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REVIEW PAPER

Iron in plant–pathogen interactions

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Abstract

Iron is an essential element for most organisms. As an indispensable co-factor of many enzymes, iron is involved in various crucial metabolic processes that are required for the survival of plants and pathogens. Conversely, excessive iron produces highly active reactive oxygen species, which are toxic to the cells of plants and pathogens. Therefore, plants and pathogens have evolved sophisticated mechanisms to modulate iron status at a moderate level for maintaining their fitness. Over the past decades, many efforts have been made to reveal these mechanisms, and some progress has been made. In this review, we describe recent advances in understanding the roles of iron in plant–pathogen interactions and propose prospects for future studies.

Keywords: Iron, iron homeostasis, pathogen, plant immunity, plant–pathogen interaction, virulence of phytopathogenic bacteria and fungi.

Introduction

One of the most important chemical characteristics of iron is its redox property, which enables iron to function as an indispensable co-factor of many enzymes involved in various crucial metabolic processes (Johnson, 2008). This function means that iron is an essential element for most organisms on Earth. Under iron-limited conditions, the growth of microbes is significantly arrested (Braun and Hantke, 2011). In plants, iron deficiency leads to reduced chlorophyll synthesis and photosynthesis, and iron-deficient plants show symptoms of chlorosis and dramatic growth defects. Conversely, excessive iron is deleterious because free iron produces hydroxyl radicals by the Fenton reaction (Fenton, 1894). The resulting hydroxyl radicals are able to oxidize lipids, proteins, and DNA, as a consequence threatening the survival of cells (Pierre, 1999). Thus, both plants and microbes have to fine-tune their iron homeostasis to maintain fitness.

In the past decades, the role of iron in plant–pathogen interactions has drawn much attention. To date, many exciting discoveries have been made in this area, especially regarding the role of iron in pathogenic bacteria–plant and fungi–plant interactions (Expert *et al.*, 1996; Boughammoura *et al.*, 2007; Johnson, 2008; Expert *et al.*, 2012; Aznar *et al.*, 2015; Aznar and Dellagi, 2015; Verbon *et al.*, 2017). In this review, we focus on iron homeostasis in plants, phytopathogenic bacteria, and fungi, and also discuss how iron contributes to plant immunity and the virulence of phytopathogenic bacteria and fungi.

Iron homeostasis in plants

Iron uptake, transport, and storage in plants

Iron is the fourth most abundant element in the Earth's crust. Despite this abundance, its bioavailability for organisms is very low in aerobic environments, because ferric iron (Fe^{3+}), which is the major valence state, reacts with oxygen to form insoluble ferric hydroxides. To assimilate iron, plants have evolved sophisticated mechanisms for the efficient uptake of iron from the environment. In principle, two efficient iron uptake strategies, known as strategy I and strategy II, have been established in plants (Marschner *et al.*, 1986; Romheld and Marschner, 1986; Romheld, 1987). All dicots and non-graminaceous plants use the strategy I mechanism, which is also named the reduction strategy. The mechanism of iron uptake in strategy I comprises three steps: acidification, reduction, and transport. Under iron-deficiency stress, protons are pumped into the rhizosphere by the H^+-ATPase AHA2 to release ferric iron from ferric hydroxides, increasing its solubility (Santi and Schmidt, 2009). Ferric iron is then reduced to ferrous iron (Fe^{2+}) by ferric reductase oxidase (FRO) on the root cell membrane (Robinson *et al.*, 1999). Once reduced, the ferrous iron is transported into the root epidermal cells by a metal transporter, IRT1 (iron-regulated transporter 1) (Vert *et al.*, 2002) (Fig. 1A). Grasses use the strategy II mechanism, which is also named the chelation strategy, to acquire iron (Romheld and Marschner, 1986). There are two steps in the strategy II mechanism: chelation and transport. Under iron-deficiency conditions, grasses release phytosiderophores (PSs), such as mugineic acids, into the rhizosphere via a transporter, TOM1. In the rhizosphere, the PSs chelate ferric iron (Takagi *et al.*, 1984; Mori and Nishizawa, 1987; Nozoye *et al.*, 2011); the resulting ferric iron–PS complexes are then transported into the roots by Yellow Stripe (YS) transporters (Curie *et al.*, 2001) (Fig. 1A).

Once in the roots, iron is transported from roots to shoots via the xylem, mainly in the form of Fe^{3+}–citrate complexes. It is also distributed throughout the plant via phloem as iron–nicotianamine complexes (Aznar *et al.*, 2015). To avoid the generation of cytotoxic hydroxyl radicals, excessive iron is compartmented into vacuoles, apoplasts, and plastids. It was reported that iron is stored in vacuoles as ferric iron (Vigani *et al.*, 2019). In apoplasts, positively charged iron is bound to negatively charged cell wall compounds, such as hemicellulose and pectin (Lei *et al.*, 2014). In plastids, iron is mainly stored in the form of the iron-storage protein ferritin and the iron-containing cofactor heme (Nouet *et al.*, 2011).

Main transcription factors of iron homeostasis in plants

Transcription factors play an essential role in iron homeostasis in plants. To date, many transcription factors involved in iron homeostasis have been discovered in *Arabidopsis thaliana*, a model plant for studying the strategy I mechanism. Most of them belong to the basic helix-loop-helix (bHLH) family, among which FIT (bHLH29), an ortholog of the tomato FER (Ling *et al.*, 2002), is a core regulator (Yuan *et al.*, 2005; Wu and Ling, 2019). As the core component, FIT directly activates the expression of *FRO2* and *IRT1* by forming heterodimers with the Ib subgroup of bHLH transcription factors (bHLH38, bHLH39, bHLH100, and bHLH101) (Yuan *et al.*, 2008; Wang *et al.*, 2013). The stability of FIT is post-transcriptionally regulated by the formation of complexes of FIT and the IVa subgroup of bHLH transcription factors (bHLH18, bHLH19, bHLH20, and bHLH25) (Cui *et al.*, 2018). More recently, the heterodimers formed by bHLH121 with bHLH IVc transcription factors (bHLH34, bHLH104, bHLH105/ILR3, and bHLH115) were identified as the upstream regulators of FIT and the Ib subgroup of bHLH transcription factors (Gao *et al.*, 2020; Lei *et al.*, 2020). However, it remains unclear whether bHLH121 and bHLH IVc regulate FIT directly or indirectly (Gao *et al.*, 2020; Lei *et al.*, 2020).

The bHLH transcription factors also play important roles in iron homeostasis in rice, a model plant for studying the strategy II mechanism (Kobayashi and Nishizawa, 2012). The bHLH transcription factor OsIRO2 is a crucial positive regulator in iron homeostasis in rice (Ogo *et al.*, 2006), functioning in the uptake, transport, and translocation of iron by regulating the expression of some iron-deficiency-induced genes (Ogo et al., 2007, 2011). Wang *et al.* (2019) found that the nuclear localization of OsIRO2 is facilitated by OsbHLH156. Further study revealed that the expression of *OsIRO2* is directly regulated by OsPRI1, OsPRI2, and OsPRI3 (Zhang *et al.*, 2017; Zhang *et al.*, 2019). It is noteworthy that OsPRI1, OsPRI2, and OsPRI3 belong to the IVc subgroup of bHLH transcription factors (Zhang *et al.*, 2019). In contrast to OsIRO2, another bHLH transcription factor, OsIRO3, plays a negative regulatory role in iron homeostasis, as plants overexpressing *OsIRO3* displayed increased sensitivity to iron-deficiency stress (Zheng *et al.*, 2010).

Iron homeostasis in plant pathogens

Iron uptake strategies in plant pathogens

Just as in plants, iron is also required for the survival of pathogens. Once a pathogen has invaded its host plant, the host plant is the only source of iron for the pathogen. To successfully infect their host plants and multiply within them, many pathogens employ multiple iron uptake systems. Generally, the iron uptake systems of phytopathogenic bacteria and fungi can be divided into high-affinity and low-affinity uptake pathways (Chu *et al.*, 2010; Haas, 2014). The high-affinity uptake pathways play important roles in acquiring iron under iron-deficiency conditions, and include the siderophore-mediated iron uptake pathway and the reductive iron assimilation (RIA) pathway (Haas *et al.*, 2008; Haas, 2014). The siderophore-mediated iron uptake pathway is similar to the chelation-based strategy II mechanism of graminaceous plants, while the RIA pathway is similar to the reduction-based strategy I mechanism of dicots and non-graminaceous plants. The low-affinity uptake pathways are mainly adopted when iron is abundant, and include the iron-containing protein (e.g. heme, ferredoxin) uptake pathway and the ferrous iron uptake pathway. Among

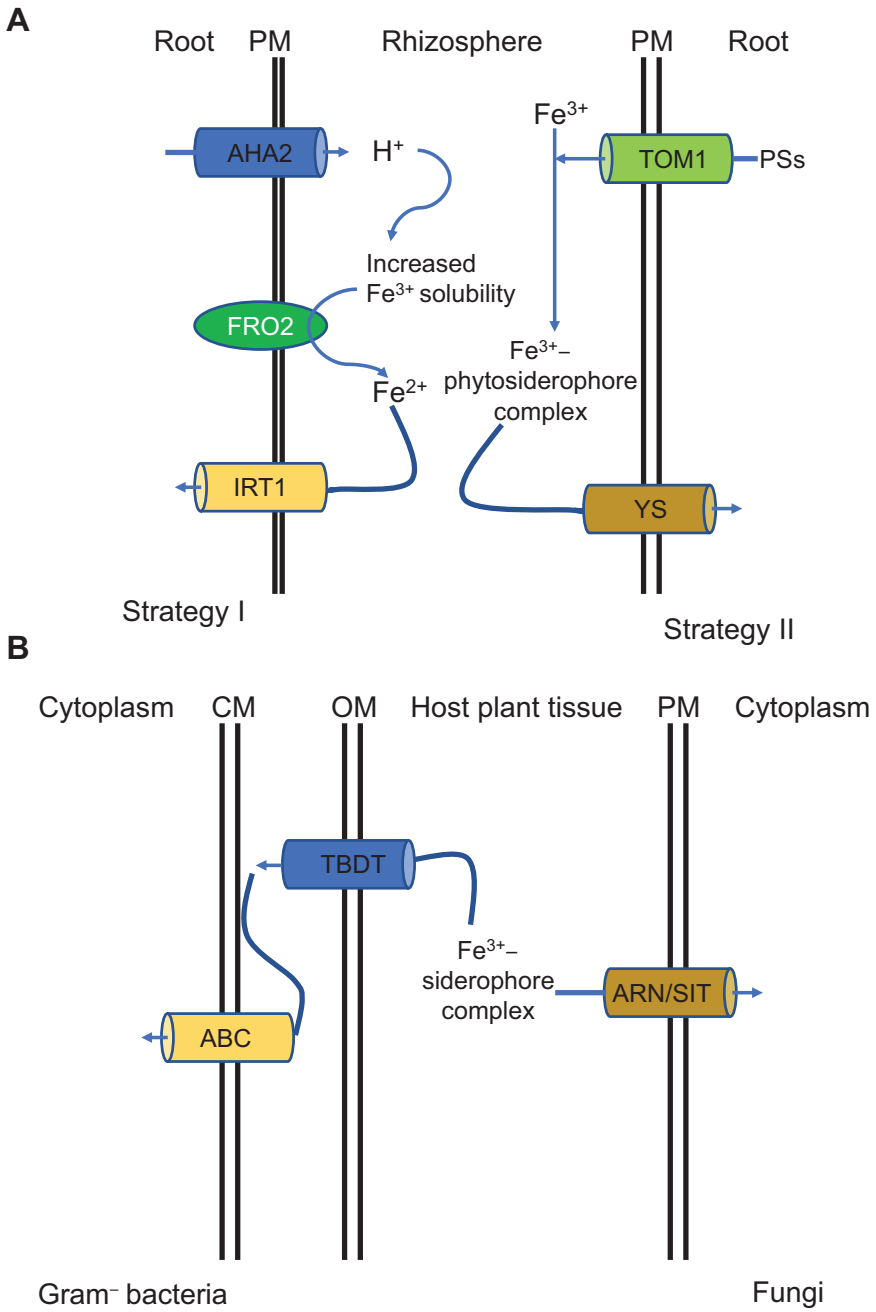

Fig. 1. Iron uptake strategies in plants and the Fe^{3+}–siderophore complex uptake system in phytopathogens. (A) Plant iron uptake strategies. In strategy I, the H^+-ATPase AHA2 located on the plasma membrane (PM) pumps protons into the rhizosphere, which increases the solubility of Fe^{3+}. FRO2 reduces Fe^{3+} to Fe^{2+}, which is then transported into the root epidermal cells by IRT1. In strategy II, phytosiderophores (PSs) (e.g. mugineic acids) are released into the rhizosphere by the transporter TOM1. After binding Fe^{3+}, the Fe^{3+}–phytosiderophore complex is transported into the roots by YS transporters. (B) The Fe^{3+}–siderophore complex uptake system in Gram-negative ($Gram^-$) bacteria and fungi. An outer-membrane-located TonB-dependent transporter (TBDT) and a cytoplasmic-membrane-located ABC transporter are required for uptake of the Fe^{3+}–siderophore uptake in $Gram^-$ bacteria. The ARN/SIT transporter is required for uptake of the Fe^{3+}–siderophore complex in fungi. CM, Cytoplasmic membrane; OM, outer membrane.

these pathways, the siderophore-mediated iron uptake pathway is the most extensively studied (Haas *et al.*, 2008).

Siderophore-mediated iron uptake pathway in plant pathogens

The siderophore-mediated iron uptake pathway is one of the most common strategies used by bacteria and fungi. Siderophores are a group of low molecular weight (500–1500 Da) ferric-iron-specific chelators produced by various bacteria and fungi under conditions of extreme iron depletion. Although siderophores show diverse structures and properties, most of them possess a peptide backbone with modified amino acid side chains, which chelate ferric iron (Chu *et al.*, 2010). Siderophores are mainly classified into three groups according to the modifications: catecholates, carboxylates, and hydroxamates (Chu *et al.*, 2010). Bacteria produce all three groups of siderophores, and catecholates are the most

common bacterial siderophores (Chu *et al*., 2010). In fungi, it was reported that hydroxamates are the main group of siderophores (Haas, 2014). Siderophores can also be divided into extracellular and intercellular forms, depending on whether they are secreted. Bacteria synthesize only the extracellular siderophores, whereas fungi can produce both extracellular and intracellular siderophores (Johnson, 2008; Haas, 2014; Khan *et al*., 2018).

More than 500 kinds of siderophores have been discovered, and half of them have been structurally characterized (Hider and Kong, 2010). Generally, a microbe produces at least one siderophore. For example, the model phytopathogenic bacterial strain *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto*), which causes bacterial speck of tomato, produces three kinds of siderophores: pyoverdine, yersiniabactin, and citrate (Jones *et al*., 2007). The plant fungal pathogen *Ustilago maydis*, which is the causal agent of smut disease in maize, generates two kinds of siderophores: ferrichrome and ferrichrome A (Eichhorn *et al*., 2006). However, some anaerobic bacteria and yeasts, such as *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *Candida albicans*, do not produce siderophores (Aznar and Dellagi, 2015).

Once ferric iron has been chelated by a siderophore, the Fe^{3+}–siderophore complex is transported into the pathogen by a siderophore uptake system. The siderophore uptake systems of bacteria and fungi are structurally different. For Gram-negative bacteria, which are surrounded by two separate membranes, the Fe^{3+}–siderophore uptake process consists of two steps. First, TonB-dependent transporters located in the outer membrane deliver the Fe^{3+}–siderophore complex from the environment into the periplasm; then, the Fe^{3+}–siderophore complex is transported into the cytoplasm via an ABC transporter located in the inner membrane (Chu *et al*., 2010) (Fig. 1B). For Gram-positive bacteria, which have one membrane, the Fe^{3+}–siderophore complex uptake is implemented in one step, which is performed through an ABC-like transport system (Chu *et al*., 2010). In fungi, Fe^{3+}–siderophore complexes are taken up by transporters belonging to the AFT1 regulon/siderophore iron transport (ARN/SIT) subfamily of the major facilitator superfamily (Heymann *et al*., 2002; Philpott, 2006) (Fig. 1B). Interestingly, this type of transporter has also been discovered in yeast, which is unable to produce siderophores. It was reported that yeast can acquire xenosiderophores (siderophores generated by other microbes) via ARN/SIT transporters (Haas, 2014). In general, each fungus has at least one ARN/SIT transporter (Johnson, 2008).

As described above, siderophores play a positive role for the survival of pathogens by promoting iron uptake. Additionally, it is noteworthy that the siderophores produced by beneficial microbes could suppress the growth of soil-borne pathogens by competing for iron, and subsequently promote plant health and growth (Leong, 1986). This phenomenon could even be used in the context of biological control.

Transcriptional regulators of iron uptake in plant pathogens

Although iron is necessary for pathogens to infect their host plants and proliferate within them, excessive iron threatens the survival of pathogens, especially in the late stage of infection. At this stage, considerable iron is released from the plant cell wall, which is degraded by enzymes secreted by the pathogen. When iron is excessive, microbes reduce their iron uptake by suppressing the production of siderophores. The protein Fur is a regulatory protein for iron uptake by bacteria. When the intracellular iron level is high, Fur, functioning as a transcriptional repressor, binds ferrous iron to form a homodimer. Then, this iron-bound homodimer binds to a common *cis*-element that exists in the promoter of many genes related to iron uptake, including the biosynthetic genes of siderophores, to suppress their expression. Thus, iron uptake is reduced (Jittawuttipoka *et al*., 2010; Troxell and Hassan, 2013). Conversely, the Fur protein loses ferrous iron and is released from its binding sites when the intracellular iron level becomes low. The biosynthesis of siderophores will then be turned on. Additionally, another novel iron-binding transcriptional regulator, XibR, has been identified from *Xanthomonas campestris* pv. *campestris* (*Xcc*). Similar to Fur, XibR acts as a transcriptional repressor of siderophore biosynthetic genes. XibR specifically binds to ferric iron but not ferrous iron. Under iron-replete conditions, the Fe^{3+}–XibR complex directly binds to the promoter of genes related to siderophore biosynthesis and suppresses their expression. Conversely, under iron-depleted conditions, free XibR functions as a transcriptional activator to activate the expression of genes related to iron storage and outer membrane receptors for enhancing iron uptake (Pandey *et al*., 2016). The dual functions of XibR indicate that the regulation of iron homeostasis in phytopathogens is complex. In fungi, two GATA-transcriptional suppressors of siderophore-biosynthetic genes, *Urbs1* and *Sre1*, have been identified in *U. maydis* (Voisard *et al*., 1993; An *et al*., 1997) and *Histoplasma capsulatum*, respectively (Chao *et al*., 2008). It is pertinent to note that ferric iron can potentially act as a co-suppressor of the biosynthetic genes of siderophores by directly binding to Sre1 protein when iron is abundant (Chao *et al*., 2008).

The role of iron in plant immunity

Iron contributes to plant immunity

Iron, as an essential nutrient, is required for the survival and virulence of plant pathogens. It is reasonable that iron-starved host plants are hostile to pathogen infection. Indeed, Kieu *et al*. (2012) showed that Arabidopsis seedlings grown under iron-deficiency conditions are more resistant to the bacterial pathogen *Dickeya dadantii* and the necrotrophic fungus *Botrytis cinerea*. However, as a critical modulator of reactive oxygen species (ROS) production, iron overaccumulation will threaten the survival of pathogens in host plants. Coinciding with this, Ye *et al*. (2014) found that an iron-sufficient status in maize suppressed the infection and biotrophic growth of *Colletotrichum graminicola*, and the most likely reason for this is that abundant iron leads to more ROS production. Furthermore, Pandey *et al*. (2018) reported that the exogenous application of iron significantly suppressed the symptoms of

Xcc disease in cabbage. Chapelle *et al.* (2015) found that excessive iron strongly induced the expression of the *ibpS* gene of *D. dadantii* during the early infection stage. The protein encoded by *ibpS* directly binds and sequesters iron to reduce toxicity and ROS production in the host plant Arabidopsis (Liu *et al.*, 2019). In sum, the iron status of host plants significantly contributes to plant immunity to pathogens, but the correlation between iron status and plant immunity is not simply linear.

Moreover, some plant secondary metabolites induced by iron deficiency have also been found to influence plant immunity. For example, the coumarins, a family of secondary metabolites secreted by roots under iron-deficient conditions, possess iron-mobilizing ability and facilitate iron uptake from alkaline soils where iron availability is low (Verbon *et al.*, 2017; Stringlis *et al.*, 2018, 2019). It has also been discovered that the coumarins have antimicrobial ability and function as defense compounds against pathogen infection in host plants (Verbon *et al.*, 2017; Beyer *et al.*, 2019). Further studies revealed that the Arabidopsis root-specific R2R3-type MYB transcription factor MYB72 is a central regulator of coumarin biosynthesis and of the systemic resistance induced by beneficial microbes (Van der Ent *et al.*, 2008; Stringlis *et al.*, 2018). Through regulating the biosynthesis of coumarins, MYB72 plays dual functions in both iron uptake responses and plant immunity (Van der Ent *et al.*, 2008; Palmer *et al.*, 2013; Stringlis *et al.*, 2018).

Iron overaccumulation and iron withholding in plant immunity

Host plants have two strategies to defend against pathogen infection through controlling the availability of iron. The first strategy is the overaccumulation of iron in the infection sites. The accumulated iron enhances the production of ROS, which physically damage pathogens. The second strategy is depriving the pathogens of iron, which significantly limits pathogen growth (Fig. 2). This iron-withholding strategy is also well documented as an effective immunity strategy in animals (Nairz *et al.*, 2010). These two strategies lead to different patterns of iron redistribution in the host plant among different pathosystems (host plant–pathogen combinations). In the wheat–*Blumeria graminis* f. sp. *tritici* (*Bgt*) and rice–*Magnaporthe oryzae* pathosystems, ferric iron is accumulated at sites of pathogen attack. The overaccumulated iron dramatically suppresses the growth of pathogens through a ROS burst produced by the Fenton reaction. Greenshields *et al.* (2007*b*) and Liu *et al.* (2007) reported the accumulation of ferric iron and a ROS burst at cell wall appositions when wheat and barley were challenged with *Bgt*, a biotrophic fungus that is the causal agent of powdery mildew. Pretreatment with the siderophore deferoxamine (DFO) revealed that iron was specifically required for the ROS burst at pathogen attack sites (Liu *et al.*, 2007). It was also found that both ferric iron and ROS (H_2O_2) focally accumulated inside and around invasive hyphae in rice leaf sheath epidermis cells inoculated with avirulent (but not virulent) *M. oryzae* (Dangol *et al.*, 2019).

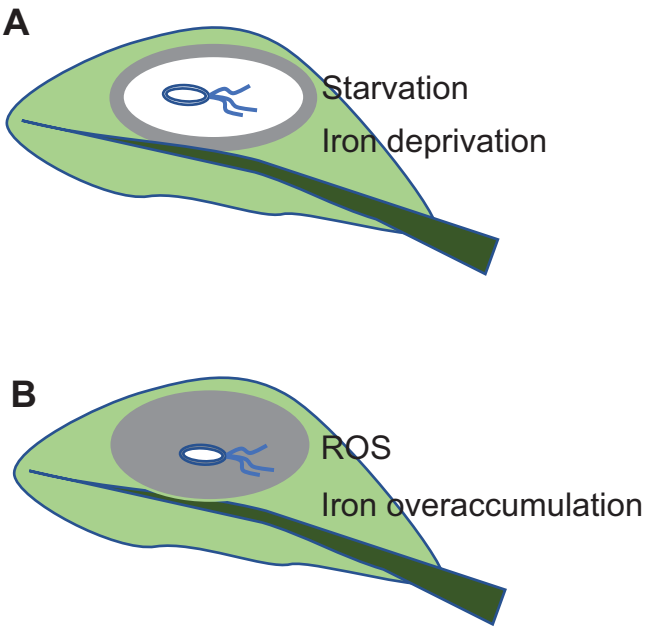

Fig. 2. Iron distribution in the host plant. (A) The host plant limits the growth of pathogens by reducing the iron content in the sites of infection. (B) The host plant suppresses pathogen infection by iron overaccumulation in the sites of infection, which enhances ROS production.

Iron redistribution and ROS accumulation led to iron- and ROS-dependent ferroptotic cell death triggered by incompatible rice–*M. oryzae* interactions, and this ferroptotic cell death could be suppressed by DFO treatment (Dangol *et al.*, 2019). It is noteworthy that actin microfilament reorganization, which contributes significantly to vesicle trafficking, is involved in the focal accumulation of H_2O_2 and ferric iron (Dangol *et al.*, 2019). Iron redistribution is not limited to the intracellular level (Greenshields *et al.*, 2007*b*; Liu *et al.*, 2007) but also occurs at the intercellular level. Liu *et al.* (2007) found that the iron concentration increased by 55% in the epidermis of wheat leaves infected with *Bgt* compared with the epidermis of healthy wheat leaves. In contrast, Aznar *et al.* (2015) reported that iron content was low in the vicinity of the infection site, whereas strong iron accumulation was found in the healthy zone around the infected tissues in the Arabidopsis–*D. dadantii* pathosystem. The low iron content was strictly correlated with bacterial cell localization in the infected tissues, indicating that Arabidopsis adopts an iron-withholding strategy to defend against infection by *D. dadantii*.

Although iron redistribution is one strategy of plant immunity, iron distribution in host plants can also be affected by siderophores secreted by pathogens. Treatment with chrysobactin or DFO, two structurally different siderophores, triggers iron accumulation in the cell wall of leaf cells in Arabidopsis (Aznar *et al.*, 2014). DFO not only modulated the distribution of iron at the cellular level, but also mediated iron uptake in Arabidopsis. Aznar *et al.* (2014) showed that infiltration of the leaf with DFO increased the iron content in roots. Thus, it would be interesting to study how host plants and pathogens synergistically or antagonistically modulate the distribution of iron in host plant cells for their benefit.

Iron homeostasis genes of host plants are involved in plant immunity

In agreement with the findings regarding iron redistribution in host plants, the expression of many iron homeostasis genes in host plants is significantly modulated by pathogens. Meanwhile, many iron homeostasis genes have been found to contribute to plant immunity. For example, the expression of *IRT1*, *FRO2*, and *AtNRAMP3* (which encodes an iron transporter located on the vacuole membrane) is up-regulated in roots by the inoculation of *D. dadantii* into Arabidopsis leaves (Segond *et al.*, 2009) (Fig. 3A). This finding is consistent with the report that the infiltration of DFO into the leaves of Arabidopsis induced the expression of *IRT1* and *FRO2* in the roots (Fig. 3A) and was associated with increased iron content in the roots (Aznar *et al.*, 2014). The studies on *D. dadantii* inoculation and DFO

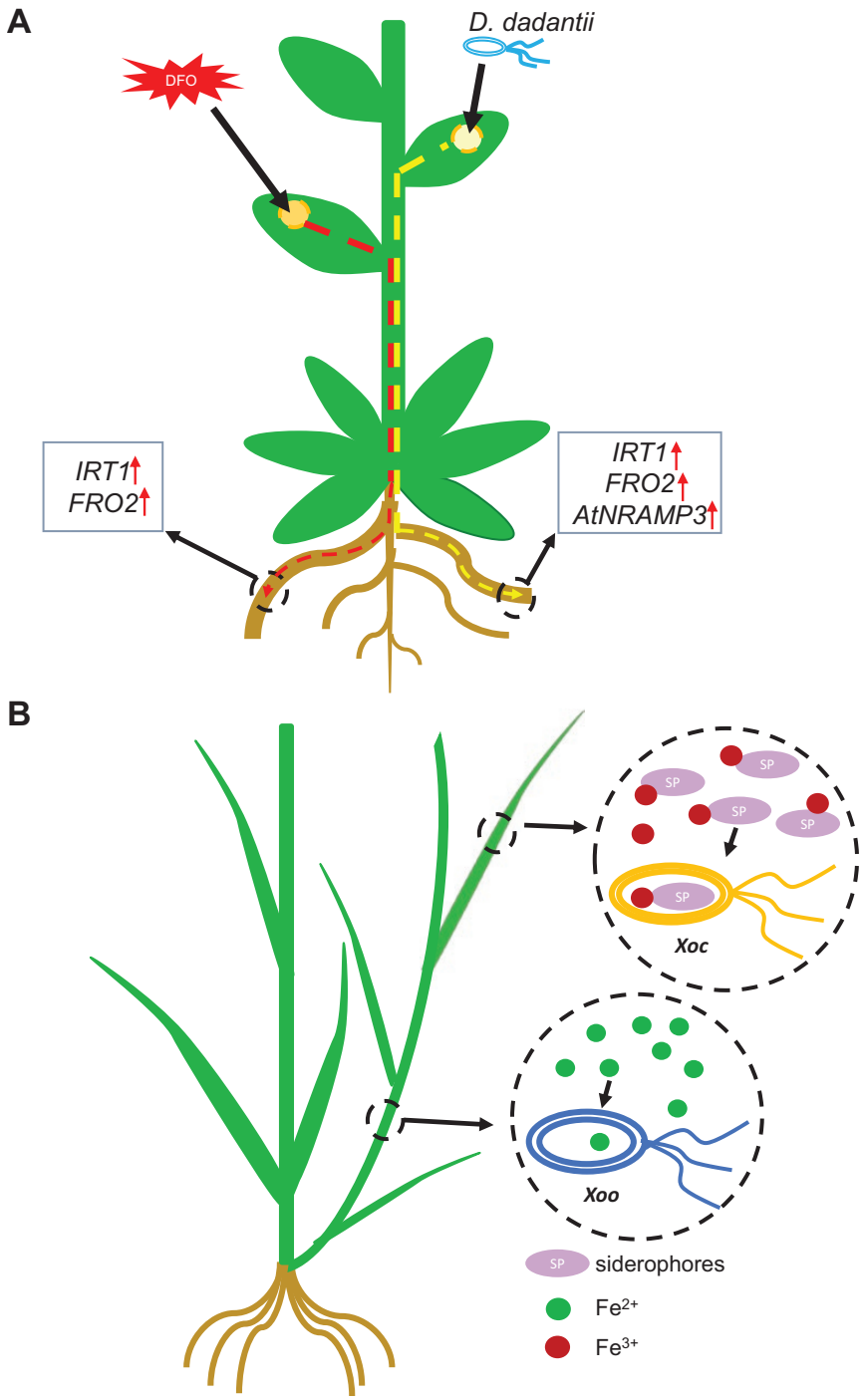

Fig. 3. Functions of iron in plant–pathogen interactions. (A) The pathogen *D. dadantii* and the siderophore DFO initiate shoot-to-root signaling reminiscent of an iron-deficiency signal in the plant. (B) The infection strategies and propagation sites of pathogens determine the iron uptake pathway. *Xoo* enters the rice leaf via hydathodes at the leaf margin and multiplies inside the xylem vessels. The full virulence of *Xoo* is dependent on direct uptake of ferrous iron, which is sufficient in the xylem. *Xoc* enters the leaf mainly through stomata and colonizes the apoplast of the parenchyma. The siderophore-mediated iron uptake pathway is required to maintain its full virulence.

treatment suggest that infection by the pathogen may activate a shoot-to-root signal reminiscent of an iron-deficiency signal in the host plant (Segond *et al.*, 2009; Aznar *et al.*, 2014) (Fig. 3A). Moreover, the expression of *AtFer1*, which encodes the iron-storage protein ferritin, is induced by *D. dadantii* infection (Segond *et al.*, 2009), and the induction of *AtFer1* expression is partially dependent on siderophore production by pathogens (Dellagi *et al.*, 2005). Interestingly, it was confirmed that *IRT1*, *NAMP3*, and *AtFer1* were involved in plant immunity (Dellagi *et al.*, 2005; Segond *et al.*, 2009; Aznar *et al.*, 2014). Pathogenicity assays showed that IRT1 and NAMP3 were positive regulators of plant immunity to *D. dadantii* (Segond *et al.*, 2009; Aznar *et al.*, 2014). Dellagi *et al.* (2005) also demonstrated that *AtFer1* positively regulates the defense response of Arabidopsis by inoculating *Atfer1* mutant plants with *D. dadantii*. In addition, the ectopic expression of the alfalfa *ferritin* gene in tobacco led to greater resistance to infection by the fungal pathogens *Alternaria alternata* and *B. cinerea* (Deak et al., 1999). Taking these findings together, it is clear that a considerable number of iron homeostasis genes significantly affect plant immunity, likely through coordinating iron homeostasis in the whole host plant to defend the plant against infection by pathogens.

Iron redistribution is likely an active immune response

Plants have a two-layer immune system to protect themselves against pathogen infection, comprising pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006). The perception of pathogen-associated molecular patterns (PAMPs) by transmembrane pattern recognition receptors triggers PTI responses (Jones and Dangl, 2006; Boutrot and Zipfel, 2017). The interception of effectors of avirulent pathogens by intracellular nucleotide-binding/leucine-rich-repeat (NLR) receptors induces the ETI, a robust resistance response that is associated with localized plant cell death, referred to as the hypersensitive response (HR) (Jones and Dangl, 2006; Cui *et al.*, 2015).

The work of Aznar *et al.* (2014) revealed that the siderophore DFO triggered typical PTI responses, such as a ROS burst and callose deposition, as well as iron redistribution. Aznar and Dellagi (2015) speculated that siderophores are likely a type of pathogen-associated molecular pattern. Thus, iron redistribution could be a type of PTI response. Iron accumulation also contributes to the ETI response. It was reported that focal accumulation of ferric iron at the infection sites in rice cells is required for HR cell death (a canonical ETI response) triggered by avirulent *M. oryzae* (Dangol *et al.*, 2019). However, virulent *M. oryzae* could suppress the iron accumulation and the HR and thus successfully colonize rice leaf cells (Dangol *et al.*, 2019). Therefore, iron redistribution in plant–pathogen interactions is likely an active immune response rather than a consequence of pathogen infection.

The bacterial iron acquisition system is impacted by plant immunity

As well as redistributing iron, host plants also employ PTI and ETI to limit the acquisition of iron by bacteria *in planta* by interfering with their iron-related gene expression, eventually inhibiting the growth of the pathogens (Nobori *et al.*, 2018). In a study using transcriptome analysis, Nobori *et al.* (2018) revealed that the siderophore biosynthetic genes of *Pto* are induced *in planta* and are suppressed by both PTI and ETI, and that more than half of the iron-responsive genes in *Pto* (69 of 133 genes) are differentially regulated by plant immunity. In line with the findings of this transcriptome study, it was found that increased content of PvdS, an extracytoplasmic function sigma factor, which positively regulates the biosynthesis of the siderophore pyoverdine, could significantly enhance the growth of bacterial pathogens in host plants by overexpression of the *pvdS* gene in the *Pto* strain AvrRpt2 (Nobori *et al.*, 2018). This provides solid evidence of host plant immunity influencing bacterial iron acquisition *in planta*. It would be interesting to study whether host plants can influence the iron acquisition system of fungal pathogens in a similar manner.

Iron uptake strategies used by pathogens when infecting host plants

It is well known that pathogens can use different iron uptake pathways for successful infection of their host plant. Many studies have found that the siderophore-mediated iron uptake pathway is essential for the full virulence of many bacterial pathogens, such as *Pantoea stewartii* subsp. *Stewartia* (the causal agent of Stewart's wilt of sweet corn) (Burbank *et al.*, 2015), *X. oryzae* pv. *oryzicola* (*Xoc*) (the causal agent of bacterial leaf streak of rice) (Rai *et al.*, 2015), and *D. dadantii* (Franza *et al.*, 2005), as well as fungal pathogens such as *Cochliobolus miyabeanus* (the causal agent of brown spot of rice) (Oide *et al.*, 2006), *Alternaria brassicicola* (the causal agent of black spot of *Brassica* species) (Oide *et al.*, 2006), *A. alternata* (the causal agent of black rot of citrus) (Chen *et al.*, 2013), and *Fusarium graminearum* (the causal agent of head blight of wheat) (Greenshields *et al.*, 2007*a*). Interestingly, both extracellular and intracellular siderophores contribute to the full virulence of the rice blast fungus *Magnaporthe grisea* and the saprophytic fungus *Aspergillus fumigatus*, based on pathogenicity assays (Hof *et al.*, 2007 2009; Haas, 2014). This discovery indicates that not only iron uptake but also intracellular siderophore-mediated iron storage contributes to the full virulence of pathogens. For the maize smut fungus *U. maydis*, the RIA pathway, but not the siderophore-mediated iron uptake pathway, is required for full virulence (Eichhorn *et al.*, 2006). Besides the high-affinity iron uptake pathways, the low-affinity iron uptake pathways are also used by many pathogens. For example, the iron-containing protein uptake pathway is employed by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum*, and the iron-containing protein ferredoxin is the iron source for *P. carotovorum* and *P. atrosepticum* (Grinter *et al.*, 2012), while the ferrous iron uptake pathway is used by *X. oryzae* pv. *oryzae* (*Xoo*), the causal agent of rice blight (Pandey and Sonti, 2010) (Fig. 3B).

It is common for pathogens to utilize more than one iron uptake pathway. Studies of mutants of pathogens deficient in different iron uptake pathways have shown that having more than one functional iron uptake pathway is essential for full

virulence. The *nps6ftr1* double mutant of the maize fungal pathogen *Cochliobolus heterostrophus*, which is unable to produce extracellular siderophores and deficient in the RIA pathway, displayed less virulence than *nps6* (an extracellular siderophore negative mutant) alone, while the *ftr1* mutant, lacking an essential component of RIA, showed wild-type-like virulence (Condon *et al*., 2014). These findings suggested that the RIA pathway acts as an important backup of siderophore-mediated iron uptake for the survival of pathogens. In wild-type strains, the role of RIA is overshadowed by the siderophore-mediated iron uptake pathway (Condon *et al*., 2014). This work suggests that systemically evaluating the pathogenesis of mutants deficient in multiple iron uptake pathways is required for clarifying the contribution of each pathway to the virulence of the pathogen.

The hemibiotrophic pathogens function as biotrophs during the early stage of infection and shift to the necrotrophic phase at the late stage of infection. The shift of parasitic lifestyle in their infectious cycle suggests that the hemibiotrophic pathogens may change their iron uptake strategies during the process of infection. Consistent with this speculation, RIA is deployed for acquiring iron during the biotrophic stage of *C. graminicola*, which causes maize leaf anthracnose and stem rot. Meanwhile, the expression of biosynthetic genes of the siderophore coprogen is drastically repressed, possibly to avoid the elicitation of host immune responses. However, the production of coprogen is required for the full virulence of *C. graminicola* in the necrotrophic stage (Albarouki and Deising, 2013; Albarouki *et al*., 2014). These findings suggest that certain pathogens may have to change their iron uptake strategies to maintain full virulence in the host plant throughout their whole life cycle. More investigations are needed to study how the phytopathogens change their iron uptake systems during the whole process of infection.

There is some evidence that the iron uptake pathway of a pathogen is determined by the infection strategy and propagation site of the pathogen in its host plant, because the forms of available iron could be different in different infection or propagation sites. *Xoo* and *Xoc*, which are closely related pathovars of *X. oryzae*, provide an example of this. *Xoo* enters the rice leaf via hydathodes at the leaf margin and multiplies inside the xylem vessels. The full virulence of *Xoo* is dependent on the ferrous iron uptake pathway rather than the siderophore-mediated iron uptake pathway, and it was found that ferrous iron is sufficient in the xylem (Pandey and Sonti, 2010). In contrast, *Xoc* enters the leaf mainly through stomata and colonizes the apoplasts of the parenchyma. The siderophore-mediated iron uptake pathway is required to maintain the full virulence of *Xoc* (Pandey and Sonti, 2010; Rai *et al*., 2015) (Fig. 3B). These findings indicate that the available forms of iron in a host plant potentially determine the iron uptake pathway of the pathogen.

Prospects

To date, many studies have revealed the overall picture of iron homeostasis in plants and phytopathogens, as well as the role of iron in plant–pathogen interactions. However, some important questions remain unanswered. First, how do the pathogens perceive the intracellular and extracellular iron status during the process of infection? Considering that the iron status of host plants may substantially determine which iron uptake pathway will be utilized by the pathogens, it will be meaningful to study how the pathogens sense the intracellular and extracellular iron status. Second, how do pathogens integrate the regulatory signaling pathways of iron homeostasis during the process of infection? As described above, a given pathogen often has more than one iron uptake system, produces more than one kind of siderophore, and harbors at least one iron transporter, which suggests that a complex regulatory signaling network controls iron homeostasis in pathogens. More effort is required to understand how pathogens integrate all of these signaling pathways. Third, how do host plants determine whether to defend against pathogens by depriving them of iron or by overaccumulating iron? To date, several lines of evidence have indicated that these two main strategies are alternatively utilized by host plants in different pathosystems. However, further study is needed to understand how the host plants choose the specific defense strategy. A better understanding of the mechanisms listed above may give a clearer picture of the roles of iron in plant–pathogen interactions and pave the way for designing new crops utilizing the properties of iron as a nutrient and in plant immunity.

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Author contributions

YL, DK, H-LW, and H-QL studied the literature and wrote the manuscript.

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