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REVIEW ARTICLE

Foliar fertilization: possible routes of iron transport from leaf surface to cell organelles

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ABSTRACT

Iron (Fe) plays a pivotal role in plant nutrition as well as human health. Foliar application is being adopted as an economic, targeted and environment friendly alternative over soil fertilization. However, dependency of foliar fertilization on crop species, environment, Fe compound and Fe status of plant makes it a complex practice. Though a large body of evidence is available on Fe movement from root to shoot, very little is known on its movement from the leaf surface (extracellular) to various organelles (intracellular). This article presents possible pathways of foliar-fed Fe including crossing leaf physical barriers to reach the apoplast, crossing the plasma membrane, moving through plasmodesmata, phloem loading and unloading and storage in intracellular compartments. We have also emphasized on various chelating agents present in intracellular environments along with membrane-bound proteins aiding in Fe movement. Further, genes involved in Fe uptake and transport from leaf surface are discussed. At organelle level, presence of importer/exporter in outer membranes or bound Fe (Fe(II) to Fe(III)) form needs to be identified. The long-distance transport of Fe in phloem supports the existence of unknown oxido-reductases. The understanding of Fe mobilization pathways from foliage to sink tissues might also assist in molecular biofortification.

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Introduction

Iron (Fe) is the fourth most abundant element on earth's crust, whose non-availability in soil is a major concern worldwide. It is one of the essential micronutrients involved in many ubiquitous metabolic processes, such as respiration, photosynthesis, sulphur (S) and nitrogen (N) assimilation and biosynthesis of DNA, lipids and hormones, most of them requiring Fe in terms of Fe-heme groups or Fe-S clusters (Balk and Lobreaux 2005). Plant Fe deficiency leads to leaf chlorosis, stunted growth and decreased Fe content in edible parts of plants, which is considered as one of the major causes of Fe-deficiency-induced anaemic people worldwide.

The success with soil-applied Fe fertilizers is limited due to low solubility and Fe-fixation, including the possibility of leaching and contaminating ground water (Abadía et al. 2011). Besides soil application, the most effective alternative method to correct plant Fe deficiency is foliar application. This takes into account the absorption capability of above-ground plant parts, such as leaves, stems, inflorescences and fruits (Fernández et al. 2013). The response of plants to foliar Fe application is influenced by physiological factors including leaf age, surface area, leaf

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expansion and source/sink status, chemical composition of stomata, cuticle and lenticels and environmental factors such as light, temperature, humidity and wind. These lead to differences in penetration, storage and re-translocation of foliar-applied nutrients (Fernández and Brown 2013). Moreover, the efficiency of foliar spray in delivering minerals to plants is influenced by the physico-chemical properties of spray solutions such as concentration, solubility, molecular weight, electric charge and pH (Fernández et al. 2013). In spite of its complexity and dependency on various factors, this 'direct-feeding' approach (bypassing soil) aids in overcoming nutrient deficiency and enhances plant's performance with a special focus on targeted areas thereby making it an economic way to correct nutritional disorders.

Many reports are available on ameliorating plant Fe deficiency via foliar application with organic and inorganic Fe compounds (Rios et al. 2016). The successful utilization of nutrients by plants is achieved by foliar adsorption, penetration through physical barriers (cuticle, aqueous and stomatal pores) to reach the leaf apoplast, uptake by leaf cellular compartments (chloroplast, mitochondria, vacuole) and finally translocation to other plant parts (flowers and seeds). Although an efficient re-translocation of Fe to other plant organs after foliar application has been shown, the information on biochemical and molecular mechanisms underlying the movement of Fe at leaf physical and cellular levels is still limited (He et al. 2013). Many authors have covered the information available on Fe movement from soil to seed but only a few have addressed its movement from leaves to other organs following foliar fertilization (Grillet et al. 2014a). In this article, we have outlined the possible pathways of Fe movement from the site of application (foliage) to the site of metabolic processes (cellular organelles) based on available information. The transporters involved in Fe movement through various cellular compartments that can be manipulated for successful molecular biofortification have been discussed. In addition, the present review reflects on the current findings in the field of foliar Fe fertilization, comparing application practices to enhance efficiency.

Iron formulations: type, concentration and efficacy

Efforts have been made to assess the efficacy of foliar-applied Fe solutions by evaluating rate of Fe absorption, re-greening capacity and its translocation from the site of application (Fernández et al. 2008; Rios et al. 2016). Iron deficiency can be treated by exogenous application of inorganic Fe compounds as well as natural or synthetic Fe chelates (Abadía et al. 2011). Contradictory results have been obtained on foliar fertilization using these compounds (Table 1). In comparison to chelates, inorganic Fe salts have shown increased efficiency of penetration and leaf re-greening (El-Jendoubi et al. 2014; Rios et al. 2016). Nevertheless, a few studies strongly recommended the use of chelates over inorganic compounds due to their enhanced mobility, while others observed no difference in their applicability and concluded their dependability on type of adjuvant-formulation used (Fernández et al. 2006). However, these studies were performed under different conditions with varying concentration and Fe formulations. Also, there might be intra-species differences in response to foliar Fe application.

In addition to above-discussed formulations, biostimulants such as humic acid (HA) have popularized as an effective Fe chelate for improving the Fe status of plants under Fe-deficient environment (Abroskin et al. 2016). Though some studies depict similar Fe translocation rate after the supply of Fe-ethylenediamine tetra acetic acid (EDTA) and Fe-HA (Nikolic et al. 2003; Sharma et al. 2018), emphasis has been given to bring into practice the use of HA and other biostimulants (fulvic acid, N-containing compounds) to increase the nutrient uptake (for review, see Tanou et al. 2017). HA can be obtained from varied sources, thereby its application results in differential response in plants. This necessitates the comparison of different humic substances to find the best sources for its efficient penetration into the plant system.

In addition to type of formulation, the concentration of spray solution containing Fe is of utmost importance as concentrated solutions might reduce the penetration rate or if penetrated, might cause toxicity to the plant (Fernández et al. 2013; Sharma et al. 2018). During cuticular penetration, high Fe(III) concentration in foliar spray competes with ionized groups (in cuticle) for water

Table 1. Brief outline of various foliar Fe fertilization studies highlighting differences in conditions under study.

Condition	Fe formulation	Fe source	Parameters studied	Soil/hydroponics	Crop	Reference
Fe sufficiency	Single	1% and 2% $FeSO_4{\cdot}7H_2O$ 0, 200, 400 and 600 ppm $FeSO_4{\cdot}H_2O$ 0, 2, 4 and 6 g/L Nano-Fe 1% $FeSO_4$ 3% $FeSO_4{\cdot}7H_2O$	Fe concentration in shoot, dry weight of shoot Plant height, grain N and yield parameters Plant height and yield parameters Yield parameters and grain Fe Yield parameters, Fe and Zn concentration in grain	Loamy calcareous Sandy Clay loam Fine loam	Soybean Wheat Wheat Wheat Wheat	Moosavi and Ronaghi (2011) Ali (2012) Armin et al. (2014) Pahlavan-Rad and Pessarakli (2009) Rakshit et al. (2014)
	Comparison	1 g Fe/L FeEDTA, FeEDDHA, FeHEEDTA,FeCDTA, FeDTPA, Glycerol + $FeCl_3$, $FeCl_3$,Fe(III)citrate, Fe(III)tartrate, Fe(III)phosphate, Ferrous ammonium sulphate, Ferbam (0.28% Fe) FeEDTA and $FeSO_4{\cdot}7H_2O$ 0.1 mM $FeSO_4$ and FeEDDHA 2 and 4 g/L Nano Fe oxide, Fe chelate (4 and 8 g/L) and $FeSO_4$ (4 and 8 g L-1)	Fe concentration in lead blade, petiole and stem Leaf Fe, P, Ca, Mg, Mn and Zn concentration Penetration rate through cuticle Grain protein, carbohydrate and Fe, chlorophyll and carotenoids content in leaves, antioxidant enzymes	Quartz sand Clay loam Silt clay loam	Citrus Strawberry *Euonymus japonicus*, tomato Wheat	Basiouny and Biggs (1971) Erdal et al. (2004) Kannan (1969) Ghafari and Razmjoo (2013)
Fe deficiency	Single	2 mM $FeSO_4$ 1% and 2% $FeSO_4$ 0, 150 g/ha Fe_2O_3 and $ZnSO_4$	Leaf chlorophyll, nutrients and photosynthetic pigments Plant height, flag leaf Zn and Fe, yield parameters Yield and seed Fe	Hydroponics and calcareous soil Micronutrient deficient soil Clay loam soil	Peach, sugar beet Wheat Wheat	El-Jendoubi et al. (2014) Naz et al. (2015) Habib (2009)
	Comparison	$FeSO_4.7H_2O$ (5 mM), FeEDTA (5 mM), Fe(III)citrate (1–15 mM), Fe-rhizoferrin (1 mM), Fe-dimerum acid (1–10 mM), Fe-coprogenhydrolysate (5 mM) $FeSO_4$ (500 mg Fe L^{-1}), diluted ascorbic, citric and sulphuric acids (2, 2 and 0.55 g L^{-1}, respectively), applied to leaves either alone or in combination with $FeSO_4$ (500 mg Fe L^{-1}), FeDTPA (119 mg Fe L^{-1}) $FeSO_4$ and FeDTPA $FeSO_4$, $Fe_2(SO_4)_3$, $FeCl_3$ and FeEDTA all containing 9 mM Fe 2 mM Fe in $FeSO_4{\cdot}7H_2O$ (pH 4),FeEDTA, FeDTPA, FeIDHA, Fe(III) citrate Fe(III)malate, Fe(III)citrate, Fe(III)DTPA, $FeSO_4$ + amino acid-polypeptide mixture	Leaf Fe Leaf chlorophyll Leaf re-greening Leaf Fe, leaf structure staining Leaf weight, area, Fe and leaf structure Leaf regreening and characterization of leaf FCR	Hoagland Calcareous soil Calcareous Hoagland Calcareous Fe deficient andsufficient soil	*Vicia faba*, tobacco, citrus Pear Kiwi fruit, peach and pear Prunus Peach Kiwi fruit	Fernàndez et al. (2005) Álvarez-Fernández et al. (2004) Tagliavini et al. (2000) Rios et al. (2016) Fernández et al. (2008) Rombolà et al. (2000)

(Schonherr 2006). These electrochemical or osmotic interactions of Fe compounds end up reducing size or causing partial dehydration of aqueous pores that lead to clogging of hydrophilic pathways. Increasing the concentration of Fe(III) immunodisuccinic acid (IDHA) and Fe(III) EDTA affected the permeability, for example, increasing the concentration of Fe(III) IDHA from 2.5 to 5.0 mM in the foliar spray reduced its penetration by 2.2-fold (Schlegel et al. 2006). High concentrations of Fe(III) in foliar sprays promote the generation of reactive oxygen species and subject the plants to oxidative stress leading to reduced growth (Sida-Arreola et al. 2015). Thus, choosing lower concentrations may be beneficial for successful foliar sprays, but this has to be tested for each plant species and type of formulation used, in order to make a balance between phytotoxicity and nutrient supply.

The efficacy of foliar feeding depends on translocation of Fe from the site of application to the sink. Experiments limited to treated leaves would not provide any fruitful insight since reports have suggested that mobility of Fe within the plant is a major concern rather than penetration (El-Jendoubi et al. 2014). Though Fe shows high reproductive mobility in various crops including wheat (*Triticum aestivum*) and muskmelon (*Cucumis melo*), it has been reported to be relatively immobile in tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and kidney bean (*Phaseolus vulgaris*) (Guzman et al. 1990; Garnett and Graham 2005). Many studies performed under Fe-sufficient conditions and may be not applicable under Fe-deficient condition, due to alteration in properties of physical barriers in Fe-deficient leaf. The available literature is not sufficient to draw conclusions with respect to the type of Fe formulation, since the response may vary with crop, environment (soil or hydroponics), Fe compound and background Fe status of plant (Fe-deficient or Fe-sufficient). The efficacy of Fe formulation should not only be judged on the basis of re-greening effect and local Fe concentration, but its translocation within the plant must also be considered for obtaining best results. Exploiting the benefits of nano-sized and other Fe formulations requires the understanding of the factors affecting their bioavailability to plants.

Entry through leaf surface

Foliar-applied Fe crosses the physical barriers to reach the leaf apoplast, and then can deeply penetrate to enter various cell organelles (Abadía et al. 2011; Pandey et al. 2013; El-Jendoubi et al. 2014). Iron enters the leaf via cuticle through cuticular cracks, aqueous/stomatal pores or other specialized epidermal structures and possibly through physical damage on leaf surface (Figure 1) (Fernández and Brown 2013; Fernández et al. 2013).

Cuticle

Plant cuticle is the foremost barrier for all aqueous solutions due to its lipoidal nature. The cutin waxes and biopolymer matrix together form the waxy layer, the cuticle proper and the cuticular and pectin layer that aids in bonding the cuticle with the outer cell wall. However, the chemical composition of the cuticle is still unclear for most plant species, limiting the understanding of Fe movement through foliage. Fernández et al. (2016) questioned the independence of cuticle from cell wall, and suggested re-assessment of its structural and chemical nature. From the available literature, it is clear that cuticle thickness and epidermal cell wall do play a pivotal role in affecting the penetration of foliar compounds. The presence of carbonyl/carboxylic and hydroxyl groups in the cuticle impart negative charges that gradually increase from epicuticular wax to the pectin layer (Gunzman et al. 2014). These negative charges facilitate the penetration and diffusion of positively charged ions (Fe(III), Fe(II)) along the concentration gradient (Mengel 2002). Along with ions, Fe might also penetrate the leaf as FeO in the case of nanoparticles (Dimkpa and Bindraban 2016). Studies showed that the use of laser light caused microscopic perforations in leaf cuticle, thereby enhancing the penetration of foliar formulation into leaf cuticle (Etxeberria et al. 2016). However, such technologies need further validation and development for large-scale application in the field.

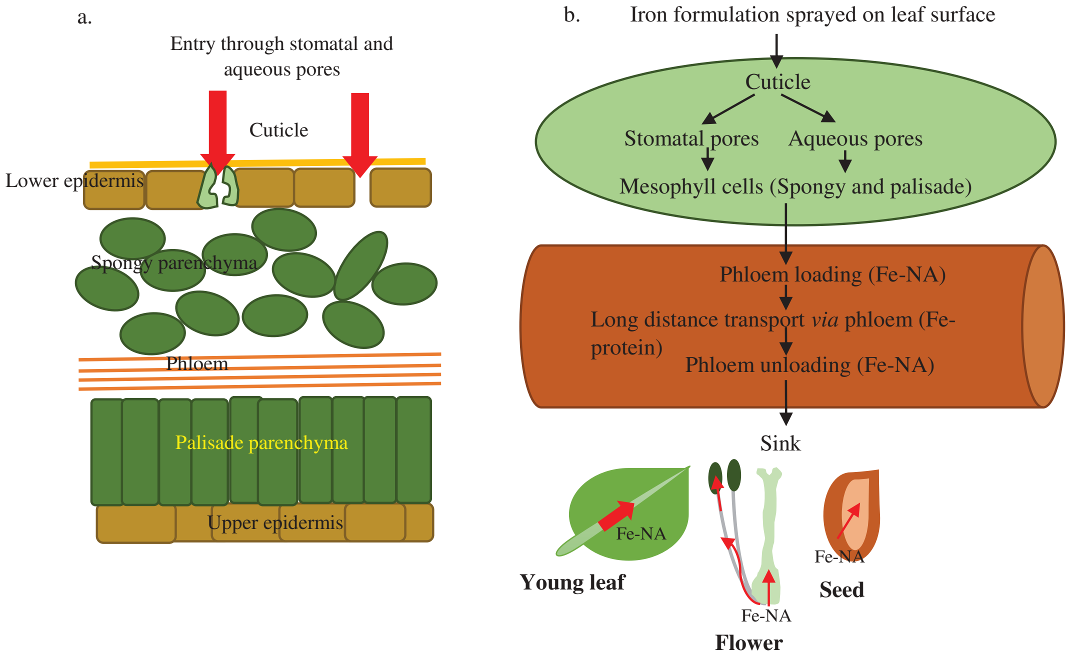

Figure 1. Schematic representation of the route of Fe after foliar spray. (a) Movement of Fe into the leaf after foliar application by crossing the cuticle through stomatal and aqueous pores and reaching the internal cells. (b) Transport of Fe to other tissues and organs (sink) through phloem loading and unloading once it enters from the leaf surface (source).

Aqueous and stomatal pores

The lipoidal make-up of cuticle paves the way for lipophilic solutes by dissolution and diffusion, while movement of various charged ions or hydrophilic polar molecules (Fe formulations) occurs by diffusion across cuticular layer through stomatal or aqueous pores (Fernández et al. 2013; Lv et al. 2019). Water-based nutrient solution sprays containing inorganic salts have been hypothesized to penetrate the cuticle through aqueous pores (Figure 1). The size of aqueous pores is dynamic, depending on hydration level and movement of water molecules, which may form unbroken water-phase from cuticle to plasma membrane (Beyer et al. 2005). The movement of ionic species has been suggested to occur through cuticular aqueous pores and the radii of these pores were estimated to lie in the range between 0.3 and 3.0 nm (Eichert and Goldbach 2008). The Van der Waals radius of Fe chelates ranges from 0.5 to 1.3 nm while it is 0.2 nm for $FeSO_4$ (Fernández et al. 2008) which is comparable to the size of aqueous pores suggesting easy penetration of Fe formulations through cuticular pathway. Since direct evidence for their physical occurrence has not been found (Tanou et al. 2017), there is still a need to reveal the chemical structure as well as biological function of these pores.

A few reports presented the uncertainty of nutrient uptake through stomatal pores without the use of surfactants (for references, see Fernández et al. 2017). However, other reports favour the uptake of dissolved ionic substances through stomata or adjacent areas under natural conditions without the requirement of any external pressure or surface active ingredients (Eichert and Goldbach 2008; Burkhardt et al. 2012). Though stomatal pores may also be covered by cuticles, its layer is thinner compared to the external cuticle which makes stomatal penetration easier than cuticular (Shahid et al. 2016). Spraying Fe formulations on lower leaf surfaces maximizes Fe penetration as stomatal density is often greater on abaxial (lower) surface compared to adaxial surface (Álvarez-Fernández et al. 2004; Eichert and Goldbach 2008; Burkhardt et al. 2012). The molecular radii of Fe(II) and Fe(III) compounds are far smaller than the size of stomatal pores (average width 3–12 µm), thus causing easy penetration through these pores (Fernández et al. 2008). The uptake of Fe(III) via stomata of leek (*Allium porrum*), asiatic dayflower (*Commelina communis*) and stonecrop (*Sedum telephium*) studied using microscopy and image analysis (Eichert and Burkhardt 2001) stressed the essentiality of this pathway for penetration of ionic solutes.

Various methods are in use to track the path of Fe movement after foliar application. With the use of Perls blue staining, the Fe uptake pathway was traced in Prunus leaf showing the beginning of Fe movement from stomatal areas and progressing towards vascular areas over time (Rios et al. 2016). Another method employed a combination of LT SEM-EDX (low temperature scanning electron microscopy energy dispersive X-ray) and Perls blue staining to compare Fe-deficient (chlorotic), Fe-recovered and Fe-sufficient leaf (El-Jendoubi et al. 2014). Interestingly, Fe was found to be distributed throughout the leaf in Fe-sufficient and Fe-recovered chlorotic leaves while it was restricted to vascular and parenchymal areas in the untreated parts of sprayed leaves. The intensity of Fe signals was also higher in Fe-sufficient and Fe-recovered chlorotic leaves when compared to the Fe-chlorotic leaves. These studies suggest that foliar-applied Fe successfully enters the treated leaves through stomatal pores and reaches various internal compartments, including spongy and palisade parenchyma and vascular bundles (Figure 1(a)). However, highly sensitive staining methods would need to be developed to track Fe movement throughout leaf internal areas as well as intracellular spaces such as apoplast and symplast.

Various iron binding forms in apoplast, symplast and phloem

The Fe efficiency in leaves depends on Fe distribution between apoplast and symplast. Several potentiometric, spectrophotometric and mass spectrometry techniques have been used to assess the chelation capability and pH-dependent stability of Fe complexes with different molecules (Rellán-Álvarez and Alvarez-Fernández 2008; Álvarez-Fernández et al. 2014; Flis et al. 2016; AlChoubassi et al. 2017). Fe(II) and Fe(III) are highly reactive species and are extremely insoluble at alkaline pH (7.0–8.0) of the cytoplasm and phloem sap and so they bind with anionic constituents and ligands such as nicotianamine (NA), deoxymugienic acid (DMA) and proteins (such as iron transport protein (ITP) (Kruger et al. 2002). Due to the pH difference, exchange reactions occur between different chelating agents to bind Fe in leaf cytosol, apoplast and phloem. At apoplastic pH 5.5, Fe prevails in Fe(III) form while Fe(II)NA exists as major Fe form in cytosol due to slightly basic or neutral pH environment (Rellán-Álvarez and Alvarez-Fernández 2008). However, some apoplastic Fe(II) may also form complex with NA available in the apoplast though Fe(II)NA is unstable at slightly acidic pH (5.5) of apoplast (Grillet et al. 2014a). In phloem, the slightly basic pH favours the uptake of Fe as Fe-NA or Fe-DMA and long-distance transport of Fe in phloem occurs via proteins or DMA that complexes preferably with Fe(III) (Inoue et al. 2008; Álvarez-Fernández et al. 2014). The movement of Fe from apoplast to symplast and through phloem has been reviewed by Rodríguez-Celma et al. (2016) and Kobayashi et al. (2019). We briefly discuss all Fe binding partners in the following sections.

Fe and nicotianamine

NA, a non-proteinogenic amino acid, is essential for long-distance transport of Fe within the plant. NA is known to chelate both Fe(II) and Fe(III), though the kinetic stability of Fe(II)NA is much higher than Fe(III)NA (Flis et al. 2016). Requirement of Fe-NA is critical for normal development of leaf, flower and seed. The Fe(III)DMA is converted to Fe(II)NA via two steps: reduction of Fe(III) followed by the chelation to NA. The reduction step can occur through plasma membrane-bound ferric chelate reductase (FCR) or by the cytoplasmic Nicotinamide adenine dinucleotide phosphate reduced (NADPH)-dependent FCR (Ishimaru et al. 2006). This could be followed by the exchange of DMA to NA as Fe(II)NA is relatively stable at high pH in phloem cells (Rellán-Álvarez and Alvarez-Fernández

2008). The essentiality of Fe-NA for the development of flowers and seeds has been confirmed using Arabidopsis, tomato (*Solanum lycopersicum*) and tobacco mutants defective in NA synthesis (Pich and Scholz 1996; Takahashi et al. 2003; Klatte et al. 2009). In comparison to non-transgenic plants, the Fe concentration increased by threefold in transgenic sweet potato (*Ipomoea batatas*) leaves overexpressing barley nicotianamine synthase (NAS) gene (Nozoye et al. 2017). Similar studies have been performed in rice and wheat engineered with Arabidopsis and rice NAS gene, confirming the role of NA in improving grain Fe concentration (Boonyaves et al. 2017; Singh et al. 2017). Thus, these studies highlighted the importance of NA in Fe distribution at cellular level or in its entry in symplastic compartment to reach various plant organs.

Fe and carboxylic acids

Several carboxylates such as malate and citrate bind metal ions and assist in their long-distance transport as Fe-carboxylate complexes. Using Arabidopsis mutants, the involvement of citrate in the lateral movement of Fe from xylem to aged leaves was revealed while NA exhibited major role in transporting Fe from phloem to young leaves (Schuler et al. 2012). The Fe-carboxylate complexes might be present in pure (single organic acid) or mixed (more than one organic acid) forms depending on the Fe and organic acid ratio but the stability of mixed Fe complexes was found to be higher than pure Fe complexes. In xylem sap of tomato and pea, several complexes of Fe(II) and Fe(III) with malate and citrate have been observed (Rellán-Álvarez et al. 2010; Flis et al. 2016). Similarly, such complexes were also observed in leaf apoplastic fluid of pear (*Pyrus communis*), beet (*Beta vulgaris*) and Arabidopsis (López-Millán et al. 2000; Larbi et al. 2010; Roschzttardtz et al. 2011). Also, the organic acids bind primarily with Fe(III) whereas Fe(II) is the preferred form for NA (Flis et al. 2016). Under Fe deficiency, the concentration of carboxylates increases to complex with extracellular Fe pool and make it available for the long-distance transport (Álvarez-Fernández et al. 2014).

Fe and deoxymugineic acid

Mugineic acids (MAs) are a class of phytosiderophores that enhance the bioavailability of Fe by chelating to Fe(III). DMA, a member of MAs, is biosynthesized from NA via the enzymes nicotianamine aminotransferase (NAAT) and deoxymugineic acid synthase (DMAS) (Wu et al. 2018). The co-expression of DMA biosynthesizing genes viz. OsNAS1-3, OsNAAT and OsDMAS1 found in the phloem companion cells in roots and leaves contributes in Fe uptake (Inoue et al. 2008; Bashir et al. 2017). Large amounts of DMA have been detected in phloem companion cells of Fe-sufficient and Fe-deficient rice leaves and its synthesis was shown to increase under conditions of Fe deficiency (Inoue et al. 2009). In rice phloem sap, DMA was found to be the major Fe(III) binding chelate form whereas both Fe(II)NA and Fe(III)DMA were involved in seed loading (Aoyama et al. 2009; Nishiyama et al. 2012; Díaz-Benito et al. 2018). Increasing DMA levels successfully enhance Fe concentration in rice embryo and endosperm (Díaz-Benito et al. 2018). Translocation of Fe from roots to youngest leaf in barley via phloem in the form of Fe(III)DMA was traced using ^{52}Fe (Tsukamoto et al. 2009). Fe(III)DMA movement from root to flag leaves and seeds by the constitutive expression of HvYS1 transporter in roots of transgenic barley (*Hordeum vulgare*) plants suggests that DMA plays a key role in the transport of Fe (Banakar et al. 2017).

Fe and nitrosyl complexes

Nitric oxide (NO) is a biologically active molecule that has a high affinity towards Fe and other transition metals. The interaction between Fe and NO forms Fe nitrosyl and dinitrosyl complexes which are important for the biological activity of NO (Borodulin et al. 2013). These are respectively formed by the reaction of NO with Fe-containing proteins or by the interaction of Fe(II) with NO and low molecular weight thiols, including glutathione (GS) and cysteine (Severina et al. 2003;

Shanmugam et al. 2015). The resulting GS-Fe-NO complex can easily diffuse through membranes or transported by a carrier (Watts and Richardson 2002). Intracellular availability of Fe is improved by nitrosylated Fe complexes which activate several molecular and physiological responses under Fe-deficient conditions (García et al. 2011). Application of NO resulted in enhanced FCR activity and reduction of Fe(III) to Fe(II) as well as increased intracellular Fe pool in Arabidopsis and cucumber (Chen et al. 2010; García et al. 2011). Further, it was shown that both NO and ethylene are involved in up-regulation of genes and transcription factors such as FRO2, IRT1, bHLH38, bHLH39 and MYB72, governing Fe-acquisition and transport in *Arabidopsis* (García et al. 2011). Application of NO significantly enhanced the Fe content and leaf chlorophyll in Fe-deficient peanut plants most probably due to improved antioxidant scavenging system and enhanced FCR and H^+-ATPase activities (Song et al. 2017), thereby confirming that NO has a role in increasing the bioavailability of Fe under Fe-deficient condition.

Fe and transport proteins

Proteins and peptides are also known to be involved in Fe transport in plants. Several Fe-binding compounds with molecular sizes ranging from 10–30 kD were found in phloem sap. Using radio-labelled Fe, it was found that nearly all Fe present in phloem of castor bean (*Ricinus communis*) seedlings was bound to ITP, a 17 kD dehydrin (Kruger et al. 2002). However, Nishiyama et al. (2012) showed that in the phloem sap of rice Fe was present as Fe(III)DMA and the recovery was only 23% using a 3 kDa membrane filter. This suggests that Fe(III)DMA complexes with high molecular weight compounds, but the identity of those is still unknown. Also, using Fe-affinity chromatography some metallothioneins and other Fe-binding proteins were found in the phloem of *Lupinus texensis* and *Brassica napus* (Lattanzio et al. 2013; Gutierrez-Carbonell et al. 2015). This suggests the presence of different proteins in various plant species for the movement of Fe in phloem. In the case of ITP, it showed preference for Fe(III) and was only weakly bound to Fe(II) (Kruger et al. 2002) suggesting an oxido-reduction system operating in phloem. The apparent lack of Fe(II) binding to proteins implies that another molecule such as NA could be essential for phloem loading and symplastic pathway while the long-distance movement of Fe occurs through Fe-protein complex.

Apoplastic Fe(III) reduction: candidates and modulators

In biological systems, Fe exists in ferrous (Fe(II)), ferric (Fe(III)) and ferryl (Fe(IV)) forms. It is well established that root to leaf transport of Fe occurs as Fe(III) (Rellán-Álvarez et al. 2010). Since Fe(II) is the metabolically active form of Fe in plants, it was assumed that Fe(III) needs to be reduced again for Fe(II) uptake from leaf apoplast to mesophyll cells. Different systems including leaf discs, plasma membrane and protoplasts have been used to study Fe uptake by leaf mesophyll tissue (Bruggemann et al. 1993; Larbi et al. 2001). Results stressed on the essentiality of reduction of Fe (III) for deep penetration into leaves (Mukherjee et al. 2006; Jain et al. 2014). This reduction process is modulated by several factors, including plasma membrane FCR activity, leaf apoplasmic pH, availability of reducing equivalents, light and citrate:Fe ratio (Flis et al. 2016).

The Fe(III) reduction system consists of a FCR of the ferric chelate reductase oxidase (FRO) family. Several reports have confirmed the presence of FCR activity in intact leaf mesophyll cells to reduce Fe(III) citrate, Fe(III) malate and Fe(III) EDTA prior to its uptake as Fe(II) (Bruggemann et al. 1993; Larbi et al. 2001). Expression of *FRO* gene has been detected in leaf, stem, rosette, silique in Arabidopsis (*FRO 5–8*) and in flower of pea (*Pisum sativum, FRO1*) (Wu et al. 2005; Mukherjee et al. 2006). Expression of *FRO* helps in reduction of Fe(III) to Fe(II) at the cell surface prior to its entry into the cytoplasm (Jeong et al. 2008; Jain et al. 2014; Gayomba et al. 2015). However, some studies failed to detect FCR activity or *FRO* expression in leaf discs raising doubts over requirement of Fe(III) reduction before its uptake by leaf cells (Schmidt 1999; Grillet et al. 2014b). This could probably be due to the localization of FRO protein in the internal leaf cells rather than on its surface or there may be existence of reduction systems other than FCR.

Photosynthetically active light stimulates both enzymatic and non-enzymatic photoreduction of Fe(III) and thereby increases movement of Fe(II) from the apoplast into leaf mesophyll cells (Bruggemann et al. 1993; Schmidt 1999; Grillet et al. 2014a). Under low light intensity, Fe(III) reduction rates were lower when compared under normal light intensity (de la Guardia and Alcantara 1996). However, the extent to which photoreduction of Fe(III) is likely to occur remains an open question. Abadía et al. (2011) stressed on the use of new mass spectrometry-based analytical tools to confirm the role of light in Fe(III) reduction.

Organic anion leakage (ascorbic acid and phenols) at the leaf wound also has the capability to induce photo- or non-photochemical reduction of Fe(III) (Larbi et al. 2001; Grillet et al. 2014b). These reductant molecules increase Fe uptake in leaves. In cowpea (*Vigna unguiculata*), the reactive oxygen species or strong blue light might mediate the reduction of Fe(III) to Fe(II) before its entry into leaf mesophyll cells (Bruggemann et al. 1993). Using radio-labelled ^{55}Fe, it was demonstrated that extrusion of ascorbate from pea embryos is essential to reduce Fe(III) bound to either citrate or malate complexes (Grillet et al. 2014b). Further experimentation is required for the successful validation of such non-enzymatic mechanisms in plants.

Leaf apoplastic pH is another important factor for cellular Fe acquisition. A range of pH optima for ferric chelates reduction were observed in leaf discs of various crops such as 6.5–6.8 in *V. unguiculata*, 6.0–6.7 or 5.5–6.0 in sugar beet, 6.0 in Faba bean and 5.0 in leaf apoplast of sunflower (Kosegarten et al. 1999; Gonzalez-Vallejo et al. 2000; Larbi et al. 2001). High apoplastic pH hinders Fe(III) reduction to Fe(II) followed by its transportation across the plasma membrane into symplast thereby decreasing the efficiency of Fe uptake in leaf mesophyll cells (Rajaie and Tavakoly 2018). The absence of optimum pH in leaf apoplast may lead to increased levels of Fe in inactive form in leaves with restricted translocation.

The citrate:Fe molar ratio is another key player controlling the stability of Fe complexes and its uptake by leaf cells (López-Millán et al. 2000; Flis et al. 2016). Under Fe deficiency, increase in citrate:Fe ratio decreases the FCR activity in sugar beet leaf apoplastic fluid by 62%. Upon Fe resupply, decrease in citrate:Fe ratio from 1700 to 50 caused 10–20% increase in the FCR activity (Larbi et al. 2010). The predominant $[FeCit_2]^{-3}$ species under Fe deficiency in contrast to $[FeCitOH]^{-1}$ under Fe sufficiency would experience a strong electrostatic repulsion with the negatively charged plasma membrane and hence cause progressive impairment of Fe acquisition mechanisms in leaf cells (López-Millán et al. 2000). Thus, uptake of Fe depends on the efficiency of FCR, which in turn is modulated by factors discussed above. The FCR activity might change under Fe-deficient or -sufficient condition owing to pH change and presence of different Fe complexes. Thus, it is necessary to study the Fe pathway under both Fe-deficient and -sufficient conditions.

Fate of Fe(III) after reduction: traversing the plasma membrane

Upon Fe reduction, the entry of Fe into the symplast takes place by crossing the plasma membrane. The symplastic environment is comprised of cytoplasm and several intracellular organelles where Fe reaches through various membrane-bound transporters *viz*. IRT (iron-regulated transporter) and OPT (oligopeptide transporter) family (Grillet et al. 2014a; Gayomba et al. 2015). The phloem can take up Fe from the surrounding tissues through the apoplast via transporters on plasma membrane or symplast via plasmodesmata.

Iron-regulated transporter

After reduction, Fe enters the leaf mesophyll cells through IRT transporters (Figure 2). In Arabidopsis, *AtIRT1* is a plasma membrane-bound transporter that mediates uptake of Fe into leaf mesophyll cells. *IRT1* and *IRT2* are expressed in both root and shoot but were mainly

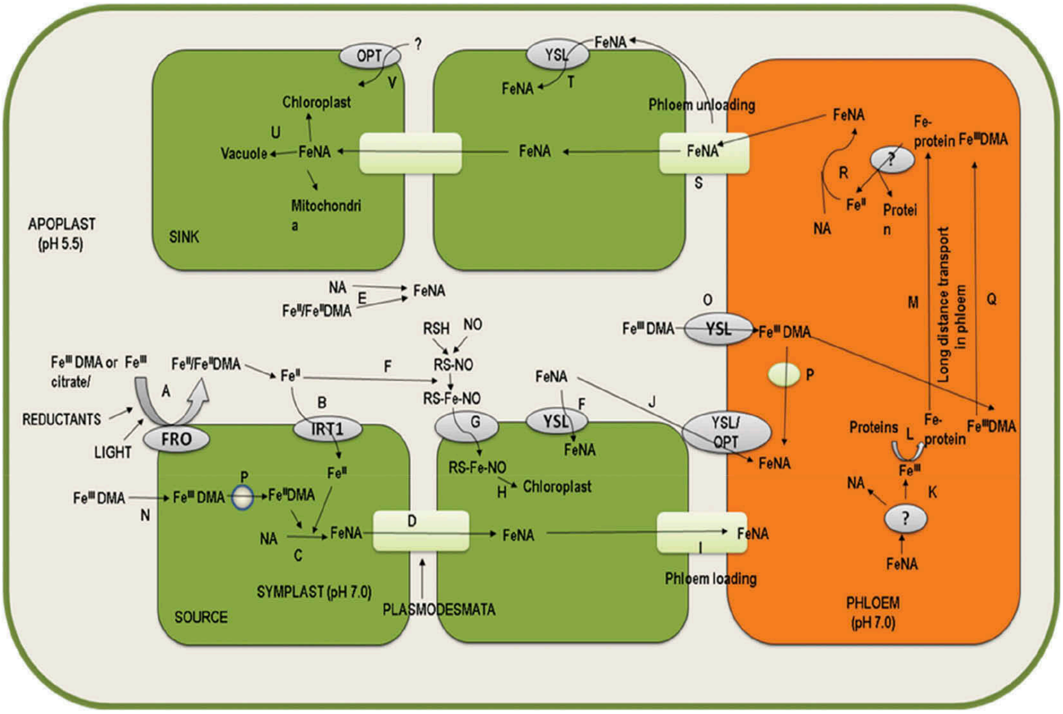

Figure 2. Intra-cellular translocation of Fe transport from source leaves (Fe fed) to sinks. This includes apoplastic uptake, movement through plasmodesmata, phloem loading and unloading and Fe movement to sink. After reaching apoplast (pH 5.5), Fe(III) is reduced to Fe(II) via FRO (ferric-chelate reductase oxidase), the light or ascorbic acid (A) and enters into the cell cytoplasm via IRT1 (iron-regulated transporter) (B). Reduced Fe is complexed with nicotianamine (NA) present in cytoplasm (C) and moves to adjacent cells via plasmodesmata (D). Fe(II) (or Fe(II)DMA) may also bind to apoplastic NA (E) to reach cell cytoplasm (F) or directly into phloem (G) via membrane-bound YSL (yellow stripe like) transporters. The reduced cytoplasmic Fe can also lead to the formation of dinitrosyl Fe complex (RS-Fe-NO) (F) that enters cytoplasm via some membrane-bound transporter (G) and moves into chloroplast (H) for further action. Cytoplasmic Fe is loaded into phloem via symplastic (I) or apoplastic pathway (J) as the Fe(II)NA complex. Unknown oxido-reductases oxidize Fe(II) to Fe(III) (K) which further binds it to proteins (L). Long-distance transport of Fe occurs in this form through phloem (M). Fe(III)DMA can directly enter mesophyll cell (N) or phloem (O) through diffusion or some membrane-bound carrier. Cytoplasmic Fe(III)DMA gets reduced to Fe(II)DMA via NADPH-dependent FCR (P). Fe(III)DMA can also contribute to the long distance movement of Fe via phloem (Q). During phloem unloading, Fe(III) is again reduced to Fe(II) and binds to NA (R) to enter sink cell via symplastic (plasmodesmata, M) or apoplastic (YSL, T) pathway, ultimately reaching cellular organelles (U). OPT (oligopeptide transporter) can also contribute in transporting Fe (V) but the chelating agent is unknown.

reported in roots under Fe deficiency (Eide et al. 1996; Bauer et al. 2004). The expression of *AtIRT1* was also detected in anther filaments of unpollinated flowers irrespective of Fe nutritional status suggesting a role in supplying Fe to developing pollen grains. Conversely, the expression of *IRT1* and *IRT2* could not be detected in Arabidopsis shoots under both Fe-deficient or-sufficient conditions (Vert et al. 2002). Later reports confirmed *AtIRT3* to function as a Fe-uptake transporter in Arabidopsis shoot (Lin et al. 2009). In poplar trees, the expression of *PtIRT3* also increased under Fe deficiency (Huang and Dai 2015). Although results suggest that IRT is indeed essential for the uptake and translocation of Fe within the plant, the expression of IRT as well as FRO is tightly regulated by a complex regulatory network of transcription factors (Gao et al. 2019). Sixteen members of basic helix-loop-helix (bHLH) transcription factors belonging to different clades interact with each other and negatively regulate FER-LIKE IRON-DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) which downstream controls induction of genes *FRO2* and *IRT1*. The bHLH encoded by FER in tomato and by OsIRO2 in rice were shown to regulate Fe uptake and translocation (Ling et al. 2002; Ogo et al. 2011). Several other Fe regulators such as,

HEMERYTHRIN MOTIF-CONTAINING REALLY INTERESTING NEW GENE AND ZINC-FINGER PROTEIN (HRZ1 AND HRZ2), IDEF, POPEYE (*PYE*) and BRUTUS (*BTS*) have been identified and functionally characterized in rice and Arabidopsis (Long et al. 2010; Kobayashi et al. 2012, 2013; Rodríguez-Celma et al. 2018). However, whether these transcriptional regulators also influence the absorption of foliar-applied Fe needs to be revealed.

Oligopeptide transporter family

Plasma membrane localized YSL (Yellow Stripe Like) transporters, belonging to the OPT family, load Fe from mature leaves to flowers and developing seeds, thus transporting Fe across cell membranes (Figure 2). In shoots of Fe-deficient maize plant, *ZmYS1* was strongly expressed and was involved in transport of Fe(III) and Fe(II) complexes with NA throughout the plant (Schaaf et al. 2004). Fe translocation from shoots to seeds takes place by *YSL1* and *YSL3* in Arabidopsis and *YSL2* and *YSL13* in rice, which is expressed strongly in vascular parenchyma cells in leaves and in various parts of seed (Koike et al. 2004; Bashir et al. 2017; Zhang et al. 2018). In addition to *OsYSL2*, upregulation of *OsYSL15* under Fe deficiency in flowers and developing seeds confirmed its role in long-distance Fe transport (Koike et al. 2004; Inoue et al. 2009). In barley, *HvYS1* selectively transports Fe while its overexpression in rice caused increased Fe uptake and translocation to shoot and seed (Banakar et al. 2017). The apoplastic uptake of Fe by YSL transporters in phloem is decided by the formation of Fe-NA or Fe-DMA complex in the apoplast. *OsYSL2* is expressed in leaf phloem companion cells as well as developing seeds of Fe-deficient rice plants allows the transport of Fe(II)NA, but not Fe(III)DMA (Koike et al. 2004). Conversely, *OsYSL15, OsYSL16* and *OsYSL18* are involved in transport of Fe(III)DMA, but not Fe(II)- and Fe(III)-NA (Aoyama et al. 2009; Inoue et al. 2009; Kakei et al. 2012). Thus, different YSL proteins are responsible for the transport of Fe complexed with NA or DMA in different species.

Another member of OPT family, OPT3, was reported to deliver Fe complexed with NA to seeds in Arabidopsis (Figure 2) (Mendoza-Conzatl et al. 2014; Zhai et al. 2014). Expression of OPT3 was observed in companion cells that transport Fe(II) from mature leaves to young leaves and seeds in Arabidopsis; however, its ability to transport naked Fe ions is not yet established (Zhai et al. 2014). Using Arabidopsis mutant, *opt3-1*, it was proved that OPT3 is essential for embryo development (Stacey et al. 2002, 2008). Besides *OPT3*, expression of other members (*AtOPT1,4, 6, 7, 8*) was comparable to *AtOPT3* and was localized in vascular tissues of vegetative and reproductive organs (Stacey et al. 2006). In a recent study, *opt3* mutation in Arabidopsis led to Fe overload in leaves and roots, preventing its further translocation to seeds (Khan et al. 2018). This highlights the role of OPT3 in Fe nutrition of developing seeds but the chemical nature of the Fe species is yet to be identified.

Molecular basis of Fe storage in cell organelles

Several metabolic processes occurring in distinct intracellular compartments require Fe at different concentrations, thus adequate amount of Fe should reach these cell organelles. Excessive Fe can be stored in the vacuole by its association with Fe storage proteins (Mary et al. 2015). Several transporters and storage proteins that participate in import, export and storage of Fe in various cellular organelles are presented in Figure 3. It was found that rice lines expressing *IRT1*, *NAS1* and ferritin (*FER*) resulted in higher accumulation of Fe in grain as compared to lines expressing only *IRT1* and *NAS1* depicting the significance of Fe storage protein ‘ferritin’ in these organelles (Khan et al. 2018).

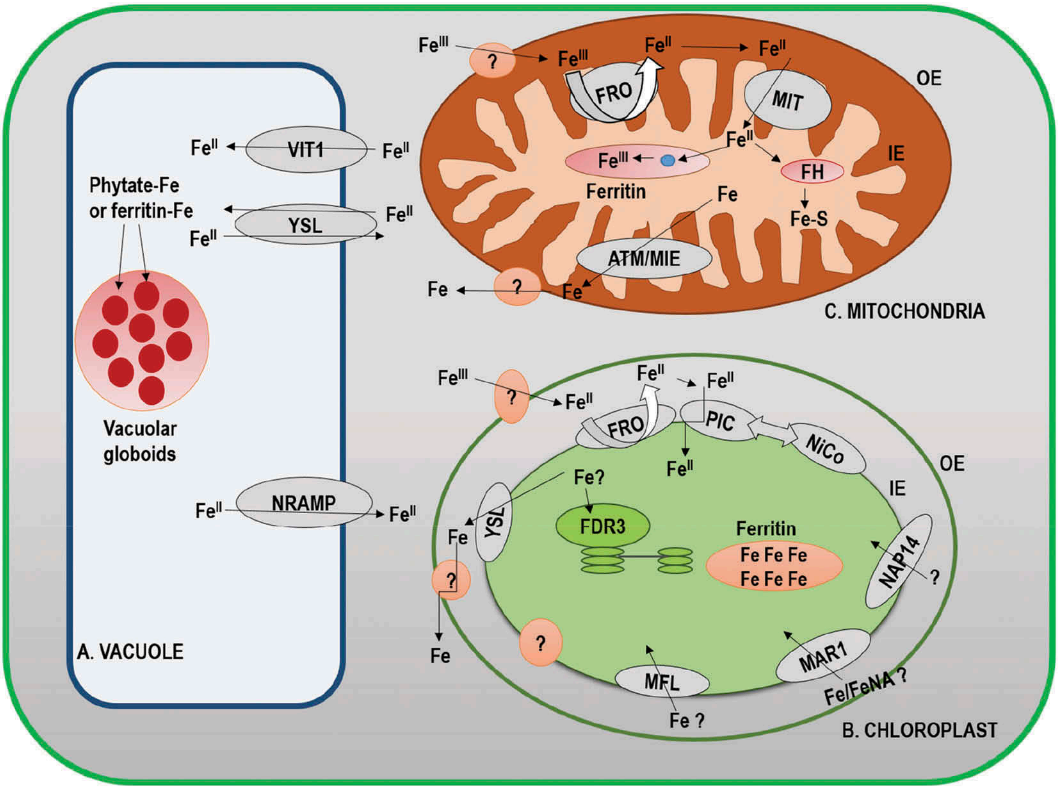

Figure 3. Sub-cellular import, export and storage of iron. (a) Vacuole entry of Fe into vacuole takes place through VIT (vacuolar iron transport) transporter. Fe may bind with ferritin or phosphate and stored as complexes internal in vacuolar globoids. NRAMP (natural resistance associated macrophage protein) releases Fe from these stored forms and exports Fe out of the vacuole. YSL (yellow stripe like) plays dual role by importing and exporting vacuolar Fe. (b) Chloroplast Fe import in outer chloroplast envelope is mediated as Fe(III) by unknown importer. FRO (ferric-chelate reductase oxidase) localized on inner envelope reduces ferric to ferrous form which is then imported into stroma by PIC (permease in chloroplast), NiCo (nickel-cobalt transporter family), NAP14 (non-intrinsic ABC transporter protein), MAR1 (multiple antibiotic resistance) and MFL (mitoferrin like) transporters. FDR 3 (Fe deficiency related) is localized in stroma or thylakoid lumen to import Fe into thylakoids. Ferritin is Fe storage protein in chloroplasts. (c) Mitochondria Fe is imported into outer mitochondrial membrane through unknown transporter followed by entry into mitochondrial inner envelope through MIT (mitochondrial iron transport) after reduction by FRO located on inner envelope. Here it is stored as Ferritin-Fe or forms Fe-S clusters using frataxin (FH). Fe is exported to the cytosol through ATM/MIE (mitochondrial ATP cassette transporter/mitochondrial iron exporters) though transporter for its export from outer membrane is unknown.

Transport and storage of Fe in vacuole

In germinating seeds, vacuole represents the initial source of Fe prior to its uptake by roots (Figure 3(a)). Arabidopsis vacuolar iron transporter (*AtVIT*) mediates Fe(II) transport into vacuole during maturation of the embryo (Kim et al. 2006). This was confirmed by using *vit1-1* mutant that failed to store Fe in seeds and embryos appropriately. Two VIT genes, *VIT1* and *VIT2*, play a pivotal role in the distribution of Fe in seeds in rice and wheat (Zhang et al. 2012; Connorton et al. 2017) resulting in enhanced seed Fe content, which can improve biofortification in these crops. The expression of *OsVIT2* was highly induced in rice under Fe excess conditions thereby detoxifying excess Fe in plant (Aung et al. 2018). Thus, *VIT* is required for loading and storing Fe in the endodermal cells of seeds which is utilized during germination.

Fe export from the vacuole is mediated by another Fe transporter called Natural resistance-associated-macrophage proteins (NRAMP3 and NRAMP4) located in the vacuoles of vascular tissues of shoots that play an essential role in mobilizing vacuolar Fe during seed germination. Functional characterization of *AtNRAMP3* proved to be crucial for Fe transport in plants. The double mutant *nramp3nramp4* seeds failed to remobilize Fe from vacuoles and showed 90% lethality during germination highlighting the involvement of NRAMP transporters in vacuolar Fe movement (Mary et al. 2015; Bastow et al. 2018). In addition to NRAMP, YSL also mediate intracellular delivery as well as long-distance transport of Fe. The transport of Fe-NA complex in and out of the vacuole is mediated by YSL4 and YSL6 (Jaquinod et al. 2007). In excess Fe condition, it is concentrated in cytoplasm and vacuole in contrast to its accumulation in cytoplasm only during Fe sufficiency and deficiency.

The Fe transported to vacuoles is stored in globoid bodies (Mary et al. 2015). In Arabidopsis seeds, vacuole globoids containing phytate constitute the major site for storing Fe due to very little ferritin reported in Arabidopsis (Ravet et al. 2009). Lanquar et al. (2005) also detected the association of Fe to vacuole globoids using microanalysis of Fe in *Arabidopsis* seeds.

Transport and storage of Fe in chloroplast

Photosynthesis, chlorophyll and heme biosynthesis are important Fe demanding metabolic processes that takes place inside chloroplasts, thereby necessitating Fe delivery to chloroplast for efficient plant productivity (Figure 3(b)). A double membrane organelle raises the question whether any difference exists among the two membranes in the form of Fe during entry or exit? The majority of Fe form identified between the two envelopes was Fe(III)citrate while the minor form was Fe-sulphur cluster (Fe_4S_4) protein (Solti et al. 2012). In intact sugar beet chloroplast, Fe(III) citrate was the preferred form than Fe(II)citrate as Fe source while in pea chloroplast inner envelope, the transport was in the form of Fe(II) (Shingles et al. 2002; Solti et al. 2012). Likewise, Müller et al. (2019) also reported that intact chloroplast isolated from *B. napus* utilizes Fe(III)citrate effectively as compared to Fe(III) or Fe(II)-NA. Many studies indicate the presence of FCR activity on chloroplast inner envelope which is strongly dependent on photosynthetic electron transport (Schagerlof et al. 2006; Solti et al. 2014; Müller et al. 2019). Out of eight members of FRO family in Arabidopsis, only *FRO7* contributes to Fe uptake by chloroplast as shown by 75% reduced FCR activity in *fro7* mutants (Jeong et al. 2008). Hence, FRO carries out the reduction of Fe(III) in the inter-envelope space, thereby preparing it to enter chloroplast matrix (Solti et al. 2014).

The permease in chloroplasts (PIC) is a chloroplast Fe importer targeted to the inner envelope (Gong et al. 2015). After reducing Fe(III), FCR feeds Fe(II) to PIC1 for its immediate transport into stroma and, hence FCR and PIC1 work in close association on the chloroplast inner envelope facing the inter-envelope space (Duy et al. 2007; Solti et al. 2014; Müller et al. 2019). *PIC1*-overexpressing lines accumulated 2.5 times more Fe in chloroplasts (Duy et al. 2011) while *pic1* mutant exhibited impaired chloroplast development, but the overall Fe levels in leaf remained unchanged (Teng et al. 2006). This was also accompanied by an increase in ferritin clusters which are contemplated to sequester excess Fe (Duy et al. 2007). PIC also interacts with another protein called NiCo (nickel-cobalt) which function together to transport Fe into the chloroplast for Fe-S cluster biogenesis (Duy et al. 2011; López-Millán et al. 2016; Müller et al. 2019).

Besides PIC and FRO, there are other Fe importers into the chloroplast. Fe complexes can enter chloroplasts through transporter, Multiple Antibiotic Resistance (MAR1), Non-intrinsic ABC transporter Protein (NAP14) and Mitoferrin-like 1 (Mfl1), which plays a major role in Fe chelation, storage and sequestration (Conte and Lloyd 2010). However, the expression of *MAR1* was found to be lower in leaves of oilseed rape (Müller et al. 2019). NAP14 is present in chloroplast inner envelope and might have a role in Fe-S cluster biogenesis, although this needs further confirmation (López-Millán et al. 2016). Mfl1 is mainly expressed in leaves and contains a predicted chloroplast targeting peptide (Tarantino et al. 2011). Another chloroplast localized Fe Deficiency Related 3 (FDR3) protein was identified in maize stroma or thylakoid lumen involved in Fe import (Han et al. 2009). The Fe export from chloroplast is mediated by YSL4 and YSL6, but their localization and ion specificity are controversial (Müller et al. 2019). The *ysl4ysl6* double mutant of Arabidopsis showed Fe accumulation in chloroplasts with a concomitant increase in ferritin (Divol et al. 2013). This report indicates that only YSL transporters are involved in exporting Fe out of the chloroplast.

The storage of Fe in chloroplast occurs in the form of ‘Ferritin’, a ubiquitous Fe storage protein consisting of large spherical 24-mer protein complexes and is functional under Fe excess condition (Briat et al. 2010). Though they do not constitute major Fe pool in Arabidopsis seeds and leaves, they are essential for protecting cells against oxidative damage (Ravet et al. 2009). In Arabidopsis, all four ferritin genes are predicted to be localized in plastids (Petit et al. 2001). The expression of *FER2* is restricted to seeds while others are expressed in shoots and flowers as proved by using Arabidopsis triple mutant (*fer1fer3fer4*) with silenced non-seed ferritin coding genes. In this mutant, Fe was translocated from stem to flowers by *FER2* resulting in higher Fe accumulation in flowers. This ultimately led to abnormal flower development and oxidative stress, though chloroplast and leaf development were not affected (Ravet et al. 2009). Also, integration of tri-omics study confirmed the importance of ferritin in the development of flowers (Sudre et al. 2013). So, chloroplast seemed to be an important Fe sink while ferritin was involved in sequestering excess Fe, thereby acting as Fe buffer and preventing excess Fe movement into the flower (Morrissey and Guerinot 2009). In Arabidopsis, ferritin accounted for only 5% of total seed Fe while in pea, ferritin was 92% of total seed Fe (Ravet et al. 2009). This implies that Fe storage is species dependent and needs further confirmation on other plant species.

Transport and storage of Fe in mitochondria

Similar to chloroplast, mitochondrial requirement of Fe is also large to carry out respiration process and synthesis of heme and Fe-S clusters (Balk and Lobreaux 2005). The excess of both electron and free Fe can be toxic highlighting the importance of maintaining mitochondrial Fe levels. This is achieved through transporters and Fe sequestering proteins in mitochondria (Figure 3(c)). Mitochondrial iron transporter (MIT1) identified in rice imports Fe and its reduced expression affected Fe-S cluster synthesis in mitochondria (Bashir et al. 2011). The essentiality of MIT1 was proved by using *mit1* knock out rice lines which displayed embryo lethal phenotype, reduction in concentration of mitochondrial Fe and chlorophyll as well as poor seed yield. This was accompanied by an up- and down-regulation of *FER* and *VIT1* expression, respectively. This evidence supports the cross-talk between mitochondria, chloroplast and vacuole for Fe homeostasis (Jain and Connolly 2013). However, further characterization of OsMIT1 is necessary for clear understanding of mitochondrial Fe homeostasis. Other members of FRO family (*AtFRO3* and *AtFRO8*), expressed in shoots, possess mitochondrial targeting sequence suggesting the reduction of Fe prior to its uptake by this cellular compartment (Mukherjee et al. 2006). The presence of Fe reduction system is essential due to oxidizing environment of mitochondrial inter-membrane space but their exact roles are still not clear (Hu et al. 2008; Abadía et al. 2011). Moreover, in rice mitochondria, the FROs have not been identified which raises the possibility of presence of a non-reductive or other transport system of Fe in these mitochondria (Jain and Connolly 2013).

Three Fe exporters homologous to yeast mitochondrial ATP cassette transporter1 (ATM1) identified in Arabidopsis were the only mitochondrial Fe exporters (Chen et al. 2007). These are localized in mitochondrial inner envelope and involved in efflux of Fe-S clusters from the mitochondrial matrix (Bernard et al. 2009). Arabidopsis plants defective in *sta1* (functional homolog of yeast *atm1*) also accumulated higher levels of non-heme and non-protein Fe as compared to wild-type mitochondria (Kushnir et al. 2001). Out of the three ATMs present in Arabidopsis, ATM3 showed functional similarity to ScATM followed by ATM1 which are suggested to be involved in Fe transport (Chen et al. 2007). However, there was uncertainty over role of AtATM3 in mitochondrial Fe homeostasis and stressed on the identification and characterization of existing and additional transporters responsible for Fe import into mitochondria (Bernard et al. 2009). Using rice mutants, it was proved that mitochondrial ATM3 was essential in Fe-S cluster assembly and Fe homeostasis (Zuo et al. 2017).

In mitochondria, two Fe-binding proteins, ferritin and frataxin (FH), were identified as Fe storage protein and are involved in sequestration of excess Fe, thereby protecting mitochondria from oxidative damage. Presence of ferritin was confirmed in mitochondria isolated from pea stems and Arabidopsis cell cultures (Zancani et al. 2004). *AtFER4* and *AtFH* were targeted to mitochondria with highest expression in flowers and floral stalk. Cell size and plasma membrane were affected in callus cultures of *fer4* mutant with an increased mitochondrial Fe concentration resulting in accumulation of reactive oxygen species (Tarantino et al. 2010). The ferroxidase activity of ferritin helps to oxidize Fe(II) to Fe(III) and store it as hydrous ferric oxides but the mechanism of Fe release from ferritin core needs to be unravelled (Arosio et al. 2009). Although *atfer4* loss-of-function mutants failed to show any distinctive phenotype but loss-of-function frataxin (*atfh*) produced increased reactive oxygen species resulting in decreased vegetative growth and seed set (Busi et al. 2006). Frataxin has an important role in embryogenesis as proved by knocking out *AtFH* gene that arrested embryo development at globular stage. However, along with its role in Fe sequestration and embryogenesis, frataxin also serves as a chaperone to deliver Fe to Fe-S cluster assembly scaffold (Gomez-Casati et al. 2018). These studies indicate that ferritin and frataxin together play an important role in Fe-S cluster biogenesis, cellular Fe homeostasis and protection against oxidative stress; however, their transport mechanism and role in mitochondrial Fe metabolism needs to be explored.

Conclusion and future prospects

In order to develop an effective foliar-applied molecule to correct Fe deficiency, or to improve translocation of Fe to sink from biofortification perspective, it is important to know the pathways of different Fe complexes and the transporters involved in its movement from leaf surface to the cell. Based on available reports, we have proposed possible pathways of Fe movement and its storage in various organelles once it is applied to the foliage (Figures 2 and 3). After physical entry, once the Fe reaches leaf apoplastic environment in Fe(III) form (as Fe-citrate or Fe-DMA), it undergoes reduction by plasma membrane FRO, light, ascorbic acid or phenolic compounds (Figure 2). The reduced Fe in the form of Fe(II)NA or RS-Fe-NO could enter the cytoplasm and be transported symplastically to adjacent cells, ultimately reaching the phloem. However, apoplastic Fe(II)NA can also directly enter phloem through OPT family proteins, including YSL and OPT3 suggesting that there is possibility to apply Fe in the Fe(II) form thus bypassing the plasma membrane reduction step. Therefore, compounds such as $FeSO_4$, Fe(II) humic acid and Fe chelated with bacteriosiderophore can be used for foliar application for better results. The chelation of Fe(II) with the chemical analogue of phytosiderophore (NA) used as foliar spray might increase the efficiency of Fe translocation within the phloem and its partitioning to other plant parts.

Once the phloem loading of Fe occurs, the Fe(II)NA is re-oxidized by unknown oxido-reductase complex to Fe(III) which then chelates with proteins helping in long-distance Fe transporter inside the phloem (Figure 2). The identification and characterization of this oxido-reductase complex which dissociates NA and Fe and re-oxidizes the latter needs further studies. Fe(III)DMA can also directly enter into phloem cells through YSL and assist in Fe movement inside phloem. Before phloem unloading, the Fe-protein complex dissociates and Fe is reduced. The phloem unloading at sink cells occurs as Fe(II)NA that ultimately reaches the cellular organelles for metabolic reactions and storage (Figure 3). Higher concentrations of Fe in the phloem can result in the suppression of root Fe-deficiency responses. Thus, it is possible to improve the absorption of foliar-applied Fe by choosing the right Fe formulation or developing new Fe chelating agent and enhance its translocation efficiency from source to sink tissue. Molecular biofortification can be achieved by manipulating genes involved in Fe transport into different cellular compartments of sink tissue to increase the concentration of Fe in storage.

Disclosure statement

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References

Abadía J, Vazquez S, Rellan-Alvarez R, El-Jendoubi H, Abadía A, Alvarez-Fernendez A, Lopez-Millan AF. 2011. Towards a knowledge-based correction of iron chlorosis. Plant Physiol Biochem. 49:471–482. doi:10.1016/j.plaphy.2011.01.026.

Abroskin DP, Fuentes M, Garcia-Mina JM, Klyain OI, Senik SV, Volkov DS, Perminova IV, Kulikova NA. 2016. The effect of humic acids and their complexes with iron on the functional status of plants grown under iron deficiency. Eurasian Soil Sc. 49:1099–1108. doi:10.1134/S1064229316100021.

AlChoubassi G, Aszyk J, Pisarek P, Bierla K, Ouerdane L, Szpunar J, Lobinski R. 2017. Advances in mass spectrometry for iron speciation in plants. Trends Anal Chem. doi:10.1016/j.trac.2017.11.006

Ali EA. 2012. Effect of iron nutrient care sprayed on foliage at different physiological growth stages on yield and quality of some durum wheat (*Triticum durum* L.) varieties in sandy soil. Asian J Crop Sci. 4:139–149. doi:10.3923/ajcs.2012.139.149.

Álvarez-Fernández A, Diaz-Benito P, Abadía A, Lopez-Millan A, Abadía J. 2014. Metal species involved in long distance metal transport in plants. Front Plant Sci. 5:1–20. doi:10.3389/fpls.2014.00105.

Álvarez-Fernández A, Garcia-Lavina P, Fidalgo C, Abadía J, Abadía A. 2004. Foliar fertilization to control iron chlorosis in pear (*Pyrus communis* L.) trees. Plant Soil. 263:5–15. doi:10.1023/B:PLSO.0000047717.97167.d4.

Aoyama T, Kobayashi T, Takahashi M, Nagasaka S, Usuda K, Kakei Y, Ishimaru Y, Nakanishi H, Mori S, Nishizawa NK. 2009. OsYSL18 is a rice iron(III)-deoxymugineic acid transporter specifically expressed in reproductive organs and phloem of lamina joints. Plant Mol Biol. 70:681–692. doi:10.1007/s11103-009-9500-3.

Armin M, Akbari S, Mashhadi S. 2014. Effect of time and concentration of nano-Fe application on yield and yield components of wheat. Int J Biosci. 4:69–75.

Arosio P, Ingrassia R, Cavadini P. 2009. Ferritins: a family of molecules for iron storage, antioxidation, and more. Biochim Biophys Acta. 7:589–599. doi:10.1016/j.bbagen.2008.09.004.

Aung MS, Masuda H, Kobayashi T, Nishizawa NK. 2018. Physiological and transcriptomic analysis of responses to different levels of iron excess stress in various rice tissues. Soil Sci Plant Nutr. 64:370–385. doi:10.1080/00380768.2018.1443754.

Balk J, Lobreaux S. 2005. Biogenesis of iron–sulfur proteins in plants. Trends Plant Sci. 10:324–331. doi:10.1016/j.tplants.2005.05.002.

Banakar R, Fernández AA, Abadía J, Capell T, Christou P. 2017. The expression of heterologous Fe (III) phytosiderophore transporter *HvYS1* in rice increases Fe uptake, translocation and seed loading and excludes heavy metals by selective Fe transport. Plant Biotech J. 15:423–432. doi:10.1111/pbi.12637.

Bashir K, Ishimaru Y, Shimo H, Nagasaka S, Fujimoto M, Takanashi H, Tsutsumi N, An G, Nakanishi H, Nishizawa NK. 2011. The rice mitochondrial iron transporter is essential for plant growth. Nat Commun. 2:1–7. doi:10.1038/ncomms1326.

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

Basiouny FM, Biggs RH. 1971. Uptake and distribution of iron in citrus. Proc Fla State Hort Soc. 84:17–22.

Bastow EL, de la Torre VSG, Maclean AE, Green RT, Merlot S, Thomine S, Balk J. 2018. Vacuolar iron stores gated by NRAMP3 and NRAMP4 are the primary source of iron in germinating seeds. Plant Physiol. 177:1267–1276. doi:10.1104/pp.18.00478.

Bauer P, Thiel T, Klatte M, Bereczky Z, Brumbarova T, Hell R, Grosse I. 2004. Analysis of sequence, map position, and gene expression reveals conserved essential genes for iron uptake in Arabidopsis and tomato. Plant Physiol. 136:4169–4183. doi:10.1104/pp.104.047233.

Bernard DG, Cheng Y, Zhao Y, Balk J. 2009. An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron–sulfur proteins in *Arabidopsis*. Plant Physiol. 151:590–602. doi:10.1104/pp.109.143651.

Beyer M, Lau S, Knoche M. 2005. Studies on water transport through the sweet cherry fruit surface: IX. Comparing permeability in water uptake and transpiration. Planta. 220:474–485. doi:10.1007/s00425-004-1354-y.

Boonyaves K, Wu TY, Gruissem W, Bhullar NK. 2017. Enhanced grain levels in rice expressing an iron regulated metal transporter, nicotianamine synthase, and ferritin gene cassette. Front Plant Sci. 8:1–11. doi:10.3389/fpls.2017.00001.

Borodulin RR, Kubrina LN, Mikoyan VD, Poltorakov AP, Shvydkiy VO, Burbaev DS, Serezhenkov VA, Yakhontova ER, Vanin AF. 2013. Dinitrosyl iron complexes with glutathione as NO and NO^+ donors. Nitric Oxide. 29:4–16. doi:10.1016/j.niox.2012.11.001.

Briat JF, Ravet K, Arnaud N, Duc C, Boucherez J, Touraine B, Cellier F, Gaymard F. 2010. New insights into ferritin synthesis and function highlight a link between iron homeostasis and oxidative stress in plants. Ann Bot. 105:811–822. doi:10.1093/aob/mcq046.

Bruggemann W, Maas-Kantel K, Moog PR. 1993. Iron uptake by leaf mesophyll cells: the role of the plasma-membrane bound ferric-citrate reductase. Planta. 190:151–155. doi:10.1007/BF00196606.

Burkhardt J, Basi S, Pariyar S, Hunsche M. 2012. Stomatal penetration by aqueous solutions- an update involving leaf surface particles. New Phytol. 196:774–787. doi:10.1111/j.1469-8137.2012.04307.x.

Busi MV, Maliandi MV, Valdez H, Clemente M, Zabaleta EJ, Araya A, Gomez-Casati DF. 2006. Deficiency of *Arabidopsis thaliana* frataxin alters activity of mitochondrial Fe–S proteins and induces oxidative stress. Plant J. 48:873–882. doi:10.1111/j.1365-313X.2006.02923.x.

Chen S, Sacnchez-Fernández R, Lyver ER, Dancis A, Rea PA. 2007. Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of *Arabidopsis* half-molecule ATP-binding cassette transporters implicated in iron homeostasis. J Biol Chem. 282:21561–21571. doi:10.1074/jbc.M702383200.

Chen WW, Yang JL, Qin C, Jin CW, Mo JH, Ye T, Zheng SJ. 2010. Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis thaliana*. Plant Physiol. 154:8100–8819. doi:10.1104/pp.110.161109.

Connorton JM, Jones ER, Rodriguez-Ramiro I, Fairweather-Tait S, Uauy C, Balk J. 2017. Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. Plant Physiol. 174:2434–2444. doi:10.1104/pp.17.00672.

Conte S, Lloyd A. 2010. The MAR1 transporter is an opportunistic entry point for antibiotics. Plant Signal Behav. 5:49–52.

de la Guardia MD, Alcantara E. 1996. Ferric chelate reduction by sunflower (*Helianthus annuus* L.) leaves: influence of light, oxygen, iron deficiency and leaf age. J Exp Bot. 47:669–675. doi:10.1093/jxb/47.5.669.

Díaz-Benito P, Banakar R, Rodriguez-Menendez S, Capell T, Pereiro R, Christou P, Abadía J, Fernández B, Alvarez-Fernández A. 2018. Iron and zinc in the embryo and endosperm of rice (*Oryza sativa* L.) seeds in contrasting 2′-deoxymugienic acid/nicotianamine scenarios. Front Plant Sci. 9:1–17. doi:10.3389/fpls.2018.00001.

Dimkpa CO, Bindraban PS. 2016. Fortification of micronutrients for efficient agronomic production: a review. Agron Sustain Develop. 36:1–27. doi:10.1007/s13593-015-0346-6.

Divol F, Couch D, Conejero G, Roschzttardtz H, Mari S, Curie C. 2013. The *Arabidopsis* YELLOW STRIPE LIKE 4 and 6 transporters control iron release from the chloroplast. Plant Cell. 25:1040–1055. doi:10.1105/tpc.112.107672.

Duy D, Stube R, Wanner G, Philippar K. 2011. The chloroplast permease PIC1 regulates plant growth and development by directing homeostasis and transport of iron. Plant Physiol. 155:1709–1722. doi:10.1104/pp.110.170233.

Duy D, Wanner G, Meda AR, von Wirén N, Soll J, Philippar K. 2007. PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron transport. Plant Cell. 19:986–1006. doi:10.1105/tpc.106.047407.

Eichert T, Burkhardt J. 2001. Quantification of stomatal uptake of ionic solutes using a new model system. J Exp Bot. 52:771–781.

Eichert T, Goldbach HE. 2008. Equivalent pore radii of hydrophilic foliar uptake in stomatous and astomatous leaf surfaces: further evidence for a stomatal pathway. Physiol Plant. 132:491–502. doi:10.1111/j.1399-3054.2007.01023.x.

Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc Natl Acad Sci USA. 93:5624–5628. doi:10.1073/pnas.93.11.5624.

El-Jendoubi H, Vazquez S, Calatayud A, Vavpetic P, Vogel-Mikus K, Pelicon P, Abadía J, Abadía A, Morales F. 2014. The effects of foliar fertilization with iron sulfate in chlorotic leaves are limited to the treated area. A study with peach trees (*Prunus persica* L. Batsch) grown in the field and sugar beet (*Beta vulgaris* L.) grown in hydroponics. Front Plant Sci. 5:1–16. doi:10.3389/fpls.2014.00002.

Erdal I, Kepenek K, Kizilgoz I. 2004. Effect of foliar iron applications at different growth stages on iron and some nutrient concentrations in strawberry cultivars. Turk J Agric For. 28:421–427.

Etxeberria E, Gonzalez P, Borges AF, Brodersen C. 2016. The use of laser light to enhance the uptake of foliar-applied substances into citrus (*Citrus sinensis*) leaves. Appl Plant Sci. 4:1–10. doi:10.3732/apps.1500106.

Fernández V, Bahamonde HA, Peguero-Pina Gil-Pelegrin E, Sancho-Knapik D, Gil L, Goldbach HE, Eichert T. 2017. Physico-chemical properties of plant cuticles and their functional and ecological significance. J Exp Bot. 68:5293–5306. doi:10.1093/jxb/erx302.

Fernández V, Brown PH. 2013. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. Front Plant Sci. 4:1–5. doi:10.3389/fpls.2013.00001.

Fernández V, Del Rio V, Abadía J, Abadía A. 2006. Foliar iron fertilization of peach (*Prunus persica* L. batsch): effects of iron compounds, surfactants and other adjuvants. Plant Soil. 289:239–252. doi:10.1007/s11104-006-9132-1.

Fernández V, Del Rio V, Pumarino L, Igartua E, Abadía J, Abadía A. 2008. Foliar fertilization of peach (*Pruniu spersica* L. btasch) with different iron formulations: effects on re-greening, iron concentration and mineral composition in treated and untreated leaf surfaces. Sci Hort. 117:241–248. doi:10.1016/j.scienta.2008.05.002.

Fernàndez V, Ebert G, Winkelmann G. 2005. The use of microbial siderophores for foliar iron application studies. Plant Soil. 272:245–252. doi:10.1007/s11104-004-5212-2.

Fernández V, Guzman-Delgado P, Graca J, Santos S, Gil L. 2016. Cuticle structure in relation to chemical composition: re-assessing the prevailing model. Front Plant Sci. 7:1–14. doi:10.3389/fpls.2016.00001.

Fernández V, Sotiropoulos T, Brown P. 2013. Foliar fertilization: scientific principles and field practices. Paris: International fertilizer industry association.

Flis P, Ouerdane L, Grillet L, Curie C, Mari S, Lobinski R. 2016. Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. New Phytol. 211:1129–1141. doi:10.1111/nph.13964.

Gao F, Robe K, Gaymard F, Izquierdo E, Dubos C. 2019. The transcriptional control of iron homeostasis in plants: a tale of bHLH transcription factors? Front Plant Sci. 10:1–8. doi:10.3389/fpls.2019.00001.

García MJ, Suárez V, Romera FJ, Alcántara E, Pérez-Vicente R. 2011. A new model involving ethylene, nitric oxide and Fe to explain the regulation of Fe-acquisition genes in Strategy I plants. Plant Physiol Biochem. 49:537–544. doi:10.1016/j.plaphy.2011.01.019.

Garnett TP, Graham RD. 2005. Distribution and remobilization of iron and copper in wheat. Ann Bot. 95:817–826. doi:10.1093/aob/mci085.

Gayomba SR, Zhai Z, Jung H, Vatamaniuk OK. 2015. Local and systemic signaling of iron status and its interactions with homeostasis of other essential elements. Front Plant Sci. 6:1–13. doi:10.3389/fpls.2015.00001.

Ghafari H, Razmjoo J. 2013. Effect of foliar application of nano-iron oxidase, iron chelate and iron sulphate rates on yield and quality of wheat. Intl J Agron Plant Prod. 4:2997–3003.

Gomez-Casati DF, Busi MV, Pagani MA. 2018. Plant frataxin in metal metabolism. Front Plant Sci. 9:1–8. doi:10.3389/fpls.2018.00001.

Gong X, Guo C, Terachi T, Cai H, Yu D. 2015. Tobacco PIC1 mediates iron transport and regulates chloroplast development. Plant Mol Biol Rep. 33:401–413. doi:10.1007/s11105-014-0758-5.

Gonzalez-Vallejo EB, Morales F, Cistue L, Abadía A, Abadía J. 2000. Iron deficiency decreases the Fe(III)-chelate reducing activity of leaf protoplasts. Plant Physiol. 122:337–344.

Grillet L, Mari S, Schmidt W. 2014a. Iron in seeds – loading pathways and subcellular localization. Front Plant Sci. 4:1–8. doi:10.3389/fpls.2013.00535.

Grillet L, Ouerdane L, Flis P, Hoang MTT, Isaure MP, Lobinski R, Curie C, Mari S. 2014b. Ascorbate efflux as a new strategy for iron reduction and transport in plants. J Biol Chem. 289:2515–2525. doi:10.1074/jbc.M113.514828.

Gunzman P, Fernández V, Garcia ML, Khayet M, Fernández A, Gil L. 2014. Localization of polysachharides in isolated and intact cuticles of eucalypt, poplar and pear leaves by enzyme-gold labelling. Plant PhysiolBiochem. 76:1–6.

Gutierrez-Carbonell E, Lattazino G, Albacete A, Rios JJ, Kehr J, Abadía A, Grusak MA, Abadía J, López-Millán AF. 2015. Effects of Fe deficiency on the protein profile of *Brassica napus* phloem sap. Proteomics. 15:3835–3853. doi:10.1002/pmic.201400464.

Guzman M, Valenzuela JL, Sanchez A, Romero L. 1990. A method for diagnosis the status of horticulture crops. II. Micronutrients. Phyton Int J Exp Bot. 51:43–56.

Habib M. 2009. Effect of foliar application of Zn and Fe on wheat yield and quality. Afr J Biotechnol. 8:6795–6798.

Han J, Song X, Li P, Yang H, Yin L. 2009. Maize ZmFDR3 localized in chloroplasts is involved in iron transport. Sci China Life Sci. 52:864–871. doi:10.1007/s11427-009-0108-2.

He W, Shohag MJI, Wei Y, Feng Y, Yang X. 2013. Iron concentration, bioavailability, and nutritional quality of polished rice affected by different forms of foliar iron fertilizer. Food Chem. 141:4122–4126. doi:10.1016/j.foodchem.2013.07.005.

Hu J, Dong L, Outten CE. 2008. The redox environment in the mitochondrial intermembrane space is maintained separately from the cytosol and matrix. J Biol Chem. 283:29126–29134. doi:10.1074/jbc.M803028200.

Huang D, Dai W. 2015. Two iron-regulated transporter (IRT) genes showed differential expression in poplar trees under iron or zinc deficiency. J Plant Physiol. 186-187:59–67. doi:10.1016/j.jplph.2015.09.001.

Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakanishi H, Mori S, Nishizawa NK. 2009. Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J Biol Chem. 284:3470–3479. doi:10.1074/jbc.M806042200.

Inoue H, Takahashi M, Kobayashi T, Suzuki M, Nakanishi H, Mori S, Nishizawa NK. 2008. Identification and localisation of the rice nicotianamine aminotransferase gene *OsNAAT1* expression suggests the site of phytosiderophore synthesis in rice. Plant Mol Biol. 66:193–203. doi:10.1007/s11103-007-9262-8.

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

Jain A, Connolly EL. 2013. Mitochondrial iron transport and homeostasis in plants. Front Plant. Sci. 4:318. doi:10.3389/fpls.2013.00348

Jain A, Wilson GT, Connolly EL. 2014. The diverse roles of FRO family metalloreductases in iron and copper homeostasis. Front Plant Sci. 5:100. doi:10.3389/fpls.2014.00100.

Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, Bourguignon J. 2007. A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. Mol Cell Proteomics. 6.3:394–412. doi:10.1074/mcp.M600250-MCP200.

Jeong J, Cohu C, Kerkeb L, Pilon M, Connolly EL, Guerinot ML. 2008. Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions. Proc Natl Acad Sci USA. 105:10619–10624. doi:10.1073/pnas.0708367105.

Kakei Y, Ishimaru Y, Kobayashi T, Yamakawa T, Nakanishi H, Nishizawa NK. 2012. OsYSL16 plays a role in the allocation of iron. Plant Mol Biol. 79:583–594. doi:10.1007/s11103-012-9930-1.

Kannan S. 1969. Factors related to iron absorption by enzymically isolated leaf cells. Plant Physiol. 44:1457–1460.

Khan MA, Castro-Guerrero NA, McInturf SA, Nguyen NT, Dame AN, Wang J, Bindbeutel RK, Joshi T, Jurisson SS, Nusinow DA, et al. 2018. Changes in iron availability in Arabidopsis are rapidly sensed in the leaf vasculature and impaired sensing leads to opposite transcriptional programs in leaves and roots. Plant Cell Environ. 41:2263–2276. doi:10.1111/pce.13192.

Kim SA, Punshon T, Lanzirotti A, Liangtao L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML. 2006. Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. Science. 314:1295–1298. doi:10.1126/science.1132563.

Klatte M, Schuler M, Wirtz M, Fink-Straube C, Hell R, Bauer P. 2009. The analysis of Arabidopsis nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses. Plant Physiol. 150:257–271. doi:10.1104/pp.109.136374.

Kobayashi T, Itai RN, Aung MS, Senoura T, Nakanishi H, Nishizawa NK. 2012. The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status. Plant J. 69:81–91. doi:10.1111/j.1365-313X.2011.04772.x.

Kobayashi T, Nagasaka S, Senoura T, Itai RN, Nakanishi H, Nishizawa NK. 2013. Iron-binding haemerythrin RING ubiquitin ligases regulate plant iron responses and accumulation. Nat Comm. 4:1–12. doi:10.1038/ncomms3792.

Kobayashi T, Nozoye T, Nishizawa NK. 2019. Iron transport and its regulation in plants. Free Radic Biol Med. 133:11–20. doi:10.1016/j.freeradbiomed.2018.10.439.

Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2004. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J. 39:415–424. doi:10.1111/j.1365-313X.2004.02146.x.

Kosegarten HU, Hoffmann B, Mengel K. 1999. Apoplastic pH and Fe^{3+} reduction in intact sunflower leaves. Plant Physiol. 121:1069–1079.

Kruger C, Berkowitz O, Stephan UW, Hell R. 2002. A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis*L. J Biol Chem. 277:25062–25069. doi:10.1074/jbc.M201896200.

Kushnir S, Babiychuk E, Storozhenko S, Davey MW, Papenbrock J, De Rycke R, Engler G, Stephan UW, Lange H, Kispal G, et al. 2001. A mutation of the mitochondrial ABC transporter Sta1 leads to dwarfism and chlorosis in the Arabidopsis mutant starik. Plant Cell. 13:89–100.

Lanquar V, Lelievre F, Bolte S, Hames C, Alcon C, Neumann D, Vansuyt G, Curie C, Schroder A, Kramer U, et al. 2005. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. EMBO J. 24:4041–4051. doi:10.1038/sj.emboj.7600864.

Larbi A, Morales F, Abadía A, Abadía J. 2010. Changes in iron and organic acid concentrations in xylem sap and apoplastic fluid of iron-deficient *Beta vulgaris* plants in response to iron resupply. J Plant Physiol. 167:255–260. doi:10.1016/j.jplph.2009.09.007.

Larbi A, Morales F, Lopez-Millan AF, Gogorcena Y, Abadía A, Moong PR, Abadía J. 2001. Technical advance: reduction of Fe(III) chelates by mesophyll leaf disks of sugar beet. Multi-component origin and effects of Fe deficiency. Plant Cell Physiol. 42:94–105.

Lattanzio G, Sofía A, Matros A, Calvete JJ, Kehr J, Abadía A, Abadía J, López-Millán A. 2013. Protein profile of *Lupinus texensis* phloem sap exudates: searching for Fe- and Zn-containing proteins. Proteomics. 13:2283–2296. doi:10.1002/pmic.201200515.

Lin YF, Liang HM, Yang SY, Boch A, Clemens S, Chen CC, Wu JF, Huang JL, Yeh KC. 2009. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. New Phytol. 182:392–404. doi:10.1111/j.1469-8137.2009.02766.x.

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

Long TA, Tsukagoshi H, Busch W, Lahner B, Salt DE, Benfey PN. 2010. The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. Plant Cell Online. 22:2219–2236. doi:10.1105/tpc.110.074096.

López-Millán AF, Duy D, Philippar K. 2016. Chloroplast iron transport proteins-function and impact on plant physiology. Front Plant Sci. 7:1–12. doi:10.3389/fpls.2016.00001.

López-Millán AF, Morales F, Abadía A, Abadía J. 2000. Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. Plant Physiol. 124:873–884.

Lv J, Christie P, Zhang S. 2019. Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges. Environ Sci Nano. 6:41–59. doi:10.1039/C8EN00645H.

Mary V, Ramos MS, Gillet C, Socha AL, Giraudat J, Agorio A, Merlot S, Clairet C, Kim SA, Punshon T, et al. 2015. Bypassing iron storage in endodermal vacuoles rescues the iron mobilization defect in the natural resistance associated-macrophage protein3natural resistance associated-macrophage protein4 double mutant. Plant Physiol. 169:748–759. doi:10.1104/pp.15.00380.

Mendoza-Conzatl DG, Xie Q, Akmakjian GZ, Jobe TO, Patel A, Stacey MG, Song L, Demoin DW, Jurisson SS, Stacey G, et al. 2014. OPT3 is a component of the iron-signaling network between leaves and roots and misregulation of OPT3 leads to an over-accumulation of cadmium in seeds. Mol Plant. 7:1455–1469. doi:10.1093/mp/ssu067.

Mengel K. 2002. Alternative or complementary role of foliar supply in mineral nutrition. Acta Hortic. 594:33–47. doi:10.17660/ActaHortic.2002.594.1.

Moosavi AA, Ronaghi A. 2011. Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a Calcareous soil. Aust J Crop Sci. 5:1550.

Morrissey J, Guerinot ML. 2009. Iron uptake and transport in plants: the good, the bad and the ionome. Chem Rev. 109:4553–4567. doi:10.1021/cr900112r.

Mukherjee I, Campbell NH, Ash JS, Connolly EL. 2006. Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper. Planta. 22:31178–31190.

Müller B, Kovács K, Pham HD, Kavak Y, Pechoušek J, Machala L, Zbořil R, Szenthe K, Abadía J, Fodor F, et al. 2019. Chloroplasts preferentially take up ferric-citrate over iron-nicotianamine complexes in *Brassica napus*. Planta. 249:751–763. doi:10.1007/s00425-018-3037-0.

Naz S, Yousaf B, Tahir MA, Qadir A, Yousaf A. 2015. Iron and zinc bio-fortification strategies in wheat crop by exogenous application of micronutrients. Food Sci Qual Manage. 35:49–54.

Nikolic M, Cesco S, Römheld V, Varanini Z, Pinton R. 2003. Uptake of iron (59Fe) complexed to water-extractable humic substances by sunflowers leaves. J Plant Nutr. 26:2243–2252. doi:10.1081/PLN-120024278.

Nishiyama R, Kato M, Nagata S, Yanagisawa S, Yoneyama T. 2012. Identification of Zn-nicotianamine and Fe-2′-deoxymugienic acid in the phloem sap from rice plants (*Oryza sativa* L.). Plant Cell Physiol. 53:381–390. doi:10.1093/pcp/pcr188.

Nozoye T, Otani M, Senoura T, Nakanishi H, Nishizawa NK. 2017. Overexpression of barley nicotianamine synthase 1 confers tolerance in the sweet potato to iron deficiency in calcareous soil. Plant Soil. 418:75–88. doi:10.1007/s11104-016-3134-4.

Ogo Y, Itai RN, Kobayashi T, Aung AS, Nakanishi H, Nishizawa NK. 2011. OsIRO2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil. Plant Mol Biol. 75:593–605. doi:10.1007/s11103-011-9752-6.

Pahlavan-Rad MR, Pessarakli M. 2009. Response of wheat plants to zinc, iron, and manganese applications and uptake and concentration of zinc, iron, and manganese in wheat grains. Commun Soil Sci Plant Anal. 40:1322–1332. doi:10.1080/00103620902761262.

Pandey R, Krishnapriya V, Bindraban PS. 2013. Biochemical nutrient pathways in plants applied as foliar spray: phosphorus and iron. VFRC Report 2013/1. Washington (DC): Virtual Fertilizer Research Center.

Petit JM, Briat JF, Lobreaux S. 2001. Structure and differential expression of the four members of the *Arabidopsis thaliana* ferritin gene family. Biochem J. 359:575–582. doi:10.1042/0264-6021:3590575.

Pich A, Scholz G. 1996. Translocation of copper and other micronutrients in tomato plants (*Lycopersicon esculentum* Mill.): nicotianamine- stimulated copper transport in the xylem. J Exp Bot. 294:41–47. doi:10.1093/jxb/47.1.41.

Rajaie M, Tavakoly AR. 2018. Iron and/or acid foliar spray versus soil application of Fe-EDDHA for prevention of iron deficiency in Valencia orange grown on a calcareous soil. J Plant Nutr. 41:150–158.

Rakshit R, Patra AK, Purakayastha TJ, Singh RD, Pathak H, Dhar S. 2014. Super-optimal NPK along with foliar iron application influences bioavailability of iron and zinc of wheat. Proc Natl Acad Sci, India, Sect B Biol Sci. 86:159–164. doi:10.1007/s40011-014-0428-2.

Ravet K, Touraine B, Boucherez J, Briat JF, Gaymard F, Cellier F. 2009. Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. Plant J. 57:400–412. doi:10.1111/j.1365-313X.2008.03698.x.

Rellán-Álvarez AJ, Alvarez-Fernández A. 2008. Formation of metal-nicotianamine complexes as affected by pH, ligand exchange with citrate and metal exchange. A study by electrospray ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 22:1553–1562. doi:10.1002/rcm.3523.

Rellán-Álvarez R, Giner-Martinez-Sierra J, Orduna J, Orera I, Angel Rodríguez-Castrillón J, Ignacio García-Alonso J, Abadía J, Álvarez-Fernández A. 2010. Identification of a tri-iron(III), tri-citrate complex in the xylem sap of iron-deficient tomato resupplied with iron: new insights into plant iron long-distance transport. Plant Cell Physiol. 51:91–102. doi:10.1093/pcp/pcp170.

Rios JJ, Carrasco-Gil S, Abadía A, Abadía J. 2016. Using Perls staining to trace the iron uptake pathway in leaves of a Prunus rootstock treated with iron foliar fertilizers. Front Plant Sci. 7:1–13. doi:10.3389/fpls.2016.00001.

Rodríguez-Celma J, Ceballos-Laita L, Grusak MA, Abadía J, López-Millán A. 2016. Plant fluid proteomics: delving into the xylem sap, phloem sap and apoplastic fluid proteomes. Biochim Biophys Acta Proteins Proteom. 1864:991–1002. doi:10.1016/j.bbapap.2016.03.014.

Rodríguez-Celma J, Green RT, Connorton JM, Kruse I, Cui Y, Ling HQ, Balk J. 2018. BRUTUS-LIKE proteins moderate the transcriptional response to iron deficiency in roots. bioRxiv [Preprint]. doi:10.1101/231365.

Rombolà AD, Brüggemann W, Tagliavini M, Marangoni B, Moog PR. 2000. Iron source affects iron reduction and re-greening of kiwifruit (*Actinidia deliciosa*) leaves. J Plant Nutri. 23:1751–1765. doi:10.1080/01904160009382139.

Roschzttardtz H, Seguela-Arnaud M, Briat J, Vert G, Curie C. 2011. The FRD3 citrate effluxer promotes iron nutrition between symplastically disconnected tissues throughout Arabidopsis development. Plant Cell. 23:2725–2737. doi:10.1105/tpc.111.088088.

Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N. 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J Biol Chem. 279:9091–9096. doi:10.1074/jbc.M311799200.

Schagerlof U, Wilson G, Hebert H, Al-Karadaghi HC. 2006. Transmembrane topology of FRO2, a ferric chelate reductase from *Arabidopsis thaliana*. Plant Mol Biol. 62:215–221. doi:10.1007/s11103-006-9015-0.

Schlegel TK, Schonherr J, Schreiber L. 2006. Rates of foliar penetration of chelated Fe(III): role of light, stomata, species, and leaf age. J Agric Food Chem. 54:6809–6813. doi:10.1021/jf061149i.

Schmidt W. 1999. Mechanisms and regulation of reduction-based iron uptake in plants. New Phytol. 141:1–26. doi:10.1046/j.1469-8137.1999.00331.x.

Schonherr J. 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. J Exp Bot. 57:2471–2491. doi:10.1093/jxb/erj217.

Schuler M, Rellán-Álvarez R, Fink-Straube C, Abadía J, Bauer P. 2012. Nicotianamine functions in the phloem-based transport of iron to sink organs, in pollen development and in pollen tube growth in *Arabidopsis*. Plant Cell. 24:2380–2400. doi:10.1105/tpc.112.099077.

Severina IS, Bussygina OG, Pyatakova NV, Malenkova IV, Vanin AF. 2003. Activation of soluble guanylate cyclase by NO donors, S-nitrosothiols, and dinitrosyl–iron complexes with thiol containing ligands. Nitric Oxide. 8:155–163.

Shahid M, Dumat C, Khalid S, Schreck E, Xiong T, Niazi NK. 2016. Foliar heavy metal uptake, toxicity and detoxification in plants: a comparison of foliar and root metal uptake. J Hazard Mater. 325:36–58. doi:10.1016/j.jhazmat.2016.11.063.

Shanmugam V, Wang Y, Tsednee M, Karunakaran K, Yeh K. 2015. Glutathione plays an essential role in nitric oxide-mediated iron-deficiency signaling and iron-deficiency tolerance in *Arabidopsis*. Plant J. 84:464–477. doi:10.1111/tpj.13011.

Sharma S, Malhotra H, Borah P, Meena MK, Bindraban P, Chandra S, Pande V, Pandey R. 2018. Foliar application of organic and inorganic iron formulation induces differential detoxification response to improve growth and biofortification in soybean. Indian J Plant Physiol. doi:10.1007/s40502-018-0412-6

Shingles R, North M, McCarty RE. 2002. Ferrous ion transport across chloroplast inner envelope membranes. Plant Physiol. 128:1022–1030. doi:10.1104/pp.010858.

Sida-Arreola JP, Sanchez-Chavez E, Avila-Quezada GD, Zamudio-Flores PB, Muniz CHA. 2015. Iron biofortification and its impact on antioxidant system, yield and biomass in common bean. Plant Soil Environ. 61:573–576.

Singh SP, Keller B, Gruissem W, Bhullar NK. 2017. Rice nicotianamine synthase 2 expression improves dietary iron and zinc levels in wheat. Theor Appl Genet. 130:283–292. doi:10.1007/s00122-016-2808-x.

Solti A, Kovacs K, Basa B, Vertes A, Sarvari E, Fodor F. 2012. Uptake and incorporation of iron in sugar beet chloroplasts. Plant Physiol Biochem. 52:91–97. doi:10.1016/j.plaphy.2011.11.010.

Solti A, Muller B, Czech V, Sarvari E, Fodor F. 2014. Functional characterization of the chloroplast ferric chelate oxidoreductase enzyme. New Phytol. 202:920–928. doi:10.1111/nph.12715.

Song YL, Dong YZ, Tian XY, Wang WW, He ZL. 2017. Effects of nitric oxide and Fe supply on recovery of Fe deficiency induced chlorosis in peanut plants. Biol Planta. 61:155–168. doi:10.1007/s10535-016-0642-2.

Stacey MG, Koh S, Becker J, Stacey G. 2002. AtOPT3, a member of the oligopeptide transporter family, is essential for embryo development in Arabidopsis. Plant Cell. 14:2799–2811.

Stacey MG, Osawa H, Patel A, Gassmann W, Stacey G. 2006. Expression analyses of *Arabidopsis* oligopeptide transporters during seed germination, vegetative growth and reproduction. Planta. 223:291–305. doi:10.1007/s00425-005-0087-x.

Stacey MG, Patel A, McClain WE, Mathieu M, Remley M, Rogers EE, Gassmann W, Blevins DG, Stacey G. 2008. The Arabidopsis AtOPT3 protein functions in metal homeostasis and movement of iron to developing seeds. Plant Physiol. 146:589–601. doi:10.1104/pp.107.108183.

Sudre D, Gutierrez-Carbonell E, Lattanzio G, Rellan-Alvarez R, Gaymard F, Wohlgemuth G, Fiehn O, Alvarez-Fernández A, Zamarreno AM, Bacaicoa E, et al. 2013. Iron-dependent modifications of the flower transcriptome, proteome, metabolome, and hormonal content in an *Arabidopsis* ferritin mutant. J Exp Bot. 10:2665–2688. doi:10.1093/jxb/ert112.

Tagliavini M, Abadía J, Rombola AD, Abadía A, Tsipouridis C, Marangoni B. 2000. Agronomic means for the control of iron deficiency chlorosis in deciduous fruit trees. J Plant Nutr. 23:2007–2022. doi:10.1080/01904160009382161.

Takahashi M, Terada Y, Nakai I, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK. 2003. Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell. 15:1263–1280.

Tanou G, Ziogas V, Molassiotis A. 2017. Foliar nutrition, biostimulants and prime-like dynamics in fruit tree physiology: new insights on an old topic. Front Plant Sci. 8:1–9. doi:10.3389/fpls.2017.00001.

Tarantino D, Morandini P, Ramirez L, Soave C, Murgia I. 2011. Identification of an *Arabidopsis mitoferrin like* carrier protein involved in Fe metabolism. Plant Physiol Biochem. 49:520–529. doi:10.1016/j.plaphy.2011.02.003.

Tarantino D, Santo N, Morandini P, Casagrande F, Braun HP, Heinemeyer J, Vigani G, Soave C, Murgia I. 2010. AtFer4 ferritin is a determinant of iron homeostasis in *Arabidopsis thaliana* heterotrophic cells. J Plant Physiol. 167:1598–1605. doi:10.1016/j.jplph.2010.06.020.

Teng YS, Su YS, Chen LJ, Lee YJ, Hwang I, Li HM. 2006. Tic21 is an essential translocon component for protein translocation across the chloroplast inner envelope membrane. Plant Cell. 18:2247–2257. doi:10.1105/tpc.106.044305.

Tsukamoto T, Nakanishi H, Uchida H, Watanabe S, Matsuhashi S, Mori S, Nishizawa NK. 2009. ^{52}Fe translocation in barley as monitored by a positron emitting tracer imaging system (PETIS): evidence for the direct translocation of Fe from roots to young leaves via phloem. Plant Cell Physiol. 50:48–57. doi:10.1093/pcp/pcn192.

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat JF, Curie C. 2002. IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. Plant Cell. 14:1223–1233.

Watts RN, Richardson DR. 2002. The mechanism of nitrogen monoxide (NO)-mediated iron mobilization from cells. NO intercepts iron before incorporation into ferritin and indirectly mobilizes iron from ferritin in a glutathione-dependent manner. Eur J Biochem. 269:3383–3392.

Wu H, Li L, Du J, Yuan Y, Cheng X, Ling HQ. 2005. Molecular and biochemical characterization of the Fe(III) chelate reductase gene family in *Arabidopsis thaliana*. Plant Cell Physiol. 46:1505–1514. doi:10.1093/pcp/pci163.

Wu TY, Gruissem W, Bhullar NK. 2018. Facilitated citrate-dependent iron translocation increases rice endosperm iron and zinc concentrations. Plant Sci. 270:13–22. doi:10.1016/j.plantsci.2018.02.002.

Zancani M, Peresson C, Biroccio A, Federici G, Urbani A, Murgia I, Soave C, Micali F, Vianello A, Macri F. 2004. Evidence for the presence of ferritin in plant mitochondria. Eur J Biochem. 271:3657–3664. doi:10.1111/j.1432-1033.2004.04300.x.

Zhai Z, Gayomba SR, Jung HI, Vimalakumari NK, Pineros M, Craft E, Rutzke MA, Danku J, Lahner B, Punshon T, et al. 2014. OPT3 is a phloem-specific iron transporter that is essential for systemic iron signaling and redistribution of iron and cadmium in Arabidopsis. Plant Cell. 26:2249–2264. doi:10.1105/tpc.114.123737.

Zhang C, Shinwari KI, Luo L, Zheng L. 2018. *OsYSL13* is involved in iron distribution in rice. Int J Mol Sci. 19:1–14. doi:10.3390/ijms19113537.

Zhang Y, Xu YH, Yi HY, Gong JM. 2012. Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. Plant J. 72:400–410. doi:10.1111/j.1365-313X.2012.05088.x.

Zuo J, Wu Z, Li Y, Shen Z, Xiangyang F, Zhang M, Ye H. 2017. Mitochondrial ABC transporter ATM3 is essential for cytosolic iron-sulfur cluster assembly. Plant Physiol. 173:2096–2109. doi:10.1104/pp.16.01760.