex has been shown to possess high affinity for iron, allowing fungi to scavenge iron at low concentrations. The purpose of ferrieductase family reductases in plant pathogenic fungi has not been investigated; however, Albarouki et al. (88) used a genetic approach to validate the importance of FET-mediated iron uptake in the maize pathogen Colletotrichum graminicola. Mutant fungi lacking the iron deficiency–induced FET protein grew as well as WT fungi on iron-sufficient media but exhibited abnormal morphology and reduced virulence on maize. The authors concluded that fungal reductive iron assimilation is important in supplying the pathogen with this critical nutrient in planta and removing potentially dangerous free iron from the environment. The corn smut fungus Ustilago maydis utilizes a high-affinity iron uptake system composed of a high-affinity iron permease and an iron multicopper oxidase. Experiments with genetic knockouts demonstrated that this system is important for virulence (84). Fungi also utilize low-affinity iron transport systems, as exemplified in rice false smut, where a probable divalent metal transporter plays a role in virulence (89).
Bacterial pathogens of mammals can “pirate” iron by specifically importing host iron-binding proteins such as transferrin, along with their bound iron (90). Some phytopathogenic bacteria utilize similar strategies. For example, Petrobacterium utilizes a “Browninan rachet” to import plant ferritin as an iron source (91). Studies such as these illuminate the complex “tug of war” for iron inside the plant and demonstrate that iron acquisition is central to pathogen success.

Mechanisms through which plants interfere with pathogen iron acquisition

Considering the importance of iron scavenging for microbial fitness and virulence, it stands to reason that hosts might have evolved mechanisms to sequester iron from pathogens during infection, resulting in a tug of war for this essential nutrient (Fig. 1B). Indeed, this “nutritional immunity” became apparent in the 1940s for animals, including humans, and is now well-established (92–94). For example, the mammalian hormone hepcidin is deployed to block iron transport and retain intracellular iron pools, particularly in macrophages (95). Concurrently, mammals utilize sidoclarin to bind pathogen siderophores to further limit their capacity for iron assimilation (96). Analogous iron sequestration strategies have not been described in plants, but some lines of evidence indicate that iron restriction is a component of plant immunity. For example, plant genes encoding iron-binding ferritins (FER) are upregulated in many plants following infection, including potato tubers during infection by the oomycete Phytophthora infestans and in Arabidopsis during infection by the bacteria D. dadantii (97, 98). Arabidopsis deficient in FER expression are more susceptible to D. dadantii. Interestingly, FER gene expression is activated by application of siderophores but is not triggered by D. dadantii deficient in siderophore production or by application of iron-bound siderophores. This suggests that FER up-regulation is triggered by the plant’s perception of iron scavenging, and not the siderophore itself. These findings indicate that Arabidopsis competes with D. dadantii for iron during infection (98). In another study, overexpression of FER in Nicotiana tabacum inhibited oxidative damage from virulence activity of necrotrophic fungal pathogens, thereby providing resistance (70). The ectopically expressed FER also prevented paraquat-induced ROS damage, suggesting the sequestration of iron to limit the Fenton reaction is effective against some pathogens. Despite these interesting results, little follow-up work has been done, especially with biotrophic pathogens, and the role of FER in plant-pathogen interactions warrants further exploration (99).

A second iron-sequestering protein with a known role in pathogen responses was also recently identified in Arabidopsis (100): Plant defensin proteins (PDF1.1, 1.2, and 1.3) are immune-regulated and inhibit the growth of fungal plant pathogens in vitro (101–104). Like FER, AtPDF1.1 binds iron at high affinity. The AtPDF1.1 gene is induced in response to pathogen invasion and is secreted into the apoplast. Arabidopsis lines in which PDF1.1 is silenced exhibit enhanced disease caused by the necrotrophic bacterium Pectobacterium carotovorum. Conversely, lines overexpressing PDF1.1 exhibit an iron deficiency response and are more resistant to P. carotovorum. The resistance to bacteria can be mitigated by exogenous application of iron, and the overexpression lines also exhibit restriction of iron in the apoplast, consistent with a role in nutritional immunity similar to that proposed for FER. However, PDF1.1 overexpression lines also exhibit a systemic activation of immune responses that appears to be mediated at least in part through a burst of ethylene production, as discussed further below. Altogether, these data indicate that PDF1.1 might have a dual role in resistance as an iron sink and as a trigger of ethylene-dependent immunity (100).

Although the above examples suggest that iron restriction is an important immune response in some pathosystems, iron does not appear to be a limiting nutrient in others. For example, iron appears to exist in the apoplast at concentrations greater than 1 μM in bacterially infected leaves, and some bacterial siderophores are dispensable for virulence (105). This enigmatic aspect is further highlighted in a study that used a transcriptomic approach, in combination with plant mutants compromised for immunity, for an unbiased survey of how bacterial gene expression is altered during growth in plants in which PTI or ETI is activated. The bacterial transcriptomic data demonstrated that 69 of 133 previously reported iron-responsive genes were reprogrammed in plants activated for PTI or ETI, compared with transcriptomes from bacteria grown in disease-susceptible plants (106). In general, bacterial genes that are repressed by iron were also repressed by plant immunity, suggesting that a component of plant immunity is to repress genes involved in iron uptake. Repressed genes included a master regulator of iron homeostasis (pyoverdine sigma factor, pvdS), and transgenic bacteria overexpressing pvdS could partially overcome the growth restriction imposed by activation of plant immunity, thereby demonstrating that reprogramming of iron-uptake genes is a relevant mechanism for suppression of bacterial growth. The mechanism through which the plant manipulates bacterial iron-related genes remains to be established but does not appear to correlate with iron concentration in the apoplast. Further exploration of this and other mechanisms through which plants interfere with pathogen iron acquisition will likely provide important insight into plant immunity along with the mechanisms and physiological relevance of iron scavenging by plant pathogens.

Iron as a tool in plant immunity

Whereas iron sequestration is proven to provide pathogen resistance in mammals and may play a similar role in plants, iron can also be a powerful weapon for the plant when wielded against pathogens. Recruitment of iron to infection sites, to exploit its redox chemistry, is a critical immune response for many plants, particularly the Poaceae. Iron-deficient maize is unable to produce ROS at Colletotrichum infection sites, and this correlates with increased susceptibility to this hemibiotrophic fungal pathogen (107). Maize recruits ferric iron to the infection site against corn powdery mildew (Blumeria graminis), and application of iron chelators decreases resistance to this pathogen (108).
The mechanistic connection between recruitment of iron and a successful immune response can be explained at least in part by iron’s capacity to produce ROS. ROS are crucial components of plant immunity with multiple roles: They can act as second messengers, transmitting perception of a pathogen to nearby cells, they can promote oxidative cross-linking of cell wall components, and they can also act as direct weapons against microbes, which lack the capacity to detoxify large quantities of free radicals (109). ROS generation begins quickly after pathogen perception and has been linked to the action of iron-containing transmembrane NADPH oxidases that generate superoxide radicals in the apoplast (109, 110). Superoxide ions act as a signal for further immune events, as precursors for additional reactive oxygen species, and as an apoplastic toxin against microbes (111). The plant cell membrane is impermeable to superoxide, but superoxide can be converted to membrane-permeable hydrogen peroxide (H₂O₂) by superoxide dismutases, some of which contain iron as a cofactor (112).

Recently, an innovative study in rice revealed a new role for iron as a central executioner of HR cell death during ETI, through a mechanism called ferroptosis (23) (Fig. 1D). Ferroptosis was first characterized in mammalian systems and is triggered by iron accumulation and concurrent loss of antioxidant protection by GSH (113). The subsequent accumulation of ROS is exacerbated by the iron-dependent Fenton reaction and leads to peroxidation of lipids, which in turn initiates cell death. Dangol et al. (23) reported multiple lines of evidence that ferroptosis is the causative mechanism for HR cell death in response to an avirulent isolate of the rice Blast pathogen *Magnaporthe oryzae*. First, Fe³⁺ ions and H₂O₂ accumulate in rice leaf sheath cells that are in proximity to *M. oryzae* infection structures, prior to HR cell death. Second, HR cell death is suppressed by treatment with an iron chelator and by chemical inhibitors of lipid peroxidation, GSH transport, and NADPH oxidase activity, all of which rendered the plant susceptible to the otherwise avirulent *M. oryzae* strain. A virulent strain of *M. oryzae* did not induce accumulation of Fe³⁺, but application of the small molecule erastin, which triggers depletion of the antioxidant GSH and induction of ferroptosis, was sufficient to induce HR-like cell death during infection by the virulent strain. These results are very interesting, because a major unresolved aspect of ETI is how HR cell death is executed following effector perception (27). A recent paper used a similar approach to provide evidence for ferroptotic cell death in *N. benthamiana*, caused by a fast-replicating mutant of tobacco mosaic virus, but it is not clear how cell death caused by the hypervirulent virus relates to HR cell death (114). Thus, it will be of great interest to determine whether ferroptosis is a trigger of HR cell death during ETI in other plant-pathogen interactions.

**Regulatory overlap between iron deficiency and pathogen stress**

One of the most important aspects of the relationship between iron homeostasis and immunity stems from evidence of regulatory connections between these processes (99, 115). A number of papers provide evidence that imposition of iron stress can also activate immune responses. One of the first clues to this connection came from experiments in which purified siderophores from bacterial phytopathogens were applied to *Arabidopsis* and shown to activate immune responses (116). The siderophore chrysobactin is required for the virulence of *Dickeya dadantii* on *Arabidopsis* and also stimulates immunity. Importantly, chrysobactin loses its immune-triggering capability when it is applied already bound to iron (117). Similarly, Aznar et al. showed that iron deficiency responses and immunity are activated in *Arabidopsis* by treatment with deferoxamine, a derivative of a bacterial siderophore, and by a synthetic siderophore, EDDHA (118). Importantly, these siderophores also fail to trigger immunity when applied in iron-bound forms (117). Thus, it seems likely that siderophores trigger plant immunity as a result of their iron-scavenging activity (i.e., via perturbation of plant iron levels) rather than molecular recognition of the siderophore itself as a PAMP. As mentioned above, a similar immune-stimulatory effect was linked to iron-scavenging activity of the *Arabidopsis* PDF1.1 protein (100). Finally, strong connections between iron deficiency responses and induced systemic resistance, triggered by beneficial rhizosphere bacteria, have been discovered recently; bacteria that stimulate ISR can also stimulate the iron deficiency response, due in part to regulatory pathways shared between these processes, which encompass ethylene, auxin, nitric oxide, and the MYB72 transcription factor. These microbes might have dual utility as biofertilizers and biopesticides. These findings have recently been reviewed elsewhere (115, 119).

How might cross-talk between iron deficiency response and immunity occur? One obvious connection lies in hormone and small-molecule signaling sectors that are employed by both responses (99, 115) (Fig. 1C). Nitric oxide (NO) and ET have been well-established as positive regulators of iron deficiency responses and as activators of immune responses (120, 121) (Fig. 1C). The immune response hormone salicylic acid is another potential connection, first noted during the experiments with application of siderophores and explored further in a notable recent paper (122), where Shen et al. imposed iron stress on *Arabidopsis* in a hydroponic system and documented that iron deficiency was accompanied by elevated levels of SA and induction of SA-responsive immune gene expression. Several *Arabidopsis* mutants deficient in SA signaling or biosynthesis were less sensitive to iron deficiency (i.e., displaying reduced chlorosis and reduced inhibition of root growth). These morphological phenotypes correlated with an increase in soluble Fe in the roots. Finally, induction of several iron homeostasis genes was disrupted in the SA mutants grown under iron-deficient conditions.

At a broad level, these observations indicate that NO, ET, and SA signaling induced by iron deficiency may regulate the expression of a range of genes through which plant immunity is controlled (123) (Fig. 1C). Recent molecular experiments have identified regulatory modules involved in iron deficiency response that also act at the intersection of hormone and ROS signaling and immune responses. For example, FIT directly regulates MYB72, a transcription factor that is induced by ET and NO, as well as microbes and iron deficiency (124, 125). In turn, MYB72 regulates systemic immunity and iron acquisition by
controlling biosynthesis of coumarins, which facilitate iron uptake from the soil. These coumarins are bioactive against fungal pathogens, while not impacting plant growth—promoting bacteria, thereby sculpting the microbiome to favor the plant (126). In addition to interacting with clade IV bHLHs and BTS-like, which controls its regulatory capacity and stability, respectively, FIT also interacts with ET signaling transcription factors EIN3 and EIL1, which promotes its stability and contributes to iron acquisition (127). Application of the ROS H₂O₂ has also been shown to stabilize FIT. However, this stabilization requires the presence of the ROS-inducible transcriptional regulator ZAT12, which is required for ROS signaling. Similar to EIN3 and EIL1, ZAT12 interacts with FIT, suggesting that ROS prevents FIT degradation through its interaction with ZAT12. However, loss of ZAT12 function leads to increased accumulation of FIT transcript abundance. The repressive effect of ZAT12 on FIT transcript abundance, and consequently Fe uptake, indicates a more complex relationship whereby oxidative stress finely tunes FIT abundance, both transcriptionally and posttranscriptionally (128, 129).

In addition to controlling the iron deficiency response by regulating FIT binding partners, ILR3 also directly represses the expression of many genes that encode proteins essential for glucosinolate synthesis in Arabidopsis (130). Glucosinolates are secondary metabolites found only in the Brassicaceae family of plants that are produced in response to wounding to protect against herbivorous insects. Consequently, PYE/ILR3 help control wounding response caused by cyst nematode infection, by modulating glucosinolate accumulation under iron deficiency (131, 132).

There is also increasing evidence that glucosinolates play a role in protection against bacterial and fungal pathogens (133), including Sclerotinia sclerotiorum, and Colletotrichum sp. (134, 135). ILR3 also regulates NEET, which transfers Fe-S clusters between proteins in intracellular organelles and the cytosol, playing a critical role in ROS accumulation (136, 137). Finally, ILR3 interacts with the Alfalfa mosaic virus coat protein and positively regulates ROS accumulation and pathogenesis-related protein 1 (PR1) mRNA, SA, and JA accumulation, providing a more direct link between iron homeostasis and disease resistance in plants (137).

BTS (Brutus), the iron-binding E3 ligases that likely posttranslationally regulate many of the transcription factors that control iron response, and the rice ortholog, OsHRZ (138), may also play a role in disease response. In Arabidopsis BTS interacts with AtVOZ1 and AtVOZ2, which are NAC transcriptional regulators that repress tolerance to abiotic stress conditions yet activate defense response to fungal and bacterial infection (139, 140). Consequently, iron conditions may impact disease response through BTS/HRZ-mediated iron sensing. These connections provide a basis to further explore the mechanisms of cross-talk between iron and immunity and ultimately to understand their adaptive significance. Another critical priority is to extend the evidence for iron-immunity cross-talk from model systems into crops, particularly those for which iron biofortification is being engineered.

Current efforts to improve iron content and bioavailability in crops

Anemia caused by iron deficiency afflicts nearly 1 billion people worldwide, with disproportionate impacts on women and children under the age of 5 (141). This disease is estimated to underpin 5% of all global disability, ranking it ninth among the Global Burden of Disease Project’s most pressing issues (142). Iron biofortification of food crops has the potential to help millions avoid anemia (143). By definition, biofortified crops contain more of a desired nutrient in edible plant tissues. Plant breeders and genetic engineers are altering the genes of agriculturally important varieties to produce food with higher iron content. Biofortification efforts have the added benefit of increasing overall plant tolerance to alkaline soils, a condition that substantially decreases soil iron bioavailability and is prevalent in over 30% of arable land worldwide. Recent achievements in iron biofortification have been reviewed recently (144, 145). Below, we summarize current progress toward this goal to set the stage for consideration of how linkages between iron and immunity might affect the capacity of biofortified crops to resist diseases.

Modern crop varieties offer decreased genetic diversity compared with that of their wild ancestors. Introgression of alleles from wild ancestors to modern crops has increased iron content, particularly in wheat (146). In addition, some important crops exhibit natural, intraspecific variation in iron content, allowing breeders to attempt iron biofortification through conventional breeding. The Consultative Group for International Agricultural Research (CGIAR) has been a global leader in biofortification and increased bioavailability through breeding (147). Leveraging available germplasm, they have achieved iron content targets in beans, millet, and other crops (148) (Rubyogo, J.-C., and Kasuga, R. (2018) Fighting iron deficiency: new improved high-iron and zinc beans released in Tanzania; http://www.pabra-africa.org/fighting-iron-deficiency-new-improved-high-iron-zinc-beans-released-tanzania/) (Accessed 04/06/20). However, in important staples, including rice, wheat, and maize, breeding efforts have been insufficient to raise iron content to desired levels. For example, the basal iron content in polished rice is typically 2 μg/g, and biofortification targets are typically 14 μg/g (149). Additionally, the use of wild germplasm to achieve biofortification targets may impose yield penalties or affect agronomic traits to preclude industrial scale adoption.

To overcome difficulties presented by biofortification through breeding, some researchers have turned to genetic engineering. With a greater understanding of plant iron metabolism, alterations in a few key genes hold the potential to greatly increase iron content and bioavailability. Vasconcelos et al. (144) organizes these efforts into four categories: increased iron storage in edible tissues, increased iron uptake and translocation, alteration of the iron deficiency response, and combinational approaches.

Overexpression of iron storage proteins has been attempted to create iron sinks in edible tissues for human consumption. For example, edible iron content in rice grains was increased as much as 3-fold by expression of soybean ferritin under an
endosperm-specific promoter (150). Expression of ferritin under constitutive promoters does not greatly alter the content in the edible tissues (151). Additional approaches have been taken to increase iron bioavailability throughout the plant through enhancement of iron uptake or iron mobilization from the roots. When the overall abundance and mobility of iron is increased, more can be transported to edible tissues. YSL2 is important for iron uptake from the soil in Strategy II plants. Nicotianamine synthase (NAS) produces nicotianamine, which acts an iron chelator and enhances transport. Overexpressing NAS or genes of the YSL family in rice, soybean, sweet potato, and wheat increases iron content, mobility, and availability (145, 152).

When soybean ferritin was expressed alone in rice, the iron accumulation was not commensurate with the increased storage capacity (153). Expression of VIT genes using endosperm-specific promoters also led to modest increases in seed iron content in rice, wheat, and cassava (145). This would suggest that general increase of iron storage in the grain is not sufficient to deliver iron to the edible tissue. Combinatorial approaches that combine increased iron mobility and iron storage have been attempted, to further increase iron content (154). The most successful examples of this have been accomplished in rice (144). Iron mobility is raised synergistically through both up-regulation of importers like YSL2 and production of iron chaperones through NAS (155). These alterations increase free iron, so when ferritin is expressed in the endosperm, the rice grains store even more iron. Trijatmiko et al. (156) use a combinatorial approach to achieve field-grown rice with 15 µg/g of bioavailable iron, reaching targets for biofortification. When Narayanan et al. (157) engineered plants to co-express both Arabidopsis IRT1 and FER1 in cassava, they observed up to an 18-fold increase in iron content, yet a 7-fold increase by overexpressing AtVIT1 alone. In other promising studies, genes for nicotianamine synthesis were co-expressed with a combination of FER and YSL, resulting in an up to 4-fold increase in iron seed content (145).

Additional promising approaches could be possible through manipulation of the regulatory components that exert major effects on the plant’s iron deficiency response. The iron deficiency response promotes additional iron uptake from the soil and mediates partitioning of iron in the plant. Overexpression of IRO2, an iron-related bHLH transcription factor, in rice improved growth on iron-deficient soils, with an incremental increase of iron in rice grains (158). The RNAi-based silencing of IRO2 diminished induction of immune-related genes following iron deficiency (PR1) (138). Overexpression of a mutated version of the Arabidopsis transcriptional regulator IDT1 (bHLH34) in tobacco caused constitutive activation of the iron deficiency response, which doubled the iron content (159). Finally, loss of function of the BTS and OsHRZ proteins in Arabidopsis and rice, respectively, increased seed iron content and increased tolerance to iron deficiency (160) yet led to increased embryo lethality in Arabidopsis (53). Such pleiotropic effects may be a consequence of biofortification efforts that involve small genetic changes in iron uptake and regulatory processes. However, our growing understanding of the regulation of iron metabolism may allow for targeted alterations that maximize effects while minimizing off-target impacts (161, 162). In exactly this context, in the next section we will discuss potential tradeoffs that manipulation of iron will have on disease resistance.

**How might biofortification impact plant disease resistance?**

Franza and Expert noted in 2012 that the interconnections between iron homeostasis and immunity hold implications for how biofortified crops could respond to pathogens (76). We agree that plants with higher levels of iron might either be more resistant or more susceptible to disease, with the outcome depending on several variables (Fig. 2). The first is the nature of the genetic modification used for iron biofortification. Strategies that are organ-specific (e.g. seed-specific overexpression of ferritin) might be less impactful than are strategies based on uptake and transport or on alteration of regulatory pathways that could have systemic effects on iron status. Another key variable is the pathogen; different pathogens infect different organs, employ different iron acquisition strategies, and have differing requirements for iron. Some pathogens are biotrophs or hemibiotrophs that are vulnerable to HR cell death (23); others are necrotrophs that induce cell death, in some cases through ROS generation (70). Plants tailor their response to various pathogens, whether sequestering iron away from the pathogen or concentrating iron at the infection site to produce ROS, consequently initiating HR cell death. Additional factors include iron availability in the soil and the composition of the microbiome, both of which could influence iron status even in crops that are biofortified.

Considering all these variables, we cannot make a single, all-encompassing prediction of whether biofortification will generally help or hurt plant resistance to disease. In some plant-pathogen interactions, iron biofortification could impede disease resistance. Most obviously, increased iron could nullify the plant’s iron sequestration mechanisms as was demonstrated for PDF1.1-dependent resistance in the interaction of Arabidopsis and Pectobacterium (100). Systemically elevated iron could also make plants more susceptible to necrotrophic pathogens that trigger ROS production and cell death. Finally, elevated iron could interfere with mechanisms through which plants utilize iron deficiency as an immune-inducing danger signal (Fig. 2). If such signals are important for disease resistance under field conditions, then elevated iron could be a major detriment to disease.

On the other hand, plant iron biofortification could be neutral in many scenarios or could even improve disease resistance. For example, overexpression of ferritin or other iron-sequestering proteins could mitigate the damage from ROS production triggered by necrotrophic fungi (demonstrated in Ref. 70) or limit availability of iron for pathogens to scavange as a nutrient. For biotrophic pathogens, systemically elevated iron could facilitate ROS generation and initiation of HR cell death, by ferroptosis or other mechanisms that would be effective in resistance (Fig. 2). This effect might be particularly beneficial for crops grown on iron-limited land, for which iron deficiency might hinder effective immunity (e.g. see Ref. 107). Excess iron could also nullify
Conclusions and future directions

Iron deficiency–related anemia in humans is a global problem that demands interventions, including the release of biofortified crops to help those in need. Staple crops biofortified for iron though breeding or genetic engineering have the potential to improve the lives of millions. Already many biofortified crops have been shown to be safe and to provide the iron needed to improve the health of those suffering from anemia (165). However, as we have outlined in this review, biofortification has the potential to influence the interactions plants have with their pathogens. Many important questions remain unanswered about the relationship between iron homeostasis and plant immunity. Through what mechanisms do plants monitor iron and utilize that signal to activate immunity? Is iron sequestration effective against diverse plant pathogens, and can it be engineered into crops? What is the role of iron in the hypersensitive response, and do other plants use ferroptosis to initiate HR cell death? How can the iron scavenging and siderophores of beneficial microbes be leveraged for crop protection?

Using model species, we seek answers to these questions using both breeding and transgenic approaches in the laboratory. In the future, it will be critically important to extend findings from model systems into crops, particularly under field conditions. We also strongly urge the incorporation of disease screening into breeding and assessment of biofortified crops to identify unintended effects on disease resistance or to identify potentially useful enhanced resistance.

Acknowledgments—We dedicate this article to Jeffery L. Dangl, for connecting and mentoring J. M. M. and T. A. L. and for nurturing the spirit of collaboration that is exemplified by this article.

Author contributions—J. H. H., T. A. L., and J. M. M. conceptualization; J. H. H. writing–original draft; J. H. H., T. A. L., and J. M. M. writing–review and editing.

Funding and additional information—This article was supported by a joint collaborative grant (to J. M. M. and T. A. L.) from the Colleges of Agriculture and Life Sciences at Virginia Tech and North Carolina State University. T. A. L. was supported by the National Science Foundation and the Biotechnology and Biological Sciences Research Council (BBSRC) (Grant NSF MCB-1517058), the United States Department of Agriculture National Institute of Food and Agriculture, the Hatch Project (Accession Number 101090), and the North Carolina State University North Carolina Agriculture and Life Sciences Research Foundation.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: ROS, reactive oxygen species; PAMP, pathogen-associated molecular pattern; PTI, pattern–triggered immunity; ETI, effector–triggered immunity; R protein, resistance protein; HR, hypersensitive response; EDDHA, ethylenediamine–di(o–hydroxyphenylacetic) acid; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; YSL, yellow stripe–like; bHLH, basic–helix–loop–helix; TF, transcription factor; FIT, Fe deficiency–induced transcription factor; NAS, nicotianamine synthase.

References
1. Camprubi, E., Jordan, S. F., Vasiliadou, R., and Lane, N. (2017) Iron catalysis at the origin of life. IUBMB Life 69, 373–381 CrossRef Medline
2. Winterbourn, C. C. (1995) Toxicity of iron and hydrogen peroxide: the Fenton reaction. Toxicol. Lett. 82–83, 969–974 CrossRef Medline
3. Becana, M., Moran, J., and Iturbe-Ormaetxe, I. (1998) Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. *Plant Cell* **20**, 137–147 CrossRef

4. Pinto, S. D. S., Souza, A. E. D., Oliva, M. A., and Pereira, E. G. (2016) Oxidative damage and photosynthetic impairment in tropical rice cultivars upon exposure to excess iron. *Sci. Agric.* **73**, 217–226 CrossRef

5. Tsai, T.-M., and Huang, H.-I. (2006) Effects of iron excess on cell viability and mitogen-activated protein kinase activation in rice roots. *Physiol. Plant.* **127**, 583–592 CrossRef

6. Samira, R., Stallmann, A., Massenburg, L. N., and Long, T. A. (2013) Iron-outing the issues: integrated approaches to understanding iron homeostasis in plants. *Plant Sci.* **210**, 259–290 CrossRef Medline

7. Kobayashi, T., Nozoye, T., and Nishizawa, N. K. (2019) Iron transport and its regulation in plants. *Free Radiol. Biomed. Med.* **133**, 11–20 CrossRef Medline

8. Cook, D. E., Mesarch, C. H., and Thomma, B. P. (2015) Understanding plant iron status as a surveillance system to detect invasion. *Annu. Rev. Phytopathol.* **53**, 541–563 CrossRef Medline

9. Michelmore, R., Coaker, G., Bart, R., Beattie, G., Bent, A., Bruce, T., Cameron, D., Daniel, J., Dinesh-Kumar, S., Edwards, R., Evans, V., den Akker, S., Gassmann, W., Greenberg, I. T., Hanley-Bowdoin, L., Harrison, R. J., et al. (2017) Foundational and translational research opportunities to improve plant health. *Mol. Plant Microbe Interact.* **30**, 515–516 CrossRef Medline

10. Katagiri, F., and Tsuda, K. (2010) Understanding the plant immune system. *Mol. Plant Microbe Interact.* **23**, 1531–1536 CrossRef Medline

11. Boller, T., and He, S. Y. (2009) Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* **324**, 742–744 CrossRef Medline

12. Gust, A. A., and Felix, G. (2014) Receptor like proteins associate with ETR1 intracellular domains and mediate mitogen-activated protein kinase activation in rice roots. *Annu. Rev. Phytopathol.* **52**, 592–617 CrossRef Medline

13. Higuchi, K., Suzuki, K., Nakanishi, H., Yamaguchi, H., Nishizawa, N.-K., Higuchi, T., and Mori, S. (1999) Cloning of nicotianamine synthase genes, novel genes directly involved in Fe (III) uptake. *Plant Physiol.* **122**, 1433–1443 CrossRef Medline

14. McPherson, S. W., and McComb, C. C. (2001) Plant plasma membrane H+-ATPases: powerhouses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 817–845 CrossRef Medline

15. Chen, Y., and Barak, P. (1982) Iron nutrition of plants in calcareous soils. *Adv. Agronomy* **35**, 217–240 CrossRef Medline

16. Chen, Y., and Barak, P. (1982) Iron nutrition of plants in calcareous soils. *Adv. Agronomy* **35**, 217–240 CrossRef Medline

17. Barak, P., and Chen, Y. (1984) Iron deficiency induced transcription factor in higher plants in mobilization and uptake of iron. *Plant Physiol. Biochem.* **22**, 385–393 CrossRef Medline

18. Takagi, S-I., Matsuura, Y., and Kakudo, M. (1978) Structure of mugineic acid, a chelator for iron in rice plants. *J. Biol. Chem.* **253**, 6848–6852 CrossRef Medline

19. Takagi, S-I., Matsuura, Y., and Kakudo, M. (1978) Structure of mugineic acid, a chelator for iron in rice plants. *J. Biol. Chem.* **253**, 6848–6852 CrossRef Medline

20. Dangol, S., Chen, Y., Hwang, B. K., and Iwa, N.-S. (2019) Iron-and reactive oxygen species-dependent ferroptotic cell death in rice-*Magnaporthe oryzae* interactions. *Plant Cell* **31**, 189–209 CrossRef Medline
JBC REVIEWS: Connections between iron homeostasis and plant immunity

46. 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 CrossRef Medline

47. Connolly, E. L., Campbell, N. H., Grotz, N., Prichard, C. L., and Guerinot, M. L. (2003) Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncofers posttranscriptional control. Plant Physiol. 133, 1102–1110 CrossRef Medline

48. Nozoye, T., Nagasaka, S., Kobayashi, T., Sato, Y., Uozumi, N., Nakanishi, H., and Nishizawa, N. K. (2015) The phytosiderophore efflux transporter TOM2 is involved in metal transport in rice. J. Biol. Chem. 290, 27688–27699 CrossRef Medline

49. Wairich, A., de Oliveira, B. H. N., Arend, E. B., Duarte, G. L., Ponte, L. R., Sperotto, R. A., Ricachensky, F. K., and Fett, J. P. (2019) The combined strategy for iron uptake is not exclusive to domesticated rice (Oryza sativa). Sci. Rep. 9, 17 CrossRef

50. Tiffin, L. O. (1970) Translocation of iron citrate and phosphorus in xylem exudate of soybean. Plant Physiol. 45, 280–283 CrossRef Medline

51. Morrissey, J., Baxter, J. R., Lee, J. L., Lü, L., Lahner, B., Grotz, N., Kaplan, J., Salt, D. E., and Guerinot, M. L. (2009) The ferroportin metal efflux proteins function in iron and cobalt homeostasis in Arabidopsis. Plant Cell 21, 3326–3338 CrossRef Medline

52. 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 CrossRef Medline

53. 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 CrossRef Medline

54. Hindi, M. N., Akmakjian, G. Z., Pivarcsi, K. L., Punshon, T., Baxter, I., Salt, D. E., and Guerinot, M. L. (2017) BRUTUS and its paralogs, BTS BTK1 and BTS BTK2, encode important negative regulators of the iron deficiency response in Arabidopsis thaliana. Metallomics 9, 876–890 CrossRef Medline

55. 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 bHLH147. Transcription factors for maintaining Fe homeostasis in Arabidopsis. Mol. Plant 13, 634–649 CrossRef Medline

56. Gao, F., Robe, K., Bettembourg, M., Navarro, N., Rofidal, V., Santoni, V., Gaymard, F., Vignols, F., Roschitztardtz, H., Izquierdo, E., and Dubos, C. (2020) The transcription factor bHLH112 interacts with bHLH1105 (ILR3) and its closest homologs to regulate iron homeostasis in Arabidopsis. Plant Cell 32, 508–524 CrossRef Medline

57. Kim, S. A., Punshon, T., Lanzirotti, A., Li, L., Alonso, J. M., Ecker, J. R., Kaplan, J., and Guerinot, M. L. (2006) Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. Science 314, 1295–1298 CrossRef Medline

58. Duy, D., Wanner, G., Meda, A. R., von Wirén, N., Soll, J., and Philipp, K. (2007) PTC1, an ancient permease in Arabidopsis chloroplasts, mediates iron transport. Plant Cell 19, 986–1006 CrossRef Medline

59. Bashir, K., Ishimaru, Y., Shimo, H., Nagasaka, S., Fujimoto, M., Takeishi, H., Tsutsuji, N., An, G., Nakanishi, H., and Nishizawa, N. K. (2011) The rice mitochondrial iron transporter is essential for plant growth. Nat. Commun. 2, 1–7 CrossRef Medline

60. Deák, M., Horváth, G. V., Davletova, S., Töörök, K., Sass, L., Vass, I., Barna, B., Király, Z., and Duditis, D. (1999) Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. Nat. Biotechnol. 17, 192–196 CrossRef Medline

61. Briat, J.-F., Duc, C., Ravet, K., and Gaymard, F. (2010) Ferritins and iron storage in plants. Biochim. Biophys. Acta 1800, 806–814 CrossRef Medline

62. Philpott, C. C. (2006) Iron uptake in fungi: a system for every source. Biochim. Biophys. Acta 1763, 636–645 CrossRef Medline

63. Sandy, M., and Butler, A. (2009) Microbial iron acquisition: marine and terrestrial siderophores. Chem. Rev. 109, 4580–4595 CrossRef Medline

64. Neillands, J. (1995) Siderophores: structure and function of microbial iron transport compounds. J. Biol. Chem. 270, 26723–26726 CrossRef Medline

65. Khan, A., Singh, P., and Srivastava, A. (2018) Synthesis, nature and utility of universal iron chelator—siderophore: a review. Microbiol. Res. 212, 103–111 CrossRef Medline

66. Franzia, T., and Expert, D. (2013) Role of iron homeostasis in the virulence of phytopathogenic bacteria: an “a la carte” menu. Mol. Plant Pathol. 14, 429–438 CrossRef Medline

67. Aznar, A., and Dellagi, A. (2015) New insights into the role of siderophores as triggers of plant immunity: what can we learn from animals? J. Exp. Bot. 66, 3001–3010 CrossRef Medline

68. Expert, D. (1999) Withholding and exchanging iron: Interactions between Erwinia spp. and their plant hosts. Annu. Rev. Phytopathol. 37, 307–334 CrossRef Medline

69. Lan, L., Deng, X., Zhou, J., and Tang, X. (2006) Genome-wide gene expression analysis of Pseudomonas syringae pv. tomato DC3000 reveals overlapping and distinct pathways regulated by hrpB and hrps. Mol. Plant Microbe Interact. 19, 976–987 CrossRef Medline

70. Occhialini, A., Cunnac, S., Reymond, N., Genin, S., and Boucher, C. (2005) Genome-wide analysis of gene expression in Ralstonia solanacearum reveals that the HrpB gene acts as a regulatory switch controlling multiple virulence pathways. Mol. Plant Microbe Interact. 18, 938–949 CrossRef Medline

71. Zhao, Y., Blumer, S. E., and Sundin, G. W. (2005) Identification of Erwinia amylovora genes induced during infection of immature pear tissue. J. Bacteriol. 187, 8088–8103 CrossRef Medline

72. Carroll, C. S., and Moore, M. M. (2018) Ironising out siderophore biosynthesis: a review of non-ribosomal peptide synthetase (nrps)-independent
siderophore synthetases. Crit. Rev. Biochem. Mol. Biol. 53, 356–381

83. Oide, S., Moedder, W., Krasnoff, S., Gibson, D., Haas, H., Yoshikoa, K., and Turgeon, B. G. (2006) NpS6, encoding a nonribosomal peptide synthetase involved in siderophore-mediated iron metabolism, is a conserved virulence determinant of plant pathogenic ascomycetes. Plant Cell 18, 2836–2853

84. Eichhorn, H., Lessing, F., Winterberg, B., Schirawski, J., Kämper, J., Müller, P., and Kahmann, R. (2006) A ferroxidation/permeation iron uptake system is required for virulence in Listeria maydis. Plant Cell 18, 3332–3345

85. Birch, L. E., and Ruddat, M. (2005) Siderophore accumulation and phytopathogenicity in Microbacterium violaceum. Fungal Genet. Biol. 42, 579–589

86. Dancis, A., Roman, D. G., Anderson, G. J., Hinnebusch, A. G., and Klausner, R. D. (1992) Ferric reductase of Saccharomyces cerevisiae: molecular characterization, role in iron uptake, and transcriptional control by iron. Proc. Natl. Acad. Sci. U. S. A. 89, 3869–3873

87. Ashworth, C., Eide, D., Van Ho, A., Bernard, P. S., Li, L., Davis-Kaplan, S., Sipe, D. M., and Kaplan, J. (1994) The Fet3 gene of S. cerevisiae encodes a multicopper oxidase required for ferrous iron uptake. Cell 76, 403–410

88. Albarouki, E., and Deising, H. B. (2013) Infection structure-specific reductive iron assimilation is required for cell wall integrity and full virulence of the maize pathogen Colletotrichum graminicola. Mol. Plant Microbe Interact. 26, 695–708

89. Zheng, M.-T., Ding, H., Huang, L., Wang, Y-h., Yu, M-N., Zheng, R., Yu, J-J., and Liu, Y-F. (2017) Low-affinity iron transport protein uvt3277 is important for pathogenesis in the rice false smut fungus Ustilaginoidea virens. Curr. Genet. 63, 131–144

90. Barber, M. F., and Eldle, N. C. (2015) Buried treasure: evolutionary perspectives on microbial iron piracy. Trends Genet. 31, 627–636

91. Gränter, R., Hay, D. J., Song, J., Wang, J., Deng, D., Dhanesakaran, V., Wilksch, J. J., Davies, M. R., Littler, D., Beckham, S. A., Henderson, I. R., Strugnell, R. A., Dougan, G., and Lithgow, T. (2018) Fusc, a member of the m16 protease family acquired by bacteria for iron piracy against plants. Proc. Natl. Acad. Sci. U. S. A. 115, E3055–E3064

92. Sels, J., Delaure, S. L., Aerts, A. M., Proost, P., Cammue, B. P., and De Bolle, M. F. (2007) Use of a pgs-mar expression system for efficient in planta production of bioactive Arabidopsis thaliana plant defensins. Transgenic Res. 16, 531–538

93. Thomma, B. P., Cammue, B. P., and Thevissen, K. (2002) Plant defensins. Planta 216, 193–202

94. Jones, A. M., and Wildermuth, M. C. (2011) The phytopathogen Pseudomonas syringae pv. tomato DC3000 has three high-affinity iron-scavenging systems functional under iron limitation conditions but dispensable for pathogenesis. J. Bacteriol. 193, 2767–2775

95. Nobori, T., Velásquez, A. C., Wu, J., Kvitko, B. H., Kremer, J. M., Wang, Y., Ho, S. Y., and Tsuda, K. (2018) Transcriptome landscape of a bacterial pathogen under plant immunity. Proc. Natl. Acad. Sci. U. S. A. 115, 1404–1409

96. Ye, F., Albarouki, E., Lingam, B. D., and von Wirén, N. (2014) An adequate Fe nutritional status of maize suppresses infection and biotrophic growth of Colletotrichum graminicola. Physiol. Plant. 151, 280–292

97. Liu, G., Greenshields, D. L., Sommya, N., Hirji, R. M., Selvaraj, G., and Wei, Y. (2007) Targeted alterations in iron homeostasis underlie plant defense responses. J. Cell Sci. 120, 596–605

98. Torres, M. A., Jones, J. D., and Daniell, G. L. (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol. 141, 373–378

99. Torres, M. A., Daniell, G. L., and Jones, J. D. (2002) Arabidopsis gpp91phox homologues AtatrbohD and AtatrbohF are required for accumulation of active oxygen intermediates in the plant defense response. Proc. Natl. Acad. Sci. U. S. A. 99, 517–522

100. McDowell, J. M., and Dangl, J. L. (2000) Signal transduction in the plant immune response. Trends Biochem. Sci. 25, 79–82

101. Marcec, M. J., Gilroy, S., Poovaiah, B. W., and Tanaka, K. (2019) Mutual interplay of Ca2+ and ROS signaling in plant immune response. Plant Cell 32, 343–354

102. Stockwell, B. R. (2018) L-5-ferroptosis death by lipid peroxidation. Free Radic. Biol. Med. 120, 57 CrossRef

103. Macharia, M., Gilroy, S., Poovaiah, B. W., and Tanaka, K. (2017) Reactive oxygen metabolism in plant immunity. Plant Physiol. 173, 356–363

104. Thomma, B. P., Cammue, B. P., and Thevissen, K. (2002) Plant defensins. Planta 216, 193–202

105. Ata, R. A., Tanaka, K., and Marcec, M. J. (2020) The role of castor oil (Ricinus communis) in plant immunity. Plant Physiol. 173, 356–363

106. Aznar, A., Chen, N. W. G., Rigual, M., Riache, N., Joseph, D., Desmaële, D., Mouillé, G., Boutet, S., Soubigou-Taconnat, L., Renou, J.-P., Thomine, S., Expert, D., and Dellagi, A. (2014) Scavenging iron: a novel mechanism during infection by manipulating the immune response and the iron status. Plant Physiol. 150, 1687–1696
Connections between iron homeostasis and plant immunity

118. Azzam, A., Patrit, O., Berger, A., and Dellagi, A. (2015) Alterations of iron distribution in Arabidopsis tissues infected by Dickeya dadantii. Mol. Plant Pathol. 16, 521–528 CrossRef Medline

119. Romera, F. I., García, M. J., Lucena, C., Martínez-Medina, A., Aparicio, M. A., Ramos, J., Alcántara, E., Angulo, M., and Pérez-Vicente, R. (2019) Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. Front. Plant Sci. 10, 287 CrossRef Medline

120. Hindt, M. N., and Guerinot, M. L. (2012) Getting a sense for signals: regulation of the plant iron deficiency response. Biochim. Biophys. Acta 1823, 1521–1530 CrossRef Medline

121. Dubois, M., Van den Broeck, L., and Inzé, D. (2018) The pivotal role of ethylene in plant growth. Trends Plant Sci. 23, 311–323 CrossRef Medline

122. Shen, C., Yang, Y., Liu, K., Zhang, L., Guo, H., Sun, T., and Wang, H. (2016) Involvement of endogenous salicylic acid in iron-deficiency responses in Arabidopsis. J. Exp. Bot. 67, 4179–4193 CrossRef Medline

123. Maurer, F., Müller, S., and Bauer, P. (2011) Suppression of Fe deficiency gene expression by jasmonate. Plant Physiol. Biochem. 49, 530–536 CrossRef Medline

124. Palmer, C. M., Hindt, M. N., Schmidt, H., Clemens, S., and Guerinot, M. L. (2010) Myb10 and myb72 are required for growth under iron-limiting conditions. PLoS Genet. 9, e1003953 CrossRef Medline

125. García, M. I., Suárez, V., Romera, F. J., Alcántara, E., and Pérez-Vicente, R. (2011) A new model involving ethylene, nitric oxide and Fe to explain the regulation of Fe-acquisition genes in strategy I plants. Plant Physiol. Biochem. 49, 537–544 CrossRef Medline

126. Stringlis, I. A., Yu, K., Feussner, K., de Jonge, R., Van Bentum, S., Van Verk, M. C., Berendsen, R. L., Bakker, P. A., Feussner, I., and Pieterse, C. M. (2018) Myb72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proc. Natl. Acad. Sci. U. S. A. 115, E5213–E5222 CrossRef Medline

127. Lingam, S., Mohrberger, J., Brumbarova, T., Potuschak, T., Fink-Straube, C., Blondet, E., Genschik, P., and 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. Plant Cell 23, 1815–1829 CrossRef Medline

128. Le, C. T. T., Brumbarova, T., Ivanov, R., Stool, C., Weber, E., Mohrberger, J., Fink-Straube, C., and Bauer, P. (2016) Zinc finger of Arabidopsis thaliana12 (ZAT12) interacts with FER-like iron deficiency-induced transcription factor (FIT) linking iron deficiency and oxidative stress responses. Plant Physiol. 170, 540–557 CrossRef Medline

129. Brumbarova, T., Le, C. T. T., Ivanov, R., and Bauer, P. (2016) Regulation of ZAT12 protein stability: the role of hydrogen peroxide. Plant Signal. Behav. 11, e113708 CrossRef Medline

130. Li, B., Gauld, A., Tang, M., Taylor-Teeples, M., Nham, N. T., Gaudinier, A., Shibata, D., McEwan, P., Okuno, T., Schulze-Lefert, P., and Takano, Y. (2010) Entry mode–dependent function of an indole glucosinolate pathway in Arabidopsis for nonhost resistance against anthracnose pathogens. Plant Cell 22, 2429–2443 CrossRef Medline

131. Neechushat, R., Conlan, A. R., Harir, Y., Song, L., Yoge, O., Eisenberg-Domovich, Y., Livnah, O., Michaeli, D., Rosen, R., Ma, V., Luo, Y., Zuris, J. A., Paddock, M. L., Cabantchik, Z. I., Jennings, P. A., et al. (2012) Characterization of Arabidopsis NRT1.1 reveals an ancient role for NRT1 protein in iron metabolism. Plant Cell 24, 2139–2154 CrossRef Medline

132. Aparicio, F., and Pallás, V. (2017) The coat protein of alfalfa mosaic virus interacts and interferes with the transcriptional activity of the bHLH transcription factor IRL3 promoting salicylic acid-dependent defence signalling response. Mol. Plant. Pathol. 18, 173–186 CrossRef Medline

133. Ogo, Y., Nakaniishi Itai, R., Nakaniishi, H., Kobayashi, T., Takahashi, M., Mori, S., and Nishizawa, N. K. (2007) The rice bHLH protein osr2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. Plant J. 51, 366–377 CrossRef Medline

134. Selote, D., Matthiási, Á., Gillikin, J. W., Sato, M. H., and Long, T. A. (2018) The E3 ligase Bratus facilitates degradation of VOZ12/2 transcription factors. Plant Cell Environ. 41, 2463–2474 CrossRef Medline

135. Nakai, Y., Nakahira, Y., Sumida, H., Takebayashi, K., Nagasa, Y., Yamasaki, K., Akiyama, M., Ohme–Takagi, M., Fujiwara, S., Shibina, T., Mitsuda, N., Fukusaki, E., Kubo, Y., and Sato, M. H. (2013) Vascular plant one-zinc-finger protein 1/2 transcription factors regulate abiotic and biotic stress responses in Arabidopsis. Plant J. 73, 761–775 CrossRef Medline

136. Kassebaum, N. J., and GBD 2013 Anaemia Collaborators, (2016) The global burden of anaemia. Hematol. Oncol. Clin. North Am. 30, 247–308 CrossRef Medline

137. Kassebaum, N. J., Jasrasaria, R., Naghavi, M., Wulf, S. K., Johns, N., Lozano, R., Regan, M., Weatherall, D., Chou, D. P., Eisele, T. P., Flaxman, S. R., Pullan, R. L., Brooker, S. J., and Murray, C. J. L. (2014) A systematic analysis of global anaemia burden from 1990 to 2010. Blood 123, 615–624 CrossRef Medline

138. Murgia, L., Arosio, P., Tarantino, D., and Soave, C. (2012) Biofortification for combating “hidden hunger” for iron. Trends Plant Sci. 17, 47–55 CrossRef Medline

139. Vasconcelos, M. W., Gruissem, W., and Bhullar, N. K. (2017) Iron biofortification in the 21st century: setting realistic targets, overcoming obstacles, and new strategies for healthy nutrition. Curr. Opin. Biotechnol. 44, 8–15 CrossRef Medline

140. Connorton, J. M., and Balk, J. (2019) Iron biofortification of staple crops: lessons and challenges in plant genetics. Plant Cell Physiol. 60, 1447–1456 CrossRef Medline

141. Kumar, A., Kapoor, P., Chanduri, V., Sharma, S., and Garg, M. (2019) Potential of Aegilops sp. for improvement of grain processing and nutritional quality in wheat (Triticum aestivum). Front. Plant Sci. 10, 308 CrossRef Medline

142. Brooks, S., and Johnson-Beebout, S. E. (2012) Contestation as continuity? Biofortification research and the CGIAR. Contested Agronomy: Agricultural Research in a Changing World (Sumberg, J., and Thompson, J., eds) Routledge, London

143. Garcia-Oliveira, A. L., Chander, S., Ortiz, R., Menkir, A., and Gedil, M. (2018) Genetic basis and breeding perspectives of grain iron and zinc enrichment in cereals. Front. Plant Sci. 9, 937 CrossRef Medline

144. Bhullar, N. K., and Gruissem, W. (2013) Nutritional enhancement of rice for human health: the contribution of biotechnology. Biotechnol. Adv. 31, 50–57 CrossRef Medline

145. Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., and Takaiwa, F. (1999) Overexpression of the barley nicotianamine synthase gene improves amino acid enrichment in cereals. Mol. Genet. Genomics 262, 429–438 CrossRef Medline

146. Drakakaki, G., Christou, P., and Stöger, E. (2000) Constitutive expression of the rice nicotianamine synthase gene improves amino acid enrichment in cereals. Mol. Genet. Genomics 262, 429–438 CrossRef Medline

147. Shiraishi, T., and Shiraishi, M. (2000) Constitutive expression of the rice nicotianamine synthase gene improves amino acid enrichment in cereals. Mol. Genet. Genomics 262, 429–438 CrossRef Medline

148. Bhullar, N. K., and Gruissem, W. (2013) Nutritional enhancement of rice for human health: the contribution of biotechnology. Biotechnol. Adv. 31, 50–57 CrossRef Medline

149. Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., and Takaiwa, F. (1999) Overexpression of the barley nicotianamine synthase gene increases iron and zinc concentrations in rice grains. Rice 2, 155–166 CrossRef
153. Qu, L. Q., Yoshihara, T., Ooyama, A., Goto, F., and Takaiwa, F. (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. *Planta* **222**, 225–233 CrossRef Medline

154. Wirth, J., Poletti, S., Aeschlimann, B., Yakandawala, N., Drosse, B., Osorio, S., Tohge, T., Fernie, A. R., Günther, D., Gruissem, W., and Sauter, C. (2009) Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnol. J.* **7**, 631–644 CrossRef Medline

155. Masuda, H., Ishimaru, Y., Aung, M. S., Kobayashi, T., Kakeishi, M., Higuchi, K., Nakani, H., and Nishizawa, N. K. (2012) Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Sci. Rep.* **2**, 543 CrossRef Medline

156. Trijateliko, K. R., Dueñas, C., Tsakirpaloglou, N., Torrizo, L., Arines, F. M., Adeva, C., Balindong, J., Oliva, N., Sapisap, M. V., Borrero, J., Rey, J., Francisco, P., Nelson, A., Nakanihi, H., Lombe, E., et al. (2016) Biofortified *indica* rice attains iron and zinc nutrition dietary targets in the field. *Sci. Rep.* **6**, 19792 CrossRef Medline

157. Narayanan, N., Beyene, G., Chauhan, R. D., Gaitán-Solís, E., Gehan, J., Butts, P., Sirintunga, D., Okwuonu, I., Woll, A., Jiménez-Aguilar, D. M., Boy, E., Grusak, M. A., Anderson, P., and Taylor, N. J. (2019) Biofortification of field-grown cassava by engineering expression of an iron transporter and ferritin. *Nat. Biotechnol.* **37**, 144–151 CrossRef Medline

158. Ogo, Y., Itai, R. N., Kobayashi, T., Aung, M. S., Nakanihi, H., and Nishizawa, N. K. (2011) Osiro2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil. *Plant Mol. Biol.* **75**, 593–605 CrossRef Medline

159. 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 CrossRef Medline

160. Kobayashi, T., Nagasaka, S., Senoura, T., Itai, R. N., Nakanihi, H., and Nishizawa, N. K. (2013) Iron-binding haemerythrin ring ubiquitin ligases regulate plant iron responses and accumulation. *Nat. Commun.* **4**, 2792 CrossRef Medline

161. Wu, H., and Ling, H.-Q. (2019) FIT-binding proteins and their functions in the regulation of Fe homeostasis. *Front. Plant Sci.* **10**, 844 CrossRef Medline

162. Urzica, E. I., Casero, D., Yamashita, H., Hsieh, S. I., Adler, L. N., Karpo-wicz, S. J., Blaby-Haas, C. E., Clarke, S. G., Loo, J. A., Pellegrini, M., and Merchant, S. S. (2012) Systems and trans-system level analysis identifies conserved iron deficiency responses in the plant lineage. *Plant Cell* **24**, 3921–3948 CrossRef Medline

163. McCall, D. S., Ervin, E. H., Shelton, C. D., Reams, N., and Askew, S. D. (2017) Influence of ferrous sulfate and its elemental components on dollar spot suppression. *Crop Sci.* **57**, 581–586 CrossRef Medline

164. Maas, F. M., van de Wetering, D. A., van Beusichem, M. L., and Bienf-a, H. F. (1988) Characterization of phloem iron and its possible role in the regulation of Fe-efficiency reactions. *Plant Physiol.* **87**, 167–171 CrossRef Medline

165. Luna, S., Lung’ahoha, M., Gahutu, J., and Haas, J. (2015) Effects of an iron-biofortification feeding trial on physical performance of Rwandan women. *Eur. J. Nutr.* **5**, 1189–1189 CrossRef