



## Iron toxicity: effects on the plants and detoxification strategies

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Received: April 17, 2021

Accepted: December 02, 2021

### ABSTRACT

Iron (Fe) is an essential micronutrient for plants, as a cofactor in multi-heme cytochromes and within iron-sulfur clusters. However, Fe can be toxic at high concentrations. Free Fe in cells can disrupt the cell redox balance toward a pro-oxidant state, generating oxidative stress. The focuses of this review were to elucidate the Fe detoxification strategies used by plants, as well as describe the Fe excess effects on the plant body and its impact on the physiological, morphological and metabolic traits. Therefore, we highlight the importance of evaluating Fe toxicity and provide a paper compilation on Fe detoxification strategies and morpho-physiological responses to excess Fe, directing further research in this segment.

**Keywords:** antioxidant defense system, Casparian strips, iron histolocalization, iron plaque, ferritin

## Introduction

Iron (Fe) is an essential micronutrient for plants (Krohling *et al.* 2016). This element is present in the form of cofactors in multi-heme cytochromes and within iron-sulfur (Fe-S) clusters (Ferousi *et al.* 2017). The Fe is required for several key biological functions in plants, such as photosynthesis, mitochondrial respiration, nitrogen fixation and metabolism, sulfur assimilation, and hormone and DNA synthesis (Balk & Pilon 2011; Ibañez *et al.* 2021). However, although Fe is highly abundant in the earth's crust, it is poorly available to plants under alkaline and oxidative conditions (Lei *et al.* 2014). According to Araújo *et al.* (2014) and Grillet & Schmidt (2014), the solubility

of Fe<sup>3+</sup> (the ferric state) decreases as pH increases, while Fe<sup>2+</sup> (the ferrous state) is easily oxidized in aerated soils, which can cause Fe deficiency in plants (Kaya *et al.* 2020).

On the other hand, when occurring in high concentrations in plant tissue (above 500 mg Fe kg<sup>-1</sup> leaf dry mass), Fe can disrupt the cell redox balance toward a pro-oxidant state, inducing alterations in the morphological, metabolic, and physiological traits of the plants and generating oxidative stress (Siqueira-Silva *et al.* 2012; Jucoski *et al.* 2013). Under Fe excess, plants adopt different strategies to prevent uptake and the free Fe in the cell from reacting with O<sub>2</sub> (Fig. 1) (Saaltink *et al.* 2017; Araújo *et al.* 2020a).

Iron toxicity can result from environmental disasters promoted by human activities associated with the Fe processing makes Fe excess an environmental problem (Xing

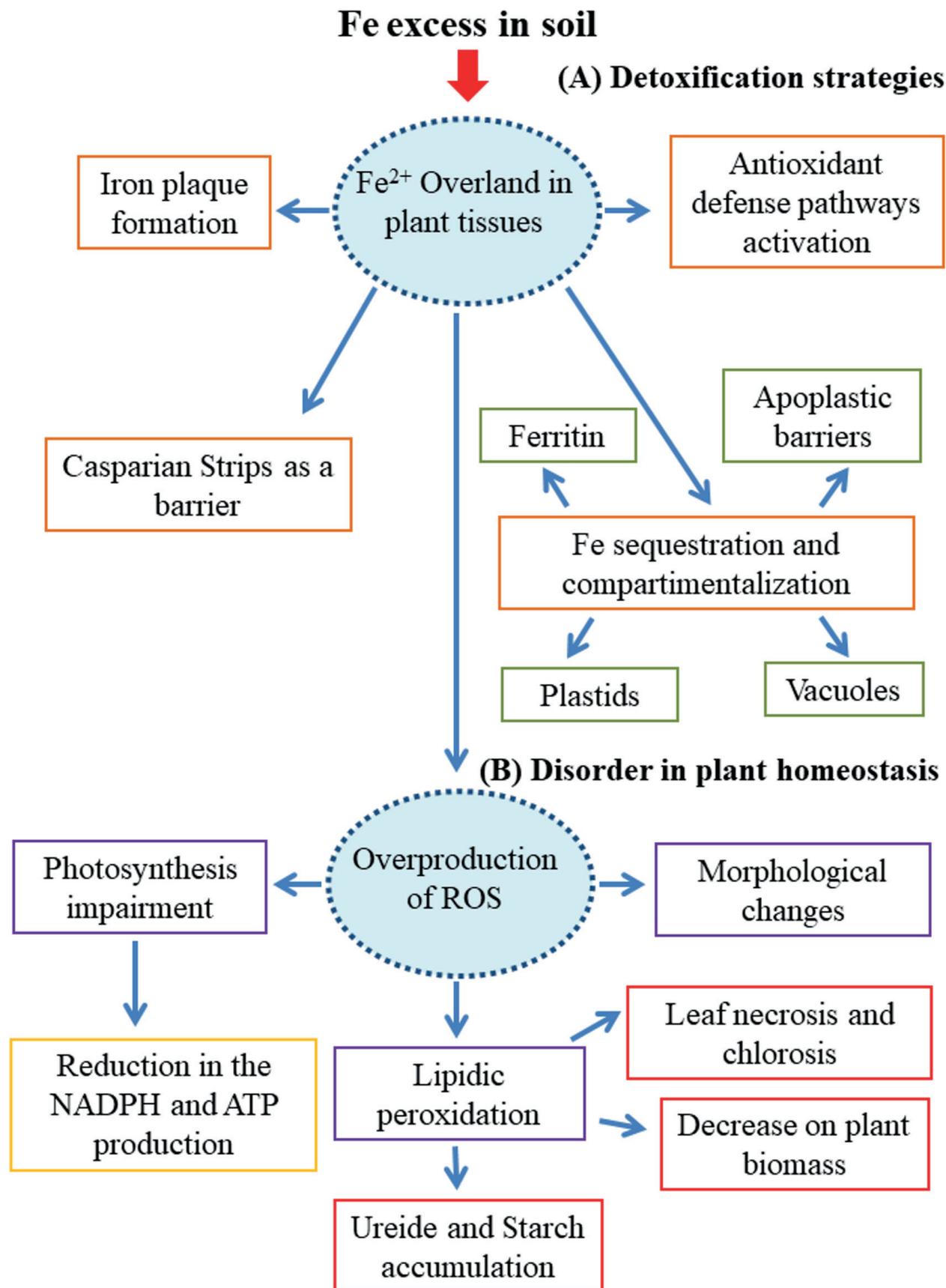
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**Figure 1.** Detoxification strategies (A) and disorder in plant homeostasis (B) in response to iron excess.



*et al.* 2010; Araújo *et al.* 2014; Cordeiro *et al.* 2019; Valeriano *et al.* 2019). Furthermore, Fe toxicity is a common problem in some areas susceptible to soil waterlogging, resulting in an exponential increase in Fe availability, especially in acid soils (Lapaz *et al.* 2020). Soil waterlogging can be caused by inappropriate irrigation, high water tables, after heavy rainfall (mainly on compacted soils with poor natural drainage), and in lowland soils (Frei *et al.* 2016; Krohling *et al.* 2016; Maranguit *et al.* 2017). Additionally, some soils naturally present high concentrations of Fe, such as the ferruginous rocky outcrops (Rocha *et al.* 2020) and acid sulfate soils with concentrations of up to 5000 mg kg<sup>-1</sup> Fe (Becker & Asch 2005).

The increased availability of Fe in waterlogged soil is due to the activities carried out by microorganisms present in the soil to maintain its metabolism through the oxidation of organic matter and their use as final electron acceptors. In this situation, aerobic microorganisms consume all the molecular O<sub>2</sub> as a final electron acceptor and then die from a lack of O<sub>2</sub>. Hence, only anaerobic and facultative anaerobic microorganisms remain in the soil. These microorganisms utilize alternative electron acceptors, preferring those that allow the highest energy yields or that are most readily available, such as Fe<sup>3+</sup> (Maranguit *et al.* 2017; Lapaz *et al.* 2020). Thus, the insoluble Fe<sup>3+</sup> oxides are reduced into a more soluble form (Fe<sup>2+</sup>), which is released into soil pore water and can result in absorption of excess Fe (Lapaz *et al.* 2020).

Therefore, this review aimed to elucidate Fe detoxification strategies used by plants and to report the most recent findings involved in these response mechanisms, in order to highlight the importance of studying Fe toxicity and show its effect on physiological, morphological and metabolic traits. This compilation should be useful to guide new research in this field of study.

### *Iron detoxification strategies used by plants*

#### *Inhibition of Fe uptake*

Mechanisms for dealing with Fe toxicity in plants may be classified into indirect or direct responses (Saaltink *et al.* 2017). The indirect response is associated with the inhibition of Fe uptake, while the direct response occurs with accumulation of free Fe in the plant (Krohling *et al.* 2016; Saaltink *et al.* 2017). In the indirect response, the iron plaque (IP) is formed on the root surface of plants exposed to Fe excess (Fig. 2F-H) (Araújo *et al.* 2020a, b), that can inhibit the absorption of Fe on the root surface after oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup> (Krohling *et al.* 2016; Araújo *et al.* 2020a). On the other hand, in plants exposed to adequate conditions of Fe availability, the IP is not formed (Fig. 2E). Thus, its available quantity in the soil will decrease, forming a smooth, regular precipitate or irregular plaque coating (Siqueira-Silva *et al.* 2012; Cheng *et al.* 2014). It is believed that IP formation is controlled by soil Fe availability and the oxidizing capacity of roots, that is, by oxygen radical

loss (Li *et al.* 2017) and/or microbiological oxidation (Wu *et al.* 2016).

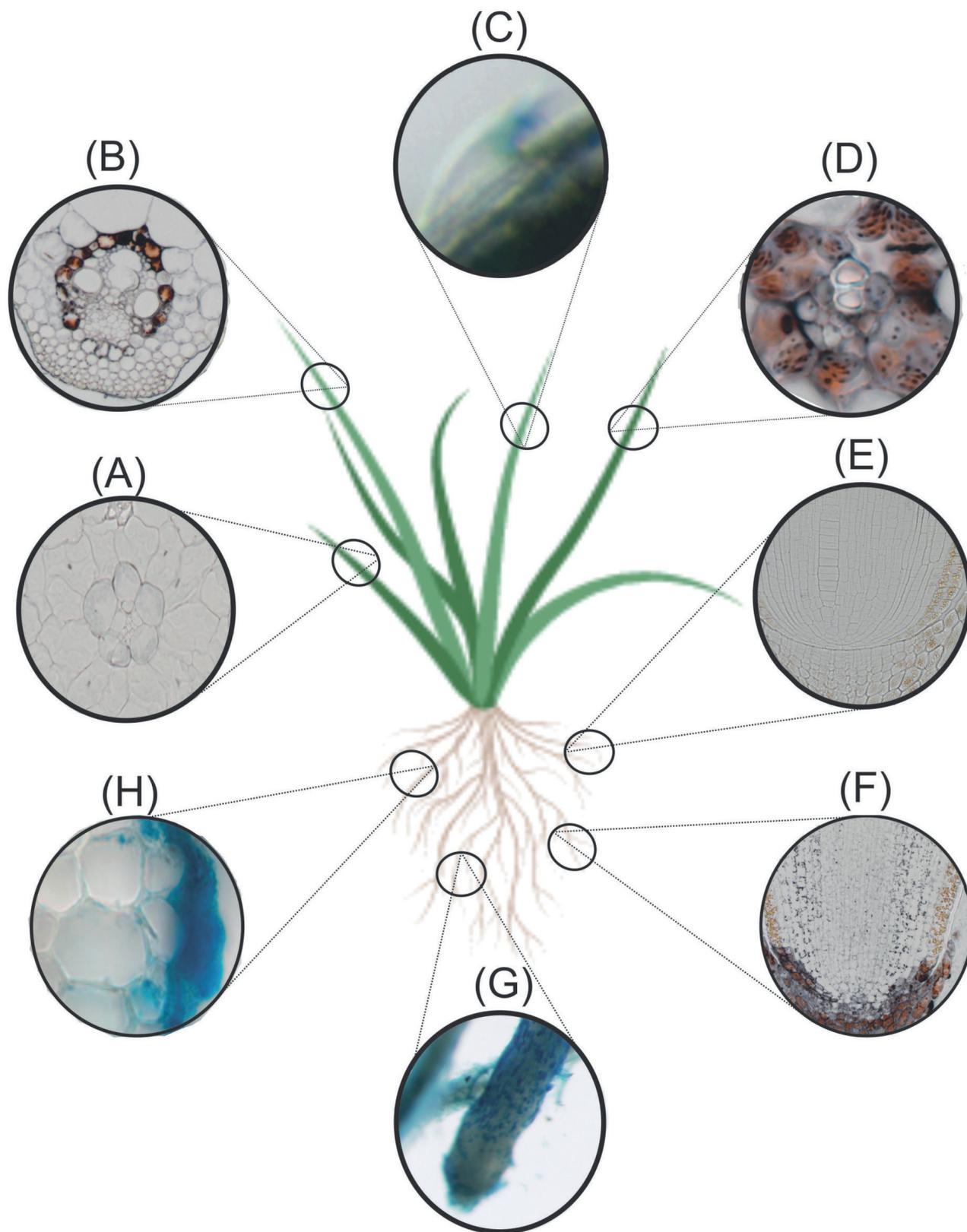
The components of the IP will depend on the biogeochemical factors in which the plant grows (Tripathi *et al.* 2014). The IP comprises a mixture of crystalline and amorphous Fe (oxyhydr) oxides, mainly Fe<sup>3+</sup> minerals such as lepidocrocite, goethite, or ferrihydrite (Pardo *et al.* 2016). Other minerals have also been reported on the root surface, forming the IP, such as jarosite in *Imperata cylindrica* (Amils *et al.* 2007) and siderite and ferric phosphate in *Typha latifolia* (Hansel *et al.* 2001).

The Fe hydroxide present in the IP can react with electrolytes, such as metals, metalloids, and nutrients, to form complexes in the IP (Cheng *et al.* 2014; Zhang *et al.* 2019). The role of the interaction of the IP with the electrolytes is controversial. Some studies have shown that the IP acts as a buffer (Tripathi *et al.* 2014). When plants lack nutrients, they are remobilized from the IP so that the roots can capture them. For example, Ye *et al.* (2001) observed in *Typha latifolia* that IP can act as a Cu reservoir. In contrast, other studies have reported that the IP acts as a physical barrier or an adsorbent that inhibits the uptake, such as As (Wu *et al.* 2016) and Cd (Huang *et al.* 2020) in rice, among others (Tripathi *et al.* 2014). These results show that further research is needed to understand whether the role of IP may vary between plants and/or is a result of different interactions of electrolytes with IP, since the -OH functional group is present in IP and has a high affinity with metals and with some ions.

#### *Role of Casparian strips in preventing Fe translocation*

In some plants, the endoderm forms an apoplastic barrier that can prevent the assimilation of metals (Siqueira-Silva *et al.* 2019). The endoderm includes the innermost cortical layer surrounding the stele. It is responsible for controlling root waterproofing by undergoing two differentiation states: (I) impregnation of cell walls with lignin (giving rise to Casparian strips), followed by (II) addition of suberin lamellae (Doblas *et al.* 2017). In hyperaccumulator plants, the importance of apoplastic barriers in preventing Fe uptake has been verified (Fig. 1A). For example, Siqueira-Silva *et al.* (2019) showed that removal of the root apex negatively influences Fe tolerance and avoidance mechanisms in *Paspalum densum* and *Echinochloa crus-galli*. Araújo *et al.* (2020a) observed that the endodermis plays a central role in the control of Fe excess in the vascular system in *P. urvillei* and *Setaria parviflora*, while in *O. sativa*, the endodermis is not such a barrier for the movement of Fe toward the stele. The different responses in the control of Fe traffic toward the stele may be explained by the fact that not all species and/or cultivars use the same mechanisms to mitigate excess Fe, including the suberization of Casparian strips. Barberon *et al.* (2016) demonstrated that suberization is substantially reduced in *IRT1* mutants with Fe deficiency in *Arabidopsis*, allowing apoplastic and transcellular Fe





**Figure 2.** Iron localization in leaves (A-D) and roots (E-H) of *Setaria viridis* treated with 0.1 mM (A, E) or 7 mM (B-D, F-H) Fe-Citrate during six days in Hoagland's solution. Fresh organs (C, G) or sections of samples fixed and embedded in resin (A, B, D-F, H) were submitted to Perls staining (C, G, H) or Perls/DAB staining (A, B, D-F). Positive staining for the presence of iron occurred in bundle sheath cells (B, D), chloroplasts (D), ferritin (D, F), vacuole (B), trichome (C), iron plaque (F-G). Source: photos taken by author Talita de Oliveira Araújo.



pathways. Suberization is mediated by the hormones abscisic acid (ABA) and ethylene, which are positive and negative regulators for this response, respectively (Curie & Mari 2017).

### *Fe sequestration and compartmentalization*

Excess Fe in the plant is a potential oxidative stress inducer (Lapaz *et al.* 2020). In view of this, internal detoxification mechanisms are used by plants as strategies to prevent Fe from reacting with O<sub>2</sub> without affecting the plant's functional demand (Siqueira-Silva *et al.* 2012; Darbani *et al.* 2013). The excess Fe is sequestered in vacuoles (Fig. 2B), plastids (Fig. 2D), and/or apoplastic compartments, away from highly sensitive intracellular sites (Araújo *et al.* 2020a), followed by compartmentalization between the various kinds of organs, including trichomes (Fig. 2C) (Thomine & Vert 2013) or restriction of the compartmentalization of Fe within the root, in order to limit the translocation of Fe toward the shoots (Müller *et al.* 2017; Bomfim *et al.* 2021). On the other hand, in plants exposed to adequate conditions of Fe availability, there was no deposition of Fe in vacuoles (Fig. 2A).

The compartmentalization of free Fe tends to dilute its quantity within plant cells. Due to its potential toxicity, Fe is translocated through the plant body associated with chelating molecules and under the proper control of redox states between the ferrous and ferric forms (Kobayashi & Nishizawa 2012). Fe<sup>2+</sup>-nicotianamine (NA) complex is mainly involved in the subcellular distribution and inter-organ partitioning of Fe by the phloem, while Fe<sup>2+</sup>-citrate is considered the main form in which Fe is transported by the xylem (Kobayashi *et al.* 2019). Iron was histolocalized in all tissues of the lateral roots of *Ipomoea pes-caprae* and *Canavalia rosea*: epidermis, including root hair, cortical parenchyma, exodermis, endodermis, pericycle, xylem, and phloem (Siqueira-Silva *et al.* 2012). In roots of *P. urvillei*, Fe is strongly histolocalized in the epidermis, aerenchyma, endodermis, pericycle, phloem, and protoxylem, whereas in *S. parviflora*, Fe is histolocalized in the epidermis, phloem, and xylem cells. The two species also showed a positive reaction for Fe histolocalization in cortex cells and in protoxylem and metaxylem cell walls (Araújo *et al.* 2015).

High Fe accumulation also occurs in the leaves in different plant tissue like spongy parenchyma cells and parenchyma cells of xylem on leaves of *Avicennia schaueriana* and *Laguncularia racemosa*, respectively (Arrivabene *et al.* 2015) and in epidermal cells of leaves of *C. hilariana* (Silva *et al.* 2017), suggests the storage of Fe in these tissues as the detoxification strategy in these species for the Fe excess. In *P. urvillei*, *S. parviflora*, *S. viridis*, and *O. sativa*, Fe accumulation was observed in different cellular compartments in the leaves. The highest Fe accumulation, common to all species, was found in the bundle sheath cells. Within these cells, Fe is highly accumulated in the central vacuole as ferric oxide (Araújo *et al.* 2020a). In *Arabidopsis*, the influx of Fe into the

vacuoles is mediated via the *FPN2* transporter (Morrissey & Gueriot 2009), while *VIT1* has a specific function in the vacuolar transport of Fe into xylem parenchyma of developing embryos (Gollhofer *et al.* 2014). The *NRAMP3* and *NRAMP4* transporters, when induced by Fe deficiency, export it out of the vacuoles (Darbani *et al.* 2013; Thomine & Vert 2013).

### *Fe sequestration as ferritin complexes*

Ferritin has a double function in plants: Fe detoxification and storage (Figs. 2D, 2F). Ferritins contain a hollow spherical shell of 24 subunits that can bind up to 4500 Fe atoms in their nucleus (Briat *et al.* 2010; Araújo *et al.* 2020a). They are present in chloroplasts and mitochondria (Nouet *et al.* 2011), which are quantitatively more important for Fe use (Thomine & Vert 2013), as well as they are also present in others plastids located in different plant tissues (Nouet *et al.* 2011), which are organelles with the greatest potential for Fe detoxification. In addition, ferritins have been found in cell walls and cytoplasm in *I. cylindrica*, amplifying the known distribution of this structure within the plant (Fuente *et al.* 2012). In the chloroplast, the reduction of Fe<sup>3+</sup> via FRO7 (ferric reductase oxidase) is required for incorporation into chloroplasts (Krohling *et al.* 2016). PIC1 is the permease that imports Fe into the chloroplast. This permease is likely a member of a larger Fe-import complex together with NiCo transporter, where Fe is bound by NiCo first and subsequently transferred to PIC1 (Duy *et al.* 2011; Müller *et al.* 2019), while permease MIT is involved in transporting Fe into mitochondria after reduction of Fe<sup>3+</sup> (Kobayashi *et al.* 2019; Malhotra *et al.* 2020).

Ferritins are encoded by four genes in *Arabidopsis* (*AtFer1* to *AtFer4*) and are regulated mainly at the transcriptional level (Briat *et al.* 2010). Fe excess and oxidative stress promote *AtFer1* gene expression through two independent and additive pathways (Briat *et al.* 2010). *AtFer3* expression in response to excess Fe is very similar to the *AtFer1* gene, while *AtFer2* is induced by ABA (Petit *et al.* 2001). However, ferritin-null mutants in *A. thaliana* are less affected by Fe excess (Ravet *et al.* 2009), despite the high Fe-buffering capacity of ferritins. This finding opens up avenues for further research on the role of these proteins in Fe detoxification mechanisms in different crops. Müller *et al.* (2017) investigated the tolerance responses to excess Fe and suggested that ferritin may contribute to growth and survival after observing a strong increase in *OsFER1* expression in the leaves of *O. sativa* cultivars. Wu *et al.* (2017) also found an increase in ferritin expression in rice cultivars with contrasting tolerance to Fe, but no genotypic differences were observed. DeLaat *et al.* (2014), studying *Phaseolus vulgaris*, found that water deficit combined with excess Fe substantially increased the expression of three ferritin genes (*PvFer1*, *PvFer2*, and *PvFer3*), but with different kinetics.



Some research has reported the role of the cell wall in response to Fe deficiency in the fixation and redistribution of Fe between roots and shoots (Lei *et al.* 2014; Ye *et al.* 2015, Zhu *et al.* 2016). These responses are related to the traits of cell wall components, in particular pectin and hemicellulose, which are highly negatively charged polysaccharides and thus represent a sink for cationic nutrients (Curie & Mari 2017). According to Fuente *et al.* (2012) and Araújo *et al.* (2020a), the cell wall contains a large pool of high Fe concentrations in the plant. Fuente *et al.* (2012) suggested that the deposit of jarosite on the cell wall in *I. cylindrica* may be related to the degradation of ferritin and phytosiderin. In *P. urvillei*, *S. parviflora*, and *O. sativa*, the chemical form of this pool of Fe in the cell wall has not been identified, whereas *S. viridis* accumulates Fe in ferritins (Araújo *et al.* 2020a). Based on reports from the literature, the characterization of biomineralized Fe deposits is limited and the signaling mechanisms involved in this process as a strategy to detoxify excess Fe have not yet been described. Hence, there is a need for further research in this segment, as well as verifying these responses in different species.

#### *Tolerance to the generation of reactive oxygen species (ROS)*

When excessive absorption and the accumulation of free Fe occur in plant tissue (Fig. 1A), the cell redox balance can be displaced to a pro-oxidant state and generate oxidative stress (Jucoski *et al.* 2013; Müller *et al.* 2017; Lapaz *et al.* 2020). Therefore, Fe excess could potentially increase the overproduction of ROS (Fig. 1B) (Pinto *et al.* 2016; Araújo *et al.* 2020b).

In this context, accumulation of Fe<sup>2+</sup> becomes highly toxic to plants cells because it catalyzes hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition, generating the hydroxyl radical (HO<sup>•</sup>), according to the Fenton reaction: Fe<sup>3+</sup> produced by this reaction can be reduced to Fe<sup>2+</sup> by the superoxide anion radical (O<sub>2</sub><sup>•-</sup>) via the Haber–Weiss reaction, allowing Fe<sup>2+</sup> to again participate in the Fenton reaction, according to the following reaction: (Becana *et al.* 1998; Lapaz *et al.* 2020). ROS can lead to peroxidation, including cell collapse and even tissue deterioration. In addition, they cause the oxidation of sugars, proteins, nucleic acids, and lipids, electron transport disruption, and enzyme inhibition/activation (Pereira *et al.* 2009; Xu *et al.* 2015).

To contain oxidative stress, plants respond by activating enzymatic antioxidant defense pathways (superoxide dismutase – SOD (EC 1.15.1.1), catalase – CAT (EC 1.11.1.6), peroxidase – POX (EC 1.11.1.7), ascorbate peroxidase – APX (EC 1.11.1.11), glutathione peroxidase – GPX (EC 1.11.1.9), and glutathione reductase – GR (EC 1.6.4.2)) and/or non-enzymatic antioxidant defense pathways (ascorbate – AA, glutathione – GSH, carotenoids, tocopherol, ubiquinol, uric acid, and lipoic acid) (Jucoski *et al.* 2013, Krohling *et al.* 2016). The combined SOD and POX enzyme activity has been established to be largely responsible for preventing Fe<sup>2+</sup>-induced oxidative stress in *O. sativa* leaves (Becker &

Asch 2005). Hence, the increasing antioxidant potential of plants is considered as one of the useful strategies to mitigate the effects of ROS overload (Ahammed *et al.* 2020). However, there is a threshold of enzyme activity; that is, the protective function of antioxidant enzymes may be limited in the face of an exorbitant production of ROS (Xing *et al.* 2010).

#### *Fe impacts on physiological, morphological and metabolic traits*

The different strategies adopted by Fe-resistant species involve mechanisms to neutralize the damage caused by the Fe presence (Fig. 1A) (Siqueira-Silva *et al.* 2012; Krohling *et al.* 2016; Kobayashi *et al.* 2019; Siqueira-Silva *et al.* 2019; Araújo *et al.* 2020a), while sensitive species, in turn, may be strongly impacted by Fe (Fig. 1B) (Neves *et al.* 2009). Iron ore industries can disturb the nearby vegetation (Silva *et al.* 2017; Silva *et al.* 2020) which deserves attention because the exposition of sensitive species to Fe leads to a decrease in biodiversity over the years (Arrivabene *et al.* 2015). The loss of the structure and function of cell membrane promoted by the lipid peroxidation due to the ROS excess promotes changes in the plant cells (Araújo *et al.* 2020b), which can compromise the anatomy of organs and their functionality, thus impair key plant processes.

Iron toxicity is a complex phenomenon and dependent on many different aspects, such as the sensitivity of the species, the plant organ, time of Fe exposure, Fe soil concentration, soil pH, exchangeable Fe content and Fe uptake and its translocation in plant body (Becker & Asch 2005; Nagajyoti *et al.* 2010; Pandey & Verma 2019). In *I. pes-caprae* roots, Siqueira-Silva *et al.* (2012) found that Fe promoted morphological changes like growth retarding, flaccidity and decreased branching, necrosis and collapse of the apex of the lateral roots. Santana *et al.* (2014) described derangement of mesophyll cells, presence of hypertrophied cells alteration on wall shape and differentiation of metaxylem elements, decreased volume of bulliform cells as an anatomical alteration in tolerant grass species to Fe excess.

The Leaf bronzing (*i.e.*, coloration caused by the accumulation of phenols in the vacuole) is commonly indicated as a typical symptom of stress caused by Fe excess (Wu *et al.* 2014; Pinto *et al.* 2016). Silva *et al.* (2017) described chlorosis, necrosis, foliar abscission and spotted necrosis, purplish spots on the leaves and an increase in the emission of new leaves completely purplish as visual symptoms found in *C. hilariana* and *Eugenia uniflora* plants growing near a Fe pelletizing factory. Moreover, Zhang *et al.* (2016) noticed that Fe toxicity can promote a global and progressive disorder in cell protoplasm, generating a deformed and shrunken appearance in the cell, which may lead toward programmed cell death.

The Fe histolocalization in plant tissues through histochemical methods is a complementary tool for studies of Fe toxicity in plants because it allows to spatially



characterize the distribution of the element in the different tissues and even organelles of the cell, showing the main sites of accumulation on the plant body (Silva *et al.* 2006; Sivaprakash *et al.* 2006). Perls/DAB method (Roschztardt *et al.* 2009) and Prussian Blue (Stevens & Chalk 1996) for instance are used to highlight iron in the plant tissues. Several authors used histolocalization techniques to show Fe accumulation in tissues such as endodermis, epidermal cells, shoots, xylem vessels, and organelles such as chloroplasts (Di Toppi *et al.* 2012; Arrivabene *et al.* 2015; Silva *et al.* 2017; Araújo *et al.* 2020a).

The impact of Fe toxicity on physiological traits can reflect a decrease in gas exchange traits and chlorophyll content, deactivation of PSII reaction center and a decrease in saturated fatty acids and increase unsaturated fatty acids in chloroplast membrane in *Pisum sativum* (Xu *et al.* 2015). Pereira *et al.* (2013) observed a decrease in photosynthesis rate in *O. sativa* due to stomatal and non-stomatal limitations, with non-stomatal limitation more severe in the most sensitive cultivar. Müller *et al.* (2017) studying *O. sativa*, found that upland cultivars displayed a mechanism to limit Fe translocation from roots to the shoots, minimizing leaf oxidative stress induced by excess Fe, while lowland cultivar invested in the increase of CO<sub>2</sub> production rate, as an alternative drain of electrons. In *Ipomoea batatas*, it was observed that exposure to excess Fe caused an increase in chlorophyll content and a decline in net CO<sub>2</sub> assimilation rate, as well as a reduction in the production of nicotinamide adenine dinucleotide (NADPH) and adenosine triphosphate (ATP) (Adamski *et al.* 2011). According to Lapaz *et al.* (2020), starch and ureide accumulation could be considered efficient biomarkers of phytotoxicity caused by soil waterlogging and Fe excess in *Glycine max*.

### Conclusions and future perspectives

Strategies against Fe toxicity were evolutionarily selected and can provide protection to the plant species to grow on Fe-rich soils. This review discussed the Fe detoxification strategies used by plants: (I) inhibition of Fe uptake through the formation of IP, (II) inhibition of Fe translocation to the stele by Casparian strips, (III) sequestration and compartmentalization of Fe in vacuoles, plastids and apoplasmic compartments that can be histolocalized through different histochemical tests, and (IV) tolerance to the generation of ROS.

We also showed that strategies can vary among species and cultivars, being pronounced to different degrees or even not important in some plants. Additionally, plants can have its homeostasis disturbed when dealing with Fe toxicity, affecting physiological, morphological and metabolic traits. In this context, a thorough understanding of Fe-excess effects on plants and their detoxification strategies should facilitate the development of new tools that allow the selection of Fe-tolerant species via conventional breeding or biotechnological strategies.

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