

Below-ground plant-soil interactions affecting adaptations of rice to iron toxicity

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March 07, 2024

Abstract

Iron toxicity is a major constraint to rice production, particularly in highly-weathered soils of inland valleys in sub-Saharan Africa where the rice area is rapidly expanding. Although there is wide variation in tolerance in the rice germplasm, progress in introgressing tolerance traits into high-yielding germplasm has been slow owing to the complexity of tolerance mechanisms and large genotype by environment effects. We review current understanding of tolerance mechanisms, particularly those involving below-ground plant-soil interactions, which to date have been less studied than above-ground mechanisms. We cover processes in the rhizosphere linked to exclusion of toxic ferrous iron by oxidation, and resulting effects on the mobility of nutrient ions. We also cover the molecular physiology of below-ground processes controlling Fe retention in roots and root-shoot transport, and also plant Fe sensing. We conclude that future breeding programs should be based on well-characterised molecular markers for tolerance traits. To successfully identify such markers, the complex tolerance response should be broken down into its components based on understanding of tolerance mechanisms, and tailored screening methods developed for individual mechanisms.

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Funding information

Biotechnology and Biological Sciences Research Council, Grant Ref. BB//R020388/1

Abstract

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Keywords

Oryza glaberrima , rice rhizosphere, root-soil interface, stress tolerance markers, submerged paddy soils

1 Introduction

Iron toxicity is a set of severely yield-limiting disorders associated with high concentrations of reduced ferrous iron (Fe(II)) in submerged lowland rice soils (Becker & Asch; 2005; Sahrawat, 2005). It is exclusively a problem of submerged soils, because the low redox potential associated with exclusion of O₂ from the soil leads to prevalence of reduced, soluble Fe(II), whereas in well-aerated soils, the dominant form is insoluble ferric Fe(III). It is particularly a problem on highly-weathered, nutrient-depleted soils rich in Fe oxides. These typify much of the current and potential rice area in sub-Saharan Africa, in contrast to the young, fertile, alluvial soils of the Asian lowlands, where most rice research has been done. Hence iron toxicity has not been a priority in much of the international rice breeding effort. However, it is a major constraint to rice in sub-Saharan Africa. Estimates of the rice area in sub-Saharan Africa affected by Fe toxicity vary from 20 to 60%, and estimated yield losses vary from 10 to 90% (Rodenburg et al., 2014; Sikirou et al., 2015). Ironically, most of the global hotspots of Fe toxicity in rice overlap with areas where Fe deficiency in human diets is acute, suggesting that currently-grown rice varieties are not effective in translocating excessively-available Fe from the soil into the grain (Frei et al., 2016).

There is large variation in tolerance of Fe toxicity in the rice germplasm, especially in the *O. glaberrima* species indigenous to West Africa (Sikirou et al., 2015), and in sub-species of *O. sativa* indigenous to Madagascar (Rakotoson et al., 2019). Modern high-yielding varieties are far more susceptible than locally-adapted but low-yielding traditional varieties. If tolerance traits in the indigenous African germplasm could be incorporated into improved varieties, this could have a huge impact on African rice productivity and the sustainable expansion of rice-based farming into new areas, and hence on overall African food security. However progress with breeding has been slow. Constraints include the complexity of the phenomenon, poor understanding of tolerance mechanisms, and a lack of reliable genetic markers for marker-assisted selection. The importance of particular mechanisms varies with the type of Fe toxicity, and there are multiple types and interactions with nutrient deficiencies.

Three distinct types of Fe toxic soil are recognised (Becker & Asch; 2005; Sahrawat, 2005):

1. acid sulphate soils in coastal plains and river deltas, in which there is also extreme acidity and Al toxicity;
2. clayey organic soils in swampy highland areas, in which the toxicity becomes acute later in the season as strongly reducing conditions develop, and so it tends to be less destructive; and
3. poor sandy to coarse-loamy soils in inland valleys, where there is upwelling of interflow water from adjacent highlands with highly-weathered soils, and the toxicity lasts throughout the growing season.

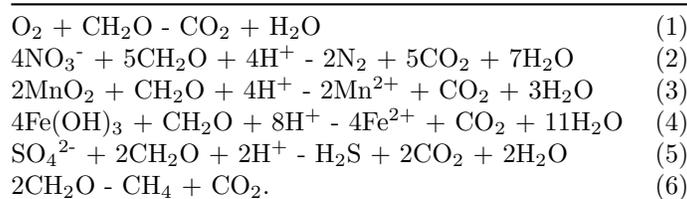
As a result of sensitivity to the local hydrology, there is often large field-scale heterogeneity in toxicity and dependence on inter-annual variability in rainfall. Further, there are often also deficiencies of mineral

nutrients, particularly P, K, Ca and Mg (Figure 1). These both compound the Fe toxicity and are exacerbated by it. Hence symptoms occur at widely differing Fe concentrations in the plant, associated with different soil types and landscape positions, and interactions with hydrology and nutrient levels.

Germplasm screening is complicated by large genotype by environment effects linked to these multiple interactions. Plant adaptations to the stress depend on complex below-ground plant-soil interactions. Hence yield losses are only weakly correlated with above-ground symptoms, though these are widely used for screening (Sikirou et al 2015). Most work on tolerance mechanisms has been done in hydroponics, far removed from field reality, and there has been limited progress with the genetics of tolerance and gene mapping (Dufey et al., 2015; Matthus et al., 2015; Melandri et al., 2021; Pawar et al., 2021; Sikirou et al., 2018). There is a need for an integrated approach to understand the mechanisms and genetics of Fe toxicity tolerance, taking account of the complex below-ground plant-soil interactions. In this review, we focus on these below-ground processes, and interactions between genotype adaptations and mineral nutrient deficiencies. Above-ground tolerance mechanisms have recently been reviewed by Aung & Masuda (2020) and Wu, Ueda, Lai & Frei (2017).

2 The soil chemistry of IRON toxicity

The following is a summary of the changes in soil chemistry following submergence for lowland rice, taken from Kirk (2004). When a soil is submerged, air is excluded and the soil quickly becomes anoxic. Microbes must then use alternative end electron acceptors in oxidizing organic compounds for their energy needs. This proceeds roughly in the sequence predicted by thermodynamics:



where CH_2O represents soil organic substrates. In the absence of O_2 Fe(III) is generally the main oxidant in the soil, its concentration typically exceeding concentrations of NO_3^- , MnO_2 or SO_4^{2-} by at least an order of magnitude. Between 1 and 20% and sometimes as much as 90% of the free Fe(III) oxides in the soil is reduced to Fe(II) over one to two months of submergence. Some of the structural Fe(III) in soil clays is also reduced. The course of soil reduction and the changes in redox and pH are therefore generally dominated by the reduction of Fe(III).

As reduction proceeds, H^+ ions are consumed in Reactions (2)–(5) and the pH tends to increase. Simultaneously CO_2 is produced, but escapes from the soil only very slowly, and it therefore accumulates to high partial pressures. The accumulation of CO_2 lowers the pH of alkaline soils and curbs the increase in pH of acid soils. As a result the pHs of most soils tend to converge following submergence in the range 6.5–7. Large concentrations of Fe^{2+} ions develop in the soil solution in the weeks following flooding, often several mM or tens of mM, balanced by HCO_3^- formed from dissolved CO_2 . The ion activity products of pure ferrous hydroxides, carbonates and other minerals are often exceeded 100-fold. Evidently precipitation of these minerals is inhibited, probably as a result of adsorption of dissolved organic matter and other solutes onto nucleation sites. However, once a sufficient supersaturation has been reached there is a rapid precipitation of amorphous solid phases containing Fe(II), which may later re-order to more crystalline forms. The exact nature of the Fe(II) compounds formed is uncertain. But there is evidence that mixed Fe(II)–Fe(III) hydroxides are formed, which have some of the observed properties of the solid phase Fe(II) found in reduced soils, including the greyish-green colours characteristic of reducing conditions.

However in highly-weathered iron toxic soils typical of inland valleys, reduction tends to be slower and the rise in pH causing precipitation of Fe(II) compounds is more gradual, so that the soil solution and exchange

complex continue to be dominated by Fe^{2+} ions (Kirk, Solivas & Alberto, 2003; Narteh & Sahrawat, 1999; Ponnampereuma, 1972). A major factor in this is that such soils generally have large reserves of acidity, and so the soil pH remains below the neutral range in which the solubility products of the Fe(II) compounds are exceeded. Also as a result of the acidity and slow reduction, concentrations of dissolved CO_2 tend to be smaller.

3 Basic adaptations to iron toxicity

3.1 Root growth

Growth of crown roots is inhibited under Fe toxicity through decreased cell elongation and division, and lateral root initiation is also inhibited (Yamauchi & Peng, 1995; Zhang et al., 2011). A smaller root surface area will restrict excessive Fe absorption, but at the cost of nutrient absorption. Root growth will also respond to the plant nutrient status (Section 5).

To the extent that there is a gradient of reducing conditions and Fe^{2+} concentration with depth through the soil, concentration of root growth at shallower depths might prevent serious Fe toxicity and allow controlled acclimation to nutritional stress (Li, Kronzucker & Shi, 2016a). Supporting this, lateral root formation on crown roots is unaffected by exposure of the crown root tip to high concentrations of Fe^{2+} . When the root tip is exposed to Fe stress, root gravitropism may change, favouring root expansion in shallower less-toxic soil.

These effects result from direct contact of the root tip with high concentrations of Fe^{2+} (Li, Kronzucker & Shi, 2016b). Ethylene and reactive oxygen species (ROS) are involved in most abiotic stress responses, but it is unknown whether ethylene acts alone or in conjunction with ROS in the response of root tips to high Fe (Li et al., 2016b). The root tip is also the primary sensing site for the effect on lateral root formation.

3.2 Root morphology and Fe^{2+} exclusion

Iron exclusion by oxygenation of the rhizosphere is a principal adaptation to Fe toxic soils. Because of the very slow diffusion of respiratory gases through water and submerged soil, rice roots must develop an efficient internal aeration system to deliver O_2 to submerged tissues and vent respiratory CO_2 in the opposite direction. As a root elongates, the cortex degrades forming a network of interconnected gas channels – aerenchyma – connected to lacunae at the shoot base (Yamauchi, Colmer, Pederson & Nakazono, 2018). Oxygen enters from the atmosphere above and diffuses through the aerenchyma to root tissues. In addition, the roots contain a barrier to radial O_2 loss in the basal zones. The barrier restricts O_2 loss, and thereby permits a greater length of root to be aerated. However, particularly in Fe toxic soils, some degree of O_2 loss to the rhizosphere is permitted to exclude Fe^{2+} by oxidizing it to Fe(III), and possibly for other aerobic processes in the rhizosphere.

Rice genotypes differ in their Fe excluding powers, due to differences in aerenchyma formation, barriers to radial O_2 loss, and enzymatic Fe oxidation power (Ando, Yoshida & Nishiyama, 1983; Engel, Asch & Becker, 2012; Mongon, Konnerup, Colmer & Rerkasem, 2014; Yamauchi et al., 2018). Aerenchyma formation is ‘constitutive’. Ethylene signalling has been implicated in the flood-induced enhancement of aerenchyma formation, with involvement also of H_2O_2 in the programmed cell death that forms the lacunae in shoots (Yamauchi et al., 2018). Wu et al. (2014) reported a QTL for Fe toxicity tolerance on Chromosome 3 associated with Fe exclusion via greater aerenchyma formation.

There is potentially a conflict between the rooting characteristics required for internal aeration and those for Fe^{2+} exclusion and efficient nutrient acquisition. Efficient nutrient uptake and Fe^{2+} exclusion are favoured by a large external root surface area (but see Section 4.2 for potential negative effects of root exclusion on nutrient uptake), whereas efficient internal aeration requires the opposite. Kirk (2003) developed a model for exploring this, based on steady-state diffusion of O_2 through a crown root and its laterals and the simultaneous consumption of O_2 in root respiration and loss to the soil. This showed, for a realistic set of parameter values, including rates of O_2 loss to the soil at typical rates of Fe^{2+} oxidation in the rhizosphere, a system of coarse, aerenchymatous, crown roots with gas-impermeable walls conducting O_2 to short, fine,

gas-permeable laterals provided the greatest absorbing surface per unit aerated root mass. This is the basic architecture of current rice genotypes.

3.3 — Membrane selectivity

Ferrous iron breaching the oxidation zone in the rhizosphere enters the root apoplast by diffusion and mass flow in the transpiration stream. To reach the xylem for transport to the shoot, Fe^{2+} ions in the apoplast must bypass the Casparian strip in the endodermis by crossing cell membranes into the symplasm. This provides a potential exclusion mechanism, at least in undamaged roots. Up to 87% of the Fe entering the root apoplast in mature plants is prevented from reaching the xylem at the endodermal barrier (Yamanouchi & Yoshida, 1981). Exclusion at the plasma membranes is strongly affected by respiration inhibitors, high Fe^{2+} concentrations, and nutrient stresses (Yoshida, 1981). Therefore, it is unlikely to be important under sustained severe toxicity (Becker & Asch, 2005) so other mechanisms of root retention must operate.

Rice is unusual in graminaceous species in possessing Fe^{2+} transporters, OsIRT1 and OsIRT2, in addition to the usual genes for the synthesis and secretion of Fe(III)-chelating phytosiderophores (Bugchio, Yamaguchi & Nishizawa, 2002; Ishimaru et al., 2006; Quinet et al., 2012). Transport of Fe^{2+} in the xylem involves complexation with nicotianamine (NA) and mugineic acid (MA), and rice possesses three NA synthase genes (OsNAS1, OsNAS2 and OsNAS3) (Inoue et al., 2003), six NA amino-transferase genes (OsNAAT1-6) (Inoue et al., 2008) and one deoxymugineic acid synthase gene (OsDMAS1) (Bashir, Ishimaru & Nishizawa, 2012). The results of microarray analyses have suggested that expression of genes involved in Fe^{2+} uptake (OsIRT1 and OsIRT2) and xylem transport are suppressed under different levels of Fe excess (Aung, Masuda, Kobayashi & Nishizawa, 2018a; Finatto et al., 2015; Quintet et al., 2012). The Fe-binding ubiquitin ligase HRZ is involved in the regulation of this process (Aung et al., 2018a).

3.4 — Retention of Fe in roots

In healthy roots, most of the Fe^{2+} absorbed is retained in the roots in metabolically-inactive forms (Yoshida, 1981). This is likely to involve sequestration in root-cell vacuoles (Moore et al., 2014; Stein, Ricachenevsky & Fett, 2014) and in the ferritin protein in plastids (Briat et al., 2010; da Silveira et al., 2009; Stein, Ricachenevsky & Fett, 2009). Rice ferritin genes (OsFER1 and OsFER2) are strongly up-regulated in roots and shoots by excess Fe (Aung et al., 2018a; Finatto et al., 2015; Quintet et al., 2012). Regulation of ferritin genes in response to Fe excess occurs at the transcriptional level (Stein et al., 2009; Briat et al., 2010), and involves regulatory pathways mediated by ABA, ROS and ethylene. However, Majerus, Bertin & Lutts (2009) showed that in *O. glaberrima* a signalling pathway leading to the induction of ferritin synthesis depended neither on ABA nor ROS.

In plants taking up chelated Fe(III), increased apoplastic pH depresses ferric chelate reductase activity, restricting Fe(III) reduction to Fe(II) and thereby restricting Fe^{2+} mobility (Kosegarten et al., 2004). Failure to regulate apoplastic pH or suppress ferric chelate reductase activity may result in uncontrolled accumulation of Fe^{2+} in shoots (Becker & Asch, 2005). Acidification of the rhizosphere as a result of Fe^{2+} oxidation and excess cation over anion intake (Section 3.1) will tend to lower the pH in the root apoplast. The cation-exchange capacity of root cell walls is also a factor. Cell-wall components with negative surface charges (pectin and hemicelluloses) possess cation exchange sites, for which Fe^{2+} ions compete with other cations (Li et al., 2016b). The cation exchange capacity will decrease as the apoplastic pH decreases.

The effectiveness of this adaptation is limited by the Fe^{2+} storage capacity of the root tissues, particularly towards the end of the growth cycle (Becker & Asch, 2005). Wu et al. (2017) studied regulation of genes controlling Fe uptake, partitioning and storage in a tolerant and a sensitive genotype in nutrient culture, and found no genotype differences in Fe concentration and speciation in different plant tissues, nor in sub-cellular partitioning genes.

3.5 — Sensing of plant Fe status

Until now, the mechanisms of Fe sensing in plants have mostly been investigated under Fe deficient conditions rather than under Fe excess. In both rice and Arabidopsis (*Arabidopsis thaliana*), a group of proteins

containing the hemerythrin motif are involved in sensing Fe deficiency. In rice, Hemerythrin motif-containing RING- and zinc-finger protein 1/2 (OsHRZ1/2), directly bind to Fe and functions as a molecular sensor for Fe status (Kobayashi et al., 2013). OsHRZ1/2 proteins negatively affect Fe deficiency responses, and knockdown of these proteins renders plants less susceptible to Fe deficiency, probably by destabilizing a basic helix-loop-helix (bHLH) transcription factor OsPRI1 (Zhang et al., 2017). Likewise, a homologue of OsHRZ1/2 in Arabidopsis, BRUTUS, negatively affects the stability of PRI1 homologues (Arabidopsis bHLH105/115) and Fe deficiency responses (Selote et al., 2015), indicating that the Fe sensing mechanisms via the hemerythrin motif-containing proteins are conserved among higher plant species.

A recent study showed that knockdown of OsHRZ2 leads to susceptibility to Fe excess stress in rice. The OsHRZ2-knockdown plants were associated with greater growth retardation, leaf bronzing and shoot Fe concentrations compared with the wild-type plants under Fe excess condition (Aung, Kobayashi, Masuda & Nishizawa, 2018b). Correspondingly, the expression of Fe deficiency-inducible genes associated with Fe uptake, such as OsNAS1/2, mugenic acid transporter OsTOM1, and Yellow Stripe-Like 15 (OsYSL15) that encodes a Fe transporter, were higher in the OsHRZ2 knockdown plants than the wild-type under the Fe excess (Aung et al., 2018b). Therefore, OsHRZ2, and probably OsHRZ1 as well, are at least partly involved in sensing excess Fe, and precise sensing of Fe status by these proteins is likely to be important for tolerance. Other components involved in sensing Fe status have been elucidated, such as the Fe-binding, graminaceous species-specific transcription factor iron deficiency-responsive elements factor 1 (IDEF1) (Kobayashi et al., 2007, 2012) and short peptides IRON MAN (IMA) that are ubiquitously found in flowering plant lineage (Grillet et al., 2018; Kobayashi, Nagano & Nishizawa, 2021). IDEF1 is classified into the ABI3/VP1 transcription factor family which binds to Fe²⁺ ion via its His-Asp repeats and Pro-rich regions (Kobayashi et al., 2012), and the overexpression of rice homologue (OsIDEF1) which leads to enhanced tolerance to Fe deficiency (Kobayashi et al., 2007). Overexpression of Fe deficiency-inducible IMA homologues in rice (OsIMA1/2) enhances the expression of many Fe deficiency-inducible Fe uptake-related genes in roots (Kobayashi et al., 2021). In Arabidopsis, IMA1-overexpression lines hyperaccumulate Fe and exhibit foliar necrotic symptoms under the standard Fe conditions, indicating Fe toxicity (Grillet et al., 2018). It remains to be clarified whether these Fe sensing mechanisms are functional under the Fe excess condition and linked with the tolerance in rice.

A recent GWAS based study on root shortening induced by excess Fe and a subsequent mutant study revealed that S-nitrosoglutathione reductase (GSNOR) is involved in sensing Fe status at the root tip (Li et al., 2019). GSNOR alleviates Fe-dependent production of nitric oxide and hydrogen peroxide, and resultant inhibition of root meristem growth. Naturally occurring polymorphism in the promoter region of GSNOR gene was associated with its expression level, and the allele of GSNOR that yielded higher expression conferred tolerance. Consistently, the knockout of rice homologues of GSNOR led to higher susceptibility to Fe excess, in terms of root elongation (Li et al., 2019). It remains to be determined if naturally occurring alleles of GSNOR in rice could be used to increase the tolerance of currently grown rice cultivars and enhance biomass production and grain yield.

4 — Effects of iron toxicity on mineral nutrition

4.1 — Root-induced change in the rhizosphere

The following root-induced changes in the soil occur in the region of the root tip and along the length of fine laterals where the bulk of nutrient uptake happens (Figure 2c).

Ferrous iron oxidation. Oxygen diffusing down through a root's aerenchyma leaks out into the soil, which has a much lower O₂ concentration. Mobile inorganic reductants in the soil are oxidized, particularly Fe²⁺ which is precipitated as Fe(OH)₃ on or near the root. As a result the concentration of Fe²⁺ near the root falls and more Fe²⁺ moves in from the bulk soil by diffusion and mass flow in the transpiration stream. This is then oxidized resulting in a zone of Fe(OH)₃ accumulation near the root (Begg, Kirk, Mackenzie & Neue, 1994). Each mol of Fe²⁺ oxidized generates 2 mol of H⁺ (Figure 2c), so the pH in the oxidation zone tends to fall. Away from the root tip, in zones where there is no release of O₂ from the roots, Fe(OH)₃ may be

re-reduced to Fe^{2+} in anaerobic respiration fuelled by organic substrates released from the roots (Benckiser, Santiago, Neue, Watanabe & Ottow, 1984). This effect may be greater under nutrient deficiencies as a result of leaky root membranes, resulting in exacerbated Fe toxicity.

CO₂ uptake into roots. Large dissolved CO₂ concentrations develop in submerged rice soils (equivalent partial pressures 5–70 kPa – Greenway, Armstrong & Colmer, 2006; Kirk et al., 2019; Ponnampertuma, 1972) because CO₂ formed in root and soil respiration escapes only slowly by diffusion through the water-filled soil pores. There is therefore a large CO₂ gradient between the soil and the aerenchyma inside the root. Hence CO₂ will enter the roots and be vented to the shoots and atmosphere by diffusion through the aerenchyma. Kirk et al. (2019) showed that CO₂ venting through rice roots can be equivalent to a third of the daily CO₂ fixation in photosynthesis. Net removal of CO₂ decreases the concentration of the acid H₂CO₃ near the root, and this may offset the acidity produced in Fe²⁺ oxidation and excess cation uptake (Affholder, Weiss, Wissuwa, Johnson-Beebout & Kirk, 2017; Begg et al., 1994).

Proton release due to excess cation over anion intake. Because the main form of plant-available N in anaerobic soil is NH₄⁺, the root absorbs an excess of nutrient cations (NH₄⁺, K⁺, Na⁺, Ca²⁺, Mg²⁺, Fe²⁺) over anions (H₂PO₄⁻, Cl⁻, SO₄²⁻). Consequently H⁺ is released by the root to maintain electrical neutrality, tending to further decrease the pH. Note that if any N is taken up as NO₃⁻ as a result of nitrification of NH₄⁺ in the rhizosphere (see below), the net acid-base change is the same because, although the root exports 2 mol less H⁺ for each mol of NO₃⁻ replacing a mol of NH₄⁺, 2 mol of H⁺ are formed in the nitrification of each mol of NH₄⁺. Note also that Si, which is taken up in large quantities by rice plants, crosses the root as the uncharged H₄SiO₄ molecule ($pK_1 = 9.46$ at 25°C).

Net changes. The net effects of these processes will depend on their rates versus rates of buffering processes in the soil. In typical, acidic Fe toxic soils, the dominant effect is likely to be a decrease in pH at the root surface due to the large amount of H⁺ produced in Fe²⁺ oxidation. Begg et al. (1994) found the rhizosphere of rice in an Fe toxic soil was acidified by two pH units from 6.5 in the soil bulk to 4.5 at the root surface. The pH profile across the rhizosphere will depend on the net rate of H⁺ generation near the root versus the rate at which the pH change is propagated away through the soil by acid-base transfer. Generally, the main acid-base pairs involved are H₃O⁺–H₂O and H₂CO₃–HCO₃⁻: H₃O⁺ ions move away from the acidification zone to the soil bulk which has a higher pH, and HCO₃⁻ towards it. The resulting pH change will be greatest in the pH range in which H₃O⁺ and HCO₃⁻ concentrations are both low (typically pH 4.5–6.0, depending on the soil CO₂ pressure), as shown in the model calculations in Figure 2d,e. Hence the pH decrease will be greater in Fe toxic soils with already low pH and relatively low dissolved CO₂.

Although Fe plaque on root surfaces is generally focused on in the literature, most of the Fe(OH)₃ is precipitated in the rhizosphere over a mm or two from the root surface (Kirk, Ahmad & Nye, 1990). The profile of Fe(OH)₃ may be banded in ‘Liesegang’ rings centred on the root axis. This happens because sorption of Fe²⁺ on the soil solid – and hence its mobility and rate of oxidation – is pH dependent, decreasing as the pH decreases. Therefore some of the Fe²⁺ locally diffuses ahead of the oxidation front towards the higher pH zone, where it is more-strongly sorbed. The total Fe profile is therefore banded.

— Effects of root-induced changes on nutrient ions in the rhizosphere

The sharp gradients in redox and pH across the rhizosphere resulting from the above changes will greatly affect its chemistry and biology, and hence nutrient and toxin uptake by roots.

Ammonium and nitrate. In some circumstances, a significant part of the N taken by rice in submerged soils is as NO₃⁻ formed by nitrification of NH₄⁺ in the rhizosphere (Kirk & Kronzucker, 2005). Studies on the kinetics of N uptake and assimilation show that lowland rice is exceptionally efficient in absorbing and assimilating NO₃⁻ compared with other plant species (Kronzucker, Siddiqi, Glass & Kirk, 2000). This is important because plant growth and yield are generally improved when plants absorb their N as a mixture of NO₃⁻ and NH₄⁺ compared with growth on either N source on its own. In Fe toxic soils, rates of nitrification in the rhizosphere are likely to be impeded both by competition for root-released O₂ with Fe²⁺ oxidation, and because the rhizosphere is acidified. The proportion of N absorbed as NO₃⁻ will be correspondingly

lowered.

There may be a further effect on N nutrition through an interaction with CO₂ venting through the roots (Kirk et al., 2019). Concentrations of dissolved CO₂ tend to be smaller in Fe toxic soils because of lower rates of microbial respiration (Section 2). But in submerged soils with high dissolved CO₂ concentrations, enhanced availability of CO₂ in the roots may have a growth stimulating effect by facilitating anaplerotic production of organic acids for amino acid synthesis (Balkos, Britto & Kronzucker, 2010; Britto & Kronzucker, 2005). This is potentially important because all the NH₄⁺ taken up by rice is assimilated into amino acids in the roots before being transported to the shoots, requiring carbon skeletons (Kronzucker et al., 2000). Rice in Fe toxic soil with lower dissolved CO₂ concentrations would not benefit from this.

Exchangeable cations. An unexplored consequence of the changes in the rhizosphere, likely to be important in Fe toxic soils, is the effect on nutrient cations such as K⁺, Ca²⁺ and Mg²⁺. The following factors will tend to decrease the concentration of nutrient cations in the soil solution where they are available for uptake by roots.

1. The overall concentration of the solution in a submerged soil is generally controlled by the concentration of bicarbonate anions (HCO₃⁻), formed from dissolved CO₂ (Kirk 2004). If the pH decreases below about 6.0, the concentration of HCO₃⁻ in solution will decrease and so the concentration of cations in solution balanced by the anions must also decrease.
2. A decrease in pH will also mean the negative charge on soil surfaces and therefore the cation exchange capacity will tend to decrease.
3. However, removal of exchangeable Fe²⁺ as it is oxidised will mean a greater proportion of surface exchange sites is occupied by non-Fe cations.

Hence the need to exclude toxic Fe²⁺ from the root by oxidizing it in the rhizosphere may impair the absorption of nutrient cations by the root. Genotypes with greater Fe oxidizing power may make things worse for themselves in Fe toxic soils deficient in nutrient cations.

Phosphate and other ions. Phosphate anions may be immobilised on iron plaque and freshly precipitated Fe(OH)₃ in the rhizosphere. On the other hand, in P-deficient soils, acid-soluble forms of P in the soil may be solubilized by acidification of the rhizosphere, resulting in increased uptake into roots (Jianguo & Shuman, 1991; Saleque & Kirk, 1995). Likewise acid-soluble forms of Zn may be solubilized in Zn-deficient soils (Kirk & Bajita, 1995). Possibly uptake of micro-nutrients is enhanced at low levels of Fe plaque as a result of a concentrating effect close to absorbing root surfaces (Kirk & Bajita, 1995; Zhang, Zhang & Mao, 1996, 1999).

4.3 — Nutrient uptake antagonisms and effects of accompanying anions

High concentrations of Fe²⁺ at the root surface may suppress uptake of other cations through antagonistic effects (Wu, Holtkamp, Wairich & Frei, 2019). Plants suffer from ionic imbalance through competition between similarly charged cations for binding and carrier sites. In submerged soils, the main anion balancing cations in solution is generally HCO₃⁻ (Section 2). A decrease in the rhizosphere pH as above and consequent decrease in HCO₃⁻ concentration means that any Fe²⁺ entering the root will be accompanied by a proportion of Cl⁻ or SO₄²⁻ rather than HCO₃⁻. If Fe²⁺ enters with HCO₃⁻, acidity generated in Fe²⁺ oxidation in the plant will be neutralized by conversion of HCO₃⁻ to CO₂, which is assimilated or lost. Whereas if it enters with a non-volatile anion, Fe²⁺ oxidation will produce the equivalent amount of free H⁺ in the plant, with damaging effects on plant tissues (van Mensvoort, Lantin, Brinkman & Van Breemen, 1985). We know of no studies exploring this effect. Yamauchi (1989) found the severity of Fe toxicity in rice in Fe toxic soils supplied with K₂SO₄ was less than that with KCl, but he attributed the effect to reduction of sulphate to sulphide in the soil and precipitation of Fe(II) sulphides, lowering the concentration of Fe²⁺ in solution and hence its uptake.

5 — Effects of mineral nutrition on iron toxicity

5.1 — Phosphorus

Severe P deficiency is uncommon in lowland rice on young alluvial paddy soils because the soil P reserves are solubilized in reductive dissolution reactions following soil submergence, and the P is therefore readily-available to the crop. It is much more common in highly-weathered soils of inland valleys (Figure 1), which typically have very low extractable P contents. Phosphorus deficiency interacts with Fe toxicity in various ways, tending to exacerbate the direct effects (Sahrawat, 2005). Phosphorus deficiency causes delayed phenological development in rice by up to a month (Dobermann & Fairhurst, 2000; Vandamme et al., 2018). In soils for which the Fe toxic conditions develop very slowly following submergence, delayed phenology may mean late season exposure to the stress that is otherwise be avoided. On the other hand, under chronic low-level exposure in acid sandy soils, slower growth might mean less Fe is accumulated in the plants and they have longer to acclimatise to it.

Phosphorus deficiency typically results in increased root: shoot ratios, and increased root surface area means increased exposure to Fe^{2+} . Kirk & Du (1997) found P deficiency in deoxygenated nutrient culture caused increased primary root porosity and an increased proportion of fine lateral roots compared to P-sufficient plants, resulting in in two-fold greater rates of O_2 release per plant. Under Fe toxic conditions this could compensate for the greater exposure to Fe^{2+} . Likewise, Fu, Yang & Shen (2014) found greater root oxidizing capacity under P deficiency. Phosphorus deficiency also results in impaired cell membrane integrity and therefore loss of organic substrates into the rhizosphere (Rose et al., 2013). This may fuel re-reduction of $\text{Fe}(\text{OH})_3$ previously oxidized by O_2 release from the root tip zone, so exacerbating Fe^{2+} toxicity (Benckiser et al., 1984).

5.2 — Potassium

The levels of potassium and other cationic nutrients such as Mg^{2+} also tend to be low in highly-weathered inland valley soils and deficiencies in rice are common. Potassium deficiency exacerbates Fe toxicity through impaired root oxidising power and Fe exclusion, decreased crown root growth and lateral root formation, and decreased Fe^{2+} storage in roots (Li, Yang & Luo, 2001; Suriyagoda, Tränkner & Dittert, 2020). It is involved in maintaining normal activity of peroxidase, which breaks down H_2O_2 formed in the Fenton reaction in response to excess Fe (Wu et al., 2017). As with P deficiency, impaired membrane permeability under K deficiency may cause leakage of organic substrates into the rhizosphere, and resulting increased reducing conditions and Fe^{2+} formation.

Relations between high Fe^{2+} and rice K^+ transporters have been investigated. OsAKT1 is thought to be the most important root K^+ transporter in rice (Golldack, Quigley, Michalowski, Kamasani & Bohnert, 2003; Li et al., 2014). Genome Wide Association Studies (GWAS) have suggested OsAKT1 is associated with shoot Fe regulation (Matthus et al., 2015). In studies with mutants lacking OsAKT1, under Fe toxic conditions root K uptake decreased and root to shoot Fe translocation increased, resulting in increased leaf bronzing, increased ROS damage, reduced chlorophyll content and impaired photosynthesis (Wu et al., 2019). Adequate K uptake protects rice plants under Fe stress via the regulation of Fe translocation from the root to shoot.

5.3 Calcium and magnesium

The factors governing Ca and Mg deficiencies, their behaviours following soil submergence, and the amounts taken up and removed by rice crops are all similar (Dobermann & Fairhurst, 2000). Likewise the root-induced changes in the soil under Fe toxicity should affect Ca and Mg similarly. Yet deficiencies of Mg in association with Fe toxicity are more common (Fig. 1). This may reflect the relative availabilities of soil amendments containing Ca and Mg. Calcium is applied in phosphate fertilizers and in lime (CaCO_3) which is more-widely available than dolomite ($\text{CaCO}_3 + \text{MgCO}_3$) or other Mg-containing soil amendments, and is widely used to correct acidity in highly-weathered soils prone to Fe toxicity.

Magnesium deficiency causes a characteristic yellow-orange coloration of the leaves (Fig. 2b), as opposed to the bronzing and dark brown streaks of direct Fe toxicity (Fig. 2a). It is induced or exacerbated by high levels of Fe^{2+} in the soil solution as a result of the root-induced change in the rhizosphere described in Sections 4.1 and 4.2, and uptake competition effects. In already-deficient soils, symptoms of Mg deficiency become apparent as the solution Fe^{2+} concentration increases, and large plants are more seriously affected

than small ones, consistent with a greater plant demand for Mg if other nutrients are less growth-limiting. Magnesium deficiency disrupts Fe²⁺ storage in leaf cell vacuoles (Kobayashi et al., 2018), and Mg is involved in defence against ROS damage (Hauer-Jákli & Tränkner, 2019). A combination of this indirect and direct Fe-toxicity produces plants with a wide range of intensities of orange symptoms and brown streaks on the leaves.

5.4 Silicon

Low Si levels are also a feature of highly-weathered acid soils. Although a ‘beneficial’ rather than an essential nutrient, it is taken up in large amounts by rice plants, and applications to rice under Fe toxic conditions have been found to reduce Fe uptake and produce fewer leaf symptoms (Becker, Ngo & Schenk, 2020; dos Santos et al., 2020; Dufey, Gheysens, Ingabire, Lutts & Bertin, 2014). Its specific role in plant metabolism is not well understood, but probably involves a stabilising effect on cell walls as an inert deposition in lignified cells and also by modulating lignin biosynthesis (Broadley et al., 2012). Becker et al. (2020) found decreased Fe²⁺ uptake on addition of Si to nutrient culture, which they attributed to enhanced formation of the root exodermal Casparian strip. Chalmardi, Abdolzadeh & Sadeghipour (2014) found Si application improved antioxidant enzyme activity under Fe toxic conditions.

6 implications for rice breeding

6.1 Tolerance within the *Oryza* gene pool

Numerous studies have screened sets of varieties or larger germplasm collections in attempts to identify donors for Fe toxicity, typically employing one of two main screening strategies. They may have been conducted in the field in Fe toxic hotspots, evaluating grain yield in addition to biomass and leaf symptoms (Melandri et al., 2021; Pawar et al. 2021; Sikirou et al. 2018), or they were designed as rapid screens in nutrient solution to which excess Fe had been added at high concentrations for short periods of time (Dufey et al. 2015; Matthus et al. 2015). From these and other studies it can be concluded that ample genetic variation for tolerance to Fe toxicity exists within the *O. sativa* gene pool (Matthus et al. 2015; Pawar et al. 2021), and that wild relatives may serve as sources of novel traits enhancing adaptation to Fe toxicity (Bierschenk et al. 2020; Wairich et al., 2021).

The indigenous African rice *O. glaberrima*, domesticated from its wild ancestor *O. barthii* about 3500 years ago (Heuer et al., 2003), includes accessions tolerant of a wide range of abiotic stresses, including Fe toxicity (Linares et al., 2002; Melandri et al., 2021; Sié et al., 2012; Sikirou et al., 2018). Sikirou et al. (2018) evaluated more than 2000 *O. glaberrima* accessions in the Africa Rice Center gene bank at multiple Fe toxic hotspots across West Africa, and identified highly tolerant accessions such as TOG 7250-A, TOG 14367, TOG 7206 and TOG 6218-B. Melandri et al. (2021) confirmed the tolerance of some of these accessions. This suggests transferring genes/alleles conferring Fe toxicity tolerance from *O. glaberrima* to high yielding *O. sativa* varieties as a promising breeding strategy. However, spikelet sterility in progenies of *O. glaberrima* and *O. sativa* interspecific crosses has been a major challenge for breeders (Ndjiondjop et al., 2018; Sié et al., 2012).

6.2 Breeding strategies

Iron toxicity in the field is rarely uniform across the larger fields needed to evaluate breeding populations and the severity of stress may vary from year to year. Where such environmental variation reduces the reliability of phenotyping, marker assisted selection rather than conventional phenotypic selection should be the breeding method of choice. Markers associated with aspects of Fe toxicity tolerance exist, identified through genome-wide associations studies (GWAS) (Matthus et al. 2015; Pawar et al. 2021; Melandri et al., 2021) or through bi-parental QTL mapping populations (Dufey et al. 2015; Rasheed et al., 2020 and references therein). To our knowledge none of these studies identified loci with large enough effects to be utilized in applied breeding.

Additionally, QTL mapping for Fe toxicity has been done in the lowland NERICA (NEw RICE for Africa) hybrids of *O. glaberrima* and *O. sativa* with the aim to identify markers linked to *O. glaberrima*-derived

tolerance loci. Two varieties (NERICA-L19 and NERICA-L43) known to be highly tolerant of Fe toxicity (Dramé et al., 2011; Ndjiondjop et al., 2018) were used to develop mapping populations with recurrent parent IR64, but again, only minor QTLs were identified (Melandri et al., 2021).

Based on QTL mapping, GWAS and transcriptome analysis, it appears that tolerance of Fe toxicity in rice is a complex trait controlled by multiple genes distributed throughout the genome (Diop et al., 2020; Melandri et al., 2021; Wairich et al., 2021). In order to successfully identify markers of utility in rice breeding, it may be necessary to break down the complex tolerance response into component traits based on the tolerance mechanisms outlined above, and to develop tailored screening methods for such individual tolerance mechanisms.

It should be noted that these should take the complexities of below-ground plant-soil interactions into account rather than focusing predominantly on the visible Fe toxicity symptoms in leaves. Nozoe, Agbisit, Fukuta, Rodriguez & Yanagihara (2004) compared selections from the same breeding population made either in the field (for grain yield) or in nutrient solution (for leaf bronzing) and showed that the yield advantage under Fe toxicity of best lines selected in the field was 45% or more over the sensitive parent IR64, whereas best lines selected in nutrient solution provided no gain in grain yield in the field.

7 Conclusions

1. There is ample variation in tolerance of Fe toxicity in both *O. sativa* and *O. glaberrima* gene pools for identifying donors and markers for breeding. However to date, no markers have been identified with large enough effects to be utilized in applied breeding programs.
2. Germplasm screening is complicated by large genotype by environment effects and weak correlation between visible symptoms and beneficial stress response strategies, including below-ground plant-soil interactions.
3. Severe nutrient deficiencies are the norm in most Fe toxic soils, and applications of N, P and K fertilizers are essential for reasonable rice yields. Toxicity symptoms become more apparent as nutrient deficiencies are alleviated.
4. Genotypes whose tolerance depends on Fe exclusion by oxidation in the rhizosphere make deficiencies of cationic nutrients (such as K and Mg) worse because of the accompanying acidification of the rhizosphere and resulting impairment of cation mobility. Genotypes with enhanced Fe storage in roots and stems may be better suited to such conditions.
5. There has been recent progress in understanding the molecular physiology of tolerance mechanisms, including below-ground processes controlling Fe retention in roots and root-shoot transport, as well as above-ground partitioning and tissue tolerance. Each of these interacts in specific ways with nutrient deficiencies.
6. To successfully identify markers for use in breeding, the complex tolerance response should be broken down into component traits based on the tolerance mechanisms outlined above, and tailored screening methods for individual tolerance mechanisms developed.

Acknowledgments

This research was funded by a grant from the UK's Biotechnology and Biological Sciences Research Council 'Mechanisms and genetics of iron toxicity tolerance in African rice' (Grant Ref. BB/R020388/1).

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Figure 1 Nutrient concentrations in rice in Fe toxic soils. Data are for leaves or shoots during maximum tillering to flowering stages at inland valleys sites in Colombia (1 site, 1 genotype, 6 nutrient treatments; Howeler 1973), Cote d'Ivoire (2 sites, 3 genotypes, 2 nutrient treatments; Sahrawat et al., 1986, 2000), Indonesia (1 site, 1 genotype, 1 nutrient treatment; Jugsujinda. & Patrick, 1993), Madagascar (1 site, 5 genotypes, 3 nutrient treatments; the authors, unpublished) and Nigeria (3 sites, 4 genotypes, 2 nutrient

treatments; Yamauchi, 1989). Boxes indicate 25th, 50th and 75th percentiles; whiskers 10th and 90th percentiles; red crosses are typical deficiency limits and Fe and Mn toxicity limits (Dobermann & Farirhurst, 2000).

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Figure 2 Root-induced changes in the rhizosphere of rice in iron toxic soil. (a) Leaf bronzing and stunted growth due to Fe toxicity. (b) Leaf bronzing and orangeing due to Fe toxicity with Mg deficiency. (c) Root effects on the soil: (1) oxidation of ferrous iron by O₂ released from the roots; (2) venting of soil CO₂ into the root aerenchyma and associated changes in soil carbonate equilibria; (3) excess intake by the root of nutrient cations (particularly ammonium, NH₄⁺) over anions and associated release of H⁺; (4) in the zone where there is no O₂ release, re-reduction of ferric iron fuelled by organic substrates (represented as CH₂O) diffusing across leaky root membranes. Note the protons (H⁺ ions) consumed or produced in these reactions will be buffered by proton-donating or -accepting groups in the soil solid. (d) Calculated pH profiles in the rhizosphere for a given flux of H⁺ due to the above root-induced changes at different initial soil pH values (dotted lines) (Kirk, 2004). (e) Sensitivity of pH changes at the root surface to important variables, varied individually from standard values as in Panel (d).