Invited review

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Essential trace metals: micronutrients with large impact

Plant iron nutrition in the long road from soil to seeds

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Highlight

The interactions between rhizosphere microbiota and plants, the seeds loading with iron represent relevant lines of research, also in wild crop relatives, for the full understanding of plant iron nutrition.

Abstract

Iron (Fe) is an essential plant micronutrient since photosynthesis, respiration, the scavenging of reactive oxygen species and many other cellular processes depend on adequate Fe levels. Nonetheless, non-complexed Fe ions can be dangerous for cells, as they can act as a pro-oxidant. Therefore, plants possess a complex homeostatic control system for safely taking up Fe from the soil, transporting it to the various cellular destinations and for its subcellular compartmentalization. At the end of the plant's life cycle, maturing seeds are loaded with the required amount of Fe for germination and early seedling establishment. In this review, we discuss recent findings on how the microbiota in the rhizosphere influence and interact with the strategies adopted by plants to take up iron from the soil. We also focus on the process of seed loading with Fe and take into account the Fe metabolism in wild crops' relatives. These aspects of plant Fe nutrition can represent promising avenues for a better comprehension of the long road of Fe from soil to seeds.

Keywords: iron, embryos, microbiota, micronutrients, plant immunity, *Pseudomonas simiae* WCS417, rhizosphere, seeds.

Abbreviations: ABA: abscisic acid; AMF: Arbuscular mycorrhizal fungi; ASC: ascorbate; BGLU42β-GLUCOSIDASE42; DMA: deoxymugeinic acid; ET: Ethylene; ETI: Effector-triggered immunity; FIT: FER-like Iron Deficiency-Induced Transcription factor; GA: Gibberellic acid; ISR: Induced systemic resistance; JA: Jasmonic acid; MAMPs: Microbe-Associated Molecular Patterns; MTI: MAMP-Triggered Immunity; NA: Nicotianamine; NO: Nitric Oxide; PAMPs: Pathogen Associated Molecular Patterns; PGPR: Plant Growth-Promoting Rhizobacteria; PGPF: Plant Growth-Promoting Fungi; PRs: Pathogenesis-related (PR) Proteins; PRRs: Pattern-Recognition Receptors; PS: Phytosiderophores; PTI: PAMP-Triggered Immunity; ROS: Reactive Oxygen Species; SA Salicylic Acid; SAR: Systemic Acquired Resistance; VOC: Volatile Organic Compound; WCS417: *Pseudomonas simiae* WCS417.

Introduction

Iron (Fe) participates in fundamental processes in plants (i.e. respiration, photosynthesis, antioxidant defenses) as well as in many biochemical pathways (e.g., hormones and secondary metabolisms) and is, therefore, an essential micronutrient (Murgia *et al.*, 2012; Kobayashi and Nishizawa, 2012; Briat *et al*., 2015; Connorton *et al*., 2017; Vigani and Murgia, 2018; Kobayashi *et al*., 2019). Iron can exert this role in various chemical forms, such as Fe-heme groups, Fe-S clusters or nitrosyl-Fe complexes (Ramirez *et al*., 2011). Nonetheless, especially when in a free non-complexed form, Fe represents a severe threat to cells due to its pro-oxidant action (Lodde *et al*., 2021). For these reasons, Fe uptake from the soil, its transport and distribution to various plant organs and tissues, its subcellular compartmentalization and seed loading with Fe are all utterly regulated processes, role of which is to ensure that plant cells receive enough Fe in the safest chemical form. Iron deficiency causes chlorosis in plants, with adverse consequences for plant health and growth, leading to yield loss (Ramirez *et al*., 2011; Vigani *et al*., 2013; Vigani and Murgia, 2018). Iron excess is also detrimental and it leads to overproduction of reactive oxygen species (ROS), damage to macromolecules, the "bronzing" symptoms, upregulation of ROS scavenging systems and downregulation of Fe uptake genes (Murgia *et al*., 2002; Arnaud *et al*., 2006; Ramirez *et al*., 2011; Aung and Masuda, 2020; Lodde *et al*., 2021). An accurate control of Fe homeostasis reduces the risk of progressive damage caused by cellular Fe excess/deficiency and it can also reduce the metabolic costs for keeping such damages under control.

Although Fe is abundant in soils, mostly present as ferric (hydro)oxides, its availability to plants is limited, due to an extremely low solubility of such oxides; for example, $Fe(OH)_3$ K_{sp} is $4x10^{-38}$ (Lindsay and Schwab, 1982; Schwertmann, 1991; Colombo *et al.,* 2014) implying that, at neutral or basic pH, the concentration of Fe(III) is extremely low. Mechanisms of plant Fe uptake from soil have been classified as either an 'acidification-reduction strategy' (Strategy I) adopted by non-graminaceous plants, or as a 'chelation strategy' (Strategy II) adopted by Graminaceae. In Strategy I plants, soil acidification by plasma membrane H^+-ATPase is followed by reduction of Fe(III) to Fe(II) and Fe(II) transport into epidermal root cells. Strategy II relies instead on the extrusion of phytosiderophores (PS) by TRANSPORTER OF MUGINEIC ACID (TOM) transporter; PS can chelate Fe(III) and the complex Fe(III)-PS is then transported into cell roots by members of the YELLOW STRIPE-LIKE (YSL) transporter family.

Such strategies are finely regulated at both transcriptional and post-transcriptional levels. As an example, the activation of Fe uptake in *Arabidopsis thaliana* plants through AHA2, FERRIC REDUCTASE OXIDASE2 (FRO2) which reduces Fe(III) to Fe(II), and IRON ROOT TRANSPORTER1 (IRT1) which transports Fe(II) into root cells, is transcriptionally regulated by the basic helix-loop-helix (bHLH) FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) (Colangelo and Guerinot, 2004; Jakoby *et al.,* 2004, Bauer *et al*., 2007). FIT activation is mediated by the ethylene-responsive transcription factors ETHYLENE INSENSITIVE3 (EIN3) and EIN3-Like1 (EIL1) (Lingam *et al*., 2011). Furthermore, other bHLH proteins (bHLH038, bHLH039, bHLH100 and bHLH101) interact with FIT and their expression increase under Fe deficiency (Wang *et al*., 2007; Wang *et al*., 2013). Most recently, the upstream regulatory role of bHLH121 (UPSTREAM REGULATOR of IRT1, URI) on the Fe homeostasis network has been unveiled and it involves the activation of various genes, among which FIT (Kim *et al*., 2019), through its interaction with bHLH105 (IRL3) (Gao *et al.,* 2020); yeast two-hybrid and chromatin immunoprecipitation (ChIP) assays, show that FIT is not a direct target of

bHLH121 (Gao *et al*., 2020).

A complex regulation of Fe uptake occurs at post-transcriptional level. IRT1 is present in endosomes/trans-Golgi network compartments (EE/TGN) and its degradation and recycling between EE/TGN and the plasma membrane are modulated by ubiquitination and monoubiquitin-dependent endocytosis (Barberon *et al*., 2011). IRT1 is ubiquitinated on the plasma membrane by the action of IRT1 DEGRADATION FACTOR1 (IDF1), a RING-type E3 ubiquitin ligase. IRT1 also mediates the transport of other metals, like Zinc (Zn), Manganese (Mn) and Cobalt (Co) which accumulate in plant tissues under Fe deficiency (Barberon *et al*., 2014; Vigani and Hanikenne, 2018). To limit IRT1-mediated metals accumulation, IDF1 facilitates its degradation through a negative feedback loop (Barberon *et al*., 2014). Such a mechanism involves other proteins, namely PHOSPHATIDYLINOSITOL-3-PHOSPHATE-BINDING PROTEIN FYVE1 and SORTING NEXIN SNX, required for IRT1 recycling in plants (Barberon *et al*., 2014; Ivanov *et al*., 2014). Due to such a recycling process, IRT1 is part of the metal sensing machinery (Dubeaux *et al*., 2018) and therefore IRT1 has been proposed to be a transceptor for metals homeostasis in plants (Cointry and Vert, 2019). Updated descriptions of both strategies and their multiple-level regulations, can be found in recent reviews (Schwarz and Bauer 2020; Gao and Dubos, 2021; Riaz and Guerinot, 2021).

The boundaries between the two Fe acquisition strategies are fading (Grillet and Schmidt, 2019); for instance, the graminaceous *Oryza sativa* (rice) adopts Strategy II, but it can also induce Fe transporters OsIRT1 and OsIRT2, which are hallmarks of Strategy I (Ishimaru *et al*., 2006; Wairich *et al*., 2019). The combination of both strategies, referred to as 'combined strategy' (CS), appears to be an adaptation to flooded soils, a situation implying oxygen O_2 depletion and a reduction of soil potential, with a consequent increase in Fe(II) concentration (Marìnes-Cunca *et al*., 2015; Wairich *et al*., 2019). Notably, Strategy I plants can also exude various compounds with iron-mobilizing properties (Palmer *et al.*, 2013; Zamioudis *et al.*, 2015; Sisó-Terraza *et al*., 2016; Stringlis *et al.*, 2019; Yu *et al.*, 2021). In particular, coumarins are secondary plant metabolites synthesized by the phenylpropanoid pathway that can promote root Fe uptake. Indeed, they are secreted by roots under Fe-deficiency, through ATP-BINDING CASSETTE G37/ PLEIOTROPIC DRUG RESISTANCE 9 (ABCG37/PDR9) transporter (Fourcroy *et al.,* 2014; Fourcroy *et al*., 2016; Ziegler *et al*., 2017) and display Fe-mobilizing, chelating and reducing properties (Schmid *et al*., 2014; Tsai and Schmidt, 2017; Ziegler *et al*., 2017; Tsai *et al*., 2018; Rajniak *et al*., 2018; Stassen *et al.*, 2021).

The transport of the various coumarin molecules is a complex and dynamic process; Robe *et al.* (2021a) investigated their pattern of accumulation in the various root cell types and they also demonstrated that coumarins can be transported from roots to aerial parts through the xylem; moreover, these authors also showed evidence that various plant species (belonging to both dicotyledons and gymnosperms) can take up coumarins from the rhizosphere (Robe *et al*., 2021a). An updated model of such a complex picture of coumarins distribution in roots and of the transcriptional regulation of their biosynthesis, is proposed by Robe *et al*. (2021b), where outstanding questions regarding the biology of coumarins are also discussed; indeed, various components are still missing from the picture, such as the identities of all the glycosyltransferases and of β-glucosidases involved, as well as the direct regulators of coumarin biosynthesis genes.

A further level of the emerging complexity in Fe acquisition from the soil is represented by the microbial communities growing in the proximity of plant roots, as they can exert beneficial or

harmful actions, favouring or inhibiting Fe uptake. Indeed, cooperation and/or competition for nutrients, including Fe, are established among the myriad of microbes living close to roots and between plants and microorganisms, in a tripartite interaction involving plants, microorganisms and Fe. Given the emerging findings on this subject, we focus first on this ''tug of war'' for iron nutrition (Herlihy *et al.*, 2020), which involves microorganisms, their colonization of plant niches and their interaction with plants. Volatile organic compounds (VOCs), emitted by plants and microorganisms, also influence the belowground interactions among plants and microorganisms and are emerging as potent regulators of these multiple interactions. VOCs-dependent microbe-plants interactions are not discussed here; readers are referred to recent publications (Zamioudis *et al.,* 2015; Delory *et al.,* 2016; Schulz-Bohm *et al.,* 2017; Garbeva and Weisskopf, 2020; Gulati *et al.,* 2020).

1) Plant Fe uptake, soil and microorganisms: the plant holobiont

Plants co-evolved with the soil microbes living in the 'rhizosphere', i.e. in the soil volume (1-3 mm in thickness), which is adherent to roots and influenced by roots' secretions. The rhizosphere is a remarkable reservoir of microbial biodiversity as it contains up to 10^{11} microorganisms per gram root (Berendsen *et al.*, 2012; Sasse *et al.*, 2018).

A dense and diversified array of microorganisms is thus present in the soil, which includes organisms belonging to Archaea, Bacteria and Eukarya domains, collectively named 'soil microbiota' (the collection of all their genomes is referred to as the 'microbiome') (Lynch and Pedersen, 2016; Trivedi *et al.*, 2020; Pascale *et al.*, 2020). The development, health and, ultimately, the phenotype of a plant is influenced by the microbial communities living in the rhizosphere and by the combined expression of both the host plant genome and its associated microbiome (Nihorimbere *et al.*, 2011; Berendsen *et al.*, 2012; Trivedi *et al.*, 2020; Pascale *et al.*, 2020). These findings led to the concept of the 'holobiont' (Vandenkoornhuyse *et al.*, 2015; Sánchez-Cañizares *et al.*, 2017; Simon *et al.*, 2019). Complex plant-microorganisms and microorganisms-microorganisms interactions occur in the rhizosphere (Trivedi *et al.*, 2020). Root exudates may shape the microbial communities living in the rhizosphere, by serving as nutrients or selective agents: the plant root microbiota are therefore different from the microbiota in the soil far away from plants, according to a phenomenon known as 'the rhizosphere effect' (Bakker *et al.*, 2013; Bakker *et al.*, 2020). In return, members of the plant microbiota may cause severe diseases, however they usually act as mutualists: plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) within the plant microbiota affect host nutrition, development and the immune system, by promoting plant growth or stimulating defense responses (Jogaiah and Abdelrahman, 2019; Verbon *et al.*, 2019; Pascale *et al.*, 2020). Beneficial root bacteria are usually a minor fraction in the rhizosphere; nonetheless, several studies have found that these bacteria may positively enhance plant yield and growth, and their pivotal role in plant life has recently been discussed (Van Loon, 2007; Ipek and Esitken, 2017; Majeed *et al.*, 2018; Compant *et al.*, 2019; do Amaral *et al.*, 2020).

Pseudomonas simiae WCS417 (syn. *Pseudomonas fluorescens* WCS417, hereafter simply WCS417) is among the PGPR studied in the greatest detail (see BOX1 for details on plant immune responses). WCS417 can promote plant growth and induce Induced Systemic Resistance (ISR) against a wide range of diseases in *A. thaliana* and other plant species (Pieterse *et al.*, 2020). WCS417 actively

colonizes roots, suppressing the local immune responses activated by its MAMPs (Stringlis *et al.*, 2018*a*) and outcompeting other microbial strains, including related sub-group members (Bakker *et al.*, 2013; Pangesti *et al.*, 2017). WCS417-ISR initiation in *A. thaliana* roots depends on plant MYB72 transcription factor (TF) and its target β-GLUCOSIDASE42 (BGLU42), which deglycosylates the coumarin scopolin (Palmer *et al.*, 2013; Zamioudis *et al.*, 2014; Zamioudis *et al.,* 2015; Verbon *et al.*, 2017; Stringlis *et al*., 2018b; Yu *et al.*, 2021). MYB72 and its paralogue MYB10 regulate the Fe deficiency regulatory cascade and are functionally redundant (Palmer *et al.*, 2013).

The phenylpropanoid pathway is often up-regulated under Fe deficiency conditions; various phenolic compounds show anti-microbial activity and can also strongly influence Fe uptake (Aznar *et al.*, 2015). Both MYB72 and MYB10 emerged as TFs required in Fe deficient roots, to adapt to low Fe levels and to regulate the biosynthesis and release of coumarins (Palmer *et al.*, 2013; Zamioudis *et al.*, 2015; Stringlis *et al.*, 2018*b*; Stringlis *et al.,* 2019; Yu *et al.*, 2021). Coumarins also emerged as important shapers of root microbiota, and they have anti-microbial potential upon pathogen infection (Voges *et al.*, 2019; Liu *et al.*, 2021).

MYB72 therefore has a dual function in both plant immunity and Fe homeostasis (Stringlis *et al.*, 2018*b*; Stringlis *et al.*, 2019). Indeed, WCS417 and Fe deficiency favour coumarins' secretion in a MYB72-dependent manner (Pieterse *et al.*, 2020 and references therein). Not only, MYB72 also represents a node of convergence between the onset of ISR by beneficial microbes, such as WCS417, and Fe deficiency response; MYB72, together with MYB10, is a direct target of bHLH121 (Gao *et al*., 2020) and its expression is itself regulated by the TFs involved in the Fe deficiency response, i.e., FIT (bHLH029) and bHLH038, under activation of the hormone Ethylene (ET). ET could therefore represent the linking molecule between ISR and Fe deficiency response, with BGLU42 and MYB72 as nodes of convergence between ISR and Fe-deficiency (Romera *et al*., 2019). Notably, WCS417 can activate an Fe deficiency response in *A. thaliana* even when Fe levels are sufficient, with consequent improved Fe nutrition and growth (Verbon *et al.*, 2019). For example, WCS417 is particularly tolerant to the antimicrobial activity of the coumarins scopolin and scopoletin, and it stimulates their secretion, to possibly favour its plant niche colonization in exchange for growth and immunity benefits for the plant (Verbon *et al.*, 2019; Yu *et al.*, 2021).

Iron-chelating compounds, the siderophores, are released not only by Strategy II graminaceous plants for Fe uptake but also by several microorganisms, in a condition of Fe deficiency (Miethke and Marahiel, 2007; Aznar *et al.*, 2014; Aznar *et al.*, 2015). Microbes indeed produce these low-molecular-weight compounds with a high affinity for Fe(III) to form Fe-siderophore complexes that are internalized by the microbial cell, thus resembling the above-mentioned plant Strategy II (Herlihy *et al.*, 2020 and references therein) and outcompeting other soil microbial strains, by making iron unavailable (Osorio Vega, 2007; Nihorimbere *et al.*, 2011). As an example, the iron-chelating fluorescent pigment pyoverdine is produced by WCS417 (Pieterse *et al.*, 2020). The density of the soil microbial population can also influence Fe uptake. Indeed, a dense microbial population causes a reduction in O_2 concentration due to its respiratory activity, accompanied by a consequent rise in carbon dioxide CO_2. Such a condition favours the conversion of Fe(III) to Fe(II) (Osorio Vega, 2007). Remarkably, WCS417 stimulates Fe deficiency responses in *A. thaliana* only when bacteria colonizing roots are in adequate amount, both under Fe-sufficient and Fe-deficient conditions (Verbon *et al.*, 2019). Moreover, the WCS417-root Fe deficiency responses are regulated by a shoot-to-root signalling system unrelated to leaf Fe status, suggesting the possible involvement of novel phloem-

mobile shoot-to-root signals and phytohormones, such as auxin (see below). Despite progress in the field, mechanisms for the induction of Fe deficiency responses by PGPR and their effect on plant Fe homeostasis are still unclear and require further investigations.

Since Fe is a limiting element in alkaline soils, it is not surprising that Fe plays a key role in the interactions between plants and non-beneficial or even pathogenic microorganisms. Fe is indeed also required by pathogens for survival: low-affinity and high-affinity strategies have been developed by phytopathogens to take up Fe from plants, and siderophores are used to sequester Fe and play a key role in microbial virulence (Franza and Expert, 2013; Aznar *et al.*, 2015; Verbon *et al.*, 2017; Liu *et al.*, 2021). Many plant genes involved in Fe homeostasis are up-regulated during pathogen attack, and biotic stresses perturb plant Fe homeostasis (Aznar *et al.*, 2015). As reported by Liu *et al.* (2021), plant Fe levels significantly contribute to plant protection against biotic stresses, as Fe withholding or Fe accumulation strategies might occur at the site of infection. The link between Fe homeostasis and plant immunity is quite complex: Fe homeostasis could contribute to the activation of ISR mediated by beneficial microbes (see Box 1). The discovery of a link between plant ISR and Fe homeostasis first occurred by the observation of a stronger induction of ISR by *Pseudomonas* spp against *Fusarium* infection in Fe deficient radish (Leeman *et al.*, 1996). Hormones may represent interesting mediators to explore, particularly ET and nitric oxide (NO), which are both implicated in Fe deficiency responses and the activation of plant immune responses (Romera *et al.*, 2019). ET levels may affect plant status in various ways, as its effects are influenced by the plant genotype, growth stage, plant organ and associated microbiota (Iqbal *et al.*, 2017; Nascimento *et al.*, 2018; Ravanbakhsh *et al.*, 2018). ET levels are affected by plant microbiota activity, and strong relations between beneficial rhizobacteria, Fe deficiency responses and ISR activation have been recently exposed (Herlihy *et al.*, 2020). As reviewed elsewhere in detail (Verbon *et al.*, 2017; Romera *et al.*, 2019), bacteria stimulating ISR could also stimulate Fe deficiency responses because of an overlap of regulatory pathways shared between the two processes, which involve several hormones such as ET, NO, auxin and the TF MYB72 (see above). Moreover, the effects of Jasmonic acid (JA) and SA on Fe nutrition as well as their roles on Fe deficiency responses have been also investigated (Kong *et al.*, 2014; Shen *et al.*, 2016; Boukari *et al.*, 2019; Kabir *et al.*, 2021). Interestingly, JA treatment increases Fe deficiency symptoms in *Arabidopsis;* indeed JA promotes FIT degradation by regulating the expression of various bHLH genes (Cui *et al*., 2018). A model describing the nodes of convergence between root Fe uptake, the rhizosphere microbiota with a highlight on WCS417, and plant immunity is presented in Figure 1.

The study of wild crops is also helpful for the elucidation of the variety of strategies activated by various plants to bypass poor soil Fe availability and the adaptation of their root apparatus together with their interaction with the rhizosphere. An example of such a field-to-lab approach was recently described in Tato *et al*., (2021): in this study, the plasticity and exudation of the roots of *Parietaria judaica* (pellitory of the wall), a wild calcicole plant growing spontaneously in an urban environment impaired in Fe availability, have been analysed; its root-associated microbiome has been also profiled. Results show that *P. judaica* roots exudate caffeoylquinic acid derivatives under calcareous conditions; they also indicate that this plant recruits beneficial soil microbes such as PGPR and phosphate solubilizers and, possibly, exclude other soil microbiota from their rhizosphere (Tato *et al*., 2021).

2) Fe transport from roots to stem and leaves and its distribution within cells

The complexity of Fe homeostasis is emerging at the soil-root interface and during Fe distribution from roots to aboveground tissues. Contents of citrate, malate and succinate are elevated in the xylem, under Fe-deficient conditions (Lopez-Millan *et al*., 2010). Iron transport in the xylem to shoots predominantly occurs as Fe (III)-citrate complexes (Durrett *et al*., 2007; Rellán-Álvarez *et al*., 2008; Rellán-Álvarez *et al*., 2010). *A. thaliana* FERRIC REDUCTASE DEFECTIVE3 (FRD3) and its rice ortholog FERRIC REDUCTASE DEFECTIVE LIKE1 (FRDL1) mediate the transport of citrate and iron to the xylem (Yokosho *et al.,* 2016). Besides FRD3, FERROPORTIN1 (FPN1) is also responsible for Fe loading into the xylem, in *A. thaliana* plants (Morrissey *et al*., 2009). Once it reaches the leaves, Fe is unloaded from the apoplastic space into the cells thanks to YSL transporters, such as AtYSL1, AtYSL2, and AtYSL3 (DiDonato *et al*., 2004; Waters *et al*., 2006).

In particular, *A. thaliana* AtYSL2 is involved in the distribution of Fe from the xylem to shoot cells (DiDonato *et al*., 2004), whereas AtYSL1 and AtYSL3 are involved in the Fe-NA translocation from senescent leaves into the inflorescences and seeds and, hence, in Fe allocation throughout the phloem. Some orthologs of such YSL transporters have also been identified in rice: OsYSL2, likely involved in the Fe(II)-Nicotianamine (NA) translocation to shoots and seeds (Ishimaru *et al*., 2010), OsYSL16 which contributes to Fe(III)-deoxymugeinic acid (DMA) allocation via the vascular bundle (Kakei *et al*., 2012) and OsYSL18 which transports Fe(III)-DMA in reproductive organs and phloem of lamina joints (Aoyama *et al*., 2009). Additionally, the OLIGOPEPTIDE TRANSPORTER 3 (OPT3) is involved in the phloematic Fe transport and it mediates Fe shoot-to-root signalling (Mondoza-Cozal *et al*., 2014; Zhai *et al*., 2014; Khan *et al*., 2018).

Fe(III) is usually reduced to Fe(II), in order to cross cellular membranes (Jain *et al.,* 2014). As already mentioned in the introduction, plants cells finely control the homeostasis of intracellular free Fe ions, to avoid the production of excess ROS, by transporting Fe(II) into vacuoles (Kim *et al.,* 2006; Sharma *et al.*, 2016) or by storing it as Fe(III) in the mineral core of the 24-mer ferritin protein cage (Briat *et al*., 2010; Lodde *et al*., 2021). *A. thaliana* possesses four ferritin isoforms (ATFER1–4) with plastidial and mitochondrial localization (Petit *et al*., 2011; Zancani *et al*., 2004; Tarantino *et al.,* 2010a; Tarantino *et al*., 2010b). The developmental and environmental regulation of *AtFer1* gene expression has been analyzed in detail, including during both natural and dark-induced senescence (Tarantino *et al*., 2003; Murgia *et al.,* 2007), as well as its dependence on the nitric oxide signalling network (Murgia *et al*., 2002; Arnaud *et al*., 2006), circadian rhythm (Duc *et al.,* 2009), phosphate homeostasis (Bournier *et al.,* 2013) and oxidative stress (Ravet *et al*., 2009a; Ravet *et al.,* 2012; Reyt *et al*., 2015).

In the cytoplasm, Fe likely forms complexes with organic acids and nicotianamine (NA) forming Fe(III)-citrate, Fe(III)-NA and Fe (II)-NA (von Wiren *et al*., 1999; Rellán-Álvarez *et al.,* 2010**;** Bashir *et al*., 2016; Flis *et al*., 2016) that would be available for uptake by chloroplasts, which are the major intracellular sink of intracellular Fe; intracellular Fe homeostasis has been recently extensively reviewed in Vigani *et al*. (2019).

Mitochondria also represent a relevant intracellular Fe sink (Vigani *et al.,* 2015). A Fe reduction-based strategy has been suggested to occur in plant mitochondria. FRO3 and FRO8 are involved in Fe(III) reduction at the mitochondrial membrane, and MITOCHONDRIAL IRON TRANSPORTERS (MIT) mediate the Fe translocation from the cytoplasm to the mitochondrial matrix (Jain and Connolly,

2013) and the knocking down of MIT impairs plant growth and metabolism in rice (Bashir *et al*., 2011; Vigani *et al*., 2016). Accordingly, *A. thaliana* MIT1 and MIT2 are involved in mitochondrial Fe import and play an essential role in cellular and mitochondrial Fe homeostasis (Jain *et al*., 2019)

Fe deficiency-induced alteration of mitochondrial functionality impacts cellular metabolism; the characterization of the mitochondrial proteome of Fe-deficient *Cucumis sativus* (cucumber) roots indeed revealed a differential protein expression of mitochondrial enzymes involved in several metabolic pathways (Vigani *et al*., 2017). Among these enzymes, formate dehydrogenase (FDH), which catalyzes the oxidation of formate ($HCOO^-$) into carbon dioxide (CO_2), deserves a particular mention; indeed, its abundance in cucumber roots depends on the Fe nutritional status of the plants (Vigani *et al*., 2017). Moreover, *Nicotiana tabacum* (tobacco) FDH overexpressing plants show altered Fe homeostasis as Fe content is reduced in their roots, stems and seeds (Murgia *et al*., 2020). A recent systems biology-oriented approach revealed that FDH might be considered a protein hub for plant nutrition (Di Silvestre *et al*., 2021).

Notably, the accumulation of FDH transcript has been documented under several unfavourable conditions, suggesting that FDH might be considered a stress-responsive enzyme in plants (Alekseeva *et al*., 2011). Most recently, evidence for FDH as part of the early response against the leaf infection by the pathogen *Xanthomonas campestris* pv *campestris* (*Xcc*) has been shown (Marzorati *et al.,* 2021): the local accumulation of formate due to a decrease in FDH expression has been proposed as a possible signal for plant defense responses to pathogen's entry through the hydathodes (Marzorati *et al*., 2021). Taken together, these findings on FDH would strongly suggest it as a possible node of the multiple interactions between plant immune responses and Fe homeostasis, as proposed in Figure 2.

3) Seed Fe loading: when, where and how

Seed development consists of various morphogenetic steps that guarantee a correct development of the embryo and of its surrounding tissues, followed by seed maturation, in which coordinated changes of its three components, i.e. the embryo, the endosperm and the surrounding maternal tissues, occur. After completion of this maturation phase, a desiccation phase guarantees the seed's entrance into a quiescent state, so that it becomes able to survive in harsh environmental conditions (Gutierrez *et al*., 2007). The final morphological extent and physiological impact of the embryo, the endosperm and the surrounding maternal tissues on the mature seed will define the final seed architecture. Although this architecture is not fixed and it is species-specific, still all the angiosperm seeds can be broadly classified according to the prevalence of the endosperm in mature seeds (endospermic, non-endospermic and perispermic seeds) (Weber *et al*., 2005; Sreenivasulu and Wobus, 2013; *Burrieza et al., 2014*)).

Seeds maturation involves quite a dense and complex interaction of signalling networks aimed at guaranteeing the loading of seeds with all the necessary nutrients (essential elements, carbohydrates, storage proteins, oils) for germination and early stages of seedlings growth (Eggert and von Wiren, 2017). Also, a balance between concentrations of abscisic acid (ABA) favouring dormancy, and gibberellic acid (GA) favouring germination is achieved (Srivastava *et al.,* 2021). ROS concentration in mature seeds is also of paramount importance and should fall within a specific

range, known as the "oxidative window" and representing the ROS range enabling imbibed seeds to germinate (Gutierrez *et al.,* 2007; Bailly *et al.,* 2019; Lodde *et al.,* 2021).

Species-specific plant architecture, including final seed architecture, will influence the mechanisms by which Fe is transferred from mother plant tissues into developing seeds; such mechanisms are quite complex as they involve the senescence of older plant parts with mobilization of Fe, its transport and distribution to the developing seed. In other words, the questions of "when" Fe is transferred from plants to seeds, "where" Fe is compartmentalized inside maturing seeds as well as inside fully matured ones and "how" Fe is mobilized from germinating seeds are of paramount relevance. Such knowledge can indeed not only allow an in-depth understanding of the physiology of Fe-loading in seeds, but it can also actively assist the various experimental approaches (breeding, genome editing, selection of relevant traits from wild crops' relatives) for the production of Fe-dense crops, so important for human nutrition (Waters and Sankaran, 2011; Murgia *et al*., 2012; Murgia *et al.*, 2013). The link between plant senescence and Fe homeostasis and its mobilization has been established by different research groups (Tarantino *et al.,* 2003; Murgia *et al*., 2007; Shi *et al*., 2012; Mari *et al*., 2019; Murgia *et al*., 2020); in particular, the timing of the onset of senescence, regulated by NAC transcription factors, influences the final seed Fe content (Ricachenevsky *et al*., 2013) (see also the following section on wild crop relatives and NAM-B1). Notably, autophagy is an essential process for Fe remobilization from vegetative parts of the plants to seeds and indeed *A. thaliana* plants defective in autophagy retain more Fe in vegetative parts and show a reduced seed Fe content (Pottier *et al.,* 2014; Pottier *et al.,* 2019).

Iron loading into seed tissues, and in particular the roles of the NA, YSL genes and OPT3, has been thoroughly reviewed by Mari *et al.* (2020) to which readers are referred. Most recently, the role of a *A. thaliana* YABBY transcription factor INNER NO OUTER (INO) as regulator of Fe loading into developing seeds has been elucidated: INO binds indeed to the promoter of NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN1 gene (*NRAMP1*), thus inhibiting its expression; such INO inhibitory effect on NRAMP1 avoids accumulation of excess Fe into developing seeds, which would therefore protecting developing embryos from Fe toxicity caused by oxidative damage (Sun et al., 2021).

An important step of the seed loading with Fe is the reduction of Fe (III) into Fe(II), which is required for Fe transport into the embryos of dicots plants, such as pea and *A. thaliana* (Grillet *et al*., 2014a; Mari *et al*., 2020). Ascorbate (ASC) is responsible for such a reductive step (Grillet *et al.,* 2014a) whereas no FRO2 homologs are apparently involved (Mari *et al*., 2020). Recently, an ASC transporter, named ATDX25, from the Multidrug And Toxic compound Extrusion (MATE) family, has been identified (Hoang *et al*., 2021). ATDX25 is expressed in flowers, seeds and seedlings and it acts as an ASC effluxer from vacuoles; its activity contributes to the Fe remobilization during germination (Hoang *et al*., 2021); to date, no evidence of ATDX25 involvement in seed Fe loading has been shown.

In a search for novel genes involved in metal uptake and transport, our research group focused on the circadian-regulated cytochrome P450 superfamily CYP82C4 gene, with expression dependent on Fe availability (Murgia *et al.,* 2011). Later, CYP82C4 enzymatic activity was clarified, being responsible for the conversion of the fraxetin into sideretin (Ranjak *et al*., 2018). CYP82C4 gene expression appeared strongly correlated with genes involved in the early Fe deficiency response, but

also with other genes not known to be involved in Fe homeostasis, at the time of publication. Among this second group of genes was At2g46750, which contains RY and IDE1-like motifs within its 1500 bp promoter region and it encodes a protein annotated as FAD-containing protein, at the time (Murgia *et al*., 2011). At2g46750 is currently annotated as L -GULONO-1,4-LACTONE OXIDASE 2 (GULLO2) and its expression, in roots of *A. thaliana* Fe deficient plants, is pH-dependent; its expression ratio at pH 7.0/pH 5.5 is quite low (0.04) (Tsai and Schmidt, 2020). GULLO2 attracted our attention because it oxidizes L-gulono-1,4 γ-lactone into ASC, with H_2O_2 as by-product; current knowledge is, however, that plants synthesize ASC, *in vivo*, solely via the D-mannose/L-galactose pathway and that the oxidation of L-gulono-1,4 γ-lactone by GULLO as the last ASC biosynthetic step, occurs in animal cells and not in plants (Smirnoff, 2018). To date, no physiological role has been assigned, *in vivo*, to GULLO2 (Eggers *et al.,* 2021; Maruta *et al*., 2010); however, unpublished findings obtained so far in our research group, by using two independent *A. thaliana gullo2* mutants would suggest its involvement in the reduction step of Fe(III) into Fe(II) in developing embryos (Murgia and coworkers, unpublished observations).

Much has been recently learned on Fe distributions within developing and mature seeds, thanks to the established Fe Perls staining technique amplified with DAB/H_2O_2 stain (Roschzttardtz *et al*., 2009; Brumbarova and Ivanov, 2014), but also thanks to the mapping of elemental distribution in embryos and seeds by micro X-ray fluorescence (μ XRF) or by Energy Dispersive X-ray Spectroscopy (EDS), in which radiation is provided by an electron beam (Lott and West, 2001; Takahashi *et al.,* 2009; Fittschen *et al.,* 2017; Cardoso *et al.,* 2018). The model plant *A. thaliana* has been one of the first species for which the seeds have been analysed by μXRF; seed analysis of wt and *vit1* mutants showed that Fe accumulates in the proximity of the provasculature (Kim e*t al.,* 2006). Further studies demonstrated that Fe is localized in the vacuoles of the endodermal cells surrounding the provascular cambium of *A. thaliana* mature seeds (Roschzttardtz *et al*., 2009; Grillet *et al*., 2014b). Analysis of Fe distribution in maturing seeds of other Brassicaceae species, i.e., *Brassica napus*, *Nasturtium officinale*, *Lepidium sativum*, *Camelina sativa*, and *Brassica oleracea*, revealed that Fe is localized in the nuclei of integument, endosperm and embryo cells and that it gradually moves to surrounding structures around the nucleus to be finally loaded into vacuoles of endodermal cells surrounding the provasculature (Ibeas *et al*., 2017). However, Fe distribution in *Vasconcellea pubescens* (mountain papaya), also in the Brassicales order, showed that seed Fe is also retrieved in cortex cells (Ibeas *et al*., 2019*).* To establish whether the pattern of Fe localization in *V. pubescens* is an exception in Brassicales or rather an indication of a wider pattern than that restricted to vacuoles of endodermal cells, more species of Eudicots belonging to orders other than Brassicales were investigated; such analyses suggested that Fe distribution has indeed a wide pattern of distribution and it can even be species- and genotype-dependent (Cvitanich et *al.,* 2010; Grillet *et al*., 2014b; Ibeas *et al*., 2019). However, another study highlighted how, among the Rosids, seed Fe is detected primarily in the endodermal cell layer of the embryo (Eroglu *et al*., 2019). In monocots, Fe is predominantly in the scutellum, aleurone layer (Ozturk *et al.,* 2009; Lemmens *et al*., 2018).Fe can also be stored in ferritin within amyloplasts, in seeds of some *Phaseolus* species (Cvitanich *et al*., 2010; Grillet *et al.*, 2014b; Moore *et al.*, 2018). These observations are important, as legume seeds have high iron content, compared to species/families such as those of *A. thaliana* itself (Murgia *et al*, 2012). During the maturation stage, seeds are loaded, besides micro and macroelements, with reserve proteins, carbohydrates and triglycerides, the proportion of which strongly depends on the species; the signalling pathways for protein and oil loading involve master regulators such as LEAFY

COTYLEDON1 (LEC1), LEC2, FUSCA3 (FUS3), ABSCISIC ACID INSENSITIVE3 (ABI3); on the other side, the lack of transcription factors orchestrating micronutrient loading, including Fe, is puzzling (Roschzttardtz *et al.*, 2020). In fact, Sun *et al*. (2020) proposed a role for ET in seed Fe loading through the signalling cascade involving its master transcriptional regulator EIN3 acting on the transcription factor ERF95, which in turn would bind to the GCC-boxes of the *AtFer1* promoter; however, ATFER2 is the only ferritin protein isoform detected so far in *A.thaliana* seeds, whereas ATFER1 has never been detected in *A.thaliana* seeds (Ravet *et al*., 2009a; Ravet *et al*., 2009b; Briat *et al*., 2010).Hence, the proposed signalling cascade ethylene-EIN3-ERF95-FER1 (Sun *et al.,* 2020) requires further experimental investigation.

Loading of Fe into the vacuole by the VIT1 transporter, during seed maturation, as well as its mobilization from the vacuole by NRAMP3 and NRAMP4 transporters, during germination, are important steps in the post-germinative phase; *A. thaliana* mutants *vit1* or *nramp3nramp4*, compromised in these two key Fe transport steps, indeed show severe chlorosis and growth arrest, under Fe deficiency (Lanquar *et al*., 2005; Kim *et al*., 2006; Bastow *et al*., 2018). Rice transporters OsVIT1 and OsVIT2 share, with *A. thaliana*, their role of Fe transport into the vacuole: notably, *osvit1* and *osvit2* mutants accumulate more Fe in seeds than the corresponding wt, whereas in the same mutants a reduction of Fe content is observed in flag leaves, thus confirming that VIT transporters regulate Fe trafficking between leaves and seeds (Zhang *et al*., 2012). Unfortunately, *osvit1* and *osvit2* also accumulate the toxic heavy metal cadmium Cd(II) when grown in contaminated paddy soils (Zhang et *al.,* 2012), thus preventing direct use of vit1 vit2 mutations for Fe biofortification approaches.

4) Wild crops relatives in the amelioration of seed Fe loading

The domestication of wild plant species, implying anatomical, morphological and genetic changes due to cultivation and selection in an anthropic environment (Charmet, 2011; Pigna and Morandini, 2017), started independently in various regions around the world between 10000 and 2000 years b.C. Domestication of wheat, which occurred in the Fertile Crescent, caused a reduction of Fe content, in both average value and variability; wild wheat, such as *Triticum boeoticum*, *Triticum urartu*, and *Triticum dicoccoides* (wild emmer), as well as primitive wheat *T. monococcum*, show a higher Fe content in grains in comparison with modern cultivars of *Triticum durum* (durum wheat) and *Triticum estivum* (bread wheat) (Cakmak *et al*., 2000; Cakmak *et al*., 2004); in particular, wheat ancestor *Triticum dicoccoides* has a higher Fe content, compared to cultivated *Triticum aestivum* and *durum* (Cakmak *et al*., 2000; Cakmak *et al*., 2004). Indeed, 825 wild emmer accessions all originating from different regions of the Fertile Crescent regions were tested for Fe content, as well as for zinc (Zn), phosphorus (P), magnesium (Mg) and sulphur (S), and they showed an average Fe content of 46 μg g^{-1} with concentrations ranging from 15 to 109 μg g^{-1}. Interestingly, these lines also showed higher Zn content but no difference in the other tested nutrients (Cakmak *et al*., 2004; Peng *et al*., 2007).

An interesting study took advantage of the archived samples from the Broadbalk Experiment, known as the “oldest continuous agricultural experiment in the world”, to investigate mineral content in the *Triticum aestivum* varieties cultivated in the last 160 years, starting from 1845 (Fan *et al*., 2008): Zn,

Fe, copper (Cu) and Mg contents remained stable until 1960, when short-straw cultivars were introduced. Since that introduction, a stable decline of mineral contents was observed, accompanied by an increase in seeds yield and harvest index, which are significant factors for the observed reduction in seeds' mineral content. Authors indeed suggest that mineral nutrition of the plants, among which Fe itself, would not catch-up with the improved redistribution of photosynthates in the short-straw cultivars (Fan *et al*., 2008). In another remarkable study, the Fe content archaeological maize kernels collected in Tarakapà Region (Atacama Desert, South America) and spanning 2000 years (according to radiocarbon dating) was analysed; obtained results show a decline in Fe content associated with the shift from ancient to more recent maize varieties.

Wild crops relatives can represent a still poorly explored reservoir of genes potentially useful for the improvement of various traits, among which are the elemental content of seeds (Charmet, 2011), including Fe itself. An illuminating example of the genetic potential of wild crops relatives is represented by the single genetic locus Gpc-B1 with Mendelian segregation and associated with a higher protein, Zn and Fe content; the Recombinant Chromosome Substitution Lines (RSLs) carrying the *T. dicoccoides* Gpc-B1 allele indeed showed a 18% higher Fe concentration, when compared with lines carrying the alternative durum allele (Distelfeld *et al*., 2007). Thanks to map-based cloning, the gene coding for a such locus has been identified and it encodes an NAC-transcription factor named NAB-B1 (Uauy *et al.,* 2006). Interestingly, the wt allele accelerates senescence and favours Fe and Zn mobilization from flag leaves into developing seeds (Uauy *et al.,* 2006; Lundström *et al*., 2017). Intriguingly, the reduction in NAM-B1 transcript by RNA interference causes a delayed senescence. Such a trait is, however, not associated with larger seeds, suggesting that the observed reduced Fe and Zn concentration in plants with reduced NAM-B1 activity is not simply due to a dilution effect in seeds, but on the inefficient remobilization of these nutrients from leaf to seeds, as the higher Fe and Zn concentrations in NAM-B1 RNA1 flag leaves demonstrate (Uauy *et al.,* 2006). According to Uauy and coworkers, wt NAM-B1 allele is present in all wild emmer accessions tested, and in the largest part of domesticated emmer accessions (*Triticum dicoccum*) whereas both the tested *T. durum* and *T. aestivum* lines lack the functional allele, as they either carry an allele with a 1-bp insertion (causing a frame shift) or a gene deletion (Uauy *et al*., 2006). In fact, some Swedish spring wheat varieties were demonstrated to carry the wt NAM-B1 allele, without showing any relevant difference in Zn and Fe content with respect to varieties carrying the null allele (Asplund *et al*., 2013; Lundström *et al*., 2017). This poses the question of the possible effect of NAM-B1 allele and of its non-functional allele, in different genetic backgrounds (Asplund *et al*., 2013). Also, the frequency of NAM-B1 allele in domesticated emmer wheat poses the question of whether the NAM-B1 gene can be considered a genuine domestication gene or, instead, a diversification gene (Lundström *et al.,* 2017). Nonetheless, a years-long analysis of the NAM-B1 wt allele and its distribution among wheats, confirms that wild crops relatives represent a large and still unexplored reservoir of potentially valuable genes that can be exploited for neo-domestication approaches (Charmet, 2011; Peng *et al.,* 2013). The annual wild species *Cicer judaicum* is, for example, the most promising wild crop relative for improvement of Fe content in chickpeas (Sharma *et al.,* 2020).

The analysis of the wild progenitor of *O. sativa*, i.e. *O. rufipogon*, allowed to ascertain that the Combined Strategy (CS) of Fe uptake preceded rice domestication (Wairich *et al*., 2019). Again, these findings support the possible use of wild rice in the improvement of CS strategy, as far as seed Fe loading is concerned.

Conclusions

The potential of beneficial rhizobacteria to activate both plant ISR defense responses and Fe uptake responses opens the possibility of using such microbial strains as biopesticides and Fe biofertilizers. Nonetheless, further studies are required to investigate the link between ISR and Fe uptake response, especially in crops under field conditions, and the various middle/long-term ecological implications that the use of such beneficial microbial strains would imply. Such studies will certainly be important for the detailed understanding of plant Fe nutrition in the field, where plants are continuously exposed to various biotic and abiotic stresses; they can also potentially impact the costs associated with reduced crop yields in alkaline soils. Thus, research on plant beneficial microorganisms and Fe nutrition appears as a very engaging field for future researchers.

The road of Fe from soil to seeds is long indeed, and it still features several question marks, one of which will be to define the precise biochemical network of transcription factors, enzymes, transporters and biochemical steps involved in moving Fe into the various seed tissues during their development and maturation. In this respect, the exploitation of genetic resources derived from Fe-dense seeds of wild crops' relatives, appears an attractive avenue to be explored in the short term.

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Authors contribution statement

IM: Conceptualization, writing – Original Draft Preparation.

FM, GV, PM: Contributions to original draft preparation

FM: Figures preparation, with contributions of IM, GV, PM.

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BOX 1: Plant immune responses

Plants are continuously exposed to the attacks of several pathogens and pests during their life; they have therefore developed diverse strategies to perceive assaulters and mount immune responses. Once plants come in contact with microbes, they firstly locally recognize characteristic features of microorganisms (e.g., lipopolysaccharides, glycoproteins, flagellin and chitin), known as microbe-associated molecular patterns (MAMPs), or pathogen-associated molecular patterns (PAMPs) when produced by pathogens. These molecules are perceived through pattern-recognition receptors (PRRs), starting downstream signalling pathways that activate the so-called 'MAMP-triggered immunity' (MTI) or 'PAMP-triggered immunity' (PTI) (Zipfel, 2008; Pieterse *et al.*, 2009; Choi *et al*., 2016; De Lorenzo *et al*., 2018; Pontiggia *et al*., 2020). A secondary major immune response, known as 'effector-triggered immunity' (ETI), is also activated when specific plant resistance proteins are produced to react against pathogens' molecules (i.e., the effectors), which are introduced in plant host cells to suppress MTI/PTI (Cui *et al.*, 2015). These two immune defense responses partially overlap, because of the accumulation of pathogenesis-related (PR) proteins that help in plant resistance. Once the defense response has been turned on at the site of the infection, a 'Systemic Acquired Resistance' (SAR), is frequently activated far away from the site of the attack to defend undamaged tissues. SAR activation is associated with an increase of the hormone salicylic acid (SA), both at the site of the infection and in distant plant organs (Fu and Dong, 2013; Klessig *et al*., 2018). Beneficial microorganisms in soils can stimulate a systemic immunity similar to SAR, known as 'Induced Systemic Resistance' (ISR) (Van Loon *et al.*, 1998; Pozo and Azcón-Aguilar, 2007; Pieterse *et al.*, 2014). In ISR, root microbiota can elevate the level of disease resistance against different pathogenic threats, activating various phytohormone signalling pathways and transferring this defense message to distant plant tissues. Jasmonic acid (JA) and ethylene (ET) are the two hormones involved in ISR signalling (Van der Ent *et al.*, 2009). Similar to SAR, ISR is activated only upon an external stress factor, so that plants can save resources; this strategy is known as 'defense priming', allowing plants to alert their immune system for future pathogen or pest attacks, thus avoiding a direct activation of defense responses. The main differences between the two systemic plant immunities are the 'priming stimulus' triggering the defense priming and the hormones involved (Conrath *et al.*, 2015; Martinez-Medina *et al.*, 2016). ISR has been described for several PGPR such as *Pseudomonas* spp., *Bacillus* spp. and *Serratia* spp.; *Pseudomonas* and *Bacillus* genera often represent the dominant group in the rhizosphere. ISR has been also described for PGPF, such as *Trichoderma* spp., *Fusarium* spp., *Serendipita* spp., and arbuscular mycorrhizal fungi AMF (Nihorimbere *et al.*, 2011; Pascale *et al.*, 2020). Nevertheless, perturbations of this 'core' root microbiota may be helpful for plants, since variations in microbial genera abundance in the rhizosphere may help plants to react against different biotic and abiotic stresses (Paasch and He, 2021 and references therein).

Figure legends

Figure 1

Schematic model of the interactions among root Fe uptake mechanisms, the microbiota present in the rhizosphere (in particular WCS417) and plant immunity responses. A Strategy I root cell and its surrounding rhizosphere are represented. Red arrows show the cascade of events occurring during Fe deficiency response (thick arrows for transport/movement; thin arrows for signalling), which are regulated by FIT1, MYB72/MYB10, BGLU72, and leading to i) Fe uptake through the coordinated activity of AHA2, FRO2 and IRT1, ii) biosynthesis of coumarins through the phenylpropanoid pathway and their release into the rhizosphere through PDR9 transporter. Both coumarins and protons act on the pool of poorly soluble Fe(hydroxy)oxides. *Pseudomonas simiae* WCS417, as well as other beneficial microorganisms, can release bacterial siderophores which also increase Fe solubility from the pool of Fe(hydroxy)oxides in the soil. Blue arrows indicate immune response pathways, such that one triggered by WCS417 and leading to suppression of MAMP-triggered immunity (evasion of host immunity), as well as those triggered by WCS417 siderophores and leading to suppression of plant pathogens. Purple arrows indicate overlapping pathways of Fe deficiency responses and immune responses; WCS417 induction of Fe deficiency responses and phenylpropanoid pathway and dependence of such induction on WCS417 concentration threshold are represented, as well as the effects of coumarins on WCS417 and pathogens. PM: plasma membrane. For further details regarding biosynthesis, transport and biological activity of coumarins refer to Robe *et al.* (2021b).

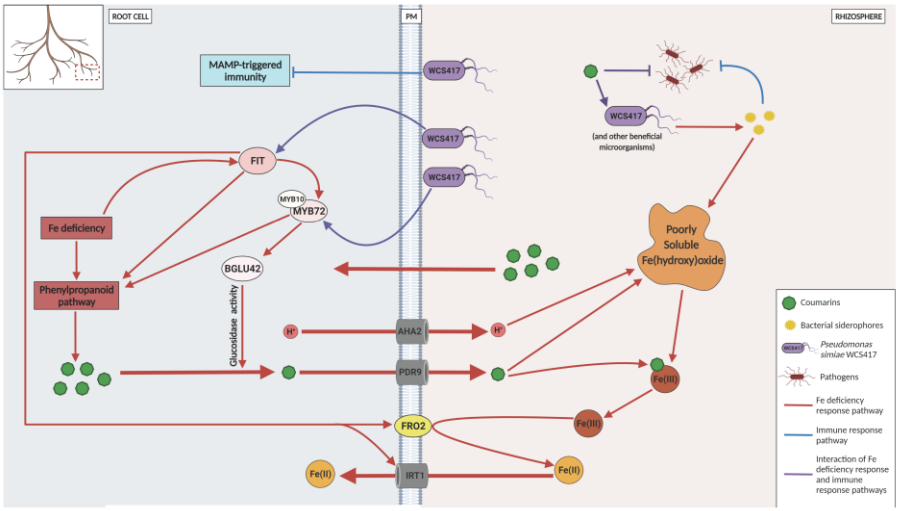
Figure 2

Formate dehydrogenase (FDH) is a protein hub for Fe plant nutrition and a node of the multiple interactions between Fe homeostasis and plant responses to abiotic and biotic stresses. Experiments conducted on roots and on aerial parts of *A. thaliana* plants (shown in the center) support the model of FDH as a hub of plant Fe nutrition, in a loop regulation with Fe homeostasis and responses against abiotic stresses. A leaf hydathode under physiological conditions (upper right), or exposed to *Xanthomonas campestris campestris Xcc* attack (lower right) are represented. Inhibition of FDH promoter activity by *Xcc* would lead to a local increase in formate concentration; such change in formate concentration, in turn, might act as a possible signal for plant defense responses to pathogen's entry (see main text and cited references for details).

Figure 3

Proposed model of Fe uptake in developing embryos. An *A.thaliana* developing seed is shown, with its embryo at the bent cotyledon stage, endosperm and the cell layers of maternal origin forming the seed coat. The possible contribution of the L-gulono-1,4 γ-lactone oxidase GULLO2 to the ASC pool, for Fe(III) reduction into Fe(II) and its subsequent transport into the developing embryos, is reported with dashed arrows. The role of the GULLO2 reaction product H_2O_2 on the endosperm and on the seed coat composition is unknown. ASC, ascorbic acid.

Figure 1



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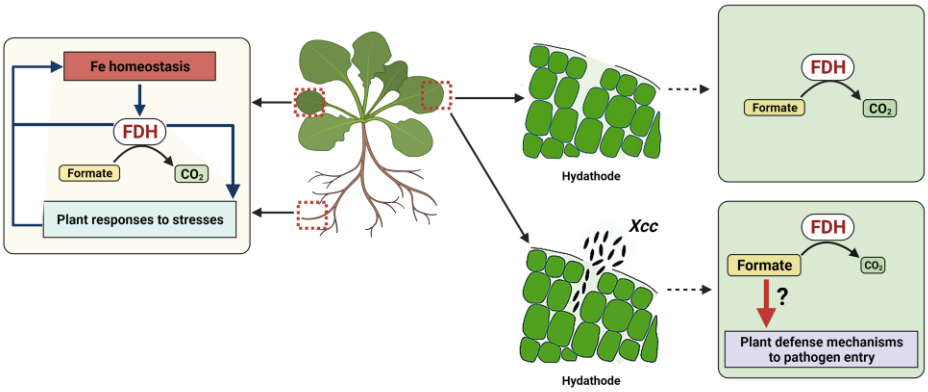

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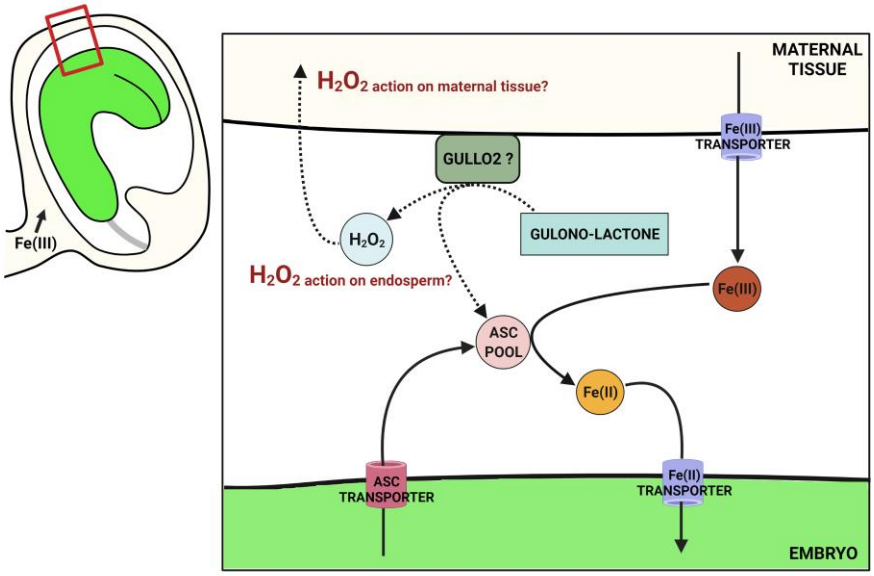

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